Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2636
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v35i2.16555

Calcium in pre-laying and laying rations on the performance and quality of laying hens' eggshell

Eliana Aparecida Rodrigues[1*], Maria Cristina de Oliveira[2], Luciana Cardoso Cancherini[3], Karina Ferreira Duarte[4], Luis Fernando Santana[1] and Otto Mack Junqueira[4]

*[1]Curso de Zootecnia, Instituto Federal do Triangulo Mineiro, Rua João Batista Ribeiro, 4000, 38064-790, Uberaba, Minas Gerais, Brazil. [2]Faculdade de Medicina Veterinária, Universidade de Rio Verde, Rio Verde, Goiás, Brazil. [3]Departamento de Zootecnia, Universidade do Estado de Mato Grosso, Pontes e Lacerda, Mato Grosso, Brazil. [4]Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil. *Author for correspondence. E-mail: eliana@iftm.edu.br*

ABSTRACT. The experiment evaluated the effect of different calcium (Ca) levels in pre-laying and laying diets on the performance and egg quality of laying hens. Two hundred and fifty-six 16-week-old Brown Hisex pullets were housed in a conventional shed. The experiment had a completely randomized design with 4 x 2 factorial arrangement (four calcium levels - 0.8, 1.3, 1.8 and 2.3% in pre-laying phase; two calcium levels - 2.5 and 3.5% in laying phase), totaling eight treatments with four repetitions of eight birds per experimental unit. The experiment started with 16-week-old hens receiving pre-laying diets provided until they were 18 weeks old. At this period, the laying diets started. Four 28-day cycles were evaluated for the following variables: production and egg weight, feed intake, feed conversion, mass of eggs and eggshell quality. Results show that a good performance was obtained with 0.8% calcium level in the pre-laying phase, whereas in the laying phase the 3.5% calcium level provided greater weight egg and better eggshell quality.

Keywords: egg mass, egg production, eggshell quality, feed intake, pullets.

Cálcio nas rações de pré-postura e postura sobre o desempenho e qualidade de casca dos ovos de poedeiras comerciais

RESUMO. O ensaio experimental objetivou avaliação do efeito de diferentes níveis de cálcio (Ca) nas rações de pré-postura e postura sobre o desempenho e qualidade dos ovos de poedeiras comerciais. Foram alojadas 256 frangas da linhagem Hisex Brown com 16 semanas de idade em galpão convencional de postura. O delineamento experimental foi inteiramente casualizado em arranjo fatorial 4 x 2 (4 níveis de cálcio - 0,8; 1,3; 1,8 e 2,3% na pré-postura e 2 níveis de cálcio - 2,5 e 3,5% na postura), totalizando oito tratamentos com quatro repetições de oito aves por unidade experimental. Iniciou-se com a ração de pré-postura às 16 semanas de idade das aves, sendo que a mesma foi fornecida até as aves atingiram 18 semanas de idade, quando então, iniciou-se com a ração de postura. Foram avaliadas durante quatro ciclos de 28 dias as seguintes variáveis: produção e peso dos ovos, consumo de ração, conversão alimentar, massa de ovos e qualidade da casca dos ovos. Através dos resultados obtidos pode-se concluir que um bom desempenho foi obtido com 0,8% de cálcio na fase de pré-postura. Na fase de postura o nível de 3,5% de cálcio promoveu maior peso de ovo e melhor qualidade de casca.

Palavras-chave: massa de ovo, produção de ovos, qualidade de casca, consumo de ração, frangas.

Introduction

Year after year laying hens are becoming more productive, largely due to great success in genetic improvements. However, this improvement in productivity has made the animals increasingly demanding in nutrition, health and management. From the nutritional point of view, one important requirement is undoubtedly that related to increased egg production and improvement in eggshell quality. The above means an increase in the contribution requirements and the balance of nutrients that comprise the egg's composition or participation in the process of its formation.

So that egg-laying may be highly productive, great sensitivity should be available with regard to variations of the diet's nutritional levels (protein, amino acids, vitamins and minerals). Calcium is a macro element because it is a critical nutrient which ensures the production of eggs with good quality eggshells and, similar to other nutrients, must have adequate levels and be well balanced in rations. However, the ability to leverage calcium in feed varies from individual to individual.

Factors such as protein level, chelates, intestinal pH (acidity favors absorption), phosphate (high Ca/P increases the formation of calcium phosphate),

free fatty acids (whose buildup in intestinal tract causes the formation of insoluble calcitic soaps and reduces the absorption of calcium) and vitamin D, are directly related to the absorption of calcium which is active in all segments of the intestine, mainly in the duodenum and jejunum, whose absorption speed is greater than that of any other ion. According to Berne and Levy (1988), cited by Rutz (1994), it is actually inferior only to that of sodium.

It is estimated that losses caused by bad eggshell quality throughout the production cycle ranges from 7 - 8% (HAMILTON; CIPERA, 1981) to 13 - 20% (ROLAND, 1988).

According to Maynard et al. (1984), calcium, which is considered the greatest factor of quality deterioration of eggshell, affects its own absorption, i.e. when the level is high, it decreases its absorption effectiveness. Clunies and Leeson (1995) have already emphasized that the intestinal absorption of calcium is amended according to the shell gland activity (uterus), i.e. when it is inactive, without the presence of egg, calcium absorption by the intestine is around 40%; when it is active, absorption efficiency reaches above 70%.

So that laying hens may develop their full productivity potential at the production stage, the pre-lay phase should be taken into consideration. Albeit short, this phase lasts about two weeks and has triggered controversy over the years with respect to calcium levels to be used at this stage. A good feeding program for the pre-lay phase should take as a basis the animals' nutritional requirements and such requirements should be evaluated so that this may occur.

At the beginning of the productive period, pullets mobilize calcium from the bones in the formation of the eggshell. The above fact may result in an increase in the incidence of cage fatigue during the later production cycle. Calcium removal from the bones in the formation of the eggshell at the beginning of the laying phase causes the pullets to be in a negative calcium balance (MORGAN; MITCHEL, 1938), which cannot be relieved by increasing the level of dietary calcium (HURWITZ; GRIMINGER, 1960). Consequently, the presence of an adequate reserve in bones at the beginning of the production cycle is crucial in reducing cage fatigue, in maintaining a good egg production and, above all, in the formation of eggs with a good eggshell quality.

In his research, Bertechini (2006) concluded that an intermediate level of calcium must be supplied to the birds in the pre-laying phase diets and discarded the same calcium level used in the egg-laying phase. The author draws attention to the fact that this intermediate level of calcium should not be administered for long periods (plus 5% egg production), as this may compromise the animals' spinal spare calcium effectively in production. However, if the pullets receive low calcium level during the pre-laying phase, those with a higher early development will consume more food to meet their calcium needs. This fact results in fatty liver, an excessive fat deposition, decreased quality of the eggshell and rise in feeding costs.

The above statements disagree with results obtained in studies by Classen and Scott (1981) who found that ingestion of intermediate levels of calcium by chickens reared during the period affected calcium storage in bone marrow 19 days before the start of the laying period and training of the eggshell during the laying period. The average level of dietary calcium ingested was 2.08 and 1.18% respectively for the initial and growing phases.

In studies conducted by Hurwitz and Bar (1970), it became clear that a pre-laying diet must be supplemented with calcium and phosphorus since supplementation tended to facilitate bone mineralization and consequently improved eggshell quality. In fact, eggshell weight per unit area increased considerably during the first month of the laying phase, although the parameters of production were not affected.

So that low calcium content (0.9 to 1.5%) could be provided to animals at the beginning of the laying period, Leeson et al. (1986) found that, although they had low eggshell quality, after the 40th egg, or rather, when all the birds had been fed with diet containing calcium, good 3.5% eggshell quality was obtained, with no harmful effects to production.

Calcium recommendations for the young birds (from 6 to 18 weeks) vary greatly, depending on the source consulted, i.e., Leeson and Summers (1997), NRC (1994) and Rostagno et al. (2011) recommend respectively 2.0-0.80, 0.80 and 0.80-0.83% calcium. However, calcium requirement may increase with age, due to the animal's ability to store calcium for the egg formation to be limited and the amount of calcium deposited in the eggshell increase slightly. Therefore, the definition of calcium needs in diets for chickens reared for laying is a challenge for nutritionists (ROLAND, 1986a and b).

Current experiment verifies which calcium levels used in pre-laying and laying phases have the best performance results in eggshell quality of laying hens.

Material and methods

Current experiment evaluates the effect of levels of calcium (Ca) for laying hens. Two hundred and fifty-

six 16-week-old Hisex Brown pullets were used. They were divided into 32 experimental plots for a period of 30 days, plus four periods of 28 days each, during 142 days in a completely randomized 4 x 2 factorial arrangement design. There were eight treatments with four replicates each and each repetition comprised eight hens. The experimental diets, iso-nutritious for the other nutrients, consisted of four levels of calcium (0.8, 1.3, 1.8 and 2.3%) in the pre-laying phase and two levels of calcium (2.5 and 3.5%) in the laying phase. The experimental diets were formulated in accordance with the requirements to each productive phase following recommendations by NRC (1994). Table 1 shows the percentage composition and nutritional levels.

Table 1. Composition of experimental diets.

Ingredients (%)	Calcium level (%)					
	Pre-lay phase				Laying phase	
	0.8	1.3	1.8	2.3	2.5	3.5
Corn	68.54	68.68	68.68	68.68	58.82	58.05
Soybean meal	18.74	18.49	18.49	18.49	28.38	28.28
Wheat bran	4.00	4.00	4.00	4.00	-	-
Limestone	0.56	1.95	3.26	4.58	5.03	7.66
Soybean oil	1.50	1.50	1.50	1.50	2.76	2.89
Di-calcium phosphate	2.19	2.02	2.02	2.02	2.05	2.05
Suppl. vit. + min.[1]	0.20	0.20	0.20	0.20	0.50	0.50
Salt	0.42	0.38	0.38	0.38	0.45	0.46
DL-methionine	-	-	-	-	0.10	0.11
Inert (Washed sand)	3.85	2.78	1.47	0.15	1.91	-
Calculated composition	100.00	100.00	100.00	100.00	100.00	100.00
Metabolizable energy (kcal kg^{-1})	2.900	2.900	2.900	2.900	2.900	2.900
Crude protein (%)	15.10	15.00	15.00	15.00	18.00	18.01
Calcium (%)	0.81	1.30	1.80	2.30	2.50	3.50
Total phosphorus (%)	0.73	0.70	0.70	0.70	0.70	0.69
Available phosphorus (%)	0.49	0.46	0.46	0.464	0.47	0.48
Met + cist. (%)	0.53	0.52	0.52	0.52	0.70	0.70
Methionine (%)	0.25	0.25	0.25	0.25	0.39	0.39
Sodium (%)	0.20	0.19	0.19	0.19	0.22	0.22

Recommended by NRC (1994). [1]Vitamin and mineral supplement-prelay: Composition per kg of product: Vit. A- 10,000 IU; Vit.D_3-2,000 IU; Vit. E-13 mg; Vit. K_3-2.5 mg; Vit. B_1 -1.5 mg; Vit. B_2 .5 mg; Vit. B_6-2 mg; Vit. B_{12}-15 mcg; Folic acid -0.8 mg; pantothenic acid-10 mg; biotin -0.1 mg; niacin -33 mg; Co-0.1 mg; Cu-6 mg; Fe-50 mg; I-1 mg; Se-0.2 mg; Mn-65 mg; Zn-50 mg; antioxidant -2 mg. Vitamin and mineral supplement-laying: Composition per kg of product: Vit. A-4,500 IU; Vit.D_3 - 2,200 IU; Vit. E-5 mg; Vit. K_3-0.5 mg; Vit. B_2 .2 mg; Vit. B_{12}-10 mcg; calcium pantothenate -2.2 mg; niacin -10 mg; coline -250 mg; Cu-75 mg; Fe-50 mg; I-0.3 mg; Se-0.1 mg; Mn-30 mg; Zn-50 mg; antioxidant -0.625 mg.

Pre-laying phase diets were provided until the end of the 17th week and subsequently replaced by laying phase diets. After the beginning of the period and at the end of each 28-day period, the following parameters were taken into account: production of eggs (% egg hen^{-1} day^{-1}), feed intake (g hen^{-1} day^{-1}), feed conversion (consumption of feed-kg $dozen^{-1}$ produced eggs and egg consumption of feed-kg kg^{-1} of egg mass), egg mass (g), percentage of eggshell, eggshell thickness (mm) and specific gravity (g mL^{-1} H_2O). The egg mass was obtained by multiplying the average weight of eggs by the percentage of the production of the same. Dead poultry and food scraps were accounted to adjust consumption, egg production and food conventions per mass and per dozen eggs.

The eggshell quality was analyzed by three eggs per plot produced over the previous two days of each experimental period. The specific gravity was calculated by using all healthy eggs produced in the previous two days of each period. Saline solutions were prepared according to Moreng and Avens (1990), with appropriate adjustments to a volume of 25 liters of water. The range of density of the solutions was 1.065 at 1.100 intervals of 0.005. Shell thickness was determined by measuring three points in the egg's equatorial region with a Mitutoyo micrometer.

Data were statistically analyzed by GLM (General Linear Model) of SAEG 4.0 program (SAEG, 1982). For the significant effect of calcium levels in the polynomial regression and pre-laying phase for the calcium levels in the laying phase, averages were compared by Tukey test at 5% probability.

Results and discussion

Table 2 shows the average results of the productive performance of layers according to the different calcium levels.

Table 2. Calcium levels on the performance of laying hens.

Calcium (%)	FI [1] (g $hen^{-1}day^{-1}$)	EP (% $hen^{-1}day^{-1}$)	EW (g)	EM (g)	FCR (kg feed kg^{-1} egg)	FCR (kg feed dz^{-1} egg)
Effects of calcium levels – pre-laying phase						
0.8	104.40	80.44	58.76	47.65	2.28	1.63
1.3	102.57	78.18	58.85	46.45	2.32	1.65
1.8	100.44	79.37	57.86	46.48	2.26	1.59
2.3	101.56	77.87	59.13	46.58	2.32	1.66
Effects of calcium levels – laying phase						
2.5	102.47	78.25	58.15b	46.11	2.35	1.65
3.5	102.01	79.68	59.14a	47.65	2.24	1.61
Values of F						
Pre-laying	0.93 ns[2]	0.43 ns	1.32 ns	0.32 ns	0.24 ns	0.53 ns
Laying	0.69 ns	0.64 ns	4.33 *[3]	1.74 ns	0.38 ns	1.20 ns
Prelay x Laying	0.52 ns	1.32 ns	0.23 ns	1.23 ns	1.06 ns	1.71 ns
CV (%)[4]	4.82	6.39	2.29	6.21	7.09	7.33

Means in columns followed by different letters are different ($p < 0.05$) by Tukey test at 5% probability. [1]Abbreviations: FI-feed intake: EP-Egg production; EW-egg weight; EM-egg mass; FCR-feed conversion ratio. [2]ns- not significant ($p > 0.05$), [3]$p < 0.05$. [4]Coefficient of variation.

Excluding the weight of the eggs, the variables under analysis were not affected ($p > 0.05$) for calcium levels provided during the pre-laying and laying periods. Although the weight of eggs was not influenced by calcium levels provided in the pre-laying phase, greater egg weight was obtained with 3.5% level of calcium in the laying phase. This result does not agree with that obtained by Castillo et al. (2004) who found no significant difference in the weight of eggs when applying five calcium levels for laying hens as from 23 weeks old.

When the level of dietary calcium was increased, Keshavarz and Nakajima (1993) and Hawes and Kling (1993) failed to notice significant change in egg weight when different levels of calcium in the diet at various stages of production were provided.

The effect of calcium levels used in the pre-laying phase under most of the performance parameters of the production phase indicated that 0.8% calcium level during the pre-lay phase was enough for a good productive performance range. These comments were contrary to one of the alternatives offered by Leeson and Summers (1997) that a 1.0% calcium level was sufficient, provided it was maintained until the hens reach 5% of egg production. Working with twenty-five 49-week-old laying hens, Araújo et al. (2011) found that a better performance was achieved by adding calcium and limestone at 4.12% thick granulometry (1.00 mm).

Table 3 demonstrates that the isolated effect of calcium levels used in the pre-laying phase was not observed for all the quality features of the eggshell ($p > 0.05$). On the other hand, best results were obtained in eggshell thickness and specific gravity at 3.5% calcium level during the laying stage. Results similar to those obtained in current experiment were reported by Castillo et al. (2004), who also concluded that hens fed at the lowest calcium level produced eggs with lower specific gravity when compared to those fed on the highest calcium level. Hartel (1989) found that increasing dietary calcium (2.0, 2.5, 3.0, 3.5, 4.0 and 4.5%) for laying hens from 22 weeks of age produced a better shell thickness with 3.0% calcium level. As from this level, average results for this characteristic do not differ statistically.

In their work with three levels of calcium (3.75, 4.15 and 4.55%) in laying phase diets, Murata et al. (2009) concluded that increases in calcium levels improved the egg weight and eggshell thickness.

Eggshell percentage reported significant interaction between calcium levels used in the pre-laying and laying phases (Table 3). Table 4 shows the unfolding of this interaction.

Table 3. Calcium levels on eggshell quality of laying hens.

Calcium levels (%)	Percent shell (%)	Shell thickness (mm)	Specific gravity (g mL^{-1} de H_2O)
	Effects of calcium levels – pre-laying phase		
0.8	9.40	0.360	1.0896
1.3	9.31	0.361	1.0898
1.8	9.30	0.356	1.0901
2.3	9.27	0.356	1.0898
	Effects of calcium levels – laying phase		
2.5	9.06	0.349 b	1.0879 b
3.5	9.57	0.368 a	1.0918 a
	Values of F		
Prelay	1.97 ns[1]	1.15 ns	0.37 ns
Laying	152.23 ★★[2]	58.76 ★★[2]	151.78 ★★[2]
Prelay x Laying	3.56 ★[3]	0.62 ns	2.10 ns
CV (%)[4]	1.26	1.95	0.08

Means in columns followed by different letters are different ($p < 0.05$) by Tukey test at 5% probability. [1]ns- not significant ($p > 0.05$); [2]$p < 0.01$; [3]$p < 0.05$. [4]Coefficient of variation.

Table 4. The deployment of significant interaction between calcium levels in the pre-laying and laying phases on eggshell percentage.

Calcium levels – laying (%)	Calcium levels - pre-lay (%)			
	0.8	1.3	1.8	2.3
2.5	9.08 B	9.00 B	9.13 B	9.06 B
3.5[1]	9.73 A	9.63 A	9.47 A	9.48 A

Means in columns followed by different letters are different ($p < 0.05$) by Tukey test at 5% probability. [1]Linear effect: $\hat{Y} = 9.8628 - 0.1845X$, $R^2 = 0.88$.

Regardless of the calcium level used in the pre-laying phase, the 3.5% level in the laying phase determined the best eggshell percentage. The 3.5% calcium level in the laying phase showed that there was a decreasing linear effect for eggshell percentage on the junction of calcium levels used in the pre-laying phase ($\hat{Y} = 9.8628 - 0.1845X$, $R^2 = 0.88$), so that an increase in calcium level in the pre-laying phase tended to decrease eggshell percentage. These results are not in accordance to those by Chowdhury and Smith (2002) who found that increasing levels (2.5, 3.0, 3.5 and 4.0%) of calcium in the diet of 30-week-old laying hens caused a linear increase in eggshell percentage.

Conclusion

A 0.8% level in the pre-laying phase was enough to ensure good performance and eggshell quality during the laying phase. In the case of the laying phase, best results were obtained with 3.5% calcium level.

References

ARAÚJO, J. A.; SILVA, J. H. V.; COSTA, F. G. P.; SOUSA, J. M. B.; GIVISIEZ, P. E. N.; SAKOMURA, N. K. Effect of the levels of calcium and particle size of limestone on laying hens. **Revista Brasileira de Zootecnia**, v. 40, n. 5, p. 997-1005, 2011.

BERNE, R. B.; LEVY, M. N. **Physiology**. 2nd ed. St Louis: Mosby Company, 1988.

BERTECHINI, A. G. **Nutrição de monogástricos**. Lavras: Editora UFLA, 2006.

CASTILLO, C.; CUCA, M.; PRO, A.; GONZÁLEZ, M.; MORALES, E. Biological and economic optimum level of calcium in white Leghorn laying hens. **Poultry Science**, v. 83, n. 6, p. 868-872, 2004.

CHOWDHURY, S. R.; SMITH, T. K. Dietary interaction of 1.4-diaminobutane (Putrescine) and calcium on eggshell quality and performance in laying hens. **Poultry Science**, v. 81, n. 1, p. 84-91, 2002.

CLASSEN, H. L.; SCOTT, T. A. Self-Selection of calcium during the rearing and early laying periods of white Leghorn pullets. **Poultry Science**, v. 61, n. 10, p. 2065-2074, 1981.

CLUNIES, M.; LEESON, S. Effect of dietary calcium level on plasma proteins and calcium flux occurring during a 24h ovulatory cycle. **Canadian Journal of Animal Science**, v. 57, n. 3, p. 439-444, 1995.

HAMILTON, R. M. G.; CIPERA, J. D. Effects dietary calcium levels during the brooding rearing, and early laying period on feed intake, egg production, and shell quality of Leghorn hens white. **Poultry Science**, v. 60, n. 2, p. 349-357, 1981.

HARTEL, H. Evaluation of the dietary interaction of calcium and phosphorus in the high producing laying hen. **British Poultry Science**, v. 31, n. 4, p. 473-494, 1989.

HAWES, R. O.; KLING, L. J. The efficacy of using prelay and early-lay rations for Brown-egg pullets. **Poultry Science**, v. 72, n. 9, p. 1641-1649, 1993.

HURWITZ, S.; BAR, A. The effect of pre-laying mineral nutrition on the development, performance and mineral metabolism of pullets. **Poultry Science**, v. 50, n. 4, p. 1044-1055, 1970.

HURWITZ, S.; GRIMINGER, P. Observation on the calcium balance of laying hens. **Journal of Agricultural Science**, v. 54, n. 3, p. 733-737, 1960.

KESHAVARZ, K.; NAKAJIMA, S. Re-evaluation of calcium and phosphorus requirements of laying hens for optimum performance and eggshell quality. **Poultry Science**, v. 72, n. 1, p. 144-153, 1993.

LEESON, S.; JULIAN, R. J.; SUMMERS. J. D. Influence of prelay and early-lay dietary calcium concentration on performance and bone integrity of Leghorn pullets. **Canadian Journal of Animal Science**, v. 66, n. 4, p. 1087-1095, 1986.

LEESON, S.; SUMMERS, J. D. **Commercial Poultry Nutrition**. 2nd ed. Canada: University Books, 1997.

MAYNARD, L. A.; LOOSLI, J. K.; HINTZ, H. F.; WARNER, R. G. **Nutrição Animal**. 3. ed. Rio de Janeiro: Freitas Bastos, 1984.

MORENG, R. E.; AVENS, J. S. **Ciência e produção de aves**. São Paulo: Livraria Roca Ltda., 1990.

MORGAN, C. L.; MITCHEL, J. H. The calcium and phosphorus balance in laying hens. **Poultry Science**, v. 17, n. 2, p. 99-104, 1938.

MURATA, L. S.; ARIKI, J.; SANTANA, A. P.; JARDIM FILHO, R. M. Níveis de cálcio e granulometria do calcário sobre o desempenho e a qualidade da casca de ovos de poedeiras comerciais. **Biotemas**, v. 22, n. 1, p. 103-110, 2009.

NRC-National Research Council. **Nutrient requirements of poultry**. 9th Revised Edition. Washington, D.C.: National Academy of Science, 1994.

ROLAND, D. A. Eggshell quality. III. Calcium and phosphorus requirements of commercial leghorns. **World's Poultry Science Journal**, v. 42, n. 2, p. 154-165, 1986a.

ROLAND, D. A. Eggshell quality. IV. Oyster shell versus limestone and the importance of particle size or solubility of calcium source. **World's Poultry Science Journal**, v. 42, n. 2, p. 166-171, 1986b.

ROLAND, D. A. Eggshell problems. Estimates of incidence and economic impact. **Poultry Science**, v. 67, n. 12, p. 1801-1803, 1988.

ROSTAGNO, H. S.; ALBINO, L. F. T.; DONZELE, J. L.; GOMES, P. C.; OLIVEIRA, R. F.; LOPES, D. C. FERREIRA, A. S.; BARRETO, S. L. T.; EUCLIDES, R. F. **Tabelas Brasileiras para Aves e Suínos**: composição de alimentos e exigências nutricionais. Viçosa: UFV, 2011.

RUTZ, F. Absorção de minerais e vitamina. In: Fundação Apinco de Ciencia e Tecnologias Avícolas. **Fisiologia da digestão e absorção das aves**. Campinas: Facta, 1994. p. 83-98.

SAEG-Sistema para análise estatística e genética. **Manual do Usuário**. Viçosa: UFV, 1982.

Received on March 28, 2012.
Accepted on June 18, 2012.