Dietary calcium and non-phytate phosphorus levels affect the performance, serum biochemical indices, and lipid metabolism in growing pullets

Q. Q. Zhang ,*,† C. Chang,* Q. Chu,* H. H. Wang,* J. Zhang,* Z. X. Yan,* Z. G. Song,† and A. L. Geng *,[1]

*Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, P. R. China; and †College of Animal Science and Technology, Shandong Agricultural University, Tai'an, Shandong, 271018, P. R. China

ABSTRACT This experiment aimed to study the effects of dietary calcium (**Ca**) and non-phytate phosphorus (**NPP**) levels on performance, serum biochemical indices, and lipid metabolism in Beijing You Chicken (**BYC**), a local chicken. A 3 × 3 factorial design was adopted, dietary Ca levels were 0.66, 0.71, and 0.76%, NPP levels were 0.25, 0.30, and 0.35%. A total of 648 ten-wk-old BYC growing pullets were randomly divided into 9 groups with 6 replicates per group, and 12 birds per replicate. Growth performance, serum biochemical indices, and lipid metabolism indicators from 10 to 16 wk were measured. The results showed as follows: 1) Dietary Ca and NPP alone did not affect growth performance, but the interaction of dietary Ca and NPP affected average feed intake (**AFI**) of growing pullets ($P < 0.05$). The AFI was the lowest for the group with 0.71% Ca and 0.25% NPP (3,550.0 g, $P = 0.036$). 2) Dietary Ca level significantly affected serum P content ($P < 0.05$); dietary NPP had an influence trend on serum Ca content ($P= 0.054$). Dietary NPP levels and the interaction of Ca and NPP significantly affected alkaline phosphatase (**AKP**) activity. 3) Dietary Ca levels significantly affected TC content and HDL-C content ($P < 0.05$). Dietary NPP level significantly affected TG content ($P < 0.05$), the TG content in 0.25% and 0.30% NPP groups was significantly lower than that in 0.35% NPP group ($P < 0.05$). The interaction of dietary Ca and NPP significantly affected TG, TC and HDL-C contents ($P < 0.05$). TG, TC, and LDL-C levels were lower and HDL-C levels were the highest in the group with 0.66% Ca and 0.25% NPP. In summary, appropriate dietary Ca level can regulate serum TG, TC, and HDL-C content. Dietary Ca and NPP levels can be adjusted in pullet phase to avoid excessive obesity during the egg-laying period. This study recommended that dietary 0.66% Ca and 0.25% NPP benefit for the lipid metabolism of BYC growing pullets without affecting the performance.

Key words: calcium, phosphorus, growth performance, biochemical index, lipid metabolism

2022 Poultry Science 102:102354
https://doi.org/10.1016/j.psj.2022.102354

INTRODUCTION

Calcium (**Ca**) and phosphorus (**P**) are 2 important nutritional minerals for skeletal development in animals and are the third most important nutrients in terms of feed cost in addition to energy and crude protein. The Ca and P requirements depend on animals' age, body composition, and genetics. The Ca is involved in neurohumoral regulation in the body and plays an important role in chondrocyte maturation (Zhang et al., 2019). The P plays a key role in chondrocyte apoptosis and provides P ions for anabolism of bone tissue such as nucleotides and phospholipids to maintain bone integrity during egg production in hens, and growth plates require appropriate P levels for maturation (Sabbagh et al., 2005; Liu et al., 2008). Ca deficiency affects the growth and development of chickens, such as swollen, lax and easily fractured bones, thinning bone density, and large bone marrow cavity, etc. Bone damage will directly affect the quality of chicken meat, such as muscle effusion (Liu et al., 2008). Non-phytate phosphorus (**NPP**) deficiency reduced egg production, inhibited bone deposition, decreased mineral density, and impaired keel bone quality (Wei et al., 2022). Román-García et al. (2010) studied that high dietary NPP levels negatively affected Ca metabolism, homoeostasis and bone quality, while low dietary NPP levels restricted growth and development. Reducing P excretion from 471 to 198 mg of each hen had no effects on laying

Received September 7, 2022.
Accepted November 15, 2022.

[1]Corresponding author: ailiangengcau@126.com

performance and egg quality, and a large amount of P pollution can be reduced every day (Ren et al. 2020). Excess Ca and P can also be detrimental to layers and affect eggshell quality (Boorman and Gunaratne, 2001). Excessive Ca in the body can interfere with absorption of P, ferrum, manganese and other mineral elements, while excessive P will combine with Ca in the intestine to form insoluble substances and reduce the absorption of Ca and P in the intestine (Chen et al., 2022). Therefore, appropriate Ca and P are essential for growth and development of chickens, and beneficial to reduce feed costs.

Appropriate levels of dietary Ca and P of different breeds of poultry at different stages are different (Cui and Che, 2001). For laying hens, when dietary Ca level was 3.45%, there was no consistent relationship between 4 levels of NPP (0.16, 0.21, 0.31, and 0.39%) and egg quality, BW, and AFI of Isha Brown laying hens aged 25 to 37 wk (Boorman and Gunaratne, 2001). The highest egg production rate was achieved with a dietary Ca addition of 2% and 0.459% NPP for 18-wk-old Hy-Line Grey commercial starter hens (Kong, 2015); For growing chickens, 1.1% Ca and 0.55% P had the best effect on growth and skeletal development of Phenix Fowl aged from 13 to 20 wk (Hao et al., 2018). The diets with 1.1% Ca and 0.65% P were more effective in promoting the growth and development of Taihang chickens aged from 13 to 20 wk (Li et al., 2021). Most studies on Ca and P in poultry focuses on optimizing P utilization because of its high cost and environmental pollution risk. Considering the nutritional, environmental, and economic factors, low phosphorus diet is recommended when the phosphorus content can meet growth needs of chickens (Li et al., 2017).

Dietary Ca can inhibit fat generation and promote fat degradation, and has different effects on weight gain, hypertension, atherosclerosis, diabetes, etc. (Zhang et al. 2019), and it also has a role in suppressing obesity by regulating adipogenesis and metabolism and influencing the gut microbiota (Das and Choudhuri, 2020).

Beijing You Chicken (**BYC**), a kind of local chicken, was widely raised for meat and eggs in northern part of China. For a long time the Ca and P requirements of BYC have been referenced to those of high producing laying hens or fast-growing broilers, and there are situations where dietary Ca and P content are too high or too low, resulting in the waste or insufficiency of Ca and P, which affects the performance of chicken. We studied the effects of dietary Ca and NPP level on performance of BYC chickens at 0 to 6 wk of age (Zhang et al., 2021), and this study continued to investigate the effects of dietary Ca and NPP levels on performance, biochemical indexes and lipid metabolism in BYC growing pullets, in order to provide a reference basis for the feed formulation of BYC.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments

The experiment was conducted at a BYC demonstration farm, Shunyi district, Beijing. A total of 648 ten-wk-old growing pullets with an average weight of 578.32 g, were fed 9 diets (6 replicates, 12 birds per replicate) arranged in a 3 × 3 factorial design. Dietary Ca levels were 0.66, 0.71, and 0.76%, non-phytate phosphrous (NPP) levels were 0.25, 0.30, and 0.35%, The settings of Ca and NPP levels were adjusted up and down according to the recommended levels in“Technical regulation of Beijing You Chicken Feed and Management”(DB11/T 1378-2016). Each group was randomly fed one of the 9 powdered diets. The composition and nutritional levels of the diets are shown in Table 1.

Growth Performance

Feed intake and body weight of each replicate cage were recorded and the average feed intake (**AFI**), body weight gain (**BWG**), feed gain ratio (**F/G**), and mortality rate were calculated at 10 to 16 wk of age.

Serum Biochemical Indexes

At the end of 16 wk, 6 birds in each replicate were randomly selected and bled (3−5 mL each bird) via the wing vein. Blood samples were centrifuged 3,000 rpm for 10 min at 4℃, and the serum was stored at −80℃ for later analysis of biochemical indexes. Serum levels of Ca, P, triglyceride (**TG**), total cholesterol (**TC**), high-density lipoprotein cholesterol (**HDL-C**), low-density lipoprotein cholesterol (**LDL-C**) content, and alkaline phosphatase (**AKP**) activity were determined by using ELISA (Multiskan FC, Thermal Fisher Scientific, Shanghai, China); serum total protein (**TP**), albumin (**ALB**), urea nitrogen (**UN**), lysozyme (**LZM**) were determined by spectrophotometer (Thermo Fisher (Shanghai) Instruments Co., LTD, Shanghai, China), and the kits were from Nanjing Jiancheng Institute of Biological Engineering (Nanjing, China).

Statistical Analysis

The data were expressed as mean and standard error between groups, and were analyzed using the General Linear Model (**GLM**) in SPSS 25.0 software to analyze the main effects and interaction of Ca and NPP. The percentage was arcsine transformed before the normality test. Significance of differences between groups was tested by Duncan’s multiple comparison test with $P <$ 0.05 as the criterion for significance of differences, and a trend was considered at 0.05< P <0.10.

RESULTS

Growth Performance

The effects of dietary Ca and NPP levels on growth performance are shown in Table 2. Dietary Ca and NPP alone did not affect growth performance, but the interaction of dietary Ca and NPP affected average feed intake (AFI) of growing pullets ($P < 0.05$). The AFI was

Table 1. Composition and nutrient level of the basal diet (air-dried base).

Item (%)	1	2	3	4	5	6	7	8	9
Calcium	0.66	0.66	0.66	0.71	0.71	0.71	0.76	0.76	0.76
Non-phytate phosphorus	0.25	0.30	0.35	0.25	0.30	0.35	0.25	0.30	0.35
Corn	65.5	65.5	65.5	65.5	65.5	65.5	65.5	65.5	65.5
Wheat bran	9	9	9	9	9	9	9	9	9
Soybean meal	20	20	20	20	20	20	20	20	20
Soybean oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calcium stone powder	1.22	1.05	0.88	1.36	1.18	1.02	1.5	1.32	1.16
Calcium hydrogen phosphate	0.67	0.98	1.28	0.67	0.98	1.28	0.67	0.98	1.28
Medical stone	1.11	0.97	0.84	0.97	0.84	0.7	0.83	0.7	0.56
Premix[1]	2	2	2	2	2	2	2	2	2
Total	100	100	100	100	100	100	100	100	100
Nutritional level									
Metabolizable energy/(MJ/kg)	11.51	11.51	11.51	11.51	11.51	11.51	11.51	11.51	11.51
Crude protein[2)]	15.15	15.12	15.16	15.14	15.13	15.17	15.16	15.17	15.14
Lysine	0.72	0.73	0.74	0.74	0.75	0.73	0.73	0.72	0.72
Methionine+Cystine	0.53	0.52	0.54	0.51	0.53	0.54	0.53	0.52	0.54
Tryptophan	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Threonine	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53
Calcium[2]	0.63	0.64	0.65	0.70	0.71	0.68	0.77	0.74	0.75
Total phosphorus	0.48	0.54	0.58	0.49	0.55	0.59	0.50	0.55	0.59
Non-phytate Phosphorus	0.24	0.29	0.36	0.25	0.29	0.35	0.23	0.29	0.34

[1]Premix of per kg diet provides: VA 6,000 IU, VD 2,800 IU, VE 20 IU, VK 2.4 mg, thiamine 2.0 mg, riboflavin 5.2 mg, Ca pantothenate 11 mg, niacin 30 mg, pyridoxine 3.6 mg, biotin 0.2 mg, folic acid 1.2 mg, VB_{12} 0.027 mg, choline 800 mg, Mn 90 mg, I 1.8 mg, Fe 100 mg, Cu 8 mg, Zn 80 mg, Se 0.30 mg.

[2]The crude protein, calcium, and non-phytate phosphorus levels are measured values, which are the average of three parallel measurements, and the rest of the nutrient levels are calculated values.

the lowest for the group with 0.71% Ca and 0.25% NPP (3,550.0 g, $P = 0.036$), which tended to have the lowest mortality rate ($P = 0.056$).

Serum Ca, P, and AKP

As shown in Table 3, dietary Ca level significantly affected serum P content ($P < 0.05$), with the lowest P content at 0.66% Ca level; dietary NPP level alone had an influence on serum Ca content at 16 wk of age ($P = 0.054$), and serum Ca content tended to increase with increasing dietary NPP level. Dietary NPP level significantly affected serum AKP activity ($P < 0.05$), which was significantly lower in the groups with 0.25% and 0.30% NPP than in 0.35% NPP group. The interaction of Ca and P significantly affected serum AKP activity ($P < 0.01$), and the lowest AKP activity was found in the group with 0.71% Ca and 0.25% NPP ($P < 0.05$).

Serum Biochemical Indices

As shown in Table 4, the effect of dietary Ca and NPP levels alone on serum TP, ALB, globulin, and UN content was not significant ($P > 0.05$), but there had a tendency for the interaction of dietary Ca and NPP affecting the LZM activity ($P = 0.094$), The LZM activities in groups with 0.66% Ca and 0.25% NPP, and 0.76% Ca and 0.35% NPP were significantly higher than that in group with 0.66% Ca and 0.35% NPP ($P < 0.05$).

Lipid Metabolism

As shown in Table 5, the TG content of 0.71% Ca group was extremely lower than that of the 0.66 and 0.76% ($P < 0.01$). Dietary Ca levels significantly affected TC content ($P < 0.01$), with 0.66% and 0.71% Ca level having extremely lower TC content than 0.76% level ($P < 0.01$). Dietary Ca level significantly affected HDL-C content ($P < 0.05$), and there was a tendency for HDL-C content to decrease as increasing of Ca level, with the highest HDL-C content in 0.66% Ca level group. Dietary NPP level significantly affected serum TG content ($P < 0.05$), and the TG content in 0.25 and 0.30% NPP groups was significantly lower than that in 0.35% NPP group ($P < 0.05$), and the NPP level had a tendency to affect the TC content ($P = 0.070$). The interaction of dietary Ca and NPP significantly affected TG, TC and HDL-C content ($P < 0.05$), the lowest TG and TC contents were found in the group with 0.71% Ca and 0.30% NPP, and the highest HDL-C content in the group with 0.66% Ca and 0.25% NPP ($P < 0.05$). TG, TC, and LDL-C levels were lower and HDL-C levels were the highest in the group with 0.66% Ca and 0.25% NPP.

DISCUSSION

Growth Performance

Ca and P are 2 important mineral elements in the body and are involved in various physiological activities of animals, for example, Ca can maintain the normal physiological functions of nerves, muscles, and heart, maintain the balance of acid and base in the body, and promote the blood clotting in wounds. P is involved in the composition of phospholipids, nucleic acids and certain enzymes . The effects of dietary Ca and NPP on performance vary depending on breeds, ages, etc.

Ding et al. (2002) suggested that the best egg shell quality was achieved at dietary Ca levels of 3.5 to 4.0% and NPP levels of 0.2% for 20-wk-old Peking Red hens. The highest egg production rate was achieved with a

Table 2. Effects of dietary Ca and NPP levels on performance of growing pullets at 10 to 16 wk of age.

Ca (%)	NPP (%)	BW (g)	AFI (g)	F/G (g:g)	Mortality rate (%)
0.66	0.25	608.70	3,706.67[ab]	6.08	4.17
0.66	0.30	569.14	3,706.46[ab]	6.51	5.56
0.66	0.35	596.11	3,605.56[ab]	6.05	0
0.71	0.25	594.72	3,550.00[b]	5.97	0
0.71	0.30	604.72	3,625.00[ab]	5.99	0
0.71	0.35	596.08	3,810.52[a]	6.39	5.56
0.76	0.25	613.03	3,805.13[a]	6.21	1.38
0.76	0.30	590.30	3,634.37[ab]	6.16	4.17
0.76	0.35	597.32	3,642.55[ab]	6.10	1.38
SEM		5.890	25.104	0.057	0.683
Main effects					
Ca	0.66	591.32	3,672.89	6.21	3.24
	0.71	598.51	3,661.84	6.12	1.85
	0.76	600.22	3,694.02	6.15	2.31
NPP	0.25	605.48	3,687.26	6.09	1.85
	0.30	588.06	3,655.28	6.22	3.24
	0.35	596.51	3,686.21	6.18	2.31
P-value					
P Ca		0.822	0.860	0.842	0.686
P NPP		0.516	0.830	0.538	0.686
P Ca×NPP	0.729	0.036	0.180	0.056	

Abbreviations: AFI, average feed intake; BW, body weight; F/G, feed gain ratio; NPP, non-phytate phosphrous.

[ab]In the same column, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different letter superscripts mean significant difference ($P < 0.05$).

dietary Ca level of 2% and a NPP level of 0.459% for 18-wk-old Hy-Line Grey commercial starter hens (Kong, 2015). Low dietary Ca content lead to an increase in body weight, and decrease in egg production (Li et al., 2022). Reducing dietary NPP level could help improve the utilization of phytate phosphorus without affecting the performance (Ding et al., 2002).

For growing chickens, increasing dietary NPP content will increase average feed intake of Ross 308 broiler chicks in grower phases (d 8−24) (Cowieson et al., 2020). Dietary 0.75% Ca and 0.35% NPP levels were better for growth performance of slow-growing yellow-feather broiler at 8 wk of age (Wang et al., 2020); dietary 1.1% Ca and 0.65% P were better for the growth and development of Taihang chickens at 13 to 20 wk age (Li et al., 2021), while this present study suggested that dietary 0.71% Ca and 0.25% NPP significantly reduced AFI in BYC growing pullets, which supported that different breeds had different Ca and P levels.

Table 3. Effects of dietary Ca and NPP levels on serum Ca, P, and AKP at 16-wk-old pullets.

Ca (%)	NPP(%)	Serum Ca (mmol/L)	Serum P (mmol/L)	Serum AKP (ng/L)
0.66	0.25	1.31	2.09	582.45[B]
0.66	0.30	1.39	1.80	585.18[AB]
0.66	0.35	1.39	2.20	585.15[AB]
0.71	0.25	1.36	2.61	580.49[BC]
0.71	0.30	1.33	2.55	582.07[B]
0.71	0.35	1.44	2.34	586.28[A]
0.76	0.25	1.41	2.09	587.42[A]
0.76	0.30	1.36	2.06	581.09[BC]
0.76	0.35	1.43	1.93	584.65[AB]
SEM		0.012	0.076	0.539
Main effect				
Ca	0.66	1.36	2.03[b]	584.26
	0.71	1.37	2.50[a]	582.95
	0.76	1.40	2.21[b]	584.38
NPP	0.25	1.36[b]	2.63	583.45[b]
	0.30	1.36[b]	2.14	582.78[b]
	0.35	1.42[a]	2.16	585.36[a]
P-value				
P Ca		0.388	0.016	0.226
P NPP		0.054	0.737	0.025
P Ca×NPP		0.220	0.635	0.002

Abbreviations: AKP, alkaline phosphatase; NPP, non-phytate phosphrous.

In the same column, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different letter superscripts ([ab]) mean significant difference ($P < 0.05$), with different capital letters ([ABC])mean extremely significant differences ($P < 0.01$).

Table 4. Effect of dietary Ca and NPP levels on serum biochemical indices at 16 wk of age.

Ca (%)	NPP (%)	TP (g/L)	ALB(g/L)	Globulin (g/L)	LZM (μg/mL)	UN (mmol/L)
0.66	0.25	69.13	11.91	57.23	11.85^{a}	0.18
0.66	0.30	64.17	13.83	55.33	7.39^{ab}	0.10
0.66	0.35	90.86	17.94	72.92	3.98^{b}	0.16
0.71	0.25	79.89	13.83	66.06	8.31^{ab}	0.17
0.71	0.30	89.21	7.79	81.41	8.39^{ab}	0.07
0.71	0.35	70.58	9.22	61.35	9.44^{ab}	0.18
0.76	0.25	97.28	8.13	89.15	7.56^{ab}	0.05
0.76	0.30	80.01	8.47	74.53	8.38^{ab}	0.15
0.76	0.35	77.41	14.09	63.32	11.89^{a}	0.13
SEM		5.436	1.036	5.506	0.729	0.017
Main effect						
Ca	0.66	74.72	14.56	60.16	7.74	0.15
	0.71	79.89	10.28	69.61	8.72	0.14
	0.76	85.89	10.23	75.67	8.94	0.11
NPP	0.25	82.10	11.29	70.81	9.24	0.13
	0.30	78.79	10.03	68.76	7.72	0.11
	0.35	79.62	13.75	65.87	8.44	0.16
P-value						
P Ca		0.793	0.134	0.635	0.731	0.681
P NPP		0.978	0.281	0.953	0.644	0.541
P Ca×NPP		0.745	0.321	0.728	0.094	0.408

Abbreviations: ALB, albumen; NPP, non-phytate phosphrous; LZM, lysozyme; TP, total protein; UN, unrea nitrogen.
In the same column, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different letter superscripts (^{ab}) mean significant difference ($P < 0.05$).

Serum Ca, P, and AKP

Serum Ca and P levels and AKP activity are important indicators to help assess the appropriateness of dietary Ca and P levels. Changes in serum Ca and P levels alter the secretion of regulatory hormones that can affect bone development. Serum Ca levels are regulated by negative feedback from parathyroid hormone and oestriol in the body and also regulate phosphorus metabolism in the kidney (Shastak et al., 2012). Ca is absorbed in the intestine into the blood and then redeposited into the bone. If plasma Ca ion or P ion is too high, the body suppresses parathyroid hormone and ossified triol, decreases intestinal and skeletal Ca ion or P ion reabsorption, and increases renal Ca and P excretion. Cui and Che (2001) found that the more Ca was added to the diet, the more Ca was deposited by the hens. Arkadiusz et al. (2020) also found that the finer the diet, the higher the Ca and P utilization, and even feeding low doses of Ca and P did not negatively affect the health of the chickens. Excessive dietary P increases parathyroid hormone release and decreases blood Ca concentrations

Table 5. Effects of dietary Ca and NPP levels on serum lipid metabolism at 16 wk of age.

Ca(%)	NPP(%)	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
0.66	0.25	0.66^{bc}	3.72^{C}	1.19	1.95^{a}
0.66	0.30	0.81^{bc}	4.66^{AB}	2.13	1.31^{bc}
0.66	0.35	0.83^{bc}	3.96^{BC}	1.76	1.35^{bc}
0.71	0.25	0.63^{cd}	4.03^{BC}	1.43	1.04^{bc}
0.71	0.30	0.57^{d}	2.80^{D}	1.37	1.42^{bc}
0.71	0.35	0.59^{d}	5.36^{A}	1.26	0.92^{c}
0.76	0.25	0.81^{bc}	5.55^{A}	1.73	1.16^{bc}
0.76	0.30	0.86^{b}	5.43^{A}	1.36	1.54^{ab}
0.76	0.35	1.20^{a}	5.25^{A}	1.10	1.14^{bc}
SEM		0.120	0.270	0.132	0.074
Main effect					
Ca	0.66	0.77^{B}	4.12^{B}	1.69	1.54^{a}
	0.71	0.59^{C}	4.07^{B}	1.35	1.13^{b}
	0.76	0.96^{A}	5.41^{A}	1.40	1.28^{b}
NPP	0.25	0.70^{b}	4.44^{ab}	1.45	1.38
	0.30	0.74^{b}	4.29^{b}	1.62	1.42
	0.35	0.88^{a}	4.86^{a}	1.37	1.14
P-value					
P Ca		0.001	0.001	0.532	0.027
P NPP		0.013	0.070	0.733	0.121
P Ca×NPP		0.030	0.001	0.478	0.048

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NPP, non-phytate phosphrous; TG, triglyceride; TC, total cholesterol.
In the same column, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different letter superscripts ($^{a\text{-}d}$) mean significant difference ($P < 0.05$), with different capital letters ($^{A\text{-}D}$) mean extremely significant differences ($P < 0.01$).

(Berndt and Kumar, 2009). Chen et al. (2022) stated that dietary Ca levels affect P absorption and, at the same time, dietary P levels affect Ca absorption. This explains the fact that dietary Ca levels influenced serum P levels, while dietary P levels influenced serum Ca levels in this study. Boorman and Gunaratne (2001) found that excessive P intake by laying hens can significantly increase serum P levels and affect egg production, indicating that P levels should not be too high. Kong (2015) found that dietary 3.75% Ca were more likely to reduce blood Ca and P levels in 18-wk-old commercial starter hens, which to some extent reduced the activity of bone metabolism and the incidence of Ca-related diseases. Li et al. (2017) found that dietary Ca is involved in stabilizing the normal physiological functions of muscles and nerves during the growing period, and if Ca ions in the body are reduced, the sensitivity of muscles and nerves will be increased, and in severe cases it will lead to convulsions. Ding et al. (2002) suggested that the use of low Ca (2.5%) diets led to an increase in AKP activity in 20-wk-old Peking Red hens, which affected osteogenesis and bone metabolism, thus affecting the normal development of bones. He and Fang (2010) showed that low dietary P or P deficiency increased serum AKP activity, and that serum AKP activity was strongly negatively correlated with bone mineral deposition in Three-yellow broiler chickens. The present study suggested that when the NPP level increases the serum Ca level tends to increase and AKP activity also increases significantly, which indicate that a NPP level of 0.25% is sufficient to meet the pullet's needs.

Serum Biochemical Indices

Serum biochemical indexes can reflect nutritional metabolism and the health status of poultry to a certain extent. The TP, ALB and UN levels can reflect the metabolic status of the dietary protein. Ding et al. (2002) suggested that a dietary Ca level of 2.5% resulted in a significant reduction in serum total protein, albumen, and globulin in 20-wk-old Peking Red layers. Lysozyme is an important small molecule protein for nonspecific immunity of the organism, and it can perform lysis immunity in vivo and has a strong antibacterial effect (Zhao et al., 2021). High levels of phosphorus will increase lysozyme activity in the body (Chang et al., 2006), however, there had no difference for lysozyme activity among the groups in the present study.

Gerlinger et al. (2021) showed that reducing dietary P levels could affect bone mineral density and trabecular bone structure of animals, but a small reduction of P level, combined with appropriate Ca levels, could help reduce the P waste and environmental pollution. This present study didn't measure the tibial indexes, but our another experiment with 4 levels of Ca and 2 levels of NPP showed that dietary 0.80% Ca and 0.40% NPP had the maximum tibial length and weight, high tibial ash, and bone Ca and P content (Zhang, 2022).

Lipid Metabolism

A preliminary mechanism by which Ca regulates fat metabolism was proposed by Zemel et al. (2000): a low Ca diet leads to increased levels of 1,25$(OH)_2$-D_3 and increased inward flow of Ca^{2+} in adipocytes, which stimulates insulin release, promotes adipogenesis, inhibits lipolysis, and reduces body heat production, ultimately leading to fat accumulation and weight gain and increased TG in adipocytes, whereas a high Ca diet has the opposite effect. Kralik et al. (2008) suggested that HDL-C prevents atherosclerosis by transporting cholesterol to the liver for metabolism, whereas LDL-C causes atherosclerosis by depositing cholesterol on the arterial walls. Thus, atherosclerosis of arteries is negatively correlated with HDL-C content and positively correlated with LDL-C content. Zhu et al. (2007) showed that low Ca diets significantly increased plasma TG, free fatty acid, TC, and LDL-C concentrations in 23-wk-old Hy-Line Brown hens at 1.2 and 2.0% Ca levels. Ma et al. (2009) suggested that normal Ca (3.6%) low P (0.15% NPP) group reduced plasma phospholipid levels and increased plasma TG, free fatty acid levels and hepatic lipoprotein lipase, hepatic lipase and hepatic total lipase activities in laying hens, which indicates that low P also causes an increase in plasma TG, etc. Li et al. (2017) indicated that there was no significant difference in growth performance between low P and low Ca when the diet was supplemented with phytase and high P and high Ca when the diet was not supplemented with phytase. The present study found that dietary Ca and NPP levels significantly affected serum TG, TC and HDL-C contents, with the lowest TG and TC contents and the highest HDL-C content at 0.66% Ca and 0.25% NPP levels, indicating that 0.66% Ca and 0.25% AP can help reduce fat synthesis. The reason is related to the chicken breed and stage, and the possible effect of Ca level itself on lipid metabolism. At present there are few studies on the effects of dietary Ca and P levels on lipid metabolism, it is interesting to regulate dietary Ca and P levels in pullet phase to avoid excessive obesity during the egg-laying period. In addition, this experiment was only conducted for 7 wk, and the long-term effects on laying hens need to be further investigated.

CONCLUSIONS

Dietary Ca and NPP levels significantly affected AFI of growing pullets. Appropriate dietary Ca level can regulate serum TG, TC, and HDL-C content. Dietary Ca and NPP levels can be adjusted in pullet phase to avoid excessive obesity during the egg-laying period. The study recommended that dietary 0.66% Ca and 0.25% NPP benefit for the lipid metabolism of BYC growing pullets without affecting the performance.

ACKNOWLEDGMENTS

The authors wish to thank BAAFS Academy Capacity Building Project (KJCX20200421), the earmarked fund for CARS (CARS-41-Z04) and Beijing Innovation Consortium of Agriculture Research System (BAIC06-2022) for providing financial supports, and the staff from Lvdudu Farm for feeding and management of the experimental birds.

DISCLOSURES

The authors declare that there is no conflict of interest.

REFERENCES

Arkadiusz, M., Ł. Monika, and N. Jan. 2020. Calcium and phosphorus and their nanoparticle forms in poultry nutrition. Worlds Poult. Sci. J. 76:328–345.

Berndt, T., and R. Kumar. 2009. Novel mechanisms in the regulation of phosphorus homeostasis. Physiology. 24:17–25.

Boorman, K. N., and S. P. Gunaratne. 2001. Dietary phosphorus supply, egg-shell deposition and plasma inorganic phosphorus in laying hens. Br. Poult. Sci. 42:81–91.

Chang, W. H., G. H. Liu, and S. H. Zhang. 2006. Effect of mulberry feed on the growth performance of broiler chickens and their plasma urea nitrogen content. China Feed. 18:3.

Chen, P., T. T. Xu, Y. F. K. He, K. L. Liu, J. F. Xie, K. Yue, C. D. Zhang, L. X. Lin, Q. Q. Cao, and S. C. Huang. 2022. Research progress on the effect of calcium and phosphorus metabolism on bone health of animals. Livestock Feed Sci. 43:39–45.

Cowieson, A. J., R. Perez-Maldonado, A. Kumar, and M. Toghyani. 2020. Possible role of available phosphorus in potentiating the use of low-protein diets for broiler chicken production. Poult Sci. 99:6954–6963.

Cui, W. G., and P. X. Che. 2001. Reasonable use of calcium and phosphorus to ensure the health of chickens. China Poult. 3:34–35.

Das, S., and D. Choudhuri. 2020. Dietary calcium regulates the insulin sensitivity by altering the adipokine secretion in high fat diet induced obese rats. Life Sci. 250:117–560.

DB11/T 1378-2016 Technical regulations of feeding and management of Beijing You Chicken. 2016. Beijing, China: Beijing Municipal Bureau of Quality and Technical Supervision.

Ding, B. A., X. G. Luo, and Y. M. Guo. 2002. Effect of dietary calcium and phosphorus levels on serum biochemical parameters and plasma mineral content in laying hens. China Feed. 14:9–11.

Gerlinger, C., M. Oster, H. Reyer, C. Polley, B. Vollmar, E. Muráni, K. Wimmers, and P. Wolf. 2021. Effects of excessive or restricted phosphorus and calcium intake during early life on markers of bone architecture and composition in pigs. J. Anim. Physiol. Anim. Nutr. (Berl). 105(Suppl 2):52–62.

Hao, Y. S., Z. Feng, G. X. Zhao, Y. Y. Zhang, S. P. Li, Y. Y. Xu, D. Liu, and L. Wang. 2018. Effets of dietary calcium and phosphorus levels on growth performance and blood biochemical indexes of the bashang long-tailed chickens in rearing period. Cereal & Feed Industry 3:47–50.

He, J., and R. J. Fang. 2010. Effect of different phosphorus levels in diets on calcium and phosphorus metabolism and nutrient digestibility of three yellow chickens. Guangdong Feed. 4:25–26.

Kong, L. X. 2015. Effect of different calcium enrichment patterns on production performance, tibia quality and egg quality of laying hens. Master thesis. Hebei Agricultural University, China.

Kralik, G., Z. Škrtić, and P. Suchý. 2008. Feeding fish oil and linseed oil to laying hens to increase the n-3 PUFA of egg yolk. Acta Vet. Brno. 77:561–568.

Li, C. Z., S. C. Tang, and Y. Wang. 2017. The role and biological effectiveness of five trace elements for chickens. Modern Anim. Husbandry Sci. Technol. 1:32–33.

Li, S. P., Y. S. Hao, G. X. Zhao, Z. H. Feng, G. Z. Liu, K. W. Ma, and Y. C. Liu. 2021. Effect of dietary calcium and phosphorus levels on growth performance and blood biochemical parameters of breeding Taihang chickens. Livestock Poult. Indust. 32:1–3.

Li, X., D. Zhang, and K. H. Huang. 2017. Available phosphorus requirement of meat chickens. Australian Rural Industries Research and Development Corporation). https://rirdc.infoservices.com.au/items/17-013 [accessed Aug. 2017].

Li, Y. F., D. Shao, and H. B. Tong. 2022. Research progress of fatty liver syndrome in laying hens. China Poult. 44:101–107 2022.

Liu, K. W., H. A. Dai, and J. C. Xing. 2008. Effects of mineral deficiency on physiological functions of poultry. Hubei J. Anim. Vet. Sci. 1:14–15.

Ma, X., L. Q. Zhu, F. H. Zhu, and Z. H. Guo. 2009. Effect of low phosphorus or low calcium high energy diets on fat metabolism in laying hens. Proceedings of the 2009 Symposium of Chinese Society of Animal Husbandry and Veterinary Medicine, Livestock Internal Medicine Branch.

Ren, Z., W. Sun, X. Cheng, Y. Liu, and X. Yang. 2020. The adaptability of Hy-line Brown laying hens to low phosphorus diets supplemented with phytase. Poult. Sci. 99:3525–3531.

Román-García, P., N. Carrillo-López, J. L. Fernández-Martín, M. Naves-Díaz, M. P. Ruiz-Torres, and J. B. Cannata-Andía. 2010. High phosphorus diet induces vascular calcification, a related decrease in bone mass and changes in the aortic gene expression. Bone 46:121–128.

Sabbagh, Y., T. O. Carpenter, and M. B. Demay. 2005. Hypophosphatemia leads to rickets by impairing caspase-mediated apoptosis of hypertrophic chondrocytes. Proc. Natl. Acad Sci. 102:9637–9642.

Shastak, Y., M. Witzig, K. Hartung, W. Bessei, and M. Rodehutscord. 2012. Comparison and evaluation of bone measurements for the assessment of mineral phosphorus sources in broilers. Poult. Sci. 91:2210–2220.

Wang, Y. B., W. W. Wang, S. Zhang, Z. Y. Gou, L. Li, X. J. Lin, Q. L. Fan, J. L. Ye, and S. Q. Jiang. 2020. Effects of dietary calcium and non-phytate phosphorus levels on growth performance and tibial characteristics of slow-growing yellow-feathered broilers aged from 29 to 56 days. Chin. J. Anim. Nutr. 32:5659–5666.

Wei, H., Y. Bi, Y. Li, H. Zhang, J. Li, R. Zhang, and J. Bao. 2022. Low dietary phosphorus impairs keel bone health and quality in laying hens. Br. Poult. Sci. 63:73–81.

Zemel, M. B., H. Shi, G. Betty, D. B. Dirienz, and P. C. Zemel. 2000. Regulation of adiposity by dietary calcium. FASEB J. 14:1132–1138.

Zhang, F., J. Ye, X. Zhu, L. Wang, and S. Wang. 2019. Anti-obesity effects of dietary calcium: the evidence and possible mechanisms. Int. J. Mol. Sci. 20:3072–3086.

Zhang, Q. Q. 2022. Study on the dietary calcium and available phosphatase levels of Beijing You Chicken during brooding and growing periods. Master thesis. Shandong Agricultural University, China.

Zhang, Q. Q., F. Zhang, X. H. Zhao, Y. Zhang, H. H. Wang, Q. Chu, Z. G. Song, and A. L. Geng. 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. Chin. J. Anim. Nutr. 33:6184–6192.

Zhao, R. W., L. P. Tan, and T. J. Liu. 2021. Research progress of lysozyme and its application. J. Qilu. Univ. Technol. 35:12–18.

Zhu, L. Q., F. H. Zhu, X. Ma, F. D. Wang, W. T. Gai, and X. F. Fu. 2007. Effect of low calcium diets on fat metabolism in laying hens. Jiangsu Agric. Sci. 4:146–150.