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Original Article

Requirement of Digestible Methionine + Cystine to Molted Laying Hens

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ABSTRACT

The present study aimed to determine the ideal requirement of digestible methionine + cystine to molted laying hens. The experimental period lasted 105 days, divided into five periods of 21 days. 144 Hisex White laying hens with 84 weeks-of-age were used. The experimental design was completely randomized with treatments constituted for six levels of digestible methionine + cystine (0.45, 0.50, 0.55, 0.60, 0.65, and 0.70%) in the diets, with four replicates of six birds each. Data collected were evaluated by polynomial regression at 5%. There was a significant effect (p<0.05) of digestible methionine + cystine on feed intake, energy intake, protein intake, egg production, and egg mass. Internal egg quality presented positive linear effect (p<0.05) on albumen height and yolk color. External egg quality was affected (p<0.05) in eggshell %, eggshell thickness and eggshell resistance, where the level of 0.60% of digestible methionine + cystine in the diets provided eggshells with better quality (higher percentage, thicker and breaking resistance). Differences (p<0.05) were also observed in glucose and triglycerides concentration, with 0.60% of digestible methionine + cystine in the diets presenting better equilibrium. Results of the present study suggested that higher levels of digestible methionine + cystine improved the performance and internal egg quality of molted laying hens. The level of 0.60% of digestible methionine + cystine provided better eggshell and equilibrium on blood biochemistry.

INTRODUCTION

Induced molt of laying hens is used by commercial egg producers to extend the productive period of their flock (Gongruttananun *et al.*, 2017). Typical molt programs cause a plumage renewal, providing changes in feed intake (Macari *et al.*, 2008; Sartório, 2011), and rejuvenation of reproductive tract, that is related to ovarian and oviducal regression (Brake & Thaxton, 1979). Feed withdrawal of various durations with photoperiod restriction is the most commonly used technique (Keshavarz & Quimby, 2002), although other alternative methods may also be used (Berry, 2003).

The main purpose of molting is to cease egg production in order for the hens to enter a nonreproductive state, which increases egg production and egg quality post molt (Webster, 2003; Donaldson *et al.*, 2005). Schmidt *et al.* (2011) reported that molt programs normally improve the quality of the eggs and extend the egg production up to 25 to 30 weeks, presenting around 85% of efficiency. According to Teixeira & Cardoso (2011), the nutritional readjustment of all nutrients of the diet is essential to a successful molt program. Studies about nutritional requirements for laying hens, especially amino acids, are frequently updated in the literature (Domingues *et al.*, 2016). However,

there is a great lack of information about requirements to molted laying hens.

The amount of protein used in diets before and after the molt has shown to affect not only feather replacement (Andrews *et al.*, 1987) but also various post molt performance factors (Harms, 1983). Amino acids are extremely important to the hens' metabolism due to having—a direct relationship with the egg production, feed efficiency, egg quality, and efficiency in the use of nitrogen (Lesson & Summers, 2001). The correct meeting of amino acids requirement increases the protein content of albumen, positively influencing the egg production, egg size, egg mass produced, and feed efficiency (Bateman *et al.*, 2008; Cupertino *et al.*, 2009; Figueredo Júnior *et al.*, 2014).

Methionine is the first limiting amino acid in poultry diets, being the precursor of cystine production, and influencing on weight and internal content of eggs (Laurentiz *et al.*, 2005; Brumano *et al.*, 2010a). General recommendations for methionine in laying hens' diets have been studied to determine an ideal requirement (Koelkebeck *et al.*, 1999; Strathe *et al.*, 2011).

Thus, the present study aimed to determine the ideal requirement of digestible methionine + cystine to molted laying hens.

MATERIAL AND METHODS

This study was conducted in the facilities of the Poultry Sector, College of Agrarian Sciences, Federal University of Amazonas, Manaus, Amazonas State, Brazil. The experimental procedures were approved by the Ethics Committee in Use of Animals (protocol number 028/2017) of Federal University of Amazonas.

The experimental period lasted 105 days divided into five periods of 21 days. The birds were subjected to an adaptation period of seven days to feed and facilities. The aviary had galvanized wire cages, trough feeders, and nipple drinkers. Throughout the experimental period, 16 hours of light/day were provided to the birds (12 hours of natural + 4 hours of artificial), with water and feed *ad libitum*. Egg collection was performed two times at day (9 a.m. and 3 p.m.). The temperature and relative humidity were recorded in the same times using a digital term-hygrometer positioned above the birds' cages, presenting average results of 30.31±0.08 °C and 77.46%, respectively.

144 Hisex White laying hens with 84 weeks-of-age were used. The birds were weighed at the beginning of the experiment in order to standardize the experimental plots, presenting a mean weight of 1.56 ± 0.03 kg. The experimental design was completely randomized with treatments constituted for six levels of methionine + cystine (0.45; 0.50; 0.55; 0.60; 0.65 and 0.70%) in the diets, with four replicates of six birds each.

For the determination of digestible sulfur amino acid requirement in the diets, they were formulated with the digestible methionine + cystine levels obeying the methionine + cystine: lysine ratios of 68, 76, 84, 91, 99 and 107, with lysine fixed at 0.653%. Levels of methionine + cystine were obtained from a basal diet deficient in methionine + cystine (0.45%) and supplemented with DL-methionine (99%). For each experimental level, the ratio of the main essential amino acids to lysine was maintained. The other nutrients in the diets met the requirements by Rostagno *et al.* (2017), with the experimental diets (Table 1) formulated by Supercrac (2004) computational software.

From the data collected during the 105 days of the experimental period, we calculated the feed intake (g/bird/day), egg production (%), feed efficiency (kg of feed used / kg of egg), feed efficiency (kg of feed used / dozen eggs), and egg mass (g). At the end of every 21 day period, four eggs from each plot were randomly selected to evaluate egg weight (g), specific gravity (g/cm³), yolk (%), albumen (%), eggshell (%), yolk height (mm), albumen height (mm), yolk color, eggshell thickness (μm), Haugh unit and eggshell resistance (N).

The eggs were stored up to one hour in room temperature and weighed using an electronic balance (0.01 g). The eggs were placed in wire baskets and immersed in buckets containing different levels of sodium chloride (NaCl) with density variations from 1.075 to 1.100 g/cm³ (interval of 0.005) to evaluate the specific gravity.

Then, the eggs were placed on a flat glass plate to determine albumen and yolk height, and yolk diameter using an electronic caliper. To separate albumen and yolk a manual separator was used. Each one was placed in a plastic cup and weighted in an analytical balance.

Eggshells were washed, dried in an oven (50 °C) for 48 hours, and weighed. Dry eggshells were used to determine the eggshell thickness using a digital micrometer. Average eggshell thickness was analyzed considering three regions: basal, meridional, and apical.

The yolk color was evaluated using a ROCHE© colorimetric fan with a scale of 1 to 15. Haugh unit was calculated using the egg weight and albumen height values in the formula $H_{unit} = 100 \times \log (H + 7.57$

Table 1 – Experimental diets composition.

	Methionine + cystine levels (%)					
Ingredients	0.45	0.50	0.55	0.60	0.65	0.70
Corn (7,88%)	68.4191	68.4388	68.4586	68.4784	68.4978	68.5175
Soybean meal (46%)	19.3914	19.3147	19.238	19.1613	19.0847	19.0080
Limestone	9.2593	9.2595	9.2596	9.2598	9.2600	9.2601
Dicalcium phosphate	1.7396	1.7404	1.7411	1.7418	1.7426	1.7433
Salt	0.5855	0.5855	0.5855	0.5856	0.5856	0.5856
PREMIX vitaminic/mineral	0.5000	0.5000	0.5000	0.5000	0.5000	0.5000
DL-Methionine	0.0335	0.0853	0.1372	0.1890	0.2408	0.2927
L-Lysine	0.0372	0.0397	0.0421	0.0446	0.0471	0.0496
L-Tryptophan	0.0271	0.0276	0.0281	0.0285	0.0290	0.0295
L-Threonine	0.0073	0.0085	0.0098	0.0110	0.0124	0.0137
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrients	Nutritional levels					
E.M, kcal.kg^{-1}	2,755.20	2,756.91	2,758.61	2,760.32	2,762.02	2,763.73
Crude Protein, %	14.500	14.500	14.500	14.500	14.500	14.500
Calcium, %	4.200	4.200	4.200	4.200	4.200	4.200
Available phosphorus, %	0.400	0.400	0.400	0.400	0.400	0.400
Sodium, %	0.250	0.250	0.250	0.250	0.250	0.250
Total methionine, %	0.269	0.320	0.371	0.422	0.473	0.523
Digestible methionine, %	0.252	0.303	0.353	0.403	0.454	0.523
Digestible met. + cys., %	0.450	0.500	0.550	0.600	0.650	0.700
Digestible lysine, %	0.653	0.653	0.653	0.653	0.653	0.653
Digestible threonine, %	0.498	0.498	0.498	0.498	0.498	0.498
Digestible tryptophan, %	0.172	0.172	0.172	0.172	0.172	0.172

[1] Guaranteedlevels per kilogramoftheproduct: Vitamin A 2,000,000 IU, Vitamin D3 400,000 IU, Vitamin E 2,400 mg, Vitamin K3 400 mg, Vitamin B1 100 mg, Vitamin B2 760 mg, Vitamin B6 100 mg, Vitamin B12 2,400 mcg, Niacin 5,000 mg, CalciumPantothenate 2,000 mg, Folicacid 50 mg, Coccidiostat 12,000 mg, Choline 50,000 mg, Copper 1,200 mg, Iron 6,000 mg, Manganese 14,000 mg, Zinc 10,000 mg, Iodine 100 mg. Selenium 40 mg. Vehicle q.s.p. 1,000 g.

– 1.7 x W0.37), where H = albumen height (mm), and W = egg weight (g).

To determine the eggshell resistance, a resistance machine located in the Materials Laboratory of the Superior College of Technology of the State of Amazonas University was used. This machine was connected to a computer, generating the power levels (represented in Newton) used to break the eggshell.

At the end of the experimental period, four birds were selected from each treatment and submitted to the analysis of serum parameters. The blood was collected from each bird through the ulnar vein and the samples immediately sent to the Laboratory of Poultry Technology, being evaluated: glucose (mg.dl^{-1}), triglycerides (mg.dl^{-1}) and cholesterol (mg.dl^{-1}) using a portable biochemical analyzer (Accucheck Trend, ROCHE©).

All data collected in this study were analyzed using the GLM procedure of SAS (Statistical Analysis System, v. 9.2) and estimates of treatments were subjected to ANOVA and subsequent polynomial regression analysis. Results were considered significant at $p \leq 0.05$.

RESULTS

There was a significant reduction ($p<0.05$) on feed intake ($y = 1.0066x^2 - 1.5745x + 105.47$; $R^2 = 0.75$), energy intake ($y = 2.8141x^2 - 4.306x + 284.05$; $R^2 = 0.78$) and protein intake ($y = 0.1788x^2 - 0.2575x + 15.193$; $R^2 = 0.79$). The level of 0.60% of digestible methionine + cystine in the diets presented lower intake (Table 2).

There was a quadratic effect ($p<0.05$) on egg production ($y = 1.0891x^2 - 1.13655x + 89.651$; $R^2 = 0.75$), and egg mass ($y = 0.7302x^2 - 0.75518x + 51.487$; $R^2 = 0.88$), where the level of 0.60% of the digestible methionine + cystine in the diets presented worse results. However, there was not effect ($p>0.05$) on feed efficiency per mass or per dozen results, as well as on viability results.

Internal egg quality (Table 3), presented positive linear effect ($p<0.05$) on albumen height ($y = 0.0846x + 15.474$; $R^2 = 0.97$) and yolk color ($y = 0.1029x + 5.66$; $R^2 = 0.93$). There was an increase in albumen height and yolk color from the increase of digestive methionine + cystine levels in the diets.

Table 2 – Feed Intake (FI), Energy Intake (EI), Protein Intake (PI), Egg Production (EP), Feed Efficiency (FE, kg/kg), Feed Efficiency (FE, kg/dz), Egg Mass (EM) and Viability (VIAB) of molted laying hens fed diets with different levels of methionine + cystine.

Variables	Methionine + cystine levels (%)						*p*-value	Effect	CV, %
	0.45	0.50	0.55	0.60	0.65	0.70			
FI, g/bird/d	101.79	99.99	96.66	89.46	95.31	97.44	0.05	Q	5.50
EI, kcal/bird/d	279.92	274.99	265.18	246.00	262.10	267.96	0.05	Q	5.50
PI, g/b/d	14.75	14.50	14.01	12.97	13.82	14.50	0.05	Q	5.52
EP, %	79.80	82.23	75.39	66.54	79.16	78.22	0.01	Q	6.31
FE, kg/kg	2.47	2.51	2.52	2.62	2.47	2.39	0.28	ns	5.26
FE, kg/dz	1.53	1.46	1.54	1.61	1.44	1.49	0.06	ns	5.02
EM, g	51.10	51.27	48.46	42.97	49.59	50.19	0.02	Q	6.98
VIAB, %	100.00	91.67	95.83	91.67	100.00	95.83	0.08	ns	7.51

CV - Coefficient of variation. p-value - Coefficient of Probability. Q - Quadratic effect. ns – non-significant.

Table 3 – Egg quality of molted laying hens fed diets with different levels of methionine + cystine.

Variables	Methionine + cystine levels (%)						p-value	Effect	CV, %
	0.45	0.50	0.55	0.60	0.65	0.70			
Egg weight, g	64.24	62.13	64.27	64.50	63.42	63.41	0.24	ns	2.26
Yolk, %	30.21	28.16	27.15	27.96	27.03	28.40	0.76	ns	11.45
Albumen, %	58.51	56.02	58.47	56.88	57.65	57.81	0.38	ns	3.17
Eggshell, %	9.20	9.41	9.54	9.74	9.59	9.43	0.01	Q	1.99
Yolk height, mm	7.33	7.02	7.00	7.10	7.15	7.23	0.71	ns	6.74
Album. height, mm	15.53	15.69	15.72	15.80	15.90	15.98	0.05	LP	1.47
Yolk color	5.75	5.81	6.03	6.11	6.20	6.22	0.05	LP	4.49
Eggshell thickness, µm	43.16	43.28	44.07	44.57	45.31	43.66	0.01	Q	1.96
Spec. Grav., g/cm^3	1,085	1,087	1,086	1,086	1,086	1,085	0.53	ns	0.14
Haugh unit	81.20	83.26	83.35	83.55	82.47	82.46	0.26	ns	1.78
Eggshell Resistance, N	34.27	35.61	40.30	42.23	35.50	35.16	0.05	Q	7.34

CV - Coefficient of variation. p-value - Coefficient of Probability. Q - Quadratic effect. PL – Positive Linear Effect. ns – non-significant.

External egg quality presented differences ($p<0.05$) in %eggshell ($y = -0.053x^2 + 0.06452x + 9.801$; $R^2 = 0.92$), eggshell thickness ($y = -0.1616x^2 + 1.991x + 45.591$; $R^2 = 0.83$) and eggshell resistance ($y = -0.9657x^2 + 6.9329x + 27.56$; $R^2 = 0.86$).The level of 0.60% of digestible methionine + cystine in the diets provided eggshells with better quality (higher percentage, thicker, and resistance).

Differences ($p<0.05$) were observed in glucose ($y = -2.6911x^2 + 2.875x + 181.63$; $R^2 = 0.95$) and triglycerides ($y = -12.345x^2 + 13.7759x + 524.46$; $R^2 = 0.78$), with 0.60% of digestible methionine + cystine in the diets presenting most balanced results (Table 4).

DISCUSSION

The increase methionine and cystine intake caused a significant effect on birds' performance. The level of 0.60% caused a significant decrease in energy and protein intake, consequently affecting the feed intake. High methionine levels reduced egg production and egg mass. Baião *et al.* (1999), and Brumano *et al.* (2010b) disagree with these results, where the authors reported that the performance improved by increasing methionine + cystine in the diets of laying hens.

The feed efficiency, although did not present significant differences in its results, reflected the reduction of feed intake and the drop of egg production

Table 4 – Glucose (GLI), Triglycerides (TRI) and Total Cholesterol (COL) of moltedlaying hens fed diets with different levels of methionine + cystine.

Variables	Methionine + cystine levels (%)						p-value	Effect	CV, %
	0.45	0.50	0.55	0.60	0.65	0.70			
GLU, mg.dl^{-1}	170.50	173.00	179.00	167.50	162.50	143.66	0.03	Q	9.29
TRI, mg.dl^{-1}	413.66	433.66	521.33	407.66	360.00	350.00	0.05	Q	15.30
COL, mg.dl^{-1}	152.00	150.00	154.00	157.00	156.00	157.00	0.08	ns	1.07

CV - Coefficient of variation. p-value - Coefficient of Probability. Q - Quadratic effect. ns - non-significant.

(Domingues *et al.*, 2016), where high methionine levels present a better equilibrium among the nutrients used and the egg production. Schmidt *et al.* (2011) reported that post molt hens normally present lower performance than birds in the first cycle, especially due to its metabolism being more susceptible to changes in diet and for presenting more difficultly to transform the diets' nutrient to eggs.

Baker *et al.* (1981b) also reported that potential improvements in molted hens' performance may be related with an increase in body weight loss of hens up to 31% of their original body weight, and better arrangement of metabolic pathways. Reductions of body weight above 35% may provide bad effects on the life of flock egg production. And the degree of improvement in post molting performance also may be associated with the number of days during which no eggs were produced, the named "rest period" (Berry, 2003).

According to Gongruttananun *et al.* (2017), the molted hens return to egg production at a slow rate after receiving re-adequate diets, and then rapidly increase during the postmolt period. Hormonal changes are typically associated with molting, where the hen responds using physical, chemical, anatomical, and physiological mechanisms at its disposal to maintain a better productive status for the longest period possible (Clarenburg, 1986; Freeman, 1987; Koelkebeck & Anderson, 2007).

However, there is not sufficient information about ideal levels of amino acids to molted hens, where the tables of requirements and manuals do not provide this information (Murakami *et al.*, 2003). We observed that the better requirements of methionine + cysteine to molted hens was higher than recommendations reported by Rostagno *et al.* (2011) for laying hens in the first cycle. The ideal use of protein and amino acids for laying hens may provide a balance in the requirement, improving performance and the quality of the eggs. And an optimal supplementation of methionine and cystine to hens' diets can increase the efficiency of protein use, affecting the egg weight, yolk, and albumen percentage (Gomez & Angeles, 2009; Del Vesco *et al.*, 2014).

Baião *et al.* (1999) reported that an increase in methionine and methionine + cystine supplementation did not present significant responses on eggshell quality. However, our results presented a different response, with significant effect in the quality of eggshell. These changes in the eggshell may be associated with an increase in egg size due to methionine and cysteine supplementation, besides metabolic changes resulting in the molting process.

Naturally, eggshell quality decreases with increasing hen age (Washburn,1982), and the incidence of cracked eggs can exceed 20% at the end of the laying period (Nys, 2001). Furthermore, the lower eggshell percentage may result in less eggshell thickness, lower internal quality, and a higher percentage of non-commercial eggs (Brumano *et al.*, 2010b). However, the quality of the eggs may be changed from the amino acid adjustment in diets, especially the eggshell. This adjustment reduces these problems observed before and after the molt (Pavan *et al.*, 2005; Brumano *et al.*, 2010a).

According to Brake & Thaxton (1979), the improvements in postmolt eggshell quality occur due to changes in the reproductive tract at the cellular level. These changes include the removal of lipid (Baker *et al.*, 1980; Baker, 1981; Baker *et al.*, 1981a), increased uptake of 1α25-dihydroxycholecal-ciferol by uterine tissue (Abe *et al.*, 1982), or the activation of some transport system such as calcium-binding protein (Berry *et al.*, 1987).

It is important to state that the eggshell quality is an essential factor to a good egg quality (Gongruttananun, 2017). A strong eggshell is significantly important for egg producers in reducing the economic losses from broken eggs (Tumova *et al.*, 2014) due to its function to provide a container for the eggs contents and protect them from bacterial contamination (Hamilton *et al.*,1979). Previous studies reported that there are 8% egg production losses due to poor eggshell quality (Nys, 2001; Abdallan *et al.*, 2009). Thus, the objective of poultry nutritionists is to formulate diets that maximize egg size and that reduce eggshell problems late in the production cycle (Schutte & DeJong, 1994; Sohail *et al.*, 2002).

CONCLUSIONS

It was concluded that higher levels of digestible methionine + cystine improved the performance and internal egg quality of molted laying hens. The level of 0.60% of digestible methionine + cystine provided better eggshell and equilibrium on blood biochemistry.

REFERENCES

Abdallah A, El-Husseiny O, Abdel-Latif K. Influence of some dietary organic mineral supplementations on broiler performance. International Journal of Poultry Science 2009;8:291-298.

Abe E, Horikawa H, Masumura T, Sugahara M, Kubota M, Suda T. Disorders of cholecalciferol metabolism in old egg-laying hens. Journal of Nutrition 1982;112:436-446.

Andrews DK, Berry WD, Brake J. Effect of lighting program and nutrition on feather replacement of molted Single Comb White Leghorn hens. Poultry Science 1987;66:1635-1639.

Bateman A, Roland DA, Bryant M. Optimal methionine + cysteine/lysine ratio for first cycle phase 1 commercial leghorns. International Journal of Poultry Science 2008;7:932-939.

Baião NC, Ferreira MOO, Borges FMO, Monti AEM. Effect of methionine levels on performance of laying hens. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 1999;51:271-274.

Baker M. The relationship between adipose accumulation and reproductive dysfunction in Gallus domesticus [thesis]. Auburn (AL): Auburn University; 1981.

Baker M, Brake J, Krista LM. Histological study of uterine lipid distribution in the laying hen. Poultry Science 1980;59:1557.

Baker M, Brake J, Krista LM. Total body lipid and uterine lipid changes during a forced molt of caged layers. Poultry Science 1981a;60:1593.

Baker M, Brake J, McDaniel GR. The relationship between body weight loss during an induced molt and post molt egg production, egg weight, and shell quality in caged layers. Poultry Science 1981b;62:409-413.

Berry W. The physiology of induced molting. Poultry Science 2003;82(6):971-980.

Berry WD, Brake J. Postmolt performance of laying hens molted by high dietary zinc, low dietary sodium, and fasting: egg production and eggshell quality. Poultry Science 1987;66(2):218-226.

Brake J, Thaxton P. Physiological changes in caged layers during a forced molt. 2. Gross changes in organs. Poultry Science 1979;58:707-716.

Brumano G, Gomes PC, Donzele JL, Rostagno HS, Rocha TC, Almeida RL. Digestible methionine + cystine level in meals for light-weight laying hens from 24 to 40 weeks of age. Revista Brasileira de Zootecnia 2010a;39:1228-1236.

Brumano G, Gomes PC, Donzele JL, Rostagno HS, Rocha TC, Mello HHC. Digestible methionine + cystine levels for light-weight laying hens from 42 to 58 weeks of age. Revista Brasileira de Zootecnia 2010b;39:1984-1992.

Clarenburg R. Syllabus for the course of veterinary physiology. 11th ed. Manhattan: Kansas State University;1986.

Cupertino ES, Gomes PC, Rostagno HS, Donzele JL, Schmidt M, Mello HHC. Nutritional requirement of methionine+cistinedigestibles for laying hens during a period of 54 to 70 weeks of age. Revista Brasileira de Zootecnia 2009;38:1238-1246.

Del Vesco AP, Gasparino E, Grieser DO, Zancanela V, Gasparin FR, Constantin J, *et al.* Effects of methionine supplementation on the redox state of acute heat stress-exposed quails. Journal of Animal Science 2014;92:806-815.

Domingues CHF, Sgavioli S, Praes MFFM, Santos ET, Castiblanco DMC, Petrolli TG, *et al.* Lysine and methionine+cystine on performance and egg qualityof laying hens: Review. PUBVET 2016;10(6):487-493.

Donalson LM, Kim WK, Woodward C, Herrera P, Kubena LF, Nisbe tDJ, *et al.* Utilizing different ratios of alfalfa and layer ration for molt induction and performance in commercial laying hens. Poultry Science 2005;84(3):362-369.

Figueiredo Júnior JP, Costa FGP, Nascimento GAJ, Santana MHM. Aspects on the use of total and digestible amino acids in diets for laying hens. Scientia Agraria Paranaensis 2014;13(3):186-197.

Freeman BM. The stress syndrome. World's Poultry Science Journal 1987;43:15-19.

Gomez S, Angeles M. Effect of threonine and methionine levels in the diet of laying hens in the second cycle of production. Journal of Applied Poultry Research 2009;18:452-457.

Gongruttananun N. Induced molt using cassava meal. 2. Effects on eggshell quality, ultrastructure, and pore density in late-phase laying hens. Poultry Science 2017;97(3):1050-1058.

Gongruttananun N, Kochagate P, Poonpan K, Yu-nun N, Aungsakul J, Sopa N. Effects of an induced molt using cassava meal on body weight loss, blood physiology, ovarian regression, and postmolt egg production in late-phase laying hens. Poultry Science 2017;96(6):1925-1933.

Hamilton RMG, Hollands KG, Voisey PW, Grunder AA. Relationship between eggshell quality and shell break-age and factors that affect shell breakage in the field – A review. Worlds Poultry Science Journal 1979;35:177-190.

Harms RH. Influence of protein level in the resting diet on performance of force rested hens. Poultry Science 1983;62:273-276

Keshavarz K, Quimby FW. An investigation ofdifferent molting techniques with an emphasis on animalwelfare. Journal Applied Poultry Research 2002;11:54-67.

Koelkebeck KW, Parsons C, Leeper R, Jin S, Douglas M. Early postmolt performance of laying hens fed a low-protein corn molt diet supplemented with corn gluten meal, feather meal, methionine, and lysine. Poultry Science 1999;78(8):1132-1137.

Koelkebeck KW, Anderson KE. Molting layers-alternative methods and their effectiveness. Poultry Science 2007;86(6):1260-1264.

Laurentiz AC, Filardi RS, Rodrigues EA, Junqueira OM, Casartelli EM, Duarte KF. Total sulfur amino acids levels for semi heavy weight laying hens after forced molt. Ciência Rural 2005;35:164-168.

Leeson S, Summers JD. Nutrition of the chicken. 4th ed. Ontario: University Books;2001.

Macari M, Furlan RL, Gonzales E. Fisiologia aplicada a frangos de corte. 2nd ed. Jaboticabal: FUNEP/UNESP; 2008.

Moreng RE, Avens JS. Ciência e produção de aves. São Paulo: Roca; 1990.

Murakami AE, Figueiredo DF, Peruzzi AZ, Franco JRG, Sakamoto MI. Sodium levels for commercial laying hens in the first and second production cycles. Revista Brasileira de Zootecnia 2003;32:1674-1680.

Nys Y. Recent developments in layer nutrition for optimising shell quality. Proceedings of 13th European Symposium on Poultry Nutrition; 2001; Blankenberge. Belgium. p. 42–52

Pavan AC, Móri C, Garcia EA, Scherer MR, Pizzolante CC. Levels of protein and sulfur amino acids on performance, egg quality and nitrogen excretion of brown egg laying hens. Revista Brasileira de Zootecnia 2005;34:568-574.

Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Oliveira RF, Lopes DC, *et al.* Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3rd ed. Viçosa: UFV; 2011.

Rostagno HS, Albino LFT, Hannas MI, Donzele JL, Sakomura NK, Costa FGP, *et al.* Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4th ed. Viçosa: UFV; 2017.

Sartório LA. Muda forçada. Avicultura Industrial 2011;1197:20-23.

Schmidt M, Gomes PC, Rostagno HS, Albino LFT, Nunes RV, Melo HHC. Nutrition levels of digestible methionine + cystine for white-egg laying hens in the second production cycle. Revista Brasileira de Zootecnia 2011;40:142-148.

Schutte JB, De Jong J. Requirement of the laying hen for sulfur amino acids. Poultry Science 1994;73:274-280.

Sohail SS, Bryant MM, Roland Sr DA. Influence of supplemental lysine, isoleucine, threonine, tryptophan, and total sulphur amino acids on egg weight of Hy-Line W-36 hens. Poultry Science 2002;81:1038-1044.

Strathe AB, Lemme A, Htoo JK, Kebreab E. Estimating digestible methionine requirements for laying hens using multivariate nonlinear mixed effect models. Poultry Science 2011;90(7):1496-1507.

Teixeira RSC, Cardoso WM. Forced molting in modern poultry production. Revista Brasileira de Reprodução Animal 2011;35:444-455.

Tumova E, Gous RM, Tyler N. Effect of hen age, environmental temperature, and oviposition time on egg shell quality and egg shell and serum mineral contents in laying and broilerbreeder hens. Czech Journal of Animal Science 2014;59:435-443.

Washburn KW. Incidence, cause, and prevention of egg shell break age in commercial production. Poultry Science 1982;61:2005-2012.

Webster AB. Physiology and behavior of the hen during induced molt. Poultry Science 2003;82:992-1002.