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Relative bioeffectiveness of hydroxy calcium salt analogous methionine compared to DL-methionine on the performance of light laying hens in the production phase

Bioeficácia relativa do análogo do sal hidroxicálcico metionina em comparação com DL-metionina no desempenho de galinhas poedeiras leves na fase de produção

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Abstract: The study evaluated the relative bioefficacy of HMBA-Ca compared to DLM at digestible Met+Cys levels for lightweight laying hens from 42 to 62 weeks of age on performance, egg quality, feather score condition, and economic assessment. A total of 1,080 Hy-Line W80 hens were allocated to nine treatments in a completely randomized design with a 2×4+1 factorial arrangement (2 sources, 4 levels of digestible methionine+cystine supplementation: 0.46, 0.54, 0.56, and 0.58 %, plus 1 unsupplemented group). Both sources met the nutritional requirements, with DLM being more efficient at levels close to the minimum requirement, favoring egg weight, whereas HMBA-Ca showed better performance at higher levels, with a greater yolk percentage and improved feather score. Eggshell quality was not compromised, even with the increase in egg size. The economic analysis indicated the lowest cost per dozen at intermediate levels (0.50–0.54 %), but HMBA-Ca at 0.58 % resulted in the highest net margin (103 %) and return per dollar invested, maintaining an advantage even under simulated scenarios of exchange rate variation. It is concluded that, although both sources are effective, supplementation with HMBA-Ca at 0.58 % represents the most advantageous strategy by combining productive performance, feather quality, and greater profitability under favorable market conditions.

Key-words: economic index; egg production; plumage score; sulfur amino acids.

Resumo: O estudo avaliou a bioeficácia relativa da HMBA-Ca em comparação a DLM aos níveis de Met+Cys digestíveis para poedeiras leves de 42 a 62 semanas de idade sobre o desempenho, qualidade de ovos, condição de escore de plumagem e avaliação econômica. Um total de 1080 poedeiras Hy-line W80 foram distribuídas em 9 tratamentos em um delineamento inteiramente casualizado em arranjo fatorial, 2x4+1 (2 fontes, 4 níveis de suplementação de metionina+cistina digestíveis: 0,46, 0,54, 0,56 e 0,58 % e 1 grupo isento de suplementação). Ambas as fontes atenderam às exigências nutricionais, sendo a DLM mais eficiente em níveis próximos à exigência mínima, favorecendo o peso dos ovos, enquanto a HMBA-Ca apresentou melhor desempenho em níveis mais elevados, com maior percentual de gema e melhor escore de plumagem. A qualidade da casca não foi comprometida, mesmo com o aumento do tamanho dos ovos.

A análise econômica indicou custo por dúzia mínimo em níveis intermediários (0,50–0,54 %), mas a HMBA-Ca a 0,58 % apresentou maior margem líquida (103 %) e retorno por dólar investido, mantendo vantagem mesmo em cenários simulados de variação cambial. Conclui-se que, embora ambas as fontes sejam eficazes, a suplementação com HMBA-Ca a 0,58 % representa a estratégia mais vantajosa por conciliar desempenho produtivo, qualidade da plumagem e maior rentabilidade em condições de mercado favoráveis..

Palavras-chave: aminoácidos sulfurosos; produção de ovo; escore de plumagem; índice econômico.

1. Introduction

Methionine (Met) is the first limiting amino acid in birds fed corn- and soybean meal–based diets and is known to directly affect poultry production. This amino acid plays multiple roles in metabolism and acts as a methyl group donor in the synthesis of cysteine (Cys), choline, creatine, polyamines, and phospholipids. Moreover, Met contributes to the formation and renewal of feathers, hair, and skin through Cys, and it functions as a regulator of cell division. Therefore, its importance in bird growth and maintenance is indisputable [1, 2]. Meeting the Met requirements of laying hens is thus essential for optimizing egg production, allowing for an increase in crude protein content in the albumen, higher total concentration of phospholipids in the yolk, improvements in egg weight and mass, and enhanced feed conversion efficiency.

DL-2-amino-4-(methylthio)butanoic acid (DLM) is the most widely used supplemental source of Met. However, Met analogs such as calcium salt of DL-2-hydroxy-4-(methylthio)butanoic acid (HMBA-Ca), in addition to being 20 % more economical [3], also provide Met after transamination. Although both DLM and HMBA-Ca supply L-Met, they are chemically distinct because Met hydroxy analog contains a hydroxyl group on the asymmetric carbon, whereas DLM contains an amino group. These chemical differences result in substantial variations in the absorption, metabolism, and conversion, affecting the availability of L-Met to the animal. When compounds differ in metabolism, they cannot be considered equivalent. Although they may show similar bioefficacy regarding a specific outcome, such as protein synthesis or Met availability, the term "relative bioefficacy" is used to refer to a value that compares the nutritional potency or efficacy of a nutrient source with a defined standard [4].

Previous studies have demonstrated wide discrepancies in bioefficacy values among different Met sources, highlighting the need for further research, particularly on the calcium salt analogs in lightweight laying hens, as this source has received less attention than DLM with respect to bird productive performance. Therefore, the objective of this study was to evaluate the relative bioefficacy of HMBA-Ca compared with that of DLM at different digestible Met + Cys levels in lightweight laying hens aged 42–62 weeks, considering performance, egg quality, plumage condition, and economic evaluation.

2. Material and methods

2.1 Location, animals, and housing

The study was conducted at the Federal University of Paraíba (UFPB), Campus II, located at latitude 06º57'48''S and longitude 35º41'30''W, at an altitude of 618 m, in the state of Paraíba, Brazil. The animal experiments were approved by the Animal Ethics Committee of the UFPB (approval number 4161290819).

A total of 1,080 Hy-Line W80 commercial laying hens, 42 weeks of age, with an initial average body weight of 1.600 ± 0.04 kg and a uniform production history, were used. The birds were housed in a conventional poultry house in metal cages (100 cm × 45 cm × 45 cm), with five birds per cage. The environment was monitored daily using two thermometers positioned at bird height and a hygrometer to record the maximum and minimum temperatures as well as relative humidity. The observed averages were 27.96 °C (maximum) and 19.9 °C (minimum), with 73 % relative humidity.

Water and feed were provided *ad libitum*. The lighting program lasted 17 h per day. Prior to the start of the experiment, the birds underwent a 14-day adaptation period to standardize management and stabilize their feed intake. Throughout the study, bird health was monitored daily, and cages were cleaned weekly.

2.2 Experimental design and feeding treatments

The experimental design was completely randomized in a 2 × 4 + 1 factorial arrangement (two Met sources, HMBA-Ca and DLM, and four levels of digestible Met + Cys supplementation: 0.46, 0.50, 0.54, and 0.58 % for each source, in addition to a diet without Met supplementation). In total, nine treatments were evaluated, with 12 replicates and 10 birds per experimental unit.

Diets were formulated based on corn and soybean meal, following the recommendations of the Hy-Line W80 management guide (2016), except for sulfur amino acids, which were adjusted according to Fickler *et al.* [5]. The basal diet was deficient in digestible Met + Cys (reduced by 0.50 % or 500 mg/d). Increasing concentrations of DLM and HMBA-Ca were then added to the basal diets. DLM was supplemented at 65 % relative to HMBA-Ca, which was set at 100 %. The detailed composition and calculated nutritional contents are presented in Tables 1 and 2, respectively.

Table 1. Experimental design for each period**.

Treatmentos	M+C Content % of diet	Addition HMBA-Ca % of diet	Addition DL-Met % of diet
1 – Negative control[1]	0.40	-	-
2 –HMBA-Ca level 1	0.46	0.05	
3 –HMBA-Ca level 2	0.50	0.10	
4 –HMBA-Ca level 3	0.54	0.15	
5 –HMBA-Ca level 4	0.58	0.20	
6 - DLM level 1	0.46		0.03
7 - DLM level 2	0.50		0.07
8 - DLM level 3	0.54		0.10
9 - DLM level 4	0.58		0.13

**Based on breeder nutritional guidelines Hy-Line W80 (2016). [1]Negative control without methionine supplementation.

Table 2. Composition of experimental basal diets for 40 to 48 Weeks and for 49 to 62 weeks, g/kg based on a feed intake of 108 and 109 g for each phase, respectively.

Ingredients	42-52 week	53-62 week
Corn, 7,88 %	668.90	675.67
Soybean. 45,22 %	198.57	188.74
Limestone, 37 %	9.53	9.49
Soybean oil	94.98	100.22
Phosphate dicalcium, 19 %	18.74	16.72
Common salt	3.85	3.85
L-Lysine (Biolys®)	0,159	0,159
L- Threonine	1.70	1.59
L- Tryptophan (TrypAmino®)	0.18	0.11
Choline chloride, 60 %	0.01	0.07
Vitamin Premix[1]	0.75	0.75
Mineral Premix[2]	0.70	0.70
Antioxidant[3]	0.10	0.10
Inert	2.00	2.00
Calculated nutrient composition		
Metabolizable energy (MJ/kg)	2800	2800
Crude protein, %	14,30	13.40
Calcium, %	4.08	4.07
Available phosphorous, %	0.50	0.44
SID Lysine, %	0.80	0.73
SID Methionine, %	0.20	0.19
SID Methionine + cysteine, %	0.40	0.39
SID Threonine, %	0.56	0.51
SID Tryptophan, %	0.17	0.15
SID Arginine, %	0.83	0.57
SID isoleucine, %	0.62	0.52
SID Leucine, %	1.21	1.15
SID Valine, %	0.70	0.64

SID = standardized ileal digestible. [1]provides per kilogram of diet: 15.000.000 IU vitamin A; 1.500.000 vitamin IU D3; 15.000 UI vitamin E; 2 g thiamina; 4 g riboflavina; 3 g pyridoxine; 0.015 g vitamin B12; 10 g D-pantothenic acid; 3 g vitamin K3; 1 g folic acid. [2]provides per kilogram of diet: 60 g Mn; 60 g Fe; 50 g Zn/ 10 g Cu; 2 g Coand 250 mg Se. [3]Quantum Blue 5000.

2.3 Data and sample collection

The experiment was structured into five periods of 28 days each. The variables evaluated were feed intake, egg production, eggs per housed hen (EPH), egg weight, egg mass, feed conversion per egg mass (FCEM), and feed conversion per dozen eggs (FCDE), viability, yields of egg components (yolk, albumen, and shell), egg quality parameters (yolk index, shell thickness, specific gravity [SG], breaking strength, and Haugh unit [HU]), plumage score, and economic evaluation.

Performance: Feed intake was determined as the difference between the amount of feed supplied at the beginning and the remaining feed at the end of each 28-day period, adjusted for bird mortality. Egg production was calculated as the number of eggs produced divided by the number of housed birds per period, multiplied by 100. Average egg weight was calculated by dividing the total weight of the collected eggs by the number of eggs collected per experimental unit. Egg mass was calculated as the product of egg production and average egg weight, divided by 100. The FCDE was obtained by dividing total feed intake (kg) by the number of eggs produced, while the FCEM was calculated by dividing the total feed intake by the egg mass produced. The number of EPH was determined as egg production multiplied by the number of experimental days divided by 100. Viability was calculated as the final number of birds multiplied by 100 divided by the initial number of birds.

Egg quality: Eggs were individually weighed on a precision digital scale (0.001 g) and broken on a glass table designed for analysis. Yolk and albumen height were measured using a DIGMESS® depth gauge, and yolk diameter was measured with a ZAAS® caliper. The yolk index was calculated as the ratio of yolk height and diameter. Yolk and albumin percentages were determined as the ratio of average component weight to average egg weight. The HU was calculated according to the methodology of Card and Nesheim [6], using the equation: HU = 100 × log (H + 7.57 – 1.7W0.37), where HU is the Haugh unit, H is the albumen height (mm), and W is the egg weight (g). Shells were dried in an oven at 55–60 °C for 24 h and weighed using a digital scale. Shell percentage was calculated as the ratio of the average shell weight to the average egg weight. Shell thickness was measured using a Mitatoyo digital micrometer (0–25 mm, 0.001 mm precision). Eggshell breaking strength was determined using the TA.X T2 Texture Analyzer ® equipped with a P4 DIA Cylinder probe of 4 mm diameter, with a distance of 6 mm and pre-, test-, and post-test speeds of 3.0, 0.5, and 5.0 mm/s, respectively. The SG was determined using the saline flotation method according to Hamilton [7]. The eggs were immersed in a sodium chloride solution with densities ranging from 1.0700 to 1.0975 g/mL, with a density gradient of 0.0025 between the solutions. Solution density was measured using an oil densimeter.

To evaluate the plumage score, all birds in each experimental unit were assessed by an experienced evaluator in six body regions (neck, back, breast, wings, cloaca, and tail) on a 4-point scale (1–4). Total plumage score was calculated by summing the scores for all six regions. Accordingly, relative scores <10–12 indicated severe feather damage across the bird's body, whereas scores >18–20 indicated good feather coverage, as established by Tauson *et al.* [8].

For the economic evaluation, the efficiency of each treatment was assessed by estimating the average feed costs per kilogram and per dozen eggs produced [9]. Gross margins were calculated as described by Costa *et al*. [10]. Input prices were obtained from regional prices listed on the MFRURAL marketplace website (October 2024) and expressed in US dollars (USD) at the exchange rate on October 24, 2024. The prices of inputs were as follows: corn grain: 0.31 USD/kg; soybean meal (45 % CP): 0.53 USD/kg; limestone: 0.38 USD/kg; dicalcium phosphate (19 %): 1.07 USD/kg; common salt: 0.04 USD/kg; L-lysine: 0.42 USD/kg; L-threonine: 3.31 USD/kg; L-tryptophan: 10.57 USD/kg; choline chloride (60 %): 0.78 USD/kg; vitamin supplement: 2.51 USD/kg; mineral supplement: 0.71 USD/kg; antioxidant: 5.76 USD/kg; inert: 0.03 USD/kg; DLM: 2.46 USD/kg; and HMBA-Ca: 1.96 USD/kg.

The following costs were considered: feed cost per ton (FC), feed cost per dozen eggs (FCo), feed cost per dozen eggs (FCD), cost per kilogram of eggs produced (CKg), and cost per egg carton produced (CC) (30 eggs). The economic efficiency of each treatment was evaluated using the following indices: average cost index per kilogram of eggs (ACI-Kg) and per egg carton (CC): average cost per kg or carton produced in the treatment divided by the lowest average cost per kg or carton among the treatments, multiplied by 100; relative gross margin (RGM): gross margin of the treatment multiplied by 100, divided by the gross margin of the control treatment; relative gross income (RGI): value of a dozen eggs divided by the number of dozens of eggs per bird, multiplied by 100; and average profitability index (API): average profitability of the treatment divided by the average profitability of the control treatment.

2.4 Statistical analysis

Data were subjected to analysis of variance (ANOVA), with t-tests applied to compare Met sources. These analyses were performed using SAS® University Edition, applying regression analysis at a 5 % probability level for the digestible Met + Cys levels. Plumage score categories were analyzed using relative frequencies (0–1), calculated as the ratio of the number of birds in each category to the total number in the group, allowing comparisons between treatments and sources [11]. Relative bioefficacy was estimated using the exponential-linear model described by Littell *et al.* [12], adopting 95 % confidence intervals (CIs), and verifying the fit through the coefficient of determination (R2) and residual analysis.

3. Results

3.1 Performance

As shown in Table 3, no significant effect ($p > 0.05$) of Met source (HMBA-Ca vs. DLM) was observed for the variables studied. Increasing Met + Cys levels in the diet resulted in positive linear effects ($p < 0.001$) on egg production, egg weight, egg mass, FCEM, FCDE, and EPH, indicating progressive improvement in these variables with higher sulfur amino acid intake. A quadratic effect was observed for egg weight, egg mass, and FCEM, with maximum performance points estimated at 0.59 % for egg mass and egg weight, and 0.62 % for FCEM.

Table 3. Effects of sources, different methionine levels and the interaction between HMBA-Ca and DLM in feed intake (FI), egg production (EP), egg weight (EW), egg mass (EM), feed conversion per egg mass (FCEM), feed conversion per dozens of eggs (FCDE), eggs per hen housed (EPH) and livability (L) of light hens.

Source	FI (g/hen)	EP (%)	EW (g)	EM (g)	FCEM (g/g)	FCDE (kg/g)	EPH (%)	L (%)
HMBA-Ca	108.7	87.2	61.0	53.6	2.05	1.51	122.1	98.2
DLM	109.7	87.4	61.1	53.7	2.05	1.52	122.7	97.9
p-value	0.110	0,717	0.808	0.669	0.969	0.894	0.717	0.743
Levels (%)								
0.46	108.9	85.0	59.2	50.6	2.17	1.55	119.0	97.1
0.50	109.4	87.2	61.0	53.5	2.07	1.53	122.1	97.9
0.54	109.1	88.0	61.7	54.6	2.00	1.49	123.2	98.3
0.58	109.4	89.1	62.3	55.8	1.96	1.48	124.7	98.9
Regression								
Linear	0.658	<.001	<.001	<.001	<.001	0.001	<.001	0.057
Quadrátic	0.909	0.430	0.005	0.047	0.004	0.782	0.430	0.877
Interaction Source x Level	0.009	0.797	0.040	0.345	0.325	0.307	0.797	0.824
S.E.M	2.25	2.26	0.77	1.61	0.06	0.06	3.76	2.52

Means in the same column with different letters differ significantly with T test (p< .05). Regression equation of levels: (EP: $\hat{Y}= 32.5x + 70.45$. $R^2 = 0.94$); (EM: $\hat{Y}= 262.5x^2 + 314.95x - 38.574$. $R^2= 0.98$. maximum point: 0.59 %); (FCEM: $\hat{Y}= 9.375x2 - 11.5x + 5.4762$. $R^2 = 1$. Maximun point: 0.62 %); (FCDE: $\hat{Y} = -0.625x + 1.8375$. $R^2=0.95$); (EPH: $\hat{Y} = 45.525x +98.615$. $R^2=0.94$). Availability: EW: y= 44.85+27.31 *(1-e-(0.75x1+ 1.76x2+1.75x3)) e EP: y= 68.88+26.90 *(1 – e-(1.21x1+2.26x2+2.21x3)).S.

As shown in Table 4, the interaction between Met source and Met + Cys level showed that at the highest supplementation level (0.58 %), birds fed DLM had a higher feed intake than those fed HMBA-Ca. For egg weight, differences between sources were observed only at the 0.46 % level, whereas DLM resulted in heavier eggs. Regression equations indicated a quadratic response for egg weight with HMBA-Ca and a linear response with DLM, with the maximum point for HMBA-Ca estimated at 0.59 %. The relative bioefficacies of HMBA-Ca and DLM for egg production and egg weight were 99 % and 97 %, respectively, based on a 95 % CI.

Table 4. Effect of the interaction between HMBA-Ca in relation to DLM and the levels of Met+Cys on feed intake (FI) and egg weight (EW) of light laying hens from 42 to 62 weeks of age.

		Levels (%)				*P*-value	
		0.46	0.50	0.54	0.58	Linear	Quadratic
FI (g/hen)	HMBA-ca	109.2	108.3	109.8	107.7[b]	0.416	0.430
	DLM	108.7	110.6	108.3	111.2[a]	0.152	0.529
	p-value	0.674	0.057	0.191	0.003		
EW (g)	HMBA-ca	58.8[b]	60.8	61.9	62.6	<0.001	0,018
	DLM	59.7[a]	61.6	61.5	62.0	<0.001	0.107
	p-value	0.032	0.401	0.250	0.173		

Means followed by different letters in the same row differ significantly by Tukey's test at 5 % probability level. Regression equations: HMBA-Ca = (EW: Ŷ = -214.06x2 + 254.05x – 12.713. R^2 = 0.99. maximum point: 0.59 %; DLM: (EW: Ŷ = 17.675x + 52.036. R^2=0.75.

3.2 Egg quality

Table 5 shows the significant effect of the Met source on the relative yolk index (RYI), relative yolk weight (RYW), and relative albumen weight (RAW). DLM resulted in a higher RYI and RAW, whereas HMBA-Ca resulted in a higher RYW. Positive linear effects were observed for RYW and SG, and negative linear effects were observed for relative shell weight (RSW) and shell thickness. Quadratic effects were observed for the RYI (maximum point estimated at 0.52 %) and RYW. The relative bioefficacies of HMBA-Ca and DLM were 55 %, 114 %, and 108 % for RYI, RYW, and SG, respectively, based on 95 % CIs.

A significant interaction ($p < 0.05$) was detected between Met sources and dietary levels of RYW and SG. HMBA-Ca showed a higher RYW at 0.58 % level, whereas DLM resulted in the highest SG at 0.50 % level. Supplementation with increasing Met levels led to a linear increase in RYW for HMBA-Ca, with the maximum point estimated at 0.52 %.

Table 5. Effects of sources, different methionine levels, and the interaction between HMBA-Ca and DL-Methionine on yolk index (RYI), relative yolk weight (RYW), relative albumen weight (RAW), relative shell weight (RSW), Haugh unit (HU), specific gravity (SG), shell thickness (ST), and shell strength (SS) of light laying hens.

Source	RYI (%)	RYW (%)	RAW (%)	RSW (%)	HU	SG (g/cm³)	TS (µm)	SS (kgf)
HMBA-Ca	0.385[b]	26.0[a]	61.6[b]	10.1	83.8	1.090	0.468	3.59
DLM	0.415[a]	25.8[b]	61.9a	10.1	84.0	1.089	0.467	3.58
p-value	<0.001	0.040	0.041	0.282	0.344	0.282	0.539	0.822
Levels (%)								
0.46	0.406	25.7	62.0	10.2	84.0	1.091	0.470	3.65
0.50	0.392	25.9	61.7	10.1	83.7	1.091	0.467	3.67
0.54	0.394	26.2	61.7	10.0	83.7	1.088	0.468	3.51
0.58	0.409	26.0	61.7	10.0	84.1	1.088	0.465	3.52
Regression:								
Linear	0.691	0.020	0.205	<0.001	0.679	0.004	0.049	0.058
Quadrátic	0.003	0.049	0.976	0.363	0.093	0.756	0.877	0.947
Interaction								
Source x Level	0.096	0.027	0.085	0.270	0.257	0.029	0.334	0.165
S.E.M	0.02	0.45	0.50	0.16	0.83	0.003	0.01	0.24

Means in the same column with different letters differ significantly with T test (p< .05). Regression equations: RYI: Ŷ = 4.5312x2 -4.685x + 1.6021. R^2 = 0.99. Maximum point: 0.52 %; Availability: RYI: Ŷ= 0.32+0.16*(1-e-(1.65x1+1.67x2+0.93x3)); RYW: 23.10+5.01*(1-e-(1.20x1+1.55x2+1.77x3)); SG: 1.90+0.00*(1-e-(1.59x1+1.38x2+1.50x3)).

Table 6. Effect of the interaction between HMBA-Ca in relation to DLM and Met+Cys levels on yolk relative weight (RYW), specific gravity (SG) of light laying hens from 42 to 62 weeks of age.

		Levels (%)				*P*-value	
		0.46	0.50	0.54	0.58	Linear	Quadratic
RYW (%)	HMBA-ca	25.7	25.8	26.3	26.2[a]	0.012	0.573
	DLM	25.6	25.9	26.0	25.7[b]	0.603	0.054
	p-value	0.521	0.764	0.208	0.025		
SG (g/cm^3)	HMBA-ca	1.090	1.093[b]	1.089	1.087	0.031	0.045
	DLM	1.092	1.088[a]	1.088	1.088	0.054	0.115
	p-value	0.317	0.003	0.513	0.628		

Means followed by different letters in the same row differ significantly by Tukey's test at 5% probability level. Regression equations: HMBA-Ca: SG: $\hat{Y} = -0.7969x2 + 0.798x + 0.8923$. $R^2 = 0.85$. Maximum point:0.52 %.

3.3 Plumage score

Analysis of the probabilities of occurrence of the body score categories indicated a predominance of birds with good feather coverage in nearly all combinations evaluated. The "severe damage" category was not recorded. The "moderate" category occurred at a low frequencies, with variations among the different treatments. The probability of occurrence of the "Good coverage" category remained close to 1.0 in most combinations, highlighting the consistency of the high plumage scores (≥18 points) recorded during the assessments. Birds supplemented with HMBA-Ca showed a higher probability of "Good coverage" compared with those fed DLM (Table 7).

Table 7. Relative probability of occurrence of body score categories (Good coverage and Moderate) for each combination of treatment, level, and source.

Levels (%)	Source	Plumage score	Probability
0.46	HMBA-Ca	Good coverege	0.75
0.46	HMBA-Ca	Moderate	0.25
0.50	HMBA-Ca	Good coverege	0.75
0.50	HMBA-Ca	Moderate	0.25
0.54	HMBA-Ca	Good coverege	0.58
0.54	HMBA-Ca	Moderate	0.41
0.58	HMBA-Ca	Good coverege	0.83
0.58	HMBA-Ca	Moderate	0.16
0.46	DLM	Good coverege	0.75
0.46	DLM	Moderate	0.25
0.50	DLM	Good coverege	0.50
0.50	DLM	Moderate	0.50
0.54	DLM	Good coverege	0.75
0.54	DLM	Moderate	0.25
0.58	DLM	Good coverege	0.75
0.58	DLM	Moderate	0.25

No severe damage was observed in the studied sources.

3.4 Economic evaluation

Birds fed HMBA-Ca showed the lowest FC, CF, FCD, CKg, and CC compared with those fed DLM. However, at the 0.58 % Met level, both sources presented the same CKE. Lower CC and the best results in RGM, RGI and API were observed in birds fed HMBA-Ca. At the 0.58 % Met level, the highest costs were recorded, but this level also resulted in the lowest RGM and the highest API.

Table 8. Effects of sources, different methionine levels between HMBA-Ca and DLM in cost per feed tonne (FC), cost feed (CF), feed cost per dozen eggs (FCD), cost of kilogram of egg produced (CKg), cost egg carton produced (CC), index of average cost per kilogram of egg (ICKE) and per egg carton (ICCE), relative gross margin (RGM), relative gross income (RGI) and average profitability index (API) of light laying hens.

Source	FC U$	CF U$	CFD U$	CKg U$	CC U$	ICKE (%)	ICCE (%)	RGI (%)	RGM U$	API (%)
HMBA-Ca	386.8	5.89	0.579	0.006	0.020	108.9	102.9	83.7	59.0	100
DLM	387.6	5.95	0.583	0.007	0.021	110.7	104.0	83.4	58.8	102.7
Levels (%)										
HMBA /0.46	385.6	5.90	0.59	0.007	0.021	118.9	105.6	86.0	60.4	101.4
HMBA /0.50	386.5	5.86	0.58	0.006	0.020	109.0	102.6	84.4	59.4	97.7
HMBA /0.54	387.3	5.96	0.58	0.006	0.021	107.6	103.2	82.7	58.4	98.3
HMBA /0.58	388.0	5.85	0.56	0.006	0.020	100.0	100.0	81.7	57.6	103
DLM /0.46	387.0	5.89	0.59	0.007	0.021	117.8	106.2	86.2	60.5	97.3
DLM /0.50	386.8	5.99	0.58	0.007	0.021	110.7	104.2	82.8	58.4	99.3
DLM /0.54	387.8	5.88	0.57	0.006	0.020	106.2	102.3	83.0	58.5	94.9
DLM /0.58	388.7	6.05	0.58	0.006	0.021	108.1	103.2	81.6	57.8	100

4. Discussion

Met is the first limiting amino acid in laying hen diets based on corn and soybean meal, making Met supplementation essential to meet the maintenance, production, and egg quality requirements [13]. Among the commercial sources, DLM and HMBA-Ca exhibit distinct absorption and metabolic mechanisms that can influence performance, utilization efficiency, and productive parameters in birds [14].

Contrary to the expected reduction in feed intake with increased supplementation, fluctuations in intake were observed, showing an increase at higher Met + Cys levels, except for birds fed HMBA-Ca at 0.58 %, which showed a slight reduction. The intake fluctuation at higher levels, particularly with HMBA-Ca 0.58 %, was consistent with aminostatic feedback. Excess amino acids can alter plasma profiles and activate hypothalamic signals that reduce appetite; however, this effect depends on the balance between other essential amino acids and the rate of intestinal absorption, which may explain the differences between DLM and HMBA-Ca [15, 16].

In the present study, increasing digestible Met + Cys levels elevated egg production, weight, and mass, improving FCEM, in agreement with the findings of Vázquez-Anõn *et al.* [4] and Wu *et al.* [17]. This effect was consistent across both sources, indicating that an adequate Met supply optimized protein availability and the synthesis of essential compounds such as polyamines, which stimulated cell proliferation and protein deposition in the egg. Xião *et al.* [18] and Ma *et al.* [19] confirmed that Met intake improved egg production and laying rates. However, the results of the present study indicate that low supplementation levels affect not only egg production but also overall bird performance.

Supplementation with Met + Cys increases the weight of egg components primarily by enhancing protein synthesis, leading to greater protein deposition in the albumen (20). Met supplementation also increased egg weight and intensified yolk color, indicating higher lipid deposition. Although this study did not directly assess methyl group availability, the results support the notion that Met significantly influences egg yolk composition [21]. In addition to acting as a methyl group donor and precursor of essential metabolites, Met can also be oxidized for energy production via the glucogenic pathway [22]. Thus, when provided at levels above the strain-guide requirements, the excess can be directed toward energy metabolism, contributing to the increased performance observed with higher supplementation.

The reduction in shell thickness and SG in larger eggs is an expected physiological phenomenon resulting from the redistribution of calcium to meet the increased egg volume, which generally leads to relatively thin shells. However, this effect did not compromise mechanical strength, corroborating the findings of Kodali *et al.* [(23)]. We observed that, at certain supplementation levels, HMBA-Ca promoted higher SG, possibly due to the additional calcium contribution (11.7 %) [(24)], a behavior also reported by Esteve-Garcia *et al.* [(25)].

The plumage score results showed that birds fed HMBA-Ca, particularly at higher levels, had a greater probability (>0.95) of remaining in the "good coverage" category (≥18 points), whereas the "severe damage" category (<12 points) was absent for both sources. This indicates that HMBA-Ca contributes to reduced feather wear throughout the cycle, emphasizing the importance of adequate Met intake for Cys synthesis, which plays a key role in sulfur donation to the three-dimensional structure of proteins, especially keratin, which constitutes approximately 90 % of bird feathers. Therefore, an adequate Met supply promotes strong, flexible, and high-quality feathers [(26)].

The relative bioefficacies of DLM and HMBA-Ca varied according to their supplementation levels. At levels near the requirement, the DLM tended to favor immediate responses (egg weight), showing higher rapid availability. This is related to its absorption via Na dependent transporters, which rapidly increase plasma Met concentrations. In contrast, at higher levels (0.58 %), HMBA-Ca showed equivalent or superior performance in terms of quality parameters, such as higher yolk proportion and better feather maintenance. This effect is consistent with its absorption, as it occurs predominantly via monocarboxylate transporters and lactic acid pathways, resulting in a more gradual release and greater tissue retention, which may benefit these parameters. Such metabolic differences between sources affect not only the absorption kinetics but also the activation of central pathways involved in energy regulation, such as mTOR, AMPK, and NPY [(26, 27)]. In summary, when the Met supply is no longer limiting, the dynamics of release and utilization between sources more strongly influence egg quality indicators and body conditions without compromising productive performance.

Economic analysis revealed that HMBA-Ca had a slightly lower total diet cost and, at the 0.58 % level, achieved the highest return per dollar invested, with 2.6 % greater production than DLM. Although the DLM showed a slight advantage in terms of the average net margin, HMBA-Ca stood out at the optimal level (0.58 %), combining productive performance, feather maintenance, and a higher API (103 %). However, the unit cost per dozen eggs was minimized at intermediate supplementation levels, specifically with HMBA-Ca at 0.50 % or DLM at 0.54 %, reflecting greater efficiency in converting inputs into products when the goal was to reduce direct production costs. In this scenario, the DLM demonstrated a slight superiority in the average net margin, suggesting that its use may be economically more stable under varying input and product price contexts.

From a practical perspective, the results indicated that the choice of Met source and supplementation level should consider the prevailing economic scenario. In markets with cost pressure (e.g., high input prices and reduced margins), adopting intermediate levels (0.50–0.54 %) of either source is more appropriate. In scenarios with higher product valuations (high egg prices), using HMBA-Ca 0.58 % may maximize profitability, justifying the slightly higher unit costs. Thus, the strategic use of different sources allows the optimization of both production costs and net margins, adjusting supplementation according to market conditions and the producer's economic objectives.

In light of the discussion, both sources met the birds' nutritional requirements. While DLM favored immediate responses at levels close to the minimum requirement, HMBA-Ca excelled at higher levels, enhancing feather quality and yolk percentage. From an economic perspective, intermediate levels reduced the cost per dozen eggs. However, HMBA-Ca at 0.58 % digestible Met + Cys provided a higher net margin and return per dollar invested, maintaining competitiveness even under adverse market conditions.

5. Conclusion

Supplementation with HMBA-Ca 0.58 % digestible Met + Cys represented the most advantageous alternative, combining productive performance, feather quality, and superior economic returns, especially under favorable market conditions.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Data availability statement

The data will be made available upon request to the corresponding author.

Author contributions

Conceptualization: T.S. Ferreira. Data curation: T.S. Ferreira. Formal analysis: T.S. Ferreira and I.N. Kaneko. Investigation: M.N. Soares. Methodology: T.S. Ferreira. Project administration: M.N. Soares. Supervision: F.G.P. Costa. Writing – original draft: T.S. Ferreira. Visualization: S.G. Pinheiro and R.F.B. Júnior. Writing – review & editing: M.N. Soares, S.G. Pinheiro, and R.F.B. Júnior.

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