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# Glutamic acid supplementation on low protein diets for laying hens

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**ABSTRACT.** With the objective of evaluating the effect of reducing crude protein (CP) and L-glutamic acid supplementation, Dekalb Brown laying hens with 34 weeks of age. The birds were distributed in six treatments in a completely randomized design, eight replications of eight birds. The control diet contained 17% CP. The second and third treatments had CP reduced by 1.5 and 3.0 percentage points (pp) of CP in relation to the control diet, respectively. In the fourth treatment, was added the same amount of glutamate of the control diet (2.982%), and the last two treatments was added 1 and 2 pp of glutamate in relation to control diet (3.982 and 4.98%), respectively. The treatment with 15.17% CP + 0.341% L-glutamic acid (T4) promoted higher egg production, and the control diet showed greater egg weight than others treatments. There was no significant effect on the variables on quality of eggs. The T4 presented values of excreted nitrogen similar to the others treatments, with lower concentration of uric acid than the control diet and lower cost in the production. In conclusion, it is recommended the treatment with 15.17% CP + 0.341% L-glutamic acid for Brown laying hens with 34 weeks of age.

Keywords: egg production, nitrogen, performance, uric acid.

# Ácido glutâmico suplementado em dietas de baixa proteína para poedeiras semipesadas

**RESUMO.** Com o objetivo de avaliar o efeito da redução da proteína bruta (PB) e suplementação de L-glutamato em poedeiras da linhagem Dekalb Brown com 34 semanas de idade, as aves foram distribuídas em seis tratamentos em um delineamento experimental inteiramente casualizado com oito repetições. A dieta controle continha 17% PB. Os segundo e terceiro tratamentos tinham redução de 1,5 e 3,0 pontos percentuais (pp) de PB em relação à dieta controle, respectivamente. No quarto tratamento, foi adicionada a mesma quantidade de glutamato da dieta controle (2,982%), e nos dois últimos tratamentos foram adicionados 1 e 2 pp de glutamato em relação à dieta controle (3,982 e 4,98%), respectivamente. O tratamento com 15,17% CP + 0,341% L-glutâmico (T4) promoveu maior produção de ovos, e a ração controle apresentou maior peso de ovos do que os outros tratamentos. Não houve efeito significativo sobre as variáveis relativas à qualidade dos ovos. O T4 apresentou valores de nitrogênio excretado semelhante aos demais tratamentos, com menor concentração de ácido úrico que a dieta controle e menor custo na produção. Em conclusão, recomenda-se o tratamento com 15,17% PB + 0,341% de ácido L-glutâmico para poedeiras semipesadas com 34 semanas de idade.

Palavras-chave: produção de ovos, nitrogênio, desempenho, ácido úrico.

## Introduction

Amino acid requirements of commercial layers have been extensively researched, because currently it is a common practice to calculate diets based on amino acid recommendations, ideal protein standard, amino acid digestibility and costs of feeding (Rocha et al., 2009). Incorrect diet formulations may cause deficiencies and/or excess depending on the variability of amino acid digestibility. As a measure to avoid this problem, researchers developed the concept of ideal protein, which implies providing nutrients in a balanced manner, using the exact balance of amino acids, in order to meet the requirements of all amino acids to maintenance and production (Furuya et al., 2005).

According to Aftab et al. (2006) the use of synthetic amino acids could reduce crude protein by10% for each phase of creating poultry. However, Dean et al. (2006), affirm that even with supplementation of essential amino acids, too much reduction of protein may lead to a situation where part of the essential amino acids is diverted to the synthesis of non-essential amino acids due to lack of non-specific nitrogen for this process, thus reducing body protein deposition and animal performance. L-glutamic acid is considered an efficient source of non-specific nitrogen, important for the maintenance of the intestinal mucosa, for being source of energy for the mucosa turnover, via the ATP produced from the Krebs cycle; is source of nitrogen for the synthesis of other amino acids and nitrogen compounds (Berres et al., 2010). It regulates the nitric oxide synthesis in specific tissues like the brain and acts as precursor of glutathione, which is an important compound in the elimination of oxidants and modulation of immune response (Li et al., 2007).

The effect of glutamate on the maintenance and improvement of intestinal quality is well described. In the intestine, this amino acid functions as oxidative fuel that has important role as an enhancer of intestinal function, promoting high turnover rate of intestinal mucosa cells (Burrin & Stoll, 2009). However, despite the great importance of this amino acid, only few studies have been conducted to evaluate its efficiency as a non-specific nitrogen source in diets for laying hens.

Table 1. Percentage and chemical composition of experimental diets

# Material and methods

The experiment was conducted in the Poultry Sector of the Department of Animal Science of the Center for Agrarian Sciences of Federal University of Paraiba, Brazil, Campus II, located in the municipality of Areia, during 140 days divided in five 28-day periods.

Four layers were housed per cage (24 x 37 x 41 cm) equipped with gutter-type feeders and nipple drinkers. The lighting program adopted consisted of 17 hours of light (artificial plus natural) plus seven hours of darkness, using a timer to control the artificial light. Feed and water were supplied for ad libitum consumption throughout the experimental period.

A total of 384 Dekalb Brown layers from 34 to 54 weeks of age were distributed in six treatments in a completely randomized design, eight replications of eight birds each.

The experimental diets for laying hens in the laying phase are listed in Table 1.

Ingredients,%		T1	T2	T3	T4	T5	T6
Corn grain		63.469	67.82	73.27	67.849	66.509	65.168
Soybean meal		22.584	18.900	13.723	18.531	18.739	18.946
Soybean oil		2.364	1.468	0.575	1.429	1.556	1.683
Dicalcium phosphate		1.550	1.575	1.605	1.579	1.583	1.586
Limestone		9.289	9.308	9.328	9.309	9.305	9.302
Common salt		0.373	0.377	0.382	0.378	0.378	0.379
DL-Methionine		0.201	0.234	0.273	0.238	0.240	0.243
L-Lysine		-	0.066	0.219	0.078	0.074	0.071
L-Valine		-	0.031	0.112	0.038	0.039	0.039
L-Isoleucine		-	0.048	0.132	0.055	0.054	0.053
L-Tryptophan		-	0.003	0.031	0.005	0.004	0.004
L-Threonine		-	-	0.059	-	-	-
-Glutamic acid		-	-	-	0.341	1.349	2.356
Choline		0.070	0.070	0.070	0.070	0.070	0.070
Mineral mix <sup>1</sup>		0.050	0.050	0.050	0.050	0.050	0.050
/itamin mix <sup>2</sup>		0.050	0.050	0.050	0.050	0.050	0.050
Potassium carbonate		-	-	0.121	-	-	-
	AV, %		(	Calculated nutrition	al composition, %		
ME, kcal kg <sup>-1</sup>	2800	2800	2800	2800	2800	2800	2800
CP, %	17.00	17.00	15.50	14.00	15.17	15.74	16.32
Calcium, %	4.200	4.200	4.200	4.200	4.200	4.200	4.200
Phosphorus available, %	0.375	0.375	0.375	0.375	0.375	0.375	0.375
Digestible met +cys, %	0.683	0.683	0.683	0.683	0.683	0.683	0.683
Digestible methionine, %	0.375	0.444	0.440	0.459	0.442	0.443	0.444
Digestible lysine, %	0.750	0.750	0.750	0.750	0.750	0.750	0.750
Digestible valine, %	0.675	0.675	0.684	0.675	0.675	0.675	0.675
Digestible isoleucine, %	0.623	0.623	0.603	0.603	0.603	0.603	0.603
Digestible threonine %	0.495	0.495	0.486	0.480	0.495	0.495	0.495
Digestible tryptophan, %	0.173	0.173	0.187	0.173	0.173	0.173	0.173
Digestible arginine, %	0.750	0.750	0.932	0.785	0.920	0.921	0.924
Digestible phenylalanine, %	0.488	0.771	0.674	0.573	0.692	0.692	0.692
Digestible histidine, %	0.218	0.421	0.387	0.338	0.356	0.364	0.38
Digestible leucine, %	0.915	1.349	1.244	1.124	1.232	1.221	1.22
odium, %	0.230	0.230	0.230	0.230	0.230	0.230	0.23
Chlorine, %	0.210	0.351	0.351	0.353	0.354	0.353	0.35
Potassium, %	0.590	0.590	0.590	0.590	0.590	0.590	0.59
Glutamate, %	-	2.982	2.670	2.318	2.982	3.982	4.98
EB, mEq kg <sup>-1</sup>	-	152.020	152.020	151.460	151.180	151.460	152.0

AV (%) – Analytical values (Rostagno et al., 2005), Table 48, p. 113); EB - Electrolytic balance; T1, 17% CP; T2, 15.5% CP; T3, 14% CP; T4, 15.17% CP + 0.341% L-Glutamic acid; T5, 15.74% CP + 1.349% L-Glutamic acid; T6, 16.32% CP + 2.356% L-Glutamic acid.<sup>1</sup>Composition kg<sup>-1</sup> of product: vitamin A – 12,000,000 IU, vitamin D<sub>3</sub> – 3,600,000 IU, vitamin B<sub>1</sub> - 2.5 g; vitamin B<sub>2</sub> - 8 g; vitamin B<sub>6</sub> - 3 g; pantothenic acid - 12 g; biotin - 0.2 g; vitamin K - 3 g; folic acid - 3.5 g; nicotinic acid - 40 g; vitamin B<sub>12</sub> - 20 mg, Se - 0.13 g; <sup>2</sup>Composition kg<sup>-1</sup> of product: Mn - 160 g, Fe - 100 g, Zn - 100 g, Cu - 20 g; Co - 2 g, I - 2 g.

The first treatment (T1) was considered the control diet and contained 17% CP, based on corn and soybean meal, supplemented with DL-methionine, and formulated so as to meet the nutritional requirements of brown-egg laying hens during the egg-laying phase with the levels of all essential amino acids reaching or slightly exceeding the recommendations of digestible amino acids for laying hens (Rostagno et al., 2005).

The second treatment (T2) was a diet with reduction of 1.5 percentage points of crude protein in relation to the Control Diet, supplemented with synthetic amino acids so as to meet the nutritional requirements of essential amino acids of birds. In the third treatment (T3), the crude protein of the diet was reduced by three percentage points in relation to control diet, and in the same manner, industrial amino acids were included to ensure that the minimum essential amino acid requirements were met.

With reduction of crude protein in the second and third treatments from 17 percentage points to 15.5 and 14%, respectively, the glutamate content, as well as the essential and non-essential amino acid levels also decreased. Thus, the subsequent treatments were supplemented with L-glutamic acid to serve as a non-specific nitrogen source of the diet.

Accordingly, in the fourth treatment (T4), was added the same amount of glutamate of the control diet (2.982%) and crude protein increased to 15.17%, supplemented with 0.341% L-glutamic acid. In the fifth (T5) and sixth (T6) treatments was added1 and 2 percentage points of glutamate in relation to control diet (3.982 and 4.98%), respectively, and the crude protein increased to 15.74 and 16.32%, respectively, supplementing, this way, with 1.35 and 2.36% L-glutamic acid.

The variables evaluated were feed intake (g bird<sup>-1</sup> day<sup>-1</sup>), final weight (kg bird<sup>-1</sup>), egg production (%), egg weight (g egg<sup>-1</sup>), egg mass (g egg<sup>-1</sup>), mass conversion produced (g g<sup>-1</sup>), feed conversion per dozen eggs (kg dozen), eggshell specific gravity (g cm<sup>-3</sup>), percentage of albumen, yolk and shell, *haugh* unit, nitrogen consumed, excreted and retained in g bird<sup>-1</sup> day<sup>-1</sup>, nitrogen retention efficiency (%), serum uric acid level (mg dL<sup>-1</sup>), and economic evaluation.

Feed intake was calculated as the difference between the amount of feed supplied and experimental leftovers, weighed at the beginning and at the end of each 28 day-period. Final weight was determined by weighing all the replicates and dividing the value by the number of birds, at the end of the experiment. Egg production was recorded per period on egg production spreadsheets daily at 15h, by dividing the total number of eggs per replicate by the number of birds.

The eggs of each replicate were weighed individually on the last three days of each 28 dayperiod for calculation of the average egg weight. Egg mass was calculated by the product of egg production and the average egg weight per replicate. Feed conversion per egg mass was calculated as the ratio between feed intake and egg mass produced.

At the end of each period, it was selected four eggs per replicate; two eggs were used for specific gravity and another two eggs were used to determine weight and percentage of yolk, albumen and shell. The percentage was calculated after manual separation of these components, and shells were oven dried at 105°C for four hours. The percentage of each of the egg components was obtained by dividing the weight of the component by the weight of the egg, and then multiplying the result by 100.

At the end of each experimental period, two eggs per replicate were immersed in different saline solutions with appropriate adjustments to a volume of 25 L of water with densities that ranged from 1.060 to 1.100 with an interval of 0.0025 g cm<sup>-3</sup>. Eggs were put in the buckets with the solutions, from the lowest to the highest density, and removed when they floated. The respective density values corresponding to the solutions of the containers were recorded. Haugh unit (HU) was determined by the formula: (HU) =  $100 \log (H + 7.57 - 1.7W^{0.37})$ , where H = height of the albumen (mm); and W =egg weight (g) (Silversides et al., 1993).. Albumen height was measured with a special micrometer. Shell thickness was measured with the aid of an analog micrometer accurate to 0.1 mm at three points along the midline of the egg, through which the arithmetic mean was calculated.

Nitrogen consumed, excreted and retained and nitrogen retention efficiency were determined by the method of total excreta collection using iron oxide as a marker. In the third period, feed intake was monitored by weighing the feed supplied, with addition of iron oxide, and leftovers at the end of the period assessed.

During this three day-period, excreta were collected in trays placed below the cages, identified, weighed twice daily (8 and 16h) and stored in a freezer at -20°C until the end of the experimental period. Subsequently, excreta were homogenized, identified and a sample of approximately 150 g per replicate was placed in aluminum foil food containers and taken to a forced-ventilation oven at  $60 \pm 5^{\circ}$ C. After drying, samples were weighed again, ground and kept in identified plastic containers until the analysis of dry matter and nitrogen in laboratory of Animal Nutrition (Animal Science Department/Agrarian Science Center/Federal University of Paraiba, Paraiba State, Brazil) (Silva & Queiroz, 2002).

The average daily of intake (NI) and excretion (NE) of nitrogen per experimental replicate were calculated by dividing the average daily input and output crude protein (CP), expressed in dry matter at 105°C, by the factor 6.25. Average daily nitrogen retention (NR) was calculated as the difference between average daily input and output of N: NR=NI - NE. The percentage of nitrogen retention efficiency was calculated by the formula 100 - (NE x 100 NI<sup>-1</sup>) according to the micro-Kjeldahl method.

At the end of the fifth period, birds were weighed for final weight, and two birds with the representative average weight of the replicate were selected for collection of approximately 10 mL of blood by cutting the jugular vein of the birds. The serum obtained after centrifugation of blood was stored at -20°C for subsequent analysis of uric acid with a commercial kit Labtest S.A. (Lagoa Santa, Minas Gerais State, Brazil), and by reading on a spectrophotometer, according to the instructions of the manufacturer.

The animals in this study sacrificed by cutting the jugular vein were used for further study on morphological analysis of intestine, liver and reproductive tract.

The cost of feed (CF) in the period was calculated by dividing of feed intake (kg ton.<sup>-1</sup>) by the cost per ton of ingredients. The dozen eggs (DE) was obtained by multiplying the percentage by 140 days of production, dividing by 100, and then by 12. The cost of feed per dozen eggs (CFDE) was obtained by dividing the cost of ingredients by DE. Gross revenue (GR) was calculated by multiplying the cost of an eggs dozen by the amount of dozen eggs produced by treatment. Gross margin (GM) was computed by reducing the GM by the feeding period (\$ bird<sup>-1</sup>). The relative gross margin (RGM) was calculated based on 100% for the first treatment, the RGM of the others treatments was calculated using the relationship between the CM of treatment x 100 divided by the previous treatment.

Statistical analyses were performed with the software Statistical Analysis System (SAS, 2004).

Data were subjected to analysis of variance and comparison of means by the tukey test at 5% probability.

### **Results and discussion**

There were significant differences between treatments for egg production and egg weight. The treatment with 15.17% of CP + 0.341% L-glutamic acid promoted higher egg production than the control diet. However, the control diet had the highest values of egg weight than the other treatments (Table 2).

**Table 2.** Effect of treatments on final weight (FW), feed intake (FI), egg production (EP), egg weight (EW), egg mass (EM), and mass conversion produced (MCP) by laying hens.

Variables	T1	T2	T3	Τ4	T5	T6	SEM
CP (%)	17.00	15.50	14.00	15.17	15.74	16.32	SEIVI
IW (kg)	1.84	1.85	1.75	1.82	1.82	1.76	2.223
FW (kg)	1.66	1.74	1.69	1.70	1.76	1.72	8.131
FI (g bird-1 day-1)	110.8	111.1	110.8	110.5	110.2	109.0	2.642
EP (%)	88.4 b	92.1 ab	92.3 ab	94.3 a	91.2 ab	91.9 ab	3.841
EW (g)	67.3 a	64.9 ab	63.9 b	64.2 b	64.0 b	63.8 b	2.524
EM (g)	61.5	59.8	59.7	60.6	59.1	58.6	3.421
MCP (g/g)	1.80	1.85	1.85	1.82	1.86	1.86	3.271

SEM (%), Standard error of the mean; CP, Crude protein; IW, Initial weight. T1, 17% CP; T2, 15.5% CP; T3, 14% of CP; T4, 15.17% CP + 0.341% L-Glutamic acid; T5, 15.74% CP + 1.349% L-Glutamic acid; T6, 16.32% CP + 2.356% L-Glutamic acid.<sup>th</sup> Means followed by different letters in linesare statistically different by tukey' test at 5% probability (p < 0.05).

Protein must be well balanced and of high quality so that the bird can maximize egg production in an economically feasible way. Egg weight is influenced by dietary protein as it is required to synthesize egg albumen, and a decrease in dietary protein may reduce the amount of albumen, leading to a smaller egg size

In this experiment, we observed that the control diet with the highest crude protein value (17%) resulted in higher egg weight in relation to the others treatments with glutamate. It was expected that supplementation with glutamate in diets with reduced CP would improve the egg weight, based on the greater amount of amino nitrogen available for the synthesis of non-essential amino acids (NEAA), sparing the essential amino acids (EAA) for protein synthesis in the animal organism.

Possibly, the reduction in egg weight has occurred due to the deficiency of some essential amino acids, probably cystine and methionine. At this stage of life, the requirement for feather cover may lead to a greater demand for cystine; in turn, methionine is required to form cystine, occurring the deficiency of two amino acids of great importance for egg weight.

However, protein reduction and supplementation of glutamic acid in the treatment with 15.17% of CP + 0.341% L-glutamic acid promoted higher egg production than the control diet, showing that laying hens of 34-54 weeks of age presents better balance of amino acids in this treatment. Although the use of non-specific nitrogen sources to meet the synthesis of nonessential amino acids cannot be fully efficient for maximum protein utilization by the bird, because some non-essential amino acids are considered essential in some periods of the life cycle of the bird.

There was no significant effect for the variables internal and external quality of eggs assessed in this study (Table 3).

**Table 3.** Effect of treatments on specific gravity (SG), percentage of shell (PS), yolk (PY) and albumen (PA), and *haugh* units (HU) of eggs by laying hens.

Variables	T1	T2	T3	T4	T5	T6	SEM
CP (%)	17.00	15.50	14.00	15.17	15.74	16.32	SEIVI
SG (g cm <sup>-3</sup> )	1.085	1.086	1.086	1.085	1.086	1.086	0.151
PS (%)	9.50	9.52	9.95	10.12	9.81	9.69	5.232
PY (%)	25.2	26.0	26.6	26.5	26.5	26.7	4.812
PA (%)	64.6	62.7	64.1	63.9	63.9	63.3	2.601
HU	96.7	95.3	95.8	96.1	95.1	96.2	2.103
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SEM (%), Standard error of the mean; CP, Crude protein. T1, 17% CP; T2, 15.5% CP; T3, 14% CP; T4, 15.17% CP + 0.341% L-Glutamic acid; T5, 15.74% CP + 1.349% L-Glutamic acid; T6, 16.32% CP + 2.356% L-Glutamic acid. No significant for analysis of variance.

There are no results in the literature with glutamic acid supplemented in diets with protein reduction involving commercial layers. In this experiment, the specific gravity, percentages of shell, yolk, and albumen and *haugh* unit of eggs of laying hens showed good values in all treatments with glutamic acid. However, the results of treatments supplemented with glutamate were similar to the Control diet.

Average values for three days of feed intake for nitrogen intake, excreted nitrogen, nitrogen retention, retention efficiency of nitrogen and serum uric acid of laying hens (Table 4).

There was a significant difference between treatments for nitrogen consumed and retained, and serum uric acid of laying hens. The control diet had higher nitrogen intake and retention than the treatments with 14.00% CP (T3) and 15.74% CP + 1.349% L-glutamic acid (T5).

However, the control diet had the highest values of acid uric in the blood compared to the other treatments studied. In sequence, the results of acid uric was higher for the control diet (T1), followed by the treatments with 16.32% CP + 2.356% of L-glutamic acid (T6) and 15.74% CP + 1.349% of L-glutamic acid (T5). The treatments with 15.5% CP (T2) and 14% CP (T3) showed the lowest results for uric acid, but not different from each other and from the treatments with 15.17% CP + 0.341% L-glutamic acid (T4).

Pavan et al. (2005) evaluated protein reduction (17, 15.5 and 14% CP) in brown-egg hens and observed that the ingestion of diets with 14% CP, reduced nitrogen excretion by 27% as compared with diets with 17% CP. In the same way, Ishibashi and Yonemochi (2003) studied amino acid requirement and protein reduction on egg production and nitrogen excretion and observed an evident drop in nitrogen excreted when dietary protein was reduced.

In this study, although without no significant effect on nitrogen excretion, it can be observed that, in relation to the control diet with 17% CP, all treatments with protein reduction (15.5, 14, 15.17, 16.32 and 15.74% CP) showed lower excretion of nitrogen (12, 14,7,10, 4%) to the environment, respectively.

Birds excrete nitrogen in small amounts of water by means of uric acid as the main product of nitrogen metabolism, it is 60-80% of nitrogen excreted in the urine, while in other species it is excreted urea. Uric acid is synthesized in the liver and kidneys, approximately 90% are excreted via tubular secretion, irrespective of tubular reabsorption of water.

Uric acid is the main catabolic product of proteins, protein nitrogen and purine in birds. Normal levels of uric acid in young birds may range from 1 to 2 mg dL<sup>-1</sup>, while laying birds have levels from 2 to 7 mg dL<sup>-1</sup>. According to Kaneko et al. (2008), in Brown Leghorn hens, the large differences in uric acid rates may be related to age, diet, hydration status of birds and species. Its concentration is elevated when the renal function is impaired, decreasing its elimination through the kidneys. There may be increased levels of uric acid, creatinine, and urea in plasma when the kidneys work at 30% capacity (Schmidt et al., 2007).

Table 4. Average values for three days of feed intake (FI) about nitrogen intake (NI), nitrogen excreted (NE), nitrogen retention (NR), retention efficiency (RE) and serum uric acid (UA) of laying hens.

Variables	T1	T2	T3	Τ4	T5	T6	- SEM
CP (%)	17.00	15.50	14.00	15.17	15.74	16.32	SEIVI
FI (g bird <sup>-1</sup> day <sup>-1</sup> )	121.5	114.2	113.3	116.5	104.5	112.0	8.132
NI (g bird <sup>-1</sup> day <sup>-1</sup> )	2.93 a	2.50 ab	2.23 b	2.52 ab	2.34 b	2.60 ab	7.931
NE (g bird <sup>-1</sup> day <sup>-1</sup> )	0.76	0.64	0.62	0.69	0.66	0.72	8.790
NR (g bird <sup>-1</sup> day <sup>-1</sup> )	2.16 a	1.87 ab	1.61 b	1.82 ab	1.68 b	1.88 ab	8.142
RE (%)	74.00	74.60	72.00	72.50	71.90	72.20	1.801
UA (mg dL <sup>-1</sup> )	3.02 a	1.67 c	1.68 c	1.94 bc	2.38 b	2.44 b	5.982

SEM (%), Standard error of the mean; CP, Crude protein. T1, 17% CP; T2, 15.5% CP; T3, 14% CP; T4, 15.17% CP + 0.341% L-Glutamic acid; T5, 15.74% CP + 1.349% L-Glutamic acid; T6, 16.32% CP + 2.356% L-Glutamic acid. <sup>Asc</sup> Means followed by different letters in lines are statistically different by *Tukey*'s test at 5% probability (p < 0.05).

In this study, it is observed that the control diet (17% CP) had the highest value of uric acid (3.02) compared to other treatments, but within the normal range for laying hens, indicating no kidney problems. However, treatments with 15.5,14, 14, 15% CP showed lower values than normal (1.67, 1.68, 1.94), respectively, due to the low protein in diet that were not sufficiently degraded to meet the requirement of laying hens in production.

The results of the economic analysis are in accordance with the performance, where the best results were observed in the treatment 4, with 15.17% CP + 0.341% L-glutamic acid (Table 5).

Table 5. Effect of treatments on the economic analysis of laying hens.

Variables	T1	T2	Т3	Τ4	T5	T6
FI, kg BIRD <sup>-1</sup>	15.52	15.55	15.52	15.47	15.43	15.26
CF, \$ ton. <sup>-1</sup>	851.73	866.56	989.99	980.83	1298.64	1616.79
CA, \$/ bird <sup>-1</sup>	13.22	1.35	1.54	1.52	2.00	2.47
ED, dz bird <sup>-1</sup>	10.31	10.74	10.77	11.00	10.64	10.72
CFED, \$ dz <sup>-1</sup>	1.28	0.13	0.14	0.14	0.19	0.23
GR, R\$ (\$ 3,97 dz <sup>-1</sup> )	40.93	42.63	42.75	43.68	42.25	42.55
GM, \$	27.72	41.29	41.22	42.16	40.24	40.08
RGM, %	100.00	148.95	148.71	152.12	145.18	144.60

T1, 17% CP; T2, 15.5% CP; T3, 14% CP; T4, 15.17% CP + 0.341% L-Glutamic acid; T5, 15.74% CP + 1.349% L-Glutamic acid; T6, 16.32% CP + 2.356% L-Glutamic acid. CF, Cost of feed; ED, Eggs Dozen; CFED, Cost of feed for eggs dozen; GR, Gross revenue; GM, Gross margin; RGM, Relative gross margin. Price of ingredients collected on Guaraves Alimentos Ltda at 7/2013. Price of dozen of eggs collected on avisite.com.br at 1/2014.

The treatment with 15.17% of CP and supplementation DL-methionine, L-lysine, Lvaline, L-isoleucine, L-tryptophan and with 0.341% L-glutamic acid for Dekalb Brown hens with 34-54 weeks age proved to be more effective with the addition of essential amino acids and glutamic acid (non-essential amino acid), it was possible a reduction of 1.83 percentage points of crude protein and improved egg production, maintenance in weight, and internal and external quality eggs with lower cost in the production of laying hens with this treatment.

#### Conclusion

It is recommended the treatment with 15.17% CP + 0.341% L-glutamic acid, as it improved the performance and the economic analysis without changing the quality of egg and amount of nitrogen excreted to the environment.

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