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RESEARCH ARTICLE

Effects of Dietary Calcium and Non-Phytate Phosphorus Intake and Genetic Line on Reproductive Performance and Calcium and Phosphorus Digestibility in Broiler Breeder Hens and their Progeny

[1,2]A.D. Magnuson, [1,3]P. Sodsee, [1,4]J. Caldas, [1,5]K. Vignale, [1,3]N. Boonsinchai, [1,6]M. Putsakum, [1]J.A. England and [1]C.N. Coon

[1]Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas 72701, USA
[2]Department of Animal Science, State University of New York at Cobleskill, Cobleskill, New York 12043, USA
[3]Charoen Pokphand Group (CP Group), Bangkok, Thailand
[4]Aviagen Incorporated, Huntsville, Alabama, USA
[5]Kemin Industries, Des Moines, Iowa, USA
[6]School of Agriculture and Cooperatives, Nonthaburi, Thailand

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Corresponding Author:
C.N. Coon,
Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas 72701, USA

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Abstract

Background and Objective: The calcium (Ca) and non-phytate phosphorus (NPP) requirements of broiler breeder hens are less well characterized than those of laying hens, and whether these requirements differ among genetic lines remains unclear. This study evaluated the effects of dietary Ca and NPP intake and genetic line on reproductive performance, egg composition and Ca and P digestibility in broiler breeder hens and their progeny.

Materials and Methods: Hens from four purelines (A to D; 40 hens per line) were individually caged and assigned to 1 of 6 diets from housing through 50 weeks of age in a 4×6 factorial arrangement. Diets contained 2.25 to 3.25% Ca with 0.25% NPP, or 3.25% Ca with 0.40% NPP. At peak feed allocation, these concentrations provided 3.08 to 4.45 g Ca and either 340 or 550 mg NPP/hen/day. Reproductive performance, egg components, hatchability and mineral digestibility were evaluated in hens, and Ca and P digestibility were subsequently evaluated in 14-day-old progeny fed a common starter diet.

Results: Genetic line affected body weight, mortality, age at first egg, egg production, egg components, hatchability, and day-old progeny weight ($p<0.05$), whereas diet affected only day-old progeny weight ($p = 0.002$). Albumen and yolk accounted for 57.4 to 59.3% and 31.8 to 34.4% of egg weight, respectively, across genetic lines, while hatchability of fertile eggs ranged from 74.0 to 86.3% ($p = 0.04$); neither trait was affected by diet. Increasing dietary Ca and NPP increased excreta Ca and P concentrations ($p<0.0001$) and decreased P digestibility in hens from 38.5 to 28.3% ($p = 0.0131$), whereas Ca digestibility was unaffected. In 14-day-old progeny fed a common starter diet, dam genetic line affected all four excreta mineral and digestibility traits ($p<0.0001$). Calcium digestibility ranged from 17.4 to 48.8% among progeny from different dam lines, whereas maternal diet affected progeny P digestibility only when the maternal diet contained 0.40% NPP.

Conclusion: Dietary Ca intake as low as 3.08 g/hen/day supported reproductive performance comparable to that achieved with higher Ca intakes under the conditions of this study. Increasing NPP intake from 340 to 550 mg/hen/day did not improve hen performance or Ca digestibility and reduced P digestibility. Differences in progeny mineral utilization were primarily associated with dam genetic line rather than maternal dietary Ca and NPP, suggesting that genetic factors may play a greater role than maternal diet in determining mineral utilization by progeny.

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INTRODUCTION

Calcium (Ca) and phosphorus (P) fulfill a wide range of structural and metabolic functions. In the broiler breeder hen, the balance between these two minerals governs bone formation and bone homeostasis, eggshell formation and a series of associated metabolic processes, while the digestibility of each is modified by the homeostatic mechanisms that maintain plasma mineral concentrations[1]. The NRC[2] recommended daily nutrient intakes for broiler breeder hens at peak production of 400 to 450 kcal metabolizable energy (ME), 19.5 g crude protein (CP), 4.00 g Ca, and 350 mg non-phytate phosphorus (NPP) per bird. Primary breeding companies express their recommendations inconsistently, with some expressed as daily intakes per bird and others as a percentage of the diet. Dietary recommendations generally range from 2750 to 2800 kcal ME/kg, 14 to 15.5% CP, 2.89 to 3.20% Ca, and 0.32 to 0.43% available P.

This inconsistency matters because feed allocation in broiler breeders is restricted and increases through the onset of lay. A fixed dietary percentage therefore delivers a variable daily intake, and two flocks fed the same dietary percentage can receive substantially different daily amounts of Ca and NPP depending on the feed management program. Expressing requirements as daily intake at a defined production point provides a more defensible basis for comparison and is the convention adopted here.

Compared with the extensive literature on commercial laying hens, there is little published work on the Ca and P requirements of broiler breeder hens and almost none that examines whether those requirements differ among genetic lines. Ekmay and Coon[3,4] reported line differences in P excretion and retention in broiler breeder hens fed a common diet and Ekmay *et al.*[5] found that purelines differed in egg production, shell weight, specific gravity, egg weight, breeder bone ash, and progeny bone ash. Selection for feed efficiency also alters mineral excretion. de Verdal *et al.*[6] reported reduced nitrogen and P excretion in broiler lines selected for higher apparent metabolizable energy efficiency and analogous line differences in mineral excretion have been described in swine[7].

The reduction of P entering the environment through land application of poultry litter has become a parallel objective to the maintenance of production. Dietary Ca and P concentrations determine how much of each mineral is excreted, and the ratio between them modifies retention[8]. Whether Ca and P requirements can be lowered without compromising reproductive performance and whether the response differs among purelines, therefore has both economic and environmental consequences.

The present study was conducted to characterize the effects of dietary Ca and NPP intake and genetic line on reproductive performance, egg components, hatchability and Ca and P digestibility in four broiler breeder purelines over a 29-week production period, as well as on the Ca and P digestibility of their 14-d-old progeny fed a common starter diet. Eggshell quality, bone homeostasis, and bone turnover markers in the same hens, as well as body composition determined by dual-energy X-ray absorptiometry, are reported in companion papers[9,10].

The present manuscript is one of three manuscripts from a single 29-wk production period, each reporting a different group of traits for the same four purelines fed the same six diets. Reproductive performance, egg components, hatchability, and Ca and P digestibility in the hens and their 14-d-old progeny are reported in the present study. Eggshell quality, bone homeostasis and the plasma bone markers bone alkaline phosphatase (BAP), tartrate-resistant acid phosphatase (TRAP) and fibroblast growth factor 23 (FGF23) are reported by Magnuson *et al.*[9]. Body composition and tibia ash of the same hens and their progeny are reported by Sodsee[10]. Because the purelines, diets, and feeding program are common across the three studies, the results can be interpreted as components of a single dataset.

MATERIALS AND METHODS

Ethics statement: The Institutional Animal Care and Use Committee of the University of Arkansas approved the study protocol (#13002), including the welfare guidelines and husbandry practices used during the experimental period. The committee is in strict compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy), the USDA Animal Welfare Act and Regulations (AWAR), the institutional Animal Welfare Assurance, and the University Policy on Animal Care and Use. The authors confirm that the ethical policies of the journal, as described on the journal's author guidelines page, have been adhered to and that appropriate ethical review committee approval was received.

Animals and handling: Pullets were reared by commercial producers and transferred to the production house at the University of Arkansas Poultry Research Farm before the onset of lay. Two hundred and forty-five pullets from purelines A and D were transferred at 20 and 22 weeks of age, respectively, and 245 pullets from purelines B and C were transferred at 21 weeks of age, together with 144 cockerels. Two production rooms were used so that the two lines transferred together

occupied the same room and received an identical lighting program from housing onward. Lines A and D were placed in one room, and lines B and C were placed in the second room. Upon arrival, pullets were placed in individual cages (47 cm high×30.5 cm wide×47 cm deep), each fitted with an individual feeder and a nipple drinker. Hens were fed individually each day and had free access to water at all times.

Pullets received 13 hrs of light per day at housing. Day length was increased to 14 hrs 10 days after housing, to 15 hrs at 5% hen-day egg production, and to 16 hrs at 60% hen-day egg production. Because the two lines sharing a room were housed together, both received the same lighting program and the same lighting changes throughout the production period.

Diets: A basal diet formulated to meet ideal protein requirements (15.4% CP, 2915 kcal ME/kg, 2.25% Ca and 0.25% NPP) was the foundation for Diets 1 to 5 (Table 1). The five diets were produced by blending the basal diet with limestone and sand so that Ca increased from 2.25 to 3.25% in 0.25% increments while NPP remained constant at 0.25%, with sand replaced by limestone on an equal-weight basis so that all other nutrients were held constant (Table 2). Diet 6 was a standard commercial breeder diet supplying 3.25% Ca and 0.40% NPP and was manufactured separately rather than blended from the basal diet. The resulting dietary Ca:NPP ratios were 9.0, 10.0, 11.0, 12.0, 13.0 and 8.1 for Diets 1 to 6, respectively.

Each diet batch was sampled and analyzed for CP, ash, crude fat, Ca, total P, and phytate P (Table 2). Supplemental Ca was supplied entirely by limestone with a mean particle size of 1500 µm and an *in vitro* solubility of 60%, determined by the method of Zhang and Coon[11]. Expressed as daily intake at peak feed allocation, the diets supplied 3.08, 3.43, 3.77, 4.11, 4.45, and 4.45 g Ca/hen/day and 340, 340, 340, 340, 340 and 550 mg NPP/hen/d, respectively.

Feeding program: The purelines were fed according to the increase in production of each line after sexual maturity rather than by calendar age. Each pureline was managed as a separate flock, and feed changes were made when that flock reached the relevant production criterion. Feed allocation was increased weekly from housing until 5% hen-day production and thereafter for every 8% increment in hen-day production until a peak allocation of 137 g/hen/day was reached at 61% production. A post-peak feed withdrawal program was then initiated separately for each line. Daily nutrient intakes across the feeding program are given in Tables S1 and S2. At peak allocation, all hens received 400 kcal ME and 21.1 g CP per bird daily. Males were fed a standard male breeder diet according to the recommendations of the primary breeder.

Table 1: Composition of the basal diet and of the commercial breeder diet used to produce the six experimental diets[a]

Ingredient (kg/tonne unless stated)	Basal	Commercial breeder
Maize	702.3	647.0
Soybean meal	222.5	229.9
Limestone	49.2	67.7
Dicalcium phosphate	8.8	18.2
Poultry fat	5.7	26.2
Sal Curb (liquid)	3.3	3.3
Sodium chloride	2.4	1.7
Alimet (DL-methionine hydroxy analogue)	1.9	2.0
Choline chloride (70%)	1.0	1.1
Sodium bicarbonate	1.0	1.0
Trace mineral premix[b]	0.8	0.8
Vitamin premix[b]	0.7	0.7
25-OH-D (HyD)	0.5	0.5
Total	1000.0	1000.0
Calculated composition		
ME (kcal/kg)	2915.00	2915.00
Crude protein (%)	15.37	15.33
Crude fat (%)	2.76	4.62
Ash (%)	8.19	10.87
Calcium (%)	2.25	3.25
Total P (%)	0.48	0.64
NPP (%)	0.25	0.41

ME: Metabolizable energy, [a]Ingredient inclusions were converted from the original formulation, expressed as pounds per 2000 lb batch, to kg/tonne, [b]Trace mineral and vitamin premixes were included at the rates shown; their composition is available from the corresponding author on request

Performance and egg component measurements: Individual body weights were recorded at housing and at 2- to 5-week intervals throughout the production cycle. Mortality was recorded daily. Egg production was recorded daily for each hen. All eggs laid were weighed individually on 2 consecutive days each week. Age at first egg, cumulative eggs per hen housed, and cumulative hen-day egg production (%) were calculated to 50 weeks of age.

All eggs weighed on the 2 collection days each week were also scanned by dual-energy X-ray absorptiometry (GE Lunar Prodigy; GE Healthcare, USA) in batches of 10 and albumen weight and yolk weight were predicted from the densitometric output using the equations of England *et al.*[12]. Predicted egg weight agreed with scale weight, with a correlation coefficient greater than 0.99 across 18,132 eggs. Albumen and yolk are reported as percentages of egg weight, together with the yolk ratio.

Artificial insemination and progeny: At 27, 37, and 47 weeks of age, all hens were artificially inseminated, and the resulting eggs were set. Semen was diluted at a ratio of 1 part semen to 2 parts Lake's high-temperature diluent, a dilution previously shown to achieve adequate fertility. Each hen was

Table 2: Blending, formulated and analyzed composition of the six experimental diets, and Ca and NPP intake at peak feed allocation[a]

Item	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Blending (kg/t)						
Basal diet	974	974	974	974	974	0
Sand	26	19.5	13	6.5	0	0
Limestone	0	6.5	13	19.5	26	0
Commercial breeder diet	0	0	0	0	0	1000
Formulated						
Calcium (%)	2.25	2.50	2.75	3.00	3.25	3.25
NPP (%)	0.25	0.25	0.25	0.25	0.25	0.40
Total P (%)	0.48	0.48	0.48	0.48	0.48	0.64
Ca:NPP ratio	9.0	10.0	11.0	12.0	13.0	8.1
Analyzed						
Crude protein (%)	17.18	17.18	16.48	16.80	17.48	16.85
Ash (%)	11.26	11.69	11.67	11.48	10.29	11.38
Crude fat (%)	5.58	5.58	5.73	5.66	5.61	5.52
Calcium (%)	2.46	2.89	3.07	3.34	3.15	3.44
Total P (%)	0.53	0.52	0.51	0.51	0.51	0.69
NPP (%)	0.28	0.26	0.28	0.29	0.27	0.42
Intake at peak feed allocation						
Calcium (g/hen/day)	3.08	3.43	3.77	4.11	4.45	4.45
NPP (mg/hen/day)	340	340	340	340	340	550

Ca: Calcium; NPP, non-phytate phosphorus, [a]Peak feed allocation was 137 g/hen/day, reached at 61% hen-day egg production. Limestone was the sole supplemental Ca source, with a mean particle size of 1500 µm and *in vitro* solubility of 60%. Proximate, Ca and phytate analyses were performed on a sample of each diet batch

Table S1: Feeding program and daily energy and protein intake per hen from housing through post-peak feed withdrawal[a]

Time of feed change	Feed allocation (g/hen/day)	ME (kcal/hen/day)	CP (g/hen/day)
Weeks after housing			
1	99	289	15.25
2	101	294	15.55
3	103	300	15.86
4	105	306	16.17
Hen-day egg production (%)			
5	107	311	16.42
13	108	315	16.66
21	111	325	17.16
29	115	334	17.65
37	119	348	18.39
45	124	362	19.13
53	131	381	20.12
61 (peak)	137	400	21.11
Weeks after peak (feed withdrawal)			
1	136	397	20.95
2	135	394	20.80
3	134	391	20.65
4	133	388	20.49
5	132	385	20.34
6	131	382	20.18
7	130	379	20.03
8	129	376	19.88
9	128	373	19.72
10	127	370	19.57
11	126	367	19.41
12	125	365	19.26
13	124	362	19.11
14	123	359	18.95

CP: Crude protein, ME: Metabolizable energy, [a]Each pureline was managed as a separate flock, and feed changes were made when that flock reached the stated criterion. Feed allocation was increased weekly from housing to 5% hen-day production and then at each 8% increment in hen-day production to peak allocation (137 g/hen/day at 61% hen-day production). The same feed allocation was used for all six diets. Energy and protein intakes were calculated from the formulated diet specification (2915 kcal ME/kg and 15.4% CP)

Table S2: Daily Ca and NPP intake per hen for each of the six diets across the feeding program[a]

	Ca (g/hen/day)						NPP (g/hen/day)	
Time of feed change	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diets 1–5	Diet 6
Weeks after housing								
1	2.23	2.48	2.72	2.97	3.22	3.22	0.25	0.40
2	2.27	2.53	2.78	3.03	3.28	3.28	0.25	0.40
3	2.32	2.58	2.83	3.09	3.35	3.35	0.26	0.41
4	2.36	2.63	2.89	3.15	3.41	3.41	0.26	0.42
Hen-day egg production (%)								
5	2.40	2.67	2.93	3.20	3.46	3.46	0.27	0.43
13	2.43	2.71	2.98	3.25	3.52	3.52	0.27	0.43
21	2.51	2.79	3.06	3.34	3.62	3.62	0.28	0.45
29	2.58	2.87	3.15	3.44	3.73	3.73	0.29	0.46
37	2.69	2.99	3.28	3.58	3.88	3.88	0.30	0.48
45	2.80	3.11	3.42	3.73	4.04	4.04	0.31	0.50
53	2.94	3.27	3.59	3.92	4.25	4.25	0.33	0.52
61 (peak)	3.08	3.43	3.77	4.11	4.45	4.45	0.34	0.55
Weeks after peak (feed withdrawal)								
1	3.06	3.40	3.74	4.08	4.42	4.42	0.34	0.54
2	3.04	3.38	3.71	4.05	4.39	4.39	0.34	0.54
3	3.02	3.35	3.69	4.02	4.36	4.36	0.34	0.54
4	2.99	3.33	3.66	3.99	4.32	4.32	0.33	0.53
5	2.97	3.30	3.63	3.96	4.29	4.29	0.33	0.53
6	2.95	3.28	3.60	3.93	4.26	4.26	0.33	0.52
7	2.93	3.25	3.58	3.90	4.23	4.23	0.33	0.52
8	2.90	3.23	3.55	3.87	4.19	4.19	0.32	0.52
9	2.88	3.20	3.52	3.84	4.16	4.16	0.32	0.51
10	2.86	3.18	3.49	3.81	4.13	4.13	0.32	0.51
11	2.84	3.15	3.47	3.78	4.10	4.10	0.32	0.50
12	2.81	3.13	3.44	3.75	4.06	4.06	0.31	0.50
13	2.79	3.10	3.41	3.72	4.03	4.03	0.31	0.50
14	2.77	3.08	3.38	3.69	4.00	4.00	0.31	0.49

Ca: Calcium, NPP: Non-phytate phosphorus, [a]Intakes were calculated from the feed allocations in Table S1 and the formulated dietary concentrations (Table 2). Diets 1 to 5 contained 2.25, 2.50, 2.75, 3.00 and 3.25% Ca with 0.25% NPP; Diet 6 contained 3.25% Ca with 0.40% NPP. Diets 1 to 5 supplied the same NPP intake at each feed change

inseminated with 0.05 mL of extended semen on 2 consecutive days. All eggs were pedigreed. Eggs were incubated, hatch residue was broken out to determine the cause of failure to hatch and all chicks were weighed individually at hatch. Progeny were reared to 14 days of age on a common commercial chick starter diet that was identical for the progeny of all hen treatments.

Every unhatched egg in the hatch residue was classified as infertile, embryonic death, or late live (a fully formed embryo dead in the shell). Hatchability was expressed both as a percentage of all eggs set and, because semen quality and quantity were poor in some lines during part of the trial, as hatch of fertile eggs, with infertile eggs excluded and late live embryos counted with live chicks. The latter basis removes a source of variation originating from the male rather than the hen and allows the four lines to be compared based on the hen's contribution alone.

Ca and P digestibility: Ca and P digestibility were determined for each batch of feed. Hens from each of the 24 line×diet treatments were fed the respective diet containing 2% Celite as a source of acid-insoluble ash (AIA) for 3 days. Excreta were collected on the third day from 8 hens per treatment, selected on the basis of least contamination by broken eggs, feathers, and feed residue, and analyzed for Ca, P and AIA. Digestibility was calculated as:

$$\text{Ca or P digestibility (\%)} = \left[1 - \frac{\text{Dietary AIA}}{\text{Excreta AIA}} \times \frac{\text{Excreta Ca or P}}{\text{Dietary Ca or P}}\right] \times 100$$

The same procedure was applied to 14-day-old progeny, with excreta collected from the progeny of each treatment.

Statistical analysis: Data were analyzed as a 4×6 factorial arrangement with genetic line, diet, and their interaction as fixed effects, using SAS (version 9, SAS Institute Inc., Cary, NC, USA). The individual hen was the experimental unit. Because lines were housed at different ages and during different calendar periods, body weight was compared only within pairs of lines housed concurrently (A with D, and B with C). Least squares means were separated using Student's *t* test, and all statements of significance are based on testing at $p \leq 0.05$.

RESULTS

Body weight, mortality, and egg production: Diet did not affect hen body weight at any age. Genetic line significantly affected body weight where concurrently housed lines could be compared ($p<0.0001$, Table S3). After 20 weeks of production, lines B and D were the heaviest (4,372 and 4,109 g, respectively) and lines C and A were the lightest (3,997 and 3,942 g, respectively). The line × diet interaction was not significant.

Cumulative mortality at 50 weeks of age differed among lines ($p = 0.03$) but not among diets (Table 3). Mortality was 16, 16, 10, and 9% for lines A, B, C, and D, respectively. Lines A and B lost significantly more hens than line D, whereas line C did not differ from any other line. Mortality ranged from 10 to 18% across diets, with no significant diet effect and no line × diet interaction.

Age at first egg differed among lines ($p<0.0001$, Table 3). Hens from lines A, B, C and D laid their first egg at 25.9, 27.1, 27.0 and 27.8 weeks of age, respectively. Line A hens laid 1.4 weeks earlier than the remaining lines, and line D hens laid later than all other lines. These differences corresponded to the age at light stimulation, which was 20 weeks for line A, 21 weeks for lines B and C, and 22 weeks for line D. Diet and the line × diet interaction had no effect.

Cumulative hen-day egg production (%) and eggs per hen housed to 50 weeks of age were affected by genetic line ($p<0.0001$) but not by diet (Table 3). Hen-day egg production

Table S3: Body weight of hens from four purelines from housing to 51 weeks of age[a,b]

(a) Lines B and C, housed concurrently

	Line B		Line C	
Hen age (week)	LSM (g)	SEM	LSM (g)	SEM
21.4	2561[a]	17.8	2333[b]	17.8
22.9	2847[a]	16.7	2587[b]	16.6
24.9	3171[a]	19.2	2891[b]	19.1
26.9	3464[a]	17.1	3133[b]	16.8
28.9	3699[a]	19.1	3366[b]	18.6
30.9	3879[a]	20.7	3516[b]	20.1
32.9	3994[a]	21.6	3616[b]	21.0
36.9	4142[a]	21.8	3729[b]	12.3
40.9	4224[a]	22.7	3840[b]	22.0
46.0	4333[a]	26.3	3953[b]	25.7
49.9	4372[a]	29.5	3997[b]	28.7

(b) Lines A and D, housed concurrently

	Line A		Line D	
Hen age (week)	LSM (g)	SEM	LSM (g)	SEM
21.1	2428	15.7	—	—
23.1	2797[a]	15.9	2483[b]	15.9
25.1	3093[a]	16.2	2869[b]	16.2
27.1	3314[a]	15.9	3195[b]	15.8
29.1	3461	17.1	3465	17.0
30.6	3534	17.8	—	—
31.1	—	—	3655	17.9
32.6	—	—	3746	19.7
33.3	3634	19.1	—	—
35.3	—	—	3851	22.2
37.1	3762	21.9	—	—
39.1	—	—	3975	24.3
41.1	3858	23.1	—	—
43.1	—	—	4050	24.0
45.3	3899	26.9	—	—
47.3	—	—	4076	24.3
49.1	3942	31.5	—	—
51.1	—	—	4109	28.0

LSM: Least squares mean, SEM: Standard error of the mean, [a]Because lines were housed at different ages and in different calendar periods, statistical comparisons were made only between lines housed concurrently (B with C, A with D) and only at ages when both lines were weighed, Genetic line, $p<0.0001$; diet, not significant; line × diet, not significant. -,not weighed at that age, [b]Means within a row not sharing a superscript differ (Student's t, $p \leq 0.05$)

Table 3: Effects of genetic line and dietary Ca and NPP on mortality, age at first egg, egg production, and egg weight in broiler breeder hens through 50 weeks of age[a,b]

Source	Mortality (%)	Age at first egg (wk)	Eggs per hen housed	Hen-day egg production (%)	Egg weight (g)
Genetic line					
A	16[a]	25.9[c]	103[ab]	50[a]	59.4[b]
B	16[a]	27.1[b]	98[b]	46[b]	61.9[a]
C	10[ab]	27.0[b]	108[a]	51[a]	62.6[a]
D	9[b]	27.8[a]	103[ab]	52[a]	59.9[b]
SEM	2.1	0.12	2.05	0.9	0.25
Diet (Ca%/NPP%)					
2.25/0.25	12	—	103	50	61.4
2.50/0.25	13	—	105	51	60.4
2.75/0.25	13	—	104	50	61.2
3.00/0.25	10	—	104	50	60.8
3.25/0.25	18	—	98	48	60.7
3.25/0.40	10	—	103	50	61.3
SEM	2.6	—	2.50	1.1	0.31
p-value					
Line	0.03	<0.0001	0.01	<0.0001	<0.0001
Diet	n.s.	n.s.	n.s.	n.s.	n.s.
Line×diet	n.s.	n.s.	n.s.	n.s.	n.s.

Ca: Calcium, n.s.: Not significant (p>0.05), NPP: Non-phytate phosphorus and SEM: Standard error of the mean, [a]Values are least squares means, n = 40 hens per line and n = 24 to 28 hens per diet, [b]Means within a column and within a source not sharing a superscript differ (p≤0.05), Least squares means for age at first egg by diet are not shown; the diet effect was not significant

Table 4: Effects of genetic line and dietary Ca and NPP on egg components of broiler breeder hens through 50 weeks of age[a-c]

Source	Albumen (% of egg weight)	Yolk (% of egg weight)	Yolk: albumen ratio
Genetic line			
A	57.38[d]	34.36[a]	0.602[a]
B	58.91[b]	32.33[c]	0.551[c]
C	59.27[a]	31.79[d]	0.538[d]
D	58.07[c]	33.49[b]	0.578[b]
SEM	0.043	0.049	0.001
Diet (Ca%/NPP%)			
2.25/0.25	58.35	33.16	0.571
2.50/0.25	58.36	33.03	0.569
2.75/0.25	58.43	32.97	0.566
3.00/0.25	58.32	33.07	0.570
3.25/0.25	58.40	32.93	0.566
3.25/0.40	58.58	32.80	0.562
SEM	0.053	0.060	0.002
p value			
Line	<0.0001	<0.0001	<0.0001
Diet	0.4553	0.0636	0.1398
Line×Diet	0.7626	0.8535	0.9126
Hen age	<0.0001	<0.0001	<0.0001
Hen age×Line	<0.0001	<0.0001	<0.0001

Ca: Calcium, NPP: Non-phytate phosphorus, SEM: Standard error of the mean, [a]Values are least squares means from repeated measures analysis, n = 40 hens per line and n = 24 to 28 hens per diet, [b]Means within a column and within a source not sharing a superscript differ (p≤0.05), [c]Albumen weight and yolk weight were predicted from dual-energy X-ray absorptiometry scans of intact eggs using the equations of England *et al*.[12]

was lower in line B hens than in the other three lines. Line B hens laid fewer eggs per hen housed than line C hens but did not differ from lines A or D. At 50 weeks of age, lines A, B, C, and D had produced 103, 98, 108 and 103 eggs per hen housed, respectively. Line A hens came into production more rapidly than the other lines and declined more rapidly from 34 to 50 weeks of age. Increasing dietary Ca from 2.25 to 3.25%, corresponding to 3.08 to 4.45 g Ca/hen/d at peak allocation, did not alter the number of eggs produced, and increasing NPP from 0.25 to 0.40% at 3.25% Ca had no effect.

Average egg weight from 21 to 50 weeks of age was affected by line (p<0.0001) but not by diet (Table 3). Eggs from lines B and C were heavier than those from lines A and D, with mean egg weights of 61.9, 62.6, 59.4, and 59.9 g for lines B, C, A, and D, respectively.

Egg components: Egg components were affected by genetic line but not by diet (Table 4). Albumen accounted for 57.4, 58.9, 59.3 and 58.1% of egg weight, and yolk accounted for 34.4, 32.3, 31.8, and 33.5% in lines A, B, C, and D, respectively.

Table 5: Effects of genetic line and dietary Ca and NPP on hatchability of eggs from broiler breeder hens artificially inseminated at 27, 37 and 47 weeks of age[a–c]

Source	Infertile (%)	Embryonic death (%)	Late live (%)	Live chick (%)	Hatch of fertile eggs (%)
Genetic line					
A	13.050[b]	21.640[a]	16.920[b]	54.880[b]	73.970[c]
B	10.640[b]	11.970[bc]	7.510[bc]	72.430[a]	86.280[a]
C	26.860[a]	11.600[c]	4.340[c]	58.460[ab]	83.970[ab]
D	7.440[b]	21.220[ab]	29.410[a]	50.200[b]	76.050[bc]
SEM	1.816	2.535	3.113	3.928	3.456
Diet (Ca%/NPP%)					
2.25/0.25	17.780	19.790	12.880	60.370	74.950
2.50/0.25	12.940	15.570	13.020	63.830	81.620
2.75/0.25	13.660	15.060	18.010	58.600	82.240
3.00/0.25	12.950	16.130	12.720	58.460	80.840
3.25/0.25	13.880	16.900	13.580	56.140	80.490
3.25/0.40	15.770	16.190	17.070	56.550	80.260
SEM	2.225	3.105	3.860	4.817	4.233
p-value					
Line	<0.0001	0.0051	<0.0001	0.0016	0.0400
Diet	0.5984	0.9146	0.8720	0.8841	0.8596
Line × Diet	0.9316	0.7431	0.9948	0.9947	0.8482

Ca: Calcium, NPP: Non-phytate phosphorus, SEM: Standard error of the mean, [a]Values are least squares means, [b]Means within a column and within a source not sharing a superscript differ ($p \leq 0.05$), [c]Infertile, embryonic death, late live and live chick are expressed as a percentage of all eggs set. Hatch of fertile eggs excludes infertile eggs and counts late live embryos with live chicks; see Materials and Methods

All four lines differed from one another for both components ($p<0.0001$). The yolk ratio followed the same order, ranging from 0.538 in line C to 0.602 in line A ($p<0.0001$). Increasing dietary Ca from 2.25 to 3.25%, or NPP from 0.25 to 0.40%, altered neither albumen percentage ($p = 0.46$), yolk percentage ($p = 0.06$), nor the yolk ratio ($p = 0.14$) and no line × diet interaction was significant.

Hen age affected every component ($p<0.0001$). Albumen percentage rose to a maximum at 34 weeks of age and was maintained to 46 weeks before declining, whereas yolk percentage moved in the opposite direction. The age × line interaction was significant for each component ($p<0.0001$).

Hatchability: Diet had no effect on any hatch outcome ($p \geq 0.60$, Table 5). Genetic line affected all hatch outcomes. Infertile eggs accounted for 26.9% of eggs set from line C hens, compared with 7.4 to 13.1% in the other three lines ($p<0.0001$). Embryonic death was highest in lines A and D (21.6 and 21.2%, respectively) and lowest in lines B and C (12.0 and 11.6%, respectively; $p = 0.0051$). Late live embryos accounted for 29.4% of eggs set from line D hens, compared with 4.3% from line C hens ($p<0.0001$). Live chicks per egg set were 54.9, 72.4, 58.5 and 50.2% for lines A, B, C, and D, respectively ($p = 0.0016$).

Expressed as hatch of fertile eggs, with infertile eggs excluded and late live embryos counted with live chicks, hatchability was 74.0, 86.3, 84.0, and 76.1% for lines A, B, C, and D, respectively ($p = 0.0400$), with line B having the highest value and line A the lowest. Diet remained without effect on this basis ($p = 0.86$) and no line × diet interaction was significant for any hatch trait. The infertility that distinguished line C on the all-eggs-set basis was therefore associated with male semen quality during part of the trial rather than with the hen or her diet, and the fertile-egg basis provides a more informative comparison of the four lines.

Day-old progeny weight: Day-old chick weight was affected by both line and diet at every hen age examined (Table 6). Chicks from line B hens were heavier than those from all other lines, whereas chicks from lines A and D were lighter than those from lines B and C. Life-of-flock means were 41.7, 44.1, 43.5, and 41.4 g for lines A, B, C, and D, respectively ($p<0.0001$).

Averaged over flock life, chicks from hens fed the 2.50% Ca diet were lighter than those from hens fed any other diet except the 2.75% Ca diet ($p = 0.002$). Life-of-flock chick weights were 43.0, 42.1, 42.6, 42.8, 42.9, and 42.8 g for Diets 1 to 6, respectively. The absence of a monotonic relationship with dietary Ca, together with the fact that the lightest chicks came from the second-lowest rather than the lowest Ca concentration, does not indicate a dose-dependent maternal Ca effect.

Significant line × diet interactions were present for chick weight at 37 weeks of hen age ($p = 0.0047$) and for the life-of-flock mean ($p = 0.0017$, Table S4). Within lines A, C, and D, diet had no effect on chick weight. Within line B, chicks from hens fed the 2.50% Ca diet were lighter than those from hens fed the 2.25% Ca diet but did not differ from those from hens fed the remaining diets.

Day-old chick weight therefore differed among lines by 2.7 g at the life-of-flock mean, a difference more than three

Table 6: Effect of genetic line and of dietary Ca and NPP on body weight of day-old progeny[a,b]

Source	27 week hen age (g)	37 week hen age (g)	47 week hen age (g)	Life of flock (g)	SEM
Genetic line					
A	38.2[b]	42.7[b]	46.8[b]	41.7[c]	0.13
B	38.3[b]	44.6[a]	48.3[a]	44.1[a]	0.12
C	38.6[ab]	44.2[a]	47.7[a]	43.5[b]	0.13
D	39.1[a]	41.1[c]	44.5[c]	41.4[c]	0.14
Diet (Ca%/NPP%)					
2.25/0.25	38.9[a]	43.7[a]	46.5[bc]	43.0[a]	0.16
2.50/0.25	38.1[b]	42.4[b]	45.7[c]	42.1[b]	0.15
2.75/0.25	38.8[ab]	43.1[ab]	46.7[ab]	42.6[ab]	0.16
3.00/0.25	38.1[b]	43.0[ab]	47.2[ab]	42.8[a]	0.15
3.25/0.25	38.9[a]	43.5[a]	47.6[a]	42.9[a]	0.17
3.25/0.40	38.4[ab]	43.3[a]	47.2[ab]	42.8[a]	0.16
p-value					
Line	0.0005	<0.0001	<0.0001	<0.0001	
Diet	0.0009	<0.0001	<0.0001	0.0020	
Line×Diet	n.s.	0.0047	n.s.	0.0017	

Ca, calcium; n.s., not significant (p>0.05). NPP: Non-phytate phosphorus; SEM, standard error of the mean, [a]Values are least squares means. [b]Means within a column and within a source not sharing a superscript differ (p≤0.05), Line×diet least squares means for 37 week hen age and for the life-of-flock mean are given in Table S4

Table S4: Line × diet least squares means for day-old progeny weight at 37 weeks of hen age and for the life-of-flock mean[a,b]

		37 week hen age		Life of flock	
Genetic line	Diet (Ca %/NPP %)	LSM (g)	SEM	LSM (g)	SEM
A	2.25/0.25	42.5[defgh]	0.39	41.2[fg]	0.33
	2.50/0.25	42.6[defgh]	0.37	41.7[fg]	0.31
	2.75/0.25	42.5[defgh]	0.47	41.3[fg]	0.33
	3.00/0.25	42.6[defgh]	0.37	41.7[fg]	0.30
	3.25/0.25	43.1[cdefg]	0.36	42.1[defg]	0.31
	3.25/0.40	43.2[bcdefg]	0.45	42.0[efg]	0.31
B	2.25/0.25	45.9[a]	0.32	45.0[a]	0.31
	2.50/0.25	43.7[bcde]	0.30	43.4[bcde]	0.28
	2.75/0.25	45.0[ab]	0.32	44.5[ab]	0.30
	3.00/0.25	43.9[bcde]	0.32	43.6[abcd]	0.31
	3.25/0.25	44.3[abcd]	0.35	43.8[abcd]	0.33
	3.25/0.40	44.7[abc]	0.30	44.6[ab]	0.29
C	2.25/0.25	44.7[abc]	0.35	44.0[abc]	0.32
	2.50/0.25	43.5[bcdef]	0.35	42.7[cdef]	0.32
	2.75/0.25	44.2[bcd]	0.33	43.4[bcde]	0.31
	3.00/0.25	43.9[bcde]	0.33	43.9[abc]	0.29
	3.25/0.25	45.3[ab]	0.37	44.2[abc]	0.33
	3.25/0.40	43.8[bcde]	0.35	42.8[cdef]	0.32
D	2.25/0.25	41.5[ghi]	0.33	41.7[fg]	0.31
	2.50/0.25	40.0[i]	0.33	40.7[g]	0.32
	2.75/0.25	40.7[hi]	0.36	41.4[fg]	0.32
	3.00/0.25	41.8[efghi]	0.46	41.8[efg]	0.34
	3.25/0.25	41.4[defghi]	0.75	41.5[fg]	0.40
	3.25/0.40	41.4[fghi]	0.53	41.7[fg]	0.36
p-value					
Line		<0.0001		<0.0001	
Diet		<0.0001		0.0020	
Line × diet		0.0047		0.0017	

Ca: calcium, LSM: Least squares mean, NPP: Non-phytate phosphorus, SEM: Standard error of the mean, [a]Chicks were hatched from eggs laid after artificial insemination at 27, 37 and 47 weeks of hen age and weighed individually at hatch. Main-effect means are given in Table 6, [b]Means within a column not sharing a superscript differ (Student's t, p≤0.05)

times the largest difference between diets. The two lines producing the heaviest chicks, B and C, were also the two lines whose progeny had the highest Ca and P digestibility (see below).

Ca and P digestibility in breeder hens: Dietary Ca and NPP strongly affected the concentration of both minerals in excreta (p<0.0001, Table 7, Fig. 1). Excreta Ca concentrations were 5.00, 5.81, 7.59, 8.23, 9.44 and 8.89% for Diets 1 to 6,

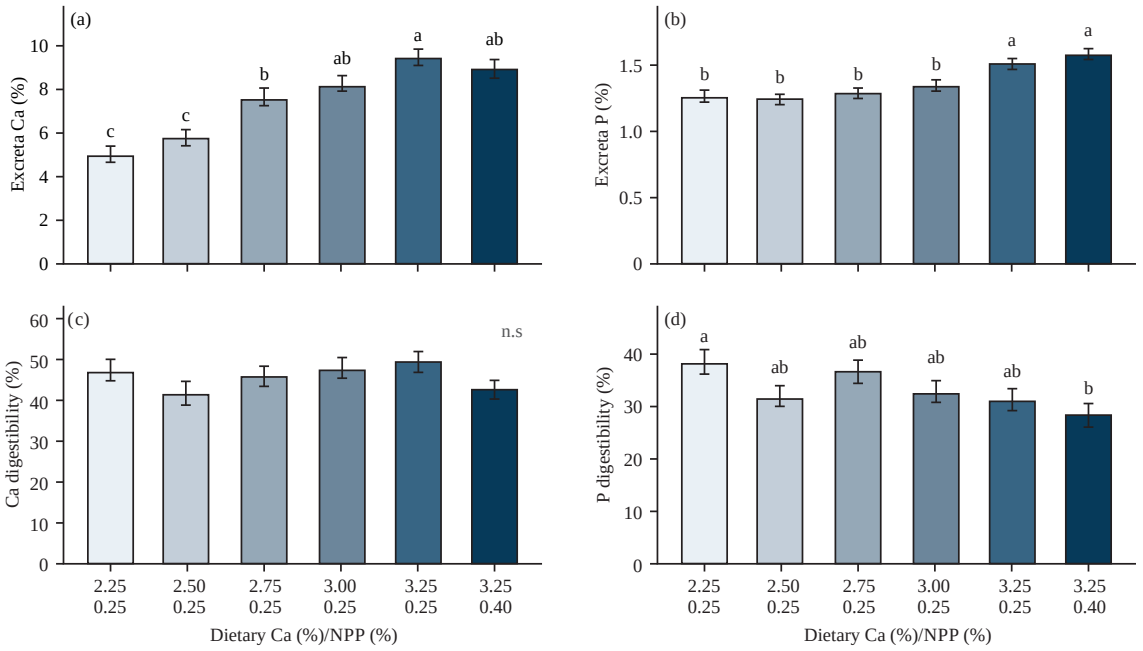


Fig. 1(a-d): Excreta Ca and P concentration and Ca and P digestibility of broiler breeder hens fed six concentrations of dietary Ca and NPP to 50 weeks of age. (a) Excreta Ca (%). (b) Excreta P (%). (c) Ca digestibility (%), (d) P digestibility (%)

Bars are least squares means with pooled standard errors; n = 32 hens per diet. Diets 1 to 5 supplied 0.25% NPP at 3.08, 3.43, 3.77, 4.11 and 4.45 g Ca/hen/d; the hatched bar is Diet 6, which supplied 4.45 g Ca and 550 mg NPP/hen/d. Bars not sharing a letter differ ($p \leq 0.05$); n.s.: not significant. Ca, calcium; NPP, non-phytate phosphorus; P, phosphorus

Table 7: Effects of dietary Ca and NPP and genetic line on excreta Ca and P concentrations and Ca and P digestibility in broiler breeder hens[a-c]

Source	Excreta Ca (%)	Excreta P (%)	Ca digestibility (%)	P digestibility (%)
Diet (Ca% NPP%)				
2.25/0.25	5.0[c]	1.27[b]	47.45	38.46[a]
2.50/0.25	5.81[c]	1.25[b]	42.03	31.83[ab]
2.75/0.25	7.59[b]	1.30[b]	46.24	36.70[ab]
3.00/0.25	8.23[ab]	1.36[b]	47.89	32.90[ab]
3.25/0.25	9.44[a]	1.52[a]	49.63	31.18[ab]
3.25/0.40	8.89[ab]	1.59[a]	42.85	28.28[b]
SEM	0.348	0.037	2.689	2.161
Genetic line				
A	7.12	1.33	47.35	32.81
B	7.51	1.41	45.66	32.81
C	7.42	1.39	47.67	34.50
D	7.93	1.40	43.38	32.77
SEM	0.284	0.030	2.196	1.765
p-value				
Diet	<0.0001	<0.0001	n.s.	0.0131
Line	n.s.	n.s.	n.s.	n.s.
Diet×Line	n.s.	n.s.	n.s.	n.s.

Ca: Calcium, n.s.: Not significant (p>0.05), NPP: Non-phytate phosphorus, P: Phosphorus, SEM: Standard error of the mean, [a]Values are least squares means, n = 8 hens per line×diet combination, [b]Means within a column and within a source not sharing a superscript differ ($p \leq 0.05$), [c]Digestibility was determined by the acid-insoluble ash marker method over a three-day collection

respectively. Hens fed the 2.25 and 2.50% Ca diets excreted less Ca than those fed any other diet, and hens fed 3.25% Ca with 0.25% NPP excreted more Ca than those fed 2.25, 2.50, or 2.75% Ca. Excreta P concentrations were 1.27, 1.25, 1.30, 1.36, 1.52, and 1.59% for Diets 1 to 6, respectively. Hens fed either of the two 3.25% Ca diets excreted more P than hens fed any lower Ca concentration.

Ca digestibility was not affected by diet, ranging from 42.0 to 49.6% with no interpretable pattern. P digestibility was affected by diet ($p = 0.0131$) and declined from 38.5% at 2.25% Ca to 28.3% at 3.25% Ca with 0.40% NPP. Genetic line affected neither excreta mineral concentration nor digestibility in the hen, although line C hens numerically had the highest Ca digestibility and line D hens had the lowest Ca digestibility

Table 8: Effects of dam dietary Ca and NPP and genetic line on excreta Ca and P concentrations and Ca and P digestibility in 14-day-old progeny fed a common starter diet[a-c]

Source	Excreta Ca (%)	Excreta P (%)	Ca digestibility (%)	P digestibility (%)
Dam diet (Ca%/NPP%)				
2.25/0.25	2.70[a]	1.30[bc]	31.82	42.44[a]
2.50/0.25	2.61[ab]	1.22[d]	32.34	44.51[a]
2.75/0.25	2.50[b]	1.19[d]	34.47	45.38[a]
3.00/0.25	2.62[ab]	1.25[cd]	33.53	44.60[a]
3.25/0.25	2.77[a]	1.33[b]	32.48	43.55[a]
3.25/0.40	2.75[a]	1.41[a]	28.88	36.17[b]
SEM	0.058	0.024	1.760	1.632
Dam genetic line				
A	3.22[a]	1.41[b]	22.10[c]	34.28[c]
B	2.18[b]	1.19[c]	40.69[b]	50.93[b]
C	1.87[c]	1.00[d]	48.83[a]	58.64[a]
D	3.35[a]	1.53[a]	17.37[c]	27.25[d]
SEM	0.047	0.020	1.437	1.332
p value				
Dam diet	0.0104	<0.0001	0.3384	0.0017
Dam line	<0.0001	<0.0001	<0.0001	<0.0001
Diet×Line	0.0389	<0.0001	0.0337	0.0003

Ca: Calcium, n.s.: Not significant (p>0.05), NPP: Non-phytate phosphorus, P: Phosphorus, SEM: Standard error of the mean, [a]Values are least squares means, [b]Means within a column and within a source not sharing a superscript differ (p≤0.05), [c]All progeny received the same commercial starter diet (Table S5). Dam line×dam diet means are shown in Fig. S1

(47.7 and 43.4%, respectively). No line×diet interactions were significant.

Ca and P digestibility in 14-day-old progeny: In progeny fed a common commercial starter diet (Table S5), genetic line of the dam affected excreta Ca and P concentrations and Ca and P digestibility (p<0.0001, Table 8, Fig. 2). Progeny of lines A and D excreted more Ca and P and had lower digestibility of both minerals than progeny of lines B and C. Calcium digestibility was 22.1, 40.7, 48.8, and 17.4% and P digestibility was 34.3, 50.9, 58.6, and 27.3% for progeny of lines A, B, C and D, respectively. Thus, the range attributable to dam line was 31.5 percentage points for Ca digestibility and 31.4 percentage points for P digestibility.

The diet fed to the dam had a smaller but detectable effect. Progeny excreta Ca (p = 0.0104), excreta P (p<0.0001), and P digestibility (p = 0.0017) all differed according to the dam's diet, whereas progeny Ca digestibility did not (p = 0.3384). The effect was concentrated in the single diet supplying 0.40% NPP. Progeny of hens fed 3.25% Ca with 0.40% NPP excreted more P (1.41%) than progeny of hens fed any other diet and had the lowest P digestibility (36.2%, compared with 42.4 to 45.4% for the five diets supplying 0.25% NPP). Across those five diets, which varied only in Ca concentration, progeny P digestibility did not differ.

The dam line×dam diet interaction was significant for all four variables (p = 0.0389, <0.0001, 0.0337 and 0.0003 for excreta Ca, excreta P, Ca digestibility, and P digestibility,

Table S5: Composition of the common commercial starter diet fed to progeny to 14 days of age[a]

Item	Broiler starter (1 to 14 day)
Ingredient (%)	
Corn, yellow	54.000
Soybean meal	37.000
Poultry fat	5.390
Defluorinated phosphate	1.920
Limestone	0.460
Alimet (methionine hydroxy analogue)	0.350
Sodium chloride	0.300
L-Lysine HCl	0.110
Choline chloride (70%)	0.110
Threonine (98%)	0.100
Coccidiostat (Monteban®)	0.080
Mineral premix	0.075
Vitamin premix	0.060
Calculated composition	
ME (kcal/kg)	3087.000
Crude protein (%)	21.500
Calcium (%)	0.900
Available phosphorus (%)	0.450
Sodium (%)	0.230
Potassium (%)	0.900
Chloride (%)	0.260
Digestible arginine (%)	1.370
Digestible lysine (%)	1.220
Digestible methionine (%)	0.610
Digestible methionine + cysteine (%)	0.900
Digestible tryptophan (%)	0.240
Digestible threonine (%)	0.800
Choline (mg/kg)	1874.000

ME: Metabolizable energy, [a]Commercial broiler starter diet supplied by Cobb-Vantress Inc, Amino acids are expressed on a digestible basis. No phytase was included, Choline was specified as 850 mg/lb (1874 mg/kg), From 9 days of age the diet was fed with 2% Celite added as the acid-insoluble ash marker

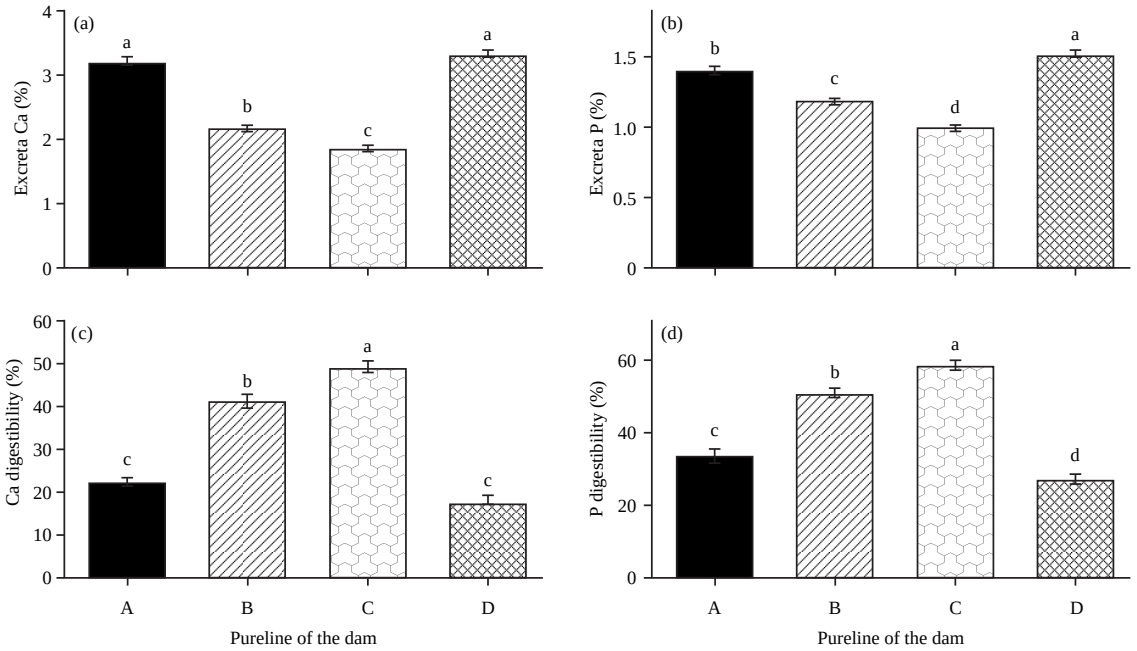


Fig. 2(a-d): Excreta Ca and P concentration and Ca and P digestibility of 14-day-old progeny fed a common commercial starter diet, according to the pureline of the dam. (a) Excreta Ca (%). (b) Excreta P (%). (c) Ca digestibility (%). (d) P digestibility (%)

Bars are least squares means with pooled standard errors. Bars not sharing a letter differ ($p \leq 0.05$). Ca, calcium; P, phosphorus

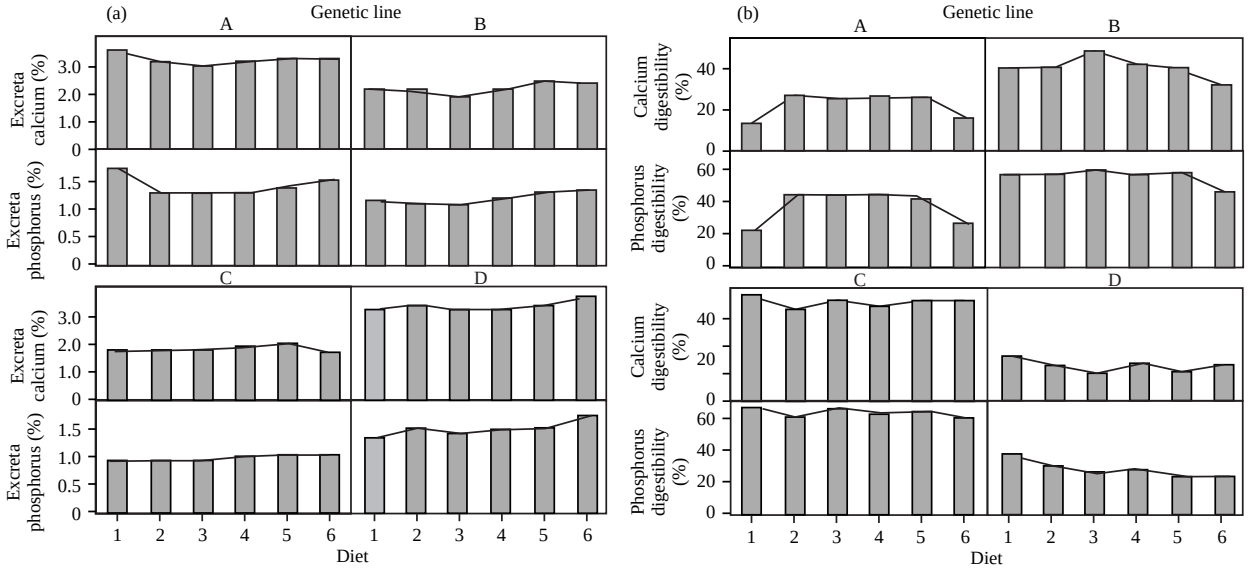


Fig. S1: Excreta Ca and P concentration and Ca and P digestibility of 14-day-old progeny by dam line and dam diet

respectively, Fig. S1). Therefore, the main effects of the dam's diet represent averages across lines and were not expressed uniformly in every line.

DISCUSSION

The principal finding of this study is a dissociation between the mineral concentration of the diet and the reproductive performance it supports. Across a range of dietary Ca concentrations from 2.25 to 3.25%, equivalent to 3.08 to 4.45 g Ca/hen/d at peak feed allocation and NPP intakes of 340 and 550 mg/hen/d, no measure of reproductive performance responded to diet except day-old chick weight, and that response was not monotonic. Genetic line, by contrast, affected every performance trait measured.

Dietary Ca and NPP intake, egg components and hatchability: The absence of an egg production response to dietary Ca as low as 2.25% is consistent with reports that broiler breeder requirements for both minerals may be lower than commonly fed. Bhanja *et al*.[13] estimated the NPP requirement for broiler breeders for optimum egg production at 0.12% (192 mg/hen/d), for optimum egg breaking strength at 0.209% (334 mg/hen/day) and for maximum bone mineralization at 0.225% (360 mg/hen/day). Ekmay and Coon[4] fed 3.25% Ca with NPP concentrations ranging from 0.20 to 0.40% and concluded that 0.20% NPP (288 mg/hen/d) had no adverse effect on egg production. Ekmay *et al*.[5] reported that breeders consuming 216 mg NPP from diets containing 0.15% NPP produced fewer eggs with lower egg weight and tibia ash and had increased mortality. The low NPP intake increased egg specific gravity, possibly because the eggs were approximately 1 g lighter. Taken together, these studies indicate that the NPP threshold needed to support egg production is between 216 and 288 mg/hen/day.

Previous research reporting decreased egg production at low dietary Ca concentrations has used Ca levels well below those evaluated in the present study. Moreki[14] reported reduced hen-day egg production from 36 weeks of age in hens fed 1.5% Ca, but not in hens fed 2.5 or 3.5% Ca. The lowest Ca concentration in the present study, 2.25%, therefore lies above the Ca threshold identified in that study. This places an important boundary on the conclusion from the present experiment: 3.08 g Ca/hen/day and 340 mg NPP/hen/day were adequate to support egg production for more than 29 weeks of lay in the pureline broiler breeders evaluated here.

Egg production is also not the trait most likely to respond first when Ca is restricted. Shell quality and skeletal integrity are more sensitive, and both are reported for these same hens by Magnuson *et al*.[9]. In that study, 2.25% Ca (3.08 g/hen/day) depressed shell quality, whereas 2.5% dietary Ca, corresponding to an intake of 3.43 g Ca/hen/day and higher Ca intakes supported maximum shell quality. The two results are complementary rather than contradictory and the practical recommendation follows from the more sensitive endpoint.

Egg composition was equally insensitive to mineral intake. Neither albumen percentage, yolk percentage, nor the yolk ratio responded to dietary Ca between 2.25 and 3.25% or to NPP between 0.25 and 0.40%. Thus, the mineral intakes tested altered neither the number of eggs a hen produced nor how those eggs were composed. The pureline differences for the same traits were consistent and, unlike the diet effects, large enough to distinguish all four lines. Pureline C produced the highest albumen percentage and lowest yolk percentage and also laid the most eggs per hen housed, whereas pureline A produced eggs with the highest yolk percentage. Because albumen and yolk proportions determine the nutrient and water reserves available to the embryo, these component differences represent an early point in the sequence at which the lines diverge in traits that could potentially carry into the progeny. Line × diet least squares means for hatch of fertile eggs are given in Table S6.

Table S6: Line × diet least squares means for hatch of fertile eggs (%) from broiler breeder hens artificially inseminated at 27, 37 and 47 weeks of age[a,b]

(a) Hatch of fertile eggs (%)

	Genetic line				
Diet (Ca %/NPP %)	A	B	C	D	Diet mean
2.25/0.25	67.51	87.46	83.23	61.61	74.95
2.50/0.25	66.13	89.02	84.86	86.47	81.62
2.75/0.25	82.45	86.47	83.75	76.30	82.24
3.00/0.25	67.67	84.00	84.78	86.89	80.84
3.25/0.25	78.10	84.84	83.12	75.91	80.49
3.25/0.40	81.94	85.89	84.09	69.12	80.26
Line mean	73.97	86.28	83.97	76.05	

Pooled SEM (cell) = 8.47

(b) Eggs set/fertile eggs (n), summed over the three hatches

	Genetic line			
Diet (Ca %/NPP %)	A	B	C	D
2.25/0.25	310/268	299/262	358/259	316/302
2.50/0.25	323/294	308/286	317/235	361/345
2.75/0.25	366/321	299/270	349/275	342/319
3.00/0.25	304/277	272/252	376/281	344/329
3.25/0.25	364/326	248/234	342/235	346/325
3.25/0.40	419/376	325/287	330/248	274/254

Ca: Calcium, NPP: Non-phytate phosphorus, SEM: Standard error of the mean, [a]Hatch of fertile eggs excludes infertile eggs and counts late live embryos with live chicks (see Materials and Methods), The experimental unit was the line × diet × hatch combination (n = 3 per cell), p-values: line, 0.0400; diet, 0.8596; line × diet, 0.8482. Line and diet main-effect means are also given in Table 5, [b]Because the line × diet interaction was not significant, cell means are not separated

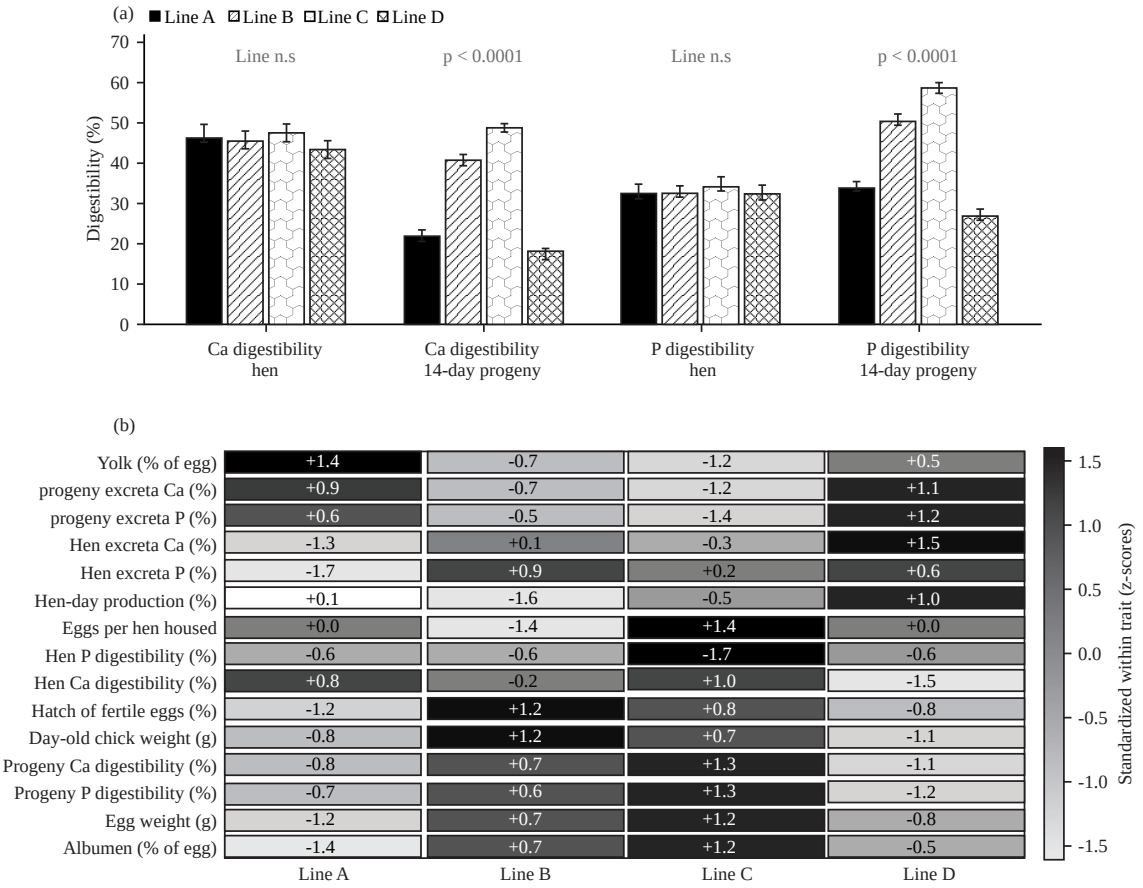


Fig. 3(a-b): Relationship between hen and progeny mineral utilization across four broiler breeder purelines, (a) Ca and P digestibility in the hen and in her 14-day-old progeny, by pureline, showing the absence of line differences in the hen against the threefold range in the progeny; bars are least squares means with pooled standard errors and (b) Hierarchically clustered heatmap of pureline means for reproductive performance, egg component, hatchability, excreta mineral and digestibility traits, standardized within trait across the four lines (z); rows are ordered by average-linkage clustering on Euclidean distance

Ca: Calcium and P: Phosphorus

Hatchability also separated the lines. On a fertile-egg basis, hatchability ranged from 74.0% for pureline A to 86.3% for pureline B, a 12-percentage-point difference, whereas none of the six diets affected hatchability of fertile eggs. The pureline differences in hatchability were therefore considerably larger than any dietary effect observed under the conditions of this study. The relative pattern among purelines for hatch of fertile eggs (B>C>D>A) was similar to that for day-old chick weight (B>C>A>D) and differed from, although still broadly corresponded with, the pattern for progeny mineral utilization (C>B>A>D, Fig. 3b). These results indicate that multiple maternal traits differed among the purelines and were associated with differences in progeny characteristics at hatch and during the subsequent 14-day period, rather than representing independent effects of dietary mineral intake.

A practical observation for the pureline breeders in the present study is that the dietary Ca and NPP intakes tested did not affect hatchability of fertile eggs, whereas genetic line was associated with substantial differences in this trait.

Mineral excretion and digestibility in the breeder hen: The excreta data show what a dietary percentage recommendation costs environmentally. Excreta Ca nearly doubled between the lowest and highest Ca diets and excreta P rose by a quarter, while egg output was unchanged. Raising NPP from 0.25 to 0.40% at constant 3.25% Ca increased excreta P without improving any production trait. The decline in P digestibility from 38.5 to 28.3% as dietary Ca rose is consistent with the formation of insoluble Ca-phosphate complexes in the intestinal lumen and with reports that high dietary Ca depresses P retention in poultry[1,4].

The solubility of the limestone used is central to this interpretation. Supplemental Ca in the present diets was supplied entirely by limestone with a mean particle size of 1500 µm and an *in vitro* solubility of 60%[11], representing a relatively soluble source rather than the coarse, slowly solubilized particles used to extend Ca release through the dark period. Manangi *et al*.[15] reported in colostomized broiler breeder hens that small, highly soluble limestone increased urinary and excreta P loss, whereas large particles of 3000 µm and above with low solubility (<40%) supported Ca and P retention. Ekmay and Coon[3,4] reached a similar conclusion regarding limestone particle size and solubility in purelines. Because digestibility calculated by the marker method is influenced by the amount of P recovered in the excreta, a highly soluble Ca source that increases P loss would decrease apparent P digestibility. Thus, the decline from 38.5 to 28.3% in P digestibility observed in the present study as dietary Ca increased is consistent with the excretory consequence of feeding a highly soluble limestone at increasing inclusion levels rather than necessarily indicating a change in the capacity of the hen to absorb P. The same reasoning applies to earlier balance work in these breeders: Ekmay and Coon[4] found no gain in P retention when NPP intake was raised above approximately 250 mg/hen/day, the amount that covers the requirement for egg production, with the remainder appearing in the excreta. Therefore, the amount of supplemental Ca fed to breeders and the solubility of the Ca source may have a substantial impact on P retention.

Ca digestibility in the hen was unaffected by diet, whereas P digestibility fell. The laying hen draws heavily on medullary bone to supply Ca for shell formation and medullary bone material is remodelled over the course of each egg-laying cycle[16]. A mineral economy dominated by this cycle of deposition and resorption is buffered against moderate changes in dietary supply, which may explain why neither diet nor genetic line altered Ca digestibility in the hens of the present study. The lack of an effect of diet on Ca digestibility may also have been related to the highly soluble limestone used in the present study. Such a Ca source may have resulted in rapid Ca availability and continued reliance on medullary bone Ca across dietary Ca concentrations, rather than allowing more efficient utilization of residual limestone from a low-solubility source retained in the gizzard.

Genetic line and mineral utilization in progeny: The progeny results were the least expected. All chicks received the same commercial starter diet, so differences in their mineral utilization cannot be attributed to differences in dietary Ca or P among progeny treatments. Ca digestibility in the progeny spanned nearly a threefold range according to the pureline of the dam, from 17.4% in pureline D progeny to 48.8% in pureline C progeny. The ranking of pureline progeny did not match the ranking in their dams because pureline differences in Ca digestibility were absent altogether in the hens (Fig. 3a). Selection appears to have altered mineral utilization in a manner that is expressed in the growing chick but masked in the mature hen. In the pureline hen, Ca and P metabolism is strongly influenced by the demands of shell formation and mobilization of minerals from medullary bone.

This interpretation is supported by independent work. de Verdal *et al*.[6] reported reduced nitrogen and P excretion in broiler lines selected for apparent metabolizable energy efficiency, and Crocker and Robison[7] found that maternal-line pigs excreted less P, Ca, Cu, Zn and Fe than paternal-line pigs. Ekmay and Coon[3] reported pureline differences in P excretion and retention in breeder hens fed a common diet containing 3.25% Ca and 0.41% NPP, so dietary variation could not have contributed to these differences. The purelines can be matched between the study reported by Ekmay and Coon[3] and the present study: the pureline C reported by Ekmay and Coon[3] is the same as pureline A in the present study. Pureline A excreted more P than the other lines in both the previous and present studies. The pureline B reported by Ekmay and Coon[3] is equivalent to pureline C in the present study. Pureline C excreted less P than the other lines in both the previous and present studies. The fact that two independent trials ranked the same genetics in the same way strengthens the evidence that genetic differences contribute to variation in mineral excretion and may indicate that mineral utilization has a heritable component. Magnuson *et al*.[17] working with parent stock breeder hens rather than pureline hens as in the present study, reached a parallel conclusion based on plasma bone turnover markers, reporting that bone alkaline phosphatase and tartrate-resistant acid phosphatase concentrations in the dam predicted bone strength and mineral ash in her progeny. The bone turnover markers measured in the progeny of these same hens provide additional evidence of pureline-associated differences in bone turnover. In 14-day-old chicks, plasma tartrate-resistant acid phosphatase was highest in the progeny of purelines A and D (6.82 and 7.38 U, respectively) and lowest in the progeny of purelines B and C (5.39 and 5.94 U, respectively, $p<0.0001$). Plasma fibroblast growth factor 23 in progeny followed a similar pureline ranking, with 752 pg/mL in line B progeny compared with 959 pg/mL in line D progeny ($p = 0.0371$), whereas the pureline dam's diet affected neither trait. The progeny that digested Ca and P least efficiently were also the progeny with the highest bone resorption marker concentrations and the strongest phosphaturic signal, consistent with a pureline difference in mineral utilization.

Table 9: Review Summary: Effect of dietary Ca and NPP for four broiler breeder purelines

Trait group	Effect of pureline	Effect of dietary Ca and NPP	Reported in
Egg production and egg components	Eggs per hen housed 98 (B) to 108 (C); albumen 57.4 (A) to 59.3% (C) of egg weight; all four lines differ	No effect of 2.25 to 3.25% Ca or of 0.40% NPP	Tables 3 and 4
Hatchability	Hatch of fertile eggs 74.0 (A) to 86.3% (B)	No effect	Table 5
Eggshell quality	Specific gravity 1.078 (A) to 1.088 (C); shell thickness 0.355 to 0.409 mm	2.25% Ca (3.08 g/hen/d) depressed shell quality; 3.43 g/hen/d and above maximal; no benefit of 550 mg NPP	Magnuson *et al.*[9]
Bone homeostasis markers	Plasma BAP highest in C (452 pg/mL); TRAP highest in A; progeny TRAP and FGF23 highest in A and D	TRAP increased on the 2.75 and 3.25% Ca diets; FGF23 highest at 2.25% Ca	Magnuson *et al.*[9]
Hen Ca and P digestibility	No line effect on either mineral	P digestibility fell from 38.5 to 28.3% as Ca and NPP rose; Ca digestibility unaffected	Table 7
Progeny mineral utilization (14 day)	Ca digestibility 17.4 (D) to 48.8% (C); P digestibility 27.3 to 58.6%	Hen's diet affected progeny P digestibility only at 550 mg NPP/hen/day	Table 8
Hen body composition and tibia ash	Body mineral 2.9 (A) to 3.8% (C) of body mass; tibia ash 56.9 to 59.8%	Mineral and tibia ash lowest at 2.25, 2.75 and 3.00% Ca; no benefit of 550 mg NPP	Sodsee[10]
Progeny body composition (day 0 and 14 d)	Day-0 tibia ash 29.6 (C) to 31.0% (B); 14-d body mineral 2.45 (A) to 2.72% (C)	No effect of the hen's diet except lean tissue proportion	Sodsee[10]

BAP: Bone alkaline phosphatase, Ca: Calcium, FGF23, fibroblast growth factor 23, NPP: Non-phytate phosphorus, P: Phosphorus, TRAP: Tartrate resistant acid phosphatase, All three manuscripts report the same pureline hens, A,B,C and D, fed the same six diets on the same feeding program

The eggshell and bone data collected from these same pureline hens place the present digestibility results in context. Magnuson *et al.*[9] reported that line C hens laid eggs with the highest specific gravity (1.088) and the thickest shells (0.409 mm) and had the highest plasma BAP concentration (452 pg/mL), whereas line A hens laid eggs with the lowest specific gravity (1.078) and the thinnest shells (0.355 mm) and had the highest plasma TRAP concentration. The 2.25% Ca diet, which resulted in a Ca intake of 3.08 g/hen/day and supported egg production and egg components in the present study, was inadequate for producing maximum shell quality in the work by Magnuson *et al.*[9], whereas Ca intakes of 3.43 g/hen/d and above supported maximum shell quality. Plasma FGF23, the phosphaturic signal, was highest in breeder purelines fed the lowest Ca diet, 2.25% Ca. Egg number was the least sensitive endpoint across the four purelines and dietary Ca and NPP treatments and should not be used alone to establish a Ca recommendation. Shell quality was the first trait to respond to low Ca intake, and the bone biomarkers changed in association with it.

Body composition measured in the same hens ranked the purelines in the same way. Sodsee[10] reported body mineral content of 2.9, 3.3, 3.8, and 3.0% of body mass and tibia ash of 56.9, 58.4, 59.8 and 57.6% for purelines A, B, C, and D, respectively. The pureline hens with the largest skeletal mineral reserve produced progeny that digested Ca and P most efficiently. Conversely, the pureline with the smallest skeletal mineral reserve was also the pureline that produced the poorest eggshells and had the highest bone resorption marker concentration. Increasing NPP intake from 340 to 550 mg/hen/day did not increase body mineral content or tibia ash in the pureline hens, which agrees with the absence of a production benefit and with the reduction in P digestibility observed in the pureline hens and their progeny. Table 9 provides a comparative summary of responses in pureline hens and their progeny across the three manuscripts.

CONCLUSION

Over a 29-week production period, dietary Ca intakes from 3.08 to 4.45 g/hen/day and NPP intakes of 340 or 550 mg/hen/day did not alter body weight, mortality, age at first egg, egg production, egg weight, egg components, or hatchability in four broiler breeder purelines. Genetic line affected all of these traits, separating the purelines by 12 percentage points in hatch of fertile eggs and by 1.9 and 2.6 percentage points in albumen and yolk proportions, respectively. Increasing dietary Ca and NPP increased the excretion of both minerals and depressed P digestibility without improving production. Mineral utilization in 14-day-old progeny fed a common starter diet differed markedly according to the pureline of the dam, indicating a genetic contribution to differences in mineral efficiency that was expressed in the growing chick. The highest dietary NPP intake in the pureline hens, 550 mg/hen/day, carried over to decrease progeny P utilization, but not progeny Ca utilization. Broiler breeder mineral recommendations are better expressed as daily intake at a defined production point than as a dietary percentage, and there is scope to reduce both dietary Ca and NPP below current commercial practice without penalty to reproductive performance, subject to the shell quality and skeletal outcomes reported separately.

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