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Effect of Digestible Threonine Level of Diet on Productive Performance, Commercial Egg Grading and Egg Quality of 55- to 61-Week-Old Laying Hens

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Abstract

The aim of this study was to establish the digestible threonine (dig. Thr) requirements and the ideal proportion between Thr and Lys for Isa Brown laying hens on productive traits and egg quality characteristics from 55- to 58- and 59- to 61 weeks of age. Increased levels of L-Thr were added to a basal diet that contained 3.7 g/kg of dig. Thr. Therefore, diets containing 3.7, 4.0, 4.3, 4.6, 4.9 and 5.2 g/kg of dig. Thr and a relation between Thr and Lys of 0.58, 0.63, 0.67, 0.72, 0.77 and 0.81, respectively were provided to laying hens. A total of 216 hens were distributed in a randomized design among the 6 experimental diets. There were 9 replicates per treatment, and 4 birds per replicate. Data were analyzed by repeated measures analysis using the MIXED procedure of SAS. The main effects (dig. Thr level and experimental period) and their interaction were studied. Neither the dig. Thr level nor the experimental period had effect on performance traits and egg quality. Also, commercial egg grading was not influenced by period. However, there was a quadratic effect ($P<0.001$) of dig. Thr level within each scale egg weight. In conclusion, diets containing more than 3.7 g/kg of dig. Thr do not improve laying hens productivity at 55- to 61-week-old.

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Introduction

According to data published by European Comission[1], the total production of eggs (for hatching and direct consumption) in the European Union in 2016 was 7.5 million tonnes, being Spain the 4th largest producer of eggs in the EU, currently with approximately 45 million hens based on egg production[2]. Thus, poultry farming is an important agricultural activity in Spain, with genetic improvement, sanitation, efficient management of production costs and nutritional programs, as well as, handling its main pillars of development.

Nowadays, the protein and amino acid sources used in birds' feeding have a special importance given its high cost. In addition, under current production conditions, the threonine (Thr) is the third limiting amino acid in maize- and soya-based feeds, mainly because cereals have a low content of it, and represents the critical point in formulations of minimum cost, due to its influence in the crude protein level of diet.

Therefore, these diets may give rise to a deficiency of Thr, being advisable to supply it by its synthetic form[3]. When synthetic amino acids are supplied and diets are formulated in terms of digestible amino acids (instead of total amino acids), it is possible to substitute maize and soya for alternative ingredients, ensuring an equivalent amount of digestible amino acids[4].

Besides that, Thr supplementation allows the reduction of crude protein level of diets, contributing to the reduction of N-excretion in the environment[3]. It is required by poultry to form body protein, collagen and elastin, as well as, the feathers. Also, being found in gastrointestinal epithelium (mucosal cells and digestive enzymes) and as a component of immunoglobulins[4].

Considering the aforementioned, this work aimed at determining the digestible Thr requirement and the Thr/Lys ratio to optimize the egg's productivity and quality in commercial Isa Brown laying hens from 55- to 58- and 59- to 61-week-old.

Material and Methods

The experiment was carried out at the Technical School of Agricultural Engineering of the Polytechnic University of Madrid in accordance with Royal decree 53/2013, which establishes the basic rules applicable to protection of experimental animals and other scientific purposes[5].

A total of 216 Isa Brown laying hens from 55 to 61 weeks of age were housed in groups of 4 birds in 54 cages of 508 x 450 x 450 mm (571.50 cm^2/bird). The cage was the experimental unit and each cage had two drinkers, and an available feeder length of 10.16 cm/ bird. The average temperature during trial was 20 ± 2° C, and the 16L:8D regimen (16 hours light : 8 hours dark). Animals received water and feed *ad libitum* throughout the experiment.

The birds consumed a basal diet containing 146.9 g/kg of crude protein, 3.7 g/kg of digestible Thr (dig. Thr) and 2,744 kcal/kg of metabolizable energy. The basal diet was supplemented with increasing levels of L-Thr so that six diets containing 3.7, 4.0, 4.3, 4.6, 4.9 and 5.2 g/kg of dig. Thr and a Thr/Lys ratio of 0.58, 0.63, 0.67, 0.72, 0.77 and 0.81, respectively. The diets were isoenergetic and isonutritic, except for dig. Thr levels and Thr/Lys ratio (Table 1). Diets did not contain antibiotics or growth promoters and were supplied for hens as flour. Each cage was randomly associated with one of the six experimental treatments with nine replicates each.

The mean daily feed intake (DFI) per replicate, initial live body weight (LBW), mean body weight gain (BWG), hatch index (HI) and mean egg weight were evaluated. The feed:gain ratio (F:G) and egg mass were calculated daily. To calculate BWG, all hens from each replicate of each treatment were weighed at the start of the trial (55 weeks), at week-58 and at the end of trial (61 weeks of age). Mortality was monitored daily. Also, the eggs were classified by commercial classes according to regulation n. 589/2008 that details the application rules of regulation n. 1234/2007 which stablishes the rules of egg's commercialization.

External and internal egg quality parameters were analyzed, including Haugh unit (HU), albumen height, yolk color, eggshell strength at break and eggshell thickness. The HU was calculated using the formula described by Haugh[6] according to which: $HU = 100 \log (h + 7.57 - 1.7 W^{0.37})$; where: h = albumen height (mm) and W = egg weight (g). The albumen height (mm) was measured with a dial micrometer gauge movement by handle rotation (B. C. AMES, model S-6428, Waltham, MA, USA) for which the eggs (albumen + yolk) were placed on a level and flat glass surface. The yolk egg color was analyzed using the

Table 1. Composition and calculated analyses of the basal diet.

Ingredients g/kg	Dieta basal (Basal diet) g/kg
Soya oil	48.03
Corn grain	225
Gluten meal	48.14
Wheat grain	192.6
Wheat bran	250
Beet pulp	20
Alfalfa	25
Sunflower meal	82.6
Phosphate monocalcium	8.46
Coarse calcium carbonate	42.15
Fine calcium carbonate	42.15
Salt	3.24
DL-Methionine	2.15
L-Lysine.HCl	4.01
Tryptophan	0.25
Red complement	0.39
Yellow complement	0.84
Premix[1]	5
Calculated values	
Metabolizable energy, kcal kg^{-1}	2,744.20
Dry matter	903.51
Ash	123.81
Crude protein	146.9
Crude fiber	59.95
Detergent neutral fiber	201.59
Sugars	28.55
Starch	327.79
Ether extract	72
Calcium	35
Phosphorous	6.843
Digestible P	3
Potassium	6.928
Sodium	1.4
Cloride	3.219
Digestible Lysine	6.4
Digestible Methionne+Cystine	6.6
Digestible Threonine	3.7
Digestible Tryptophan	1.415
Linoleic acid	34.918

[1] Supplementation of vitamins, minerals and additives per kg of product: Vitamin A = 1,800,000 I.U.; Vitamin D_3 = 370,000 I.U., Vitamin E = 150 mg, Vitamin K_3 = 10 mg, Vitamin B_2 = 80 mg, Vitamin B_{12} = 110 µg; Calcium pantothenate = 80 mg; Methionine = 13 g; Niacin = 200 mg, Choline chloride = 2,500 mg, Se = 3 mg; Cu = 120 mg , Fe = 1,000 mg, Zn = 1,475 mg, Mn = 1,236 mg, I = 13 mg, Antioxidant (B.H.T.: butylated hydroxytoluene) = 600 mg; F (maximum) = 500 mg; Excipient q.s. = 2,000 g.

Roche® colorimetric fan method[7].

For the egg texture testing, the equipment model TA-XT2i (Stable Micro Systems Ltd., Godalming, UK) was used connected to a computer and controlled by specific software. The thickness of eggshell (µm) was measured using a Mitutoyo America Corp.â (Aurora, IL, USA) digital micrometer (model 293 MDC-MX) at 3 points above the egg midline, followed by the calculation of arithmetic mean.

Data were analyzed by repeated measures analysis using the MIXED procedure of SAS[8] and significant effects were considered when $P<0.05$. Linear and quadratic polynomial contrasts were used to determine the dig. Thr optimal levels.

Results

The interaction between Thr-level and the experimental period was not significant for any of the studied parameters, and because of that, only the results for the main effects are shown. Neither the Thr-level nor the experimental period had any effect on the studied variables (Table 2) from hens of 55- to 61-week-old. The average daily values of DFI, BWG, F:G, HI, egg weight and egg mass were 128 g/day, 16.32 g, 2.31 g/g, 84.2%, 66.8 g and 56.4 g, respectively. It should be mentioned that DFI and BWG were higher for the 55- to 58-wk-old hens than for those of 59- to 61-wk-old. However, the F:G remained constant (2.3 g/g).

Neither the Thr-level in diet nor the experimental period influenced the egg quality parameters ($P>0.05$) (Table 3). The obtained average values were 6.94 mm and 6.75 mm for the albumen height, and 89.7 and 88.7 for the HU of hens of 55 to 58 weeks of age and, 59 to 61 weeks of age, respectively. Also, an average value of 5.28 kg/cm^2 and 5.09 kg/cm^2 was obtained for the eggshell strength at break and, 342 µm and 339 µm for the eggshell thickness of 55- to 58- and 59- to 61-wk-old hens, respectively.

However, a quadratic effect ($P<0.001$) of the Thr level in diet was observed on the commercial classification of eggs above 63 g. Thus, the L-egg class was affected by Thr-levels according to the quadratic regression estimate ($y = -12.897x^2 + 114.51x - 180.69$, $R^2 = 62\%$) with the maximum point of 4.44 g/kg L-Thr for egg production within L-class, a percentage of 73.63% of eggs within L-class.

The XL-egg class also had the effect ($P<0.01$) of the Thr-levels according to the quadratic regression estimate ($y = 11.94x^2 - 105.1x + 242.09$, $R^2 = 59\%$), considering the minimum for XL-egg class production at 4.4 g/kg and 11.86% of XL-egg class estimated. The requirement of Thr for obtaining large eggs (L- and XL-egg classes) was 563.2 mg for the average feed intake of 128 g/bird/day.

Discussion

Sá et al.[3] unlike the results of our trial, verified an effect of dig. Thr levels on egg production of 34- to 50-wk-old semi-heavy hens and, Escobar Alvarez[9] found effects of dig. Thr levels as responsible for greater egg production (91.72%) of laying hens from 33- to 41-wk-old. The egg weight in our study varied from 67.0 to 67.4 g for levels of dig. Thr of 3.7 to 5.2 g/kg, corresponding to a Thr intake of 473.6 to 665.6 mg and, corroborates the findings of Sá et al.[3], where different levels of dig. Thr also did not affect the egg weight and of Leeson and Summers[10] that reported the Thr supplementation (5.3 to 6.7 g/kg) in diets with high-protein content (220 g/kg crude protein) did not affect productivity or egg's weight.

On the other hand, Faria et al.[11] observed an increase on egg weight from 55.18 g with diet containing 3.5 g/kg to 58.36 g with diet containing 5.3 g/kg dig. Thr, concluding that low levels of Thr in diet reduced egg weight of 31- to 38-wk-old light laying hens. Besides that, the same authors found that diets containing more than 5.3 g/kg dig. Thr increased the feed intake and egg production, as well as, the daily egg mass; however, they did not observe any effect on body weight of 45- to 52-wk-old hens when diets were supplemented with 4.5 or 5.3 g/kg of dig. Thr. Besides that, Teixeira et al.[12], evaluating levels of dig. Thr for light laying hens noticed an improvement on egg weight of hens fed diets containing 6.1 g/kg of dig. Thr.

Likewise, the daily egg mass per bird was not improved neither by higher than 3.7 g/kg Thr levels (or 473.6 mg of dig. Thr with a feed intake of 128 g/bird/day) nor by the evaluated periods, showing higher values for periods 55 to 59-wk and 59 to 61-wk of age in comparison to the results reported by Valério et al.[13], that used levels of 0.510 to 0.635 mg of dig. Thr for the daily egg mass per bird of 40.4 g; although the birds in our trial were older (55 to 61-wk-old) than those of Valério's et al.[13].

Escobar Alvarez[9] obtained results of 57.09 and 56.51 g for the daily egg mass per bird after 744 mg and 775 mg of dig. Thr in diet. And, according to Rostagno et al.[14], the nutritional requirement of dig. Thr

Table 2. Effect of dietary digestible threonine (dig. Thr) content on productive performance of Isa Brown hens from 55- to 58-wk-old and 59- to 61-wk-old.

Dig. Thr (g/kg)	Egg mass (g)	Mean egg mass	Hatch index (%)	*DFI (g)	IBW (g)	DWG (g)	F:G (kg/kg)
n	9	9	9	9	9	9	9
3.7	56.3	67.0	84.0	128	2,123	2.41	2.29
4.0	57.2	66.4	86.2	130	2,084	14.6	2.29
4.3	57.2	67.6	84.8	128	2,102	26.2	2.25
4.6	55.4	66.2	83.8	127	1,994	18.5	2.33
4.9	56.2	67.0	83.8	128	2,063	13.2	2.33
5.2	55.7	67.4	82.6	128	2,098	23.0	2.35
n	54	54	54	54	54	54	54
Period (wk)							
55 to 58	56.7	66.9	84.6	129	-	22.1	2.32
59 to 61	56.0	66.8	83.8	127	-	10.5	2.30
RSD[1]	2.11	0.81	3.35	6.69	10.5	64.9	0.16
P-values							
Dig. Thr	0.98	0.67	0.96	0.99	0.15	0.73	0.93
Period	0.15	0.62	0.22	0.17	-	0.37	0.51
Dig. Thr x period	0.39	0.13	0.23	0.73	-	0.53	0.62

[1]RSD: residual standard deviation. DFI = daily feed intake; IBW = initial body weight; DWG = daily weight gain; F:G = feed:gain ratio.

Table 3. Effect of digestible threonine content in the different diets on egg quality and grading of laying hens in period I (55- to 58-wk-old) and II (59- to 61-wk-old).

	Digestible threonine (g/kg)						Period		RSD[1]	*P-values*[2]		
	3.7	4	4.3	4.6	4.9	5.2	I	II		L	C	P
n	9	9	9	9	9	9	54	54				
ES*	5.44	4.88	5.38	4.99	5.47	4.98	5.28	5.09	0.98	0.87	0.89	0.15
ET	349	340	344	333	341	334	342	339	29.2	0.56	0.63	0.49
YC	10.2	10.2	9.7	10.3	10.2	10	10.2	10	0.86	0.46	0.47	0.14
AH	6.16	7.23	6.85	6.63	7.54	6.67	6.94	6.75	1.24	0.11	0.12	0.26
HU	85.1	91.5	89.1	88.4	92.9	88.1	89.7	88.7	7.35	0.08	0.09	0.35
TEC	98.4	97.9	97.1	96	98.7	98.5	97.7	98	-	0.068	0.064	0.65
TBE	1.58	2.09	2.85	3.98	1.28	1.54	2.28	2	-	0.067	0.064	0.64
Egg grade (%)												
> 73	16.8	11.4	14.5	7.48	15.5	18.2	14.7	13.9	-	0.0013	0.001	0.56
63 to 73	64.8	74	72.1	74.6	67.2	67.8	70.2	69.7	-	0.001	0.001	0.78
53 to 63	15.6	11.7	9.67	12.3	15.4	11.2	12	13.2	-	0.076	0.082	0.36
< 53	0.4	0.13	0	0.19	0	0	0.06	0.17	-	0.82	0.88	0.47

* Eggshell strength (ES, kg/cm^2); Eggshell thickness (ET, µm); Yolk color (YC); Albumen height (AH, mm); Haugh units (HU); Total eggs classified (TEC); Total broken + shell-less eggs (TBE, %). [1]RSD: residual standard deviation. [2]The interaction treatment x period were not significant (P>0.05) for all traits; L: linear, C: quadratic and P: polynomial effects.

is 596 mg for semi-heavy hens with daily egg mass of 55 g and body weight of 1.6 kg; what means 25% more than the recommended in our assay (473.6 mg).

In the same way, the F:G ratio was not influenced by the different Thr levels in the diet or by the evaluated periods, being in agreement with Teixeira et al.[12], who used levels of 3.7 to 7.9 g/kg of dig. Thr in diet provided to 44-wk-old laying hens and found no effect (P>0.05) on the F:G per dozen eggs. Therefore, a dig. Thr/dig. Lys ratio of 58% in diet of our trial did not present significant differences, so the addition of dig. Thr showed no improvements on F:G.

Nunes et al.[15] determined the nutritional requirements of Thr for semi-heavy hens from 50- to 66-wk-old using five levels of dig. Thr: 4.6, 4.9, 5.2, 5.5, 5.8 g/kg and reported no effects of the dig. Thr levels on internal egg quality parameters (yolk and albumen index, specific gravity, yolk percentage, thickness or shell weight per surface area).

Besides that, the results of Schmidt et al.[16] with semi-heavy hens of 79- to 95-wk-old agreed with our results, and did not present differences on variables of Haugh unit, hatch index, albumen index, as well as, on performance parameters (feed intake and egg weight). For these authors, the recommendation for semi-heavy hens from 79 to 95 weeks of age should be 509 mg/bird.

For adult birds, amino acid deficiency results in the catabolism of body protein, especially those present in skeletal muscle. In the case of hens that have reached their physical maturity and are in production phase, the problem is aggravated by the great demand of protein for egg synthesis. Sá et al.[3] evaluating 34- to 50-wk-old semi-heavy hens observed that HU presented a quadratic effect (P<0.01) on Thr levels and its requirement was estimated at 4.76 g/kg dig. Thr in diet of semi-heavy hens what corresponds to a Thr-daily feed intake/bird of 530 mg. But in a similar trial, Valério et al.[13] evaluating dig. Thr levels ranging from 5.1 to 6.35 g/kg in diet did not observe effects on internal egg quality characteristics. According to these authors, the level of 5.1 g/kg dig. Thr was sufficient to attend the production and internal egg quality of light- and semi-heavy hens.

The minimum dig. Thr levels found in our study (4.41 g/kg equivalent to the average intake of 564.48 mg/bird) for L-egg class production and (a dig. Thr of 4.44 g/kg equivalent to the average daily dig. Thr intake of 583 mg) for XL-egg class production are lower compared to the recommendations of Rostagno et al.[14] and Rostagno et al.[17] (diets with 6.96 g/kg of total Thr and 5.96 g/kg of dig. Thr for laying hens with average daily feed intake of 105 g, daily egg mass of 55 g and 1.60 kg of body weight). Although, for other evaluated characteristics, higher levels than 3.7 g/kg, equivalent to a dig. Thr intake of 473.6 mg (corresponding to a dig. Thr/dig. Lys ratio of 58% and an average feed intake of 128 g) did not improve the productivity and quality traits of laying hens.

The NRC[18] recommends diets with dig. Thr levels of 5.2 g/kg for semi-heavy layers with an average daily feed intake of 110 g, and FEDNA[19] recommends levels of 5.0 g/kg total Thr and of 4.3 g/kg dig. Thr for laying hens older than 45 weeks of age. In the study of Sá et al.[3] with increasing levels of dig. Thr (4.10, 4.45, 4.80, 5.15 and 5.5 g/kg) in diet with 16% of crude protein for 34- to 50-wk-old light laying hens, the recommended dig. Thr level was 5.1 g/kg or a daily dig. Thr intake of 583 mg. Thus, considering the mentioned above, the differences found among authors could be due to lineages used, environment, management, and changes in production systems[20].

Conclusion

The results suggest that 55- to 61-week-old Isa Brown laying hens fed diet containing an amount greater than 3.7 g/kg dig. Thr, equivalent to a dig. Thr intake of 473.6 mg did not improve their productivity and quality traits; however, for a higher percentage of L- and XL-egg classes, the level of dig. Thr in diet should be greater than the recommended level of 4.4 g/kg.

Conflict of Interest Statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and there are no other persons who satisfied the criteria for authorship.

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References

1. European Commission for agriculture and rural development - EC (September 25, 2017). https://ec.europa.eu/agriculture/eggs/presentations_en
2. GAIN REPORT USA (August 24, 2015). https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Spain%E2%80%99s%20Poultry%20Meat%20and%20Egg%20Production%20Report%202015_Madrid_Spain_10-28-2015.pdf
3. Sá, L.M., Gomes, P. C, Cecon, P. R, Rostagno, H. S., and D'Agostini, P. (2007) Nutritional requirement of digestible threonine for light-weight and semi-heavy laying hens in the period from 34 to 50 weeks old. R. Bras. Zootec. 36, 6, 1846-1853
4. Sakomura, N.K, and Rostagno, H. S. (2007) Métodos de pesquisa em nutrição de monogástricos. Fundação de Apoio à Pesquisa, Ensino e Extensão - FUNEP, Jaboticabal, BR
5. Boletín oficial del estado – B.O.E. (2013). Real Decreto 53/2013, de 1 de febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia. B.O.E. 34, 11370-11421 https://www.boe.es/diario_boe/txt.php?id=BOE-A-2013-1337
6. Haugh, R.R. (1937) The Haugh unit for measuring egg quality. U. S. Egg & Poultry Mag. 43, 552-555
7. Roche (1987) The roche yolk colour fan: directions for use. Basel: F. Hoffman La Roche.
8. SAS Institute Inc. (1990) SAS STATs User´s Guide. version 6, 4th Ed., SAS Institue Inc. Cary, NC, USA
9. Escobar Alvarez, A.V.F. (1998) Efecto del nivel de L-treonina en dietas para gallinas de postura comercial durante el período de la semana 33 a la 41 de edad. 1998. Bachelor Monograph, Universidad de San Carlos de Guatemala, San Carlos, GUA
10. Leeson, S, and Summers, J. D. (2005). Comercial Poultry Nutrition, 3rd Ed., University Press, Nottingham, UK
11. Faria, D.E., Harms, R. H., and Russel, G. B. (2002) Threonine requirement of commercial laying hens fed a corn-soybean meal diet. Poult. Sci. 81, 6, 809-814
12. Teixeira, E.N.M., Vilar da Silva, J. H., Silva, E. L., Lima, M. R., Ribeiro, M. L. G., and Rocha, J. K. P.

(2005) Exigência de treonina digestível para poedeiras leves e semipesadas. In: Conferência Apinco de Ciência e Tecnologia Avícolas. Proceedings...Suppl. 7. p.131. FACTA, Santos, BR

13. Valério, S.R., Soares, P. R.; Rostagno, H. S., Almeida e Silva, M., Albino, L. F. T., Lana, G. R. Q., Goulart, C. C., and Kill, J. L. (2000) Determination of Threonine nutritional requirement for white-egg and brown-egg laying hens. R. Bras. Zootec. 29, 2, 518-524
14. 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., and Euclides, R. F. (2011) Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3rd Ed., Universidade Federal de Viçosa, Minas Gerais, BR
15. Nunes, R.V., Agustini, M. A. B., Takahashi, S. E., Murakami, A. E., Souza, C., Schneiders, S. E., and Polese, C. (2015) Nutritional requirement of digestible threonine for brown-egg laying hens from 50 to 66 weeks of age. Semina: Ciênc. Agrár. 36, 6, 3877-3886
16. Schmidt, M., Gomes, P. C., Rostagno, H. S.; Albino, L. F. T., Nunes, R. V., and Cupertino, E. S. (2010) Nutritional requirement of digestible threonine to brown-egg laying hens on the 2nd cycle of production. R. Bras. Zootec. 39, 5, 1099-1104
17. Rostagno, H.S., Albino, L. F. T., Donzele, J. L., Gomes, P. C., Oliveira, R. F., Lopes, D. C., Ferreira, A. S, and Barreto, S. L. T. (2005) Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 2nd ed., Universidade Federal de Viçosa, Minas Gerais, BR
18. National Research Council – NRC (1994) Nutrient requirement of poultry. 9th Ed., National Academy Press, Washington, DC
19. Fundación Española para el desarrollo de la nutrición animal - FEDNA (2008) Necesidades nutricionales para avicultura: pollos de carne y aves de puesta. Lázaro, R., Mateos, G.G. (Eds), Universidad Politécnica de Madrid, Madrid, ES
20. Jordão Filho, J., Silva, J. H. V., Silva, E. L., Ribeiro, M. L. G., Costa, F. G. P., and Rodrigues, P. B. (2006) Lysine requeriment of semi-heavy laying hens during the peak of egg production. R. Bras. Zootec. 35, 4, 1728-1734