Asian-Aust. J. Anim. Sci.
Vol. 20, No. 6 : 933 - 938
June 2007

www.ajas.info

Requirement of Non-phytate Phosphorus in Synthetic Broiler Breeder Diet

S. K. Bhanja[1, *], V. R. Reddy, A. K. Panda[1], S. V. Rama Rao[1] and R. P. Sharma[1]
Department of Livestock Production and Management College of Veterinary Science
Rajendranagar, Hyderabad 500 030 India

ABSTRACT : An experiment was conducted to study the laying performance, shell quality, bone mineralization, hatchability of eggs and performance of progeny (weight at day one and 14 d of age, P content in day old chick, leg score and survivability of chicks) of synthetic broiler breeders fed different levels of non-phytate phosphorus (NPP). Six levels of NPP (1.2, 1.8, 2.4, 3.0, 3.6 and 4.2 g/kg diet) at a constant calcium (Ca) level (30 g/kg) in a maize-soya-deoiled rice bran based diet were tested. Levels of dicalcium phosphate, shell grit and deoiled rice bran were adjusted to achieve the desired levels of NPP and Ca. Each level of NPP was fed with a weighed quantity of feed (160 g/b/d) to 40 female broiler breeders from 25 to 40 weeks of age housed in individual cages. Each bird was considered as a replicate. Egg production, feed/egg mass, body weight, egg weight, shell weight, shell thickness, egg specific gravity, serum Ca content and tibia breaking strength were not influenced ($p>0.05$) by the variation in dietary NPP levels tested. Increasing the dietary levels of NPP did not influence the hatchability of eggs, phosphorus (P) contents both in egg yolk and day old chick, chick body weight at day one and 14 d of age, leg score and survivability of chicks up to 14 d of age. Maximum response ($p\leq0.01$) in shell breaking strength, tibia ash and serum inorganic P contents were observed at NPP levels of 2.09, 2.25 and 3.50 g per kg diet, respectively. The retention of Ca increased, while the P retention decreased ($p\leq0.01$) with increasing dietary levels of NPP. Though maximum responses in shell breaking strength, bone ash and serum inorganic P were observed at NPP higher than 1.2 g/kg diet, the broiler breeder performance in terms of egg production, shell quality, hatchability of eggs and progeny performance and their survivability was not influenced by dietary NPP concentrations. It is concluded that synthetic broiler breeders maintained in cages do not require more than 1.2 g NPP/kg diet with a daily intake of 192 mg NPP/b/d during 24 to 40 weeks of age. (**Key Words :** Non Phytate Phosphorus, Requirement, Synthetic Broiler Breeder)

INTRODUCTION

Phosphorous (P) an essential and critical mineral in laying hens' diet. P does not exist in 'pure' phytic acids (PA) form, but always as a salt with divalent cations (phytate). PA in vegetable protein ingredients is not available to poultry due to lack or insufficient amount of phytase in their digestive system. About two thirds of total P (TP) in vegetable feed ingredients is in phytate form (NRC, 1994). However, phytin phosphorus (PP) content in feed ingredient may range from 32 to 75% of the TP (Eeckhout and DePaepe, 1994). The variation in PP content and the activity of endogenous phytase present in plant feed ingredients cause considerable variation in the availability of P from PP (Jongbloed et al., 1991), which may result in wide variation in available P content in the diet. Therefore, analysis of NPP gives precise estimates of the P requirements for poultry.

Several investigations were undertaken with White Leghorn layers (Roush et al., 1986; Keshavarz and Nakajima 1993; Rama Rao et al., 1999; Keshavarz, 2003; Panda et al., 2005) to determine the optimum requirement of NPP in diet. However, only few reports are available on the P requirement of broiler breeders (Harms et al., 1964; Wilson et al., 1980; Wilson and Harms, 1984). Because of the high cost of P sources and the public concern over the contribution of poultry excreta to environmental pollution, the P requirement of breeder hens need to be reinvestigated Therefore, the present experiment was undertaken to study the effects of different levels of NPP in broiler breeder diet on their egg production, shell quality, hatchability of eggs and performance of their offspring.

* Corresponding Author: S. K. Bhanja. E-mail: skbhanja65@rediffmail.com
[1] Project Directorate on Poultry, Rajendranagar, Hyderabad 500 030, India.
Received January 16, 2006; Accepted May 30, 2006

MATERIALS AND METHODS

Birds and management

Female line synthetic broiler breeders developed for high egg production for 18 generations were used in this study. Day old female chicks were fed *ad lib* breeder starter diet up to 6 weeks of age. From 7th week onwards, they were maintained on quantitative feed restriction till the day of experimentation. At 24 weeks of age, two hundred and forty birds having uniform body weight (2,900±48 g) were randomly distributed into individual California type cages (37.5 cm×30 cm×30 cm). Each cage was considered as a replicate, which was fitted with an individual feeder and a nipple waterer. A continuous 16 h light per day was provided using incandescent bulbs. All the birds were kept under uniform managemental conditions throughout the experimental period. The temperature of the house varied between 36-38°C during the entire experimental period.

Experimental diets

The proximate constituents, calcium (Ca), TP (AOAC, 1990) and PP (Haugh and Lantzscha, 1983) content in the feed ingredients were determined. The NPP content was calculated by subtracting the PP from TP. Six experimental diets were formulated based on maize-soybean-deoiled rice bran. The analysed nutrient composition for protein, Ca and TP and published values (NRC, 1994) for AME and amino acids were considered while formulating the diets. The diets were formulated to contain 1.2, 1.8, 2.4, 3.0, 3.6 and 4.2 g NPP/kg diet (Table 2). The levels of dicalcium phosphate and shell grit were altered to obtain the desired levels of NPP. A constant level of Ca (about 30 g/kg) was maintained in all the diets. Each diet was assigned randomly to 40 hens. The birds were reared on the experimental diets up to 40 weeks of age. A weighed quantity of feed (160 g/bird/d) was offered daily during forenoon (6-8 am).

Response criterion

Egg production and egg weight : Individual bird egg production and feed intake were recorded daily. All the eggs were weighed daily throughout the experimental period and the egg mass was calculated. Individual body weight was recorded at the beginning (24 weeks) and end of the experimental period (40 weeks).

Egg shell quality : Twelve eggs were randomly chosen from each NPP level during the last three consecutive days of every 28-days period to determine the specific gravity (Densitometer, Mettler-Toledo, ISO-14001, Switzerland), shell weight, shell thickness and shell breaking strength (Universal Testing Machine, EZ test, 120891-04, Japan). The eggshells were dried for 24 h at room temperature and were weighed. The shell thickness was measured at three different locations (middle, broad and narrow end) using a micrometer gauge (Mitutoyo code 7027, Japan).

Serum bio-chemical parameters

At the end of each 28-d period, 2 ml of blood were collected from five birds in each treatment from brachial vein during afternoon (2.30 to 3.39 PM). Subsequently serum was separated and the levels of Ca (AOAC, 1990) and inorganic P (Fiske and Subba Row, 1925) in the serum were estimated.

Yolk phosphorous

During each 28-d period, four eggs were randomly selected from each treatment during the last three consecutive days to determine the P content in egg yolk (AOAC, 1990).

Retention of TP and Ca

A 3-day metabolic trial involving total collection of faeces was conducted at 40 weeks of age to determine the retention of Ca and P. Three birds from each treatment group were selected at random and were acclimatized in metabolic cages for three days under similar feed intake. The dry matter (feed) intake and dried excreta voided were determined. The samples of feed and excreta were analyzed for Ca and TP (AOAC, 1990) and the retention of the nutrients were expressed per mg/g nutrient intake.

Tibia characteristics

At the end of the experiment (40 wks of age), three birds from each treatment were selected at random and sacrificed by cervical dislocation. Both the tibia were freed from soft tissue and diaphysis and then defatted by soaking in petroleum ether for 48 h and dried at 100°C for 12 h. Both the tibia were used for determination of bone ash and bone strength. Tibia breaking strength was determined by Universal Testing Machine (EZ test, 120891-04, Shimadzu, Japan). Broken pieces of both tibia from a bird were ashed at 600+30°C for 12 h for estimation of bone ash (AOAC, 1990).

Hatchability of eggs and performance of the progeny

Hatchability of eggs laid by broiler breeders fed different levels of NPP were determined at 270 days of age. All the hens in each treatment were inseminated with pooled semen from broiler breeder males of the same hatch (age). Eggs were collected through the 3rd to 8th day following insemination and incubated to determine the hatchability. All eggs laid in each treatment were triplicated and maintained during hatching. The chicks hatched were wing banded and grown up to 14 d of age in stainless steel battery brooders under uniform managemental condition to determine the survivability, body weight gain and leg abnormality (Watson et al., 1970), if any. Three replicates

Table 1. Analyzed chemical composition (g/kg dry matter) of feed ingredients

Ingredient	Dry matter	Crude protein	Ether extract	Crude fibre	Calcium	Total phosphorus	Phytin phosphorus
Maize	893.3	115.0	39.1	18.0	0.22	2.40	1.93
Soybean meal	898.3	505.5	10.9	29.0	5.98	5.00	4.08
Deoiled rice bran	897.8	172.8	5.9	147.8	2.14	12.60	9.10
Oyster shell grit	999.7				396.0		
Dicalcium phosphate	978.2				220.0	177.0	

Table 2. Composition of diets (g/kg), varying in NPP levels, fed to broiler breeder females (24-40 weeks)

Ingredient	NPP (g/kg diet)					
	1.2	1.8	2.4	3.0	3.6	4.2
Maize	636.0	637.8	639.6	641.4	643.2	642.0
Soybean meal	255.0	255.0	255.0	255.0	255.0	255.0
De oiled rice bran	13.0	9.8	6.6	3.4	0.2	-
Common salt	2.0	2.0	2.0	2.0	2.0	2.0
Dicalcium phosphate	-	4.0	8.0	12.0	16.0	20.0
Oyster shell grit	88.0	85.4	82.8	80.2	77.6	75.0
DL-methionine	1.4	1.4	1.4	1.4	1.4	1.4
Sodium bicarbonate	1.6	1.6	1.6	1.6	1.6	1.6
Choline chloride 50%	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix [1]	1.0	1.0	1.0	1.0	1.0	1.0
Trace mineral premix[2]	1.0	1.0	1.0	1.0	1.0	1.0
						1,000 total
Nutrient composition						
Analysed value (g/kg)						
Crude protein	169.1	169.0	168.5	168.3	168.0	167.8
Total phosphorus	3.1	3.7	4.2	5.1	5.8	6.6
Non phytin phosphorus	1.2	1.8	2.5	3.1	3.7	4.3
Calcium	29.6	31.2	31.8	30.3	30.4	29.2
Calculated value						
Metabolisable energy (MJ/kg)	11.46	11.43	11.41	11.38	11.34	11.39
Lysine	8.5	8.4	8.4	8.4	8.4	8.4
Methionine	4.1	4.1	4.1	4.1	4.1	4.1
Methionine+cystine	7.0	7.0	7.0	7.0	7.0	7.0

[1] Vitamin premix provided (mg/kg diet): Vitamin A, 15,000 IU; D, 3,000 ICU; E, 100 mg; K, 4 mg; thiamin, 2 mg; riboflavin, 15 mg; pyridoxine, 8 mg; cyanocobalamine, 0.25 mg; niacin, 60 mg; Pantothenic acid, 25 mg; Folic acid, 2.5 mg; Biotin, 0.2 mg.
[2] Trace mineral premix provided (mg/kg diet): Manganese, 100; Zn, 90; Iron, 40; Copper, 10; Iodine, 1; Selenium, 0.2.

were maintained per each treatment. The leg score given were 1- for normal hock joint, 2- slight amount of swelling of tibio-metatarsal joint, 3- marked degree of swelling of the joint, 4- swelling with slight amount of slipping of the Achilles tendon and 5- for swelling combined with marked degree of slipping of the tendon from its condoyles.

Total phosphorus in chicks

Three chicks were randomly chosen from each treatment on day 1 and were killed with chloroform. Subsequently, the carcass of individual bird was dried in the hot air oven at 100°C, ground and P content of individual chick was determined (Fiske and Subba Row, 1925).

Statistical analysis

The data obtained (individual bird's data was considered as an experimental unit) was subjected to the regression analysis of the treatment means by using linear and non linear equations. However, no response could be predicted. Then the data were subjected to polynomial analysis (Snedecor and Cochran, 1980). The response in the criterion considered were fitted by polynomial equation in the form of $y = a+bx+cx^2+dx^3$ when the R^2 of a criterion was significant, the requirement of NPP for the maximum response of that particular trait was calculated.

RESULTS AND DISCUSSION

Phytin phosphorus content

The PP content in maize, soyabean meal and deoiled rice bran were 80.4, 81.6 and 72.2% of the TP, respectively (Table 1). These values are relatively higher than the values reported (Eeckhout and DePaepe, 1994; NRC, 1994; Rama Rao et al., 1999). The variation in the PP content might be

Table 3. Egg production, shell quality, tibia mineralisation, retention and serum concentration of Ca and P in broiler breeders fed different levels of NPP

Attribute	NPP (g/kg)						N
	1.2	1.8	2.4	3.0	3.6	4.2	
Egg production	62.70	64.69	62.61	61.45	65.94	67.55	40
(number/100 b)	(1.73)	(1.89)	(2.30)	(2.06)	(2.16)	(1.72)	
Egg weight	55.58	55.75	55.81	55.49	57.73	55.85	40
(g)	(0.51)	(0.47)	(0.75)	(0.67)	(0.81)	(0.58)	
Feed/egg mass	4.79	4.66	5.06	5.13	4.48	4.40	40
	(0.16)	(0.17)	(0.33)	(0.33)	(0.20)	(0.14)	
Final body weight	3,456	3,557	3,586	3,489	3,465	3,497	40
(g)	(43)	(48)	(48)	(60)	(60)	(43)	
Tibia breaking strength	69.04	89.87	131.06	94.97	82.07	104.96	3
(Newton)	(16.32)	(20.55)	(11.86)	(5.35)	(7.44)	(7.70)	
Tibia ash	58.95	60.77	62.28	62.80	60.99	63.94	3
(g/100 g)	(1.19)	(0.95)	(0.18)	(0.49)	(1.20)	(0.27)	
Serum Ca	15.24	13.87	15.48	15.40	15.28	15.56	20
(mg/dl)	(0.56)	(0.44)	(0.78)	(0.58)	(0.73)	(0.64)	
Serum inorganic P	5.08	6.09	5.83	6.66	6.80	6.39	20
(mg/dl)	(0.36)	(0.36)	(0.31)	(0.28)	(0.32)	(0.28)	
Retention of Ca	158	181	418	319	400	403	3
(mg/g intake)	(72)	(29)	(24)	(65)	(56)	(34)	
Retention of P	650	561	265	330	281	149	3
(mg/g intake)	(4.9)	(31)	(54)	(10)	(60)	(44)	

Values given in the parenthesis are the standard error (+) of the respective mean.

Table 4. Effect of level of NPP in broiler breeder diet on hatchability of eggs, P content in yolk and in chick leg abnormality score and body weight of chicks from female parents fed different levels of NPP

Attribute	NPP (g/kg)						N
	1.2	1.8	2.4	3.0	3.6	4.2	
Hatchability	75.00	80.67	76.67	69.33	76.00	68.00	3
(number/100 egg set)	(5.27)	(2.87)	(5.16)	(3.85)	(5.41)	(6.02)	
Egg yolk P	745	744	730	728	750	734	4
(mg/100 g)	(23)	(19)	(16)	(17)	(23)	(17)	
Chick P	6.6	6.7	6.6	6.7	6.5	6.7	3
(g/kg)	(0.2)	(0.2)	(0.3)	(0.2)	(0.2)	(0.2)	
Body weight	42.75	43.35	42.70	42.65	43.40	43.23	3
(g (1-d))	(0.44)	(0.32)	(0.24)	(0.34)	(0.45)	(0.29)	
Body weight	250	245	258	261	248	267	3
(g (14-d))	(3.08)	(5.82)	(3.45)	(2.71)	(3.37)	(1.65)	
Leg score*	1.07	1.05	1.07	1.09	1.08	1.09	4
	(0.02)	(0.02)	(0.03)	(0.03)	(0.02)	(0.04)	
Mortality	0.98	2.76	2.43	1.75	1.94	1.78	
(No/100 chicks)							

Values given in the parenthesis are the standard error (+) of the respective mean.

* 1. Normal hoc joint; 2. Slight swelling of hoc joint; 3. Marked degree of swelling of the joint; 4. Swelling of the joint with slight amount of slipping of the Achilles tendon and 5. Swelling with marked degree of slipping of the tendon Achilles.

due to the variation in the plant cultivar, soil chemistry, dose of phosphatic fertilizer, climate etc. (Reddy et al., 1982; Hopkins et al., 1989).

Laying performance

The performance of broiler breeders fed different levels of NPP is presented in Table 3 and 4. The regression coefficient (R^2), correlation and the predicted NPP requirements for various production traits are presented in Table 5. The hen housed egg production, feed/egg mass, egg weight, body weight, egg specific gravity (1.072-1.075), shell weight (8.71-9.27%), shell thickness (0.397-0.413 mm), tibia breaking strength (82.07-131.06 Newton) and serum Ca concentration were not influenced (p>0.05) by the NPP content in the diet (1.2 to 4.2 g/kg diet; 192 to 672 mg/b/d). The fertility and hatchability of eggs, P content both in egg yolk and in day-old chick, chick body weight at day 1 and 14 d age and leg score of chicks hatched out from broiler breeders were similar on different levels of NPP in diet (1.2 to 4.2 g/kg).

Table 5. Regression coefficients, correlation, and predicted NPP requirement for different production parameters in broiler breeders (25 to 40 weeks of age) fed different NPP levels

Trait	a	b	c	d	R^2	n	NPP*
HH egg production	55.51	12.03	-5.77	0.862	0.020	40	
Feed/egg mass	4.47	0.08	0.151	0.042	0.021	40	
Final body weight	-85.57	793.13	-278.08	30.93	0.017	40	
Egg weight	61.78	-8.92	3.82	-0.482	0.041	40	
Shell breaking strength	-15.08	52.70	-20.17	2.412	0.128[a]	12	
Tibia length	139.38	-10.30	3.96	-0.489	0.045	3	
Tibia strength	-223.69	386.36	-141.68	16.230	0.360	3	
Tibia ash	38.07	27.77	-10.31	1.226	0.564[a]	3	2.25
Ca retention	-191.29	348.99	-64.44	3.537	0.524[a]	3	#
P retention	1547.24	-1059.2	319.35	-34.693	0.807[a]	3	#
Serum Ca	19.81	-6.87	2.80	-0.338	0.018	20	
Serum P	4.33	0.39	0.348	-0.077	0.122[a]	20	3.501
Hatchability	145.76	-70.27	24.168	-2.4883	0.633*	3	
Yolk P	823.62	-99.96	35.54	-3.97	0.017	4	
Chick P	0.58	0.16	-0.063	0.007	0.091	3	
Chick body weight	0.27	-0.023	0.006	-0.0006	0.204	3	
Leg score	0.98	0.008	0.049	-0.011	0.103	4	

* Predicted NPP requirement; [a] ($p\leq0.01$). # Relation is lineally proportional.

The requirement of NPP for broiler breeder females in cages for the above criterion thus appears to be not more than the minimum level employed (1.2 g/kg diet; 192 mg/b/d). The NPP intake of 192 mg/b/d is considerably lower than the calculated NPP requirement of 275 and 319 mg/b/d, as reported by Wilson and Harm (1984) and Wilson et al. (1980), respectively. The higher P requirements suggested by these two groups of authors may be due employment of higher levels of P as the minimum level in their studies.

The variation observed in P requirements in these studies may be due to variation in Ca content in the diet. The levels of Ca used in the reported studies vary greatly from 22.5 (Wilson et al., 1980) to 31.0 g/kg diet (Wilson and Harms, 1984). Higher levels of Ca in WL pullet diet (1.5 to 2.5%) depressed the P utilization and thus increasing (6 to 8 g TP/kg diet) P requirement (Evans and Carver, 1942).

The requirement of NPP to egg type breeders was higher (1.50 to 1.80 g/kg diet) than the values reported in the present study (O'Rourke et al., 1954; Waldroup et al., 1967; Nageswara et al., 2001). White Leghorn breeders require 165 mg NPP/b/d, and 200 mg/egg assuming NPP requirement 1.5 g/kg feed, feed intake of 110 g and egg production of 80%. In the present study, the NPP requirement per egg is worked out at 300 mg/egg with a requirement of 1.29 g NPP/kg diet, 160 g feed intake and 65% egg production. This is higher than the requirement of NPP for WL breeders.

The predicted NPP requirement for shell breaking strength is 2.09 g/kg (Table 5). Thus the requirement of P for shell breaking strength is higher than the required minimum for optimum egg production. Similar to these findings the earlier reports (Roush et al., 1986; Leeson et al., 1993; Rama Rao et al., 1999) also recommended higher P levels for better shell quality compared to optimum egg production.

The predicted NPP requirement for the maximum bone mineralisation was 2.25 g/kg diet (Table 5). Singsen et al. (1962) also reported increased bone mineralisation with increase in dietary NPP from 1.6 to 2.6 g/kg diet. The lower bone mineralisation at P levels less than 2.25 g/kg diet (Table 5) might be due to resorption of P from the bones to maintain the optimum shell quality. It is worth to mention that the breeders did not exhibit any symptoms of lameness and produced eggs with better shell quality when they were fed NPP less than 2.25 g/kg diet. Though the bone ash content was reduced ($p\leq0.01$) at lower P level in the diet, deposition of P in yolk and P content in day old chick and their subsequent performance of chicks were not affected. Other investigators (Waldrop et al., 1975; Rama Rao et al., 1999) also observed higher dietary P requirement for bone ash content than for optimum growth.

Retention of Ca increased ($p\leq0.01$), while retention of P decreased ($p\leq0.01$) with increase in level of NPP in the diet (Table 5). These findings are in line with the findings of earlier studies with broilers (Temperton and Cassidy, 1964; Rama Rao et al., 1999).

Hatchability and performance of progeny

The hatchability was not influenced by the level of NPP in the diet (Table 5), indicating that the similar requirement of NPP for both egg production and hatchability. Waldroup et al. (1967) reported higher P requirement for hatchability (1.93 g/kg diet) compared to optimum egg production (1.62 g/kg diet). In the present study, dietary variation in NPP concentration did not influence the P concentration in egg yolk and day -old chick and leg abnormalities in 14-d old chicks. The P concentration in egg yolk and 1-d old chick were not altered due to variation in the NPP content in the diet (Table 5). Since, the deposition of P in egg yolk did not differ due to variation in dietary NPP content, the hatchability, P content in day -old chick and chick leg score were similar (Table 4) among different levels of NPP tested in the present study. Earlier reports (O'Rourke et al., 1954; Harms et al., 1964) also indicated no difference in P content in egg and mineralisation of chick bones from parents fed various levels of P.

There may be P release from phytate degradation in adult birds and thus, as a result the actual NPP requirement may be higher. However, considering the egg production, shell quality, hatchability of eggs, P content in egg yolk and day old chick and body weight and survivability of chicks, it is concluded that broiler breeders need 1.2 g NPP with a daily intake of 192 mg NPP/b/d. The predicted requirements of NPP are 2.09 g/kg diet for shell breaking strength and 2.25 g/kg diet tibia ash content.

REFERENCES

OAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists (Arlington, USA, Association of Official Analytical Chemists).

Crowley, T. A., M. W. Pasvogel, A. R. Kemmerer, M. G. Vavich and A. A. Kurnick. 1961. Effects of soft phosphate and dicalcium phosphate on reproductive performance and egg quality. Poult. Sci. 40:74-80.

Eeckhout, W. and M. Depaepe. 1994. Total phosphorus, phytate phosphorus and phytase activity in plant Feed stuffs. Anim. Feed Sci. Technol. 47:19-29.

Evans, R. J. and J. S. Carver. 1942. The calcium and phosphorus requirements of Single Comb white Leghorn Pullets. Poult. Sci. 21:469.

Fiske, C. H. and Y. Subba Row. 1925. The colorimetric determination of phosphorus. J. Biol. Chemist. 66:375-400.

Harms, R. H., C. B. Ammerman and P. W. Waldrop. 1964. The effects of supplemental phosphorus in the breeder diet upon hatchability of eggs and bone composition of chicks. Poult. Sci. 43:209-212.

Haugh, W. and H. J. Lantzsch. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric. 34:1423-1426.

Hopkins, J. R., A. J. Ballantyne and J. L. O. Jones. 1989. Dietary phosphorus for laying hens. In: Recent Developments in Poultry Nutrition, Cole, (Ed. D. J. A. and W. Haresign), Butter Worths London, pp. 231-238.

Jongbloed, A. W. H. Everst and P. A. Kemme. 1991. Phosphorus availability and requirements of pigs. In: (Ed. W. Haresign and D. J. A. Cole), Recent Advances in Animal Nutrition. Butterworths, London, pp. 65-80.

Keshavarz, K. and S. Nakajima. 1993. Re-evaluation of calcium and phosphorus requirements of laying hens for optimum performance and egg shell quality. Poult. Sci. 72:144-153.

Keshavarz, K. 2003. The effect of different levels of nonphytate phosphorus with and without phytase on the performance of four strains of laying hens. Poult. Sci. 82:71-91.

Leeson, S., J. D. Summers and L. Caston. 1993. Response of brown egg strain layers to dietary calcium and phosphorus. Poult. Sci. 72:1510-1514.

NRC. 1994. Nutrient requirements of Poultry. 9th Revised Edition, National Research Council, USA.

O'rourke, W. F., H. R. Bird, P. H. Phillips and W. W. Cravens. 1954. The effect of low phosphorus rations on egg production and hatchability. Poult. Sci. 33:1117-1122.

Panda, A. K., S. V. Rama Rao, M. V. L. N. Raju and S. K. Bhanja. 2005. Effects of Dietary Non Phytate Phosphorus Levels on Egg Production, Shell Quality and nutrient retention in White Leghorn layers. Asian-Aust. J. Anim. Sci. 18(8):1171-1175.

Reddy, N. R., S. K. Sathe and D. K. Salunkhe. 1982. Phytates in legumes and cereals. Advances in Feed Res. 28:1-91.

Roush, W. B., M. Mylet, J. L. Rosenberger and J. Derr. 1986. Investigation of calcium and available phosphorus requirement for laying hens by response surface methodology. Poult. Sci. 65:964-970.

Salmon, A. J., M. S. Ali and J. Mcginnis. 1969. Effect of level and sources phosphorus and different levels of productivity on phosphorus utilization by laying hens. Poult. Sci. 48:1004-1009.

Singsen, E. P., A. H. Spandorf, L. D. Matterson, J. A. Serafin and J. J. Tlustohowicz. 1962. Phosphorus in the nutrition of the adult hen 1. Minimum phosphorus requirements. Poult. Sci. 41:1401-1414.

Snedecor, G. W. and W. G. Cochran. 1980. Statistical Methods, Iowa State University Press. America, I.A.

Temperton, H. and J. Cassidy. 1964. Phosphorus requirements of Poultry 1. The utilization of phytin phosphorus by the chicks as indicated by balance experiments. Br. Poult. Sci. 5:75-80.

Waldroup, P. W., R. J. Mitchell and Z. B. Johnson. 1975. The Phosphorus needs of a young broiler chicks in relationship to dietary nutrient density level. Poult. Sci. 54:436-441.

Waldroup, P. W., C. F. Simpson, B. L. Damron and R. H. Harms. 1967. The effectiveness of plant and inorganic phosphorus in supporting egg production in hens and hatchability and bone development in chick embryo. Poult. Sci. 46:659-664.

Watson, L. T., C. B. Ammerman, S. M. Miller and R. H. Harms. 1970. Biological assay of inorganic manganese for chicks. Poult. Sci. 49:1548-1554.

Wilson, H. R. and R. H. Harms. 1984. Evaluation of nutrient specifications for broiler breeders. Poult. Sci. 63:1400-1406.

Wilson, H. R., E. R. Miller, R. H. Harms and B. L. Damron. 1980. Hatchability of chicken eggs as affected by dietary phosphorus and calcium. Poult. Sci. 59:1284-1289.