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Article

Dietary phosphorus and feeding regimens modulate egg production, quality, and serum parameters in laying hens aged 61-80 weeks

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Abstract: Effective management of nutrients and feeding practices is essential to sustain egg production, maintain egg quality, and support the health of aging laying hens while optimizing resource use. This study examined the influence of dietary available phosphorus (AP) and quantitative feeding levels (QFL) on egg production, egg quality, and serum biochemical traits in Lohman Brown laying hens. A total of 540 hens, 60 weeks old, were assigned in a 2×3 factorial design, combining two AP levels (0.32% and 0.45%) with three feeding levels (90%, 95%, and 100% of standard intake). Each treatment included five replicates of 18 hens, and the experiment lasted 20 weeks following a 7-day acclimation period. Results showed that production performance was significantly affected by feeding level ($P < 0.01$), whereas AP levels had no significant impact. Egg production declined as feed restriction increased ($P < 0.01$), though no significant difference occurred between the 95% and 100% feeding groups. Egg weight, daily egg mass, and feed conversion ratio also decreased linearly with higher restriction ($P < 0.01$). Most egg quality traits were unaffected, except yolk color, which was greater at 0.45% AP ($P < 0.01$). Serum albumin decreased in the 90% feeding group ($P < 0.05$) but remained similar between 95% and 100% feeding levels. No significant interactions were detected between AP and QFL for serum biochemical indices. Overall, feeding hens at 95% of the recommended intake with 0.32% AP effectively maintained egg production, quality, and health, indicating that moderate feed restriction combined with adequate phosphorus can reduce feed costs without compromising performance in late-phase laying hens.

Keywords: feed restriction; nutrient utilization; Lohmann brown hens; performance traits; blood biochemistry

1. Introduction

Cost of production is consisted to be a crucial factor for sustaining productivity in aged laying hens while maintaining feed cost and waste of nutrients. Feed represents the largest single expense in poultry production, accounting for more than two-thirds of the total cost of production (Abdurofi *et al*., 2017). With the continuous rise in per-kilogram feed prices and other poultry utensils without any proportional increase in egg prices. Among the feed nutrients, phosphorus (P) is an expensive mineral, it crucial to manage costs and nutrient excretion to minimize environmental impact (Baradaran *et al*., 2021). It became being a major challenge to optimize production performance of laying hens while minimizing dietary costs (Rao *et al*., 2025). In response researchers and nutritionist has followed various strategies to minimize feed cost and improve profitability including limiting the intake of specific nutrients as dietary energy or protein and regulating the consumption of balanced diet (Nóbrega *et al*., 2022).

Quantitatively restricted feeding is considered such strategy to control body weight and metabolic rate during the laying period of hens, thereby improve feed efficiency and minimize the feed cost. However, restricted feeding often results in reduced egg production, egg weight, egg mass or a combination of these factors, though its effects can vary depending on the strain of hens and the level of restriction (Anene *et al*., 2023). Quantitative restricted feeding in laying hens may be helpful to maintain body weight, prevent overheating, limit health risk as well as minimize the egg production cost (Okongwu *et al*., 2025). Earlier research indicated that limiting feed intake in laying hens markedly decreased egg production, while egg weight and quality remained largely unchanged (Moreira *et al*., 2012). Additionally, alterations in feeding schedules during the late laying phase were found to negatively impact both the number of eggs produced and hen-day egg production (Liu *et al*., 2023). Conversely, other studies reported that restricting feed did not significantly influence egg production, egg weight, or egg quality in commercial laying hens (Osman *et al*., 2010).

Phosphorus is an essential nutrient for laying hens, playing a critical role not only in egg production and eggshell quality but also in supporting various metabolic processes in the body. Both deficient and excessive levels of dietary available phosphorus (AP) can negatively impact performance and compromise eggshell integrity (Dijkslag *et al*., 2023; Rana *et al*., 2023). The precise phosphorus requirements of laying hens remain variable, with reported needs ranging from 2.0 to 3.5 g of nonphytate phosphorus per kilogram of feed (Bello and Korver, 2019; Pongmanee *et al*., 2020). Other studies suggest that hens at different production stages require no more than 2.2 g AP per kilogram of diet (Rodehutscord *et al*., 2023). Eggshell quality issues are particularly common in older hens, often resulting in economic losses for producers (Lee *et al*., 2016; Fathi *et al*., 2019; Cheng and Ning, 2023; Sekeroglu *et al*., 2024). Reductions in dietary phosphorus have been shown to decrease eggshell quality (Jing *et al*., 2018), whereas other research reported that AP levels between 0.15 and 0.41% did not significantly affect egg production or shell quality (Li *et al*., 2018).

The precise phosphorus requirements of laying hens have been debated in recent years, with most research focusing on early and peak production stages. Studies on older, late-phase hens are limited, and the combined effects of dietary phosphorus levels and restricted feeding have not been thoroughly investigated. Considering these gaps, this study was designed to determine whether moderate feed restriction alongside optimized phosphorus supplementation could lower feed costs without compromising performance, egg quality, or overall hen health. The findings offer practical insights for managing feed and phosphorus in late-phase laying hens, suggesting that this strategy can sustain productivity and egg quality while improving cost efficiency.

2. Material and Methods

2.1. Ethical approval

No ethical approval was required to conduct the research.

2.2. Study area and periods

The study was conducted at the Poultry Research Farm within the Department of Animal Science at Jeonbuk National University, Korea from May 2023 to September 2023 (Figrue 1). The methodologies of the research was performed following the approved guidelines of Animal Care and Use Committeeof Jeonbuk National University.

2.3. Birds management, experimental design and diets

A total of 540 Lohman Brown laying hens at 60 weeks of age were allocated to a completely randomized design using a 2×3 factorial arrangement, which included two levels of available phosphorus (0.32% and 0.45%) and three quantitative feeding levels (90%, 95%, and 100%), forming six experimental treatments. Each treatment was replicated five times, with 18 hens per replicate. The birds were housed in A-type three-tiered cages, with two hens per wire cage, and allowed a 7-day acclimation period prior to the start of the 20-week experimental trial. Feed was offered as mash according to the Lohman Brown breeder's manual at 115 g per hen per day. The hens were kept in a controlled environment with a temperature of 22±3 °C and a lighting schedule of 16 hours light and 8 hours dark throughout the experiment. Any dead birds were replaced with spare hens subjected to the same treatment conditions. The basal diet provided 2750 kcal/kg of metabolizable energy, 15% crude protein, 4.30% calcium, 0.32% available phosphorus, and essential amino acids, including 0.31% methionine and 0.75% lysine. Experimental diets were adjusted to contain either 0.32% or 0.45% AP while maintaining a constant calcium level of 4.5%, using limestone (38.5% Ca) and monodicalcium phosphate (18% Ca and 21% P) as mineral sources (Table 1).

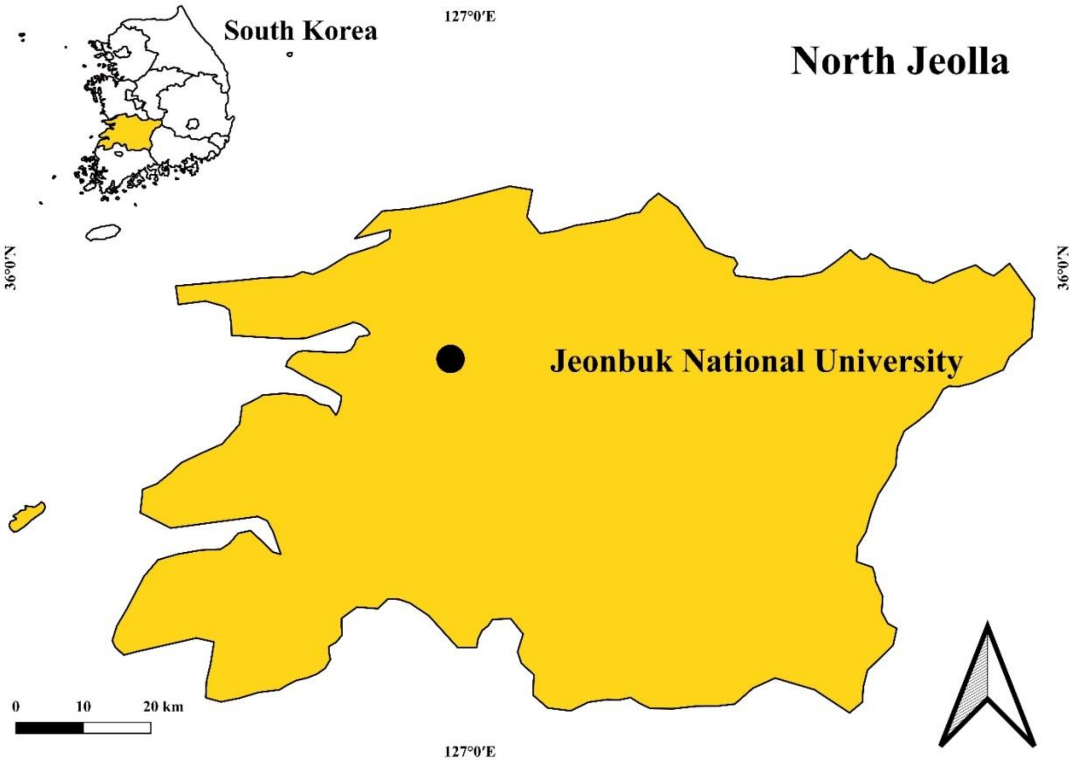

Figure 1. The study was conducted in the Jeonbuk National University, Korea.

Table 1. Composition of ingredients and calculated nutritional values of the basal diet.

Ingredients	**Amount (%)**
Corn	54.24
Wheat HRW	7.00
Wheat bran	7.66
Soybean meal (44%)	18.92
Limestone	10.08
MDCP	1.45
Salt	0.30
Vit-Min premix	0.30
DL-methionine	0.05
Calculated analysis	**Values**
Metabolizable energy (kcal/kg)	2750.00
Crude protein (%)	15.00
Lysine (%)	0.75
Methionine (%)	0.31
Cysteine (%)	0.28
Threonine	0.55
Calcium (%)	4.30
Available phosphorus (%)	0.32
Vitamin and other minerals (per kg feed)	**Values**
Vitamin A (IU)	5500
Vitamin D3 (IU)	11001
Vitamin E (mg)	11
Vitamin B12 (mg)	0.0066
Vitamin K3 (mg)	1.10
Riboflavin (mg)	4.40
Pantothenic acid (mg) (calcium pantothenate 1.96 mg)	11

Table 1. Contd.

Vitamin and other minerals (per kg feed)	Values
Cl (mg)	190.96
Folic acid (mg)	0.55
Pyridoxine (mg)	2.20
Biotin (mg)	0.11
Thiamine (mg)	2.20
Ethoxyquin (mg)	125
Cu (mg)	10
Fe (mg)	60
I (mg)	0.46
Mn (mg)	120
Zn (mg)	100

2.4. Productive performance of laying hens

Daily records of egg production, including broken and cracked eggs, were maintained for each treatment group, while egg weight was measured every other day, excluding damaged or abnormal eggs. Egg production percentage, feed conversion ratio (FCR), and egg mass were assessed over five consecutive 28-day periods from 61 to 80 weeks of age. FCR was calculated as grams of feed consumed per gram of egg produced, and egg mass was computed by multiplying the average egg weight by the corresponding egg production rate.

2.5. Evaluation of egg quality

Throughout the trial, a random sample of 30 eggs per treatment (five eggs per replicate) was collected every four weeks to evaluate egg quality traits, including shell color, shell thickness, shell breaking strength, albumen height, Haugh unit, and yolk color. The mean values were calculated for each trait. Eggshell strength was measured using an egg multi-tester (QC-SPA, Technical Services and Supplies, TSS, UK). Each egg was individually weighed and gently cracked onto a glass plate to determine albumen height, Haugh unit, and yolk and shell color using a semi-automated egg multi-tester (QCM+, TSS, UK). Shell thickness was measured at three distinct locations after carefully removing the inner membrane, using a micrometer (Digimatic, Series 293-330, Mitutoyo, Japan), and the mean value was calculated. All evaluations of egg quality followed the methodology outlined by Rana *et al*. (2023).

2.6. Serum properties evaluation

At the end of the 80th week, two hens from each replicate (totaling eight per treatment) were randomly chosen for evaluation of serum biochemical parameters. About 3–4 mL of blood was drawn from each hen’s wing vein using 5 mL disposable syringes and placed into non-heparinized tubes. The samples were centrifuged at 3000 rpm for 15 minutes at 4 °C to separate the serum, which was then refrigerated until further analysis. Biochemical analyses, including albumin (ALB), triglycerides (TG), cholesterol (CHOL), high-density lipoprotein (HDL), total protein (TP), glucose (GLU), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), were conducted using a Konelab 20 analyzer (Thermo Fisher Scientific, Vantaa, Finland) according to the manufacturer’s guidelines.

2.7. Statistical analysis

The gathered data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure in SAS software (version 9.1, 2009; SAS Institute, Cary, NC, USA). Treatment means were compared using Duncan’s multiple range test, and differences were considered statistically significant at $P < 0.05$.

3. Results and Discussion

3.1. Productive performance of laying hens

The combination of feeding levels and dietary available phosphorus significantly influenced egg production rate, egg weight, daily egg mass, and feed conversion ratio. Hens receiving diets with either 0.32% or 0.45% AP and provided 95% or 100% feeding access exhibited similar egg production rates, with no statistical difference between these groups. In the study, dietary 0.45% AP did not have any effect on performance probably because the AP requirement had been reached with the tested level 0.32%. In this study, supplementing 0.45% dietary AP did not improve performance, likely because the hens’ phosphorus requirement was already met at 0.32%

AP. This suggests that excess phosphorus in the diet does not further enhance laying performance. Comparable outcomes were stated by Bello and Korver *et al*. (2019), who found no differences in laying performance when AP levels increased from 0.22 to 0.36% in hens aged 30 to 70 weeks. In a similar study, Pongmanee *et al*. (2020) reported that aged laying hens fed diets with 0.22% or 0.45% AP showed no differences in performance traits, suggesting that 0.22% AP is adequate for maintaining optimal production, which aligns with the results of the current study. Egg production percentage was significantly affected by restricted feeding levels ($P < 0.01$), although hens with 95% and 100% feeding access showed statistically similar averages (Table 2).

Table 2. Influence of dietary available phosphorus (AP) and quantitative feeding levels (QFL) on the production performance of laying hens aged 61 to 80 weeks.

AP (%)	QFL (%)	Egg Production (%)	Egg weight (g)	Daily egg mass (g)	FCR	Broken egg (%)	Cracked egg (%)
61-80 weeks							
0.32	90	78.10[c]	61.80[c]	48.26[c]	2.14[c]	0.29	0.95
	95	78.89[ab]	62.35[bc]	49.18[b]	2.23[b]	0.63	1.57
	100	79.32[a]	63.46[a]	50.34[a]	2.29[a]	0.46	0.84
0.45	90	77.84[c]	62.13[bc]	48.36[c]	2.14[c]	0.26	0.56
	95	78.35[bc]	62.69[b]	49.12[b]	2.22[b]	0.34	0.64
	100	78.86[ab]	63.91[a]	50.39[a]	2.28[a]	0.51	0.76
Main effect							
AP (%)	0.32	78.77	62.54	49.26	2.22	0.46	1.12
	0.45	78.35	62.91	49.29	2.21	0.37	0.65
QFL (%)	90	77.97[b]	61.96[c]	48.31[c]	2.14[c]	0.27	0.75
	95	78.62[a]	62.52[b]	49.15[b]	2.23[b]	0.49	1.11
	100	79.09[a]	63.69[a]	50.37[a]	2.28[a]	0.48	0.80
SEM		0.14	0.18	0.19	0.01	0.07	0.13
***P* value**							
AP × QFL		0.003	0.0001	<0.0001	<0.0001	0.69	0.27
AP		0.12	0.31	0.94	0.85	0.54	0.07
QFL		0.001	<0.0001	<0.0001	<0.0001	0.40	0.51

FCR= feed conversion ratio; SEM= standard error of means; [a,b,c] within a column, means that do not share a common superscript are significantly different ($P < 0.05$).

A progressive decline in egg production was observed as feed restriction became more severe, which aligns with the findings of Olawumi (2014) and Anene *et al*. (2023), who reported that mild or temporary quantitative feed restriction did not compromise production performance in hens older than 40 weeks. Moreira *et al*. (2012) noted a significant drop in laying percentage when hens were subjected to feed restriction of 8% or more compared to ad libitum feeding. In this study, egg weight, daily egg mass, and feed conversion ratio also declined linearly with increasing feed limitation. These reductions may be due to restricted daily intake of energy, calcium, and phosphorus. Egg weight decreased proportionally with stricter feed restriction, consistent with Olawuni *et al*. (2012) reported no significant effect. Higher feed intake likely contributed to greater egg weight in less restricted groups. Egg mass, determined by multiplying egg production by the average egg weight, declined linearly as feed restriction increased, indicating decreases in both egg quantity and size. Similarly, feed conversion ratio declined alongside decreasing egg mass. There were no significant variations in the proportions of broken or cracked eggs among the treatments, nor were there effects from dietary AP levels or quantitative feeding levels. Consistent with these results, feed access between 90 and 100% and dietary AP levels of 0.2–0.3% did not affect eggshell quality traits in aged hens, indicating that moderate feed restriction or dietary AP was sufficient to maintain shell quality throughout the late laying time (Rana *et al*., 2022; Eltahan *et al*., 2023; Gu *et al*., 2025).

3.2. Egg quality

Yolk color was significantly influenced ($P < 0.01$) by the treatments and dietary AP levels, while other egg quality traits were not influenced by QFL, AP, or their interaction. Eggs from hens fed 0.45% AP or higher QFL exhibited significantly deeper yolk color ($P < 0.01$) compared to those fed 0.32% AP. This change in yolk pigmentation may result from enhanced carotenoid absorption or deposition, or possibly from increased blood phosphorus levels due to higher dietary AP, leading to the more intense yolk color observed in this study.

Table 3. Influence of dietary available phosphorus (AP) and quantitative feeding levels (QFL) on egg quality in laying hens at 80 weeks of age.

AP (%)	QFL (%)	Eggshell color	Albumen height (mm)	Haugh Unit	Yolk color	Eggshell breaking strength (kg/cm^2)	Eggshell thickness (mm)
0.32	90	26.00	8.74	92.55	4.75[b]	2.54	0.367
	95	26.06	8.88	93.69	4.63[b]	2.65	0.371
	100	25.63	8.64	92.31	4.81[b]	2.65	0.372
0.45	90	26.56	9.03	94.19	5.69[a]	2.63	0.372
	95	26.31	8.91	93.64	5.56[a]	2.61	0.373
	100	25.94	8.73	92.08	5.69[a]	2.71	0.375
Main effect							
AP (%)	0.32	25.90	8.75	92.85	4.73[b]	2.61	0.370
	0.45	26.27	8.89	93.31	5.65[a]	2.65	0.373
QFL (%)	90	26.28	8.89	93.37	5.22	2.58	0.369
	95	26.19	8.89	93.67	5.09	2.63	0.372
	100	25.78	8.68	92.20	5.25	2.68	0.373
SEM		0.31	0.11	0.57	0.10	0.06	0.003
***P* value**							
AP × QFL		0.97	0.93	0.87	0.001	0.98	0.99
AP		0.54	0.55	0.69	<0.0001	0.76	0.56
QFL		0.78	0.69	0.55	0.81	0.78	0.86

SEM= standard error of means; [a,b] within a column, means that do not share a common superscript are significantly different ($P < 0.05$).

Phosphorus plays a vital role in several physiological processes, including maintaining osmotic and acid-base balance, regulating energy and amino acid metabolism, supporting hormone synthesis and release, and facilitating cell replication and differentiation (Li *et al*., 2016). In this study, dietary AP levels did not influence other egg quality traits, including albumen height, Haugh unit, shell strength, and shell thickness. These outcomes support with Park *et al*. (2024), who testified that egg quality remained unchanged when hens received nonphytate phosphorus ranging from 0.25 to 0.45%. Similarly, Nie *et al*. (2013) observed that small increments of nonphytate phosphorus (0.20 to 0.40%) did not influence egg quality in laying hens. Additionally, no significant impact of feed restriction on egg quality were noted, aligned with the results of Moreira *et al*. (2012), which showed that restricted feeding did not alter egg quality traits in hens.

3.3. Serum indices

Serum albumin concentrations did not differ significantly among the treatments ($P < 0.01$) or between the various feeding levels ($P < 0.05$). Within the QFL groups, the lowest albumin level was recorded in hens receiving 90% of the feed (2.19 g/dl), while hens fed at 95% and 100% had higher concentrations (2.35 and 2.32 g/dl, respectively), with no significant difference between these two groups (Table 4). The variation in albumin levels may be attributed to the restricted diet, which could have limited metabolizable energy, protein, or other essential nutrients, thereby reducing total serum protein and causing a linear decline in albumin concentration as feed restriction intensified.

Table 4. Impact of dietary available phosphorus (AP) and quantitative feeding levels (QFL) on serum biochemical parameters of 80-week-old laying hens.

AP (%)	QFL (%)	ALB (g/dl)	TP (mg/dl)	GLU (mg/dl)	TG (mg/dl)	CHOL (mg/dl)	HDL (mg/dl)	AST (IU/l)	ALT (IU/l)
0.32	90	2.28[a]	6.75	303.12	1455.04	148.66	8.16	154.89	1.35
	95	2.34[a]	6.64	317.17	1947.88	159.10	14.02	185.90	0.62
	100	2.24[ab]	11.34	342.37	2885.14	199.56	16.64	151.88	1.26

Table 4. Contd.

AP (%)	QFL (%)	ALB (g/dl)	TP (mg/dl)	GLU (mg/dl)	TG (mg/dl)	CHOL (mg/dl)	HDL (mg/dl)	AST (IU/l)	ALT (IU/l)
0.45	90	2.10[b]	5.93	313.00	1355.23	127.90	5.76	151.36	0.47
	95	2.37[a]	7.66	323.30	2067.17	185.18	10.55	125.99	1.02
	100	2.41[a]	7.05	315.16	1501.97	152.92	26.81	151.35	1.09
Main effect									
AP (%)	0.32	2.29	8.24	320.89	2096.02	169.11	12.94	164.22	1.06
	0.45	2.29	6.88	317.15	1641.45	155.33	14.37	142.90	0.86
QFL (%)	90	2.19[b]	6.34	308.06	1405.13	138.28	6.96	153.13	0.91
	95	2.35[a]	7.15	320.24	2007.52	172.14	12.29	155.94	0.82
	100	2.32[a]	9.20	328.76	2193.55	176.24	21.72	151.61	1.17
SEM		0.03	0.60	4.93	177.78	8.70	2.75	7.91	0.13
***P* value**									
AP × QFL		0.01	0.11	0.32	0.11	0.18	0.29	0.46	0.35
AP		0.90	0.27	0.71	0.21	0.44	0.80	0.18	0.43
QFL		0.04	0.14	0.23	0.17	0.15	0.08	0.98	0.56

SEM= standard error of means; [a,b] within a column, means that do not share a common superscript are significantly different ($P < 0.05$).

Consistent with the present study, Ibigbami *et al*. (2014) reported that serum protein and albumin levels declined as feed restriction increased and dietary nutrient density decreased (Panda *et al*., 2024). Similarly, Ding *et al*. (2016) observed lower serum concentrations of albumin, triglycerides, and total cholesterol in laying hens when dietary energy or protein levels were reduced. In this study, the interaction between AP and QFL, as well as their individual effects, did not significantly affect other serum parameters, including cholesterol, high-density lipoprotein, glucose, triglycerides, aspartate aminotransferase, alanine aminotransferase, and total protein. However, increased feed restriction during the laying period numerically reduced total cholesterol, high-density lipoprotein, and triglyceride levels. These outcomes support Mellouk *et al*. (2018), who noted that restricted feeding decreased plasma triglyceride, cholesterol, and glucose concentrations, and with Panda *et al*. (2024), who reported that lowering dietary nutrient density reduced serum triglyceride and total cholesterol, supporting the outcomes of the current study.

4. Conclusions

The findings of this study indicate that implementing moderate feed restriction alongside a dietary phosphorus level of 0.32% from 61 to 80 weeks of age is sufficient to maintain satisfactory egg production and quality, suggesting that moderate restriction can help reduce production costs in aged laying hens. Higher dietary phosphorus did not provide additional benefits, and severe feed restriction negatively impacted both productivity and health. Additional studies are required to evaluate the long-term impact of moderate feed restriction combined with optimized phosphorus levels on bone integrity, eggshell quality, and hormonal regulation in laying hens.

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Data availability

All data of the study are available in the text and tables of this manuscript.

Conflict of interest

None to declare.

Authors' contribution

Md Masud Rana: conceptualization, writing original draft, design of the study, methodology, investigation, data curation, formal analysis, reviewing and editing; Md Sazedul Karim Sarker: conceptualization, methodology, formal analysis, critically reviewing and editing; Md Yousuf Ali: statistical analysis, methodology, writing original draft, reviewing and editing; Md Razibul Hassan: methodology, investigation, data curation, reviewing and proofreading; Md Aftabuzzaman: methodology, data curation, reviewing and proofreading. All authors have read and agreed to the published version of this manuscript.

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