Animal 17 (2023) 101022

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Dietary non-phytate phosphorus requirements for optimal productive and reproductive performance, and egg and tibial quality in egg-type duck breeders

W.G. Xia [a,1], K. Abouelezz [a,b,1], X.B. Huang [a], K.C. Li [a], W. Chen [a], S. Wang [a], Y.N. Zhang [a], C.L. Jin [a], M.M.M. Azzam [c], C.T. Zheng [a,*]

[a] Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Key Laboratory of Animal Nutrition and Feed Science (South China) of Ministry of Agriculture, State Key Laboratory of Livestock and Poultry Breeding, Guangdong Public Laboratory of Animal Breeding and Nutrition, Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, China

[b] Department of Poultry Production, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt

[c] Animal Production Department, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 10 May 2023
Revised 14 October 2023
Accepted 17 October 2023
Available online 25 October 2023

Keywords:
Duck breeder
Egg incubation
Egg production
Inorganic phosphorus
Tibial characteristics

ABSTRACT

Optimal dietary non-phytate phosphorus (**NPP**) is essential in poultry to maximise productive and reproductive performance, along with indices of egg and bone quality. This study aimed to establish the NPP requirements of egg-type duck breeders aged from 54 to 80 weeks on the following traits: egg production, egg incubation, egg quality, tibial characteristics, reproductive organ, plasma indices, and the expression of genes related to phosphorus absorption. Longyan duck breeders aged 54 weeks (n = 300) were randomly allotted to five treatments, each containing six replicates of 10 individually caged birds. Birds were fed corn-soybean meal-based diets containing 0.18, 0.25, 0.32, 0.38, and 0.45% NPP/kg for 27 weeks. The tested dietary NPP levels did not affect egg production or egg quality indices. The hatchling weight of ducklings increased (quadratic, $P < 0.01$) as dietary NPP level increased, and the highest value occurred with 0.25% NPP. The number of large yellow follicles (**LYF**), and the relative weights of LYF and ovary showed linear and quadratic responses to dietary NPP levels; the lowest number and relative weight of LYF occurred with 0.38% NPP, and the lowest ovarian weight was obtained with 0.25% NPP. There were no differences in tibial length, breaking strength, and mineral density in response to dietary NPP levels. In contrast, tibial content of Ca increased (linear, $P < 0.01$) with dietary NPP levels increasing from 0.18 to 0.45%, and the tibial content of P increased at 0.32% NPP and the higher dietary NPP levels. Plasma concentration of P showed a quadratic ($P < 0.05$) response to the dietary NPP levels, where the highest value was seen at 0.38% NPP. In conclusion, dietary NPP levels from 0.18 to 0.45% had no effects on egg production, and egg and tibial quality of duck breeders. The duck breeders fed a diet with 0.25% NPP showed the highest hatchling weight of their offspring, while those fed 0.38% NPP had the lowest number and relative weight of LYF. These results indicated that the diet with 0.25% NPP can be used in egg-type duck breeders to improve the hatchling weight of their offspring, without adverse effects on their productivity. The regression model indicated that the maximal hatchling weight of ducklings was obtained from duck breeders fed the diet with 0.30% NPP.

Implications

Poultry diets should be balanced for around 38 nutrients, including phosphorus. In poultry, phosphorus in the highly available forms of inorganic phosphorus as non-phytate phosphorus is essential for maintaining normal metabolic and structural functions. The results indicated that the diet containing 0.25% non-phytate phosphorus can be used in egg-type duck breeders to improve the hatchling weight of their offspring, without adverse effects on their productivity. The results obtained from the current study are useful for feed formulators to enhance the health and productivity of laying duck breeders.

* Corresponding author.
E-mail address: zhengchuntian@gdaas.cn (C.T. Zheng).

[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.animal.2023.101022
1751-7311/

Introduction

Phosphorus (**P**) is an important nutrient for normal functions allied to metabolic and structural maintenance in animals. In the plant-derived feed ingredients, the digestible forms of P usually account for only 30–40% of the total P, while the remaining P is present as phytate P. This form of P is poorly utilised by monogastric animals due to the lack of phytase activity in their digestive systems to hydrolyse phytate P. Therefore, diets added with highly available forms of inorganic P as non-phytate P (**NPP**) are essential to achieve optimal egg-laying performance, egg quality, and bone mineralisation in laying birds (Keshavarz and Nakajima, 1993; Snow et al., 2004). Much is known about the NPP requirements of laying hens which range from 0.15 to 0.30% without phytase supplementation (Boling et al., 2000; Usayran et al., 2001; Bar et al., 2002; Jing et al., 2018). The National Research Council (NRC, 1994) recommended that the NPP requirement of brown laying hens is 0.25% (or 250 mg/hen per day) during the peak laying phase. Though Commercial Poultry Nutrition (2004) suggested an available P level of 0.38% for Pekin duck (meat-type) breeders, little is known about the NPP needed by breeders of layer-type ducks.

The Longyan duck, which has an approximate adult BW of 1.5 kg and high egg production (280–300 eggs/duck per year), is a popular laying breed, especially in southern China (Xia et al., 2015). Due to the varied genetic capacities for egg production and characteristics, and hatching performance, existing findings of dietary NPP requirement of laying hens or the recommendation for Pekin ducks are not suitable for laying duck breeders. In recent years, this laboratory has been evaluating the CP, metabolisable energy, amino acids, minerals, and vitamins requirements of the highly productive laying duck breeders in a systematic program to improve their productive efficiency and feeding standards (Ruan et al., 2018; Xia et al., 2019a and 2019b; Zhang et al., 2020; Wang et al., 2022). It is hypothesised that evaluating graded levels of dietary NPP will help in defining the optimal level for egg-type duck breeders, essential for maximising their reproductive performance. Therefore, this study aimed to estimate the ideal level of dietary NPP for maximal productive and reproductive performance, egg characteristics, and tibial quality in Longyan duck breeders.

Material and methods

Animals and dietary treatments

Three hundred 54-week-old Longyan duck breeders with comparable BW and the same genetic background, in terms of the parental generation, were randomly allocated to five dietary treatments, each containing six replicates of 10 birds, and they were studied over the following 27 weeks. Ducks were provided the same ration of 160 g/bird per day, in two equal portions at 0700 and 1500 hours. The treatments were 0.18, 0.25, 0.32, 0.38, or 0.45% NPP added as dicalcium phosphate to a corn-soybean meal basal diet; zeolite powder was used as a filler. The metabolisable energy, CP, lysine, methionine, threonine, and Ca in experimental diets were balanced to maintain equal levels (Table 1). The diets in pellet form were formulated to provide the necessary amount of nutrients to Longyan ducks, based on previous findings from this laboratory for this breed. The ducks were housed in the same room at 27.3 °C (22.5–30.5 °C) and 80.6% relative humidity (72.5–85%) in individual galvanised steel battery cages (measuring 40 × 40 × 40 cm) equipped with feeders and nipple drinkers, and provided with 15 hours of 10 lx artificial lighting and 9 hours of dark daily.

Tissue sampling and storage

At the end of the experiment (after feeding for 27 weeks), two ducks were randomly chosen from each replicate (12 birds/treatment) after fasting for 12 hours. Heparinised blood samples were collected into 5-mL vacuum tubes from the wing vein. Plasma was obtained by centrifugation at 4 °C (1 200*g*) for 10 minutes, and stored at −20 °C. The ducks were killed by cervical dislocation and dissected to collect samples of tibiae, oviduct, ovary, right kidney, and mucosa from duodenum and jejunum. The samples of the right kidney, duodenum, and jejunal mucosa were then snap-frozen in liquid nitrogen (−196 °C), and held at −80 °C until further analysis.

Oviduct and ovary-related indices

As described by Xia et al. (2017), the samples of oviducts, ovaries, and large yellow follicles (**LYFs**) with diameters ≥8 mm were separated and weighed. The numbers of LYF were recorded, and their weights were each presented as percentages of ovarian weight. Oviductal weight and ovarian weight were expressed, relative to live BW.

Measurements of productive performance

Feed intake was calculated by the difference between the amounts of added and refused feed on a per-replicate basis. The number of total produced eggs was counted for each replicate, and each egg was separately weighed. Egg production, egg weight, egg mass (g egg/bird per day), feed intake, and feed conversion ratio (g feed/g egg) were obtained on a per-replicate basis and expressed as averages for the whole experimental period of 27 weeks, as mentioned by Xia et al. (2022).

Measurements of egg quality traits

The egg quality traits were measured, as described by Xia et al. (2017). Three eggs were collected randomly from each replicate every 4 weeks to evaluate egg quality. At every collection time, the average of the 18 eggs was calculated per treatment to determine the quality traits involving eggshell strength, eggshell thickness, yolk colour, and Haugh unit. The relative weights of shell, yolk, and albumen were recorded individually and presented as proportions of egg weight. The specific gravity of eggs was measured with NaCl solutions ranging in specific gravity from 1.064 to 1.100, as described by Keshavarz and Nakajima (1993).

Measurements of egg incubation indices

The egg incubation indices were evaluated as reported by Xia et al. (2020). At 76 weeks of age, all the egg-laying duck breeders were artificially inseminated on the 1st and 4th day with 100 μL of fresh semen that was diluted 1:1 (v/v) with 0.9% saline. Forty eggs per replicate, with no cracks, soft shells, double yolks, or dirtiness, were collected for 6 days from the 3rd day following the first insemination. After weighing on a replicate basis, the collected eggs were incubated at 37.2–38.0 °C and 60–75% relative humidity for 28 days. Egg fertility was determined by candling on the 7th day of incubation. After 28 days of incubation, hatched and healthy hatched ducklings as well as eggs that failed to hatch were recorded. Hatchability was expressed on the basis of fertile eggs. The weight of hatchlings was recorded on a per-replicate basis.

Table 1
Composition and nutrient values of the experimental diets (as fed basis) of laying duck breeders aged from 54 to 80 weeks.

Item	Non-phytate P level, %				
	0.18	0.25	0.32	0.38	0.45
Ingredients, %					
Corn	52.4	52.4	52.4	52.4	52.4
Soybean meal	26.1	26.1	26.1	26.1	26.1
Wheat bran	9.83	9.83	9.83	9.83	9.83
Limestone	9.36	9.08	8.81	8.57	8.30
Dicalcium phosphate	0.28	0.71	1.13	1.49	1.92
DL-Methionine	0.15	0.15	0.15	0.15	0.15
Salt	0.30	0.30	0.30	0.30	0.30
Premix[1]	1.00	1.00	1.00	1.00	1.00
Zeolite powder	0.58	0.43	0.29	0.16	0
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis[2], %					
Metabolisable energy, kcal/kg	2 500	2 500	2 500	2 500	2 500
DM	89.3	89.5	89.8	89.4	89.9
CP	18.00	18.00	18.00	18.00	18.00
Ca	3.58	3.61	3.62	3.63	3.62
Total P	0.44	0.48	0.52	0.54	0.58
Non-phytate P	0.18	0.25	0.32	0.38	0.45
Total Lysine	0.95	0.95	0.95	0.95	0.95
Total Methionine	0.40	0.40	0.40	0.40	0.40
Total Methionine + Cysteine	0.70	0.70	0.70	0.70	0.70
Total Threonine	0.65	0.65	0.65	0.65	0.65

[1] The premix provided the following per kg of diet: Vitamin A 12 000 IU, Vitamin D_3 1 800 IU, Vitamin E 26 mg, Vitamin K_3 1.0 mg, Vitamin B_1 3.0 mg, Vitamin B_2 9.6 mg, Vitamin B_6 6.0 mg, Vitamin B_{12} 0.03 mg, chloride choline 500 mg, nicotinic acid 25 mg, D-pantothenic acid 28.5 mg, folic acid 0.6 mg, biotin 0.15 mg, Fe 50 mg, Cu 10 mg, Mn 90 mg, Zn 90 mg, I 0.5 mg, Se 0.4 mg.
[2] Measured values of Ca and total P content. Other nutrient levels are calculated values.

Measurements of tibial characteristics

The tibial characteristics were determined as previously described (Xia et al., 2019b). The collected pairs of tibiae were weighed after removing any adherent muscle and tendons. To determine the tibial ash, the left tibiae were boiled in water for 6 minutes, de-fatted by soaking in diethyl-ether for 96 hours, dried in an oven to a constant weight, and ashed in a muffle furnace at 550–600 °C for 24 hours. Ca and P content in the tibial ash were measured by procedure number 942.05 described in AOAC (1990). The bone mineral density of right tibiae was determined with an X-ray osteodensitometer (Lunar Prodigy, General Electric Company, Fairfield, CT). The breaking strength of the right tibiae was then measured at mid-length using a Food Texture Analyzer (TMS-Pro, Food Technology Corporation, West Sussex, VA).

Determinations of plasma biochemical variables

Plasma concentrations of Ca, P, and alkaline phosphatase (**ALP**) were measured using kits purchased from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China). Concentrations of calcitonin (**CT**) and parathyrin (**PTH**) in plasma were determined using ELISA kits purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd, Shanghai, China. All measurements in plasma were made in duplicate.

Transcript abundance of genes related to sodium/phosphorus transporter

Methods for total RNA isolation, complementary DNA generation, and real-time quantitative PCR in the frozen samples of the duodenum, jejunum, and kidney were noted in Xia et al. (2022). Briefly, the primer sequences (5′-3′) of the targeted genes were as follows: forward primer (**F**): AGGGCTTTTGCTGGTGCTAC and reverse primer (**R**): AGGGCTCCGTGATGATTTTC for type IIa sodium-dependent phosphate cotransporter (*NaPi-IIa*, accession number: AF297188.1), F: TCGGTCCGTTCACTCTGTTG and R: GCCACGTTGCCTTTGTGATT for type IIb sodium-dependent phosphate cotransporter (*NaPi-IIb*, accession number: NM_204474.1), F: ATGTCGCCCTGGATTTCG and R: CACAGGACTCCATACCCAAGAA for β-actin (accession number: NM_001310421.1). Samples were assayed in triplicate, and the SDs of threshold cycle (**Ct**) values were ≤0.5. The relative transcript abundance of targeted genes was determined using the ΔΔCt method.

Statistical analysis

Replicate was considered as the experimental unit (n = 6) unless stated otherwise; the average of each replicate was calculated from two sampled birds. All data from the effect of dietary NPP level were analysed using the GLM procedure of SAS (version SAS 9.1., SAS Institute, 2004, Cary, NC), and the orthogonal comparisons were conducted to test the linear and quadratic impacts of dietary NPP levels. The probability level of 0.05 was used to identify significance. Once a significant quadratic response ($P < 0.05$) was observed, a quadratic regression equation ($Y = c + bX + aX^2$) based on 95% of the maximum or minimum responses was used to estimate the ideal NPP requirement (Corzo et al., 2006; Xia et al., 2019a), where c is the intercept, a and b represent the coefficients of the quadratic and linear terms, respectively.

Results

Productive performance

Effects of dietary NPP level (0.18–0.45%), fed for 27 weeks, on egg-laying performance of 54-week-old Longyan duck breeders are shown in Table 2. The indices of productive performance including egg-laying rate, egg weight, egg mass, feed intake, and feed conversion ratio of duck breeders at the age of 54 to 80 weeks were not influenced by the tested levels of dietary NPP.

Table 2
Effects of dietary 0.18–0.45% non-phytate P (NPP) fed for 27 weeks on egg-laying performance in 54-week-old Longyan duck breeders.[1]

Variables	NPP level, %					SEM	*P*-value[2]		
	0.18	0.25	0.32	0.38	0.45		NPP	L	Q
Egg production, %	74.1	75.6	74.2	71.4	75.2	3.72	0.936	0.864	0.809
Egg weight, g	67.7	67.6	67.8	66.7	67.1	0.56	0.586	0.232	0.850
Egg mass, g/bird/d	50.1	51.0	50.3	47.7	50.3	2.38	0.884	0.709	0.861
Feed intake[3], g/d	158	156	154	153	155	1.8	0.203	0.085	0.119
Feed conversion ratio, g feed/g egg mass	3.21	3.07	3.07	3.27	3.11	0.156	0.853	0.979	0.787

[1] Each value is the mean of six replicates.
[2] NPP = treatment effect; L = linear; Q = quadratic.
[3] As fed basis.

Egg quality

As shown in Table 3, there were no differences observed in egg quality indices due to dietary NPP levels except for the relative weight of yolk and albumin, and the breaking strength of eggshell. The level of 0.32% NPP showed the lowest relative weight of yolk ($P = 0.062$) and breaking strength of eggshell ($P = 0.087$), and the highest relative weight of albumin ($P = 0.062$).

Egg incubation indices

The tested dietary NPP levels did not influence the egg incubation indices of duck breeders aged 80 weeks, except for hatchling weight. The hatchling weight of ducklings exhibited a quadratic response to dietary NPP levels, where the highest value occurred with 0.25% NPP. The dietary NPP level of 0.30% was defined as the optimal level for egg-type duck breeders aged 54–80 weeks according to the regression model of hatchling weight in relation to dietary NPP levels (Table 4).

Reproductive organs indices

As described in Table 5, the LYF number and the relative weights of LYF and ovary showed linear and quadratic responses to dietary NPP levels; the lowest LYF number and relative weight of LYF occurred with 0.38% NPP, and the lowest ovarian weight was seen with 0.25% NPP. The level of 0.25% NPP showed the highest relative weight of oviduct ($P = 0.059$).

Tibial characteristics

The results presented in Table 6 showed that the tibial content of Ca increased (linear or quadratic, $P < 0.01$) as dietary NPP levels increased, and tibial content of P was low at 0.18% and 0.25% NPP and increased ($P < 0.01$) at higher NPP levels. The tibial length, breaking strength, mineral density, and ash content were not influenced by dietary NPP levels.

Chemical analysis of plasma

Except for the plasma concentration of P, other plasma chemical indices including concentration of Ca, ALP, CT and PTH in duck breeders aged 80 weeks were not affected by the tested dietary NPP levels (Table 7). Plasma concentration of P showed a quadratic ($P < 0.05$) response to the dietary NPP levels, with the greatest value being obtained at 0.35% NPP.

Transcript abundance of genes related to phosphorus absorption

Effects of dietary 0.18–0.45% NPP fed for 27 weeks on the mRNA expression levels of Na/Pi transporters in the intestine and kidney in 54-week-old Longyan duck breeders are shown in Table 8. The transcript abundances of Na/Pi transporter in the intestine and kidney of duck breeders at the age of 80 weeks were not affected by the tested dietary NPP levels.

Discussion

The tested dietary NPP levels from 0.18 to 0.45% did not influence the egg-laying performance and egg quality of duck breeders from 54 to 80 weeks of age, but increased the hatchling weight of the offspring. The hatchling weight in the present study increased by 3.1 g in ducklings from duck breeders fed the diet with 0.25% NPP than those given 0.18% NPP, and the highest hatchling weight was reached at 0.30% NPP according to the regression model (Table 5). Shell provides most of the calcium required for the skeletal mineralisation of the embryos, whereas P is derived from the yolk and albumen in the development of embryos in pigeons (Hart et al., 1992). In a previous study, it was found that the increase in hatchling weight was accompanied by an increase in the length of keel with maternal intake of NPP up to about 390 mg/g diet (Xia et al., 2023), which was attributed to the increase in the content of phosphorus in yolk and albumen. In laying hens aged 30–70 weeks, NPP concentration from 0.22 to 0.36% of feed did not exhibit differences in egg production performance, egg/feed ratio, and egg quality indices (Bello and Korver, 2019). This goes in line with the findings of Pongmanee et al. (2020) in hens aged 25 to 37 weeks with dietary NPP concentration increasing from 0.22 to 0.45% in phase 1 and 0.19 to 0.38% in phase 2. These above-mentioned studies indicated that no positive effect was obtained on egg-laying performance and egg quality of laying birds fed diets containing more than 0.18% NPP. However, Wei et al. (2022) showed that egg production of hens aged 26–36 weeks increased as dietary NPP levels increased from 0.17% up to 0.34%. Besides, Jing et al. (2018) suggested that a diet containing 0.15% NPP was sufficient to maintain the health and laying performance of hens at 22–34 weeks of age. The differences in estimated minimum NPP requirements among the studies may have resulted from the differed ingredient composition of the diet with different P fractions and variable P utilisation for aged laying birds.

During the process of eggshell formation, a high concentration of plasma phosphorus is physiologically required to maintain normal egg-laying performance. Dietary intake or bone mobilisation, in the form of hydroxyapatite (99% Ca and 80% P) which often occurs in aged laying birds, could provide and fulfil Ca requirement with increasing concentration of plasma P (Hurwitz and Bar, 1965). In the current study, the highest tibial and plasma P concentrations were reached when the ducks were fed the diets containing 0.32% and 0.38% NPP, respectively, with a constant Ca level of 3.6%, while tibial Ca linearly increased and was pronounced at 0.45% NPP treatment. This finding suggests that using 0.32% NPP in the diet is an adequate level for maximal eggshell formation in egg-type duck breeders, while there were no variations in tibial contents of ash and the mineral density, consistent with Jing et al. (2018). But, con-

Table 3
Effects of dietary 0.18–0.45% non-phytate P (NPP) fed for 27 weeks on egg quality traits in 54-week-old Longyan duck breeders.[1]

Variables	NPP level, %					SEM	*P*-value[2]		
	0.18	0.25	0.32	0.38	0.45		NPP	L	Q
Specific gravity, g/cm³	1.085	1.083	1.084	1.083	1.085	0.001	0.706	0.855	0.358
Shell, %	8.97	8.96	8.96	9.19	9.13	0.111	0.410	0.120	0.748
Yolk, %	32.2	32.5	32.0	33.0	32.4	0.24	0.062	0.174	0.687
Albumin, %	58.8	58.4	59.1	58.0	58.4	0.26	0.062	0.136	0.919
Egg shape index, %	73.4	74.7	73.7	73.5	73.6	0.55	0.489	0.692	0.446
Breaking strength, kg	38.5	36.0	34.6	35.5	37.0	0.99	0.087	0.286	0.010
Shell thickness, μm	300	297	293	301	299	3.50	0.613	0.893	0.324
Yolk colour	3.84	3.86	3.82	3.80	3.66	0.127	0.828	0.325	0.542
Haugh unit	77.0	78.6	77.9	73.8	77.9	1.44	0.175	0.508	0.769

[1] Each value is the mean of six replicates.
[2] NPP = treatment effect; L = linear; Q = quadratic.

Table 4
Effects of dietary 0.18–0.45% non-phytate P (NPP) fed for 23 weeks on the egg incubation indices in 76-week-old Longyan duck breeders.[1]

Variables	NPP level, %					SEM	*P*-value[2]		
	0.18	0.25	0.32	0.38	0.45		NPP	L	Q
Fertility of set eggs, %	80.7	83.2	83.3	80.3	89.9	2.85	0.161	0.100	0.309
Hatchability of fertile eggs, %	80.1	80.0	83.9	85.7	82.2	2.47	0.435	0.220	0.340
Healthy duckling, %	97.8	99.2	96.5	96.0	98.1	1.36	0.494	0.566	0.498
Hatchling weight[3], g	37.3	40.4	39.4	39.2	38.2	0.67	0.045	0.793	0.009
Set egg weight, g	67.1	68.1	67.0	67.0	65.9	0.49	0.081	0.038	0.119
Duckling weight/Set egg weight, g/g	56.3	57.8	58.2	59.7	58.2	0.95	0.222	0.081	0.188

[1] Each value is the mean of six replicates.
[2] NPP = treatment effect; L = linear; Q = quadratic.
[3] Regression equation based on dietary NPP level (%); quadratic equation: Y (Hatchling weight) = 28.4 + 72.4 × (NPP) − 113 × (NPP)^2, R^2 = 0.71, *P*-value <0.05 yielded the optimised dietary NPP value of 0.30%.

Table 5
Effects of dietary 0.18–0.45% non-phytate P (NPP) fed for 27 weeks on ovarian and oviductal indices in 54-week-old Longyan duck breeders.[1]

Variables[2]	NPP level, %					SEM	*P*-value[3]		
	0.18	0.25	0.32	0.38	0.45		NPP	L	Q
LYF number	5.50	5.40	4.75	4.00	5.17	0.298	0.013	0.041	0.041
LYF weight/ovarian weight, %	71.8	79.5	45.7	42.2	61.7	6.84	0.004	0.015	0.048
Ovary weight, g/kg BW	42.6	45.6	26.0	27.8	38.3	2.79	< 0.001	0.008	0.002
Oviduct weight, g/kg BW	31.2	35.9	24.7	31.0	36.3	2.86	0.059	0.569	0.096

[1] Each value is the mean of six replicates.
[2] LYF = large yellow follicle.
[3] NPP = treatment effect; L = linear; Q = quadratic.

Table 6
Effects of dietary 0.18–0.45% non-phytate P (NPP) fed for 27 weeks on tibial characteristics in 54-week-old Longyan duck breeders.[1]

Variables	NPP level, %					SEM	*P*-value[2]		
	0.18	0.25	0.32	0.38	0.45		NPP	Linear	Quadratic
Tibial length, mm	97.3	97.7	97.5	97.0	96.8	0.63	0.890	0.428	0.583
Breaking strength, N	134	141	141	135	139	5.7	0.852	0.833	0.583
Mineral density, g/cm³	0.27	0.29	0.26	0.30	0.29	0.018	0.584	0.447	0.941
Ash content, %	58.6	58.7	58.3	60.3	60.2	0.72	0.191	0.053	0.446
Ca, % ash	35.2	35.4	35.3	35.3	35.9	0.15	0.018	0.010	0.094
P, % ash	16.1	16.0	16.3	16.2	16.3	0.06	0.022	0.864	0.885

[1] Each value is the mean of six replicates.
[2] NPP = treatment effect; L = linear; Q = quadratic.

sidering the big size of egg (heavy but low in specific gravity), it is not surprising that the lowest shell-breaking strength was found in the duck breeders fed the diet with 0.32% NPP. The lowest recommended level of dietary NPP derived from our results exceeds the NRC (1994) suggestion of 0.25% NPP for brown laying hens and is less than the recommendation of 0.38% NPP for Pekin duck breeders in the Commercial Poultry Nutrition (2004).

Parathyrin, produced from cells of the parathyroid glands, promotes intestinal P absorption and reduces the efficiency of renal P reabsorption (Berndt and Knox, 1992). Previous studies demonstrated that the transporters, *NaPi-IIa* primarily expressed in the kidneys, and *NaPi-IIb* mostly found in the brush-border membranes of the jejunum and duodenum (Marks et al., 2010; Tenenhouse, 2007), were transcriptionally altered by changes in

Table 7

Effects of dietary 0.18–0.45% non-phytate P (NPP) fed for 27 weeks on chemical analysis of plasma in 54-week-old Longyan duck breeders[1].

Variables[2]	NPP level, %					SEM	P-value[3]		
	0.18	0.25	0.32	0.38	0.45		NPP	L	Q
Ca, mmol/L	1.35	1.53	1.34	1.39	1.70	0.105	0.108	0.106	0.210
P, mmol/L	2.05	2.76	2.40	2.98	2.74	0.218	0.050	0.031	0.251
ALP, U/L	9.20	9.68	9.40	9.69	9.75	1.371	0.998	0.801	0.958
CT, pg/mL	4.39	5.36	5.62	4.62	4.31	0.418	0.109	0.499	0.019
PTH, pg/mL	7.86	10.0	9.96	10.0	9.68	0.633	0.111	0.083	0.053

[1] Each value represents the mean of six replicates.
[2] ALP = alkaline phosphatase; CT = calcitonin; PTH = parathyrin.
[3] NPP = treatment effect; L = linear; Q = quadratic.

Table 8

Effects of dietary 0.18–0.45% Non-phytate P (NPP) fed for 27 weeks on the mRNA expression levels of Na/Pi transporters in the intestine and kidney in 54-week-old Longyan duck breeders.[1]

Gene[2]	NPP level, %					SEM	P-value[3]		
	0.18	0.25	0.32	0.38	0.45		NPP	L	Q
Duodenum									
NaPi-IIb	1.03	1.03	0.59	0.61	0.85	0.150	0.118	0.123	0.102
Jejunum									
NaPi-IIb	1.32	1.53	1.66	1.06	2.35	0.497	0.455	0.326	0.446
Kidney									
NaPi-IIa	0.95	0.89	1.51	1.23	1.62	0.543	0.837	0.341	0.999

[1] Each value represents the mean of six replicates.
[2] *NaPi-IIa* = type IIa sodium-dependent phosphate cotransporter; *NaPi-IIb* = type IIb sodium-dependent phosphate cotransporter.
[3] NPP = treatment effect; L = linear; Q = quadratic.

plasma PTH concentration (Murray et al., 2013; Saddoris et al., 2010). In addition, PTH is the most important regulator of Ca and P levels in the blood and within the bones (Kemi et al., 2010). In previous studies, it was demonstrated that a high NPP diet with a low Ca to P ratio increased PTH secretion and caused an ongoing process in bone reabsorption in rats (Hernandez et al., 1996) and humans (Almaden et al., 1998). On the contrary, being an inhibitor of bone reabsorption, CT could lead to a decreased plasma Ca concentration (Matsuda et al., 2006). The release of PTH, targeting the skeleton, the kidneys, and the intestine, is stimulated by low blood Ca levels (Katsumata et al., 2004). In the present study, no differences neither in plasma concentration of PTH, CT and Ca, renal transcription of *NaPi-IIa* nor duodenal and jejunal transcription of *NaPi-IIb* were seen in duck breeders with a range of dietary NPP. This suggested that dietary NPP levels ranging from 0.18 to 0.45% did not affect the intestinal P absorption and renal P reabsorption. However, this is inconsistent with the study of Nari and Ghasemi (2020) in broilers and Zhu et al. (2018) in geese, who found that the concentration of PTH in serum decreased as increasing dietary NPP levels. High levels of dietary P could inhibit the transfer of P from bone storage pools to blood (Marks et al., 2010). This partly contributes to the increase of P and Ca content in tibiae of duck breeders treated with high dietary NPP levels, especially with 0.45%. However, due to the coordinated actions of the endocrine factors such as PTH and CT, the high plasma concentration of P was found in response to the dietary NPP levels ranging from 0.25 to 0.45% in the present study.

Considering the results of egg-laying performance, along with the number and relative weight of LYF, it is suggested that the dietary NPP requirement without negative effects on follicle development and egg production in duck breeders is 0.25% with 3.6% Ca. Dietary Ca to P ratio could 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 associated with a decline in egg-laying performance (Reyer et al., 2021). It has been demonstrated that deficiencies, excesses, or imbalances in Ca and NPP result in variations in the absorption of these two minerals from the intestine (Proszkowiec-Weglarz and Angel, 2019). In laying ducks, low Ca, and high phosphorus in the diet could decrease the absorption of Ca, and Ca deficit suppresses follicle selection via a mechanism involving the cyclic adenosine monophosphate-mediated signalling pathway (Chen et al., 2020). Proper care, therefore, must be taken to reduce interactions between Ca and P that may be inimical to their absorption.

In conclusion, in egg-type duck breeders, dietary NPP levels from 0.18 to 0.45% had no effects on egg production, and egg and tibial quality. The duck breeders provided a diet with 0.25% NPP showed superior hatchling weight of their offspring, while those fed 0.38% NPP had the lowest LYF number and relative weight of LYF. These results indicated that the diet with 0.25% NPP can be used in egg-type duck breeders to improve the hatchling weight of their offspring, without adverse effects on their productivity. The regression model indicated that the maximal hatchling weight of ducklings was obtained from duck breeders fed the diet containing 0.30% NPP.

Ethics approval

All of the procedures employed in this study were approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences with the approval number of "GAASIAS-2019-020".

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies

The authors did not use any artificial intelligence-assisted technologies in the writing process.

Author ORCIDs

W.G. Xia: https://orcid.org/0000-0002-4995-9146.
K. Abouelezz: https://orcid.org/0000-0002-6141-1095.
X.B. Huang: https://orcid.org/0000-0002-7883-8503.
K.C. Li: https://orcid.org/0000-0001-7711-4864.
W. Chen: https://orcid.org/0000-0003-0260-816X.
S. Wang: https://orcid.org/0000-0001-6569-2413.
Y.N. Zhang: https://orcid.org/0000-0002-1967-9477.
C.L. Jin: https://orcid.org/0000-0003-4475-4595.
M.M.M. Azzam: https://orcid.org/0000-0001-9736–5468.
C.T. Zheng: https://orcid.org/0000-0002-1967-9477.

Author contributions

Conceptualisation, **C.T. Zheng**; methodology, **W.G. Xia and K. Abouelezz**; software, **W.G. Xia**; validation, **W.G. Xia and K. Abouelezz**; formal analysis, **W.G. Xia**; investigation, **W.G. Xia, K. Abouelezz, X.B. Huang, K.C. Li, W. Chen, S. Wang, Y.N. Zhang, C.L. Jin**; resources, **C.T. Zheng**; data curation, **W.G. Xia and K. Abouelezz**; writing—original draft preparation, **W.G. Xia and K. Abouelezz**; writing—review and editing, **W.G. Xia, K. Abouelezz, C.T. Zheng and A.A.A Azzam**; project administration, **C.T. Zheng and W. Chen**; funding acquisition, **C.T. Zheng**.

Declaration of interest

None.

Acknowledgements

W. Bruce Currie (Emeritus Professor, Cornell University) and Md Touhiduzzaman Sarker (Postdoctoral Scholar, Institute of Animal Science, Guangdong Academy of Agricultural Sciences) made suggestions on presentation of this manuscript.

Financial support statement

This work was funded by the National Key Research and Development Programs of China (Grant No. 2022YFD1300503, 2018YFE0128200), the China Agriculture Research System of MOF and MARA (Grant No. CARS-42-13), the Modern Agricultural Industry Technology System Innovation Team of Guangdong Province (Grant No. 2023KJ137), the Science and Technology Program of Guangdong Province (Grant No. 2021A0505050003), the Special Fund for Scientific Innovation Strategy-Construction of High Level Academy of Agricultural Sciences (Grant No. R2020PY-JX008, 202106TD, 201810B), the Opening project for State Key Laboratory (Grant No. 2021GZ05, YDWS202106), the Foreign Expert Project (Grant No. QNL20200130001), Jiangmen Science and Technology Program (Grant No. 2020030102870009145), and Egyptian Ministry for Scientific Research, The Science, Technology & innovation Funding Authority (Grant No. 39382). The acknowledgment was extended to Researchers Supporting Project Number (RSPD2023R731), King Saud University (Riyadh, Saudi Arabia).

References

Almaden, Y., Hernandez, A., Torregrosa, V., Canalejo, A., Sabate, L., Fernandez Cruz, L., Campistol, J.M., Torres, A., Rodriguez, M., 1998. High phosphate level directly stimulates parathyroid hormone secretion and synthesis bu human parathyroid hormone secretion and synthesis by human parathyroid tissue in vitro. Journal of the American Society of Nephrology 9, 1845–1852.

Association of Official Analytical Chemists (AOAC), 1990. Official methods of analysis. AOAC International, Arlington, VA, USA.

Bar, A., Razaphkovsky, V., Vax, E., 2002. Re-evaluation of calcium and phosphorus requirements in aged laying hens. British Poultry Science 43, 261–269.

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. Poultry Science 98, 4848–4859.

Berndt, T., Knox, F., 1992. Renal regulation of phosphate excretion. In: Seldin, D., Giebish, G. (Eds.), The Kidney: Physiology and Pathophysiology. Raven Press, New York, NY, USA, pp. 2511–2532.

Boling, S.D., Douglas, M.W., Shirley, R.B., Parsons, C.M., Koelkebeck, K.W., 2000. The effects of various dietary levels of phytase and available phosphorus on performance of laying hens. Poultry Science 79, 535–538.

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.

Commercial Poultry Nutrition, 2004. Feeding Programs for Ducks & Geese. Nottingham University Press, Nottingham, England.

Corzo, A., Dozier 3rd, W.A., Kidd, M.T., 2006. Dietary lysine needs of late-developing heavy broilers. Poultry Science 85, 457–461.

Hart, L.E., Ravindran, V., Young, A., 1992. Accumulation of calcium and phosphrus in pigeon (Columba livia) embryos. Journal of Comparative Physiology B-Biochemical Systems and Environmental Physiology 162, 535–538.

Hernandez, A., Concepcion, M.T., Rodriguez, M., Salido, E., Torres, A., 1996. High phosphorus diet increases prespro PTH mRNA independent of calcium and calcitriol in normal rats. Kidney International 50, 1872–1878.

Hurwitz, S., Bar, A., 1965. Absorption of calcium and phosphorus along the gastrointestinal tract of the laying fowl as influenced by dietary calcium and egg shell formation. Journal of Nutrition 86, 433–438.

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. Poultry Science 97, 2400–2410.

Katsumata, S., Masuyama, R., Uehara, M., Suzuki, K., 2004. Decreased mRNA expression of the PTH/PTHrP receptor and type II sodium-dependent phosphate transporter in the kidney of rats fed a high phosphorus diet accompanied with a decrease in serum calcium concentration. Bioscience, Biotechnology, and Biochemistry 68, 2484–2489.

Kemi, V.E., Karkkainen, M.U., Rita, H.J., Laaksonen, M.M., Outila, T.A., Lamberg-Allardt, C.J., 2010. Low calcium: phosphorus ratio in habitual diets affects serum parathyroid hormone concentration and calcium metabolism in healthy women with adequate calcium intake. British Journal of Nutrition 103, 561–568.

Keshavarz, K., Nakajima, S., 1993. Re-evaluation of calcium and phosphorus requirements of laying hens for optimum performance and eggshell quality. Poultry Science 72, 144–153.

Marks, J., Debnam, E.S., Unwin, R.J., 2010. Phosphate homeostasis and the renal-gastrointestinal axis. American Journal of Physiology-Renal Physiology 299, 285–296.

Matsuda, M., Yamamoto, T.A., Hirata, M., 2006. Ca^{2+}-dependent regulation of calcitonin gene expression by the transcriptional repressor DREAM. Endocrinology 147, 4608–4617.

Murray, R.D., Holthouser, K., Clark, B.J., Salyer, S.A., Barati, M.T., Khundmiri, S.J., Lederer, E.D., 2013. Parathyroid hormone (PTH) decreases sodium-phosphate cotransporter type IIa (NpT2a) mRNA stability. American Journal of Physiology-Renal Physiology 304, 1076–1085.

Nari, N., Ghasemi, H.A., 2020. Growth performance, nutrient digestibility, bone mineralization, and hormone profile in broilers fed with phosphorus-deficient diets supplemented with butyric acid and Saccharomyces boulardii. Poultry Science 99, 926–935.

National Research Council (NRC), 1994. Nutrient Requirements of Poultry. National Academics Press, Washington, DC, USA.

Pongmanee, K., Kuhn, I., Korver, D.R., 2020. Effects of phytase supplementation on eggshell and bone quality, and phosphorus and calcium digestibility in laying hens from 25 to 37 wk of age. Poultry Science 99, 2595–2607.

Proszkowiec-Weglarz, M., Schreier, L.L., Miska, K.B., Angel, R., Kahl, S., Russell, B., 2019. Effect of early neonatal development and delayed feeding post-hatch on jejunal and ileal calcium and phosphorus transporter genes expression in broiler chickens. Poultry Science 98, 1861–1871.

Reyer, H., Oster, M., Ponsuksili, S., Trakooljul, N., Omotoso, A.O., Iqbal, M.A., Murani, 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.

Ruan, D., Fouad, A.M., Fan, Q., Xia, W., Wang, S., Chen, W., Lin, C., Wang, Y., Yang, L., Zheng, C., 2018. Effects of dietary methionine on productivity, reproductive performance, antioxidant capacity, ovalbumin and antioxidant-related gene expression in laying duck breeders. British Journal of Nutrition 119, 121–130.

Saddoris, K.L., Fleet, J.C., Radcliffe, J.S., 2010. Sodium-dependent phosphate uptake in the jejunum is posttranscriptionally regulated in pigs fed a low-phosphorus diet and is independent of dietary calcium concentration. Journal of Nutrition 140, 731–736.

Snow, J.L., Douglas, M.W., Koelkebeck, K.W., Batal, A.B., Persia, M.E., Biggs, P.E., Parsons, C.M., 2004. Minimum phosphorus requirement of one-cycle and two-cycle (molted) hens. Poultry Science 83, 917–924.

Statistical Analysis Systems Institute, 2004. SAS User's Guide: Statistics, version 9.1. SAS Institute Inc., Cary, NC, USA.

Tenenhouse, H.S., 2007. Phosphate transport: molecular basis, regulation and pathophysiology. Journal of Steroid Biochemistry and Molecular Biology 103, 572–577.

Usayran, N., Farran, M.T., Awadallah, H.H., Al-Hawi, I.R., Asmar, R.J., Ashkarian, V.M., 2001. Effects of added dietary fat and phosphorus on the performance and egg quality of laying hens subjected to a constant high environmental temperature. Poultry Science 80, 1695–1701.

Wang, S., Huang, L.S., Zhang, Y.N., Chen, W., Huang, X.B., Xia, W.G., Li, K.C., Wang, S. L., Zheng, C.T., 2022. Effects of dietary vitamin E supplemental level on laying performance, egg quality and antioxidant performance of breeding laying ducks. Chinese Journal of Animal Nutrition 34, 5777–5784.

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. British Poultry Science 63, 73–81.

Xia, W.G., Zhang, H.X., Lin, Y.C., Zheng, C.T., 2015. Evaluation of dietary calcium requirements for laying Longyan shelducks. Poultry Science 94, 2932–2937.

Xia, W.G., Abouelezz, K.F.M., Fouad, A.M., Chen, W., Ruan, D., Wang, S., Azzam, M.M. M., Luo, X., Fan, Q.L., Zhang, Y.N., Zheng, C.T., 2019a. Productivity, reproductive performance, and fat deposition of laying duck breeders in response to concentrations of dietary energy and protein. Poultry Science 98, 3729–3738.

Xia, W.G., Chen, W., Abouelezz, K.F.M., Azzam, M.M.M., Ruan, D., Wang, S., Zhang, Y. N., Luo, X., Wang, S.L., Zheng, C.T., 2019b. Estimation of calcium requirements for optimal productive and reproductive performance, eggshell and tibial quality in egg-type duck breeders. Animal 13, 2207–2215.

Xia, W.G., Chen, W., Abouelezz, K.F.M., Ruan, D., Wang, S., Zhang, Y.N., Fouad, A.M., Li, K.C., Huang, X.B., Zheng, C.T., 2020. The effects of dietary Se on productive and reproductive performance, tibial quality, and antioxidant capacity in laying duck breeders. Poultry Science 99, 3971–3978.

Xia, W.G., Abouelezz, K.F.M., Makled, M.N., Wang, S., Chen, W., Zhang, Y.N., Elokil, A. A., Wang, S.L., Huang, X.B., Li, K.C., Zheng, C.T., 2022. Effects of dietary substitution of peanut meal for soybean meal on egg production, egg quality, oxidative status, and yolk fatty acid profile in laying ducks. Animal 16, 100652.

Xia, W., Fouad, A.M., Chen, W., Ruan, D., Wang, S., Fan, Q., Wang, Y., Cui, Y., Zheng, C., 2017. Estimation of dietary arginine requirements for Longyan laying ducks. Poultry Science 96, 144–150.

Xia, W.G., Huang, X.B., Wang, S.L., Wang, S., Zhang, Y.N., Li, K.C., Chen, W., Zheng, C. T., 2023. Effects of dietary low non-phytate phosphorus levels and phytase on productive performance, eggshell quality, hatchability in egg-type duck breeders and skeleton growth in their offspring. Chinese Journal of Animal Nutrition 35, 892–902.

Zhang, Y.N., Wang, S., Li, K.C., Ruan, D., Chen, W., Xia, W.G., Wang, S.L., Abouelezz, K. F.M., Zheng, C.T., 2020. Estimation of dietary zinc requirement for laying duck breeders: effects on productive and reproductive performance, egg quality, tibial characteristics, plasma biochemical and antioxidant indices, and zinc deposition. Poultry Science 99, 454–462.

Zhu, Y.W., Wang, C.Y., Wen, J., Wang, W.C., Yang, L., 2018. Effect of dietary high non-phytate phosphorus level on growth performance and metabolism of calcium and phosphorus in Lionhead geese. Animal Feed Science and Technology 236, 115–121.