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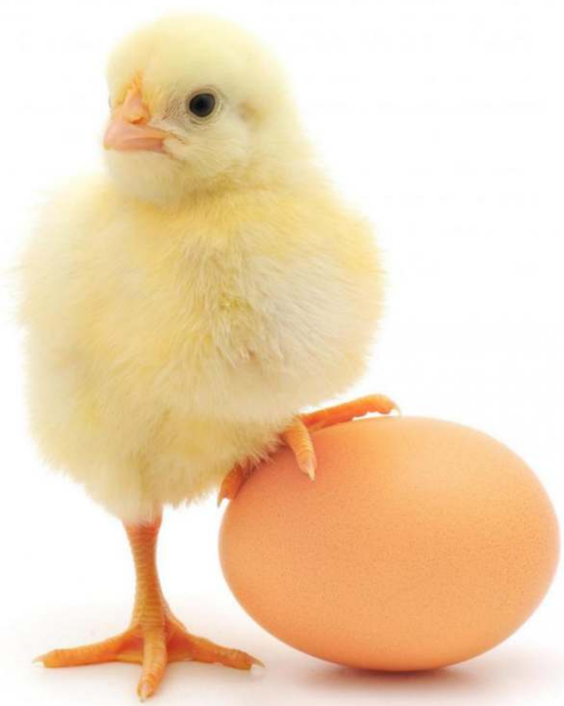

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The Effect of Limestone Particle Size on the Performance of Three Broiler Breeder Purelines

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Abstract: A 40-wk study was conducted to assess the effects of limestone particle size on the production performance, skeletal integrity and Ca/P balance of three broiler breeder purelines. A flock of LINE A, LINE B and LINE C broiler breeders were reared according to Cobb 500 guidelines (Cobb-Vantress, 2005) and transferred to a production house at 21 wks of age. Each line was split into two groups consisting of 66, 68 and 62 hens for the LINE A, LINE B and LINE C lines, respectively and fed a diet that differed only in the inclusion of either large (3489.7 microns; 38.5% solubility) or small particle (185.5 microns; 58.8% solubility) limestone. Production performance, egg quality, breeder skeletal integrity, reproductive performance, progeny performance and Ca/P balance were monitored. LINE B produced the most eggs per hen housed and highest egg number, followed by LINE C and LINE A, respectively. Egg weight, specific gravity and shell weight were also superior in LINE B compared to the other purelines. The % P retention did not differ across purelines, but Ca retention was also highest in LINE B. Breeder skeletal integrity and progeny bone quality were higher in LINE C. The addition of large particle limestone in lieu of small particles did not provide a consistent effect in production performance but significantly improved shell quality (P = 0.0013), bone quality (P = 0.0341). The large genetic differences in purelines compared to parent stock may uniquely alter the effects of particulate calcium on production performance; yet large particle limestone provided the boost in shell quality and bone quality reported by others. In summary, LINE A, LINE B and LINE C purelines utilize Ca and P differently and have unique genetic potential for hatching egg production, egg shell quality, breeder bone ash and progeny bone ash.

Key words: Limestone, egg production, egg shell quality

INTRODUCTION

The effect of Ca particle size on performance, P utilization and shell quality has garnered much interest in broiler breeder nutrition. Early studies in laying hens have reported an improvement in eggshell quality and bone ossification characteristics when a particulate source of Ca is utilized (Guinotte, 1987). Cheng and Coon (1990) found similar results in layer hens fed limestone of lower solubility. It is believed that retention of particulate calcium in the crop makes available calcium later on during eggshell formation and consequently reduces the amount of bone mobilization. Zhang and Coon (1994) were able to confirm that large particle limestone is retained for a longer period of time in the gizzard of commercial layers. When marine shells were fed to broiler breeder hens and compared to ground limestone it was found that marine shells improved egg wt, 1-d chick wt, yield stress and elastic deformation of tibias from 1-d old chicks (Guinotte and Nys, 1991). Manangi and Coon (2006) found a tendency for large particle limestone to reduce P excretion in broiler breeder hens along with an improvement in bone ash and specific gravity.

Recent field observations of broiler breeder purelines indicated that some purelines seemed to have poorer shell quality than other lines (Chet Wiernuscz, personal communication). Several factors influence shell quality, including the source and particle size of limestone. This study attempts to assess the validity of field observations and to establish the normal production parameters of these lines as well as test the effect of calcium particle size for purelines.

MATERIALS AND METHODS

Stock and management: A flock of 540 broiler breeders chicks consisting of three Cobb pure lines (LINE A, LINE B and LINE C) were raised in 2.38 m x 1.83 m floor pens from day old utilizing the Cobb Breeder Management Guide (Cobb-Vantress, 2005) as a reference for all management conditions. The starter diet was fed from 0-4 wk of age, the grower diet was fed from 4 wk until 20 wk, the prebreeder was fed from 20 wk until 25 wk and experimental diets from 25 wk through termination at 65 wk. The flock was fed *ad libitum* for the first 2 wk. From 2-4 wk, all birds were fed restricted amounts of feed every day. After 4 wk, all birds were switched to a skip-a-

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Table 1: Percent composition of breeder diets

Ingredient	Experimental diet (25-65 wk) (%)
Corn	63.200
Soybean meal, 47%CP	24.000
Dicalcium phosphate	1.620
Limestone[1]	7.500
Termin-8[2]	0.050
Sodium chloride	0.350
Poultry fat	2.510
L-Lysine HCl	0.070
Choline Cl-70%	0.090
Mineral premix[4]	0.060
Copper sulphate	0.050
Vitamin premix[5]	0.100
Ethoxyquin	0.020
Nutrient	
ME (kcal/kg)	2915.000
CP (%), calculated	16.000
CP (%), analyzed[6]	15.900
Lysine (%)	0.890
Methionine (%)	0.470
Crude fat (%)	4.960
Calcium (%)	3.250
Total phosphorous (%), analyzed[6]	0.607
Non-Phytate Phosphorus (%), analyzed[6]	0.410

[1]Two different particle sizes (small, x_{50} = 183.1 µm; large, x_{50} = 3704.26 µm) utilized; one for each experimental diet.
[2]50% Propionic acid. (Kemin Industries, Inc., Des Moines, Iowa).
[3]Alimet-Methionine Hydroxy Analog (Novus Int., St. Charles, Missouri).
[4]Provided per kg of diet: Mn, 180 mg; Zn, 150.6 mg; Fe, 20.16 mg; Cu, 2.04 mg; I, 1.26 mg; Se, 0.3 mg.
[5]Provided per kg of diet: Vitamin A, 13200 IU; Vitamin E, 66 IU; Vitamin D_3, 4950 ICU; Niacin, 74.25 mg; D-panthothenic acid, 33 mg; Riboflavin, 19.8 mg; Pyridoxine, 5000 mg; Thiamine, 3.3 mg; Menadione, 3.3 mg; Folic acid, 3.3 mg; Biotin, 0.33 mg; Vitamin B_{12}, 0.0297 mg.
[6]Corrected to 90% DM

day feeding program. Feed allocation was based on breeder recommended guidelines to reach target BW. Birds were weighed weekly by pen in order to adjust feed allocation to ensure target BW was met. At 21 wk, 124 LINE C birds, 132 LINE A birds and 136 LINE B birds were transferred to a production house and housed individually in breeder cages. Cages (47 cm high, 30.5 cm wide and 47 cm deep) were each equipped with an individual feeder and nipple drinker. Birds were fed individually and provided with free access to water at all times. At 24 wk, birds were put on an everyday feeding system and birds from each line were randomly assigned to one of 2 experimental diets that differed only in the limestone particle size. Mean particle size of the small particle limestone (Unical, ILC Resources, Des Moines, Iowa) was 185.5 microns (58.8% solubility), whereas mean of the large particle limestone (SBB, ILC Resources, Des Moines, Iowa) was 3489.7 microns (38.5% solubility). Composition of all diets can be seen in Table 1.

Production and reproduction performance: Production and reproductive performance, eggshell quality, parental and progeny skeletal structure were all monitored through 65 wk of age. Egg production was recorded daily and egg weights were recorded four days a week. All soft shelled, double yolk and cracked eggs were recorded and were not considered settable. Eggs per Hen Day (EHD) were defined as Eggs per Hen Housed (EHH) corrected for mortality. Peak egg production was determined as a five day rolling average. Shell quality was determined by specific gravity twice a week using the flotation method (Bennett, 1992). Hens were artificially inseminated beginning at 38 wk and the insemination was repeated approximately at six-week intervals through 60 wk. Twenty-five randomly selected hens from each treatment were inseminated with 1 x 10^6 cells/50 µl and settable eggs were collected for a six-day period. Semen was collected from broiler breeder males using the abdominal massage method, as described by Burrows and Quinn (1937). Semen was pooled and sperm cell concentration determined using an IMV Micro-Reader I, using an optical density of 381 nm (King *et al.*, 2000). Semen was diluted to 1 x 10^6 cells/50 µl using Beltsville Poultry Semen Extender to ensure all hens were inseminated with the same number and volume of sperm cells. The fertility and hatchability of fertile eggs were determined 4 times during the 40 wk study. Progeny weight was recorded at day of hatch. Progeny were euthanized via CO_2 asphyxiation and tibia samples were collected. Both tibia per bird were collected at day one and then pooled to ten tibia per replicate with a total of 10 replicates per treatment and used for analysis. Samples are representative of the entire insemination period. Samples of breeder hen tibia were collected at 65 weeks after CO_2 asphyxiation; 10 sample hens were utilized, respectively. Tibia for both progeny and breeder were stored at -20°C until analysis. Tibia were cut length-wise, oven dried and ashed in ceramic crucibles for 16 h at 600°C to determine bone ash. Total Ca and P were determined by Inductively Couple Plasma Emission Mass Spectrometry (ICP-MS) as described by Leske and Coon (1999).

A retention study was conducted at 41 weeks to assess Ca and P retention. Test diets were mixed with 2% celite as an acid insoluble ash marker. Birds were acclimated for 3 days to the celite diets and fed a meal of 137g; after which all excreta and eggs were collected during a 24-h test period. Total nutrient retention percentage was defined as: (total intake - total excreted)/total intake x 100. Retention was confirmed by determination of the acid insoluble ash. Egg Ca and P were not factored into the calculation of total P retention percentage. Retention was confirmed by determination of acid insoluble ash.

Statistical analysis: A two-factor factorial design was utilized with three genetic lines and two limestone particle sizes. Data was analyzed on JMP 7 (SAS Institute, Cary, North Carolina) utilizing GLM to determine overall treatment effects and interaction effects, when effects were significant a student's t test was performed to determine the differences between means and trend contrasts to determine line shape. Correlations were estimated by the REML method. All statements of significance are based on testing at p≤0.05.

RESULTS

LINE B produced more EHH and more settable eggs than LINE C and LINE A (Table 2). No significant particle size effect on EHD was seen, however a significant difference in the number of settable eggs was seen. No interaction effect was seen between genetic line and limestone particle size. Peak production was highest in LINE B and lowest in LINE A, while peak production was nearly identical between breeders fed large or small particle limestone. LINE B reached sexual maturity earlier than the other lines. No effect was seen for age at sexual maturity by limestone particle size. Sixty-five wk bone ash was higher in LINE C followed by LINE B and LINE A, respectively. A significant limestone particle size effect was seen for 65 wk breeder tibia ash. No interaction effect was seen.

A significant interaction effect between genetic line and limestone particle size was noted for egg wt. LINE B produced a heavier egg than the other two lines (Table 3); limestone particle size did not exert a strong effect on egg wt. Specific gravity was highest in LINE B followed by LINE C and LINE A. A significant interaction effect was seen for specific gravity as well. Differences seen in specific gravity due to limestone particle size were dependent on which genetic line was being considered. Shell wt, another evaluation method for assessing shell quality, revealed similar interaction effects. The eggs from LINE B had significantly higher shell wt than eggs from LINE C and LINE B. No statistical differences in hatch of fertile eggs were seen; nonetheless, a wide range of hatchability existed. LINE A hens fed small particle Ca produced eggs with 100% hatchability, yet LINE C hens fed the same small particle Ca produced eggs with the lowest hatchability at 77.2%. The intake of total P was held constant across all treatments. There were no statistical differences for % total P retention by genetic line or limestone particle size and no interactions. Total excreta P was numerically higher in LINE C breeder hens however no statistical differences were found due to genetic line or limestone particle size (Table 4). No statistical differences in Ca retention were seen across genetic lines (P = 0.0853) or by limestone particle size (P = 0.1039). Total egg P content did not differ between lines or by limestone particle size.

Table 2: Broiler breeder performance of three purelines fed different particle sizes of limestone[1,2]

	Line				Limestone particle size			L*P
	A	B	C	P	Large	Small	P	P
Age at sexual maturity (d)	200.93±1.38^{A}	192.58±1.28^{B}	194.75±1.40^{B}	<0.0001	196.93±1.12	195.25±1.09	0.284	0.1469
Eggs per HD	128.72±3.32^{C}	164.17±3.08^{A}	142.64±3.39^{B}	<0.0001	141.53±2.70	148.82±2.63	0.0542	0.8268
Settable eggs per HD	126.11±3.32^{C}	160.88±3.08^{A}	138.75±3.39^{B}	<0.0001	138.14±2.70	145.69±2.63	0.0460	0.8478
Peak production (%)	66.4	82.4	79.2	n/a	75.3	76.7	n/a	n/a
65-wk parental bone Ash (%)	49.15±1.66^{B}	50.66±1.66^{B}	59.95±1.66^{A}	0.0002	55.41±1.36	51.1±1.36	0.0341	0.6639
Mortality (%)	22.89±3.54	13.49±3.39	16.25±3.77	0.15	18.06±3.04	17.03±2.79	0.8035	0.4648

[A-E]Means within a row that do not share a letter are significantly different (p≤0.05). [1]n=66, 68 and 62 hens per treatment for line A, B and C, respectively. [2]Values are presented as means±SEM for the entire 40-week production period. L*P = Line*particle

Table 3: Egg quality 1 d progeny weight and % bone ash from three purelines fed different limestone particle sizes[1]

	LINE A		LINE B		LINE C			Line	Particle size	L*P
	Large	Small	Large	Small	Large	Small	SEM	P	P	P
Egg weight (g)	62.3^{C}	63.0^{B}	63.6^{A}	63.2^{B}	63.0^{B}	63.0^{B}	0.067	<0.0001	0.2948	<0.0001
Specific gravity	1.078^{B}	1.076^{E}	1.082^{A}	1.082^{A}	1.077^{D}	1.078^{C}	0.0001	<0.0001	0.0013	<0.0001
Shell weight (g)	5.40^{B}	5.09^{C}	5.66^{A}	5.78^{A}	5.29^{BC}	5.33^{B}	0.075	<0.0001	0.4214	0.0094
Hatch of fertile (%)	94.9	100.0	95.0	84.7	87.0	77.2	10.44	0.2246	0.4767	0.6298
1-day progeny weight (g)	45.5	43.8	46.9	46.7	48.1	44.8	4.18	0.4891	0.7872	0.8315
1-day progeny bone ash (%)	43.4	43.1	39.5	36.4	42.0	42.6	1.46	<0.0001	0.3135	0.1947

[A-E]Means within a row that do not share a letter are significantly different (p≤0.05).
[1]Values are presented as means±SEM for the entire 40-week production period. L*P = Line*Particle

Table 4: Ca and P balance in broiler breeder purelines fed different limestone particle sizes[1,2]

	Line					Limestone particle size				L*P
	A	B	C	SEM	P	Large	Small	SEM	P	P
Total feed P (mg)	830.00	830.00	830.00	n/a	n/a	830.00	830.00	n/a	n/a	n/a
Total excreta P (mg)	664.51	630.41	719.59	44.1	0.1887	689.48	653.52	36.8	0.3355	0.8016
Total P retention (%)	19.94	24.05	13.30	5.3	0.1887	16.93	21.26	4.4	0.3355	0.8016
Total egg P (mg)	103.41	103.85	103.94	4.6	0.9960	103.90	103.57	3.6	0.9483	0.5884
Total egg P (ppm)	10750.10	10638.30	10887.70	76.2	0.0783	10832.63	10684.77	76.2	0.1391	0.8697
Total excreta Ca (g)	3.68	3.14	3.38	3.7	0.0853	3.24	3.56	0.1	0.1039	0.9766
Total Ca retention (%)	16.78	28.93	23.63	0.2	0.0853	26.72	19.50	3.1	0.1039	0.9766

[1]Values are presented as means ± SEM for breeders at 41 wk of age. [2]N = 10 for each pureline. L*P = Line*particle

DISCUSSION

The results of the study show clear genetic differences in nutrient utilization and performance. LINE B outperformed the other lines in nearly every performance criteria measured. LINE B produced 164 total eggs per Hen Day (HD) and 161 settable eggs per HD. Cobb 500 broiler breeder hens fed the same basal diet with an inclusion of only large particle size limestone (3489.7 microns, 38.5% solubility) and managed under identical conditions produced 169 eggs per HD (Ekmay and Coon, 2008). Egg wt, specific gravity and 1-d progeny wt for LINE B were also comparable to same performance traits collected from Cobb 500 hens (Ekmay and Coon, 2008) and superior to same performance from LINE C and LINE A. Progeny bone ash from Line B was similar to that found from Cobb 500 hens and was the one criterion that was surpassed by both LINE C and LINE A. LINE A was the poorest performer during the 40 wk production period. LINE A had consistently lower egg wt, specific gravity and 1-d progeny wt. In comparing commercial laying hens to broiler breeders, it is often understood that breeders produce fewer total eggs and therefore do not endure the same nutrient pressure that layers do. The same shell quality issues seen in layers are not seen as often for breeders. The diminished production in LINE A, however, did not coincide with adequate shell quality. The diminished production performance of LINE A also produced equally poor shell characteristics. This is indicative of reduced genetic production potential in LINE A. LINE C had a tendency to fall in between LINE A and LINE B in terms of performance.

The addition of particulate limestone to the diet has produced mixed results for improving egg production in laying hens. The addition of 50% oyster shell along with limestone for supplying the calcium requirement has been shown to increase egg production in broiler breeders and caged layers (Van Wambeke and DeGroote, 1986; Ahmad and Balander, 2003). Guinotte (1987) pointed out that only 13 of 51 studies have showed a positive effect on egg production with particulate calcium. In the present study, purelines fed diets containing only large particle calcium did not show an increase in egg production. Purelines fed only large particle calcium tended to produce less eggs (P = 0.054) than purelines fed only smaller calcium particles. The reason for the slight reduction in egg number for the purelines fed only the large particle calcium is not understood. The purelines may have less demand for dietary calcium to produce shell mass during the production period and feeding the same quantitative amount of large calcium particles may lead to excessive physiological calcium. The % Ca retention was higher for purelines fed the large particles (P = 0.1039) indicating less loss through the digesta and kidney but this may be caused by the significant improvement in bone ash. The purelines fed large particle calcium showed an improvement in shell quality and skeletal quality. Shell characteristics in commercial laying hens have consistently shown an improvement to particulate limestone (Zhang and Coon, 1994; Ahmad and Balander, 2003; Roland, 1986). Safaa *et al*. (2008) found that brown layers fed limestone in a particulate form showed improved shell quality but the large particle limestone exerted its greatest effect on skeletal quality. Similarly, Manangi and Coon (2006) found that including large particle limestone in the diet of 32 wk old Cobb 500 broiler breeders over a period of 6 wk improved tibia ash and specific gravity. In a sister study to the work presented here, inclusion of large particle limestone in the diets of Cobb 500 breeders through 40 wks of age produced same number of hatching eggs but significantly improved specific gravity and egg weight (Ekmay and Coon, 2009) compared to breeders fed small particle limestone. The findings for purelines are consistent with these reports. As mentioned earlier, reduced egg production in purelines may limit the nutrient requirement and the reduced production potential may overshadow any positive effect particulate limestone may induce.

The % total P retention for LINE A and LINE B was similar to % P retention previously seen in Cobb 500 hens. It was determined that Cobb 500 hens fed 0.4% NPP and 3.25% Ca in the form of large particle limestone retained 22.5% of total P (Ekmay and Coon, 2008). Also in another study reported by Ekmay and Coon (2009), Cobb 500 breeders fed the exact same two particulate calcium sources had a 22.81 and 28.40 % total P retention (P = 0.1339) for small and large particulate Ca, respectively. In present experiment, LINE A and LINE B retained 19.94 and 24.05% of total P, respectively. Line C had a lower % total P retention along with an increased P excretion compared to LINE A and LINE B. The overall productive performance of LINE A seems to indicate that P availability is not a limiting factor for egg production, yet shell quality remained poor. Large particle limestone has been shown to reduce total P excretion (Ekmay and Coon, 2009) for Cobb 500 breeders, however no differences were obtained among the purelines for total P excretion by particle size.

LINE A retained only 16.78% of total Ca compared to 28.93% and 23.63% for Line B and Line C, respectively. The relatively low breeder bone ash in LINE A indicates that at least a portion of the excreted Ca originates from bone. This would also suggest that LINE A does not efficiently deposit calcium during eggshell formation as evidenced by the poor shell quality. The poor shell quality seen in LINE C appears to originate differently than from LINE A. LINE C hens had substantially better bone ash than LINE A, indicating that LINE C did not mobilize bone reserves as readily for eggshell formation. LINE C also had the lowest % total P retention which may also indicate dietary Ca was utilized more for producing egg shell with less dietary P being retained as new bone. These observations may also explain the strong negative correlation between breeder bone ash and progeny bone ash in LINE C.

In summary, feeding large particle limestone improved breeder bone ash as well as specific gravity. The effects of particulate limestone on production appear to be masked by genetic predispositions. Broiler breeder purelines each have a unique set of production performance potential. LINE C and LINE A produced significantly less hatching eggs with a lower projected shell mass output than LINE B however both lines produced poorer egg shell quality than LINE B. The research indicates the purelines are handling calcium and phosphorus differently and may have unique dietary Ca and P requirements.

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