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Níveis de metionina+cistina digestíveis para aves de reposição leves de uma a seis semanas de idade

Digestible methionine+cystine levels for white-egg layers aged one to six weeks

Niveles de metionina+cistina digestibles para pollitas de remplazo blancas de una a seis semanas

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Resumo

Objetivou-se determinar a exigência nutricional de metionina+cistina (M+C) digestíveis para aves de reposição leves de 1 a 6 semanas de idade. Utilizou-se um delineamento inteiramente ao acaso com cinco níveis de metionina+cistina, seis repetições e 30 aves por unidade experimental (UE). Os tratamentos dietéticos consistiram em cinco dietas suplementadas com DL-Metionina que resultou em cinco níveis de metionina+cistina digestíveis 80% (0.516%), 90% (0.578%), 100% (0.640%), 110% (0.702%), and 120% (0.764%), baseado nas tabelas brasileiras de exigências nutricionais. Foram avaliados dados de desempenho, sorologia e histologia. Houve comportamento quadrático para consumo de ração, conversão alimentar, deposição de glicogênio hepático, atividade enzimática da alanina aminotransferase, gama-glutamiltransferase e níveis séricos da creatinina e albumina em função dos níveis de M+C digestíveis, sendo as exigências para estas características: 89.78% (0.575%), 114.33% (0.732%), 86.50% (0.554%), 100% (0.640%), 100.40% (0.643%), 104.30% (0.668%), and 111.88% (0.716%), respectivamente. Recomenda-se a utilização de 0,732% de metionina+cistina digestíveis, que corresponde a um consumo de 151.20 mg/ave/dia, e uma relação com a lisina de 83%, para aves de reposição leves de 1 a 6 semanas de idade.
Palavras-chave: Exigência nutricional; Desempenho; Aminoácidos sulfurosos.

Abstract

The aim of this study was was to determine the nutritional requirements of digestible methionine+cystine (M+C) for white-egg layers aged one to six weeks**.** A completely randomized design with five methionine+cystine levels, six replicates, and 30 birds per experimental unit was adopted. Dietary treatments consisted of five diets supplemented with DL-Methionine with resulted in five levels of digestible methionine + cystine, 80% (0.516%), 90% (0.578%), 100% (0.640%), 110% (0.702%), and 120% (0.764%), based on Brazilian tables of nutritional requirements. Performance, serological blood, and histological data were evaluated. Feed intake, feed conversion, hepatic glycogen deposition, enzymatic activity of alanine aminotransferase and gamma-glutamyl transferase, and serum creatinine and albumin levels had showed a quadratic response to the levels of digestible M+C, with the respective requirements: 89.78% (0.575%), 114.33% (0.732%), 86.50% (0.554%), 100% (0.640%), 100.40% (0.643%), 104.30% (0.668%), and 111.88% (0.716%). Increasing levels of methionine+cystine elevated the relative liver weight and the deposition of hepatic glycogen, in addition to promote higher growth in pullets, with better body weight and body weight gain and feed conversion ratio. Our findings suggest that 0.732% digestible methionine+cystine is recommended, which corresponds to an intake of 151.20 mg/bird/d and a Met+Cys:Lys ratio 83%, for light pullets from one to six weeks.

Keywords: Nutritional requirement; Performance; Sulfur amino acids.

Resumen

El objetivo de este estudio fue determinar los requerimientos nutricionales de metionina + cistina digestibles (M+C) para pollitas de remplazo blancas de una a seis semanas. Se adoptó un diseño completamente al azar con cinco niveles de metionina + cistina, seis réplicas y 30 aves por unidad experimental. Los tratamientos dietéticos consistieron en cinco dietas suplementadas con DL-Metionina que dieron como resultado cinco niveles de metionina + cistina digeribles, 80% (0.516%), 90% (0.578%), 100% (0.640%), 110% (0.702%), y 120% (0.764%), basado en tablas brasileñas de requerimientos nutricionales. Se evaluaron el rendimiento, la sangre serológica y los datos histológicos. El consumo de alimento, la conversión alimenticia, el depósito de glucógeno hepático, la actividad enzimática de alanina aminotransferasa y gamma-glutamil transferasa, y los niveles séricos de creatinina y albúmina mostraron una respuesta cuadrática a los niveles de M + C digestible, con los requisitos respectivos: 89.78% (0.575 %), 114.33% (0.732%), 86.50% (0.554%), 100% (0.640%), 100.40% (0.643%), 104.30% (0.668%) y 111.88% (0.716%). Los niveles crecientes de metionina + cistina elevaron el peso relativo del hígado y la deposición de glucógeno hepático, además de promover un mayor crecimiento en las pollitas, con un mejor peso corporal y ganancia de peso corporal y relación de conversión alimenticia. Nuestros hallazgos sugieren que se recomienda 0.732% de metionina + cistina digerible, lo que corresponde a una ingesta de 151.20 mg / ave / día y una relación Met + Cys: Lys 83%, para pollitas de una a seis semanas.

Palabras clave: Requerimiento nutricional; Desempeño; Aminoácidos azufrados.

1. Introduction

Among the essential nutrients directly influencing birds' performance is methionine, the noteworthy first limiting amino acid to these animals when corn- and soybean meal-based diets are supplied. Methionine also performs several functions in the organism of birds and affects the immune system, the deposition of protein, the lipid metabolism, and the energy metabolism (Zhu et al., 2019; Figueiredo Jr et al., 2020; Jankowski et al., 2020).

Some other functions of methionine include the donation of methyl radicals, precursors of the biosynthesis of cysteine, which, bound to the pairs by a disulfide bond, forms cystine, which is why nutritional recommendations are expressed as methionine+cystine (Bin et al., 2017).

The proper methionine nutritional level not only boost productive performance rates, tissue formation, and the functioning of physiological systems, but also maximizes the preparation of pullets for the subsequent developer and egg-laying phases. However, there is little information about pullets, especially regarding amino acids. This may influence the maximization of production by birds in the phase subsequent to their growth, i.e., the egg-laying phase, because it is clear that egg production in the laying phase will be closely linked to its period of development.

In this sense, Rostagno et al. (2017) have recently recommended 0.86% digestible methionine+cystine values for the starter phase (one to four weeks of age), and Dekalb White (2009) manual recommended levels of 0.75% digestible methionine+cystine for white-egg pullet layers from 1 to 5 weeks of age. D'Agostini et al. (2017), in turn, recommended 0.70% digestible methionine+cystine in diets for pullets aged one to six weeks.

Therefore, the aim of the current study was to determine the requirement of digestible methionine+cystine for light pullets aged one to six weeks.

2. Material and Methods

This paper was an experimental research of a quantitative nature, which was used as a collection instrument the testing technique. According to Pereira et al. (2018) the quantitative method generates a set of data that can be analyzed using mathematical techniques, such as percentages, statistics and probabilities, numerical methods, analytical methods and generation of equations and / or mathematical formulas applicable to some process.

The experiment was conducted in Poultry Sector, Department of Animal Science at Federal University of Paraiba. The project had ethical approval from the Animal Use and Care Committee of the Federal University of Paraíba, Brazil, under protocol number 149/2015. The total experimental period was 42 d, comprising the bird starter phase, from one to six weeks of age.

A total of 900 Dekalb White pullets from the 1st day of age with an average initial live weight of 35.94 ± 0.10 g were housed in 1.0 × 1.5 m experimental pens. The floor was covered with sugarcane bagasse bedding, and each pen contained an incandescent 100 W light bulb for heating the birds in the first weeks of life, a tubular feeder, and a pendulum chick drinker. Water and feed were available ad libitum, and the vaccination and lighting programs adopted were those suggested for the sanitation challenge in the region and by guide of the lineage, respectively.

The experiment comprised a completely randomized design, with five treatments and six replicates with 30 pullets per experimental unit. Treatments consisted of five experimental diets, a basal diet was formulated to meet the requirements of all nutrients, according to recommendations of Rostagno et al. (2011), except for methionine, which was supplemented by DL-Methionine (0.00, 0.062, 0.124, 0.186, and 0.248%), resulting in five levels of digestible methionine+cystine (0.516, 0.578, 0.640, 0.702 and 0.764%), which corresponded to 80, 90, 100, 110, and 120%, respectively, of the recommendations of Rostagno et al. (2011), shown in Table 1.

Table 1- Ingredients and chemical composition of the experimental diets.

Ingredients	Rostagno et al. (2011)[2]				
	0.516	0.578	0.640	0.702	0.764
Corn	75.065	75.065	75.065	75.065	75.065
Soybean meal, 45%	18.838	18.838	18.838	18.838	18.838
Corn gluten, 60%	1.862	1.862	1.862	1.862	1.862
Dicalcium phosphate	1.801	1.801	1.801	1.801	1.801
Limestone	1.121	1.121	1.121	1.121	1.121
Salt	0.406	0.406	0.406	0.406	0.406
L-lysine	0.194	0.194	0.194	0.194	0.194
L-threonine	0.034	0.034	0.034	0.034	0.034
DL-methionine	0.000	0.062	0.124	0.186	0.248
Choline chloride, 60%	0.070	0.070	0.070	0.070	0.070
Vitamin premix[3]	0.050	0.050	0.050	0.050	0.050
Mineral premix[4]	0.050	0.050	0.050	0.050	0.050
Antioxidant[5]	0.010	0.010	0.010	0.010	0.010
Inert[6]	0.500	0.438	0.376	0.314	0.252
Total	100	100	100	100	100
Nutrient density-calculated					
Crude protein (%)	18.00	18.00	18.00	18.00	18.00
Calcium (%)	0.940	0.940	0.940	0.940	9.40
Available phosphorus (%)	0.437	0.437	0.437	0.437	0.437
Metabolizable energy (kcal/kg)	2.900	2.900	2.900	2.900	2.900
Arginine (%)	0.937	0.937	0.937	0.937	0.937
Isoleucine (%)	0.604	0.604	0.604	0.604	0.604
Lysine (%)	0.876	0.876	0.876	0.876	0.876
Met + Cys (%)	0.516	0.578	0.640	0.702	0.764
Threonine (%)	0.587	0.587	0.587	0.587	0.587
Tryptophan (%)	0.158	0.158	0.158	0.158	0.158
Sodium (%)	0.180	0.180	0.180	0.180	0.180
Chlorine (%)	0.290	0.290	0.290	0.290	0.290
Potassium (%)	0.610	0.610	0.610	0.610	0.610
Analyzed composition					
Crude protein (%)	18.01	17.87	17.82	18.02	17.34
Methionine, %	0.40	0.41	0.43	0.52	0.56
Lysine, %	0.75	0.76	0.74	0.75	0.76

[2]Diets formulated based on digestible amino acids [3] Inorganic mineral premix per kg: Mn - 20 g; Fe - 10 g; Zn - 13.7 g; Cu - 2.5 g; Se - 0.063 g; I - 0.19 g; excipient quantity sufficient for 500 g.[4]Vitamin premix per kg: vit. A - 15,000,000 IU; vit. D_3 - 1,500,000 IU; vit. E - 15,000 IU; vit. B_1 - 2.0 g; vit. B_2 - 4.0 g; vit B6 - 3.0 g; vit. B_{12} - 0.015 g; nicotinic acid - 25 g; pantothenic acid - 10 g; vit. K_3 - 3.0 g; folic acid - 1.0 g; zinc bacitracin - 10 g; selenium - 250 mg.[5]Antioxidant (BHT) - 10 g; excipient quantity sufficient for 1.000 g; [6]Washed sand. Source: own research.

The studied variables were final body weight (FBW, g/bird), weight gain (BWG, g/bird), feed intake (FI, g/bird), feed conversion (FCR, g/g), methionine+cystine intake (MCI, mg/bird/d), measurements of the weights of eviscerated carcass (EVC, g/bird), liver (LIV, g/bird), and spleen (SPL, g/bird, hepatic glycogen deposition (HCD, Ishak's score), and serological analysis of alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), gamma-glutamyl transferase (GGM, U/L), creatinine (CRE, mg/dL), albumin (ALB, g/dL), and serum protein (PTN, g/dL). Histological analysis of liver was also performed, and the growth curve of the pullets was built.

The diets samples were analyzed for DM by placing duplicate samples in a drying oven at 105◦C for 24 h (AOAC, 1990). Nitrogen content of feed samples were determined on a 0.25 g sample with a combustion analyzer (Leco model FP-2000 N analyzer, St. Joseph, MI) using EDTA as a calibration standard, with CP being calculated by multiplying percentage N by 6.25. For AA analysis, samples were prepared by 6 N HCL hydrolysis for 24 h at 110◦C followed by neutralization with 4 mL 25% (wt/vol) NaOH, and then cooled to room temperature. Afterward, sodium citrate buffer was added and the mixture was equalized to a 50 mL volume (AOAC 1990; method 982.30). Methionine and cysteine (sulfur containing amino acids) were analyzed by performic acid oxidation at 0◦C, followed by acid hydrolysis. The amino acids (AOAC 1990; method 982.30) in the hydrolyzate were determined by an AA analyzer (Biochrom 30. 30 plus, Biochrom Ltd, Cambridge, United Kingdom).

At the end of the experiment (42 d), ten birds were slaughtered per plot for serological, histological analyses and measurement of organs. Serological analyses were performed using a VetTest Blood Chemistry Analyzer (IDEXX Laboratories, Westbrook, Maine). For the histological analyses, fragments of the digestive system (liver) were immersed in methacarn fixative solution (60% methanol, 30% chloroform, and 10% acetic acid) for 12 h, performing the standard histological procedure.

For the optical microscopy, fragments were included in paraplast and subsequently sectioned into series of 5 µm thickness. The following histological staining methods were performed: hematoxylin and eosin and Periodic Acid Schiff (PAS). Photomicrographs were captured with a micro-camera coupled to an Olympus BX-51 microscope (Olympus, Tokyo, Japan), and images were digitalized on the KS 400.3 software (Carl Zeiss Vision GmbH, Germany).

To evaluate hepatic glycogen deposition, an assessment score was assigned to each analyzed material (ten birds), considering the degree of positivity to PAS staining, as follows:

0 (no glycogen deposition), 1 (little glycogen deposition), 2 (medium glycogen deposition), 3 (elevated glycogen deposition), and 4 (large glycogen deposition), according to Ishak's semi-quantitative scoring system (Ishak et al., 1995). Assessments were undertaken by the same histologist.

Concerning the growth curve, the pullets live weight was measured weekly in the period of one to six weeks of age, totaling five weightings, and the obtained data were submitted to the equation models proposed by Von Bertalanffy (1957), Brody (1945), Gompertz (1825), Logistic (Verhulst, 1845), and Richards (1959), using the GOSA Statistical Software, which gave the best adjustment of mathematical equations. Akaike's Information Criterion (AIC) (Akaike, 1987) was used to choose the mathematical model that best fit the pullets age-weight data on the growth curve.

The statistical analyses of the evaluated traits were performed using the SAS software (SAS, 2011). The digestible methionine+cystine requirement was estimated by regression analyses.

3. Results and Discussion

The data of variables, FBW, BWG and MCI fitted the linear regression models better, but variables FI and FCR adjusted better to the quadratic regression. The points of minimum found for FCR was 114.33% (0.732%) and the maximum point for FI were 89.78% (0.575%), estimated by the derivative of the regression equations (Table 2).

Table 2- Effects of treatments on final live weight (FBW, g/bird), weight gain (BWG, g/bird), feed intake (FI, g/bird), feed conversion (FCR, g/g) and Met+Cys intake (MCI, mg/bird/d) in hens aged one to six weeks.

Met+Cys	Met:Lys*	FBW	BWG	FI[1]	FCR[2]	MCI
80% (0.516%)	59	288.2	252.4	848.23	3.36	104.21
90% (0.578%)	66	319.7	283.7	851.89	3.00	117.24
100% (0.640%)	73	316.1	280.3	846.94	3.02	129.06
110% (0.702%)	80	341.4	305.4	867.11	2.84	144.93
120% (0.764%)	87	358.6	322.6	896.43	2.78	163.06
P-value						
Linear		P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Quadratic		0.977	0.977	0.052	0.054	0.015
SEM		0.032	0.083	0.095	0.013	0.033
CV (%)		2.32	2.60	2.87	3.63	2.78

*Methionine+cystine:lysine ratio (%). CV = coefficient of variation; [1]$Y = 14.204X^2 – 163.81X + 1317.8$, $R^2 = 0.96$; [2]$Y = 0.0743X^2 – 1.1643X + 7.3499$, $R^2 = 0.91$. Source: own research.

The optimum digestible methionine+cystine value estimated by FCR is similar to the 0.750% recommended by the Dekalb White (2009) guide for the phase from one to six weeks of age, but bottom than the 0.859% described by Rostagno et al. (2017).

Likewise, Silva et al. (2009) utilized methionine+cystine levels for pullets aged one to four weeks varying from 0.650 to 0.850%, and a significant effect was observed on FI, BWG, and FCR. The optimum amino acid levels found in this study were 0.800% and 0.790% for BWG and FCR, respectively.

The methionine+cystine levels utilized in this experiment were not sufficient to cause an effective reduction in feed intake to reward for the excess amino acids in the diet. According to Gietzen et al. (1998), there is evidence that alterations in the levels of limiting amino acids in relation to the total amino acids in the diet cause an imbalance detected in the prepiriform cortex of the brain, followed by behavioral alterations such as reduction in feed intake.

According to Keshavarz and Nakajima (1995), body weight has been considered the major factor for the egg weight in the laying phase, stated that for every 45 g of body weight below the expected weight, sexual maturity may be delayed by 3 to 3.5 d.

Additionally, Leeson & Summers (1997) emphasized the importance of maximizing weight gain until maturity, because the BW affect not only the egg weight, but delay onset of lay too.

According to D'Agostini et al. (2017), during the growth phase, it is essential to supply nutrients that meet the requirements for maintenance and gain, thereby ensuring the proper development of the immune and reproductive systems and body structure, which in turn provides greater productivity in the laying phase and the formation of uniform birds, which ultimately makes the activity more profitable.

Sulfur amino acids play an essential role in the performance and maturity of hens, as these acids are able to regulate the expression of numerous genes and thus control the metabolism of nutrients, cell functions, and the protein synthesis (Tesseraud et al., 2008). Thus, optimal levels of dietary methionine is not only required for normal growth, but also for proper cellular and molecular functions (Fagundes et al., 2020).

A linear effect of the levels of digestible methionine+cystine ($P < 0.05$) was also found on EVC, LIV, SPL, and liver yield (Table 3). The highest supplementation of methionine+cystine in the diet was considered the most efficient in terms of absolute and relative weights of the analyzed variables.

Bunchasak & Silapasorn, (2005) demonstrated that methionine supplementation decreased the weight of the liver and the hepatic protein, but elevated the synthesis of fat in the liver of layers reared in tropical conditions.

The opposite was observed in this study, wherein the digestible methionine+cystine levels increased the absolute and relative weights of the birds' liver. Nevertheless, the above previous studies found these results with adult birds, and in the current study the tested birds were under growth, for which no scientific reports exist yet.

Table 3- Effects of treatments on the absolute (g) and relative (%) weights of eviscerated carcass (EVC), liver (LIV), and spleen (SPL), of hens aged one to six weeks.

Treatment	Met:Lys*	EVC	LIV	SPL
Met+Cys			Absolute weight (g)	
80% (0.516%)	59	250.00	9.610	0.819
90% (0.578%)	66	286.38	10.858	1.041
100% (0.640%)	73	266.88	11.160	1.035
110% (0.702%)	80	285.63	11.594	1.138
120% (0.764%)	87	297.50	12.789	1.114
P-value				
Linear		0.017	P<0.01	0.021
Quadratic		0.811	0.988	0.265
SEM		1.53	1.22	1.63
CV (%)		12.20	11.35	24.83
Met+Cys			Yield (%)	
80% (0.516%)	59	77.53	2.98	0.25
90% (0.578%)	66	77.81	2.95	0.28
100% (0.640%)	73	76.20	3.22	0.30
110% (0.702%)	80	77.08	3.13	0.31
120% (0.764%)	87	77.19	3.34	0.29
P-value				
Linear		0.530	P<0.01	0.158
Quadratic		0.418	0.765	0.196
SEM		0.02	0.023	0.04
CV (%)		2.57	8.84	20.22

*Methionine+cystine:lysine ratio (%).CV= coefficient of variation. Source: own research.

In addition to the higher LIV, the absolute values of EVC and SPL were also higher, demonstrating the faster development of birds that received increasing levels of digestible methionine+cystine.

Another important result was the increasing linear response shown by SPL with the levels of digestible methionine+cystine, which may indicate improvement of the immune system, since the weight of this organ is related to immunity and this organ is responsible for the processing of antigens (Ag), production of immunoglobulins (IgM), and the deposition and maturation of T lymphocytes (Turletti et al., 2008), corroborating studies that demonstrate this beneficial activity of methionine in the immune system (Kalvandi et al., 2019; Zhu et al., 2019).

In their first weeks of age, supplementation of methionine+cystine increased hepatic glycogen deposition (HGD), which was higher in the treatment that met 120% (0.764%) of the requirements of digestible methionine+cystine. The point of minimum found for HGD was 86.50% (0.554%), estimated by the derivative of the regression equation (Table 4).

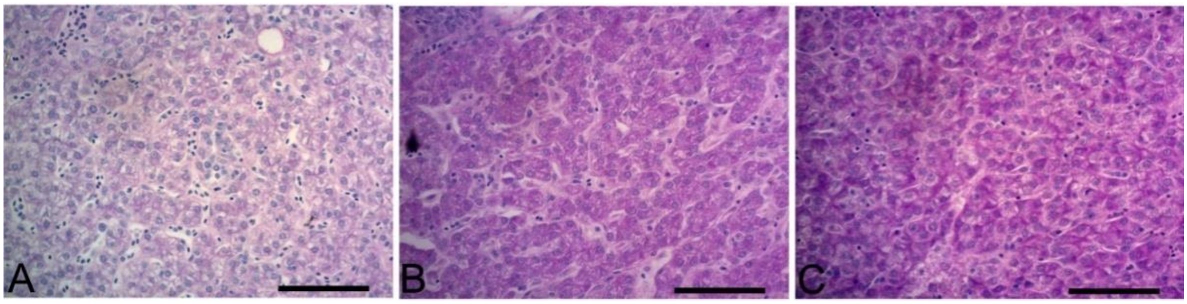
Table 4- Modified Ishak's semi-quantitative scoring system for positivity to Periodic Acid Shiff (PAS) staining as indicative of stocked hepatic glycogen in six-week-old white-egg layers supplemented with levels of methionine+cysteine.

Treatment	Met:Lys*	Ishak's Score					
Met+Cys		0	1	2	3	4	Mean
80% (0.516%)	59	1	7	2	0	0	1.1
90% (0.578%)	66	1	8	1	0	0	1.0
100% (0.640%)	73	1	8	1	0	0	1.0
110% (0.702%)	80	0	0	3	7	0	2.7
120% (0.764%)	87	0	0	1	7	2	3.1
P-value							
Linear							P<0.01
Quadratic							P<0.01
SEM							0.001
CV (%)							26.62

*Methionine+cystine:lysine ratio (%). CV = coefficient of variation; $Y = 0.0019X^2 – 0.3287X + 14.98$, $R^2 = 0.88$. Source: own research.

The hepatic glycogen deposition was verified by the higher positivity to Periodic Acid Schiff staining (Figure 1), and no hepatic steatosis was detected in the studied treatments. This deposition of hepatic glycogen can be seen in the higher frequency of pink coloration in the image.

Figure 1- Photomicrographs of liver of white-egg layers aged one to six weeks supplemented with levels of methionine+cystine. (a) liver of white-egg layers representing the treatment with 100% (0.640%) of digestible methionine+cystine. (b) liver of white-egg layers on the treatment with 110% (0.702%) of digestible methionine+cystine. (c) liver of white-egg layers on the treatment with 120% (0.764%) of digestible methionine+cystine. Bar: 100µm. Periodic Acid Schiff staining.



Source: own research.

The hepatic deposition glycogen indicates an energy reserve, which in practical terms of poultry farm situation, allows a beneficial effect for the pullets as they could start laying phase in a better physiological state, through better formation, development and maturation of the reproductive system, and consequently an early age at first egg, and persistence of production. Liu et al. (2019) found that dietary levels of methionine can alter the levels of mRNA of genes related to the metabolism of glucose and lipids in the liver of laying hens, which in high amounts may result in a condition of hypercholesterolemia in birds (Ognik et al., 2020). The increase in the levels of methionine+cystine had a quadratic effect on variables ALT, GGM, CRE, and ALB (Table 5), whose maximum obtained values were 100% (0.640%), 100.40% (0.643%), 104.30% (0.668%) and 111.88% (0.716%), respectively. Regarding the serum protein levels (PTN), they showed a linear effect ($P < 0.01$) caused by the levels of digestible methionine+cystine.

Despite the linear response of the absolute and relative weights of the liver and the glycogen stock to the levels of digestible methionine+cystine, there were no biochemical alterations related to the activity of hepatic enzymes and creatinine, considering the standards for that species and age (Kaneko et al., 2008). Therefore, the hepatic changes observed in this study, increased LIV and HGD — did not cause unfavorable biochemical alterations, which might compromise the health and productive performance of birds in the laying phase.

Table 5- Effects of treatments on the activity of enzymes alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), gamma-glutamyl transferase (GGM, U/L) and serum creatinine (CRE, mg/dL), albumin (ALB, g/dL) and protein (PTN, g/dL) in hens aged one to six weeks.

Met+Cys	Met:Lys*	ALT[1]	AST	GGM[2]	CRE[3]	ALB[4]	PTN
80% (0.516%)	59	1.75	169.63	22.25	0.275	1.35	3.03
90% (0.578%)	66	1.88	187.25	30.38	0.365	1.61	3.19
100% (0.640%)	73	2.88	181.88	33.25	0.529	1.63	3.58
110% (0.702%)	80	2.38	151.88	30.13	0.505	1.83	4.04
120% (0.764%)	87	1.50	193.75	23.25	0.333	1.72	3.91
P-value							
Linear		1.0000	0.5249	0.6738	0.0070	0.0003	0.0010
Quadratic		0.0086	0.3207	0.0001	0.0001	0.0562	0.5712
SEM		0.21	0.47	0.33	0.01	0.02	0.032
CV (%)		40.73	10.26	12.88	18.97	12.92	18.60

*Methionine+cystine:lysine ratio (%). CV (%) = coefficient of variation; [1]$Y = -0.0025X^2 + 0.5X - 22.425$, $R^2 = 0.73$; [2]$Y = -0.0257X^2 + 5.1604X - 225.9$, $R^2 = 0.99$; [3]$Y = -0.0005X^2 + 0.1043X - 4.8412$, $R^2 = 0.87$; [4]$Y = -0.0004X^2 + 0.0895X - 3.2422$, $R^2 = 0.90$. Source: own research.

Albumin is a soluble protein that comprises approximately half of the protein from the blood serum. And, similarly to the findings of this study, Yalçin et al. (2011) stated that the ALB and PTN levels increased as the supplementation of methionine+cystine in the birds' diet was increased. Because ALB and PTN are elements produced in the liver, these results also corroborate the previous ones, in which better liver functioning was observed with greater supplementation of methionine+cystine.

Likewise, Jankowski et al. (2020) found an increase in plasma protein and albumin levels, as there was an increase in methionine levels in the diets. This is explained by the fact that albumin is responsible for 55-60% of the total plasma protein, being composed of 585 amino acids arranged in helices that are retained by 17 sulfur-containing particles disulfide bridges: cystine, cysteine and Methionine.

To build the growth curve, the data of the growth variable of birds aged one to six weeks fitted the mathematical models proposed by Gompertz and Richards better; however, the parameters estimated by the Gompertz function showed the lowest AIC (1362.937) (Table 6), and consequently best described of the average growth curves of hens aged one to six weeks (Figure 2).

Table 6- Properties of the non-linear models utilized in the growth curve of hens aged one to six weeks.

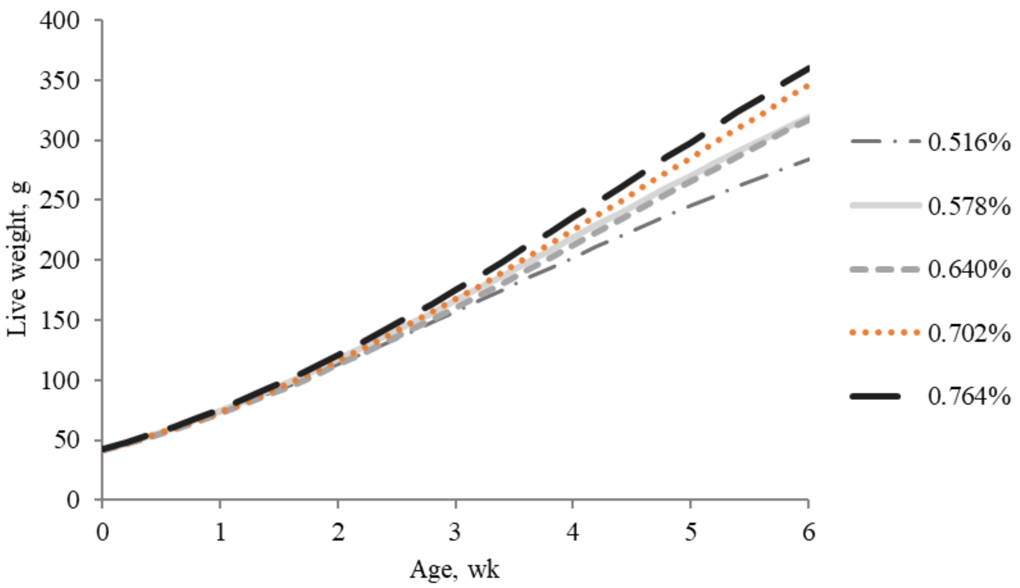
MODEL	EQUATION	AIC
Von Bertalanffy	$Y = A(1- Be^{-Kt})\ 3 + \varepsilon$	1393.359
Brody	$Y = A(1- Be^{-Kt}) + \varepsilon$	1393.453
Gompertz	$Y = Ae^{-Be-Kt} + \varepsilon$	1362.937
Logistic	$Y = A(1+ Be^{-Kt})\ -1 + \varepsilon$	1386.629
Richards	$Y = A(1- Be^{-Kt})^{M} + \varepsilon$	1367.328

Source: own research.

Cuello et al. (2018) worked with performance, growth, and productive traits of pullets, and determined the estimates of sexual maturity rate and body growth of birds through equation adjustments to the mathematical model proposed by Gompertz. Likewise, Neme et al. (2006) studied growth curves of different pullets strains and found maturity and growth rates based on the parameters estimated by the Gompertz function.

The application of the growth curve is aimed at modeling the response pattern of data such as age-weight throughout the life of an animal (Freitas, 2005), and the Gompertz model is the most widely employed to obtain the growth curve of birds and to study nutritional "trends" and requirements during growth (Hruby, 1994).

Figure 2- Adjusted growth curve according to the Gompertz model.



Source: own research.

4. Conclusion

Increasing dietary methionine+cystine levels elevated the relative liver weight and the deposition of hepatic glycogen. In addition, they promoting higher growth in pullets, with better body weight and body weight gain and feed conversion ratio.

Therefore, 0.732% digestible methionine+cystine level is recommended, corresponding 151.20 mg/bird/d and a Met+Cys:Lys 83% for light pullets aged one to six weeks.

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