Int. J. Poult. Sci., 2026, 25: 230-243 | DOI: 10.66920/ijps.2026.230.243

International Journal of Poultry Science

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RESEARCH ARTICLE

Effects of Genetic Line, Dietary Calcium and Non-Phytate Phosphorus and Age on Body Composition and Tibia Ash of Broiler Breeder Hens and their Progeny Determined by Dual-Energy X-ray Absorptiometry

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Key words:
Body composition, bone mineral content, dual-energy X-ray absorptiometry, progeny, pureline, tibia ash

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Cite this Article:
Magnuson, A.D., P. Sodsee, J. Caldas, K. Vignale and N. Boonsinchai *et al.*, 2026. Effects of genetic line, dietary calcium and non-phytate phosphorus and age on body composition and tibia ash of broiler breeder hens and their progeny determined by dual-energy X-ray absorptiometry. Int. J. Poult. Sci., 25: 230-243.

Abstract

Background and Objective: Body composition of broiler breeder hens changes dynamically throughout the rearing and laying periods, with alterations in lean tissue, fat and mineral reserves supporting growth and egg production. Broiler breeders utilize dietary calcium (Ca) and phosphorus (P) for eggshell formation, egg production and maintenance of skeletal integrity. However, limited information is available regarding the effects of dietary Ca and non-phytate phosphorus (NPP) on body mineral dynamics in broiler breeder purelines and their progeny. Therefore, this study evaluated the effects of genetic line, dietary Ca and NPP and age on body composition and tibia ash of broiler breeder hens and their progeny using dual-energy X-ray absorptiometry (DEXA).

Materials and Methods: A total of 960 pureline broiler breeder hens, comprising 240 hens from each of four purelines (A, B, C and D), were fed six experimental diets containing increasing Ca concentrations in 0.25% increments, ranging from 2.25 to 3.25% Ca with 0.25% NPP, or 3.25% Ca with 0.40% NPP. These diets provided approximately 3.08 to 4.45 g Ca and either 340 or 550 mg NPP/hen/d at peak allocation. Ninety-six hens, comprising 24 hens per pureline and four hens per diet, were scanned using DEXA at 4-week intervals from 21 to 51 weeks of age. Progeny from the four purelines were scanned at hatch and 14 d of age. Body mineral content, fat mass, lean tissue mass and tibia ash were evaluated.

Results: Pureline, dietary treatment and age significantly affected body mineral content, fat mass, lean tissue mass and tibia ash of the broiler breeder hens ($p \leq 0.0003$). Line C hens had the highest body mineral content (134 g; 3.8% of body mass), tibia ash (59.8%) and fat mass (726 g), whereas line A hens had the lowest body mineral content (99 g; 2.9%) and tibia ash (56.9%). Absolute body mineral content was lowest at 21 weeks of age, before sexual maturity and remained relatively stable thereafter. In contrast, body mineral content expressed as a percentage of body mass decreased from 3.8 to 2.9% as fat and lean tissue accumulated. Mineralization of the progeny followed the same line-specific ranking observed in the dams. At 14 days of age, progeny from line C dams had the highest body mineral proportion (2.72%), whereas progeny from line A dams had the lowest (2.45%; $p<0.0001$). Increasing dietary NPP from 340 to 550 mg/hen/d did not increase body mineral content or tibia ash in the pureline hens.

Conclusion: Genetic line and age substantially influenced body composition and skeletal mineralization of broiler breeder hens and their progeny, whereas increasing dietary NPP within the tested range did not increase body mineral content or tibia ash. The consistent line-specific ranking of mineralization between breeder hens and their progeny suggests a genetic contribution to mineral utilization and deposition. These findings, together with companion studies demonstrating similar line differences in eggshell quality and progeny mineral utilization, indicate that genetic line should be considered when evaluating mineral requirements and skeletal development in broiler breeder populations.

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INTRODUCTION

A substantial literature addresses how the growth pattern of the broiler breeder pullet during rearing affects subsequent reproductive performance and some of it suggests a critical body weight or body composition for the initiation of sexual maturity. Robinson and Robinson[1] selected pullets in three weight ranges at 21 weeks of age and found that the lightest hens reached sexual maturity later and laid fewer eggs, although body weight at sexual maturity did not differ among groups. The three groups differed in absolute water, fat, protein and ash content at 21 and 62 weeks of age and in the proportional contribution of ash at 21 weeks and of water, fat, protein and ash at 62 weeks.

Dietary manipulation during rearing produces comparable effects. Bennett and Leeson[2] fed pullets three dietary energy concentrations and found more fat, more protein and more fat-free mass at first egg in those fed the highest energy concentration. van Emous *et al*.[3] reported that lower dietary protein during rearing increased the abdominal fat pad and decreased breast muscle in 20-week-old pullets and that pullets reared on a higher growth curve had more abdominal fat at 20 weeks but no difference in breast muscle or abdominal fat by 40 weeks. Sun *et al*.[4] compared *ad libitum* with restricted feeding in hens of similar body weight at 21 weeks and found higher carcass protein and lower carcass fat in restricted hens at every age sampled, with *ad libitum* feeding detrimental to egg production.

Far less is known about how body composition changes during the reproductive phase itself. Dixson[5] reported body composition of female parent stock at 5-week intervals from 5 to 50 weeks of age while comparing methods of determination and observed fluctuations across that period. However, the hens were brought in from the field at different ages and from different farms rather than followed individually and no prediction equations were applied to adjust the densitometric values against proximate analysis.

Dual-energy X-ray absorptiometry (DEXA) offers a means of repeatedly measuring the same individual. Early applications in poultry required anesthesia or sacrifice and 25 to 30 min per scan[5,6]. Current instruments can scan a conscious bird under mild restraint in approximately 5 min, making longitudinal measurement of individual hens through an entire laying cycle practical.

The objectives of the present study were to determine whether four broiler breeder purelines differ in body mineral, fat and lean tissue accretion and in tibia ash during lay; whether dietary Ca and NPP intake alters those traits; how body composition changes with age in individually followed hens from 21 to 51 weeks of age and whether the line differences observed in the hen are reproduced in her progeny, measured as day-0 chick tibia ash and as the body composition of 14-day-old chicks reared on a common starter diet.

The hens described here were part of a single 29-week trial whose outcomes are reported in three companion manuscripts, each covering a different set of traits in 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-day-old progeny are reported by Magnuson *et al*.[7]. 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 Sodsee[8]. Body composition and tibia ash of the same hens and their progeny are reported in the present study. Because the purelines, diets and feeding program are common to all three manuscripts, the results can be viewed as components of a single dataset and the present paper draws on the other two wherever they inform the interpretation of body composition.

MATERIALS AND METHODS

Ethics statement: All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Arkansas (protocol 13002). Birds were managed in accordance with internationally accepted principles for the care and use of agricultural animals.

Birds, housing and diets: Birds, housing, lighting, diets and the feeding program were as described for the companion performance study[7] and are summarized in the present study. Two hundred and forty-five pullets of each pureline were reared by commercial producers and transferred to the University of Arkansas Poultry Research Farm, with lines A and D transferred at 20 and 22 weeks of age, respectively and lines B and C at 21 weeks. 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 in the second. Hens were kept in individual cages (47 cm high×30.5 cm wide×47 cm deep) with an individual feeder and nipple drinker, fed individually each day and given free access to water. Lighting began at 13 hrs at housing and increased to 14 hrs 10 days later, 15 hrs at 5% hen-day egg production and 16 hrs at 60% 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.

Forty pullets from each line were assigned to each of the six diets in a 4×6 factorial arrangement. A basal diet containing 2,915 kcal ME/kg and 15.4% CP with 0.25% NPP was blended with limestone and sand to provide 2.25, 2.50, 2.75, 3.00 and 3.25% Ca (Diets 1 to 5) and a commercial breeder diet containing 3.25% Ca and 0.40% NPP was fed as received (Diet 6). Supplemental Ca was supplied entirely by limestone with a mean particle size of 1,500 µm and an *in vitro* solubility of 60%, determined by the method of Zhang and Coon[9]. Composition is given in Tables 1 and 2. The purelines were fed according to the increase in production of each line after sexual maturity rather than by calendar age, so each line 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 at every 8% increment in hen-day production until a peak allocation of 137 g/hen/d was reached at 61% production, at which point all hens received 400 kcal ME and 21.1 g CP per bird daily. At peak feed allocation, the diets supplied 3.08 to 4.45 g Ca/hen/d and 340 or 550 mg NPP/hen/d. Daily energy, protein, Ca and NPP intakes across the feeding program are given in Table S1.

Hen selection and body composition determination: Ninety-six hens were selected at housing to be scanned repeatedly throughout the production cycle so that changes within each individual could be followed. Twenty-four hens were chosen from each line, all with a housing body weight within one standard deviation of the line mean and four hens per line were assigned to each of the six diets.

Hens were scanned with a GE Lunar Prodigy dual-energy X-ray absorptiometer. Body mineral content, fat tissue, lean tissue and total body mass were derived from the densitometric output using regression equations previously

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

Ingredient (kg/t unless stated)	Basal	Commercial breeder
Maize	702.3	647.00
Soybean meal	222.5	229.90
Limestone	49.2	67.70
Dicalcium phosphate	8.8	18.20
Poultry fat	5.7	26.20
Sal Curb (liquid)	3.3	3.30
Sodium chloride	2.4	1.70
Alimet (DL-methionine hydroxy analogue)	1.9	2.00
Choline chloride (70%)	1.0	1.10
Sodium bicarbonate	1.0	1.00
Trace mineral premix	0.8	0.80
Vitamin premix	0.7	0.70
25-OH-D3 (HyD)	0.5	0.50
Total	1000.0	1000.00
Calculated composition		
ME (kcal/kg)	2915	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

Ingredient inclusions were converted from the original formulation, expressed as pounds per 2,000 lb batch, to kg/t, Trace mineral and vitamin premixes were included at the rates shown; their composition is available from the corresponding author on request

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

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

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 1,500 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, protein, Ca and NPP intake per hen from housing through post-peak feed withdrawal[a]

(a) Feed allocation and energy and protein intake

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

(b) Ca and NPP intake (g/hen/day)

	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

Table S1: Continue

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

Abbreviations: Ca: Calcium, CP: Crude protein, ME: Metabolizable energy, NPP: Non-phytate phosphorus, [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, Intakes were calculated from the feed allocation and the formulated diet specification (2915 kcal ME/kg and 15.4% CP; Diets 1 to 5, 2.25 to 3.25% Ca with 0.25% NPP; Diet 6, 3.25% Ca with 0.40% NPP; Table 2)

established in this laboratory against proximate analysis[10] (r^2 = 0.9895 to 0.9996, p<0.0001). Mineral, fat and lean values were also expressed as a proportion of total body mass. Tibia ash was predicted from the same scans. Scans were performed at 4-week intervals from 21 to 51 weeks of age. Birds were scanned conscious under mild restraint; the room was illuminated with green lamps, under which hens remained calm for the duration of the scan and no anesthesia was used.

Progeny measurements: All hens were artificially inseminated at 27, 37 and 47 weeks of age and the resulting eggs were pedigreed, set and hatched as described by Magnuson *et al*.[7]. The right tibiae of day-old chicks were collected and ashed by laboratory analysis and these values are reported as day-0 chick tibia ash. Progeny were reared to 14 days of age on a single commercial starter diet, identical for the progeny of every hen treatment and were then scanned by DEXA under the same protocol used for the hens. Body mineral, fat and lean tissue were derived using the prediction equations of Salas *et al*.[10] and are expressed as proportions of total body mass. Because every chick received the same diet, differences among progeny groups cannot be attributed to differences in the chick's own dietary Ca or P and instead reflect differences associated with the pureline or diet of the dam.

Statistical analysis: Body composition data were analyzed as repeated measures on the individual hen, with genetic line, diet, age and their interactions as fixed effects. Progeny traits, measured once per bird, were analyzed as a 4×6 factorial with dam line, dam diet and their interaction as fixed effects, using SAS (version 9, SAS Institute Inc., Cary, NC, USA). The individual hen was the experimental unit. Least squares means were separated by Student's *t* test and significance was declared at $p \leq 0.05$. Linear responses of body mineral content to dietary Ca and dietary NPP concentration were tested within line by regression, with lack of fit assessed for each response.

RESULTS

Genetic line, diet and age each affected body mineral content, body fat, lean tissue, total body mass and tibia ash, whether expressed in grams or as a proportion of total body mass, with the single exception that diet did not affect total body mass (Table 3). Least squares means for the effects of genetic pureline, age and diet are shown in Fig. 1-3, respectively. The line×diet and line×age interactions were significant for every trait. Neither the diet×age nor the line×diet×age interaction was significant for any trait. The corresponding traits in the progeny of these hens, measured at hatch and at 14 days of age, are reported below.

Effect of genetic line: Body mineral content differed among all four lines, both in grams and as a proportion of body mass (p<0.0001, Table 4, Fig. 1). Mineral content was 99, 128, 134 and 111 g for lines A, B, C and D, respectively, equivalent to 2.9, 3.3, 3.8 and 3.0% of total body mass. Line C hens had the highest and line A hens the lowest mineral content. The distribution of individual mineral content was also wider in lines B and C than in lines A and D, whose individual values clustered more narrowly around their respective means.

Body fat differed among lines (p<0.0001). Total body fat was 566, 539, 726 and 536 g for lines A, B, C and D, respectively, or 16.1, 14.1, 20.5 and 14.3% of body mass. Line C hens carried more fat than every other line, whereas lines B and D carried less fat than lines A and C.

Lean tissue differed among all lines in grams (p<0.0001), at 2,786, 3,211, 2,584 and 3,087 g for lines A, B, C and D, respectively. Line B hens had the most lean tissue and line C hens the least. Expressed proportionally, lines B and D did not differ from one another (82.3 and 82.0%, respectively) and both exceeded lines A and C (79.4 and 73.1%, respectively).

Total body mass did not differ between lines A and C (3,509 and 3,538 g, respectively), both of which were lighter than lines B and D. Line B hens were heavier than line D hens (3,887 and 3,762 g, respectively, p<0.0001). Body weight of the four purelines from housing to 51 weeks of age is given in Table S2.

Tibia ash differed among all four lines (p<0.0001), at 56.9, 58.4, 59.8 and 57.6% for lines A, B, C and D, respectively. The ranking of lines for tibia ash matched the ranking for body mineral content, with tibia ash highest in line C and lowest in line A.

Table 3: Significance of genetic pureline, diet, age and their interactions for body composition and tibia ash of broiler breeder hens from 21 to 51 weeks of age

Source	BMC (g)	Fat (g)	Lean (g)	Total mass (g)	Tibia ash (%)	BMC (%)	Fat (%)	Lean (%)
Line	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Diet	<0.0001	<0.0001	0.0002	0.1046	0.0003	<0.0001	<0.0001	<0.0001
Line×diet	0.0003	<0.0001	<0.0001	<0.0001	0.0037	0.0184	<0.0001	<0.0001
Age	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Line×age	<0.0001	<0.0001	<0.0001	0.0249	<0.0001	0.0107	<0.0001	<0.0001
Diet×age	0.0587	n.s.	n.s.	n.s.	0.1717	0.1173	n.s.	n.s.
Line×Diet×age	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

BMC: Body mineral content, n = 96 hens, 24 per line, 4 per line×diet combination, each scanned at eight ages and Percentage traits are expressed as a proportion of total body mass

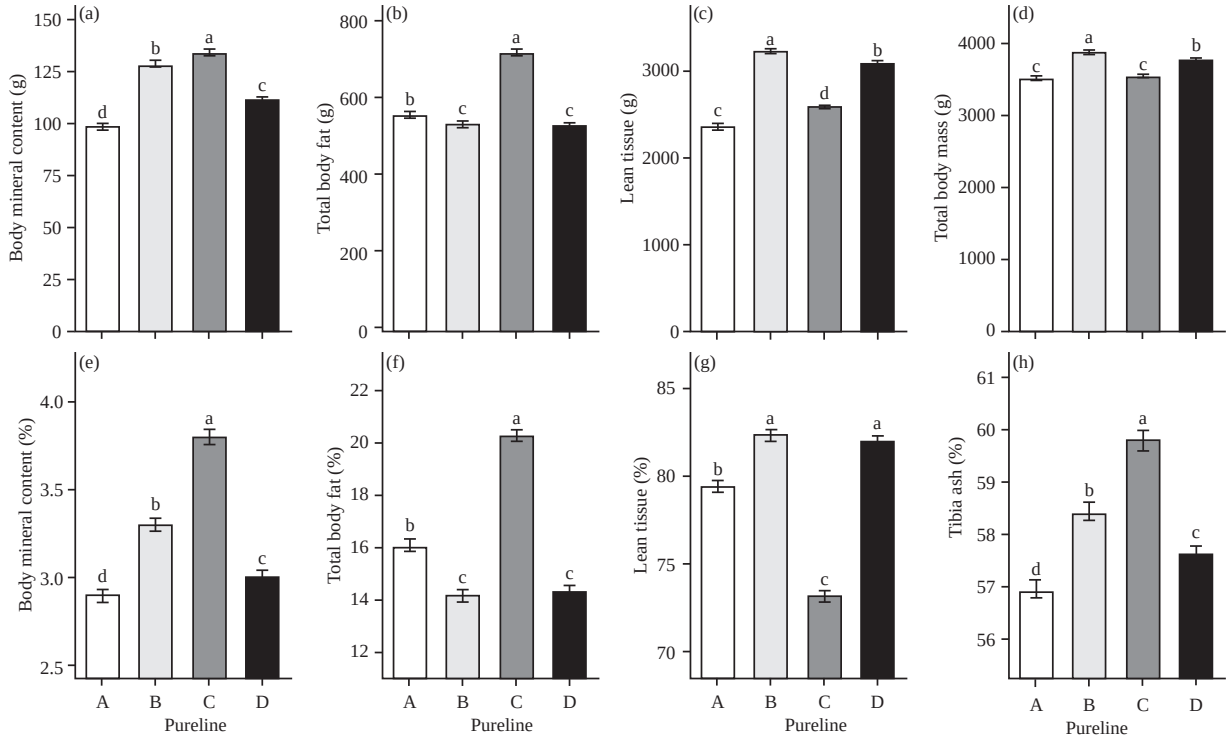


Fig. 1(a-h): Body composition and tibia ash of broiler breeder hens from four purelines, (a) Body mineral content (g), (b) Total body fat (g), (c) Lean tissue (g), (d) Total body mass (g), (e) Body mineral content (% of total body mass), (f) Total body fat (%), (g) lean tissue (%) and (h) tibia ash (%)

Bars are least squares Means±SEM over eight scanning ages, n = 24 hens per line. Bars not sharing a letter differ ($p \leq 0.05$), Axes in panels (e) to (h) are truncated to resolve the line differences. Tibia ash values are dual-energy X-ray absorptiometry predictions

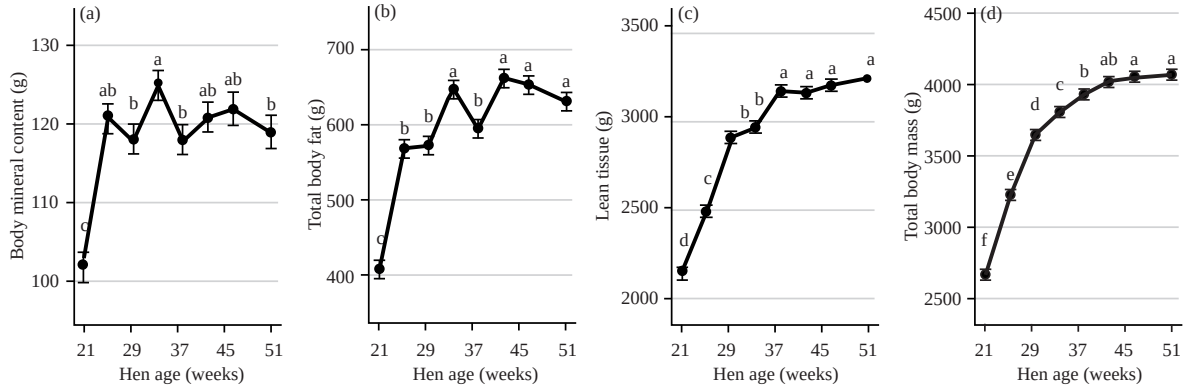


Fig. 2(a-h): Continue

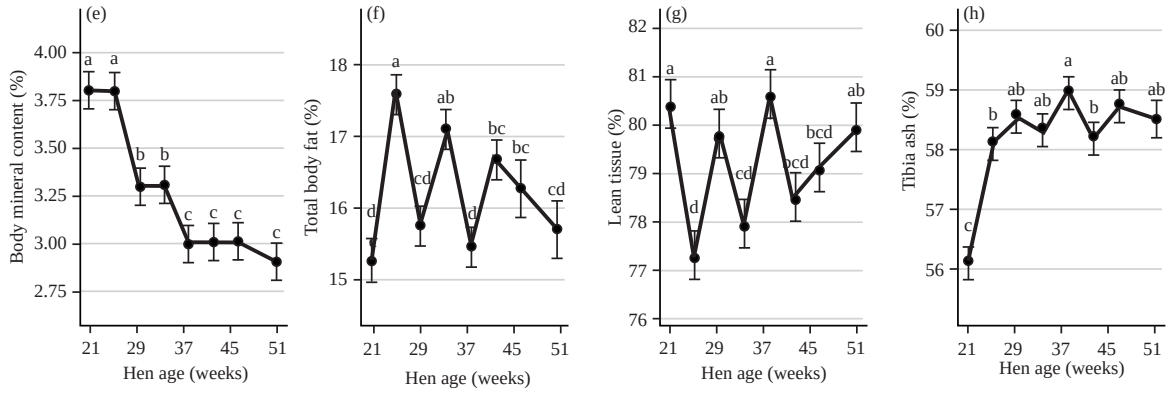


Fig. 2(a-h): Change in body composition and tibia ash of broiler breeder hens across the laying cycle, (a) Body mineral content (g), (b) Total body fat (g), (c) Lean tissue (g), (d) Total body mass (g), (e) Body mineral content (%), (f) Total body fat (%), (g) Lean tissue (%) and (h) Tibia ash (%)

Points are least squares means±SEM at four-week intervals from 21 to 51 weeks of age, pooled over line and diet; n = 96 hens at each age. Points not sharing a letter differ ($p \leq 0.05$). Tibia ash values are dual-energy X-ray absorptiometry predictions

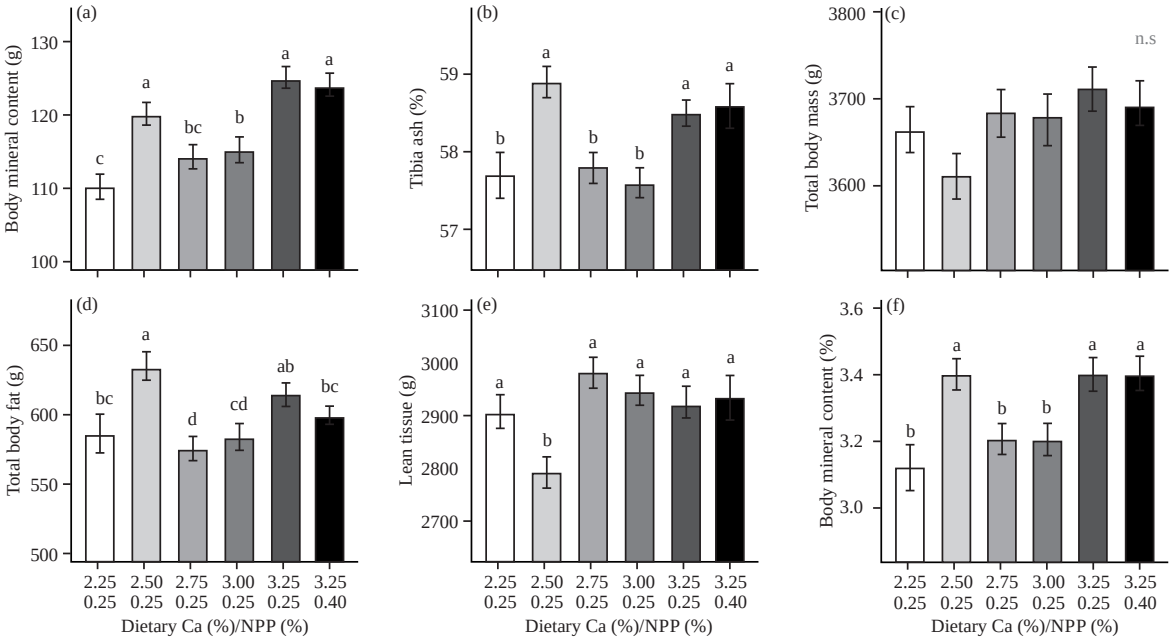


Fig. 3(a-f): Effect of dietary Ca and NPP on body composition and tibia ash of broiler breeder hens, (a) Body mineral content (g); (b) Tibia ash (%), (c) Total body mass (g), (d) Total body fat (g), (e) Lean tissue (g), (f) Body mineral content (%)

Bars are least squares Means±SEM over eight scanning ages, n = 16 hens per diet. Diets 1 to 5 supplied 0.25% NPP at 3.08 to 4.45 g Ca/hen/day, the hatched bar is Diet 6 (4.45 g Ca and 550 mg NPP/hen/day), Bars not sharing a letter differ ($p \leq 0.05$), n.s.: No effect of diet and axes are truncated to resolve the treatment differences

Effect of dietary Ca and NPP: Diet affected body mineral content, fat and lean tissue and tibia ash but not total body mass (Table 5 and Fig. 3). Body mineral content was 110, 120, 114, 115, 125 and 124 g for Diets 1 to 6, respectively and hens fed the 2.50% Ca, 3.25% Ca/0.25% NPP and 3.25% Ca/0.40% NPP diets had greater mineral content than hens fed the 2.25, 2.75, or 3.00% Ca diets ($p<0.0001$). Tibia ash followed the same pattern, at 57.7, 58.9, 57.8, 57.6, 58.5 and 58.6% for Diets 1 to 6, respectively ($p = 0.0003$).

Body fat was affected by diet ($p<0.0001$). Hens fed the 2.50% Ca diet had more fat, at 631 g and 17.6% of body mass, than hens fed any other diet except the 3.25% Ca with 0.25% NPP diet. Lean tissue was correspondingly lowest in hens fed the 2.50% Ca diet (2,795 g), which differed from every other diet ($p = 0.0002$). Total body mass was unaffected by diet, ranging from 3,610 to 3,711 g ($p = 0.10$).

Body mineral content and tibia ash of hens fed 3.25% Ca with 0.25% NPP did not differ from those of hens fed 3.25% Ca

Table S2: 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 (wk)	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 4: Effect of genetic pureline on body composition and tibia ash of broiler breeder hens

Line	BMC (g)	SEM	Fat (g)	SEM	Lean (g)	SEM	Total mass (g)	SEM
A	99[d]	1.34	566[b]	8.57	2786[c]	24.39	3509[c]	21.89
B	128[b]	1.32	539[c]	8.46	3211[a]	24.06	3887[a]	21.59
C	134[a]	1.31	726[a]	8.37	2584[d]	23.82	3538[c]	21.38
D	111[c]	1.34	536[c]	8.56	3087[b]	24.36	3762[b]	21.86
Line	BMC (%)	SEM	Fat (%)	SEM	Lean (%)	SEM	Tibia ash (%)	SEM
A	2.9[d]	0.04	16.1[b]	0.24	79.4[b]	0.34	56.9[d]	0.20
B	3.3[b]	0.04	14.1[c]	0.23	82.3[a]	0.34	58.4[b]	0.20
C	3.8[a]	0.04	20.5[a]	0.23	73.1[c]	0.33	59.8[a]	0.20
D	3.0[c]	0.04	14.3[c]	0.24	82.0[a]	0.34	57.6[c]	0.20

BMC: Body mineral content, Values are least squares means over eight scanning ages, n = 24 hens per line. Means within a column not sharing a letter differ ($p \leq 0.05$) and Standard errors for percentage traits are reported on the percentage scale

with 0.40% NPP, indicating that increasing NPP intake from 340 to 550 mg/hen/day conferred no additional skeletal mineral benefit at this Ca concentration.

Effect of age: Age affected every trait (Fig. 2, $p<0.0001$, Table 6). Body mineral content in grams was lowest at 21 weeks (102 g) and thereafter fluctuated between 118 and

Table 5: Effect of dietary Ca and NPP on body composition and tibia ash of broiler breeder pureline hens

Diet (Ca %/NPP %)	BMC (g)	SEM	Fat (g)	SEM	Lean (g)	SEM	Total mass (g)	SEM
2.25/0.25	110^{c}	1.67	590^{bc}	10.69	2909^{a}	30.4	3663	27.3
2.50/0.25	120^{a}	1.59	631^{a}	10.15	2795^{b}	28.9	3610	25.9
2.75/0.25	114^{bc}	1.62	557^{d}	10.39	2981^{a}	29.6	3686	26.5
3.00/0.25	115^{b}	1.63	576^{cd}	10.44	2947^{a}	29.7	3680	26.7
3.25/0.25	125^{a}	1.57	609^{ab}	10.07	2929^{a}	28.6	3711	25.7
3.25/0.40	124^{a}	1.66	588^{bc}	10.63	2942^{a}	30.2	3694	27.2
Diet (Ca %/NPP %)	BMC (%)	SEM	Fat (%)	SEM	Lean (%)	SEM	Tibia ash (%)	SEM
2.25/0.25	3.1^{b}	0.05	16.2^{b}	0.3	79.3^{b}	0.4	57.7^{b}	0.3
2.50/0.25	3.4^{a}	0.05	17.6^{a}	0.3	77.3^{c}	0.4	58.9^{a}	0.2
2.75/0.25	3.2^{b}	0.05	15.3^{c}	0.3	80.6^{a}	0.4	57.8^{b}	0.2
3.00/0.25	3.2^{b}	0.05	15.9^{bc}	0.3	79.7^{ab}	0.4	57.6^{b}	0.2
3.25/0.25	3.4^{a}	0.05	16.5^{b}	0.3	78.8^{b}	0.4	58.5^{a}	0.2
3.25/0.40	3.4^{a}	0.05	16.0^{bc}	0.3	79.6^{ab}	0.4	58.6^{a}	0.3

BMC: Body mineral content. Values are least squares means over eight scanning ages, n = 16 hens per diet (4 per line), Means within a column not sharing a letter differ ($p \leq 0.05$), Diets 1 to 5 supplied 3.08, 3.43, 3.77, 4.11 and 4.45 g Ca/hen/day and 340 mg NPP/hen/day at peak feed allocation; Diet 6 supplied 4.45 g Ca and 550 mg NPP/hen/d. Percentage-trait standard errors are reported on the percentage scale, as in Table 4

Table 6: Effect of age on body composition and tibia ash of broiler breeder pureline hens scanned repeatedly from 21 to 51 weeks

Age (weeks)	BMC (g)	SEM	Fat (g)	SEM	Lean (g)	SEM	Total mass (g)	SEM
21	102^{c}	1.79	406^{c}	11.46	2140^{d}	32.61	2659^{f}	29.27
25	121^{ab}	1.80	567^{b}	11.51	2496^{c}	32.75	3227^{e}	29.39
29	118^{b}	1.82	573^{b}	11.62	2912^{b}	33.07	3641^{d}	29.68
33	125^{a}	1.84	646^{a}	11.75	2976^{b}	33.44	3808^{c}	30.01
37	118^{b}	1.85	595^{b}	11.86	3179^{a}	33.75	3934^{b}	30.30
41	121^{ab}	1.88	663^{a}	12.07	3167^{a}	34.34	4017^{ab}	30.82
45	122^{ab}	1.97	653^{a}	12.60	3210^{a}	35.85	4046^{a}	32.18
51	119^{b}	2.04	631^{a}	13.09	3255^{a}	37.23	4060^{a}	33.42
Age (week)	BMC (%)	SEM	Fat (%)	SEM	Lean (%)	SEM	Tibia ash (%)	SEM
21	3.8^{a}	0.1	15.3^{d}	0.3	80.4^{a}	0.5	56.1^{c}	0.3
25	3.8^{a}	0.1	17.6^{a}	0.3	77.3^{d}	0.5	58.1^{b}	0.3
29	3.3^{b}	0.1	15.8^{cd}	0.3	79.8^{ab}	0.5	58.5^{ab}	0.3
33	3.3^{b}	0.1	17.1^{ab}	0.3	78.0^{cd}	0.5	58.3^{ab}	0.3
37	3.0^{c}	0.1	15.3^{d}	0.3	80.6^{a}	0.5	59.0^{a}	0.3
41	3.0^{c}	0.1	16.7^{bc}	0.3	78.5^{bcd}	0.5	58.2^{b}	0.3
45	3.0^{c}	0.1	16.3^{bc}	0.4	79.1^{bcd}	0.5	58.7^{ab}	0.3
51	2.9^{c}	0.1	15.7^{cd}	0.4	79.9^{ab}	0.5	58.5^{ab}	0.3

BMC: Body mineral content. Values are least squares means, n = 96 hens scanned at each age, Means within a column not sharing a letter differ ($p \leq 0.05$), Percentage-trait standard errors are reported on the percentage scale, as in Table 4

125 g, peaking at 33 weeks. Expressed as a proportion of body mass, mineral content was highest at 21 and 25 weeks (3.8%) and declined to 3.0% by 37 weeks and 2.9% by 51 weeks. Because absolute mineral content was essentially stable after 25 weeks, whereas fat and lean tissue continued to accumulate, this proportional decline reflects dilution rather than mineral loss.

Body fat in grams rose from 406 g at 21 weeks to 646 g at 33 weeks, decreased to 595 g at 37 weeks and increased again to 663 g at 41 weeks. The reduction coincides with peak egg production and the associated peak in nutrient demand. Proportional body fat fluctuated between 15.3 and 17.6%, with the highest values at 25 and 33 weeks and the lowest at 21, 29, 37 and 51 weeks.

Lean tissue increased from 2,140 g at 21 weeks to 3,255 g at 51 weeks, with significant increments to 25, 29 and 37 weeks and nonsignificant increases thereafter. Proportional lean tissue varied inversely with proportional fat. Total body mass increased throughout the laying period, from 2,659 g at 21 weeks to 4,060 g at 51 weeks, with lean gain exceeding fat gain overall. Tibia ash was lowest at 21 weeks (56.1%) and fluctuated between 58.1 and 59.0% thereafter.

Line × age interaction: Lines differed in the pattern as well as the magnitude of compositional change ($p<0.0001$ for all traits except total body mass, $p = 0.0249$ and proportional mineral content, $p = 0.0107$). All lines gained mineral content from 21 to 25 weeks. Thereafter, line B hens continued to gain mineral content to 33 weeks and then declined, line D hens remained stable and mineral content in line A hens declined through 40 weeks. Absolute mineral content and body fat of line C hens fluctuated more across the cycle than those of any other line.

Lean tissue gain from 21 to 51 weeks was 959, 1,446, 1,081 and 1,179 g for lines A, B, C and D, respectively. Lines B

Table 7: Day-0 tibia ash and body composition of 14-day-old progeny reared on a common commercial starter diet, by pureline and diet of the dam

Source	Day-0 tibia ash (%)	Total body mass (g)	Lean (%)	Fat (%)	Mineral (%)
Dam pureline					
A	29.91[b]	330.00[b]	87.92[a]	4.97[c]	2.45[c]
B	30.96[a]	366.30[a]	85.26[d]	8.45[a]	2.59[b]
C	29.55[b]	327.40[b]	86.69[c]	6.40[b]	2.72[a]
D	30.71[a]	292.40[c]	87.15[b]	5.54[c]	2.49[bc]
SEM	0.221	4.302	0.176	0.211	0.032
Dam diet (Ca %/NPP %)					
2.25/0.25	30.50	326.30	86.56[ab]	6.56	2.54
2.50/0.25	30.21	318.20	86.40[b]	6.70	2.61
2.75/0.25	30.37	334.40	86.87[ab]	6.22	2.61
3.00/0.25	30.31	331.90	87.02[a]	6.04	2.53
3.25/0.25	30.07	334.20	86.89[ab]	6.24	2.54
3.25/0.40	30.23	329.30	86.81[ab]	6.27	2.55
SEM	0.269	5.272	0.200	0.259	0.032
p-value					
Dam line	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Dam diet	0.9333	0.3122	0.0392	0.5044	0.3274
Dam line×dam diet	n.d.	0.8931	0.0706	0.1084	0.6082

Ca: Calcium; NPP: Non-phytate phosphorus. n.d.: Not determined, SEM: Standard error of the mean, Values are least squares means, Means within a column not sharing a letter differ ($p \leq 0.05$). Lean, fat and mineral are expressed as a proportion of total body mass at 14 days of age. All progeny received the same starter diet, so differences among groups are attributable to the dam, The dam line×dam diet interaction was not tested for day-0 tibia ash; cell means are given in Table S3.

Table S3: Day-0 chick tibia ash (%) by dam line and dam diet[a,b].

	Dam pureline			
Dam diet (Ca%/NPP%)	A	B	C	D
2.25/0.25	29.68±0.55 (15)	30.88±0.44 (17)	29.35±0.38 (18)	31.77±0.53 (23)
2.50/0.25	30.09±0.46 (19)	30.58±0.62 (18)	29.55±0.41 (20)	30.62±0.40 (25)
2.75/0.25	30.36±0.68 (21)	30.68±0.62 (19)	29.36±0.42 (18)	30.95±0.48 (30)
3.00/0.25	28.73±0.51 (19)	31.84±0.45 (21)	30.07±0.33 (17)	30.59±0.50 (23)
3.25/0.25	30.24±0.39 (16)	—	29.73±0.58 (19)	29.60±0.73 (18)
3.25/0.40	30.20±0.48 (25)	—	29.24±0.48 (19)	30.49±0.32 (23)
Dam line LSM[c]	29.91[b]	30.96[a]	29.55[b]	30.71[a]

Ca: Calcium, LSM: Least squares mean, NPP: Non-phytate phosphorus, [a]Values are means±SE (number of hens sampled), Each sample consisted of the pooled right tibiae of day-old chicks from one hen, ashed in the laboratory. -, no sample for that combination, [b]Line B chicks from dams fed Diets 5 and 6 were not sampled, so the dam line × dam diet interaction was not tested, [c]Dam line least squares means from Table 7 (dam line, $p<0.0001$, dam diet, $p = 0.9333$). Means not sharing a superscript differ (Student's t, $p \leq 0.05$)

and C continued to accrue lean tissue throughout, whereas lines A and D ceased to gain lean tissue after 37 weeks and added mass principally as fat. Total body mass at 51 weeks was 3,844, 4,202, 4,045 and 4,147 g for lines A, B, C and D, respectively.

Tibia ash of lines B and C rose from 21 to 33 weeks, after which line C continued to increase and line B declined. Tibia ash for purelines A and D increased to 29 weeks, tended to decline to 41 weeks and rose again to 45 weeks.

Line×diet interaction: Body mineral content responded linearly to dietary Ca concentration in lines A ($r^2 = 0.040$, $p = 0.0056$) and D ($r^2 = 0.073$, $p = 0.0002$) but not in lines B or C, for which there was significant lack of fit to a linear model ($p = 0.0005$ and $p = 0.0024$, respectively). Body mineral content of line B hens responded to increased dietary NPP concentration ($r^2 = 0.036$, $p = 0.0094$), whereas lines A, C and D did not. The line×diet interaction was significant for body fat and lean tissue ($p<0.0001$) but no linear response to dietary Ca was detected for either trait in any line.

Progeny body composition and day-0 tibia ash: Day-0 chick tibia ash differed with the pureline of the dam ($p<0.0001$) but not with her diet ($p = 0.9333$), at 29.9, 31.0, 29.6 and 30.7% for the progeny of lines A, B, C and D, respectively (Table 7 and Fig. 4a). Means for each dam line×dam diet combination are given in Table S3. At 14 days of age, chicks from line B dams were the heaviest (366 g) and chicks from line D dams the lightest (292 g, $p<0.0001$).

Body mineral expressed as a proportion of body mass ranked the progeny in the same order as their dams (Fig. 4c, f): 2.72, 2.59, 2.49 and 2.45% for the progeny of lines C, B, D and A, respectively ($p<0.0001$), with progeny from pureline C having the highest body mineral proportion and progeny from

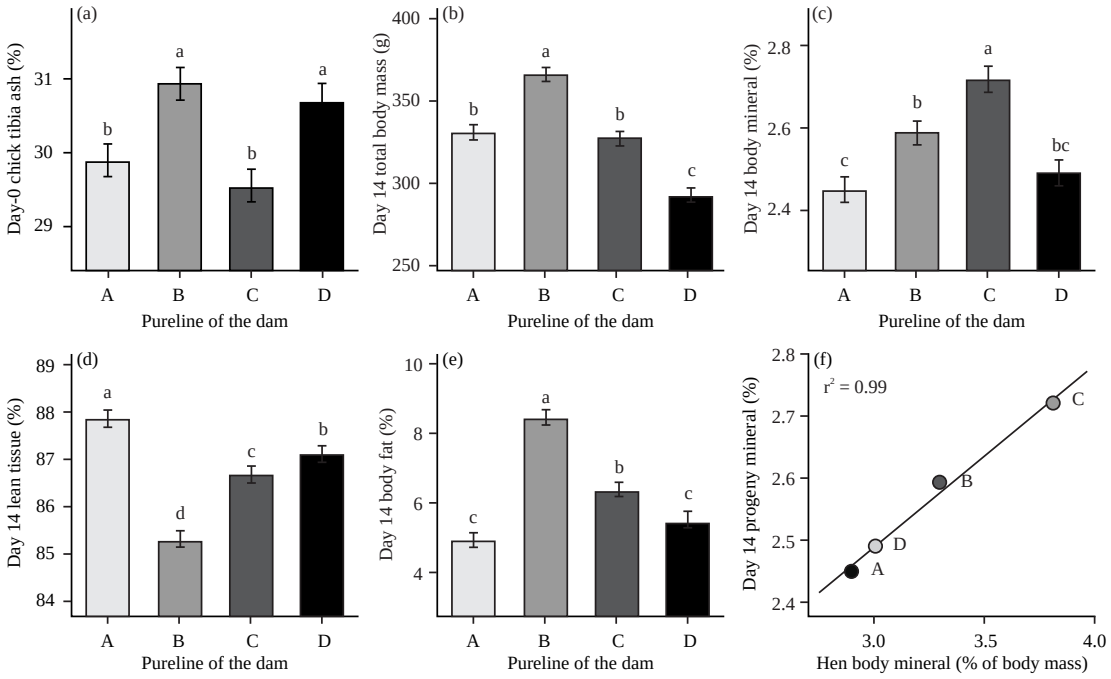


Fig. 4(a-f): Body composition of the progeny of four broiler breeder purelines and its relationship to the dam, (a) Day-0 chick tibia ash (%), (b) 14-day total body mass (g), (c) 14-day body mineral (%), (d) 14-day lean tissue (%), (e) 14-day body fat (%), each by the pureline of the dam and (f) 14-day progeny body mineral against hen body mineral, one point per pureline, with the least squares regression line

Bars are least squares means±SEM and bars not sharing a letter differ ($p \leq 0.05$). All progeny received the same commercial starter diet and the dam's diet affected no progeny trait except lean tissue proportion. Axes are truncated to resolve the line differences

pureline A having the lowest. The dam body mineral percentage for purelines C and A was also the highest and lowest, respectively. Progeny fat was highest in chicks from line B dams (8.45%) and lowest in chicks from line A dams (4.97%), whereas the proportion of lean tissue showed the opposite pattern, ranging from 85.26% in line B progeny to 87.92% in line A progeny ($p<0.0001$ for both). The dam's diet produced no effect on progeny traits except the proportion of lean tissue, which varied over a range of 0.6 percentage points with no relation to dietary Ca (86.40 to 87.02%; $p = 0.0392$). No dam line×dam diet interaction was significant for any progeny trait measured at 14 days (Table 7).

DISCUSSION

The present study produced three principal findings. Broiler breeder purelines differ substantially and consistently in how they partition body mineral, fat and lean tissue during lay. Body mineral content in absolute terms is established before the onset of lay and then remains nearly constant, so that its proportional contribution to body mass declines steadily as the hen continues to gain body weight. Dietary Ca and NPP concentrations influence skeletal mineral status without altering total body mass, with no apparent benefit from increasing NPP intake above 340 mg/hen/day.

The pureline differences are large. Line C hens carried 35% more body mineral than line A hens and nearly three percentage points more tibia ash, while simultaneously carrying the most fat and the least lean tissue of any line. The ranking of pureline hens for body mineral content and tibia ash matched their ranking for eggshell quality[8], with line C having the best eggshell quality and line A producing the poorest eggshell quality. This correspondence is consistent with the conventional interpretation that the mineral reserve available for shell formation constrains shell quality and the present findings indicate that body mineral content measured by DEXA before or during early lay could serve as a non-destructive screening trait for parent stock selection. Ekmay *et al.*[11] reached a related conclusion based on bone ash, reporting that pureline hens differed in bone ash and that hens with a propensity to produce progeny with high or low bone ash could be identified, although bone ash was not correlated with shell quality in that study.

The progeny data support the same conclusion. Chicks from line C dams had the highest proportion of body mineral at 14 days of age, whereas chicks from line A dams had the lowest, reproducing the ranking of their dams for body mineral content and tibia ash even though all chicks were reared on the same starter diet. Furthermore, the dam's diet affected no progeny trait other than the proportion of lean tissue. A line difference that persists into a generation fed a common diet is consistent with a genetic contribution to differences in mineral accretion rather than a nutritional effect, a prerequisite for body mineral content measured by DEXA to be useful as a selection trait.

Day-0 chick tibia ash is the one progeny trait that does not follow this pattern. It was lowest in the progeny of line C (29.6%), the line with the greatest body mineral content and highest in the progeny of lines B and D. A day-old chick has been dependent on yolk reserves rather than feed, so its bone ash reflects the nutrients supplied by the egg during incubation rather than the chick's subsequent response to dietary nutrients. The 14-day value, measured after 2 weeks on a common starter diet, is therefore the more informative index of the line's mineral accretion and day-0 bone ash should not be used alone to rank purelines for progeny bone quality.

Egg composition ranked the lines in the same way. Magnuson et al.[7] reported that the same four purelines differed in every egg production component measured. Albumen accounted for 59.3% of egg weight in line C and 57.4% in line A, whereas yolk accounted for 31.8 and 34.4%, respectively. Line C also produced the most eggs per hen housed, while none of these components responded to dietary Ca or NPP. Line C hens also laid eggs with the best shell quality[8]. Thus, the hen with the greatest body mineral content laid the most albumen-rich, best-shelled eggs and produced 14-day-old chicks with the greatest proportion of body mineral. Body composition, egg components and progeny composition appear to reflect an underlying line difference.

Body composition in the context of eggshell quality and bone turnover: The line ranking for body mineral content is reproduced, trait for trait, in the eggshell and bone data collected from these same hens. Sodsee[8] 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, a marker of bone resorption. Line C carried 35% more body mineral and nearly three percentage points more tibia ash than line A in the present study. The pureline hen with the largest skeletal mineral reserve produced the best-shelled eggs and had the lowest resorptive signal, an association consistent with the conventional interpretation of medullary bone function.

The dietary results converge in the same way. Body mineral content and tibia ash were lowest in hens fed 2.25% Ca in the present study and Sodsee[8] found that the same 2.25% Ca diet (3.08 g Ca/hen/d at peak) decreased specific gravity and shell thickness, whereas intakes of 3.43 g/hen/day and above supported maximum shell quality. Plasma FGF23, the phosphaturic signal, was also highest on that diet. Increasing NPP intake from 340 to 550 mg/hen/day did not improve skeletal mineral status or shell quality and in the digestibility study, the increased NPP intake decreased P digestibility in the hen from 38.5 to 28.3% and in her progeny from 42.4 to 36.2%, while improving no production trait[7]. Three independent sets of measurements therefore identify the same lower bound for dietary Ca and the same absence of benefit from the higher NPP intake.

The solubility of the limestone that delivered the supplemental Ca is part of this picture. Supplemental Ca in these diets came entirely from limestone with a mean particle size of 1,500 µm and an in vitro solubility of 60%[9], a readily soluble source rather than the coarse, slowly solubilized particle used to extend Ca release through the dark period. Manangi *et al.*[12] reported in colostomized broiler breeder hens that small, highly soluble limestone increased urinary and excreta P loss, whereas particles of 3,000 µm and above with low solubility (<40%) supported Ca and P retention and Ekmay and Coon[13] reached the same conclusion regarding limestone particle size in purelines. A highly soluble source releases its Ca early in the digestive tract, where it may be less well matched to the overnight demand for shell calcification and may be more likely to precipitate dietary P; the hen may then draw on medullary bone reserves to meet Ca requirements, while P is excreted. This mechanism is consistent with the present skeletal data. Elevating dietary Ca from 2.25 to 3.25% increased body mineral content by 15 g and tibia ash by less than one percentage point, gains far smaller than the 35 g and nearly three percentage points separating lines A and C, while the same Ca increase decreased P digestibility from 38.5 to 28.3% in the companion digestibility study[7]. Thus, feeding a more highly soluble Ca source added little skeletal mineral while increasing P loss to the environment. Whether a denser, less soluble, larger-particle limestone would produce a larger skeletal response at the same Ca intake warrants further study.

Change with age, nutrient partitioning and methodological comparison: Absolute body mineral content increased sharply from 21 to 25 weeks, the interval spanning the onset of lay and the deposition of medullary bone and then remained between 118 and 125 g for the following 26 weeks.

Table 8: 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	Magnuson *et al.*[7], Tables 3 and 4
Hatchability	Hatch of fertile eggs 74.0 (A) to 86.3% (B)	No effect	Magnuson *et al.*[7], 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	Sodsee[8]
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	Sodsee[8]
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	Magnuson *et al.*[7], Table 7
Progeny mineral utilization (14 day)	Ca digestibility 17.4 (D) to 48.8% (C); P digestibility 27.3 to 58.6%	Dam's diet affected progeny P digestibility only at 550 mg NPP/hen/d	Magnuson *et al.*[7], 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%; fat and lean tissue differ among all lines	Mineral and tibia ash lowest at 2.25, 2.75 and 3.00% Ca; no benefit of 550 mg NPP	Tables 3 to 6; Fig. 1 to 3
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 dam's diet except lean tissue proportion	Table 7; Fig. 4

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 hens, purelines A to D and fed the same six diets on the same feeding program

Proportional mineral content nonetheless fell from 3.8 to 2.9%. A study reporting only the proportional value could conclude that hens progressively demineralize during lay, the absolute values show that they do not and that the proportional decline reflects dilution by continued fat and lean tissue accretion. Both measures should therefore be reported and repeated measurement of the same hens, as in the present study, allows the two patterns to be distinguished.

Fat and lean tissue accretion diverged among lines in a way that has practical consequences. Lines B and C continued to add lean tissue through 51 weeks, whereas lines A and D ceased adding lean tissue after 37 weeks and added mass predominantly as fat. Since all lines were fed the same energy and protein intake under a common feeding program, this divergence reflects differences in nutrient partitioning rather than differences in intake and it implies that a single post-peak feed withdrawal program may not produce the same body composition outcome across lines. Sun *et al.*[4] showed that restricted feeding increased carcass protein and lowered carcass fat relative to *ad libitum* feeding; the present data suggest that the magnitude of that effect may depend on genotype.

The dietary effects were smaller than the genetic pureline effects. Body mineral content and tibia ash were greatest in hens fed 3.25% Ca at either NPP concentration and in pureline hens fed 2.50% Ca. The NPP comparison was made at constant Ca. Hens fed 340 mg NPP/hen/d had body mineral content and tibia ash similar to those of hens fed 550 mg/hen/d. Given the environmental cost of excreted P and the absence of any production benefit from the higher NPP intake 7, 340 mg NPP/hen/d was adequate to support skeletal mineral status under the conditions of the present study.

Comparison with earlier DEXA work is limited by methodology. Dixson[5] reported lower values than those found in the present study but applied no prediction equations to adjust densitometric output against proximate analysis, a correction that has repeatedly been shown to be necessary[10,14-16]. The hens reported by Dixson[5] were also sourced from the field at different ages and farms rather than followed individually, so genuine longitudinal change could not be separated from between-flock variation.

CONCLUSION

Broiler breeder purelines differ markedly in body mineral, fat and lean tissue accretion during lay and in the trajectory of these changes with age. Body mineral content is established by 25 weeks of age or sexual maturity and remains nearly constant thereafter, so that the proportional contribution of mineral to body mass declines through lay as fat and lean tissue accumulate; reporting only the proportional value would misrepresent this pattern as progressive demineralization. The ranking of purelines for body mineral content and tibia ash corresponded to their ranking for eggshell quality and egg components and was reproduced in the body mineral proportion of their 14-day-old progeny reared on a common starter diet. These findings suggest that dual-energy X-ray absorptiometry could support non-destructive screening of skeletal mineral status in parent stock and that differences in mineral accretion among purelines

may have a genetic component that is expressed in their progeny. Increasing NPP intake from 340 to 550 mg/hen/day did not increase body mineral content or tibia ash, indicating that the lower intake was adequate for skeletal mineral status under the conditions of the present study. The causes of the compositional fluctuations observed across the laying cycle warrant further investigation. Together with the companion studies, these results show that pureline separated every group of traits measured in this flock, from egg production and shell quality through hen and progeny mineral utilization to body composition and tibia ash, whereas dietary Ca and NPP affected mainly the mineral-dependent endpoints, with no benefit from increasing NPP intake above 340 mg/hen/day (Table 8).

ACKNOWLEDGMENT

The authors thank Cobb-Vantress Inc. for continued support of this research program and the staff of the University of Arkansas Poultry Research Farm for their care of the birds and assistance with scanning.

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