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Lysine and Methionine + Cystine for Laying Hens During the Post-Molting Phase

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ABSTRACT

One experiment was conducted to evaluate the effect of using different lysine and methionine + cystine levels on body weight recovery, performance, and egg quality of laying hens during the post-molting period. In this trial, 432 Isa Brown layers, with 72 weeks of age, were distributed in 54 cages according to a completely randomized design with six treatments and nine replicates of eight birds each. During the resting period, six diets with different digestible lysine and methionine + cystine levels were used, as follows: 0.48% digestible lysine and 0.43% methionine + cystine; 0.48% digestible lysine and 0.47% methionine + cystine; 0.48% digestible lysine and 0.52% methionine + cystine; 0.56% digestible lysine and 0.50% methionine + cystine; 0.56% digestible lysine and 0.56% methionine + cystine; 0.56% digestible lysine and 0.62% methionine + cystine. Data were submitted to analysis of variance and means were compared at by Tukey's test at 5% probability level. The different lysine and methionine + cystine levels in the diets fed during the resting period significantly influenced layer performance. The diet containing 0.56% lysine and 0.56% methionine + cystine promoted higher egg weight eggs during the second production cycle.

INTRODUCTION

Modern layer strains have high genetic potential for egg production, and may produce eggs for more than one laying cycle by inducing molting. Forced molting is defined as a set of management practices that cause stress, inducing birds to stop producing eggs and the partial molting of the feathers (Roland & Brake, 1982). The objective of forced molting is to allow the reproductive system of layers to rest for some time in order to recover its production capacity and to increase the longevity of layers for further 25 to 30 weeks, as well as to improve egg quality, reducing losses caused by poor eggshell quality (Rodrigues, 2005).

Good egg production during the second laying cycle is only obtained when adequate nutrition is provided during the resting period to ensure the recovery of body components and rapid return to lay (Mendonça & Lima, 1999). One of the most important nutrients in this phase is protein, particularly sulfur amino acids. The optimal performance of laying hens demands protein supply based on the requirements of essential amino acids, which most important are lysine and methionine + cystine (Mendonça, 1996).

Lysine is used for the synthesis of egg and body protein, and any deficiency results in egg production losses (Hiramoto *et al.*, 1990).

In addition, sulfur amino acid levels may influence internal egg quality parameters, such as Haugh units and albumen index (Bertechini *et al.*, 1995; Narvaez-Solarte, 1996).

This study aimed at evaluating the effects of different dietary digestible lysine and methionine + cystine levels supplied during the post-molting period and second laying cycle on body recovery, performance, egg quality, and morphometrics of the reproductive system, liver, and pancreas of commercial layers.

MATERIALS AND METHODS

The experimental period included the phases of molting (14 days), rest (28 days) and second laying cycle (four periods of 28 days each). Layers were selected according to body weight and egg production. In the trial, 432 commercial Isa Brown layers with 72 weeks of age were distributed, according to a completely randomized experimental design, into six treatments with nine replicates of eight birds each, totaling 54 experimental units.

Birds were offered water and feed *ad libitum* during the entire experimental period. During molting, a 12 hour light/day lighting program was adopted. During rest, light was increased 15 min/day until 17 hours/ day were reached, and this was maintained during the second laying cycle.

Molt was induced by including 3,000 ppm zinc oxide in a diet, containing 0.43% calcium and 0.02% sodium, that was fed for 14 days.

During rest, the following digestible lysine and methionine + cystine levels were supplied (Table 1): 0.48% digestible lysine and 0.43% digestible methionine+cystine (90% amino acid ratio); 0.48% digestible lysine and 0.47% digestible methionine+cystine

Table 1 - Ingredient and nutritional composition of the experimental diets supplied during the resting period.

Ingredients (%)	0.48% lysine			0.56% lysine		
	0.43% met+cys	0.47% met+cys	0.52% met+cys	0.50% met+cys	0.56% met+cys	0.62% met+cys
Corn grain	77.67	77.63	77.61	77.60	77.57	77.54
Soybean meal 45%	10.85	10.87	10.90	10.93	10.96	10.99
Wheat midds	8.72	8.68	8.62	8.52	8.44	8.38
Calcitic limestone	1.16	1.16	1.16	1.16	1.16	1.16
Dicalcium phosphate	1.10	1.10	1.10	1.10	1.11	1.11
Vitamin and mineral supp.*	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.00	0.06	0.11	0.09	0.16	0.22
L-lysine HCl	-	-	-	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated levels						
Metabolizable energy (kcal/kg)	2.900	2.900	2.900	2.900	2.900	2.900
Crude protein (g/kg)	12.99	12.99	12.99	12.99	12.99	12.99
Calcium (%)	0.780	0.780	0.780	0.780	0.780	0.780
Available phosphorus (%)	0.310	0.310	0.310	0.310	0.310	0.310
Total phosphorus (%)	0.538	0.538	0.538	0.538	0.538	0.538
Sodium (%)	0.150	0.150	0.150	0.150	0.150	0.150
Total lysine (%)	0.550	0.550	0.551	0.631	0.631	0.631
Dig. Lysine (%)	0.480	0.480	0.480	0.560	0.560	0.560
Total methionine	0.244	0.284	0.335	0.314	0.375	0.436
Dig. methionine (%)	0.220	0.260	0.310	0.290	0.350	0.411
Total met+cys (%)	0.493	0.533	0.583	0.563	0.623	0.683
Dig. met+cys (%)	0.430	0.470	0.520	0.500	0.560	0.620
Total threonine	0.492	0.492	0.492	0.492	0.492	0.492
Dig. threonine (%)	0.414	0.414	0.414	0.415	0.415	0.415
Total tryptophan (%)	0.134	0.134	0.134	0.134	0.134	0.134
Dig. tryptophan (%)	0.121	0.121	0.121	0.121	0.121	0.121
Total valine (%)	0.603	0.603	0.603	0.603	0.603	0.603
Dig. valine (%)	0.521	0.521	0.521	0.521	0.521	0.521

*Supplied per kg feed: 8,000 IU vitamin A, 1,800 IU vitamin D3, 12 mg vitamin E, 2 mg vitamin K3, 1 mg vitamin B1, 4 mg vitamin B2, 1 mg vitamin B6, 10 mcg vitamin B12, 0.40 mg folic acid, 0.04 mg biotin, 28 mg niacin, 11 mg calcium pantothenate, 6 mg Cu, 0.10 mg Co, 1 mg I, 50 mg Fe, 65 mg Mn, 45 mg Zn, 0.21 mg Se, 500 mg choline chloride 50%, 60 mg Coxistac® 12% , 12 mg antioxidant.

(100% amino acid ratio); 0.48% digestible lysine and 0.52% digestible methionine+cystine (110% amino acid ratio); 0.56% digestible lysine and 0.50% digestible methionine+cystine (90% amino acid ratio); 0.56% digestible lysine and 0.56% digestible methionine+cystine (100% amino acid ratio); 0.56% digestible lysine and 0.62% digestible methionine+cystine 110% amino acid ratio).

During the second laying cycle, a diet based on corn and soybean meal was formulated according to the recommendations of Rostagno *et al.* (2005) with 17% crude protein, 4.2% calcium, and 0.23% sodium.

During molting, the following performance parameters were evaluated: feed intake, egg production, body weight loss, days until laying stopped, and livability.

During the resting period, the following performance parameters were evaluated: feed intake, egg production, days to return to lay (50 – 60% egg production), days to lay the first and the tenth egg, body weight recovery, feed conversion ratio (kg feed/dozen eggs), and livability [(total number of birds – number of dead birds during forced-molting)/total number of birds x 100).

During the second production cycle, the following performance parameters were evaluated at the end of each period: feed intake, egg production, feed conversion ratio (kg feed per kg eggs and per dozen eggs), average egg weight (total weight of eggs laid/ number of eggs laid, per experimental unit), egg mass (egg production percentage x average egg weight). Egg quality was also evaluated as to: Haugh unit (100 log (h + 7.57 – 1.7 W 0.37), according to Brant *et al.* (1951), where: H = albumen height in mm, W = egg weight in g, eggshell percentage and thickness, and egg specific gravity. Egg specific gravity was determined immersing the eggs in NaCl solutions, which densities ranged between 1,065 and 1,100 g/cm^3 at 0.005 gradients, as proposed by Moreng & Avens (1990).

At the end of the molting period and of the resting period, four birds per treatment were sacrificed by neck dislocation. Birds were previously fasted for eight hours and weighed immediately before sacrifice to allow subsequent calculation of relative ovary, oviduct, liver, and pancreas weights.

The obtained data were submitted to analysis of variance using the General Linear Model (GLM) procedure of SAS statistical package (SAS Institute, 2002). Means were compared by the test of Tukey at 5% probability level.

RESULTS AND DISCUSSION

The layers submitted to forced molting by the supply of zinc oxide presented 35.51g/hen/day feed intake, 14.94% relative egg production, body weight loss of 14.60% on day 7 and 17.46% on day 14 of the molting period, 4.61 days until laying stopped, and 98.60% livability.

According to Baker *et al.* (1983), in order to obtain good performance during the second laying cycle, layers need to lose 25-30% of their body weight, allowing for ovary regression. In the present study, the method applied to induce molting resulted in lower body weight loss (17.46%). However, the dietary inclusion of zinc oxide to induce molting promotes adequate body

Table 2 – Mean, coefficient of variation (CV) and probability of performance parameter evaluated during the resting period.

Parameters								
Treatments Lys met+cys	Feed intake (g/hen/day)	Egg production (%/hen/day)	Return to lay (days)	1st egg (days)	10th egg (days)	Body weight recovery (%)	Feed conversion ratio (kg/dz)	Livability (%)
0.47 0.43	93.32 D	23.27	27.83	6.66	11.66	15.31	5.28	95.83
0.47 0.47	95.87 CD	24.84	27.80	6.00	11.50	15.25	4.76	97.91
0.47 0.52	97.68 BCD	28.66	27.00	6.16	12.00	19.81	4.12	97.91
0.56 0.50	101.62 AB	27.55	28.20	5.16	10.66	17.89	4.48	97.91
0.56 0.56	104.78 A	25.58	27.50	5.66	11.83	20.19	5.31	93.50
0.56 0.62	99.08 BC	25.61	27.50	5.33	10.66	13.19	4.73	95.83
Probability	<0.0021	0.4459	0.5795	0.4704	0.4438	0.5089	0.4039	0.7737
CV (%)	4.40	18.40	3.95	24.17	12.65	23.53	22.94	6.10

weight loss and reduces mortality rates because feed is not withdrawn, thereby reducing bird stress.

During the post-molting period, feed intake significantly increased (p<0.05) when hens were fed the diet with 0.56% digestible lysine and 0.56% digestible methionine+cystine (daily intakes of 586.77 mg/hen/day of both digestible lysine and methionine+cystine), which, however, was statistically different from the feed intake of those fed 0.56% digestible lysine and 0.50% digestible methionine+cystine (daily intakes of 559.07 and 508.77 mg/hen/day digestible lysine and methionine+cystine, respectively; Table 2). The influence of lysine dietary level on feed intake may have been a response to the change in the diet amino acid profile when lysine level increased. According to Andriguetto *et al.* (2003), amino acid imbalance in a diet results in specific changes in blood amino acid levels, affecting appetite.

These results are different from those obtained by Latshaw (1976) in a study to determine optimal lysine requirements for white layers. He supplemented a basal diet containing 0.57 lysine with 0, 0.05, 0.10, or 0.15% L-lysine, and did not observe any influence of these levels on egg production or feed intake; only egg weight was positively affected.

The findings obtained in the present study relative to methionine+cystine levels are consistent with those of Waldroup and Hellwing (1995), who observed lower feed intake in Leghorn hens fed lower methionine+cystine levels added to a basal diet based on corn and soybean meal.

Austic (1986) mentions that severe dietary methionine deficiency reduces methionine blood level, which signals the central nervous system to active the mechanisms responsible for feed intake reduction.

There was no influence of treatments on the other evaluated parameters (p>0.05).

During the second laying cycle, egg weight was significantly affected (p<0.05) by treatments (Table 3). Hens fed the diet with 0.56% digestible lysine (509.88 mg/hen/day) and 0.56% digestible methionine+cystine (509.88mg/hen/day) presented higher egg weight, which, however, was not significantly different from that obtained with hens fed the diet containing 0.56% digestible lysine (514.47 mg/hen/day) and 0.62% digestible methionine+cystine (569.59 mg/hen/day).

The higher egg weight obtained with the diet containing 0.56% lysine and 0.56% methionine+cystine may be explained by the adequate supply of the amino acid requirements during the resting period. Although hens fed the diets containing lower lysine and methionine + cystine levels produced lighter eggs, the other parameters evaluated during the second laying cycle were not affected.

These results are consistent with those of Harms and Russell (2003), who observed that egg weight increased with increasing dietary methionine + cystine levels. Laurentiz *et al.* (2005) evaluated total sulfur amino acid levels for semi-heavy layers after forced molting and also found that the lowest TSAA level supplied (0.45%) after molting reduced egg weight relative to the other TSAA levels (0.60% and 0.75%).

However, the results of the present study are different from the findings of Schmidt *et al.* (2009), who did not find any influence of the supply of

Table 3 – Mean, coefficient of variation (CV) and probability of performance parameter evaluated during the second laying cycle (average of the four evaluated periods).

	Parameters					
Treatments Lys met+cys	Feed intake (g/hen/day)	Egg production (%)	Feed conversion ratio (kg/dz)	Feed conversion ratio (kg/kg)	Egg weight (g)	Egg mass (g/day)
0.47 0.43	93.75	60.56	1.88	2.42	64.62 B	39.13
0.47 0.47	89.67	60.19	1.88	2.43	64.94 B	39.08
0.47 0.52	91.66	61.47	1.80	2.33	64.21 B	39.47
0.56 0.50	91.90	59.38	1.87	2.41	64.57 B	38.34
0.56 0.56	91.05	62.58	1.76	2.13	68.60 A	42.92
0.56 0.62	91.87	60.37	1.84	2.33	65.80 AB	39.72
Probability	0.9275	0.7634	0.5470	0.0649	< 0.0006	0.1200
CV (%)	6.85	6.28	6.70	7.18	3.24	6.23

[A,B] Means followed by different letter as statistically different.

Table 4 – Mean, coefficient of variation (CV) and probability of the egg quality parameters evaluated during the second laying cycle.

	Parameters			
Treatments Lys met+cys	Haugh units	Eggshell (%)	Eggshell thickness (mm)	Egg specific gravity (g/cm³)
0.47 0.43	96.26	9.52	0.40	1.087
0.47 0.47	96.03	9.58	0.40	1.088
0.47 0.52	94.57	9.42	0.40	1.086
0.56 0.50	96.18	9.23	0.40	1.086
0.56 0.56	97.04	8.98	0.39	1.084
0.56 0.62	96.53	8.97	0.39	1.086
Probability	0.4490	0.3686	0.4040	0.2761
CV (%)	1.48	2.74	2.65	0.16

different digestible lysine levels (0.55, 0.60, 0.65, 0.70, and 0.75%) to layers during the second laying cycle.

Silva *et al.* (2000) also fed different digestible lysine and sulfur amino acid levels to commercial layers and did not find any differences in performance parameters.

Lysine and methionine + cystine levels supplied during the post-molting period did not affect (p>0.05) internal or external quality parameters of the eggs produced during the second laying cycle (Table 4). Although 0.56% lysine and 0.56% methionine + cystine promoted higher egg weight, their eggshell quality was not affected.

These results are consistent with those obtained by Shafer *et al.* (1996), who did not find any effect of increasing SAA levels on eggshell percentage or weight. Feeding 0.517, 0.569, 0.624, 0.679, or 0.734% digestible methionine + cystine to semi-heavy layers, Sá *et al.* (2007) also found no effect of SAA levels on egg quality.

On the other hand, Cupertino (2006) observed decreasing eggshell percentage as digestible methionine+cystine levels increased in the diet, as well as Carey *et al.* (1991). According to Harms *et al.* (1998), egg weight and eggshell quality present high negative correlation, and when dietary methionine+cystine levels promote an increase in egg weight, and there are no changes in eggshell deposition, eggshell quality is compromised. However, this effect was not observed in the present study.

After molting, relative organ weights were: 0.35% for the ovary, 1.21% for the oviduct, 1.77% for the liver, and 0.15% for the pancreas. Similar results were observed by Berry & Brake (1991), who compared fasting to the dietary supply of 2,000 ppm zinc oxide

Table 5 – Mean, coefficient of variation and probability of the evaluated organ relative weights obtained after the resting period.

	Parameters			
Treatments Lys met+cys	Ovary (%)	Oviduct (%)	Liver (%)	Pancreas (%)
0.47 0.43	1.90	2.71	2.40	0.205
0.47 0.47	2.55	3.46	2.37	0.205
0.47 0.52	2.62	2.96	2.43	0.195
0.56 0.50	2.82	3.21	2.32	0.197
0.56 0.56	2.18	2.53	2.15	0.225
0.56 0.62	1.90	2.99	2.61	0.210
Probability	0.5094	0.3704	0.7495	0.8536
CV (%)	29.97	20.81	16.24	16.74

as forced-molting method until 30% weight loss, and 1.18% oviduct weight relative to live weight when zinc oxide was used. One of the most important factors of induced molting is body weight loss, resulting in follicle development arrest and atresia (Araújo *et al.* 2007), which will ensure optimal performance during the second laying cycle.

Relative organ weights evaluated when hens were sacrificed after the post-molting period were not influenced by the treatments (Table 5), showing that the amino acid levels supplied were sufficient to recover the weights of the reproductive organs, liver, and pancreas, thereby promoting good results during the second laying cycle.

Mean, coefficient of variation and probability of the evaluated organ relative weights obtained after the resting period.

CONCLUSIONS

Based on the results of the present study, it is recommended to supply 0.56% digestible lysine and 0.56% digestible methionine + cystine (daily intakes of 509.88 mg/hen/day) to commercial layers during the post-molting period, because these levels promote higher egg weight without affecting eggshell quality during the second laying cycle.

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