Dietary supplementation of increasing levels of DL-methionine and L-lysine in hypoproteic diets for laying hens improves productivity and egg quality

La suplementación dietética de niveles crecientes de DL-metionina y L-lisina en dietas hipoproteicas para gallinas ponedoras mejora la producción y calidad del huevo

Fecha de recepción: 18 de agosto de 2014
Fecha de aceptación: 22 de diciembre de 2014

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Abstract

To evaluate the effect of dietary supplementation of DL-methionine and L-lysine in hypoprotein diets on productivity and egg quality, a total of 240 White Leghorn laying hens (Hybrid $_{L}$-33) of 26 weeks during laying peak period were placed for 49 days, according to completely randomized design with four treatments and 10 repetitions. Dietary treatments consisted of a control diet (no amino acid supplementation) (T0) and supplementation of 0.03% DL-Met and 0.04% L-Lys (T1); 0.06% DL-Met and 0.08% L-Lys (T2) and 0.09% DL-Met and 0.12% L-Lys (T3). At 26 and 33 weeks of age, 30 eggs per treatment were collected to determine internal and external egg quality parameters.

The experiment diets did not affect the viability and feed intake. Supplementation of essential amino acids, especially the T3 improved egg weight (54.30 to 58.30 g), laying rate (76.89 to 85.45%) and mass conversion (2.52 to 2.11 kg/kg) ($P < 0.05$). At 33 week, the albumin height (7.21 to 8.23 mm), Haugh units (86.70 to 91.15) ($P < 0.05$) and shell surface (67.10 to 69.20 cm^2) increased by the effect of supplementation of DL-Met and L-Lys. The results allow recommending the dietary supplementation of 0.09% DL-Met and 0.12% L-Lys in hypoprotein diets to improve productivity and egg quality in laying hens.

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Key words*:* Hypoprotein diet, DL-methionine, L-lysine, Laying Hen, Egg Productivity, Egg Quality.

Resumen

Para evaluar el efecto de la suplementación dietética de DL-metionina y L-lisina en dietas hipoproteicas sobre producción y calidad del huevo, un total de 240 gallinas ponedoras White Leghorn (Híbrido L-33) de 26 semanas durante el pleno pico de puesta, se ubicaron durante 49 días, según diseño completamente aleatorizado con cuatro tratamientos y 10 repeticiones. Los tratamientos consistieron en un control (sin suplementación de aminoácidos) (T0) y la suplementación de DL-Met 0.03: L-Lys 0.04 % (T1); DL-Met 0.06: L-Lys 0.08 % (T2) y DL-Met 0.09: L-Lys 0.12 % (T3). A las 26 y 33 semanas de edad, se recolectaron 30 huevos por tratamientos para determinar los indicadores de calidad externa e interna del huevo. Las dietas experimentales no afectaron la viabilidad y el consumo de alimento. La suplementación dietética de los aminoácidos esenciales, sobre todo el T3 mejoró el peso del huevo (50.3 a 54.3 g), intensidad de puesta (46.89 a 55.45%) y la conversión masal (3.49 a 4.46 kg/kg) ($P<0.05$). En la semana 33, la altura de la clara densa (7.21 a 8.23 mm), unidades Haugh (87.70 a 92.15) ($P<0.05$) y la superficie de la cáscara (55.24 a 59.84 cm2) mejoraron por efecto de la suplementación de DL-Met y L-Lys. Los resultados obtenidos permiten recomendar la suplementación de dietética de DL-Met 0.09 y L-Lys 0.12 % en dietas hipoproteicas para gallinas ponedoras para mejorar la producción y calidad del huevo.

Palabras clave: dieta hipoproteica, DL-metionina, L-lisina, gallina ponedora, producción de huevo, calidad del huevo.

Introduction

The poultry farming, particularly egg production is a complex and dynamic system, by the amount of productive links involved in it and the elements involved to obtain the final result (1). Currently the amino acids play a key role in the nutrition of monogastric animals; have been studied different needs maintenance and production in birds, especially in commercial laying hens, where the nutritional wear is so demanding (2, 3).

Since several years, the amino acid requirements have been published for birds. Some of them were proposed for chicks in the initial phase, broilers and laying hens. In the majority of cases, the authors estimate the needs of some essential amino acids in separate experiments and then calculate the needs of other amino acids using body composition or feathers (4, 5).

Generally, lysine is used as amino acid of reference and the needs of the other essential amino acids are expressed as percentage of lysine. However, this affirmation is discussed today, because it minimizes the value of the concentration thereof, their relationship and antagonism. Many factors can influence the needs of amino acids such as chemical composition of the feedstuffs, type of feed, feed presentation form, particle size, ambient temperature, sex and age, which can alter feed intake and thus, amino acids intake (3, 6).

The use of synthetic amino acids in diets not only corrects efficiently and quickly the deficit of this nutrient in the raw materials in birds, also it helps to reduce the emission of nitrogen to the environment as concept of "ideal protein" (7). Moreover, at present some essential amino acids such as L-arginine and L-lysine have been used as nutraceutical supplements for controlling enteric processes, reduce pathogenic microflora and the harmful lipids and improve growth performance (5, 8).

On the other hand, has been studied the relationship of the contributions of amino acids (especially essential) in diets, with the aim of increasing the biological responses of birds, the ratio of sulfur amino acids: lysine is one of the most studied (3, 5). The main recommendations are in a ratio of 0.50 to 0.90 (3). In this sense, Faria *et al*. (9), Vieira *et al*. (5) & Martínez *et al*. (10) recommend a close relationship between these amino acids to maximize production and egg weight, Vieira *et al*. (5) have recommended a ratio of 0.77, with good results in growing birds. Despite these researches, it is not enough information available about the effect of supplementation of these amino acids in hypoprotein diets for laying hens.

Furthermore, lysine is main amino acid linked to the egg production, synthesis of hormones and the immune system of birds and the sulfur amino acids are the most important in egg weight and connective tissue growth (4). The aim of this study was to evaluate the effect of dietary supplementation of DL-methionine and L-lysine in hypoprotein diets on productivity and egg quality in laying hens.

Materials And Methods

Location of the experimental area: The experiment was carried out in the Poultry Unit "San Andrés" situated at the outskirts of the municipality of Holguín, belonging to the Eastern region of Cuba. Mean relative humidity was 72% and average minimum and maximum temperatures were 23 and 31.7ºC, respectively (data from the Meteorological Station of Holguín, Cuba).

Amino Acids, birds and treatments: To dietary supplementation of amino acids, 10 kg of DL-methionine (Met) and L-lysine (Lys) were collected of the feed mill with a purity of 98%. A total of 240 White Leghorn laying hens (Hybrid $_{L}$-33) of 26 weeks during laying peak period were placed for 49 days, according to completely randomized design with four treatments, 10 repetitions and six birds per repetition.

Dietary treatments consisted of a control diet (no amino acid supplementation) (T0) and supplementation of 0.03% DL-Met and 0.04% L-Lys (T1); 0.06% DL-Met and 0.08% L-Lys (T2) and 0.09% DL-Met and 0.12%L-Lys (T3). Hypoprotein diets were prepared with 15.50 % of crude protein, based on corn and soybean cake meal according to the recommendations of UECAN (11) (Table 1).

It took into account the requirements of the birds for supplementation and relation of synthetic amino acids used (11).

Experimental conditions: The experimental unit was a 60 x 80 cm metallic cage, where 8 hens were assigned. Birds received 105 g of feed/hen/d. Water was supplied ad libitum by three nipple drinkers per cage. Sixteen hours of illumination were provided daily. There was no adaptation period to the experiment (12). No medicine or therapeutic veterinary care was offered during the experimental stage.

Productive indicators: Initial and final weighing of the laying hens was individually performed at 26 and 33 weeks of age, through a digital SARTORIUS (model BL 1500) balance, with precision ± 0.10 g. Egg weight was taken twice weekly to 30 eggs/treatment, between 8:30 and 9.30 a.m. and average weight was estimated. Feed intake was determined daily by the offer and rejection method.

For determining laying rate, total egg production/week/treatment was considered. One egg/d/bird housed was assumed as 100%. Mass conversion was calculated through feed consumed, egg weight per replication and number of eggs laid. At the end of the experiment, the viability was also calculated.

Percentage of eggs not fit for consumption (cracked, without an eggshell and broken) was calculated by the formula: % of eggs not fit for consumption (ENFC) = #ENFC* 100/eggs fit for consumption.

Table I. Dietary ingredients and nutrient levels in diets (as fed)

Ingredients	*(%)*
Corn meal	57.85
Soybean cake meal	28.00
Vegetable oil	1.19
Salt	0.25
DL-Methionine	0.19
Dicalcium phosphate	1.63
Calcium carbonate	8.84
Copper sulfate	0.05
Premix[1]	1.00
Zeolite	1.00
Contributions	*(%)*
Metabolizable energy (MJ/kg)	11.51
Crude protein	15.50
Calcium	3.80
Total phosphorus	0.63
Available phosphorus	0.40
Methionine plus cystine	0.63
Lysine	0.70
Crude fiber	2.66

[1]Each kg contains: vit. A, 10 x 10^6 I.U.; vit.D_3, 1.5 x 10^6 I.U.; vit.K_3, 2100 mg; vit.E, 10000 mg; thiamin, 800 mg; riboflavin. 2500 mg; pantothenic acid, 10000 mg; pyridoxine, 2500 mg; folic acid, 250 mg; biotin, 100 mg; vit. B_{12}, 15 mg; manganese, 60000 mg; copper,8000 mg; iron, 60000 mg; zinc, 50000 mg; selenium, 200 mg; iodine, 800 mg; cobalt, 500 mg; Antioxidant 100%, 125000 mg.

External and internal egg quality indicators: At 26 and 33 weeks of the experiment, 30 eggs/treatment were sampled to determine the external quality indicators (weight, thickness, eggshell surface) and internal quality indicators (albumin height and of the yolk, Haugh units and yolk color). A Russian slide gauge with ± 0.01 mm precision was used for measuring the eggshell thickness at the egg's equator and at the upper and lower poles. The shell surface was established according to Carter's (1975) formula: Area= $3.9782W^{0.7056}$, where W is the egg weight (g). The albumin height and of the yolk was measured with a height gauge with ± 0.01 mm accuracy (Maver). The records of Haugh units were calculated by the relationship between the egg weight and the albumin height: HU = 100 log ($H-1.7w^{0.37}+7.6$), where: HU are the Haugh units, H is the albumin height and W is the egg weight. The yolk color was determined by Roche's range of 15 colors.

Statistical analysis: Data were processed by analysis of variance (Anova) of simple classification, in completely randomized design. In the necessary cases Duncan's test was applied to determine mean differences, according to the statistical software SPSS version 12.1. The percentage of cracked eggs and without an eggshell was analyzed by comparison of proportions in the statistical software COMPARPRO (13).

Results And Discussion

Table 2 shows the effect of dietary supplementation of DL-Met and L-Lys on productivity and egg quality in laying hens. The viability, feed intake and eggs unfit showed no significant differences ($P > 0.05$) among treatments, with low incidence of this last indicator. However, the laying rate (76.89 to 85.45%), egg weight (54.30 to 58.30g) and mass conversion (2.52 to 2.11 kg/kg) improved with supplementation of these amino acids, especially with T3 ($P < 0.05$).

A diminution of crude protein and consequently true protein decreases the concentration of lysine and methionine (4) (Table I). Likewise, these amino acids are limiting in birds, a deficiency in the diets depresses the main production indicators (10). According to Aguilar *et al*. (6), an intake of lysine efficient indicates a good balance of this amino acid in the basal diet. In this sense, Rostagno *et al*. (3), found an increase on production and mass conversion, by adding synthetic amino acids on laying hen diets. Faria and Dos Santos (14) found similar results when they added increasing levels of L-Lys on diets.

Faria and Dos Santos (14) and Aguilar *et al*. (6) have reported that lysine is the essential amino acid most significant on the egg production; as well as involved in growth and tissue repair. It is observed that egg production was progressive, which indicated the deficiency of this amino acid in the diet.

On the other hand, an adequate supply of protein and amino acids in the diets of laying hens is essential for producing a good egg size (15). According to the NRC (4) and Aguilar *et al*. (6) methionine is a sulfur amino acid that may synthesize cysteine by biological processes and sulfur donated by homocysteine, being the most important amino acid in egg weight. In this sense, Faria *et al*. (9) found an increase on egg weight, by supplementation of increasing levels of methionine plus cysteine on laying hen diets similar to our results (Table II). It is noted that methionine is deficient in the soybean meal and corn (4); therefore supply it artificially in the diet of laying hens is an everyday norm.

There have been many studies on the relationship of the essential amino acids in birds, Roland *et al*. (16) indicated that there is a close relationship between dietary level of sulfur amino acids and lysine. The same authors report an increase in production, feed intake and egg weight by using a ratio of 0.75 to 0.90 Met/Lys, respectively, as well as Faria and Dos Santos (14) recommend a ratio Met/Lys of 0.80. It is important to emphasize that these researches were developed with isoproteic-diets, no information available on the relationship of these amino acids in hypoprotein diets for laying hens, a ratio of 0.75 Met/Lys with increasing levels of these synthetic amino acids on diets was efficient to increase egg productivity.

At 26 weeks of the experiment the indicators of egg quality did not show significant differences ($P > 0.05$) among treatments (Data not shown), as well as at 33 weeks the shell thickness did not change($P > 0.05$) by effect of experimental treatments (Table II), which justifies an adequate intake of calcium, phosphorus and other minerals in diets supplemented with synthetic amino acids. However, the shell surface increased with dietary supplementation of L-Lys and DL-Met (Table II), demonstrating that these amino acids directly influences in this indicator related to the egg weight.

Albumin levels increased proportionally with supplementation of DL-Met and L-Lys and with egg weight ($P < 0.05$; Table II); Novak and Scheideler (17) indicated similar results. According to Whitehead (18) the amount of albumen depends on the balance of amino acids composed by the protein in the diet. A deficiency in lysine or methionine reduces albumen weight and decreases the concentration of all free amino acids. This shows that supplementation of DL-Met and L-Lys can deter-

mine the albumin height composed by protein and water. It is noted that not it found researches about the ratio of these two amino acids and its influence on internal egg quality, our research showed that increasing levels of DL-Met and L-Lys with a ratio of 0.75 in hypoprotein diets for laying hens is valid for increase the egg quality (Table II).

The Haugh units improved with supplementation of DL-Met and L-Lys on diets ($P<0.05$), especially with T3, due to increased egg weight and albumin height (Table II). Tallarico *et al*. (19) and Martínez *et al*. (10) found similar results by increasing these two quality parameters. This indicator is important to know the nutritional and sanitary quality of the egg (20), also influenced by other factors such as sampling, temperature and storage time. According to Suk and Park (21) prolonged egg storage reduces a unit/day the Haugh units.

The yolk height showed no variation with supplementation of synthetic amino acids ($P>0.05$; Table II); but it was observed that as it advanced age of hens, egg yolks were more prominent (Data not shown); this could be determined by egg weight which increased in the experimental weeks (Data not shown) (Table II), although other factors also influence such as the content of lipids in the diet, productive stage and race (22).

Table II. Effect of dietary supplementation of DL-methionine and L-lysine in hypoproteic diets on productivity and egg quality in laying hens

	Dietary supplementation of DL-Met and L-Lys				
Items	T0	T1	T2	T3	SEM± Sig.
Viability (%)	100.00	100.00	100.00	100.00	
Feed intake (g/ave/day)	105.00	105.00	105.00	105.00	
Laying rate (%)	76.89^{c}	82.00^{b}	85.34^{a}	85.45^{a}	1.57*
Egg weight (g)	54.3^{b}	54.00^{b}	55.30^{b}	58.30^{a}	0.300
Mass conversion (kg/kg)	2.52^{a}	2.37^{b}	2.23^{c}	2.11^{d}	0.046***
Cracked eggs (%)	0.10	0.07	0.07	0.10	0.520
Broken eggs (%)	0.04	0.03	0.04	0.03	0.041
Shell-less eggs (%)	0.04	0.03	0.03	0.03	0.037
Egg weight (g)	54.84^{c}	54.94^{c}	56.30^{b}	57.30^{a}	0.462*
Shell thickness (mm)	0.43	0.44	0.44	0.43	0.007
Shell surface (cm^2)	67.10^{c}	67.20^{c}	67.78^{b}	69.20^{a}	0.210*
Album in height (mm)	7.21^{b}	8.10^{a}	8.13^{a}	8.23^{a}	0.140*
Yolk height (mm)	9.72	9.53	9.71	9.70	0.170
Haugh units	86.70^{b}	91.64^{a}	91.43^{a}	91.15^{a}	1.021**

$^{a,b,c.}$ Means with different superscripts in the same row are different ($P<0.05$) *$P<0.05$; **$P<0.01$; ***$P<0.001$.

The results obtained allow to recommend dietary supplementation of 0.09% DL-Met and 0.12% L-Lys with a ratio of 0.75 in hypoprotein diets for laying hens to improve laying rate, egg weight, massal conversion, albumin height and Haugh units.

Acknowledgements

Thanks are given for their collaboration to the executive staff and technicians of the laying hen farm "San Andrés" for the development of this research.

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