



## Inclusion of leucaena leaf hay in the diet of laying hens during the growing phase

Alexsandro Nunes de Oliveira\*, Ednardo Rodrigues Freitas, Carlos Eduardo Braga Cruz, Thales Marcel Bezerra Figueira, Germano Augusto Jerônimo do Nascimento and Raffaella Castro Lima

Departamento de Zootecnia, Centro de Ciências Agrárias, Universidade Federal do Ceará, Rua Campus do Pici, s/n, Bloco 810, 60440-554, Fortaleza, Ceará, Brazil. \*Author for correspondence. E-mail: alexsandro.n.o@gmail.com

**ABSTRACT.** Current experiment evaluated the effects of inclusion of leucaena leaf hay (LLH) on the performance and nutrient digestibility of diets for laying hens during the growth phase (14-18 weeks). Ninety pullets (Rhode Island Red and New Hampshire) were distributed in a completely randomized design with three treatments (0%, 5% and 10% inclusion of LLH) and five replicates, with six birds. Feed intake ( $\text{g bird}^{-1} \text{day}^{-1}$ ), weight gain ( $\text{g bird}^{-1} \text{day}^{-1}$ ), feed conversion ( $\text{kg kg}^{-1}$ ), metabolizable energy intake ( $\text{kcal bird}^{-1} \text{day}^{-1}$ ), intake of crude protein ( $\text{g bird}^{-1} \text{day}^{-1}$ ), coefficients of dry matter (CDDM) and gross energy (CDCE), nitrogen digestibility (CDN), apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen (AMEn) were evaluated. The inclusion of LLH did not statistically influence CDN, AME and AMEn of diet. However, this inclusion significantly affected CDDM and CDCE, resulting in lower CDDM and CDCE with inclusion of 10%. Whereas the use of nutrients by chicks fed on diets with the inclusion of LLH allowed the same amount of metabolizable energy, inclusion of up to 10% of LLH diet during the growth phase (14-19 weeks) of laying hens (Rhode Island Red and New Hampshire) may be recommended.

**Keywords:** alternative food, poultry, fiber, leucaena, nutrition.

## Inclusão de feno da folha de leucena na ração de poedeiras na fase de crescimento

**RESUMO.** O experimento avaliou os efeitos da inclusão do feno da folha de leucena (FFL) sobre o desempenho e digestibilidade dos nutrientes das rações para poedeiras em crescimento (14 a 18 semanas de idade). Utilizaram-se 90 frangas (Rhode Island Red e New Hampshire), distribuídas em delineamento inteiramente casualizado com três tratamentos (0%, 5% e 10% de inclusão do FFL) e cinco repetições de seis aves. Avaliou-se o consumo de ração ( $\text{g ave}^{-1} \text{dia}^{-1}$ ), o ganho de peso ( $\text{g ave}^{-1} \text{dia}^{-1}$ ), a conversão alimentar ( $\text{kg kg}^{-1}$ ), a ingestão de energia metabolizável ( $\text{kcal ave}^{-1} \text{dia}^{-1}$ ), a ingestão de proteína bruta ( $\text{g ave}^{-1} \text{dia}^{-1}$ ) e os coeficientes de digestibilidade da matéria seca (CDMS), da energia bruta (CDEB), do nitrogênio (CDN), energia metabolizável aparente (EMA) e energia metabolizável aparente corrigida para nitrogênio (EMAn). A inclusão de FFL não influenciou estatisticamente CDN, EMA e EMAn da ração. Entretanto, influenciou significativamente os CDMS e CDEB, resultando em menor CDMS e CDEB com inclusão de 10%. Considerando que o aproveitamento dos nutrientes da ração pelas aves alimentadas com a inclusão do FFL possibilitou o mesmo valor de energia metabolizável pode-se recomendar a inclusão de até 10% do FFL na ração de crescimento (14 a 19 semanas de idade) para poedeiras (Rhode Island Red e New Hampshire).

**Palavras-chave:** alimento alternativo, aves, fibra, leucena, nutrição.

### Introduction

A feed program with three diets differentiated by decrease of nutrition levels in proportion to growth is highly common in the feed management of laying hens during the growing phase. This is especially true for recommendations with regard to metabolizable energy and protein.

The northeastern region of Brazil is characterized by rich vegetation that may be used in animal feed. However, several plants may not be included in animal diets mainly due to their fibers and anti-nutritional

factors that impair digestibility of proteins and decrease the absorption of minerals and vitamins, besides increasing energy requirements (COSTA et al., 2007).

According to Hetland et al. (2005), fibrous feed stimulates activity and its retention time in the gizzard. Further, Roberts et al. (2007) reports that fiber has negative effects on the better use of nutrients due to production increase of endogenous matter and to decrease in digestive enzyme activity, coupled to morphological and physiological changes in the digestive tract. On the other hand, González-Alvarado

et al. (2007) reported that the traditional idea that poultry should receive diets with scanty fiber to avoid decrease in feed digestibility and performance must change. They insisted that moderate inclusion of fiber in the diet may be an asset in the performance of poultry.

The leucaena (*Leucaena leucocephala*) is a perennial leguminous plant featuring high protein levels in the hay derived from its leaves. It has also an excellent potential of pigments due to its high xanthophyll contents (D'MELLO; ACAMOVIC, 1989; LOPES et al., 2014). However, tannin, trypsin inhibitors, toxic factors such as a non protein amino acid,  $\beta$ -[N-(3-hydroxy-4-oxopyridyl)]- $\alpha$ -amino ropionic (mimosine) (HUSSAIN et al., 1991) and fiber level may restrict the use of the hay in poultry feed. According to Dilger et al. (2004), the use of ingredients with high fiber level in poultry diets may reduce the digestibility of nutrients and increase nitrogen excretion. Meulen et al. (1984) reported that the concentration of 5% mimosine in broilers' diet decreased intake and affected the performance of the fowls, even though the effects of mimosine on the animals' performance are still unclear.

The inclusion of hay from leucaena leaves in the diet of domestic laying hens may be an alternative to decrease feed costs and a source of pigment of the egg yolk of laying hens treated within a semi-intensive regime (BHATNAGAR et al., 1996), together with products that do not contain carotenoid hues in their composition, such as sorghum.

Current research evaluates the effects of the inclusion of leucaena leaves hay (LLH) on performance, nutrient digestibility and metabolizable energy rates of diets for laying hens during the growth phase (between the 14<sup>th</sup> and 19<sup>th</sup> week).

## Material and methods

The assay was performed at the Poultry Sector of the Department of Animal Science of the Universidade Federal do Ceará, Fortaleza, Ceará State, Brazil, on the littoral zone, altitude 15.49 m above sea level, at 3°43'02" S and 38°32'35" W. Ninety (Rhodes Island Red e New Hampshire) laying hens, 14 weeks old, were distributed in an entirely randomized design with three treatments and five replications, with 6 hens, during 42 days. Factors under analysis comprised three types of leucaena leaves hay (LLH) at 0%, 5% and 10% levels.

The birds were first weighed and selected with uniform mean weight, for experimental parcels, following Sakomura and Rostagno (2007), and placed in galvanized wire cages (50 x 50 x 45 cm) with a galvanized feeder and a drinking trough with nipples.

Temperature and relative air humidity in the shed were measured daily throughout the experimental period respectively with a maximum-minimum thermometer and psychrometer. Data were registered daily and readings taken at 8:00 am and 4:00 pm. During the entire experimental phase (14<sup>th</sup> to 19<sup>th</sup> week) the laying hens were given diet *ad libitum* and kept under a natural light from September to October.

Diets were formulated according to nutrition recommendations for semiweighed poultry by Rostagno et al. (2005) and to feed composition rates proposed by the same authors, except LLH, and the amino acid composition according to D'Mello and Acamovic (1989). During the experimental phase the diets were deliberately iso-nutrients with 2.800 kcal EMAn kg<sup>-1</sup> and 15% crude protein in natural matter (Table 1).

**Table 1.** Percentage and nutritional composition for experimental diet.

Ingredient	LLH level (%)		
	0	5	10
Corn	61.23	60.87	60.47
Soyabean meal (45%)	15.23	17.44	19.83
Wheat meal	20.00	13.35	6.55
Monobasic calcium phosphate	0.91	0.98	1.05
Limestone	1.92	1.65	1.39
Common salt	0.31	0.31	0.31
Vitamin and Mineral Supplement <sup>1</sup>	0.40	0.40	0.40
Leucaena leaf hay	0.00	5.00	10.00
Total	100.00	100.00	100.00
Calculated nutritional composition			
Metab. Energy (kcal kg <sup>-1</sup> )	2.800	2.800	2.800
AFD (%)	6.11	6.44	6.77
NFD (%)	17.20	17.48	17.72
Crude Fiber (%)	3.80	3.89	3.95
Available phosphorus (%)	0.31	0.31	0.31
Calcium (%)	1.00	1.00	1.00
Total lysine (%)	0.70	0.78	0.87
Met. + total cystine (%)	0.53	0.54	0.56
Total methionine (%)	0.25	0.26	0.27
Crude protein (%)	15.00	15.00	15.00
Sodium (%)	0.15	0.15	0.15
Total Threonine (%)	0.57	0.59	0.61
Total tryptophan (%)	0.17	0.22	0.26

<sup>1</sup>Guarantee levels per kg of the product: vit. A 10,000,000UI, Vit. D 2,000,000 UI, Vit. E 30,000 UI, Vit. K 3.0 g, Thiamin 2.0 g, Riboflavin 2.0 g, Pyridoxin 6.0 g, Cobalamin 1.5 g, Pantothenic acid 12 g, Folic acid 1.0 g, Biotin 1.0 g, Niacin 50 g, BHT 5.0 g, Copper 20 g, Iron 100 g, Iodine 2.0 g, Manganese 160 g, Selenium 0.25 g, Zinc 100 g, vehicle q.s.p. 1,000 g.

Diet intake was measured by weighing diet given at the start and at the final period of the experiment, minus the surplus at the end. Diet intake (g hen<sup>-1</sup> day<sup>-1</sup>) for each experimental unit was obtained.

Since hens were weighed at the start and at the end of the experimental period, weight gain of the laying hens (g hen<sup>-1</sup> day<sup>-1</sup>) was calculated by previous data and the duration of the experiment.

Total excreta collection lasted four consecutive days, undertaken after 28 days in which hens were fed on experimental diets. Markings with 1% ferrous oxide placed in the diet at the first and last day of the collection showed their origin from experimental

diets. The non-marked excreta were collected during the first day and the marked one on the last day. Collections, undertaken twice a day in the early morning and in the late afternoon, were conditioned in plastic bags and frozen at -20° C.

After thawing at room temperature the excreta of each replication were homogenized for sampling. Samples were taken to the Animal Nutrition Laboratory (LANA) of the Animal Science Department of the Universidade Federal do Ceará for pre-drying in an air-forced buffer at 55°C for 72 hours. They were then ground in a knife mill with a 16 mesh sieve with 1 mm and conditioned in labeled plastic bags for determination of dry matter (DM), nitrogen (N) and crude energy (CE), following methodology by Silva and Queiroz (2002).

Based on laboratory results, the digestibility coefficients of dry matter (CDDM), nitrogen (CDN), crude energy (CDCE) and the rates of apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) were calculated.

Performance results were determined by intake of diet (g hen<sup>-1</sup> day<sup>-1</sup>), weight gain (g hen<sup>-1</sup> day<sup>-1</sup>), feed conversion (kg kg<sup>-1</sup>). Ingested AMEn (kcal hen<sup>-1</sup> day<sup>-1</sup>) and CP (g hen<sup>-1</sup> day<sup>-1</sup>) were calculated from data of diet composition, diet intake by laying hens during the experiment and AMEn rates determined in the metabolism assay.

Performance and digestibility data were analyzed by ANOVA of SAS (2002) and means compared by SNK test at 5% probability.

**Results and discussion**

The performance of laying hens during growth phase (Table 2) revealed that the inclusion of leucaena leaf hay in the diet did not affect significantly (p > 0.05) the variables evaluated diet intake (g hen<sup>-1</sup> day<sup>-1</sup>), weight gain (g hen<sup>-1</sup> day<sup>-1</sup>), feed conversion (kg kg<sup>-1</sup>), ingestion of metabolized energy (kcal hen<sup>-1</sup> day<sup>-1</sup>) and ingestion of crude protein (g hen<sup>-1</sup> day<sup>-1</sup>).

**Table 2.** Performance of laying hens in the growth phase fed on diets with different levels of leucaena leaf hay (LLH).

Variables	Treatment			Mean	CV <sup>1</sup> (%)	Effects
	0% LLH	5% LLH	10% LLH			
Diet intake (g hen <sup>-1</sup> day <sup>-1</sup> )	68.80	68.60	67.00	68.13	6.19	NS <sup>2</sup>
Weight gain (g hen <sup>-1</sup> day <sup>-1</sup> )	11.00	10.00	9.40	10.13	6.20	NS
Feed conversion (kg kg <sup>-1</sup> )	6.55	7.17	7.60	7.10	6.74	NS
Ingested AMEn (kcal hen <sup>-1</sup> day <sup>-1</sup> )	196.77	192.92	184.23	190.99	6.35	NS
Ingested CP (g hen <sup>-1</sup> day <sup>-1</sup> )	12.20	12.10	13.17	12.49	6.37	NS

<sup>1</sup>CV= Coefficient of variation; <sup>2</sup>NS = not significant statistical effect (p > 0.05).

Reports in the literature on harmful effects by increasing LLH inclusion in diets for the performance of laying hens have been associated to the negative effects of an increase in fiber and antinutritional factors, such as mimosine and tannins occurring in the plant. Hussain et al. (1991) evaluated LLH inclusion at 5, 10, 15 and 20% levels in diets for broilers from 1 to 35 days of age and reported that only the highest inclusion level decreased intake, weight gain and feed conversion.

Bhatnagar et al. (1996) tested LLH inclusion levels 5, 10 and 20% in the diets of commercial laying hens and registered that a significant decrease in egg production, weight and mass of eggs and high diet intake occurred only at 20% inclusion level. On the other hand, Oliveira et al. (2000) evaluated two species of leucaena (*L. leucocephala* and *L. cunningham*) at level up to 6% inclusion in the diet and reported a significant decrease in weight gain in 1 to 21-day-old fowls.

Consequently, the feasibility of employing up to 10% LLH verified in current research agreed with evaluations by Hussain et al. (1991) and Bhatnagar et al. (1996) respectively for broilers and laying hens. However, the above differed from reports by Oliveira et al. (2000) that 6% of leucaena hay impaired the performance of broilers. It should be underscored that variations may occur in the chemical composition and in the amount of nutritional factors in leucaena due to type of cultivar, soil, climate of the cultivated area (D'MELLO; ACAMOVIC, 1989). Differences may exist at LLH inclusion levels recommended for this plant in poultry diets.

As a rule, the performance of the laying hens in current research may be considered adequate since it is similar to the performance expected of commercial laying hen strains within the same phase. Hy-line do Brasil management handbook shows that Hy-line Brown hens at this phase have, at an average, an intake of 75.00 (g hen<sup>-1</sup> day<sup>-1</sup>), gain weight of 10.86 (g hen<sup>-1</sup> day<sup>-1</sup>), feed conversion of 6.96 and metabolizable energy of 204.20 (kcal hen<sup>-1</sup> day<sup>-1</sup>).

Analysis of data on the coefficients of digestibility and metabolizable energy of growing laying hens were analyzed (Table 3) showed that there was no significant effect of LLH inclusion on CDN, AME and AMEn rates. However, significant decrease in CDDM and CDCE rates was verified, with the lowest rates on the increase of 10% LLH inclusion in the diet.

Decrease of CDDM and CDCE rates caused by adding 10% LLH in the diet may be attributed to an increase in excretion of dry matter by laying hens fed on diets with a higher proportion of fibers. The

above occurs because the fibrous fraction is only scantily digested in the digestion tract and consequently its proportion in the excretion increases.

**Table 3.** Coefficients of digestibility and metabolizable energy of laying hens in the growth phase fed on diets with LLH.

Variables <sup>1</sup>	Treatments <sup>2</sup>			Mean	CV <sup>3</sup> (%)
	0% LLH	5% LLH	10% LLH		
CDDM (%)	72.96a	71.60 ab	70.07b	71.55	2.28
CDN (%)	51.88a	47.27 <sup>a</sup>	45.38a	48.18	10.52
CDCE (%)	76.32a	74.86ab	73.28b	74.82	1.90
AME (kcal kg <sup>-1</sup> DM)	3.341a	3.285 <sup>a</sup>	3.255a	3.293	1.90
AMEn (kcal kg <sup>-1</sup> DM)	3.204a	3.166 <sup>a</sup>	3.116a	3.162	1.72

<sup>1</sup>CDDM = Coefficient of digestibility of dry matter; CDN = Coefficient of digestibility of nitrogen; CDCE = Coefficient of digestibility of Crude Energy; AME = Apparent metabolizable energy; AMEn = Apparent metabolizable energy corrected for Nitrogen. <sup>2</sup>Means followed by same small letters in the line do not differ statistically by SNK test ( $p < 0.05$ ); <sup>3</sup>CV = Coefficient of variation.

Several metabolic and physiological effects in the laying hens' organism have been proposed to increase fiber level in the diet, among which may be mentioned increase in the rate of endogenous excretion and volume occupied by the feed cake in the digestion track, dilution of some diet nutrients, reduction of the activity of digestion enzymes and of the feed pathway through the gastro-intestinal tract (ARAÚJO; SILVA 2008). Dunkley et al. (2007) reported that the diet's fiber may change microbial activity in the intestine, passage rate and digestion efficiency. These effects may have negative digestibility effects on the laying hens' nutrients.

The fibrous fraction raises passage rate, endogenous losses (SAKAMURA; ROSTAGNO, 2007), an undesirable chelation with tannins that decrease the availability of the mineral elements and astringent activity on the intestinal proteolytic activity (RAMOS et al., 2007; ARRUDA et al., 2010a). On the other hand, joint effects of antinutritional factors in leucaena, such as tannins, saponins, galactomannan gums, trypsin inhibitors and mimosine may reduce the digestibility of the dry matter and protein of the laying hens' diet (D'MELLO; ACAMOVIC, 1989).

The coefficients of metabolizable energy of leucaena hay represent the influence of the chemical-energetic composition of the leguminous plant on the capacity of the better use of nutrients by the laying hens (ARRUDA et al., 2010a; LOPES et al., 2014). The above shows that there is an interactivity involving maturity and physiological adaptation of the digestion tract of the laying hens when fed on diets with leucaena hay (GONZÁLEZ-ALVARADO et al., 2007).

Arruda et al. (2010b) reported that a 20% inclusion of leucaena hay in the diet of domestic hens did not have a significant decrease in protein and fibrous fractions. On the other hand, energy was affected during the growing phase.

Results show that an increase in fiber levels and antinutritional factors with up to 10% LLH inclusion in growth diet was not enough to impair the use of diet nutrients of laying hens to the point of significantly affecting the metabolizable energy rates of the diets.

## Conclusion

Since a better use of diet nutrients by laying hens fed on diets with LLH provided the same metabolizable energy rate, the inclusion of up to 10% LLH in growth diet (14 to 19 weeks old) may be recommended for (Rhodes Island Red e New Hampshire) laying hens.

## References

- ARAÚJO, D. M.; SILVA, J. H. V. Enzimas exógenas em dietas contendo farelo de trigo e outros alimentos alternativos para aves: revisão. **PUBVET**, v. 2, n. 47, ed. 58, Art. 346, 2008. Available from: <[http://www.pubvet.com.br/artigos\\_det.asp?artigo=346](http://www.pubvet.com.br/artigos_det.asp?artigo=346)>. Access on: June 5, 2014.
- ARRUDA, A. M. V.; FERNANDES, R. T. V.; OLIVEIRA, J. F.; FILGUEIRA, T. M. B.; FERNANDES, D. R.; GALVÃO, R. J. D. Valor energético de feno de forrageiras do semiárido para aves Isa Label. **Acta Veterinária Brasileira**, v. 4, n. 2, p. 105-112, 2010a.
- ARRUDA, A. M. V.; MELO, A. S.; OLIVEIRA, V. R. M.; SOUZA, D. H.; DANTAS, F. D. T.; OLIVEIRA, J. F. Avaliação nutricional do feno de leucena com aves caipira. **Acta Veterinária Brasileira**, v. 4, n. 3, p. 162-167, 2010b.
- BHATNAGAR, R.; MEENA, K.; VERMA, S. V. S. Effect of dietary *Leucaena* leaf-meal (LLM) on the performance and eggs characteristics in White Legorn hens. **Indian Journal of Animal Sciences**, v. 66, n. 12, p. 238-260, 1996.
- COSTA, F. G. P.; OLIVEIRA, C. F. S.; BARROS, L. R.; SILVA, E. L.; NETO, R. C. L.; SILVA, J. H. V. Valores energéticos e bromatológicos dos feno de jureminha, feijão bravo e maniçoba para aves. **Revista Brasileira de Zootecnia**, v. 36, n. 4, p. 813-817, 2007.
- DILGER, R. N.; SANDS, J. S.; RAGLAND, D.; ADEOLA, O. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. **Journal of Animal Science**, v. 82, n. 3, p. 715-724, 2004.
- D'MELLO, J. P. F.; ACAMOVIC, T. Leucaena leucocephala in poultry nutrition. A review. **Animal Feed Science Technology**, v. 26, p. 1-28, 1989.
- DUNKLEY, K. D.; DUNKLEY, C. S.; NJONGMETA, N. L.; CALLAWAY, T. R.; HUME, M. E.; KUBENA, L. F.; NISBET, D. J.; RICKE, S. C. Comparison of In Vitro fermentation and molecular microbial profiles of high-fiber feed substrates incubated with chicken cecal inocula. **Poultry Science**, v. 86, n. 5, p. 801-810, 2007.

- GONZÁLEZ-ALVARADO, J. M.; JIMÉNEZ-MORENO, E.; LÁZARO, R.; MATEOS, G. G. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. **Poultry Science**, v. 86, n. 8, p. 1705-1715, 2007.
- HETLAND, H.; SVIHUS, B.; CHOCT, M. Role of insoluble fiber on gizzard activity in layers. **Journal of Applied Poultry Research**, v. 14, p. 38-46, 2005.
- HUSSAIN, J.; SATYANARAYANA REDDY, P. V. V.; REDDY, V. R. Utilisation of leucaena leaf meal by broiler. **British Poultry Science**, v. 32, p. 131-137, 1991.
- LOPES, I. R. V.; FREITAS, E. R.; NASCIMENTO, G. A. J.; VIANA NETO, J. L.; CRUZ, C. E. B.; BRAZ, N. M. Inclusão de feno de folhas de leucena e de cunhã na ração de poedeiras. **Arquivos de Zootecnia**, v. 63, n. 241, p. 183-190, 2014.
- MEULEN, U. Ter; PUCHER, F.; SZYSKA, M.; EL-HARITH, E. A. Effects of administration of *Leucaena* meal on growth performance and mimosine accumulation in growing chicks. **Archiv Fur Geflugelkunde**, v. 48, n. 2, p. 41-44, 1984.
- OLIVEIRA, P. B.; MURAKAMI, A. E.; GARCIA, E. R. M.; MACARI, M.; SCAPINELLO, C. Influência de fatores antinutricionais da Leucena (*Leucaena leucocephala* e *Leucaena cunningham*) e do feijão Guandu (*Cajanus cajan*) sobre o epitélio intestinal e o desempenho de frangos de corte. **Revista Brasileira de Zootecnia**, v. 6, n. 29, p. 1759-1769, 2000.
- RAMOS, L. S. N.; LOPES, J. B.; FIGUEIRÊDO, A. V.; FREITAS, A. C.; FARIAS, L. A.; SANTOS, L. S. Metabolizabilidade dos nutrientes em frangos de corte alimentados com rações com diferentes níveis da polpa de caju desidratada. **Revista Científica de Produção Animal**, v. 9, n. 2, p. 137-145, 2007.
- ROBERTS, S. A.; XIN, H.; KERR, B. J.; RUSSELL, J. R.; BREGENDAHL, K. Effects of dietary fiber and reduced crude protein on nitrogen balance and egg production in laying hens. **Poultry Science**, v. 86, n. 8, p. 1716-1725, 2007.
- 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. **Tabela brasileira para aves e suínos**, composição de alimentos e exigências nutricionais. 2. ed. Viçosa: UFV, 2005.
- SAKOMURA, N. K.; ROSTAGNO, H. S. **Métodos de pesquisa em nutrição de monogástricos**. Jaboticabal: Funep, 2007.
- SAS Institute. **SAS users guide: Statistics**. Version 9. Cary: SAS, 2002.
- SILVA, D. J.; QUEIROZ, A. C. **Análise de alimentos: métodos químicos e biológicos**. 3. ed. Viçosa: UFV, 2002.

Received on September 6, 2013.

Accepted on March 17, 2014

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.