104 (2025) 105055

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Dietary calcium and non-phytate phosphorus levels affect performance, follicular development, and egg quality of native chicken at peak laying period
Calcium and non-phytate phosphorus affect performance

L.F. Cheng [a,b], Q.Q. Zhang [a], W.Y. Zhao [a], C. Chang [a], X. Wang [a], Z.X. Yan [a], J. Cao [a], H.G. Liu [a], A.L. Geng [a,*]

[a] Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China
[b] College of Animal Science and Technology, Shandong Agricultural University, Tai'an, Shandong, 271018, China

ARTICLE INFO

Keywords:
Calcium
Phosphorus
Performance
Follicular development
Egg quality

ABSTRACT

The experiment aimed to study effects of dietary calcium **(Ca)** and non-phytate phosphorus (**NPP**) levels on performance, follicular development, egg quality and serum biochemical indices in native bird-*Beijing You Chicken* (**BYC**). A 3 × 3 factorial design was adopted, dietary Ca levels were 2.8 %, 3.2 % and 3.6 %, dietary NPP levels were 0.33 %, 0.38 % and 0.43 %. A total of 972, 28-wk-old BYC laying hens were randomly divided into 9 groups with 4 replicates per group, and 27 birds per replicate. The trial was conducted after 2 wks of pre-adaptation. The performance was determined during 30~35 wks, 36~41 wks and 30~41 wks; follicular development, egg quality and serum biochemical indices were determined at 41 wks of age. The results showed that dietary Ca and NPP levels alone and the interaction affected feed egg ratio (**FER**) of BYC during 30~35 wks ($P < 0.05$), the FER was the lowest (2.65±0.05) and the egg-laying rate was the highest (77.45±3.19 %) in the group with 3.6 % Ca and 0.43 % NPP. Dietary 0.33 % NPP and 0.43 % NPP increased the number of small yolk follicles ($P < 0.05$). Dietary 3.6 % Ca deepened the yolk color (**YC**) ($P < 0.001$), dietary NPP level affected the YC, Haugh unit (**HU**), relative yolk weight (**RYW**) and relative albumen weight (**RAW**) ($P < 0.05$). The interaction of dietary Ca and NPP levels affected eggshell color (**ESC**) ($P < 0.05$), YC ($P < 0.001$), and relative eggshell weight (**RESW**) ($P < 0.001$), also had the trend to affect eggshell strength (**ESS**) ($P = 0.073$), albumen height (**AH**) ($P = 0.077$) and HU ($P = 0.055$). 4) 0.43 % NPP had a trend to affect malondialdehyde (**MDA**) ($P = 0.064$). In summary, dietary Ca and NPP levels alone and the interaction did not affect egg production of BYC, and FER in the group with 3.6 % Ca and 0.43 % NPP seemed to be the lowest during 35~41 wks. Dietary NPP may be related to pre-graded follicular development. The recommended dietary Ca level is 3.6 % and NPP level is 0.43 % for BYC during the peak laying period. The Ca/NPP ratio in the range of 8.39~9.67 may be beneficial to laying performance and egg quality of laying hens.

Introduction

Calcium (**Ca**) and phosphorus (**P**), as essential mineral elements in animals, participate in many physiological and productive activities and play an irreplaceable role (Ren et al., 2023a). Dietary Ca is directly involved in eggshell formation (McDowell, 2003), while non-phytate phosphorus (**NPP**), as an effective form of phosphorus utilization, is closely related to normal metabolic and structural functions (Elser, 2012; Reyer et al., 2021). The demand for Ca and NPP varies significantly among different breeds and physiological stages of poultry. Jing et al. (2018) suggested that dietary 0.15 % NPP was sufficient to maintain the health and laying performance of hens at 22-34 wks of age. Wei et al. (2022) showed that egg production of hens aged 26-36 wks increased as dietary NPP levels increased from 0.17 % up to 0.34 %. Insufficient Ca and P can lead to reduced body functions, slow growth and even stagnation, while excessive Ca and P can impair the growth

* Corresponding author at: No. 9 Shu-Guang Garden Mid-Road, Haidian District. Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China.

E-mail address: ailiangengcau@126.com (A.L. Geng).

https://doi.org/10.1016/j.psj.2025.105055
Received 14 January 2025; Accepted 15 March 2025
Available online 16 March 2025

and development of poultry (Ren et al., 2020; Webster, 2004). Laying hens, especially those with high laying rates, have additional requirements for mineral homeostasis and nutrient flow during laying. With the increase of age and egg production of laying hens, dietary Ca and P supplementation is necessary to ensure the production of high-quality eggs and prevent osteoporosis (Ren et al., 2020). The NRC (1994) suggested that the Ca requirement is 3.25 % and NPP requirement is 0.25-0.45 % for laying hens; the Feeding Standard of Chicken in China (NY/T 33-2004, 2004) indicated that Ca and total P requirements are 3.5 % and 0.60 % respectively for laying hens. Studies have shown that although low P diet (0.11 % NPP) is beneficial to old laying hens (69~78 wks), it significantly reduces the laying rate of young laying hens (34 wks) (Ren et al., 2023a; Teng et al., 2020).

Beijing You Chicken (**BYC**) is a dual-purpose chicken in China, its excellent meat and egg quality are popular with consumers and has been developed rapidly in recent years (Geng et al., 2018), but its nutritional requirements are not perfect. The existing nutritional recommendations for BYC are mostly based on commercial high-yield laying hens (such as Hy-line Brown and Roman Brown), while local varieties may have different demand patterns for Ca and NPP due to their slow growth rate and long laying cycle (Teng et al., 2020). We have studied the effects of dietary Ca and NPP levels on performance of BYC in brooding period (Zhang et al., 2021) and growing period (Zhang, et al., 2023), this experiment aimed to study the effects of dietary Ca and NPP levels alone and in interaction on egg laying performance, follicle development, egg quality and serum biochemical indices of BYC at peak laying period, so as to provide a theoretical basis for precision nutrition regulation of local chicken in the future.

Materials and methods

Experimental design and birds

The experiment was conducted at a BYC demonstration farm, Shunyi district, Beijing. A 3 × 3 factorial design was adopted, dietary Ca levels were 2.8 %, 3.2 %, and 3.6 %, dietary NPP levels were 0.33 %, 0.38 %, and 0.43 %. A total of 972, 28-wk-old BYC laying hens were randomly divided into 9 groups with 4 replicates per group, and 27 birds per replicate. The trial was conducted after 2 wks of pre-adaptation. The settings of dietary Ca and NPP levels were adjusted up and down according to "Technical code of practice of feeding and management of *Beijing-You Chicken*" (DB11/T 1378-2023, 2023).

The nutritional composition of the diets is shown in Table 1. The hens were housed in 3-layer cages, fed ad libitum, and managed under normal feeding conditions with a photoperiod of 16 h (6:00~22:00).

Egg laying performance

The number of live birds, dead birds, the number of eggs, the weight of eggs, and the number of abnormal eggs in each replicate were recorded daily. Feed was measured each week and feed intake was counted for each replicate group. The weekly average feed intake (**AFI**), egg mass (**EM**), egg-laying rate, feed to egg ratio (**FER**) and mortality were calculated during 30~35 wks, 36~41 wks and 30~41 wks.

Follicular development

At the end of 41 wks of age, one hen was randomly selected from each replicate, four hens each group, euthanized by cervical dislocation after 12 h of feed deprivation. The size and number of follicles were observed. Follicles with a diameter of >1 mm were exfoliated, weighed, and measured with an electronic vernier calipers. Follicles were divided into graded follicles (≥12 mm), large yellow follicles (9~11 mm), and small yellow follicles (6~8 mm) according to the diameter. Count the number of follicles at each level (the slaughter performance, serum hormone indicators are not listed in this article).

Egg quality

At the end of 41 wks, 7~8 fresh eggs were randomly chosen from each replicate, totaled 30 eggs for each group. Egg weight (**EW**), Haugh unit (**HU**), albumen height (**AH**), yolk color (**YC**), egg grade (**EG**), eggshell strength (**ESS**), eggshell thickness (**EST**), eggshell color (**ESC**), egg shape index (**ESI**), relative yolk weight (**RYW**), relative eggshell weight (**RESW**), and relative albumen weight (**RAW**) were measured and calculated within 24 h. The indicators were measured according to our previous report (Geng et al., 2018).

Table 1
Composition and nutrient levels of basal diets (air-dry basis).

Items	Groups								
	1	2	3	4	5	6	7	8	9
Ingredients (%)									
Corn	66.70	64.60	62.44	66.40	64.35	62.21	66.10	64.00	61.90
Soybean meal	22.45	22.50	22.69	22.50	22.50	22.69	22.55	22.60	22.75
Tamari protein powder 60	0.60	0.85	1.00	0.60	0.85	1.00	0.60	0.85	1.00
Soybean oil	0.12	0.80	1.50	0.22	0.90	1.60	0.32	1.00	1.70
Monocalcium phosphate	0.98	0.99	1.00	1.22	1.23	1.23	1.46	1.47	1.47
Limestone powder	7.15	8.26	9.37	7.06	8.17	9.27	6.97	8.08	9.18
Premix[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient levels									
ME/(MJ/kg)	11.499	11.500	11.499	11.499	11.502	11.503	11.499	11.500	11.503
CP	16.001	16.010	16.019	16.000	15.990	16.001	16.000	16.009	16.004
Lysine	0.831	0.831	0.833	0.832	0.830	0.833	0.833	0.832	0.834
Methionine	0.450	0.452	0.452	0.450	0.451	0.452	0.450	0.451	0.452
Methionine + Cystine	0.720	0.721	0.721	0.720	0.720	0.721	0.720	0.721	0.720
Ca[2]	2.801	3.202	3.604	2.803	3.203	3.600	2.804	3.205	3.601
Total phosphoru	0.535	0.533	0.531	0.585	0.583	0.579	0.635	0.633	0.629
NPP	0.330	0.331	0.331	0.380	0.381	0.379	0.431	0.431	0.429
Ca/NPP	8.49	9.67	10.89	7.38	8.41	9.49	6.51	7.44	8.39

1) The premix provided the following per kg of the diet: VA 7.200 IU, VD 3,360 IU, VE 20 IU, VK 2.9 mg, thiamine 2.4 mg, riboflavin 6.2 mg, calcium pantothenate 13 mg, niacin 36 mg, pyridoxine 4.3 mg, Biotin 0.24 mg, folic acid 1.5 mg, VB12 0.032 mg, choline 800 mg, Mn 100 mg, I 1.8 mg, Fe 100 mg, Cu 8 mg, Zn 90 mg, Se 0.30 mg.
2) ME=metabolizable energy; CP=crude protein; Ca=Calcium; NPP= non-phytate phosphorus.
3) Ca and NPP are measured values, the rest of the nutrient levels are calculated from the data provided by Feed Database in China (2013).

Serum biochemical indices

At the end of 41 wks, 3 hens per replicate were selected and bled via the wing vein (5 mL) after a fast of 12 h. Blood samples were centrifuged 3 000 rpm for 10 min at 4°C, and the serum was stored at −80°C for analysis of biochemical indices, including serum Ca, P, alkaline phosphatase (**AKP**), high-density lipoprotein cholesterol (**HDL-C**), low-density lipoprotein cholesterol (**LDL-C**), malondialdehyde (**MDA**), total cholesterol (**TC**), glutamate and total antioxidant capacity (**T-AOC**). The kits were from Nanjing Jiancheng Bioengineering Institute Co. Ltd (Nanjing, China), and were measured by spectrophotometer (Evolution 60, Thermo Fisher, Shanghai, China).

The study was performed in accordance with local ethical guidelines and approved by the institutional animal care and use committee (IHVM11-2103-4).

Statistical analysis

The general linear model in SPSS 25.0 software for Windows (SPSS Inc. Chicago, IL) was used to analyze main effects and the interaction of Ca and NPP. Duncan's test was used to test the significance of the differences between groups, with $P < 0.05$ as the significance, $0.05 \le P < 0.10$ as the trend of difference, and $P < 0.001$ as the highly significant difference. The results of egg-laying performance and follicular development were expressed as mean ± standard deviation and the egg quality and serum biochemical indices were expressed as mean and standard error between groups. The percentage was arcsine transformed before the normality test.

Results

Egg laying performance during 30∼35 wks

As shown in Table 2, dietary Ca and NPP levels alone and the interaction affected the FER of BYC during 30∼35 wks ($P < 0.05$), the FER was the lowest in the group with 3.6 % Ca and 0.43 % NPP (2.65 ±0.05). Increasing dietary Ca levels and NPP levels had a trend to affect the EM ($P = 0.082$, $P = 0.081$), and the highest EM was found in 3.6 % Ca group (4 406.53 g) and 0.43 % NPP group (4 376.11 g). The interaction of dietary Ca and NPP levels had a trend to affect egg-laying rate of BYC ($P = 0.089$), the egg-laying rate seemed to be the highest in the group with 3.6 % Ca and 0.43 % NPP (77.45±3.19 %).

Egg laying performance during 36∼41 wks

As shown in Table 3, dietary Ca and NPP levels alone and the interaction had no effects on egg laying performance during 36∼41 wks ($P > 0.05$).

Egg laying performance during 30∼41 wks

As shown in Table 4, increasing dietary Ca levels had a trend to affect EM during 30∼41 wks ($P = 0.050$), the EM was the highest in 3.6 % Ca group (4 258.15 g), and the lowest in 2.8 % Ca group (4 038.13 g); Increasing dietary Ca levels had the trend to affect the FER ($P = 0.061$) and mortality ($P = 0.056$). Dietary NPP levels alone had no effects on egg production during 30∼41 wks ($P > 0.05$). The interaction of dietary Ca and NPP levels had a trend to affect the AFI ($P = 0.082$) and FER ($P = 0.083$). At 2.8 % Ca, increasing NPP level did not influence the AFI during 30-41 wks. At 3.2 % and 3.6 % Ca, the AFI was decreased, respectively. At 2.8 % Ca, the FER was decreased when NPP level increased from 0.33 % to 0.38 %, but at 3.2 % and 3.6 % Ca, the FER was not affected. The group with 3.6 % Ca and 0.43 % NPP seemed to have the lowest FER (2.69) during 30∼41 wks.

Table 2
Effects of dietary calcium and non-phytate phosphorus on egg laying performance of *Beijing You Chicken* during 30-35 wks.

Items		AFI (g/d)	EM (g)	Egg-laying rate (%)	FER (g:g)	Mortality (%)
Ca (%)	NPP (%)					
2.8	0.33	91.21 ±2.78	4207.92 ±128.74	74.04 ±2.28	2.83 ±0.13[ab]	0.25±0.50
3.2	0.33	99.33 ±2.91	4433.75 ±229.43	76.32 ±4.40	2.80 ±0.15[ab]	0.00±0.00
3.6	0.33	98.37 ±2.16	4316.67 ±275.62	73.55 ±4.30	2.85 ±0.15[ab]	0.00±0.00
2.8	0.38	98.73 ±2.43	3993.75 ±164.75	68.78 ±1.99	3.08 ±0.11[c]	0.00±0.00
3.2	0.38	96.07 ±3.44	4151.25 ±385.19	72.67 ±6.15	2.84 ±0.16[ab]	0.50±0.58
3.6	0.38	100.44 ±4.08	4339.58 ±216.56	75.68 ±3.02	2.84 ±0.10[ab]	0.25±0.50
2.8	0.43	101.01 ±0.62	4365.00 ±241.46	76.08 ±3.27	2.84 ±0.14[ab]	0.25±0.50
3.2	0.43	97.63 ±3.64	4200.00 ±200.68	72.45 ±3.07	2.89 ±0.08[b]	0.00±0.00
3.6	0.43	97.61 ±2.31	4563.33 ±128.18	77.45 ±3.19	2.65 ±0.05[a]	0.00±0.00
SEM		0.48	38.59	0.62	0.02	0.06
Main effects						
Ca (%)	2.8	98.98	4188.89	72.67	2.92[b]	0.17
	3.2	97.67	4261.67	73.82	2.84[ab]	0.17
	3.6	98.81	4406.53	75.56	2.78[a]	0.08
	P-value	0.490	0.082	0.238	0.039	0.795
NPP (%)	0.33	98.30	4319.44	74.64	2.83[ab]	0.08
	0.38	98.41	4161.53	72.38	2.92[b]	0.25
	0.43	98.75	4376.11	75.33	2.79[a]	0.08
	P-value	0.925	0.081	0.145	0.042	0.409
Ca × NPP	P-value	0.123	0.243	0.089	0.040	0.199

Note: Ca=calcium; NPP= non-phytate phosphorus; AFI=average feed intake; EM=egg mass; FER=feed to egg ratio.

Follicular development at 41 wks of age

As shown in Table 5, the number of mature follicles in dietary 3.2 % Ca had a trend to be higher than that in 2.8 % Ca and 3.6 % Ca groups ($P = 0.076$); dietary 0.33 % NPP and 0.43 % NPP significantly increased the number of small yolk follicles ($P = 0.002$); The interaction of dietary Ca and NPP levels had no effects on the follicle development ($P > 0.05$).

Egg quality at 41 wks of age

As shown in Table 6, dietary Ca level significantly deepened the YC, the 3.6 % Ca was the deepest ($P < 0.001$); dietary NPP levels affected the YC, HU, RYW and RAW ($P < 0.05$). The YC was increased with the increasing NPP levels, the highest YC was found in 0.43 % NPP (8.17), and the lowest in 0.33 % NPP (6.40) ($P < 0.001$); The interaction of Ca and NPP affected the ESC, YC and RESW ($P < 0.05$); The HU was lower in 0.38 % NPP than in 0.33 % NPP ($P < 0.05$), but had no difference with 0.43 % NPP; The RYW was higher in 0.38 % NPP than in 0.33 % NPP ($P < 0.05$), but had no difference with 0.43 % NPP. The RAW was higher in 0.33 % NPP than in other two NPP groups ($P < 0.001$). Dietary 0.33 % NPP tended to increase the AH ($P = 0.057$). At 0.43 % NPP, increasing Ca level did not influence the YC. However, at 0.33 % and 0.38 % NPP, the YC was higher at 3.6 % and 3.2 % Ca, respectively, resulting in an interaction between Ca and NPP level ($P < 0.001$). It was like ESC ($P < 0.001$) and RESW ($P < 0.001$). The Ca × NPP interaction had the trend to affect ESS ($P = 0.073$), AH ($P = 0.077$) and HU ($P = 0.055$).

The ESC was higher (lighter in color) in the groups with 3.6 % Ca and 0.33 % NPP, 2.8 % Ca and 0.38 % NPP, 2.8 % Ca and 0.43 % NPP, 3.2 %

Table 3
Effects of dietary calcium and non-phytate phosphorus on egg laying performance of *Beijing You Chicken* during 36-41 wks.

Items		AFI (g/d)	EM (g)	Egg-laying rate (%)	FER (g:g)	Mortality (%)
Ca (%)	NPP (%)					
2.8	0.33	93.98 ±1.63	4063.75 ±191.50	62.50 ±3.90	2.77 ±0.06	1.00±0.82
3.2	0.33	96.09 ±2.79	3979.17 ±219.44	61.11 ±2.92	2.88 ±0.13	1.50±1.29
3.6	0.33	93.44 ±2.46	4120.54 ±287.39	60.37 ±2.45	2.79 ±0.15	0.75±0.96
2.8	0.38	91.14 ±3.62	3627.92 ±123.74	55.57 ±3.47	3.01 ±0.11	1.50±1.00
3.2	0.38	92.71 ±3.67	3989.17 ±215.17	61.46 ±1.92	2.74 ±0.08	1.00±0.82
3.6	0.38	94.58 ±0.84	4096.25 ±162.78	60.37 ±1.63	2.87 ±0.08	0.00±0.00
2.8	0.43	92.93 ±3.25	3970.42 ±385.33	58.74 ±5.40	2.90 ±0.20	0.25±0.50
3.2	0.43	91.88 ±4.11	3991.25 ±372.18	60.72 ±3.85	2.79 ±0.12	1.25±0.96
3.6	0.43	91.37 ±3.33	4112.50 ±308.68	60.89 ±4.02	2.74 ±0.27	0.75±0.50
SEM		0.50	44.37	0.48	0.03	0.14
Main effects						
Ca (%)	2.8	92.68	3887.36	58.94	2.89	0.92
	3.2	93.56	3986.53	61.10	2.80	1.25
	3.6	93.13	4109.76	60.54	2.80	0.50
	P-value	0.779	0.142	0.301	0.227	0.109
NPP (%)	0.33	94.50	4054.49	61.33	2.81	1.08
	0.38	92.81	3904.44	59.13	2.87	0.83
	0.43	92.06	4024.72	60.12	2.81	0.75
	P-value	0.147	0.358	0.315	0.495	0.604
Ca × NPP	*P-value*	0.382	0.447	0.249	0.160	0.208

Note: Ca=calcium; NPP= non-phytate phosphorus; AFI=average feed intake; EM=egg mass; FER=feed to egg ratio.

Ca and 0.43 % NPP.

Serum biochemical indices at 41 wks of age

As shown in Table 7, there were no effect of dietary Ca and NPP levels alone and the interactions on serum biochemical indices ($P > 0.05$). The 0.43 % NPP had a trend to affect MDA ($P = 0.064$).

Discussion

Egg laying performance and Ca, P levels

The Ca and P as two essential elements for bone and eggshell formation, are particularly important for improving egg production in laying hens (Jlali et al., 2023). There had different responses of different breeds of chickens to dietary Ca and P concentrations (Reyer et al., 2021; Sommerfeld et al., 2020). Feed consumption of DeKalb XL pullets increased as dietary Ca or P levels increased during peak production (Frost and Roland, 1991). Attia et al. (2020) suggested that 4 % Ca significantly reduced feed conversion ratio of H&N Brown laying hens in the late laying period. Dietary 0.35 % NPP was favorable to production performance and tibia development of Roman laying hens (Sun, 2016); dietary 0.18 % NPP caused a decrease in egg production in 34-wk-old Roman white laying hens (Teng et al., 2020). In laying hens aged 30-70 wks, dietary NPP from 0.22 to 0.36 % had no significant effects on laying performance (Bello and Korver, 2019). A low NPP (0.11 %) was beneficial for egg production and bone health for laying hens during 69~78 wks (Ren et al., 2023b), but dietary NPP levels (0.12 %, 0.17 %, 0.22 %, 0.27 %, 0.32 %, 0.37 %, and 0.42 %) had no effects on laying performance of 40-wk-old Hy-line Brown laying hens (Ren et al., 2020).

Table 4
Effects of dietary calcium and non-phytate phosphorus on egg laying performance of *Beijing You Chicken* during 30-41 wks.

Items		AFI (g/d)	EM (g)	Egg-laying rate (%)	FER (g:g)	Mortality (%)
Ca (%)	NPP (%)					
2.8	0.33	95.60 ±1.49	4135.83 ±136.21	68.27 ±2.92	2.80 ±0.09	1.25±0.50
3.2	0.33	97.71 ±2.15	4206.46 ±143.35	68.72 ±3.17	2.83 ±0.11	1.50±1.29
3.6	0.33	95.90 ±2.31	4218.60 ±242.24	66.96 ±2.92	2.82 ±0.13	0.75±0.96
2.8	0.38	94.93 ±2.61	3810.83 ±143.17	62.17 ±2.71	3.05 ±0.11	1.50±1.00
3.2	0.38	94.39 ±0.85	4070.21 ±209.00	67.06 ±2.19	2.79 ±0.12	1.50±0.57
3.6	0.38	97.51 ±2.21	4217.92 ±186.71	68.02 ±2.25	2.86 ±0.08	0.25±0.50
2.8	0.43	96.97 ±1.50	4167.71 ±305.82	67.41 ±4.27	2.86 ±0.16	0.50±0.58
3.2	0.43	94.75 ±3.24	4095.63 ±260.73	66.59 ±3.15	2.84 ±0.10	1.25±0.96
3.6	0.43	94.49 ±2.79	4337.92 ±193.75	69.17 ±3.44	2.69 ±0.15	0.75±0.50
SEM		0.37	39.95	0.51	0.02	0.14
Main effects						
Ca (%)	2.8	95.83	4038.13^{a}	65.95	2.90	1.08^{ab}
	3.2	95.62	4124.10^{ab}	67.46	2.82	1.42^{b}
	3.6	95.97	4258.15^{b}	68.05	2.79	0.58^{a}
	P-value	0.928	0.050	0.168	0.061	0.056
NPP (%)	0.33	96.40	4186.97	67.98	2.82	1.17
	0.38	95.61	4032.99	65.75	2.90	1.08
	0.43	95.40	4200.42	67.72	2.80	0.83
	P-value	0.522	0.114	0.241	0.109	0.584
Ca × NPP	*P-value*	0.082	0.401	0.139	0.083	0.497

Note: Ca=calcium; NPP= non-phytate phosphorus; AFI=average feed intake; EM=egg mass; FER=feed to egg ratio.

Table 5
Effects of dietary calcium and non-phytate phosphorus on follicular development of 41- wk-old *Beijing You Chicken.*

Items		Number of large yellow follicles	Number of small yellow follicles	Number of mature follicles
Ca (%)	NPP (%)			
2.8	0.33	2.60±2.07	0.60±0.55	0.40±0.55
3.2	0.33	3.80±1.10	1.00±0.71	0.60±0.89
3.6	0.33	3.60±1.14	1.00±0.00	0.40±0.55
2.8	0.38	3.80±1.92	0.40±0.55	0.20±0.45
3.2	0.38	3.40±1.95	0	0.80±0.45
3.6	0.38	3.40±1.95	0.40±0.55	0.20±0.45
2.8	0.43	4.20±0.45	0.60±0.55	0.60±0.55
3.2	0.43	3.80±1.30	1.20±0.45	1.00±0.00
3.6	0.43	4.40±0.55	0.80±0.45	0.60±0.55
SEM		0.22	0.07	0.08
Ca (%)	2.8	3.53	0.53	0.40
	3.2	3.67	0.73	0.80
	3.6	3.80	0.73	0.40
	P-value	0.889	0.433	0.076
NPP (%)	0.33	3.33	0.87^{b}	0.47
	0.38	3.53	0.27^{a}	0.40
	0.43	4.13	0.87^{b}	0.73
	P-value	0.327	0.002	0.213
Ca × NPP	*P-value*	0.692	0.168	0.919

Note: Ca=calcium; NPP= non-phytate phosphorus.

Table 6
Effects of dietary calcium and non-phytate phosphorus levels on egg quality of 41-wk-old *Beijing You Chicken*.

Items		EW (g)	ESS (kg/cm^{2})	EST (mm)	ESC	AH (mm)	YC	HU	RYW (%)	RESW (%)	RAW (%)	ESI	EG
Ca (%)	NPP (%)												
2.8	0.33	45.35	3.46	0.30	43.16^{a}	5.12	5.76^{b}	74.77	0.31	0.14^{b}	0.56	1.35	2.75
3.2	0.33	46.71	3.55	0.31	45.21^{ab}	5.08	4.80^{a}	74.70	0.31	0.13^{a}	0.56	1.32	2.81
3.6	0.33	47.78	3.35	0.30	52.06^{c}	4.87	8.38^{d}	72.18	0.31	0.13^{a}	0.56	1.33	2.67
2.8	0.38	46.78	3.48	0.31	51.19^{c}	4.41	7.49^{c}	68.46	0.32	0.14^{b}	0.54	1.33	2.50
3.2	0.38	46.05	3.45	0.31	46.90^{abc}	4.69	8.15^{d}	70.93	0.32	0.13^{a}	0.55	1.33	2.53
3.6	0.38	46.15	3.52	0.32	50.03^{bc}	4.73	7.41^{c}	70.69	0.32	0.14^{b}	0.55	1.34	2.73
2.8	0.43	46.92	3.77	0.30	50.30^{c}	5.33	8.31^{d}	75.92	0.32	0.13^{a}	0.55	1.32	2.66
3.2	0.43	45.95	3.30	0.30	50.27^{c}	4.98	8.16^{d}	73.98	0.31	0.14^{b}	0.55	1.33	2.60
3.6	0.43	46.94	3.56	0.31	47.44^{abc}	4.39	8.05^{d}	68.28	0.31	0.13^{a}	0.56	1.34	2.69
SEM		0.26	0.04	0.002	0.58	0.07	0.06	0.57	0.001	0.001	0.002	0.003	0.03
Main effects													
Ca (%)	2.8	46.35	3.56	0.30	48.22	4.95	7.21^{a}	73.34	0.32	0.13	0.55	1.34	2.64
	3.2	46.24	3.46	0.31	47.46	4.92	7.12^{a}	73.26	0.31	0.13	0.56	1.32	2.65
	3.6	46.95	3.47	0.31	49.85	4.66	7.96^{b}	70.84	0.31	0.13	0.56	1.34	2.70
	P-value	0.464	0.576	0.104	0.211	0.188	<0.001	0.120	0.315	0.158	0.209	0.207	0.691
	0.33	46.61	3.45	0.30	46.81	5.02	6.40^{a}	74.03^{b}	0.31^{a}	0.13	0.56^{b}	1.33	2.74
NPP (%)	0.38	46.33	3.49	0.31	49.37	4.61	7.72^{b}	70.33^{a}	0.32^{b}	0.13	0.55^{a}	1.34	2.59
	0.43	46.60	3.56	0.30	49.34	4.90	8.17^{c}	73.08^{ab}	0.31^{ab}	0.13	0.55^{a}	1.33	2.65
	P-value	0.875	0.540	0.336	0.124	0.057	<0.001	0.013	0.019	0.394	<0.001	0.378	0.119
Ca × NPP	*P-value*	0.317	0.073	0.405	0.003	0.077	<0.001	0.055	0.378	<0.001	0.655	0.241	0.306

Note: Ca=calcium; NPP=non-phytate phosphorus; EW=egg weight; HU=Haugh unit; AH=albumen height; YC=yolk color; EG=egg grade; ESS=eggshell strength; EST=eggshell thickness; ESC=eggshell color; ESI=egg shape index; RYW=relative yolk weight; RESW=relative eggshell weight; RAW= relative albumen weight.

Table 7
Effects of dietary calcium and non-phytate phosphorus on serum biochemical indices of 41-wk-old *Beijing You Chicken*.

Items		Ca (mmol/L)	P (mmol/L)	AKP (IU/L)	HDL (mmol/L)	LDL (mmol/L)	MDA (nmol/mL)	T-CHO (mmol/L)	GLU (mmol/L)	T-AOC (mmol/L)
Ca (%)	NPP (%)									
2.8	0.33	1.74	1.09	45.74	4.13	4.66	3.00	3.66	10.78	0.43
3.2	0.33	1.74	1.32	17.94	2.84	3.54	5.33	2.16	11.18	0.59
3.6	0.33	1.76	1.14	9.31	3.74	3.44	7.05	2.60	9.70	0.42
2.8	0.38	1.76	1.19	13.58	4.29	4.27	9.52	2.53	9.32	0.44
3.2	0.38	1.78	0.87	21.18	6.80	4.78	6.45	3.01	10.02	0.48
3.6	0.38	1.72	0.81	11.42	5.76	3.37	1.75	2.04	10.57	0.55
2.8	0.43	1.71	1.75	14.43	2.05	3.48	4.25	1.31	9.21	0.34
3.2	0.43	1.69	1.47	14.84	2.44	3.37	35.26	1.94	7.79	0.44
3.6	0.43	1.81	0.96	16.74	2.94	4.31	15.03	2.16	10.58	0.54
SEM		0.02	0.09	6.82	0.83	0.27	2.30	0.28	0.23	0.02
Main effects										
Ca (%)	2.8	1.74	1.35	24.58	3.49	3.71	5.15	2.50	9.77	0.40
	3.2	1.74	1.22	17.99	4.03	3.73	11.40	2.37	9.66	0.50
	3.6	1.76	0.97	12.49	4.15	3.91	5.09	2.27	10.28	0.50
	P-value	0.855	0.293	0.796	0.944	0.823	0.191	0.949	0.555	0.176
	0.33	1.75	1.18	24.33	3.57	4.31	6.60	2.81	10.55	0.48
NPP (%)	0.38	1.75	0.96	15.39	5.62	4.14	5.91	2.53	9.97	0.49
	0.43	1.74	1.39	15.34	2.48	2.91	9.14	1.80	9.19	0.44
	P-value	0.953	0.154	0.815	0.289	0.813	0.064	0.421	0.134	0.712
Ca × NPP	*P-value*	0.740	0.538	0.786	0.903	0.507	0.165	0.494	0.121	0.462

Note: Ca=calcium; NPP=non-phytate phosphorus; AKP=alkaline phosphatase; HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; MDA=malondialdehyde; TC=total cholesterol; T-AOC=total antioxidant capacity (T-AOC).

In this study, it was found that the group with 3.6 % Ca and 0.43 % NPP significantly decreased FER of BYC during 30~35 wks, and high Ca level (3.6 %) had an increasing trend on egg mass, which was consistent with the study of Attia et al. (2020) in H&N Brown laying hens. High Ca may optimize feed utilization by promoting calcification efficiency of eggshell gland and reducing energy consumption (Yu et al., 2019). Dietary Ca and NPP levels did not significantly affect performance between 36 and 41 wks of age in this study, suggesting that BYC laying hens are less sensitive to mineral requirements at peak laying period, which may be related to changes in reproductive axis hormone secretion or adaptation in Ca and P metabolism (Li, 2022).

Reyer et al. (2021) used Lohmann Selected Leghorn (LSL) and Lohmann Brown (LB) hens at peak laying period to study the effects of four dietary groups with standard or reduced levels of Ca or P or both, and found that LSL and LB appeared to adopt different molecular pathways and exhibit different Ca, P requirements, which may also apply to BYC laying hens at peak laying period.

It was found that dietary NPP level alone had a limited effect on laying performance in this study, but its interaction with Ca tended to modulate FER and AFI. For example, an increase in NPP reduced FER at low Ca (2.8 %), while an increase in NPP inhibited AFI at high Ca (3.6 %). This may be related to the imbalance of Ca/NPP ratio affecting intestinal absorption efficiency: when the Ca/NPP ratio is too high (e.g. 3.6 % Ca/0.33 % NPP=10.89), excess Ca may form insoluble phosphate and reduce the bioavailability of phosphorus (Dijkslag et al., 2021), which was similar to Reyer et al. (2021): dietary Ca/P ratio can affect the digestion and absorption of Ca and P in the gastrointestinal tract of birds, and disturb the homeostasis of Ca and P in hens, and lead to a decrease in egg-laying performance. Therefore, optimizing the Ca/NPP ratio (8.39~9.67 in this study) may be a key to balance laying performance.

Follicular development

Egg production in laying hens is primarily determined by the processes that control follicular growth and development, including primitive follicle recruitment, pre-graded follicular development, follicle selection, and graded follicle maturation (Johnson, 2015). Calcium is required for follicular growth and selection in birds. For example, in calcium-deficient medium, germ layer development was completely arrested in quail oocytes, whereas Ca supplementation induced germ layer development in 20.5 % of oocytes (Mizushima et al., 2007); the Ca^{2+}content in the granulosa cells of the pre-graded follicles of hens was significantly increased during ovulation compared to forced molting birds (Laporta et al., 2011).

Compared with the Ca-adequate control (containing 3.6 % Ca), egg-type ducks fed 0.38 % Ca during the depletion period had significantly decreased numbers of hierarchical follicles and total ovarian weight, which were accompanied by reduced egg production (Chen et al., 2020). Ca-deficient diets (containing 1.8 % or 0.38 % Ca) also negatively affect follicle selection of laying ducks, probably by activating cAMP/PKA/ERK1/2 signaling pathway. In this present study, the number of mature follicles was increased in 3.2 % Ca group, suggesting that moderately high Ca levels may mediate the maturation of follicles by activating Ca signal in the ovary and promoting the proliferation in follicular granulosa cells through cyclic adenosine monophosphate (cAMP) pathway (Liu, 2021).

Another study on egg-type duck breeders found that dietary NPP levels from 0.18 % to 0.45 % influenced several reproductive parameters, including the number of large yellow follicles and the relative weights of reproductive organs (Xia et al., 2023). In the present study, dietary 0.33 % and 0.43 % NPP significantly increased the number of small yellow follicles, suggesting that NPP may be involved in pre-graded follicular development, which may be related to the effect of phosphorus metabolism and function, its related mechanism needs to be further studied.

Ca, P level and egg quality

The Ca and P are two important nutrients for maintaining optimal bone quality and egg quality (Matuszewski, et al., 2020). Calcium is crucial in the formation of eggshells: Ca makes up about 38 % of the eggshell (McDowell, 2003). Insufficient calcium content in the diet of laying hens may affect eggshell quality (Castillo et al., 2004). Large amounts of dietary Ca are required for eggshell and yolk formation during egg production (Bar, 2009). Pre-laying diets decreased the incidence of egg cracking and increased shell strength and egg weight with increasing Ca level (de Juan et al., 2023).

The eggs laid on high dietary Ca-P levels were significantly thicker than those laid on diets with low Ca-P levels (Dijkslag et al., 2021). Dietary 4.0 % Ca helped improve the eggshell thickness of H&N Brown Nick laying hens at late laying period (Attia et al., 2020). Yu et al. (2019) found that eggshell strength was significantly reduced at all stages of egg production in low Ca-P diets. Compared with dietary 3.0 % Ca, dietary 3.5 % Ca significantly improved eggshell strength in late laying hens (Zhang, 2021). This present study indicated that dietary Ca and NPP alone had no effects on eggshell thickness and eggshell strength, indicating the current levels meet the requirement of the laying hens, but the interaction of dietary Ca and NPP levels had the trend to affect the eggshell strength. The effect may be due to different responses for Ca/NPP ratio in native laying hens and high-yield laying hens.

Eggshell color and yolk color are two important factors that affect the preference and choice of eggs by consumers all over the world. Usually, the eggshell color is related to breed or genetic factors, and yolk color is greatly affected by nutritional factors. There was no correlation between eggshell color and yolk color (Aygun, 2014). In the process of egg formation, the pigment that affects the eggshell color is mainly protoporphyrin, which is synthesized by the shell gland of hens, and deposited in the outer and upper layers of the eggshell. In the present study, the eggshell color was higher (lighter in color) in groups with 3.6 % Ca and 0.33 % NPP, 2.8 % Ca and 0.38 % NPP, 2.8 % Ca and 0.43 % NPP, 3.2 % Ca and 0.43 % NPP, the corresponding Ca/NPP ratio were 10.89, 7.38, 6.51, 7.44, respectively, indicating that too high or too low Ca/NPP ratio could lead to lighter eggshell color, this may be related to the competitive inhibition of protoporphyrin synthase activity by inappropriate Ca (Matuszewski et al., 2020).

Pelicia et al. (2009) studied the effect of four dietary Ca levels (3.0 %, 3.5 %, 4.0 % and 4.5 %) and four NPP levels (0.25 %, 0.30 %, 0.35 % and 0.40 %) in the diet of Hisex Brown layers aged 90 and 108 wks (after molting), and found yolk color was linearly increased with increasing dietary Ca levels, but was not affected by NPP levels. Eltahan et al. (2023) reported that dietary NPP levels (0.20 %, 0.25 %, 0.30 %) did not affect eggshell color and yolk color of Hy-line Brown laying hens at 76 and 80 wk. In this present study, yolk color was deepened in 3.6 % Ca, 0.43 % NPP alone, and the interaction of Ca and NPP, the reason may be related to their promoting the deposition of fat-soluble pigments such as lutein in yolk (Zhang, 2021). The related lutein content in egg yolk and the mechanisms need further investigation in the future.

Response of serum biochemical indices

Serum biochemical indices are direct reflection of an animals' metabolism and health status. Different breeds have different growth and metabolic characteristics due to their genetic backgrounds and therefore exhibit different serum biochemical indices (Song et al., 2023). Li (2022) found that low dietary Ca increased serum ALP levels. Xu et al. (2020) adjusted P content of laying hens' diets and found that P intake of the low-phosphorus group was reduced due to insufficient dietary P content. We investigated serum biochemical indices of BYC growing pullets (10~16 wks of age) (Zhang et al., 2023), and found that dietary Ca level significantly affected serum P content, dietary NPP level had an influence on serum Ca content at 16 wks of age, and serum Ca content tended to increase with increasing dietary NPP level. In the present study, serum Ca and P content was not affected by dietary Ca and NPP levels, the reason may be due to the increased demand for Ca and P caused by increased egg production during peak laying period, and their own neutralization. The present study showed that dietary Ca and NPP levels alone and the interactions had no effect on serum biochemical indices at 41-wk-old BYC, which is consistent with a previous report (Zhang et al., 2023). This may be due to the dynamic regulation of intestinal absorption and bone mobilization to maintain blood calcium/phosphorus homeostasis in laying hens (Li, 2022). In addition, serum MDA increased in the 0.43 % NPP group, suggesting that high-phosphorus diet may slightly increase oxidative stress, but it did not reach a significant level, which was consistent with the results of Zhang et al. (2023) in growing *Beijing You chicken*.

In summary, dietary Ca and NPP levels alone and the interaction did not affect egg production of BYC, and FER in the group with 3.6 % Ca and 0.43 % NPP seemed to be the lowest during 35~41 wks. Dietary NPP may be related to pre-graded follicular development. The recommended dietary Ca level is 3.6 % and NPP level is 0.43 % for BYC during the peak laying period. The Ca/NPP ratio in the range of 8.39~9.67 may be beneficial to laying performance and egg quality of laying hens.

Declaration of competing interest

The authors declared that we have no conflicts of interest to this work.

Acknowledgements

The authors wish to thank Reform and Development Project of BAAFS (XMS202514), China Agriculture Research System of MOF and MARA (grant number CARS-41-Z04), National Natural Science Funds (32472959), BAAFS Academy Capacity Building Project (KJCX20200421), Beijing Innovation Consortium of Agriculture Research System (BAIC06-2024) for financial support, and the staff from Lvdudu Farm for feeding and management of the experimental birds.

References

Attia, Y.A., Al-Harthi, M..A., Abo El-Maaty, H.M., 2020. Calcium and cholecalciferol levels in late-phase laying hens: effects on productive traits, egg quality, blood biochemistry, and immune responses. Front. Vet. Sci. 7, 389.
Aygun, A., 2014. The relationship between eggshell colour and egg quality traits in table eggs. Indian J Anim. Res. 48, 290–294.
Bar, A., 2009. Calcium homeostasis and vitamin D metabolism and expression in strongly calcifying laying birds. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 152, 477–490.
Bello, A., Korver, D.R., 2019. Long-term effects of buttiauxella sp. phytase on performance, eggshell quality, apparent ileal Ca and P digestibility, and bone properties of white egg layers. Poult. Sci. 98, 4848–4859.
Castillo, C., Cuca, M., Pro, A., González, M., Morales, E., 2004. Biological and economic optimum level of calcium in White Leghorn laying hens. Poult. Sci. 83, 868–872.
Chen, W., Xia, W.G., Ruan, D., Wang, S., Abouelezz, K.F.M., Wang, S.L., Zhang, Y.N., Zheng, C.T., 2020. Dietary calcium deficiency suppresses follicle selection in laying ducks through mechanism involving cyclic adenosine monophosphate-mediated signaling pathway. Animal 14, 2100–2108.
DB11/T 1378-2023, 2023. Technical Code of Practice of Feeding and Management of Beijing-You Chicken. Beijing Bureau of Market Supervision, Beijing, China.
de Juan, A.F., Scappaticcio, R., Aguirre, L., Fondevila, G., García, J., Cámara, L., Mateos, G.G., 2023. Influence of the calcium and nutrient content of the prelay diet on egg production, egg quality, and tibiae mineralization of brown egg-laying hens from 16 to 63 wk of age. Poult. Sci. 102, 102491.
Dijkslag, M.A., Kwakkel, R..P., Martin-Chaves, E., Alfonso-Carrillo, C., Walvoort, C., Navarro-Villa, A., 2021. The effects of dietary calcium and phosphorus level, and feed form during rearing on growth performance, bone traits and egg production in brown egg-type pullets from 0 to 32 weeks of age. Poult. Sci. 100, 101130.
Elser, J.J., 2012. Phosphorus: a limiting nutrient for humanity? Curr. Opin. Biotechnol. 23, 833–838.
Eltahan, H.M., Cho, S.., Rana, M.M., Saleh, A.A., Elkomy, A.E., Wadaan, M.A.M., Alagawany, M., Kim, I.H., Eltahan, H.M., 2023. Dietary exogenous phytase improve egg quality, reproductive hormones, and prolongs the lifetime of the aging Hy-Line brown laying hens fed nonphytate phosphorus. Poult. Sci. 102, 102895.
Feed Database in China, 2013. Tables of Feed Composition and Nutritive Values in China. Feed Database in China, Beijing.
Frost, T.J., Roland Sr, D.A., 1991. The influence of various calcium and phosphorus levels on tibia strength and eggshell quality of pullets during peak production. Poult. Sci. 70, 963–969.
Geng, A.L., Zhang, Y.., Zhang, J., Wang, H.H., Chu, Q., Liu, H.G., 2018. Effects of lighting pattern and photoperiod on egg production and egg quality of a native chicken under free-range condition. Poult. Sci. 97, 2378–2384.
Jing, M., Zhao, S., Rogiewicz, A., Slominski, B.A., House, J.D., 2018. Assessment of the minimal available phosphorus needs of laying hens: implications for phosphorus management strategies. Poult. Sci. 97, 2400–2410.
Jlali, M., Hincelin, C., Francesch, M., Rougier, T., Cozannet, P., Ozbek, S., Ceccantini, M., Yavuz, B., Preynat, A., Devillard, E., 2023. A novel bacterial 6-phytase improves productive performance, precaecal digestibility of phosphorus, and bone mineralization in laying hens fed a corn-soybean meal diet low in calcium and available phosphorus. J. Poult. Sci. 60, 2023019.
Johnson, A.L., 2015. Ovarian follicle selection and granulosa cell differentiation. Poult. Sci. 94, 781–785.
Laporta, L., Micera, E., Surdo, N.C., Moramarco, A.M., Di Modugno, G., Zarrilli, A., 2011. A functional study on L-type calcium channels in granulosa cells of small follicles in laying and forced molt hens. Anim. Repro Sci. 126, 265–270.
Li, X., 2022. Effects of Low Calcium or Low Phosphorus Diets and Immune-challenge on Bone Metabolism in Layer-type Chickens. Master's Thesis. Shandong Agricultural University, Tai'an, China.
Liu, J.G., 2021. Functions of Calcium Signaling in Mediating Follicular Development in Laying Ducks and Its Mechanism. PhD Diss. Hunan Agricultural University, Changsha, China.
McDowell, L.R., 2003. Minerals in Animal and human Nutrition, 2nd ed. Elsevier science, Amsterdam, the Netherlands, p. 644. Page.
Matuszewski, A., Łukasiewicz, M., Niemiec, J., 2020. Calcium and phosphorus and their nanoparticle forms in poultry nutrition. World's Poult. Sci. J. 76, 328–345.
Mizushima, S., Takagi, S., Ono, T., Atsumi, Y., Tsukada, A., Saito, N., Shimada, K., 2007. Possible role of calcium on oocyte development after intracytoplasmic sperm injection in quail (Coturnix japonica). J. Exp. Zool. 307, 647–653.
National Research Council, 1994. Nutrient Requirements of Poultry, 9th rev ed. Natl Acad Press, Washington, DC, pp. 19–34. Pages.
NY/T 33-2004, 2004. Feeding standard of chicken. Ministry of Agriculture. PRC.
Pelicia, K., Garcia, E.A., Faitarone, A.B.G., Silva, A.P., Berto, D.A., Molina, A.B., Vercese, F., 2009. Calcium and available phosphorus levels for laying hens in second production cycle. Braz. J. Poult. Sci. 11, 39–49.
Ren, Y., Zhao, T., Zhang, K., Zhu, Z., Li, L., Li, Y., Jiang, S., Jiao, N., Yang, W., 2023a. Effect of replacing dicalcium phosphate with mono-dicalcium phosphate to supplement phosphorus on laying performance, phosphorus-calcium metabolism and bone metabolism of aged laying hens. Front. Vet. Sci. 10, 1196334.
Ren, Y., Liu, Y., Jiang, K., Li, L., Jiao, N., Zhu, Z., Zhang, K., Jiang, S., Yang, W., Li, Y., 2023b. Effects of low-phosphorus diets supplemented with phytase on the production performance, phosphorus-calcium metabolism, and bone metabolism of aged Hy-Line Brown laying hens. Animals (Basel) 13, 1042.
Ren, Z., Yan, J., Hu, Q., Liu, X., Pan, C., Liu, Y., Zhang, X., Yang, X., Yang, X., 2020. Phosphorus restriction changes the expression of fibroblast growth factor 23 and its receptors in laying hens. Front Physiol 11, 85.
Reyer, H., Oster, M., Ponsuksili, S., Trakooljul, N., Omotoso, A.O., Iqbal, M.A., Murán, E., Sommerfeld, V., Rodehutscord, M., Wimmers, K., 2021. Transcriptional responses in jejunum of two layer chicken strains following variations in dietary calcium and phosphorus levels. BMC Genomics 22, 485.
Sommerfeld, V., Huber, K., Bennewitz, J., Camarinha-Silva, A., Hasselmann, M., Ponsuksili, S., Seifert, J., Stefanski, V., Wimmers, K., Rodehutscord, M., 2020. Phytate degradation, myo-inositol release, and utilization of phosphorus and calcium by two strains of laying hens in five production periods. Poult Sci 99, 6797–6808.
Song, H., Li, W., Li, Y., Zhai, B., Guo, Y., Chen, Y., Han, R., Sun, G., Jiang, R., Li, Z., Yan, F., Li, G., Liu, X., Zhang, Y., Tian, Y., Kang, X., 2023. Genome-wide association study of 17 serum biochemical indicators in a chicken F2 resource population. BMC Genomics 24, 98.
Sun, F., 2016. Effects of Dietary Phosphorus and the Ratio of Calcium to Phosphorus on Performance and Phosphorus Uptake of Laying Hens. Master's Thesis. Hunan Agricultural University, Changsha, China.
Teng, X., Zhang, W., Xu, D., Liu, Z., Yang, N., Luo, D., Wang, H., Ge, M., Zhang, R., 2020. Effects of low dietary phosphorus on tibia quality and metabolism in caged laying hens. Prev. Vet. Med. 181, 105049.
Webster, A.B., 2004. Welfare implications of avian osteoporosis. Poult. Sci. 83, 184–192.
Wei, H., Bi, Y., Li, Y., Zhang, H., Li, J., Zhang, R., Bao, J., 2022. Low dietary phosphorus impairs keel bone health and quality in laying hens. Brit. Poult. Sci. 63, 73–81.
Xia, W.G., Abouelezz, K.., Huang, X.B., Li, K.C., Chen, W., Wang, S., Zhang, Y.N., Jin, C. L., Azzam, M.M.M., Zheng, C.T., 2023. Dietary non-phytate phosphorus requirements for optimal productive and reproductive performance, and egg and tibial quality in egg-type duck breeders. Animal 17, 101022.
Xu, D., Teng, X., Guo, R., Shen, X., Wan, M., Li, G., Zhang, R., Ge, M., 2020. Metabonomic analysis of hypophosphatemic laying fatigue syndrome in laying hens. Theriogenology 156, 222–235.
Yu, H., Yang, P.F., Chai, X., Mi, X.M., Meng, Y.Q., Zhao, R.Y., 2019. Effects of organic acids and prebiotics on the performance and eggshell quality of laying hens. China Feed 16, 44–49.
Zhang, Y.J., 2021. A Preliminary Study on the Effect of Disequilibrium Feeding on Eggshell Quality of Laying Hens in the Later Stage of Laying and Its Mechanism. Master's Thesis. Guangxi University, Nanning, China.
Zhang, Q.Q., Zhang, F.., Zhao, X.H., Zhang, Y., Wang, H.H., Chu, Q., Song, Z.G., Geng, A. L., 2021. Effects of dietary calcium and available phosphorus levels on growth performance and serum biochemical indexes in *Beijing You Chicken* new line aged from 0 to 6 weeks. Chinese J. Anim. Nutr. 33, 6184–6192.
Zhang, Q.Q., Chang, C.., Chu, Q., Wang, H.H., Zhang, J., Yan, Z.X., Song, Z.G., Geng, A. L., 2023. Dietary calcium and non-phytate phosphorus levels affect the performance, serum biochemical indices, and lipid metabolism in growing pullets. Poult. Sci. 102, 102354.