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## Abstract

This study evaluated the effects of graded dietary calcium (Ca) and available phosphorus (AvP) regimens on production performance, egg quality, skeletal integrity, and mineral utilization efficiency in Hy-Line Brown® laying hens from 16 to 70 weeks of age, to estimate phase-specific optimal Ca and AvP levels. Experimental diets were formulated in two feeding phases (Phase 1: weeks 16-48; Phase 2: weeks 50-70). Within each phase, five Ca-AvP regimens representing approximately −20%, −10%, standard, +10%, and +20% deviations from the phase-specific recommendations were prepared and designated as CaP80, CaP90, CaP100, CaP110, and CaP120, respectively. A total of 180 pullets were allocated to treatments in a completely randomized design with six replicates per treatment and six hens per replicate. Results revealed that growth performance and egg production were unaffected by dietary Ca and AvP regimens throughout the experimental period. At 70 weeks of age, eggshell quality traits, including shell thickness and shell strength, were highest ($p < 0.05$) in hens fed CaP120; however, values in CaP90 and CaP100 were comparable. Tibial breaking strength and tibial mineral concentrations (Ca and P) showed similar responses, with no differences among CaP90, CaP100, CaP110, and CaP120 treatments. In contrast, ileal digestibility of crude ash, Ca, and P was improved ($p < 0.05$) in hens fed CaP80 and CaP90 diets. Regression analyses further refined dietary mineral recommendations. Linear-plateau and quadratic-plateau models indicated that higher Ca-AvP regimens were required to maximize skeletal mineralization and eggshell quality, with phase-specific optima ranging from 3.53-3.58% Ca and 0.30% AvP in Phase 1 (week 48) and 3.64-4.05% Ca and 0.29-0.32% AvP in Phase 2 (week 70). In contrast, quadratic regression analyses of ileal nutrient digestibility showed maximal absorption of crude ash, Ca, and P at lower dietary concentrations, typically 3.05-3.20% Ca and 0.26-0.27% AvP in Phase 1 and 3.09-3.53% Ca and 0.25-0.28% AvP in Phase 2. These findings support precision, phase-specific mineral feeding strategies that maintain performance and skeletal health while improving mineral utilization efficiency and reducing P oversupply in commercial layer production.

**Keywords:** Available phosphorus, calcium, laying hens, regression models, tibial mineralization

# Introduction

Calcium (Ca) and phosphorus (P) are essential macro minerals in laying hens, required for eggshell formation, skeletal integrity, and key metabolic processes such as muscle contraction, nerve transmission, and enzyme activation [1-3]. These two minerals are closely interrelated, and an imbalance (either a deficiency or excess) of one can impair the absorption and utilization of the other. Their interaction plays a critical role in regulating the metabolism and homeostasis of both nutrients [4-6]. Therefore, their dietary concentrations must be carefully maintained to ensure optimal physiological function and productivity.

The high egg-laying rate of commercial hens (about one egg per day) demands extensive Ca mobilization for eggshell calcification and P for essential metabolic functions [5]. Laying hens secrete approximately 2.4 g of Ca into the uterine fluid to form an average eggshell, of which around 70% is supplied through intestinal absorption of dietary Ca, while the remaining 30% is mobilized from bone resorption [7]. This underscores the importance of adequate dietary Ca intake in sustaining eggshell formation and preventing bone depletion. Inadequate Ca intake reduces laying rate, and prolonged deficiency has been linked to decreased egg production, poor eggshell quality, and an increased risk of osteoporosis [1,8-10]. Phosphorus is likewise critical, as it supports energy metabolism (ATP generation) and plays a key role in follicle maturation and ovulation in hens. An inadequate supply of available P (AvP) can disrupt normal ovulation, lead to irregular laying cycles, and contribute to bone disorders [11-13]. However, excessive Ca or P intake can cause reciprocal disruption of mineral homeostasis, consequently reducing productivity and egg quality, impairing skeletal health, increasing feed costs, and elevating P excretion into the environment [14-16]. Hence, optimizing dietary levels of these minerals is critical for sustaining egg production, maintaining shell quality, and preventing skeletal disorders in commercial laying hens. Nutrition guidelines for laying hens, therefore, typically reflect these mineral requirements, and commercial layer diets are commonly formulated with relatively high Ca levels (3.25-4.00%) and moderate AvP (0.25-0.45%) to achieve a Ca: AvP ratio ranging from approximately 9.5:1 to 13:1 [17-19]. In the local context, the Korean Feeding Standard for Poultry [20] recommends levels near the upper limit of this range, with 3.80-3.90% Ca and 0.31-0.32% AvP (approximately 12-13:1 Ca: AvP ratio).

Despite well-established nutritional guidelines, there remains a need to evaluate how moderate, phase-specific deviations from recommended dietary Ca and AvP levels influence layer performance and physiology across extended laying cycles. Most previous Ca-P studies have been limited to short-term feeding periods or focused on isolated parameters (i.e., egg production or bone traits) [1,10,11], often without modeling nutrient requirements. In contrast, the present study uniquely examined the effects of graded dietary Ca and AvP levels formulated as ±10% and ±20% deviations from the Korean Feeding Standard for Poultry [20] on growth, egg production, egg quality, tibial bone traits, and ileal nutrient digestibility in Hy-Line Brown® laying hens reared from 16 to 70 weeks of age. To estimate the optimal Ca and AvP levels that support key physiological functions, we employed three regression modeling approaches: linear-plateau, quadratic-plateau, and quadratic. These models were applied to the data to derive biologically meaningful estimates of Ca and AvP requirements. We hypothesized that moderate reductions in dietary Ca and AvP would not impair performance, egg quality, or skeletal integrity, and that nutrient utilization efficiency would be maximized below current recommendation levels. The outcomes of this study aim to refine mineral nutrition guidelines for commercial layers and to contribute to more sustainable, welfare-conscious feeding strategies under extended production conditions.

## Materials and methods

All experimental procedures, animal care, and handling practices adhered to the institutional guidelines for the ethical use of animals in research and were approved by the Animal Ethics Committee of Chungnam National University (Protocol Number: 202304A-CNU-025). Further, the layer hens were reared and cared for according to the Hy-Line Brown management guide [18].

### Layer hens, housing, and management

A total of 180 Hy-Line Brown pullets (16 weeks of age) were sourced from a single commercial flock and used throughout the 54-week experimental period. At allocation, hens had uniform initial body weights (1125.11 ± 10.48 g) and were randomly assigned to five dietary treatments (CaP80, CaP90, CaP100, CaP110, CaP120), with six replicate cages per treatment and six birds per cage (n=36 hens/treatment). Hens were housed in raised, enriched cages equipped with perches, nipple drinkers (two per cage), and a front-mounted metal feed trough. Environmental conditions were controlled according to commercial layer management standards, maintaining a temperature of 20-22 °C and relative humidity of 55-65%. A 16 h light:8 h dark lighting schedule was applied throughout the study. Hens had *ad libitum* access to drinking water, and feed was supplied on a restricted basis (110 g/hen/day) in accordance with Hy-Line Brown management recommendations [18]. The experiment consisted of two production phases: phase 1 (16-48 weeks), followed by a 2-week transition period (48-50 weeks) where all birds received a standard commercial diet, and phase 2 (50-70 weeks). Mortality was monitored and recorded daily basis.

## Experimental design and dietary treatments

The experiment was conducted using a completely randomized design with five iso-caloric and iso-nitrogenous corn-soybean meal based diets formulated to meet the nutrient requirements of the NRC [17] and Korean Feeding Standard for Poultry [20]. Experimental diets were provided over two feeding phases: Phase 1 (weeks 16-48) and Phase 2 (weeks 50-70). Five graded dietary Ca and AvP regimens were formulated within each phase and designated as CaP80, CaP90, CaP100, CaP110, and CaP120. The absolute Ca and AvP concentrations corresponding to each treatment were phase-specific.

For Phase 1 (weeks 16-48), the dietary Ca and AvP concentrations (% of diet) were: CaP80 (3.04:0.26), CaP90 (3.42:0.29), CaP100 (3.80:0.32), CaP110 (4.18:0.35), and CaP120 (4.56:0.38). For Phase 2 (weeks 50-70), the respective concentrations were: CaP80 (3.12:0.25), CaP90 (3.51:0.28), CaP100 (3.90:0.31), CaP110 (4.29:0.34), and CaP120 (4.68:0.37). The CaP100 diets adhered to the standard recommendations for each phase, as outlined in the Korean Feeding Standard for Poultry [20]. The CaP90 and CaP110 diets were formulated to represent ~10% reductions and increases, respectively, while CaP80 and CaP120 represented ~20% deviations from the phase-specific reference. These deviations were selected to simulate practically achievable variations in mineral formulation under commercial conditions and to identify tolerable thresholds without compromising productivity or mineral utilization. Dietary Ca and AvP concentrations were modified by adjusting the inclusion levels of limestone and mono-calcium phosphate. The corresponding dietary Ca: AvP ratio was relatively stable to isolate the effects of total mineral supply. All diets were offered in mash form and included 0.30% chromium oxide (Sigma-Aldrich, USA) as an indigestible marker for ileal digestibility assessment. Ingredient composition and calculated nutrient contents for each feeding phase are detailed in Table 1.

## Growth performance parameters

Individual body weights were recorded at the start of the experiment (week 16) and at week 70. Body weight gain (BWG) per hen was calculated as the difference between the final and initial weights.

## Productive performance parameters

The daily number of eggs produced from each cage was counted, and egg weight was measured. Hen-Day Egg Production (HDEP; %), egg mass (g/hen/day), and mortality corrected feed conversion ratio (FCR) were calculated on a weekly basis for each treatment according to Oketch et al. [21], as follows:

$$\text{HDEP (\%)}=\frac{\text{Total number of eggs produced during a week}}{\text{Total number of hens available in the same week}}\times 100\%$$

$$\text{Egg mass}=\frac{\text{Average egg weight}\times\text{HDEP}}{100}$$

$$\text{FCR}=\frac{\text{Feed intake}}{\text{Egg mass}}$$

### Egg quality parameters

Egg quality assessments were conducted at the end of weeks 48 and 70. From each replicate cage, five eggs were randomly collected (30 eggs per treatment) and evaluated within 24 h of collection. Spoiled or damaged eggs were excluded from the analysis. Eggshell breaking strength was measured using a texture analyzer (TA.XTplusC, Stable Micro Systems, Surrey, UK). Albumen height and Haugh unit were subsequently determined using an egg multi-tester (TSS QCM+ Range, York, UK), which includes an integrated albumen-height gauge and an automated Haugh-unit calculator. Yolk color was evaluated using the DSM Yolk Color Fan (scale 1 = light yellow to 15 = dark orange). Shell thickness was then measured with a digital micrometer (Mitutoyo Digimatic MDC-MX Series, Illinois, USA) at three locations on each egg (sharp end, equator, and blunt end) with shell membranes intact, and the mean of the three measurements was recorded.

### Slaughter, sample collection, and Tibia bone measurements

At 48 and 70 weeks, one bird per replicate cage with body weight closest to the treatment mean was selected. Hens were euthanized by carbon dioxide asphyxiation, and sacrificed hens were necropsied to obtain ileal digesta samples. Collected digesta samples were stored in −20 °C freezing conditions until further analysis for ileal nutrient digestibility. Before analysis, the samples were thawed and oven-dried at 55 °C for 24 hours, followed by fine grinding and strained through a < 0.75 mm sieve (ZM 200 Ultra-Centrifugal Mill, Retsch GmbH & Co., Haan, Germany) as described by Nawarathne et al. [22]. Ileal nutrient analyses of digesta samples followed AOAC [23] procedures. Dry matter was determined using the air-oven method (103 °C for 16-18 h; AOAC 934.01). Crude protein content was analyzed using the Kjeldahl method, with nitrogen values multiplied by 6.25 (AOAC 984.13). Gross energy was measured using an adiabatic bomb calorimetric method. Ash content was determined by incinerating pre-dried samples at 550 °C for 4 h in a muffle furnace (AOAC 942.05). Ca and P concentrations were determined using AOAC wet-ash procedures (AOAC 968.08 and AOAC 965.17) with minor modifications. $Cr_2O_3$ concentrations in feed and digesta were analyzed using the method of Fenton and Fenton [24]. Ileal nutrient digestibility of each nutrient was calculated using the standard marker ratio equation:

$$\text{Ileal nutrient digestibility (\%)}=100-\left[100\times\frac{(\text{Marker in the diet}\times\text{Nutrient in the digesta})}{(\text{Marker in the digesta}\times\text{Nutrient in the diet})}\right]$$

Tibia bones from both legs were carefully dissected, cleaned of soft tissues, and processed for mechanical and proximate measurements. Physical characteristics of the tibia were evaluated using the left tibia, whereas the right tibia was retained for proximate analysis. Tibia weight (g) was recorded using an analytical balance (OHAUS Explorer E12140, Ohaus Corp., USA). Tibia length (mm; proximal to distal epiphysis) and tibia width (mm; midpoint of the shaft) were measured using a digital vernier caliper, with three repeated measurements taken to minimize error. Tibia volume ($cm^3$) was determined using the water displacement method by immersing each bone in a known volume of water and calculating displacement [25]. Tibia density ($g/cm^3$) was subsequently computed as the ratio of tibia weight to tibia volume. Further, tibia breaking strength was determined using a texture analyzer (TA.XTplusC, Stable Micro Systems, Surrey, UK). Left tibiae were positioned horizontally on a custom three-point bending rig with the mid-shaft aligned beneath the loading probe. A constant compression speed of 5 mm/s was applied to the dorsal surface of the bone until structural failure occurred. The maximum force (N) recorded at the point of fracture was taken as the tibia breaking strength. De-fatted right tibiae were analyzed for crude ash, Ca, and P content using the procedures described previously by Abdelqader et al. [25].

**Statistical analysis**

All data were analyzed using one-way ANOVA in a completely randomized design using SPSS 29.0 (IBM Corp., Armonk, NY, USA). When significant treatment effects were detected, means were separated using Tukey's multiple-range test, with $p < 0.05$. The experimental unit was the cage for growth performance and productive traits, the individual egg for egg quality measurements, and the individual bird for tibia mechanical properties, tibia proximate composition (ash, Ca, and P), and ileal nutrient digestibility. Regression analyses were performed using the Nutritional Response Model (NRM) version 1.3 [26] to estimate optimal dietary Ca and AvP concentrations. Linear-plateau and quadratic-plateau models were applied for parameters that exhibited both linear and/or quadratic significance and a biological response consistent with a plateau pattern. For response variables that followed a curvilinear trend without plateau behavior, a quadratic regression model was used. Parameters with non-significant trends or inconsistent patterns were not subjected to regression analysis.

# Results

## Growth and productive performance

Productive performance outcomes were not affected by dietary Ca and AvP levels (Table 2). Hen-day egg production (%) remained stable across the 24-48, 50-70, and 24-70 week periods. No significant trends were observed in egg weight or egg mass across any phase. Egg weight remained between 62.91 and 67.29 g, while egg mass ranged from 50.66 to 58.55 g/hen/day. Feed conversion ratio (FCR), calculated based on egg mass, also exhibited no significant linear or quadratic response to CaP level.

## Egg quality parameters

As summarized in Table 3, several egg quality traits were significantly influenced by dietary Ca and AvP levels, particularly those associated with albumen and shell quality. At week 48, both albumen height and Haugh unit showed significant quadratic effects ($p < 0.05$), though not linear trends. Hens receiving CaP80 and CaP120 produced significantly higher albumen height and Haugh unit values than those receiving CaP90 ($p < 0.05$). Yolk color, shell thickness, and shell breaking strength at week 48 were not influenced by dietary CaP, and no significant linear or quadratic trends were observed. By week 70, albumen quality continued to respond to dietary CaP levels. Albumen height and Haugh unit were affected (overall $p < 0.05$), but no linear or quadratic trends were observed. Notably, CaP120 yielded the highest albumen height and Haugh unit values. Shell quality parameters responded more consistently to dietary CaP levels in late production (Week 70). Shell thickness exhibited both linear ($p < 0.05$) and quadratic ($p < 0.05$) responses, with thickness increasing progressively as CaP levels increased. Likewise, shell breaking strength increased with dietary CaP (overall $p < 0.05$), driven primarily by a strong linear trend ($p < 0.05$). CaP120 resulted in the greatest shell thickness and breaking strength, indicating improved shell quality at higher CaP inclusion levels during late lay.

## Tibia bone mechanical properties

As shown in Table 4, dietary Ca and AvP levels did not influence any tibial mechanical traits at 48 weeks of age, including bone weight, length, width, volume, density, and breaking strength. No linear or quadratic trends were observed for any parameter during this phase. However, by 70 weeks of age, tibia breaking strength was affected by dietary CaP (overall $p < 0.05$). While reporting a significant linear ($p < 0.05$) pattern with no quadratic patterns, the highest tibia breaking strength was recorded in hens fed CaP120 (2622.55 N), which differed from CaP80 (1972.76 N), the lowest performing group ($p < 0.05$). Intermediate treatments (CaP90-CaP110) exhibited comparable breaking strengths. Other tibia parameters (i.e., weight, length, width, volume, and density) remained unaffected by dietary CaP at 70 weeks, with no significant linear or quadratic trends detected.

**Tibia bone proximate composition**

The effects of dietary Ca and AvP levels on tibia bone proximate composition at weeks 48 and 70 are presented in Table 5. At week 48, tibia Ca and P concentrations increased ($p < 0.05$) with higher dietary Ca and AvP levels. Both parameters displayed significant linear ($p < 0.05$) and quadratic ($p < 0.05$) responses, indicating that tibia mineralization followed both linear and quadratic-plateau patterns. The lowest Ca and P values were observed in hens fed CaP80 (8.70% and 3.09%, respectively), whereas the highest were recorded at CaP120 (11.50% and 4.82%). In contrast, crude ash content remained unaffected by dietary treatment and exhibited no significant regression trends. A similar pattern was observed at 70 weeks. Tibia Ca and P concentrations increased progressively with dietary Ca and AvP level ($p < 0.05$), accompanied by significant linear ($p < 0.05$) and modest quadratic ($p < 0.05$ for Ca) trends. Crude ash content again remained unaffected, with no regression pattern observed.

**Ileal nutrient digestibility**

Ileal digestibility of crude ash, Ca, and P was influenced ($p < 0.05$) by dietary Ca and AvP levels at both week 48 and week 70 (Table 6). At week 48, crude ash ($p < 0.05$), Ca ($p < 0.05$), and P ($p < 0.05$) digestibility declined as dietary Ca and AvP levels increased. These parameters exhibited significant linear ($p < 0.05$ for all) and quadratic ($p < 0.05$) trends and were best described using quadratic regression models. The highest digestibility values were observed in the CaP80 and CaP90 groups, whereas CaP120 consistently yielded the lowest values. Digestibility of energy and crude protein was unaffected, with no discernible regression patterns. The same trends persisted at week 70. Digestibility of crude ash, Ca, and P (all $p < 0.05$) decreased with increasing dietary Ca and AvP levels. Linear and quadratic effects remained significant for all three parameters ($p < 0.05$ for both). Notably, hens fed CaP120 exhibited the lowest digestibility for all three components. Moreover, energy and protein digestibility remained statistically unchanged across treatments.

**Dietary Ca and AvP requirement estimation based on linear-plateau and quadratic-plateau models**

The dietary Ca and AvP requirements for optimal tibia mineralization and shell quality parameters in Hy-Line® layer chickens were estimated by linear-plateau and quadratic-plateau regression models (Table 7; Fig. 1 and 2). Only tibia Ca and P content (weeks 48 and 70) and shell thickness (week 70) exhibited significant linear and quadratic responses, making them suitable for broken-line regression modeling. Both regression models showed excellent fit ($R^2 \geq 0.97$) across all traits. For tibia Ca content, the estimated requirement based on linear-plateau models ranged from 3.490% to 3.490% Ca and 0.296% to 0.296% AvP across weeks 48 and 70, while quadratic-plateau models suggested slightly higher levels of 3.716-3.668% Ca and 0.296-0.310% AvP. Similarly, for tibia P content, estimates from linear models ranged from 3.464% to 3.928% Ca and 0.293% to 0.312% AvP, whereas quadratic models proposed levels between 3.601% and 4.162% Ca and 0.304% to 0.330% AvP. Shell thickness at week 70 followed the same trend, with plateau values achieved at 3.640% Ca and 0.290% AvP (linear-plateau) and 3.899% Ca and 0.310% AvP (quadratic-plateau). Recommended Ca and AvP levels were derived from both models to support practical application. Phase-specific Ca and AvP recommendations required to maximize tibial mineral deposition were identified. To achieve the highest tibial Ca content, the estimated optimal dietary Ca and AvP concentrations ranged from 3.579% Ca and 0.303% AvP during Phase 1 (week 48) to 3.640% Ca and 0.290% AvP during Phase 2 (week 70). In contrast, dietary levels required to maximize tibial P content were slightly higher, with optimal Ca and AvP concentrations ranging from 3.533% Ca and 0.299% AvP in Phase 1 to 4.045% Ca and 0.321% AvP in Phase 2, depending on the response variable and regression model applied.

**Dietary Ca and AvP requirement estimation based on quadratic regression models**

Quadratic regression analysis was applied to selected parameters that showed statistically significant ($p < 0.05$) linear and/or quadratic trends but did not conform to linear-plateau or quadratic-plateau models. The estimated Ca and AvP requirements, associated equations, and $R^2$ values are presented in Table 8, with model fit visualized in Fig 3. Ileal crude ash digestibility was significantly influenced by dietary Ca and AvP levels at both 48 and 70 weeks. Digestibility peaked at intermediate nutrient levels (3.203% Ca and 0.273% AvP at week 48; 3.444% Ca and 0.275% AvP at week 70), with a decline observed at higher inclusion rates, particularly in CaP120. Strong model fits were observed ($R^2 = 0.97$ and 0.92, respectively). In addition, ileal Ca digestibility also followed a quadratic trend. At week 48, maximum Ca digestibility was estimated at 3.127% Ca and 0.267% AvP ($R^2 = 0.87$), while at week 70, the optimal level decreased to 3.087% Ca and 0.247% AvP ($R^2 = 0.82$). Digestibility declined beyond these concentrations. Ileal P digestibility showed similar patterns. At week 48, optimal digestibility corresponded to 3.048% Ca and 0.261% AvP ($R^2 = 0.98$), while at week 70, it corresponded to 3.525% Ca and 0.281% AvP ($R^2 = 0.97$).

It is noteworthy that only parameters listed in this and the preceding regression section were suitable for modeling. Other traits, such as tibia strength (week 70), albumen height and Haugh unit (week 48), and yolk color (week 70), although significant in ANOVA, exhibited inconsistent or non-regression-conforming patterns. These were therefore not subjected to further regression analysis, and corresponding nutrient requirements could not be determined. Moreover, for ileal Ca digestibility at week 70, the quadratic regression model predicted optimal Ca and AvP levels beyond the concentration range tested in this study, indicating extrapolated estimates.

## Discussion

Although Ca sources such as limestone and oyster shell are relatively inexpensive and readily available, P is considered the third-costliest nutrient (after energy and protein) in poultry diets and a major contributor to environmental pollution [27,28]. Therefore, even modest adjustments to the Ca and AvP levels in the layer diet can have substantial economic and environmental implications. To address this, the current study evaluated the effects of varying dietary Ca and AvP concentrations on productive performance, egg quality, tibial bone characteristics, and nutrient digestibility in Hy-Line Brown hens from 16 to 70 weeks of age. The results highlight the impact of Ca and AvP modifications on key performance indicators and physiological responses across the laying cycle.

The current study demonstrated that moderate adjustments (±10-20%) in dietary Ca and AvP from the Korean Feeding Standard for Poultry (2022) had no significant effect on the body weight, weight gain, FCR, or laying performance of Hy-Line Brown hens from 16 to 70 weeks of age. These findings align with prior research by Jing et al. [14], Pelicia et al. [29], Cufadar et al. [30], Świątkiewicz et al. [31], and Bello et al. [32], who similarly observed that layer hens tolerate moderate dietary variations in Ca (3.0-4.5%) and AvP (0.10-0.40%) without compromising growth or productivity. The biological rationale behind this tolerance lies in the highly efficient endocrine regulation of mineral homeostasis in poultry. Plasma Ca and P concentrations are tightly maintained through the actions of 1,25-dihydroxyvitamin $D_3$, parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and calcitonin. These hormones work synergistically to adjust intestinal absorption, renal reabsorption, and bone mobilization of Ca and P in response to dietary fluctuations [5,7]. Consequently, even when dietary inputs vary, homeostatic mechanisms can buffer plasma mineral levels, sustaining key physiological functions and egg production until more extreme imbalances are reached. Additionally, body weight and egg production are more directly influenced by energy and amino acid intake than by mineral supply once baseline requirements are met [33,34]. Thus, the absence of significant differences in growth or egg output across the CaP80 to CaP120 treatments suggests that all tested levels fell within a functional "homeostatic safe zone" that is adequate to prevent deficiency but not excessive enough to impair nutrient utilization. These results provide further evidence that small deviations in Ca and AvP from standard recommendations may offer flexibility in diet formulation without compromising hen performance, supporting both biological resilience and cost-effective feed strategies.

Although Ca and P homeostasis in laying hens is tightly regulated through endocrine control mechanisms, modest fluctuations in dietary Ca and AvP can influence egg quality traits prior to measurable changes in production or skeletal metrics. This reflects the role of the egg as a sensitive physiological endpoint, often responding earlier to mineral imbalances as hens attempt to maintain systemic homeostasis through altered resource allocation [5]. Consistent with this, the present study observed diet-dependent effects on albumen quality, as measured by albumen height and Haugh unit. Both parameters exhibited non-linear responses to dietary Ca and AvP, with reductions evident in intermediate treatments (e.g., CaP90 at week 48 and CaP110 at week 70), whereas the CaP120 group consistently maintained higher values at both time points. These patterns contrast marginally with earlier reports that found no significant effects on albumen height or Haugh unit under moderate dietary mineral variation [14,16,29,35], possibly due to narrower treatment ranges or different experimental durations. The elevated albumen quality in the CaP120 treatment may reflect improved structural stabilization of albumen proteins through $Ca^{2+}$ binding, supporting previous observations that high dietary Ca (0.60-4.18%) can enhance albumen height in chicken and pigeon eggs [36,37]. Despite these variations, all Haugh unit values remained within the U.S. Grade AA threshold (≥72) [38], suggesting that moderate Ca and AvP deviations do not compromise commercial egg quality. The age-related decline in albumen height and Haugh unit from week 48 to 70 aligns with the expected degradation of ovomucin-rich protein matrices over time [39], highlighting the physiological basis for declining albumen freshness in older hens. Yolk color is an important determinant of consumer acceptability that is primarily modulated by carotenoid content [3,40]. In the present study, yolk color was marginally enhanced in the CaP120 group at week 70, despite all diets containing identical basal pigment sources. Previous studies have reported similar effects, where diets with elevated Ca (2.8-4.5%) and AvP (0.25-0.43%) yielded deeper yolk pigmentation [3,29], potentially via improved absorption and deposition of fat-soluble pigments (i.e., lutein) or altered hepatic yolk precursor transport [41]. Nevertheless, all yolk color scores fell within the DSM Yolk Color Fan's consumer-acceptable range (7-10) [42], suggesting limited practical impact on marketability.

Eggshell thickness and strength directly affect egg market value, as greater shell thickness and strength reduce breakage losses during transportation and storage [43]. Moderate deviations in dietary Ca levels can influence eggshell thickness and strength, with higher levels generally resulting in thicker (or heavier) and stronger shells [1,29,35,44]. In contrast to other egg quality parameters, shell quality parameters (including thickness and breaking strength) exhibited a robust positive response to dietary Ca and AvP levels, particularly at 70 weeks. Both traits increased linearly with increasing mineral supply, and shell thickness additionally satisfied the model-fitting criteria for broken-line linear and quadratic-plateau regression (Table 7; Fig. 2). This reflects the higher mineral demands during the late laying period, as aging hens experience reduced intestinal Ca absorption and diminished mobilization from medullary bone due to decreased responsiveness to 1,25-dihydroxyvitamin $D_3$ and PTH, ultimately impairing Ca homeostasis and shell deposition capacity [2,5,6]. Increased dietary Ca may partially overcome this by stimulating uterine expression of Ca-binding proteins such as calbindin-D28k, enhancing Ca transport into the shell gland lumen [45]. Based on the linear and quadratic-plateau models, dietary levels of 3.77% Ca and 0.30% AvP can be recommended for optimal shell thickness, as further increases beyond these levels did not result in significant improvements, indicating a plateau response. Although the effects of P remain less clearly defined, previous research has suggested that modest reductions in AvP (from 0.36% to 0.22%) may improve Ca utilization for eggshell formation [16,45]. However, such effects were not definitively supported in the present study. These inconsistent findings and the proposed mechanisms underlying AvP effects on eggshell quality highlight the need for further investigation. Importantly, eggshell thickness values of about 0.3-0.4 mm and shell strength values exceeded 3.5 kgf (approximately 35 N) across all treatments, indicating commercial suitability regardless of dietary mineral concentration [46]. The observed changes in egg quality parameters, especially those that aligned with regression-predicted optima, underscore the biological sensitivity of egg traits to dietary mineral balance and illustrate how hens prioritize physiological homeostasis through early adjustments in egg composition before skeletal or production outcomes are impacted.

Our findings are consistent with previous reports [8,9,14,44,47,48], suggesting that moderate changes in dietary Ca and AvP (±20%) are unlikely to affect tibial mechanical parameters, including tibia weight, length, width, volume, and density. These results indicate that hens can physiologically compensate for moderate variations in Ca and AvP levels, maintaining stable bone size and density. Chicken bones are primarily composed of Ca, and laying hens exhibit adaptive mechanisms to maintain skeletal integrity during egg production despite variations in dietary mineral supply [2,7]. Specifically, hens maintain a dynamic equilibrium via the medullary bone, which serves as a labile Ca reserve that is mobilized when dietary Ca is insufficient for eggshell formation, while excess dietary Ca can be stored without immediately altering structural bone mass [5-7]. This adaptive regulation likely explains the absence of observable changes in most tibial mechanical parameters in the present study. However, a significant effect on tibia breaking strength was observed at week 70, with higher dietary Ca and AvP levels resulting in stronger tibiae.

Measurement of tibia breaking strength is a reliable and straightforward method for assessing overall skeletal health, bone quality, and severity of osteoporosis, which directly affects animal welfare. In this test, a perpendicular force is applied to the mid-diaphysis of the tibia, which consists of cortical and medullary bone tissues, to determine the minimum force required to induce fracture [32]. Several studies have corroborated our observations, reporting that moderate increases in dietary Ca and AvP result in stronger tibiae in old hens [1,8,32]. While the medullary bone serves as a readily mobilizable Ca reservoir, the cortical bone provides the primary mechanical strength of the skeleton [5,7]. During the laying period, hens mobilize Ca from structural bone and deposit it into the medullary bone to meet the increased Ca demand for eggshell formation, which can gradually reduce cortical bone strength [32]. When dietary Ca or mineral supply is inadequate, resorption of medullary bone is increased while its redeposition is reduced, resulting in weaker and more fragile bones [5,8]. This mechanism is consistent with our findings, as the lowest tibia breaking strength was observed in hens fed the CaP80 diet, whereas the highest strength was recorded in hens receiving the CaP120 diet (linear $p < 0.05$). Moreover, with advancing age and continued egg production, bone strength in laying hens naturally declines, and by approximately 70 weeks of age, hens undergo considerable bone loss that can compromise skeletal integrity due to estrogen decline, reduced osteoblast activity, and increased osteoclast activity following the post-peak laying phase [35,49]. Consequently, moderate reductions in dietary Ca and AvP may have minimal effects in younger hens (week 48); however, they can lead to measurable bone weakening in older hens, as reflected by reduced tibia breaking strength in the present study

Moderate adjustments in dietary Ca (2.97-4.50%) and AvP (0.37-0.61%) have been reported to alter tibial Ca and P concentrations in both younger (22-31 weeks) and older laying hens (60-72 weeks), with higher dietary levels leading to increased mineral deposition in bone [41,50], which is in agreement with our present findings. Despite significant variations in Ca and P concentrations, crude ash content of the tibia remained statistically unchanged across treatments at both week 48 and 70. This observation has been similarly noted in previous studies [14,35,48], and supports the concept that total ash represents a broader matrix of bone constituents beyond specific mineral fractions. Once the baseline mineral requirements are met, additional dietary Ca and AvP appear to affect the proportions of mineralized bone (i.e., Ca and P content), without significantly altering the overall ash mass. Mineral homeostasis mechanisms mediated by endocrine regulators such as 1,25-dihydroxyvitamin $D_3$, PTH, and FGF23 enable hens to maintain relatively stable circulating Ca and P concentrations despite moderate fluctuations in intake. These mechanisms orchestrate intestinal absorption, renal reabsorption, and skeletal mobilization, and thus help prioritize mineral allocation to either eggshell formation or skeletal deposition depending on physiological demand [5]. Our results confirm this adaptive capacity, as hens fed higher Ca and AvP diets exhibited greater tibial Ca and P retention, likely because surplus mineral supply exceeding the requirements for shell calcification can be deposited in bone [50]. However, age-related biological variation also played a key role. Tibial Ca and P concentrations were consistently lower at week 70 compared with week 48, supporting previous reports that bone mineral content tends to decline with advancing age due to skeletal demineralization and structural deterioration [49]. This reduction may be attributed to increased bone porosity and a shift in mineral partitioning toward sustained eggshell production in older hens.

Accurate estimation of nutrient requirements is highly dependent on the selection of an appropriate statistical model, as different models can yield divergent requirement values. While the linear-plateau model often provides a satisfactory statistical fit, it may underestimate optimal requirements by failing to account for physiological variability among hens, whereas the quadratic-plateau model generally estimates higher requirements [26]. Accordingly, the present study adopted an integrated approach by averaging the estimates derived from both models. Linear- and quadratic-plateau regression analyses were applied to tibial Ca and P concentration data at weeks 48 and 70 to determine dietary Ca and AvP requirements for optimal tibial mineralization for each phase (Table 7; Fig. 1). The estimated dietary Ca and AvP requirements for maximizing tibial Ca content were 3.58% Ca and 0.30% AvP during Phase 1 (week 48) and 3.64% Ca and 0.29% AvP during Phase 2 (week 70). Corresponding requirements for maximizing tibial P content were 3.53% Ca and 0.30% AvP in Phase 1 and 4.05% Ca and 0.32% AvP in Phase 2. Both regression models indicated that tibial mineral deposition increased with increasing dietary Ca and AvP concentrations up to a threshold, beyond which further increases did not result in additional benefits. It is important to note that this study maintained a fixed Ca: AvP ratio across all treatments. As such, the regression estimates reflect optimal concentrations within this fixed-ratio framework rather than identifying a true optimal ratio. Determination of the ideal Ca: AvP ratio would require independent manipulation of Ca and AvP, which was beyond the scope of the current design. Notably, these estimated requirements were generally lower than the current recommendations of the Korean Feeding Standard for Poultry [20] (3.80% Ca and 0.32% AvP for Phase 1; 3.90% Ca and 0.31% AvP for Phase 2), with the exception of the requirement for tibial P content during Phase 2. Collectively, these findings suggest that moderate reductions in dietary Ca and AvP may be implemented without compromising skeletal mineral status, thereby supporting more precise nutrient formulation and reducing the risk of mineral oversupply. Direct comparison with previous Ca-P requirement studies is limited by differences in modeling approaches, study duration, hen strains, and diet formulation strategies. Many earlier studies have not applied plateau-type regression models or evaluated full-cycle feeding phases (16-70 weeks), which may affect the comparability of results. Nevertheless, our findings contribute to the refinement of Ca and AvP recommendations by providing model-based estimates tailored to extended production conditions.

Ileal digestibility is a critical indicator of nutrient utilization efficiency and environmental sustainability in laying hens. In the present study, increasing dietary Ca and AvP levels was associated with a marked decline in the digestibility of crude ash, Ca, and P, particularly at the highest inclusion levels, consistent with prior findings in layers [9,51]. Hens fed CaP80 and CaP90 exhibited the highest digestibility values for these minerals, suggesting that moderate reductions in dietary Ca and AvP below current recommendations (Cap100) may enhance nutrient absorption while minimizing excretory losses. Physiologically, intestinal absorption of Ca and P is governed by active transport mechanisms that are sensitive to dietary supply. When AvP intake exceeds physiological needs, expression of key transporter proteins (i.e., sodium-phosphate cotransporter type IIb and calbindin) is downregulated [52], limiting mineral uptake. In addition to transporter downregulation, higher dietary Ca and AvP levels may reduce mineral digestibility through physical–chemical interactions in the gut lumen. Excess Ca can bind with dietary P to form insoluble Ca-P complexes that are poorly absorbed in the small intestine [2,28]. Moreover, the fixed Ca:P ratio may influence intestinal mineral dynamics, as imbalances can impair the efficiency of nutrient uptake [4-6]. Additionally, saturation of active transport pathways, particularly at supraphysiological mineral intakes, may further constrain absorption efficiency [6]. Together, these mechanisms likely contributed to the observed reductions in ileal Ca and P digestibility at higher dietary inclusion levels. This may explain the suppressed digestibility values observed in hens fed diets exceeding 3.80%/3.90% Ca and 0.30%/0.31% AvP. These observations are particularly relevant for P management, as excessive P excretion contributes to environmental pollution. Musilova et al. [51] previously suggested that the AvP requirement in layers may be lower than the 0.25% NRC [17] recommendation, further supporting the rationale for refining modern nutrient guidelines. In this context, our results further suggest that the AvP level recommended by the Korean Feeding Standard for Poultry [20] of 0.31-0.32% may be higher than necessary and could be reduced without adversely affecting productivity or P digestibility. To better quantify optimal Ca and AvP concentrations for enhancing mineral utilization, quadratic regression models were applied to ileal crude ash, Ca, and P digestibility data (Table 8; Fig. 3). Quadratic regression was selected as the most appropriate modeling approach because the response variables exhibited significant quadratic trends without reaching a clear plateau, thereby precluding the application of linear- or quadratic-plateau models for biologically meaningful requirement estimation. The regression analyses indicated that maximal ileal crude ash digestibility was achieved at 3.20% Ca and 0.27% AvP during Phase 1 (week 48) and 3.44% Ca and 0.28% AvP during Phase 2 (week 70). Maximum ileal Ca digestibility occurred at 3.13% Ca and 0.27% AvP in Phase 1 and 3.09% Ca and 0.25% AvP in Phase 2, whereas maximal ileal P digestibility was observed at 3.05% Ca and 0.26% AvP in Phase 1 and 3.53% Ca and 0.28% AvP in Phase 2. Collectively, these estimates indicate that optimal mineral absorption occurred at dietary Ca and AvP concentrations below current recommendations of the Korean Feeding Standard for Poultry (2022) (3.80% Ca and 0.32% AvP for Phase 1; 3.90% Ca and 0.31% AvP for Phase 2), suggesting that dietary Ca and AvP levels could be reduced by approximately 13-14% without compromising intestinal mineral utilization. Based on these findings, practical dietary levels of approximately 3.13% Ca and 0.27% AvP for Phase 1 and 3.35% Ca and 0.26% AvP for Phase 2 may be proposed to support efficient ileal mineral digestibility while minimizing excess mineral excretion. Overall, these results support the implementation of precision mineral nutrition strategies aimed at improving feed efficiency and reducing the environmental footprint of layer production systems. While moderate reductions in dietary Ca and AvP improved mineral digestibility, potential long-term effects on skeletal health warrant consideration, particularly during extended laying cycles. Age-related declines in estrogen, osteoblast activity, and calcium absorption capacity predispose hens to skeletal demineralization and osteoporosis [7,8,49,50], especially beyond 70 weeks of age. Consequently, inadequate mineral supply during this period may exacerbate cortical bone loss and compromise medullary bone regeneration, increasing fracture risk. Our findings at week 70, where tibial breaking strength declined under the CaP80 regimen, align with this risk, highlighting the need to balance improved mineral utilization with skeletal integrity in aging hens. Further research is needed to evaluate whether precision feeding strategies can sustain bone health beyond 70 weeks without increasing dietary mineral load.

In conclusion, moderate reductions (10-20%) in dietary Ca and AvP below current Korean Feeding Standard recommendations did not compromise growth, egg production, or overall egg quality in Hy-Line Brown hens from 16 to 70 weeks of age. A 10% reduction (CaP90) maintained comparable values for shell quality, albumen traits, and bone strength, while enhancing the ileal digestibility of crude ash, Ca, and P. Regression analyses further refined dietary mineral recommendations across feeding phases. Phase-specific requirements for maximizing tibial mineralization and shell thickness ranged from 3.53-3.58% Ca and 0.299-0.303% AvP during Phase 1 (week 48) and 3.64-4.05% Ca and 0.29-0.32% AvP during Phase 2 (week 70). In contrast, quadratic regression analyses of ileal nutrient digestibility indicated that maximal absorption of crude ash, Ca, and P occurred at comparatively lower dietary concentrations, typically between 3.05-3.20% Ca and 0.26-0.27% AvP in Phase 1 and 3.09-3.53% Ca and 0.25-0.28% AvP in Phase 2, with digestibility declining at higher mineral inclusion levels and no clear plateau response observed. These results underscore the need for phase-specific dietary adjustments that balance skeletal integrity with efficient nutrient utilization. The improved digestibility at reduced mineral levels highlights opportunities to minimize oversupply and mitigate environmental P output. Future studies should evaluate the long-term and economic effects of these strategies under commercial conditions.

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## Data tables

**Table 1.** Ingredients and calculated nutrient compositions of experimental diets (%, as-fed basis)

| Item | Phase 1 diet (week 16-48)[1] | | | | | Phase 2 diet (week 50-70)[2] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CaP80 | CaP90 | CaP100 | CaP110 | CaP120 | CaP80 | CaP90 | CaP100 | CaP110 | CaP120 |
| ***Ingredients (%)*** | | | | | | | | | | |
| Corn | 62.80 | 60.54 | 58.27 | 56.05 | 53.82 | 61.43 | 61.56 | 61.71 | 62.23 | 61.74 |
| Soybean meal | 20.76 | 21.14 | 21.52 | 21.88 | 22.24 | 19.08 | 18.12 | 17.16 | 16.28 | 15.24 |
| Corn gluten meal | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | - | 1.00 | 2.00 | 3.00 | 4.00 |
| Wheat bran | - | - | - | - | - | 5.92 | 4.40 | 2.84 | 0.92 | - |
| Canola meal | - | - | - | - | - | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| limestone | 7.68 | 8.65 | 9.62 | 10.59 | 11.56 | 8.28 | 9.34 | 10.41 | 11.48 | 12.55 |
| Mono-calcium phosphate | 0.76 | 0.90 | 1.04 | 1.16 | 1.28 | 0.64 | 0.80 | 0.92 | 1.08 | 1.20 |
| Salt | 0.40 | 0.40 | 0.41 | 0.41 | 0.41 | 0.42 | 0.42 | 0.42 | 0.43 | 0.40 |
| Tallow | 1.48 | 2.26 | 3.04 | 3.82 | 4.60 | 0.32 | 0.40 | 0.52 | 0.52 | 0.76 |
| Choline chloride (50%) | 0.09 | 0.09 | 0.09 | 0.09 | 0.09 | 0.08 | 0.09 | 0.10 | 0.11 | 0.12 |
| DL-Methionine (98%) | 0.22 | 0.22 | 0.22 | 0.22 | 0.23 | 0.20 | 0.19 | 0.19 | 0.18 | 0.17 |
| L-lysine-sulfate (65%) | 0.21 | 0.20 | 0.19 | 0.18 | 0.17 | 0.03 | 0.08 | 0.13 | 0.17 | 0.22 |
| Vitamin-mineral premix[3] | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.3 | 0.30 | 0.3 | 0.30 |
| Chromium oxide | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| ***Calculated values*** | | | | | | | | | | |
| Metabolizable energy (kcal/kg) | 2,849 | 2,849 | 2,849 | 2,850 | 2,851 | 2,701 | 2,701 | 2,701 | 2,700 | 2,700 |
| Crude protein (%) | 17.50 | 17.50 | 17.50 | 17.50 | 17.50 | 16.5 | 16.50 | 16.51 | 16.50 | 16.51 |
| Calcium (%) | 3.04 | 3.42 | 3.80 | 4.18 | 4.56 | 3.12 | 3.51 | 3.90 | 4.29 | 4.68 |
| Total phosphorus (%) | 0.47 | 0.50 | 0.53 | 0.56 | 0.58 | 0.49 | 0.51 | 0.53 | 0.55 | 0.58 |
| Available phosphorus (%) | 0.26 | 0.29 | 0.32 | 0.35 | 0.38 | 0.25 | 0.28 | 0.31 | 0.34 | 0.37 |
| Lysine (%) | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 |
| Methionine (%) | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.41 | 0.42 | 0.42 | 0.42 | 0.42 |
| Methionine + Cystine (%) | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.71 | 0.71 | 0.71 | 0.71 | 0.71 |
| Threonine (%) | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 | 0.62 | 0.62 | 0.62 | 0.62 | 0.62 |
| Sodium (%) | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.18 | 0.17 | 0.17 | 0.17 | 0.17 |

[1] Five different percentage ratios of Calcium and available Phosphorous (Ca: P%) as: 1) 80 CaP (3.04:0.26%); 2) 90 CaP (3.42:0.29%); 3) 100 CaP (3.80:0.32%); 4) 110 CaP (4.18:0.35%); and 5) 120 CaP (4.56:0.38%).

[2] Five different percentage ratios of Calcium and available Phosphorous (Ca: P%) as: 1) 80 CaP (3.12:0.25%); 2) 90 CaP (3.51:0.28%); 3) 100 CaP (3.90:0.31%); 4) 110 CaP (4.29:0.34%); and 5) 120 CaP (4.68:0.37%).

[3] Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 21 mg; vitamin K3, 2.4 mg; vitamin b1, 1.2 mg; vitamin b2, 4.8 mg; vitamin b6, 2.4 mg; vitamin b12, 20 µg; niacin, 15 mg; pantothenic acid, 10 mg; folic acid, 0.3 mg; Mn, 72 mg; Zn, 60 mg; Fe, 24 mg; Cu, 4.5 mg; I, 1 mg; Co, 0.15 mg; Se, 0.2 mg

.

**Table 2.** Effects of moderate adjustments in dietary calcium and available phosphorus on growth and productive performance of Hy-Line® laying hens1).

| Parameter | Treatment2) | | | | | SEM3) | *p*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | CaP80 | CaP90 | CaP100 | CaP110 | CaP120 | | Linear | Quadratic | Overall |
| ***Growth performance*** | | | | | | | | | |
| Initial body weight (Week 16) | 1124.72 | 1148.56 | 1119.25 | 1115.22 | 1117.78 | 10.487 | 0.528 | 0.845 | 0.858 |
| Final body weight (Week 70) | 2110.75 | 2095.33 | 2103.42 | 2136.00 | 2014.00 | 17.639 | 0.220 | 0.201 | 0.338 |
| Body weight gain (g/hen) | 986.03 | 946.77 | 984.17 | 1020.78 | 896.22 | 13.548 | 0.179 | 0.066 | 0.634 |
| ***Productive performance*** | | | | | | | | | |
| Hen day egg production (%) | | | | | | | | | |
| Week 24-48 | 82.29 | 83.14 | 84.00 | 87.71 | 85.38 | 1.258 | 0.254 | 0.751 | 0.713 |
| Week 50-70 | 78.51 | 78.95 | 80.38 | 87.02 | 78.59 | 1.926 | 0.560 | 0.455 | 0.617 |
| Week 24-70 | 80.57 | 81.23 | 82.35 | 87.39 | 82.28 | 1.524 | 0.395 | 0.657 | 0.673 |
| Egg weight (g) | | | | | | | | | |
| Week 24-48 | 65.01 | 65.01 | 62.91 | 64.79 | 66.50 | 0.453 | 0.388 | 0.058 | 0.172 |
| Week 50-70 | 65.34 | 67.06 | 66.47 | 67.29 | 64.47 | 0.525 | 0.688 | 0.084 | 0.393 |
| Week 24-70 | 65.49 | 65.77 | 64.71 | 66.05 | 64.73 | 0.369 | 0.638 | 0.802 | 0.715 |
| Egg mass (g/hen/day) | | | | | | | | | |
| Week 24-48 | 53.50 | 54.05 | 52.85 | 56.83 | 56.78 | 0.839 | 0.129 | 0.575 | 0.426 |
| Week 50-70 | 51.27 | 52.94 | 53.42 | 58.55 | 50.66 | 1.315 | 0.637 | 0.196 | 0.359 |
| Week 24-70 | 52.50 | 53.64 | 53.27 | 57.71 | 53.88 | 1.008 | 0.353 | 0.554 | 0.543 |
| Feed conversion ratio | | | | | | | | | |
| Week 24-48 | 2.06 | 2.05 | 2.11 | 1.94 | 1.95 | 0.033 | 0.150 | 0.529 | 0.401 |
| Week 50-70 | 2.17 | 2.11 | 2.13 | 1.88 | 2.21 | 0.056 | 0.722 | 0.308 | 0.406 |
| Week 24-70 | 2.11 | 2.07 | 2.11 | 1.91 | 2.06 | 0.041 | 0.390 | 0.674 | 0.525 |

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

2)Five graded dietary Ca and AvP regimens were formulated and designated as CaP80, CaP90, CaP100, CaP110, and CaP120. For Phase 1 (weeks 16-48), the Ca and AvP concentrations (% of diet) were: CaP80 (3.04:0.26), CaP90 (3.42:0.29), CaP100 (3.80:0.32), CaP110 (4.18:0.35), and CaP120 (4.56:0.38). For Phase 2 (weeks 50-70), the corresponding Ca and AvP concentrations were: CaP80 (3.12:0.25), CaP90 (3.51:0.28), CaP100 (3.90:0.31), CaP110 (4.29:0.34), and CaP120 (4.68:0.37).

3)Pooled standard error of mean.

**Table 3.** Effects of moderate adjustments in dietary calcium and available phosphorus on different egg quality parameters of Hy-Line® laying hens 1).

| Item | Treatment 2) | | | | | SEM 3) | *p*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | CaP80 | CaP90 | CaP100 | CaP110 | CaP120 | | Linear | Quadratic | Overall |
| ***Week 48*** | | | | | | | | | |
| Albumen height (mm) | $12.05^{b}$ | $10.89^{a}$ | $11.27^{ab}$ | $11.27^{ab}$ | $12.04^{b}$ | 0.131 | 0.696 | 0.001 | 0.013 |
| Haugh unit | $106.46^{b}$ | $101.23^{a}$ | $103.70^{ab}$ | $102.78^{ab}$ | $106.15^{b}$ | 0.608 | 0.826 | 0.006 | 0.027 |
| Yolk color | 7.17 | 7.13 | 7.03 | 7.23 | 7.33 | 0.058 | 0.297 | 0.249 | 0.572 |
| Shell thickness (mm) | 0.36 | 0.37 | 0.36 | 0.36 | 0.37 | 0.003 | 0.519 | 0.532 | 0.251 |
| Shell breaking strength (N) | 48.31 | 51.58 | 50.35 | 51.49 | 51.00 | 0.790 | 0.355 | 0.431 | 0.686 |
| ***Week 70*** | | | | | | | | | |
| Albumen height (mm) | $7.21^{ab}$ | $8.28^{b}$ | $8.20^{b}$ | $6.80^{a}$ | $8.35^{b}$ | 0.186 | 0.523 | 0.810 | 0.016 |
| Haugh unit | $81.92^{ab}$ | $88.24^{b}$ | $87.49^{ab}$ | $76.04^{a}$ | $89.67^{b}$ | 1.374 | 0.721 | 0.720 | 0.007 |
| Yolk color | $7.05^{a}$ | $7.60^{ab}$ | $6.95^{a}$ | $7.25^{ab}$ | $8.00^{b}$ | 0.099 | 0.021 | 0.087 | 0.003 |
| Shell thickness (mm) | $0.37^{a}$ | $0.40^{b}$ | $0.41^{b}$ | $0.41^{b}$ | $0.41^{b}$ | 0.004 | 0.001 | 0.006 | < 0.001 |
| Shell breaking strength (N) | $56.51^{a}$ | $58.35^{ab}$ | $63.25^{abc}$ | $65.79^{bc}$ | $67.15^{c}$ | 0.977 | < 0.001 | 0.659 | 0.001 |

1) Values are the mean of six replicates per treatment; $^{a-c}$ Values in a row with different superscripts differ significantly ($p < 0.05$) when the overall treatment effect was significant.

2) Five graded dietary Ca and AvP regimens were formulated and designated as CaP80, CaP90, CaP100, CaP110, and CaP120. For Phase 1 (weeks 16-48), the Ca and AvP concentrations (% of diet) were: CaP80 (3.04:0.26), CaP90 (3.42:0.29), CaP100 (3.80:0.32), CaP110 (4.18:0.35), and CaP120 (4.56:0.38). For Phase 2 (weeks 50-70), the corresponding Ca and AvP concentrations were: CaP80 (3.12:0.25), CaP90 (3.51:0.28), CaP100 (3.90:0.31), CaP110 (4.29:0.34), and CaP120 (4.68:0.37).

3) Pooled standard error of mean.

**Table 4.** Effects of moderate adjustments in dietary calcium and available phosphorus on different tibia bone mechanical parameters of Hy-Line® laying hens1).

| Item | Treatment2) | | | | | SEM3) | *p*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | CaP80 | CaP90 | CaP100 | CaP110 | CaP120 | | Linear | Quadratic | Overall |
| ***Week 48*** | | | | | | | | | |
| Tibia weight (g) | 12.33 | 13.33 | 12.00 | 13.67 | 13.50 | 0.237 | 0.095 | 0.717 | 0.080 |
| Tibia length (mm) | 120.83 | 121.83 | 119.33 | 122.17 | 123.00 | 0.650 | 0.322 | 0.369 | 0.463 |
| Tibia width (mm) | 6.79 | 7.34 | 7.07 | 7.22 | 7.10 | 0.065 | 0.247 | 0.087 | 0.087 |
| Tibia volume (cm$^3$) | 8.83 | 10.17 | 9.17 | 10.50 | 11.00 | 0.321 | 0.061 | 0.796 | 0.166 |
| Tibia density (g/cm$^3$) | 1.45 | 1.35 | 1.34 | 1.30 | 1.23 | 0.038 | 0.096 | 0.954 | 0.528 |
| Tibia breaking strength (N) | 2470.77 | 2533.38 | 2608.86 | 2695.03 | 2746.62 | 94.548 | 0.322 | 0.989 | 0.903 |
| ***Week 70*** | | | | | | | | | |
| Tibia weight (g) | 12.63 | 12.57 | 12.54 | 13.45 | 12.91 | 0.195 | 0.312 | 0.983 | 0.570 |
| Tibia length (mm) | 118.33 | 121.00 | 118.17 | 121.67 | 121.33 | 0.672 | 0.163 | 0.952 | 0.279 |
| Tibia width (mm) | 7.28 | 7.11 | 7.21 | 7.46 | 7.04 | 0.073 | 0.804 | 0.609 | 0.441 |
| Tibia volume (cm$^3$) | 8.83 | 9.00 | 9.67 | 9.00 | 9.17 | 0.178 | 0.610 | 0.391 | 0.655 |
| Tibia density (g/cm$^3$) | 1.44 | 1.41 | 1.30 | 1.51 | 1.41 | 0.029 | 0.835 | 0.419 | 0.281 |
| Tibia breaking strength (N) | 1972.76$^{a}$ | 2177.76$^{ab}$ | 2227.44$^{ab}$ | 2285.35$^{ab}$ | 2622.55$^{b}$ | 66.591 | 0.002 | 0.579 | 0.026 |

1)Values are the mean of six replicates per treatment; $^{a\text{-}b}$ Values in a row with different superscripts differ significantly ($p < 0.05$) when the overall treatment effect was significant.

2)Five graded dietary Ca and AvP regimens were formulated and designated as CaP80, CaP90, CaP100, CaP110, and CaP120. For Phase 1 (weeks 16-48), the Ca and AvP concentrations (% of diet) were: CaP80 (3.04:0.26), CaP90 (3.42:0.29), CaP100 (3.80:0.32), CaP110 (4.18:0.35), and CaP120 (4.56:0.38). For Phase 2 (weeks 50-70), the corresponding Ca and AvP concentrations were: CaP80 (3.12:0.25), CaP90 (3.51:0.28), CaP100 (3.90:0.31), CaP110 (4.29:0.34), and CaP120 (4.68:0.37).

3)Pooled standard error of mean.

**Table 5.** Effects of moderate adjustments in dietary calcium and available phosphorus on different tibia bone proximate parameters of Hy-Line® laying hens1).

| Item | Treatment2) | | | | | SEM3) | $p$-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | CaP80 | CaP90 | CaP100 | CaP110 | CaP120 | | Linear | Quadratic | Overall |
| ***Week 48*** | | | | | | | | | |
| Crude ash (%) | 29.27 | 29.33 | 30.25 | 30.97 | 31.13 | 0.347 | 0.134 | 0.999 | 0.282 |
| Ca (%) | 8.70$^{a}$ | 10.80$^{b}$ | 10.98$^{bc}$ | 11.08$^{bc}$ | 11.50$^{c}$ | 0.203 | < 0.001 | < 0.001 | < 0.001 |
| P (%) | 3.09$^{a}$ | 4.55$^{b}$ | 4.57$^{b}$ | 4.76$^{b}$ | 4.82$^{b}$ | 0.138 | < 0.001 | < 0.001 | < 0.001 |
| ***Week 70*** | | | | | | | | | |
| Crude ash (%) | 21.72 | 22.92 | 24.00 | 24.75 | 25.77 | 0.606 | 0.125 | 0.888 | 0.254 |
| Ca (%) | 7.75$^{a}$ | 9.58$^{b}$ | 9.63$^{b}$ | 9.84$^{b}$ | 10.01$^{b}$ | 0.201 | < 0.001 | 0.013 | < 0.001 |
| P (%) | 2.44$^{a}$ | 3.24$^{ab}$ | 3.60$^{b}$ | 3.65$^{b}$ | 3.78$^{b}$ | 0.136 | < 0.001 | 0.081 | 0.004 |

1)Values are the mean of six replicates per treatment; $^{a-c}$ Values in a row with different superscripts differ significantly ($p < 0.05$) when the overall treatment effect was significant.

2)Five graded dietary Ca and AvP regimens were formulated and designated as CaP80, CaP90, CaP100, CaP110, and CaP120. For Phase 1 (weeks 16-48), the Ca and AvP concentrations (% of diet) were: CaP80 (3.04:0.26), CaP90 (3.42:0.29), CaP100 (3.80:0.32), CaP110 (4.18:0.35), and CaP120 (4.56:0.38). For Phase 2 (weeks 50-70), the corresponding Ca and AvP concentrations were: CaP80 (3.12:0.25), CaP90 (3.51:0.28), CaP100 (3.90:0.31), CaP110 (4.29:0.34), and CaP120 (4.68:0.37).

3)Pooled standard error of mean.

**Table 6.** Effects of moderate adjustments in dietary calcium and available phosphorus on ileum nutrient digestibility of Hy-Line® laying hens[1].

| Parameter | Treatment[2] | | | | | SEM[3] | *p*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | CaP80 | CaP90 | CaP100 | CaP110 | CaP120 | | Linear | Quadratic | Overall |
| ***Week 48*** | | | | | | | | | |
| Energy (%) | 67.12 | 68.42 | 68.04 | 67.09 | 67.36 | 0.234 | 0.608 | 0.181 | 0.274 |
| Crude protein (%) | 67.03 | 62.07 | 68.25 | 66.94 | 66.72 | 0.884 | 0.487 | 0.781 | 0.212 |
| Crude ash (%) | $80.67^{c}$ | $85.00^{c}$ | $75.47^{bc}$ | $64.68^{ab}$ | $52.67^{a}$ | 2.491 | < 0.001 | 0.005 | < 0.001 |
| Ca (%) | $77.68^{bc}$ | $85.07^{c}$ | $75.01^{b}$ | $57.30^{a}$ | $53.48^{a}$ | 2.444 | < 0.001 | 0.001 | < 0.001 |
| P (%) | $84.56^{b}$ | $84.00^{b}$ | $80.81^{b}$ | $70.82^{ab}$ | $64.07^{a}$ | 2.185 | < 0.001 | < 0.001 | 0.002 |
| ***Week 70*** | | | | | | | | | |
| Energy (%) | 53.90 | 54.67 | 51.79 | 58.98 | 55.08 | 1.014 | 0.349 | 0.931 | 0.260 |
| Crude protein (%) | 59.39 | 52.70 | 57.63 | 55.68 | 57.77 | 1.194 | 0.975 | 0.298 | 0.474 |
| Crude ash (%) | $65.02^{c}$ | $73.54^{d}$ | $64.43^{c}$ | $53.18^{b}$ | $44.31^{a}$ | 2.050 | < 0.001 | < 0.001 | < 0.001 |
| Ca (%) | $68.20^{bc}$ | $74.76^{c}$ | $62.55^{b}$ | $47.23^{a}$ | $46.57^{a}$ | 2.225 | < 0.001 | 0.016 | < 0.001 |
| P (%) | $65.92^{bc}$ | $73.55^{c}$ | $71.74^{c}$ | $62.76^{b}$ | $52.72^{a}$ | 1.616 | < 0.001 | < 0.001 | < 0.001 |

[1] Values are the mean of six replicates per treatment; $^{a-c}$ Values in a row with different superscripts differ significantly ($p < 0.05$) when the overall treatment effect was significant.

[2] Five graded dietary Ca and AvP regimens were formulated and designated as CaP80, CaP90, CaP100, CaP110, and CaP120. For Phase 1 (weeks 16-48), the Ca and AvP concentrations (% of diet) were: CaP80 (3.04:0.26), CaP90 (3.42:0.29), CaP100 (3.80:0.32), CaP110 (4.18:0.35), and CaP120 (4.56:0.38). For Phase 2 (weeks 50-70), the corresponding Ca and AvP concentrations were: CaP80 (3.12:0.25), CaP90 (3.51:0.28), CaP100 (3.90:0.31), CaP110 (4.29:0.34), and CaP120 (4.68:0.37).

[3] Pooled standard error of mean.

**Table 7.** Estimated calcium and available phosphorus requirements for optimal shell thickness and tibia mineralization of Hy-Line® laying hens using linear- and quadratic-plateau regression models.

| **Parameter** | Plateau model equation | Estimated requirement (%)1) | | $R^2$-value | Recommendation (%; per phase)2) | |
|---|---|---|---|---|---|---|
| | | Ca | AvP | | Ca | AvP |
| Shell thickness [Week 70; Phase 2] | | | | | | |
| LP | $Y = 0.41-0.30(0.93-x)$ | 3.640 | 0.290 | 0.99 | 3.770 | 0.300 |
| QP | $Y = 0.41-1.01(0.99-x)^2$ | 3.899 | 0.310 | 0.99 | | |
| Tibia Ca [Week 48; Phase 1] | | | | | | |
| LP | $Y = 11.19-20.98(0.92-x)$ | 3.490 | 0.296 | 0.97 | 3.579 | 0.303 |
| QP | $Y = 11.19-90.93(0.97-x)^2$ | 3.668 | 0.310 | 0.97 | | |
| Tibia Ca [Week 70; Phase 2] | | | | | | |
| LP | $Y = 9.83-18.32(0.91-x)$ | 3.563 | 0.284 | 0.98 | 3.640 | 0.290 |
| QP | $Y = 9.83-89.06(0.95-x)^2$ | 3.716 | 0.296 | 0.98 | | |
| Tibia P [Week 48; Phase 1] | | | | | | |
| LP | $Y = 4.72-14.57(0.91-x)$ | 3.464 | 0.293 | 0.98 | 3.533 | 0.299 |
| QP | $Y = 4.72-74.65(0.95-x)^2$ | 3.601 | 0.304 | 0.98 | | |
| Tibia P [Week 70; Phase 2] | | | | | | |
| LP | $Y = 3.72-5.83(1.01-x)$ | 3.928 | 0.312 | 0.97 | 4.045 | 0.321 |
| QP | $Y = 4.71-17.80(1.07-x)^2$ | 4.162 | 0.330 | 0.99 | | |

1) Estimated dietary Ca and AvP requirements based on linear-plateau (LP) and quadratic-plateau (QP) regression analyses.

2) Recommended Ca and AvP levels per phase based on LP and QP models for each parameter.

**Table 8.** Estimated dietary calcium and available phosphorus requirements for ileal nutrient digestibility and shell strength of Hy-Line® laying hens based on quadratic regression analysis.

| **Parameter** | Quadratic regression equation | Estimated requirement (%)[1) ] | | $R^2$-value |
|---|---|---|---|---|
| | | Ca | AvP | |
| Crude ash digestibility [Week 48; Phase 1] | $Y = -241.56x^2+406.82x-88.73$ | 3.203 | 0.273 | 0.97 |
| Crude ash digestibility [Week 70; Phase 2] | $Y = -263.71x^2+465.65x-136.56$ | 3.444 | 0.275 | 0.92 |
| Ca digestibility [Week 48; Phase 1] | $Y = -214.90x^2+353.65x-64.72$ | 3.127 | 0.267 | 0.87 |
| Ca digestibility [Week 70; Phase 2] | $Y = -125.25x^2+179.71x+7.90$ | 3.087 | 0.247 | 0.82 |
| P digestibility [Week 48; Phase 1] | $Y = -137.13x^2+220.11x-3.38$ | 3.048 | 0.261 | 0.98 |
| P digestibility [Week 70; Phase 2] | $Y = -303.7x^2+570.21x-195.10$ | 3.525 | 0.281 | 0.97 |

1)Estimated Ca and AvP requirements based on quadratic regression analysis for each parameter.

## Figures

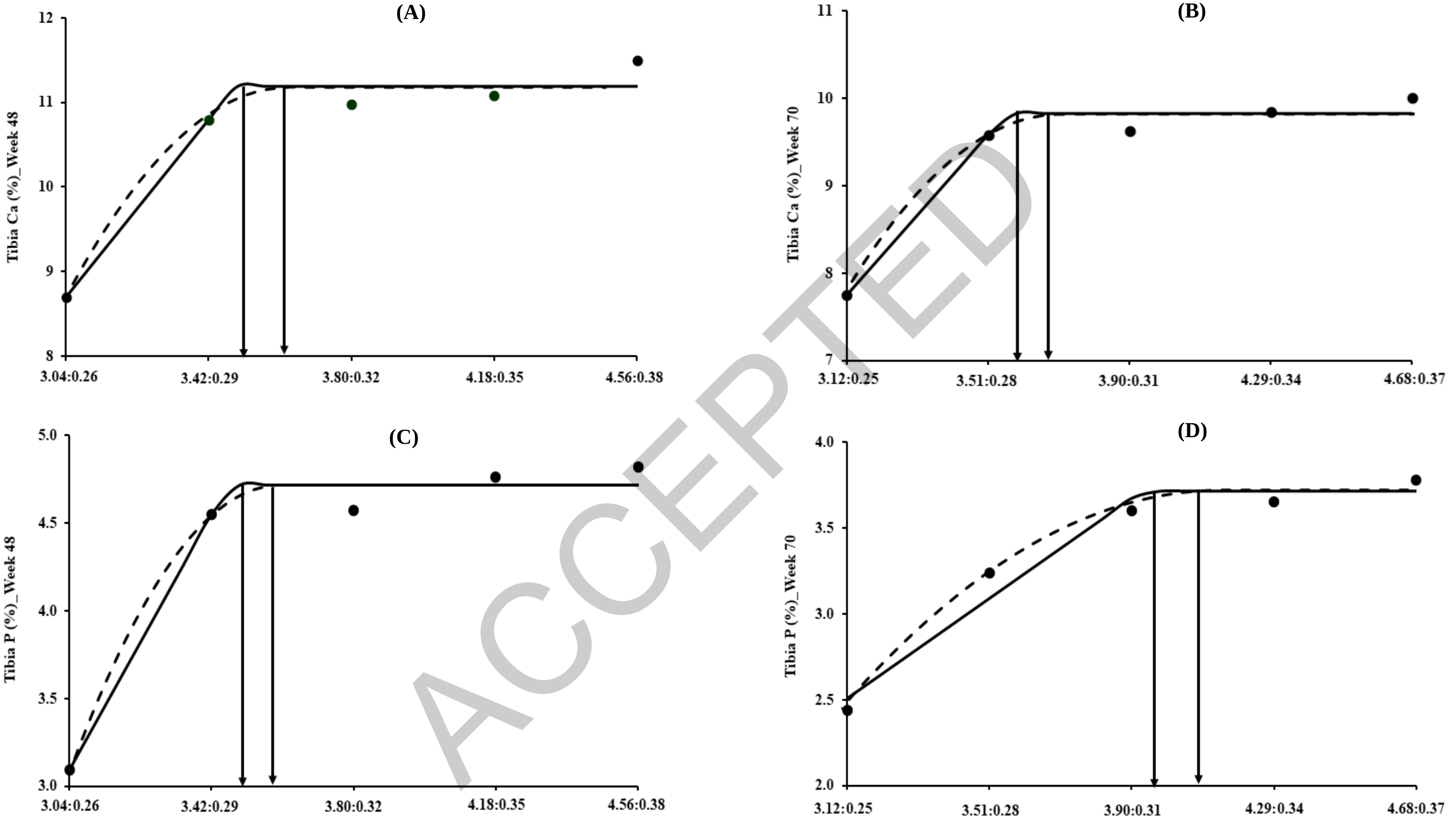

**Fig. 1.** Estimated dietary calcium and available phosphorus requirements for Hy-Line® laying hens based on tibia mineralization data from weeks 48 and 70, as determined by linear-plateau (solid line) and quadratic-plateau (dashed line) models. (A) Tibia Ca content at week 48, (B) Tibia Ca content at week 70, (C) Tibia P content at week 48, and (D) Tibia P content at week 70. Data points (●) represent the least squares means of dietary treatments (n=6). Arrows indicate the estimated nutrient requirement values calculated by each model.

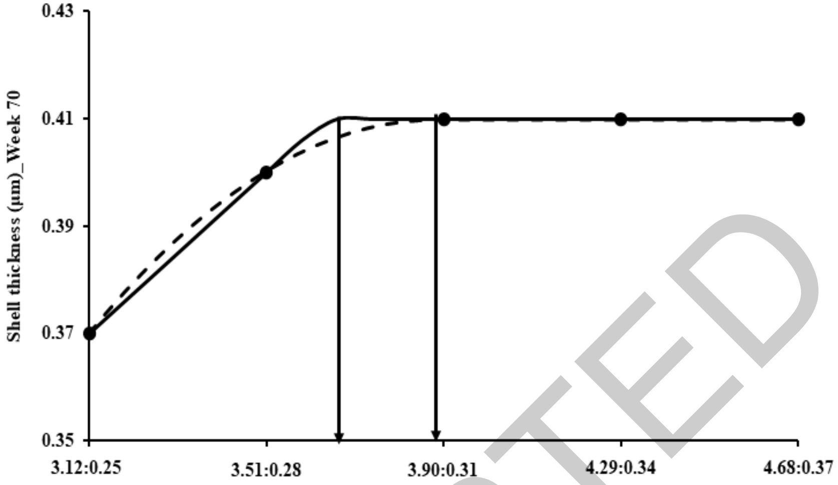

**Fig. 2.** Estimated dietary calcium and available phosphorus requirements for Hy-Line® laying hens based on shell thickness at week 70, as determined by linear-plateau (solid line) and quadratic-plateau (dashed line) models. Data points (●) represent the least squares means of dietary treatments (n=30). Arrows indicate the estimated requirement values for calcium and available phosphorus derived from each model.

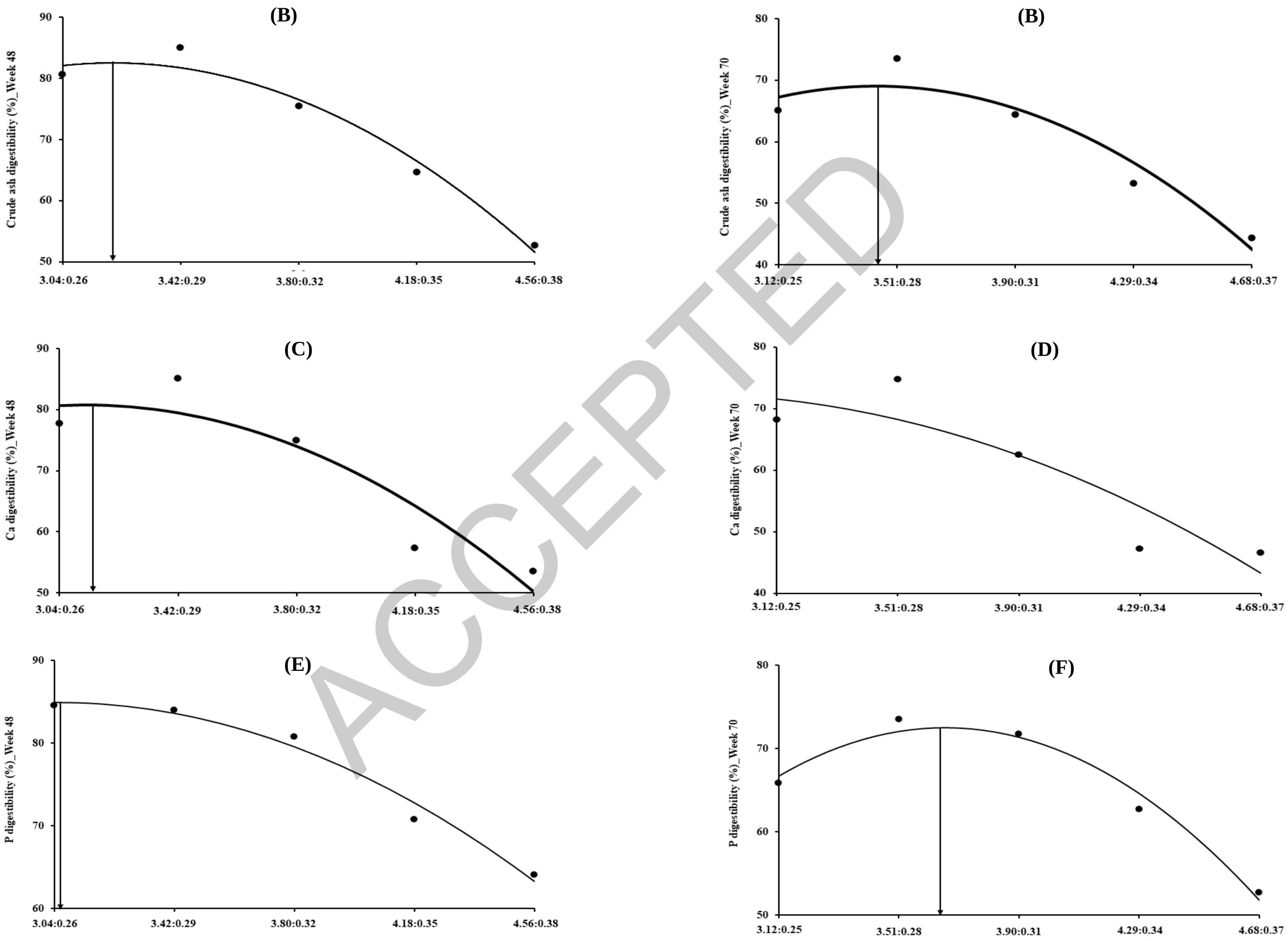
(B)
Crude ash digestibility (%)_Week 48
90
80
70
60
50
3.04:0.26
3.42:0.29
3.80:0.32
4.18:0.35
4.56:0.38
(B)
Crude ash digestibility (%)_Week 70
80
70
60
50
40
3.12:0.25
3.51:0.28
3.90:0.31
4.29:0.34
4.68:0.37
(C)
Ca digestibility (%)_Week 48
90
80
70
60
50
3.04:0.26
3.42:0.29
3.80:0.32
4.18:0.35
4.56:0.38
(D)
Ca digestibility (%)_Week 70
80
70
60
50
40
3.12:0.25
3.51:0.28
3.90:0.31
4.29:0.34
4.68:0.37
(E)
P digestibility (%)_Week 48
90
80
70
60
3.04:0.26
3.42:0.29
3.80:0.32
4.18:0.35
4.56:0.38
(F)
P digestibility (%)_Week 70
80
70
60
50
3.12:0.25
3.51:0.28
3.90:0.31
4.29:0.34
4.68:0.37

**Fig. 3.** Estimated dietary calcium and available phosphorus requirements for Hy-Line® laying hens based on quadratic regression analysis of ileal nutrient digestibility. (A) Ileal crude ash digestibility at week 48, (B) Ileal crude ash digestibility at week 70, (C) Ileal Ca digestibility at week 48, (D) Ileal Ca digestibility at week 70, (E) Ileal P digestibility at week 48, and (F) Ileal P digestibility at week 70. Data points (●) represent least squares means of dietary treatments (n=6). Arrows indicate the estimated requirement values for calcium and available phosphorus derived from each model.