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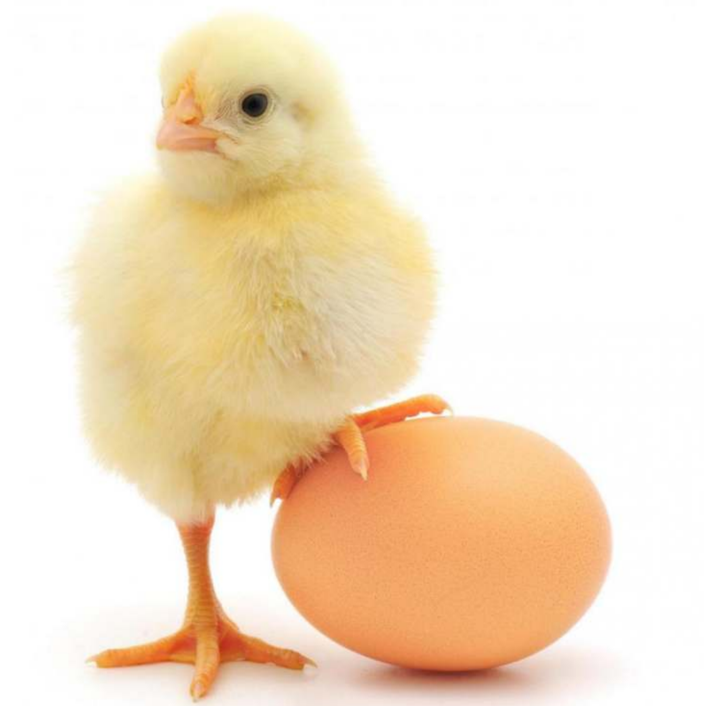

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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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An Examination of the P Requirements of Broiler Breeders for Performance, Progeny Quality and P Balance 2. Ca Particle Size

R.D. Ekmay and C.N. Coon
University of Arkansas, Center of Excellence for Poultry Science, Fayetteville, Arkansas 72701, USA

Abstract: A 2 x 5 factorial production and balance study with 90 broiler breeders was performed to assess the effects calcium particle size and NPP levels. Cobb 500 broiler breeders, 24 wk of age, were fed 4.68 g Ca intake at peak using 2 particle sizes of dietary calcium carbonate (185.5 microns; 58.8% solubility and 3489.7 microns; 38.5% solubility) and 5 levels of dietary %NPP (0.2% to 0.4% NPP in 0.05% increments; corresponding to a daily intake of 288, 360, 432, 504 and 576 mg at peak intake). Egg production, specific gravity and egg wt were monitored from 24 to 40 wk of age and tibia relative strength at 45 weeks. A retention study was performed at 31 wk of age to determine Ca and P balance. No differences were noted in breeder bone integrity due to NPP intake, though eggs per hen housed and egg shell quality were affected. The breeders fed 288 mg NPP produced the largest number of eggs. The % P retention showed a positive linear response to increasing dietary NPP for breeders fed large particle limestone; but no response in hens fed small particle limestone. The amount of P excreted was increased with P intake but was minimized for hens fed large particle limestone. The amount of Ca excreted was significantly increased with increasing P intake. There was a significant linear increase in excreta Ca and linear decrease in % Ca retention for breeders fed increasing P intake along with small particle calcium but the amount of Ca excreted and % Ca retention was not statistically impacted by particle size. Feeding breeders large particle calcium carbonate increased the egg weight but did not significantly improve shell quality or tibia bone strength. The increased egg weight response for breeders fed large particle calcium carbonate in this short term experiment may have reduced the opportunity for large particle Ca to significantly improve egg shell quality. It can be concluded that particulate Ca sources can improve breeder performance and that dietary levels as low as 0.20% NPP (288 mg/day NPP intake) can be fed without impacting breeder performance; however dietary levels of ≥0.25%NPP (360 mg/day NPP intake) ensure adequate skeletal integrity.

Key words: Breeders, Ca particle size, Ca and P retention, performance

INTRODUCTION

The effects of Ca particle size on performance and P utilization for commercial layers has created interest in broiler breeder nutrition. Early studies with laying hens have been reported an improvement in eggshell quality and ossification characteristics when a particulate source of Ca was utilized (Guinotte, 1987). Cheng and Coon (1990b) found similar results in layer hens fed limestone of increasing size; which corresponded to lower Ca solubility. It is believed that retention of particulate calcium in the gizzard makes calcium available later on during eggshell formation and consequently reduces the amount of bone mobilization. Zhang and Coon (1994), who reported an improved eggshell, were able to confirm that indeed large particle limestone is retained for a longer period of time in the gizzard of commercial laying hens. When marine shells were fed to broiler breeder hens and compared to ground limestone it was found that marine shells improved egg weight, 1-d progeny wt, yield stress and elastic deformation of tibiae from day old progeny (Guinotte and Nys, 1990).

Previous research for the purpose of determining the NPP requirements of broiler breeder hens showed that lowering daily intakes to 288 mg NPP at peak during a 40 wk production study did not impair egg production and progeny performance but the breeder bone ash at 45 and 65 wk was significantly reduced (Ekmay and Coon, 2010a). Breeders fed 360 mg NPP at peak did not show the significant reduction in skeletal integrity (Ekmay and Coon, 2010a). How Ca particle size affects the NPP requirements of broiler breeder hens has not been investigated. It has been shown in broilers that decreasing NPP will increase P retention but has a negative impact on Ca retention (Viveros *et al.*, 2002). Kornegay *et al.* (1996) showed that maintaining a Ca: total P ratio of 2:1 in broilers while increasing the level of NPP improves total P retention. Chandramoni *et al.* (1998) showed that increasing dietary P concentration increased absolute P retention but had no specific effect on Ca intake or output of laying hens. Manangi and Coon (2006) recently showed that breeders fed large particle calcium carbonate during a six wk balance study had significantly improved bone ash and egg shell quality

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

and the large Ca particles showed a strong tendency (p = 0.15) to reduce excreta P.

The present study was conducted to determine the impact of NPP intake and limestone particle size on breeder production, progeny performance, shell quality and Ca and P retention.

MATERIALS AND METHODS

Breeder management: Ninety-four 21-wk old broiler breeder pullets, reared in floor pens, were transferred to a production house and individually caged. Cages (47 cm high, 30.5 cm wide, 47 cm deep) were each equipped with an individual feeder and nipple drinker. Breeder pullets were photostimulated with 13h light: 11h dark at 21 wk until 15h light: 9h dark at 24 wk, based on the Cobb Breeder Management Guide (Cobb-Vantress, 2005). Hens were fed individually and provided with free access to water at all times. At 24 wk, all birds were put on an everyday feeding system and 90 breeders were randomly assigned to a treatment in a 2 x 5 factorial arrangement with limestone particle size and NPP % as factors, nine hens per treatment. Phosphorus levels ranged from 0.2% to 0.4% NPP in 0.05% increments; and corresponded to a daily intake of 288, 360, 432, 504 and 576 mg at peak intake. Dietary P was provided by feed grade dicalcium phosphate-18.5% P (PCS Sales (SA), Inc, Northbrook, Illinois). Mean particle size of the small particle calcium carbonate (Unical) was 185.5 microns (58.8% solubility) and mean large particle calcium carbonate (SBB) was 3489.7 microns (38.5% solubility). Composition of experimental diets can be seen in Table 1. Production performance and eggshell quality were monitored through 40 wk of age and breeder skeletal integrity at 45 wk of age. Egg production and mortality was recorded daily and egg weights were recorded four days a week. All soft shelled, double yolk and cracked eggs were recorded. Eggs per hen day were defined as eggs per hen housed corrected for mortality. Peak egg production was determined on a five day rolling average. Shell quality was determined by specific gravity once a week using the flotation method. Samples of breeder hen tibia were collected at 45 weeks. Tibiae 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). Bone strength was determined with an Instron 4502 and reported as relative strength (breaking force divided by diameter).

Ca and P retention: A balance study was conducted at 31 wk to assess Ca and P retention. Test diets were mixed with 2% celite as an acid insoluble ash marker.

Table 1: Composition (%) of experimental diets

Ingredient					
Corn	64.27	64.27	64.27	64.27	64.27
Soybean meal	23.80	23.80	23.80	23.80	23.80
Dicalcium Phosphate	0.48	0.76	1.03	1.30	1.57
Limestone[1]	8.10	8.10	8.10	8.10	8.10
Mold Curb[2]	0.05	0.05	0.05	0.05	0.05
Salt	0.34	0.34	0.34	0.34	0.34
Poultry fat	2.13	2.13	2.13	2.13	2.13
L-Lysine HCl	0.07	0.07	0.07	0.07	0.07
Choline	0.10	0.10	0.10	0.10	0.10
Mineral premix[3]	0.06	0.06	0.06	0.06	0.06
Copper sulphate	0.05	0.05	0.05	0.05	0.05
Vitamin premix[4]	0.10	0.10	0.10	0.10	0.10
Ethoxyquin	0.02	0.02	0.02	0.02	0.02
Nutrients					
ME (kcal/kg)	2915.00	2915.00	2915.00	2915.00	2915.00
CP calculated	0.16	0.16	0.16	0.16	0.16
Lysine	0.89	0.89	0.89	0.89	0.89
Methionine	0.47	0.47	0.47	0.47	0.47
Crude fat	4.18	4.18	4.18	4.18	4.18
Ca (calculated)	3.25	3.25	3.25	3.25	3.25
Total P (calculated)	0.40	0.45	0.50	0.55	0.60
Total P (analyzed)	0.42	0.46	0.51	0.56	0.63
NPP (calculated)	0.20	0.25	0.30	0.35	0.40
NPP (analyzed)	0.21	0.24	0.32	0.35	0.42

[1]Limestone was replaced with two different particle sizes for two experimental diets. Mean large particle limestone size was 3489.7 micron; mean small particle limestone size was 185.5 micron.

[2]50% Propionic acid. Kemin Industries, Inc., Des Moines, Iowa.

[3]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.

[4]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

Table 2: Production performance through 40 wk for broiler breeder hens fed a unique combination of limestone (large or small particle) and dietary NPP (0.20-0.40%) from 21 through 45 wk. n = 9 hens per treatment. Values are presented as means ± SEM for the entire 20-week production period

Limestone particle size	Dietary NPP (%, mg/d)	Age at sexual maturity (d)	Eggs per hen housed	Egg weight (g)	Specific gravity	45 wk Breeder tibia relative strength
Large		184.43±1.30	81.11±1.75	58.04±0.26	1.07983±0.0004	7.755±0.289
Small		182.35±1.32	80.95±1.77	57.00±0.26	1.07919±0.0004	7.348±0.219
	0.20% (288)	180.94±2.10	85.82±2.81	57.26±0.56	1.08073±0.0006	6.941±0.414
	0.25% (360)	183.83±2.03	80.78±2.73	57.61±0.57	1.08074±0.0006	7.326±0.409
	0.30% (432)	185.44±2.03	73.89±2.73	58.01±0.58	1.07916±0.0006	7.448±0.399
	0.35% (504)	180.44±2.03	84.61±2.73	57.15±0.55	1.07865±0.0006	8.235±0.409
	0.40% (576)	186.56±2.16	80.25±2.89	57.74±0.61	1.07856±0.0007	7.808±0.396
p-values						
	Particle size	0.2465	0.9875	0.0039	0.2224	0.2992
	P level	0.1541	0.0271	0.3933	0.0076	0.1805
	P level*Particle size	0.9032	0.775	0.7244	0.2464	0.1497

Birds were acclimated for 3 days to the celite diets and fed a meal of 142 g; after which all excreta were collected during a 24-h test period. Total Ca and P were determined by ICP; Ca and P retention was defined as: (intake-excretion)/intake.

Statistical analysis: Data was analyzed on JMP 7 (SAS Institute, Cary, North Carolina) using the least squares procedure 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 the best-fit line. All statements of significance are based on testing at $p\leq0.05$.

RESULTS

The number of eggs per hen housed was affected by NPP intake (p = 0.0271) with hens fed 0.30% NPP producing the fewest eggs (Table 2). Breeders fed 0.20% (288 mg NPP/day at peak) produced the largest number of eggs. There were no differences in egg production due to particle size (p = 0.9875). No mortality was observed from 24 to 40 wk of age, therefore eggs per hen day did not differ from eggs per hen housed. Large particle limestone induced heavier eggs compared to small particle limestone (p=<0.0001), however no NPP effect was noted. Specific gravity was not affected by particle size however reducing dietary NPP intake improved specific gravity (p = 0.0076). Relative bone breaking strength was not affected by either NPP intake (p = 0.1805) or by limestone particle size (p = 0.2992).

The total amount of P excretion was affected by the level of dietary NPP intake (p = 0.0002): reaching a maximum P excretion for hens fed 0.40% NPP (Table 3). Feeding breeders large particle limestone decreased the daily P excreted by approximately 50 mg but statistically there was no significant difference in the amount of P excreted (p = 0.0863) due to calcium particle size. No significant interaction effect was seen (p = 0.0919). The increases in excreted P due to increases in dietary NPP were not proportional increases. A large range in P retention was seen in hens fed large particle limestone. The % P retention in breeder hens fed large particle limestone ranged from 16.70% to 40.14%; though no significant differences were seen due to P level. The % P retention in hens fed small particle limestone ranged from 16.84% to 29.09%. No interaction effect between limestone particle size and NPP was determined. Particle size did not exert any statistically significant effect on % P retention. The deposition of egg P was not affected by the amount of dietary P (p = 0.5662). However, limestone particle size significantly increased the amount of P deposited in breeder eggs. Large particle limestone increased the amount deposited in the egg independent of P level (p = 0.0276) with a near significant interaction effect (p = 0.0596).

The absorption and utilization of Ca followed a similar result pattern to that of % P retention. Excretion of Ca was highest in hens fed 0.30% NPP (432 mg NPP/day at peak) with both large and small particle limestone. In general, excretion of Ca was higher at higher levels of P: those above 0.30% NPP (p = 0.0081). Particle size did not exert an influence in the amount of Ca excreted and there was no interaction effect. The % Ca retention showed a high overall variation, but two different retention patterns emerged from the particle size used. In hens fed large particle limestone, overall % Ca retention fell to a minimum for breeders fed 0.30% NPP before increasing for hens fed higher levels of daily NPP. Hens fed small particle limestone showed a linear decrease in Ca retention with increasing dietary NPP intake (p = 0.0115). Despite these differences, no overall statistical differences were noted due to particle size or P intake.

DISCUSSION

The high cost of P and its environmental impact has created an incentive into further evaluating the P requirement of broiler breeders. In a similar study, breeder hens fed 288 mg NPP/day at peak maintained equal egg production through 65 wk of age compared to

Table 3: Ca and P retention for 31 week-old broiler breeder hens fed unique combination of limestone (large or small particle) and dietary NPP (0.20-0.40%) from 21 through 45 wk. n = 9 hens per treatment. Values are presented as means ± SEM for the entire 20-week production period

Limestone particle size	Dietary NPP (%, mg/d)	Total feed P (mg)	Total excreta P (mg)	Total P retention (%)	Total egg P (mg)	Total egg P (ppm)	Total feed Ca (g)	Total excreta Ca (g)	Total Ca retention (%)
Large		763.72	538.86±19.60	28.40±2.48	131.42±05.98	7931.67±55.89	4.62	3.36±0.28	27.25±06.02
Small		763.72	589.63±21.54	22.81±2.72	109.25±06.63	7248.88±58.43	4.62	3.19±0.31	30.96±06.61
	0.20% (288)	605.49	479.63±30.29	20.79±3.83	115.66±09.49	7485.22±85.22	4.62	2.32±0.43	49.65±09.30
	0.25% (360)	653.20	504.96±35.19	22.69±4.45	130.99±10.73	7758.99±90.63	4.62	2.94±0.50	36.20±10.80
	0.30% (432)	789.17	549.73±35.19	30.34±4.45	114.50±10.73	7652.36±98.30	4.62	3.81±0.50	17.38±10.80
	0.35% (504)	799.32	606.78±30.05	24.09±3.80	124.31±09.17	7683.16±87.67	4.62	3.64±0.43	21.12±09.22
	0.40% (576)	971.42	680.13±31.68	30.12±4.00	116.20±09.66	7371.42±89.62	4.62	3.64±0.45	21.19±09.72
p-values									
	Particle size	NA	0.0863	0.1339	0.0276	<0.0001	NA	0.4352	0.68
	P level	NA	0.0002	0.3456	0.5662	0.0193	NA	0.0081	0.1097
	Particle size*P level	NA	0.0919	0.1352	0.0596	0.6969	NA	0.7725	0.6551
	Linear-small	NA	<0.0001	0.7776	0.4009	0.6732	NA	0.0115	0.0115
	Linear-large	NA	0.0418	0.0154	0.3525	0.1473	NA	0.2890	0.2890

breeders fed higher levels of dietary NPP (Ekmay and Coon, 2010a). Similar results were found in the present study. No significant differences were found between four of the five diets. Plumstead *et al.* (2007) showed that reducing P intake in broiler breeders improved production performance. Plumstead also reported a drop in fertility from 97 to 95%, but an increase in total chicks produced per hen. Several authors (Keshavarz, 2000; Boling *et al.*, 2000) have found no difference in egg production for breeder hens or laying hens fed low levels of NPP. Therefore, it can be concluded that P is not limiting for egg production. Several authors have previously reported that excess P has detrimental effects on shell quality (Sibbald and Hamilton, 1977; Miles *et al.*, 1982). However, no differences were seen in shell quality in the present study nor was it seen in others (Ekmay and Coon, 2010a; Keshavarz, 2000; Boling *et al.*, 2000). When dietary Ca or P is limiting for egg formation, whether due to dietary insufficiency or time of feeding, the difference is made up by mobilization of bone reserves. Research appears to indicate that mobilization occurs regardless of NPP sufficiency and dietary NPP serves to replenish bone reserves (Ekmay *et al.*, 2009; Ekmay and Coon, 2010a). Medullary bone is the main source of labile calcium (and its counter ion PO_4^-) for eggshell formation; however, it does not provide the level of strength trabecular and cortical bone provide. The switch from structural bone to labile bone does not always cause a decrease in bone ash, since medullary bone itself can maintain bone ash. Previous studies (Ekmay and Coon, 2010a) have indicated that reducing dietary P reduces skeletal integrity of the breeder hen. No statistical differences in relative tibia strength were seen due to NPP intake; however, this may be due to particle size effects (p = 0.1497).

Large particle limestone did not increase eggs produced. No conclusive evidence of an effect on egg production by particulate calcium has been found in previous studies. Van Wambeke and DeGroote (1986) found an increase in egg number in breeder hens fed over 50% oyster shell, however, Guinotte (1987) pointed out that only 13 of 51 studies showed a positive effect on egg production when feeding dietary particulate calcium. Guinotte and Nys (1990) reported an improvement in egg weight and shell index when marine shells were used. The increase in egg weight for breeders fed large particle limestone in the present study is in agreement with the findings by Guinotte and Nys. Cheng and Coon (1990a) failed to report an increase in egg weight in layer hens fed particulate limestone. Retention of limestone in the gizzard makes available calcium for eggshell formation at a later time. Several authors have reported an improvement in shell quality in hens fed particulate calcium (Zhang and Coon, 1994; Guinotte and Nys, 1990; Roland, 1986). Breeders in the present study fed large particle limestone did not improve shell quality. This may be partly due to the increased egg weight observed with hens fed large particle limestone. Purelines fed large particle limestone showed an improvement in shell quality and bone strength as well (Ekmay and Coon, 2010b). Cheng and Coon (1990a) showed an improvement in bone strength for commercial layers fed large particle calcium (US Screen number $\leq$18). However, breeder hens in the present study did not show the same improvement in tibia strength in hens fed particulate calcium.

Several authors (Leske and Coon, 1999; Manangi and Coon, 2006; Chandramoni *et al.*, 1998; Keshavarz, 2000) have reported an improvement in the absolute amount P retained with increased dietary P. In the present study, the % total P retention diminished with increasing dietary P. Previously, a threshold was determined at 360 mg NPP/d, above which, there was a significant increase in total P excretion and a reduction in % total P retention (Ekmay and Coon, 2010a). Breeders fed increasing levels of dietary NPP for the younger breeder hens in the present study show increased levels of P excretion but do not show the same retention response. Overall, P retention showed no discernable pattern with increasing dietary NPP. As such, no statistical differences were noted. However, a closer examination of P retention for breeders fed large and small particle limestone separately reveals that particle size effects how P is utilized. Breeders fed large particle limestone with increasing NPP intake show a positive linear response for % P retention ($p = 0.0154$) but no linear response for % Ca retention ($p = 0.2890$). Breeders fed small particle limestone showed no linear relationship to increased NPP intake ($p = 0.7776$) but a negative linear response for Ca retention ($p = 0.0115$). Previous findings in older breeders hens fed large particle limestone with the same dietary NPP range revealed that P retention had a negative linear response, not a positive one as indicated in this study. The only difference between the two studies was age of the breeder females. The aforementioned study was conducted at 52 weeks, while the present retention study was conducted at 31 weeks. Decreases in P retention with age have been observed in layer hens (Scheideler and Sell, 1987; Ceylan *et al.*, 2003). Broiler breeder hens were at peak production at 31 weeks in present study.

The high rate of production and the hen's young age may be determinants of P absorption and utilization. The deposition of egg P in the present study confirms this shift in nutrient utilization. The amount of P deposited in the egg by 52 week-old hens (Ekmay and Coon, 2010a) was lower than in present study with 31 week-old hens. Hens on a diet that included large particle limestone deposited more egg phosphorus compared to hens on a diet consisting of small particle limestone at 31 weeks. Therefore, the positive linear response to P retention with large particle Ca for the 31 wk old breeder may be due to increased P deposition into the egg and may explain the loss of a distinct P excretion threshold as observed in 52 wk old breeders. The mechanism by which P is deposited into the egg is poorly understood. The deposition of P into egg (yolk) was assumed to be a continuous process, with small variation in the amount deposited at any given time (Kebreab *et al.*, 2009). The amount of P deposited was also thought to be limited to only the requirement. The vast majority of P in the egg is found either as phospholipids or as phosvitin. The differences between Ca and P retention can also be explained due to the dynamics of bone turnover. Bone mobilization is limited when NPP in the diet is deficient (Ekmay *et al.*, 2009). With higher levels of bone mobilization at higher NPP intakes, more Ca becomes available than what is needed. If only a set amount of the small particle limestone is retained, the excess Ca arising from bone mobilization would amount to higher levels of Ca excreted. Large particle limestone may limit the large imbalances caused by continual bone turnover on P homeostasis and optimize conditions for P incorporation *in the young hen*. The improved P retention seen with large particle limestone may provide additional P for incorporation into phospholipids and phosphoproteins.

In summary, neither calcium particle size nor dietary % NPP conclusively impacted production in the short term broiler breeder study. Feeding large particle limestone improved egg specific gravity. Evidence suggests that Ca particle size, dietary NPP and possibly age influences Ca and P retention.

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