Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Apr - Jun 2011 / v.13 / n.2 / 157-162

Technical Note

Nutrient Balance of Layers Fed Diets with Different Calcium Levels and the Inclusion of Phytase and/or Sodium Butyrate

Author(s)

Vieira MM[3]
Kessler AM[1]
Ribeiro AML[3]
Silva ICM[2]
Kunrath MA[2]

1 UFRGS Professor.
2 Graduate student. Post-Graduation. Program in Animal Science, UFRGS.
3 UFRGS Professor.

Mail Adress

Alexandre de Mello Kessler
UFRGS Professor
Av. Bento Gonçalves 7712,
91.540-000. Porto Alegre, RS, Brazil.

E-mail: akessler@ufrgs.br

Keywords

Bacterial phytase, brown layers, Ca and P, metabolizability, organic acids.

Submitted: October/2009
Approved: February/2011

ABSTRACT

In this study, Hisex Brown layers in lay were evaluated between 40 and 44 weeks of age to evaluate the inclusion of bacterial phytase (Ph) and sodium butyrate (SB) to diets containing different calcium levels (CaL). Performance, average egg weight and eggshell percentage, in addition to nutrient metabolizability and Ca and P balance were evaluated for 28 days. Birds were distributed according to a completely randomized experimental design with a 3x2x2 factorial arrangement, with three calcium levels (2.8, 3.3, 3.8%); the addition or not of phytase (500PhU/kg) and the addition or not of sodium butyrate (20mEq/kg), composing 12 treatments with eight replicates of one bird each. There was no additive effect of phytase or SB on the evaluated responses. Feed intake and feed conversion ratio were influenced by CaL, with the best performance obtained with 3.3% dietary Ca. Ca balance was positively affected by dietary Ca, and P balance by the addition of phytase. Ca dietary concentration, estimated to obtain Ca body balance, was 3.41%, corresponding to an apparent retention of 59.9% of Ca intake.

INTRODUCTION

Calcium (Ca) requirement estimate for optimal egg production and eggshell quality is 3.25% (NRC, 1994; Rama-Rao *et al*., 2003); however, Chandramoni & Sinha (1998) and Bar *et al*.(2002) suggest higher requirements, of about 3.6% Ca. Keshavarz *et al*. (2003) found even higher needs, of around 3.77g Ca/day for optimal eggshell synthesis, whereas Leeson & Summer (2005) suggest 4.2% Ca, which increases up to 4.6% dietary Ca as birds age.

Dietary Ca is essential for eggshell synthesis, and its needs are affected by the stage of eggshell formation (Etches, 1987). Layers actively absorb dietary Ca for eggshell formation along the entire gastrointestinal tract (Sugiyama *et al*., 2007). Whereas absolute Ca retention increases with increasing dietary Ca levels, the efficiency of the absorption process is inversely related to dietary Ca levels (Chandramoni & Sinha, 1998).

Better egg production was verified in laying hens fed corn-soybean diets supplemented with microbial phytase (Um & Paik, 1999), which allowed the reduction of dietary phosphate levels with no negative effect on egg production and quality (Camps & Pérez, 2004). However, other authors did not find any significant influence of microbial phytase supplementation on commercial layer performance (Casartelli, *et al*. 2005; Liebert *et al*., 2005), although it has already been shown that the effect of phytase supplementation can be changed by dietary Ca levels (Lim *et al*., 2003).

Organic acids had a positive influence on egg production (Gama *et al*., 2000). Organic acids may also positively affect the intestinal mucosa

(García *et al*., 2007) and have a beneficial effect on the intestinal health of poultry (Pirgozliev *et al*., 2008). The addition of sodium butyrate to poultry diets may stimulate the growth of the duodenal mucosa (Hu & Guo, 2007) and may improve dietary energy availability (Pirgolzliev *et al*. 2008), thereby improving nutrient absorption.

Nevertheless, the interaction between phytase and organic acids in layers diets is still poorly elucidated. This study aimed at evaluating the addition of sodium butyrate and phytase addition to diets containing different calcium levels on the performance and apparent Ca and P retention brown layers.

MATERIALS AND METHODS

In this study, Hisex Brown layers in lay were evaluated between 40 and 44 weeks of age to evaluate the inclusion of bacterial phytase and sodium butyrate to diets containing different calcium levels.

Birds were housed in individual cages in an environmentally-controlled room since 36 weeks of age and were offered water and feed *ad libitum* and submitted to a lighting program of 16h of light daily.

Diets were formulated according to the levels proposed by Rostagno *et al*. (2005) for brown layers. The diets were based on corn and soybean meal and contained different Ca levels (CaL) and addition or not of sodium butyrate (SB) and bacterial phytase (Ph – Quantum® 2500, ABEnzymes) (Table 1). Birds were distributed according to a completely randomized experimental design with a 3x2x2 factorial arrangement, with three calcium levels (2.8, 3.3, 3.8%); the addition or not of 500PhU/kg of phytase (as recommended by the manufacturer); and the addition or not of 20mEq/kg sodium butyrate, composing 12 treatments with eight replicates of one bird each, totaling 96 experimental units (Table 2). Available phosphorus level was estimated as 0.40% in all experiment diets, which required the reduction of total phosphorus content in diets containing phytase equivalent to the enzyme contribution of 0.13% available P/kg diet, according to the manufacturer.

Total excreta collection was performed during the entire experimental period (40 to 44 weeks of age) to calculate nutrient metabolizability and mineral balance responses. Comparative slaughter of bird carcasses in the beginning and at the end of the experimental period was used to obtain body nutrient balance responses. Analyses of dry matter, ash, and nitrogen in the diets, excreta and carcasses, and of crude fat in the carcasses were performed according to the methods of the AOAC (1993). Gross energy of diets, excreta, and carcasses was determined using an isoperibolic bomb calorimeter (IKA WERKE, model C2000). Ca and P analyses in feeds and excreta were carried out using atomic absorption spectrophotometry and colorimetry, according to the specifications of Tedesco *et al*. (1995).

Table 1 – Basal diet composition and calculated nutritional composition of the diets fed to brown layers between 40 and 44 weeks of age.

Ingredients	**%**
Basal diet	
Corn	58.73
Soybean meal	25.37
Vegetable fat	3.40
Salt	0.47
DL-Methionine, %	0.24
Choline-Cl, %	0.07
L-Lysine HCl, %	0.03
Mineral premix[1]	0.06
Vitamin premix[2]	0.03
Treatments[3]	11.60
Calculated composition	
Metabolizable energy (kcal/kg)	2900
Crude protein (%)	16.6
Digestible methionine (%)	0.46
Digestible lysine (%)	0.75
Digestible methionine+cystine (%)	0.70
Digestible threonine (%)	0.60
Digestible tryptophan (%)	0.17
Digestible arginine (%)	0.91
Sodium (%)	0.20
Chloride (%)	0.31
Potassium (%)	0.70
Calcium (%)[4]	2.8; 3.3; 3.8
Total phosphorus (%)[4]	0.48; 0.61
Available phosphorus (%)	0.40

1 - Addition per kg diet: selenium 0.3 mg; iodine 0.7 mg; iron 40 mg; copper 10 mg; zinc 80 mg; manganese 80 mg; 2 - Addition per kg diet: Vit A 8000 IU; Vit D3 2000 IU; Vit E 30 mg; Vit K 2 mg; Vit B1 2 mg; Vit B2 6 mg; Vit B6 2.5 mg; Vit B12 0.012 mg; biotin 0.08 mg; pantothenic acid 15 mg; niacin 35 mg; folic acid 1 mg. 3 - Calcium, sodium butyrate and phytase levels according to treatment. 4 - Different according to experimental diets.

The following performance parameters were evaluated for the 28d experimental period: daily feed intake, feed conversion ratio per dozen eggs and per egg mass, egg production, average egg weight, and average eggshell percentage (dried at 105 °C). The metabolizability of dry matter (DMM), organic matter (OMM), mineral matter (MMM), crude protein (CPM) and gross energy (GEM) expressed as intake percentage; Ca balance (CaB) and P balance (PB) expressed in absolute (g/day) and relative (intake percentage) values; Ca and P intake and excretion in g/day; Ca eggshell content (CaShell) on g/day and intake percentage (CaShell%) and Ca body balance in mg/day (CaBB = Ca intake – Ca excretion – Ca in the eggshell) were evaluated. In addition, the nutrient body balance of minerals (MBB), protein (PBB), fat (FBB) were calculated and expressed in g/day, as well as and energy body balance (EBB), expressed in kcal/day.

Table 2 – Composition of the experimental diets containing different calcium levels and the addition or not of phytase and/or sodium butyrate fed to brown layers between 40 and 44 weeks of age.

Calcium levels (%)	**2.8**	**2.8**	**3.3**	**3.3**	**3.8**	**3.8**
Total phosphorus (%)	0.61	0.48	0.61	0.48	0.61	0.48
Ingredients (%)						
Calcitic limestone	6.48	6.92	7.87	8.31	9.27	9.70
Dicalcium phosphate	1.73	1.00	1.73	1.00	1.73	1.00
Kaolin	2.79	3.06	1.40	1.67	-	0.28
Phytase	-	0.02	-	0.02	-	0.02
Sodium butyrate/ Starch[1]	0.60	0.60	0.60	0.60	0.60	0.60

1 - In each diet 0.6% sodium butyrate (20meq/kg sodium butyrate at 29% encapsulated with medium-chain fatty acids) was added in replacement of corn starch.

The statistical model was submitted to analysis of variance using the GLM procedure of SAS statistical package (1999). The means of the main factors and interactions were compared by the test of Student-Newman-Keuls at 5% probability levels. The effects of dietary calcium levels were tested by analysis of regression.

RESULTS AND DISCUSSION

The statistical analysis of performance responses (Table 3) showed that the treatments influenced only feed intake, feed conversion ratio and average egg weight ($p<0.05$). There were no significant interactions for feed intake and feed conversion ratio ($p>0.05$). As to the main effects, only dietary calcium levels affected performance (Table 3). The level of 3.3% Ca resulted in lower feed intake, and considering that egg production was similar among treatments, feed conversion ratio was better. This result is consistent with those of Keshavarz (2003), who observed that 3.34% dietary calcium resulted in adequate performance and eggshell quality in layers after 45 weeks of age, while Chandramoni & Sinha (1998) found that 3.6g/day resulted in optimal daily egg production during lay.

Average egg weight was significantly affected by the interaction among the three studied factors ($p<0.005$): at 3.8% Ca and in when both additives were present in the diet, egg weight was statistically higher (68.2g) than in treatment containing only Ph (64.6g) or SB (64.7g), but similar to the diet with no additives (67.0g), which therefore does not confer any practical application to this interaction. On the other hand, the treatments did not affect egg production, differently from the findings of Um and Paik (1999), who used phytase, and of Gama *et al*. (2000), who used an organic acid blend (fumaic, lactic, citric, and ascorbic acids). Eggshell percentage was not affected by the studied factors, which is in agreement with the results of Lichovnicova (2007), but not with those of Casartellli *et al*. (2005).

There was no effect of sodium butyrate or phytase on calcium balance (Table 4). However, dietary Ca levels affected Ca intake, with a linear and positive effect on relative Ca balance (in g) and on Ca content in the excreta. Calcium levels affected CaB both in g/day and as intake percentage. Linear equations of Ca balance and excretion showed that Ca excretion increased 0.89 g/day, whereas Ca balance increased 0.41 g/day per one percentage

Table 3 – Feed intake (FI), feed conversion ratio (FCR), egg production (EPr) (EPr), average egg weight (AEW) and average eggshell percentage (ES%) of brown layers fed diets containing different calcium levels and the addition or not of phytase and/or sodium butyrate between 40 and 44 weeks of age.

	FI(g/d)	**FCR(kg/dz)**	**FCR(kg/kg)**	**EPr(%)**	**AEW(g)**	**ES%**
Calcium levels (CaL)						
2.8%	117.9a*	1.379a	1.767^{a}	96.0	65.37	9.57
3.3%	112.3b	1.288b	1.645b	97.4	65.35	9.66
3.8%	119.8a	1.390a	1.746^{a}	97.0	66.13	9.71
Sodium butyrate (SB)						
0	116.0	1.344	1.718	96.5	65.18	9.60
20 meq/kg	117.4	1.361	1.721	97.1	66.04	9.70
Phytase (Ph)						
0	118.4	1.379	1.751	96.3	65.23	9.68
500 PhU/kg	115.0	1.325	1.687	97.3	66.00	9.62
Probability						
CaL	0.028	0.015	0.048	0.465	0.424	0.691
SB	0.536	0.591	0.947	0.596	0.113	0.498
Ph	0.161	0.083	0.140	0.266	0.160	0.674
CaLxSB	0.644	0.721	0.786	0.438	0.717	0.856
CaLxPh	0.759	0.866	0.984	0.178	0.894	0.295
SBxPh	0.459	0.183	0.093	0.189	0.368	0.582
CaLxSBxPh	0.671	0.604	0.060	0.804	0.005	0.486
SEM	10.963	0.143	0.200	4.499	3.849	0.650

*Means followed by different letters in the same column are significantly different (SNK $p<0.05$).

point of Ca increase in the diet. Therefore, there was a decreasing effect on Ca retention efficiency as Ca dietary levels increased, which was also found by Chandramoni & Sinha (1998). Moreover, Ca deposition in the eggshell, in g, was similar among diets, which is consistent with the results of Lichovnicova (2007), but the efficiency of Ca deposition in the eggshell as a percentage of intake decreased as dietary Ca increased. Eggshell formation was maintained with the lowest dietary Ca levels, but at the expense of bone Ca, resulting in a negative body Ca balance, as shown by the data (CaBB).

The results of the present study show important features of Ca metabolism in layers and its effects on Ca requirements: at low dietary levels, birds maximize dietary Ca utilization, reducing their excretion. As dietary Ca increases, it higher availability is directly related to higher Ca excretion and, at the same time, bone loss is prevented. In the present experiment, when egg production was maintained, Ca body balance was the best indicator of dietary calcium requirements. The equation Ca body balance = -1.065 + 0.312*CL ($p<0.001$; $R^2= 0.255$) indicated that level of 3.41% dietary Ca is necessary to optimize Ca body balance. When this level is applied in the equation Ca% in the eggshell = 122.46 – 18.326*CL ($p<0.001$; $R^2= 0.691$), the efficiency of Ca retention in the egg was 59.9%, and apparent Ca retention was 59%, as determined by the equation Ca balance % = 88.667 – 8.673*CL ($p<0.001$; $R^2= 0.194$). These values are similar to that of 54.2% proposed by Georgievskii (1982) for 3.08 g/d intake, of 57.8% measured by Lichovnicova (2007) with a diet containing 4.08% Ca; and of 56.1% estimated by Kebreab (2009) with a diet with 3.5% Ca. Even considering that different dietary Ca levels were used in the different experiments and that Ca levels affect the efficiency of apparent retention, the value close to 60% possibly represent the maximum physiological limit of dietary Ca utilization with no impairment of body functions.

The addition of phytase significantly influenced P balance, leading to lower P intake and excretion, consequently improving the efficiency of P retention, although not significantly ($p<0.063$) (Table 5). Better efficiency of Ca and P retention was observed by Um & Paik (1999) with the use of phytase. However, in the present experiment, P balance (PB) was lower, which was expected because the diets containing phytase had lower total P content. According to Liebert *et al*. (2005), the addition of phytase to layer diets did not affect P excretion, whereas Casartelli *et al*. (2005) found reduced P, Ca and N excretion with 1000 PhU/kg. Considering that the level of available P was similar among the experimental diets, the results confirm the

Table 4 – Ca intake, excretion and balance expressed in g/day and as intake percentage, Ca in the eggshell (g/day and intake percentage) and Ca body balance (mg/day of brown layers fed diets containing different calcium levels and the addition or not of phytase and/or sodium butyrate between 40 and 44 weeks of age.

	Ca intake(g/d)	Ca excr^1 (g/d)	CaB^2 (g/d)	CaB^3 (%)	CaES^4 (g)	CaES^5 (%)	CaBB^6 (mg/d)
Ca level							
2.8%	3.26^a	1.15^a	2.11^a	64.75^a	2.33	71.72^a	-184.6^a
3.3%	3.93b	1.60b	2.32b	59.34^a	2.37	60.85b	-49.3b
3.8%	4.53c	1.99c	2.54c	56.19b	2.43	53.46c	128.2c
Sodium butyrate							
0	3.86	1.55	2.31	60.45	2.35	62.65	-30.4
20 meq/kg	3.95	1.61	2.34	59.73	2.40	62.02	-51.8
Phytase (Ph)							
0	3.89	1.57	2.32	59.96	2.38	62.54	-32.1
500 FTU/kg	3.91	1.59	2.33	60.21	2.37	62.13	-50.0
Probability							
CaL	0.001	0.001	0.001	0.001	0.151	0.001	0.001
SB	0.188	0.351	0.704	0.654	0.250	0.734	0.625
Ph	0.759	0.805	0.956	0.875	0.823	0.843	0.622
CaL x SB	0.440	0.523	0.965	0.776	0.946	0.439	0.869
CaL x Ph	0.644	0.414	0.462	0.515	0.454	0.971	0.454
SB x Ph	0.219	0.199	0.924	0.309	0.315	0.997	0.244
CaL x SB x Ph	0.510	0.252	0.161	0.200	0.274	0.592	0.513
SEM	0.316	0.325	0.301	7.375	0.178	5.179	0.225

*Means followed by different letters in the same column are significantly different (SNK $p<0.05$). 1 - Ca excretion = -1.355 + 0.8856*CaL ($p<0.001$; $R^2= 0.570$). 2 - Ca balance = 0.979 + 0.412*CaL ($p<0.001$; $R^2= 0.257$). 3 - Ca balance % = 88.667 – 8.673*CaL ($p<0.001$; $R^2= 0.194$). 4 - Ca in the eggshell g = 2.045 + 0.099*CaL ($p<0.04$; $R^2= 0.052$). 5 - Ca in the eggshell % = 122.46 – 18.326*aCL ($p<0.001$; $R^2= 0.691$). 6 - Ca body balance = -1.065 + 0.312*CaL ($p<0.001$; $R^2= 0.255$).

positive action of phytase both on the excretion and on the efficiency of P retention, showing that these diets promoted the utilization of phosphorus derived from phytate and made available by phytase. Table 5 shows that neither calcium levels nor sodium butyrate influenced P balance responses.

Table 5 – P intake (PInt), P excretion (PExcr) and P balance (PB), expressed in mg/day and P balance expressed as intake percentage of brown layers fed diets containing different calcium levels and the addition or not of phytase and/or sodium butyrate between 40 and 44 weeks of age.

	PInt (mg/d)	PExcr (mg/d)	PB (mg/d)	PB (%)
Calcium level (CaL)				
2.8%	630.4	404.6	225.7	35.97
3.3%	645.7	421.4	224.3	34.67
3.8%	645.0	422.1	228.9	34.70
Sodium butyrate (SB)				
0	633.6	411.1	222.9	35.29
20 meq/kg	646.8	421.1	225.7	34.93
Phytase (Ph)				
0	715.4a*	471.1a	244.3ª	34.05
500 FTU/kg	565.4b	360.7b	204.3b	36.17
Probability				
CaL	0.430	0.232	0.958	0.547
SB	0.244	0.297	0.728	0.750
Ph	0.001	0.001	0.001	0.063
CaL x SB	0.479	0.968	0.373	0.464
CaL x Ph	0.575	0.805	0.579	0.698
SB x Ph	0.342	0.326	0.897	0.672
CaL x SB x Ph	0.589	0.974	0.382	0.509
SEM	0.052	0.045	0.039	5.251

*Means followed by different letters in the same column are significantly different (SNK p<0.05).

Nutrient metabolizability responses were not different as a function of the main effects studied (Table 6). In addition, there was no interaction among CaL, Ph or SB on nutrient utilization.

Nutrient body balance was not affected by the different diets, but, in average, there was negative body balance of minerals and protein with all experimental diets, independently of the tested factors. This somewhat reflects the high egg production observed during the experimental period, when birds used their mineral and protein body reserves for egg production, but presented, compensatorily, body fat and energy gain.

CONCLUSION

Phytase and sodium butyrate did not affect the performance or nutrient metabolizability of layers in lay. Phytase improved P apparent retention. The lowest dietary Ca level promoted the best Ca apparent retention, but resulted in negative Ca body balance. The estimated concentration of dietary Ca for Ca body balance was 3.41% de Ca.

REFERENCES

Association of Official Agricultural Chemistis. Official methods of analysis. 16th ed. Washington; 1993. 1160p.

Bar A, Razaphphkovsky V, Vax E. Re-evaluation of calcium and phosphorus requirements in aged laying hens. British Poultry

Table 6 – Metabolizability of dry matter (DMM), organic matter (OMM), mineral matter (MMM), crude protein (CPM) and gross energy (GEM), and body balance of minerals (MBB), protein (PBB), fat (FBB) and energy (EBB) of brown layers fed diets containing different calcium levels and the addition or not of phytase and/or sodium butyrate between 40 and 44 weeks of age.

	DMM %	OMM %	MMM %	CPM %	GEM %	MBB mg/d	PBB mg/d	FBB g/d	EBB kcal/d
Calcium level (CaL)									
2.8%	72.07	77.23	41.28	47.86	80.13	-348	-96.8	2.28	20.9
3.3%	72.01	77.14	41.42	46.87	79.75	-308	-236	2.35	20.6
3.8%	72.30	77.59	40.38	46.60	79.97	-277	-12.1	2.86	26.6
Sodium butyrate (SB)									
0	71.90	77.06	40.80	47.16	79.59	-293	-36.1	2.31	21.4
20 meq/kg	72.35	77.59	41.25	47.06	80.31	-331	-200	2.66	23.7
Phytase (Ph)									
0	72.02	77.12	41.46	46.82	79.77	-301	-303	2.40	20.5
500FTU/kg	72.24	77.52	40.60	47.40	80.13	-324	60.4	2.57	24.6
P									
CaL	0.903	0.742	0.756	0.578	0.782	0.435	0.802	0.509	0.421
SB	0.413	0.279	0.704	0.927	0.120	0.366	0.517	0.402	0.581
Ph	0.693	0.421	0.475	0.584	0.435	0.616	0.185	0.719	0.320
CaLxSB	0.850	0.694	0.665	0.677	0.837	0.462	0.634	0.582	0.390
CaLxPh	0.771	0.878	0.409	0.979	0.901	0.150	0.987	0.381	0.416
SBxPh	0.346	0.450	0.235	0.615	0.328	0.161	0.978	0.853	0.899
CaLxSBxPh	0.624	0.829	0.124	0.483	0.761	0.176	0.560	0.091	0.240
SEM	2.56	2.30	5.59	4.901	2.14	0.19	1.22	1.87	17.89

Science 2002; 43:261–269.

Casartelli EM, Junqueira OM, Laurentiz AC, Filardi RS, Lucas Junior J, Araújo LF. Effect of phytase in laying hen diet whith different phosphorus sources. Revista Brasileira de Ciência Avícola 2005; 7(2):93-98.

Chandramoni SBJ, Sinha RP. Effect of dietary calcium and phosphorus concentration on retention of these nutrients by caged layers. British Poultry Science 1998; 39:544-548.

Camps DM, Pérez J. Utilización de fitasa en la dieta para ponedoras White Leghorn. Revista Cubana de Ciência Avícola 2004; 28:1-4.

Etches RJ. Calcium logistics in the laying hen. Journal of Nutrition 1987; 117:619-628.

Gama NMSQ, Oliveira MBC, Santin E, Berchieri Jr A. Ácidos orgânicos em rações de poedeiras comerciais. Ciência Rural 2000; 30(3):499-502.

García V, Catalá-Gregori P, Hernandéz F, Megías MD, Madrid J. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. Journal of Applied Poultry Research 2007; 16:555–562.

Hu Z, Guo Y. Effects of dietary sodium butyrate supplementation on the intestinal morphological structure, absorptive function and gut flora in chickens. Animal Feed Science and Technology 2007; 132:240-249.

Keshavarz KA. Comparison between cholecalciferol and 25-OH-cholecalciferol on performance and eggshell quality of hens fed different levels of calcium and phosphorus. Poultry Science 2003; 82:1415–1422.

Leeson S, Summer J. Comercial poultry nutrition. 3rd ed. Guelph: University Books; 2005. 411p.

Lichovnikova M. The effect of dietary calcium source, concentration and particle size on calcium retention, eggshell quality and overall calcium requirement in laying hens. British Poultry Science 2007; 48(1):71-75.

Lim HS, Namkung H, Paik IK. Effects of phytase supplementation on the performance, egg quality, and phosphorous excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorous. Poultry Science 2003; 82:92–99.

Liebert F, Htoo JK, Sünder A. Performance and nutrient utilization of laying hens fed low-phosphorus corn-soybean and wheat-soybean diets supplemented with microbial phytase. Poultry Science 2005; 84:1576–1583.

National Research Council. Nutrient requirements of poultry. 9th ed. Washington, D.C.: National Academic Press; 1994. 155p.

Pirgozliev V, Murfhy TC, Owens B, George J, McCann MEE. Fumaric and sorbic acis as additives in broiler feed. Research in Veterinary Science 2008; 84:387-394.

Rama Rao SV, Panda AK, Raju MVLN, Shyam Sunder G, Praharaj NK. Requirement of calcium for commercial broilers and white leghorn layers at low dietary phosphorus levels. Animal Feed Science and Technology 2003; 106:199–208.

Rostagno HS, Albino LFT, Donzelle JL, Gomes PC, Oliveira RF, Lopes DC, Ferreira AS, Barreto SLT. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 2° ed. Viçosa: UFV, Departamento de Zootecnia; 2005. 186p.

SAS Institute. SAS/STAT user’s guide. Cary, NC: SAS Institute; 1999. 943p.

Sugiyama T, Kikuchi H, Hiyama S, Nishizawa K, Kusuhara S. Expression and localisation of calbindin D28k in all intestinal segments of the laying hen. British Poultry Science 2007; 48(2): 233-238.

Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ. Análises de solos, plantas e outros materiais. 2° ed. Porto Alegre: Departamento de Solos da UFRGS; 1995. 174p.

Um JS, Paik IK. Effects of microbial phytase supplementation on egg production, eggshell quality, and mineral retention of laying hens fed different levels of phosphorus. Poultry Science 1999; 78: 75-7.