DOI: 10.5216/cab.v12i3.2535

GLUTAMINE IN DIET OF LAYING HENS SUBMITTED TO HEAT STRESS AND THERMONEUTRALITY

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ABSTRACT

The aim of this study was to evaluate the effect of glutamine supplementation of the diet on intestinal mucosa morphology, performance, and egg quality of commercial laying hens, submitted to heat stress and thermoneutral conditions. In this study, 96 (Isa Babcock) laying hens at 35 weeks of age were used and distributed in a completely randomized design according to a 2x2 factorial arrangement, with two levels of ambient temperature (thermoneutral and hot) and two levels of glutamine in the diet (0.0 and 1.0% of inclusion), in 6 replicates of 4 hens per box. Feed intake, daily egg production, feed conversion per kilogram of eggs, and egg quality were obtained in two periods of 28 days each. Heat stress decreased egg production and quality, and glutamine supplementation improved egg quality and feed conversion. The heat and glutamine supplementation provided an increase in calliciform cells quantity in duodenum and ileum, respectively. Significant morphological modifications in the intestinal mucosa of laying hens were not found.

KEYWORDS: egg quality; glutamine; intestinal morphology; thermal stress.

GLUTAMINA NA DIETA DE POEDEIRAS LEVES SUBMETIDAS AO ESTRESSE PELO CALOR E À TERMONEUTRALIDADE

RESUMO

Com este trabalho objetivou-se verificar o efeito da suplementação com glutamina na dieta sobre a morfologia da mucosa intestinal, o desempenho e a qualidade de ovos de poedeiras comerciais leves, submetidas a condições de estresse pelo calor e termoneutralidade. Foram utilizadas 96 poedeiras da linhagem Isa Babcok, com 35 semanas de idade, distribuídas em delineamento inteiramente casualizado, no esquema fatorial 2x2, com duas temperaturas ambientes (termoneutra e quente) e 2 níveis de glutamina na dieta (0,0 e 1,0% de inclusão), com seis repetições de quatro aves por tratamento. O consumo de ração, produção diária de ovos, peso e massa de ovos, conversão alimentar por quilograma de ovos e qualidade dos ovos foram obtidos em dois períodos de 28 dias. Ao final do experimento foram abatidas quatro aves/tratamento para avaliação do peso de órgãos e morfologia intestinal. O estresse pelo calor diminuiu o desempenho e a qualidade dos ovos e a suplementação com glutamina melhorou a qualidade dos ovos e a conversão alimentar. O calor promoveu aumento na quantidade de células caliciformes no duodeno, enquanto a suplementação com glutamina provocou esse aumento no íleo. Não foram encontradas modificações morfológicas representativas na mucosa intestinal das poedeiras.

PALAVRAS-CHAVE: estresse térmico; glutamina; morfologia intestinal; qualidade de ovos.

INTRODUCTION

Environmental factors such as high temperature and air relative humidity may be a challenge to poultry production. It is known that high temperatures reduce food intake impairing performance. The higher the temperature, the lower the loss of sensible heat by the bird due to lower thermal gradient between the environment and the bird's body. As a result, homeostatic mechanisms of body temperature control are activated, so that the animal does not develop hyperthermia. Heat stress is a factor that may alter intestinal morphology, hence, the ability of nutrients digestion and absorption by the birds (MACARI et al., 2002).

The number of enterocytes, microvilli height and density and the membrane structure determine the dimension of the digestion surface and intestinal absorption, thus, the higher the villi, the better the performance of birds (FERRER et al., 1995).

The development of the intestinal mucosa depends on the height and density increase of intestinal villi, which corresponds to an increase in the number of epithelial cells (enterocytes, goblet and enteroendocrine cells), thus on the increase of the digestive and absorptive capacity of the intestine. This development is due, primarily, to two associated cytological events: cellular renewal (UNI et al. 1998; APPLEGATE et al., 1999) and cellular loss, which normally occurs at the apex of the villi. The balance between these two processes determines constant turnover (migration-extrusion synthesis), i.e., maintenance of the size of the villi and, therefore, of the digestive capacity and intestinal absorption. When there is imbalance in the cellular renewal rate in the intestine due to a stimulus, changes in height and density of villi and microvilli occur.

Currently, amino acids such as glutamine, which plays an important role as an energy source for the development of mucosa (WINDMUELLER & SPAETH, 1974), have been used in the attempt of reducing atrophy of the intestinal mucosa (PIERZYNOWSKI & SJODIN, 1998). Its effect on the reconstitution of the intestinal mucosa after some injury has been investigated because this amino acid is the major metabolite that nourishes the enterocytes (VASCONCELOS & TIRAPEGUI, 1998; PADOVESE, 2000).

MAIORKA et al. (2000), by studying broilers, showed that the addition of 1.0% glutamine in the diet was able to increase the size of the villi in the duodenum and ileum, when evaluated on the first seven days of life, also that glutamine has trophic effects on intestinal mucosa, increasing its functional capacity, providing better bird performance due to greater ability to digest and absorb nutrients from the diet.

Glutamine stimulates intestinal cellular proliferation (KANDIL et al. 1995; FISHER DA SILVA et al., 2007), which could result in an increase of the absorptive surface of the gastrointestinal tract mucosa. Thus, the addition of glutamine may be an alternative to improve the development of the intestinal mucosa.

This work aimed to verify the effect of glutamine supplementation in the diet on the morphology of the intestinal mucosa, performance and egg quality of laying hens submitted to conditions of heat stress and thermoneutrality.

MATERIAL AND METHODS

The experiment was conducted at the Veterinary Medicine and Animal Science School, UNESP - Universidade Estadual Paulista - Botucatu Campus, in the bioclimatic chamber. We used 96 Isa Babcok laying hens, at 35 weeks of age, housed in 24 laying cages with four birds per compartment. Water and feed were provided *ad libitum*, with weekly control of feed intake. The birds were submitted to a photoperiod of 17 hours of light daily.

No temperature control, which varied according to the temperature of the day, was carried out in the thermoneutral chamber. The hot chamber was heated by electric heaters during the day and the resistances were turned off at night, allowing the room to cool down gradually, simulating the daily temperature variation in a hot region. The data on temperature, relative humidity, mortality and number of eggs were recorded daily.

The birds were distributed in a completely randomized design, in a 2x2 factorial arrangement, with two ambient temperatures (thermoneutral and hot) and two levels of glutamine in the diet (0.0 and 1.0% of inclusion), with six replicates of four birds per cage.

The ration was formulated as recommended by ROSTAGNO et al. (2005) for laying hens, which included 1.0% glutamine, replacing 1.0% of corn in the diet (Table 1).

Performance data of the birds were obtained in two periods of 28 days each, and expressed as mean rate during the total period of 56 days. We evaluated feed intake per bird, the average daily production of eggs and feed conversion per kilogram and per dozen eggs produced.

Table 1. Composition and nutritional value of feed calculated with and without the inclusion of glutamine in the diet

	Glutamine inclusion levels, %	
Ingredients	1.0	0.0
Corn	60.423	61.423
Soybean meal	26.218	26.218
Soybean oil	2.155	2.155
Dicalcium phosphate	1.735	1.735
Limestone	7.727	7.727
DL - methionine	0.192	0.192
Vitamin Supplement[1]	0.100	0.100
Mineral Supplement[2]	0.100	0.100
Salt	0.350	0.350
L-glutamine[3]	1.000	0.000
Total	100	100
Calculated values		
Metabolizable energy, kcal/kg	2,869	2,850
Crude protein, %	17.590	17.500
Methionine, %	0.465	0.465
Lisine, %	0.882	0.882
Methionine + cystine, %	0.750	0.750
Calcium, %	3.500	3.500
Available phosphorus, %	0.420	0.420

[1]*Vitamin supplement Postura Multimix* ® (per kg of product) - vit A: 7,000,000 UI; vit D3: 2,000,000 IU; vit E: 5000 mg; vit K3: 1600 mg; vit B2: 3000 mg; vit B12: 8000 mcg; niacin: 20,000 mg; pantothenic acid: 5000 mg; antioxidant: 15,000 mg; excipient QSP 1.000g.
[2]*Mineral Supplement Multimeral Multimix* ® (per kg of product) - Cu: 8000 mg; Fe: 50,000 mg, Mn: 70,000 mg, Zn: 50,000 mg; I: 1200 mg; Se: 200 mg; Excipient QSP 1.000g.
[3] *L-Glutamina Ajinomoto Biolatina ®:* 92% purity.

The assessment of egg quality was carried out by the proportion of internal content (albumen and yolk percentage), evaluation of egg yolk color (comparison of the yolk color with Roche® colorimetric range – evaluation of albumen quality (Haugh units) and of shell quality (shell percentage and specific gravity).

The specific gravity of the eggs was determined by the use of salt solutions at increasing concentrations. Haugh unit values were calculated by the following logarithmic formula: HU = 100 log (H + 7.57 - 1.7 $W^{0.37}$), where H = albumen height, in millimeters, and W = egg weight, in grams.

At 56 days of experiment, after a 12-hour fasting, one bird per cage was weighed and euthanized by dislocation of the craniocervical joint, totaling four birds per treatment, for collecting the following organs: heart, liver, proventriculus, gizzard, pancreas, small intestine and large intestine. Liver and heart were weighed immediately after being removed. Proventriculus and gizzard were opened and weighed after contents removal. Small and large intestines were separated by sections where the duodenum emerges from the gizzard and at the beginning of the cecum, then they were weighed and measured. Intestine length was taken as the length of the colon and rectum plus the length of the cecum. Pancreas was weighed after being removed from the small intestine.

For histological analysis two 3.0-cm segments were collected from duodenum, jejunum and ileum, cut transversely and longitudinally, opened at the mesenteric border, washed and extended by the tunica serosa, and fixed in 10% formalin solution for a period of 24 hours. They were subsequently washed in running water and stored in 70% ethanol, then dehydrated in an ascending series of alcohols, cleared in xylene and embedded in *Paraplast Plus*® slides of each segment were prepared with seven-micrometers-thick cuts, which were stained with periodic-acid Schiff (PAS).

Later, with the aid of an optical microscope connected to a Leica image analyzer *(Image-Pro Plus version 4.5.0.27)* and a computer, villi height and perimeter, crypt depth were measured and enterocytes and goblet cells of the duodenum, jejunum and ileum were counted, to determine the goblet cells/enterocytes relation. The height measurements were done from the basal region of the villi, coincident with the upper portion of the crypts, to its apex. The measurement of the perimeter was carried out by passing the edge of the entire villus where the microvilli are found (CARRIJO et al., 2005). The crypts were measured from its base to the crypt:villus transition region.

The statistical analysis was performed by analysis of variance (ANOVA) using the GLM procedure of SAS (1996) at a significance level of 5%.

RESULTS AND DISCUSSION

The average minimum and maximum temperatures for thermoneutral chamber were 23°C and 28°C, respectively, and the average minimum and maximum temperatures for the hot chamber were 27°C and 32°C, respectively.

There was no significant interaction between the level of glutamine and the temperature for any of the performance traits evaluated, as well as no effect of glutamine supplementation on feed intake, percentage of laying, weight and egg mass was found (Table 2). However, glutamine supplementation improved ($P < 0.05$) the results of

feed conversion (FC) per kilogram and per dozen eggs, which is probably due to better food digestion and absorption, as shown by WU et al. (1996), who found 25% improvement in feed conversion for pigs supplemented with glutamine in the diet.

Table 2. Mean values of production during the experimental period of 56 days according to the addition of glutamine (G) to the diet and ambient temperature (T)

Variables	L-Glutamine, %			Temperature, °C			G x T	CV
	0.0	1.0	P	Thermoneutral	hot	P	P	(%)
Feed intake, g	99.51	96.45	0.509	105.73 a	90.23 b	0.003	0.856	11.35
Laying, %	82.77	86.88	0.130	90.07 a	79.57 b	0.001	0.298	7.51
Egg weight, g	58.82	58.74	0.914	59.28	58.28	0.194	0.284	3.10
Egg mass, g	48.71	50.99	0.151	53.38 a	46.32 b	0.000	0.143	7.48
FC/kg eggs	2.07 a	1.92 b	0.036	1.99	2.00	0.876	0.129	7.75
FC/dozen eggs	1.46 a	1.34 b	0.027	1.42	1.38	0.503	0.187	8.55

[a, b] Means in line followed by different letters differ (P <0.05) by F test

Ambient temperature did not affect egg weight, feed conversion per kilogram and per dozen eggs (Table 2). However feed intake, laying percentage and egg mass decreased with increasing temperature, according to LEESON et al. (1996), who verified that long periods of heat stress decreased egg production and weight.

There was interaction (P <0.05) between the level of glutamine and the temperature for specific gravity and egg shell weight (Table 3), which indicates that, for both hot and thermoneutral ambient temperature, glutamine supplementation increased the percentage of egg specific gravity and shell. Temperature increase resulted in an augmentation in the percentage of egg shell and specific gravity in birds that did not receive glutamine, which is contrary to effect found by LEESON et al. (1996), who showed that heat stress in laying hens reduced egg shell quality.

Glutamine supplementation did not affect yolk percentage, although it reduced albumen percentage. There was no effect of temperature on the percentage of yolk and albumen. There was no interaction between glutamine supplementation and ambient temperature for the percentage of shell, yolk height, albumen height, color and Haugh unit (Table 4).

Table 3. Effects of interaction between the level of glutamine and ambient temperature on shell weight (%) and specific gravity (g / L)

		Temperature		
	L-glutamine, %	Thermoneutral	Hot	Means
Shell, %	0.0	9.02bB	9.58aB	9.30
	1.0	9.87A	9.98A	9.93
Means		9.44	9.78	
Specific gravity, g/cm^3	0.0	1.085bB	1.090aB	1.087
	1.0	1.092A	1.093A	1.092
Means		1.088	1.091	

[a, b] Means in line followed by different lowercase letters, differ by the F test (P <0.05).
[A, B] Means in column followed by different capital letters, differ by the F test (P <0.05).

Glutamine supplementation did not affect Haugh unit, but changed (P <0.05) yolk color on colorimetric range, causing the darkening of the yolk. This fact is probably due to improved absorption of nutrients in the birds´ intestine, consequently, to better absorption of carotenoids, which are responsible for a darker color of the yolk. According to BISCARO & CANNIATTI-BRAZACA (2006), consumers from South American countries prefer eggs with yellow-orange yolk.

Hot ambient temperature decreased (P <0.05) Haugh unit and produced a lighter yolk color, showing a decrease in egg quality.

Table 4. Mean values of egg quality according to the inclusion of glutamine (G) to the diet and ambient temperature (T)

Variables	L-Glutamine, %			Temperature, °C			G x T	CV
	0.0	1.0	P	Thermoneutral	Hot	P	P	(%)
Specific gravity, g/cm^3	1087	1092	0.000	1088	1091	0.000	0.008	0.56
Yolk, %	25.15	25.40	0.247	25.48	25.06	0.052	0.528	7.26
Albumen, %	65.56a	54.76b	0.003	65.07	65.16	0.072	0.120	3.09
Egg shell, %	9.30	9.93	0.000	9.45	9.78	0.000	0.013	8.18
Yolk height, mm	17.98	18.08	0.332	18.23a	17.81b	0.000	0.975	5.28
Albumen height, mm	7.26	7.31	0.708	7.61a	6.95b	0.000	0.758	14.68
Color	6.67a	7.06b	0.003	7.00a	6.72b	0.026	0.749	16.09
Haugh unity	84.66	84.70	0.958	86.81a	82.55b	0.000	0.560	8.40

[a, b] Means in line followed by different letters differ (P <0.05) by F test

As shown in Table 5, there was no interaction between glutamine supplementation and room temperature for villi perimeter, crypt depth and goblet/enterocytes relation in the duodenum. Villi perimeter, crypt depth and goblet/enterocytes relation were not affected (P> 0.05) by the inclusion of glutamine or by ambient temperature, except for an increase (P <0.05) in the goblet/enterocytes relation as the temperature rose.

There was no effect of glutamine supplementation and ambient temperature nor effect of these two factors on villi perimeter, crypt depth and goblet/enterocytes relation in the jejunum (Table 5).

Glutamine supplementation resulted in an increase in the goblet/enterocytes relation of villi in the ileum. There was no effect of ambient temperature nor interaction between glutamine supplementation and ambient temperature on the morphometric characteristics of the ileum (Table 5).

Table 5. Crypt perimeter and depth and goblet cells/enterocytes relation in the villi of the duodenum, jejunum and ileum according to the addition of glutamine (G) to the diet and ambient temperature (T)

Variables	L-Glutamine, %			Temperature, °C			G x T	CV
	0.0	1.0	P	Thermoneutral	Hot	P	P	(%)
Duodenum								
Perimeter, μm	2686.48	2641.00	0.801	2598.97	2728.51	0.475	0.206	16.35
Crypt depth, μm	67.67	73.49	0.483	62.42	78.74	0.059	0.442	28.24
Goblet cells / enterocytes, %[1]	26.97	29.15	0.148	26.00a	30.12b	0.011	0.052	11.58
Jejunum								
Perimeter, μm	1930.81	1713.20	0.166	1766.88	1877.13	0.475	0.304	20.37
Crypt depth, μm	65.59	71.12	0.507	60.81	75.90	0.081	0.969	29.45
Goblet cells / enterocytes, %[1]	30.90	34.70	0.054	31.43	34.17	0.156	0.100	13.83
Ileum								
Perimeter, μm	1277.17	1135.28	0.260	1100.55	1311.89	0.100	0.250	24.85
Crypt depth, μm	58.25	58.21	0.994	55.72	60.75	0.355	0.524	21.86
Goblet cells / enterocytes, %[1]	34.73a	40.10b	0.012	36.61	38.22	0.416	0.102	12.12

[a, b] Means in line followed by different letters differ (P <0.05) by F test

[1] Counting of 500 enterocytes and all goblet cells per bird.

The intestinal maturation of the birds occurs in the first weeks of life, which may explain why there was no effect of glutamine on intestinal morphology during the studied period, because the birds have already presented intestinal maturation (UNI et al., 1995). Some authors (MAIORKA et al. 2000; MURAKAMI et al., 2007. in birds; ABREU et al., 2010, in pigs), working with glutamine supplementation to the diet, observed the development of intestinal mucosa in the first weeks of life showing the beneficial effect of glutamine in the early stages of life.

Under this experiment conditions, intestinal development was not affected, which is probably due to the fact that the intensity of stress used was not very severe (temperatures between 27 and 32°C).

Despite the reduction in feed intake, the intestinal mucosa of birds and organ weights were not affected (Table 6), indicating the need for increased heat stress in birds to affect such characteristics, regarding especially organs morphology, as shown by OLIVEIRA NETO et al. (2000).

There was no interaction between glutamine supplementation and ambient temperature for the variables analyzed (Table 6). Supplemetation did not affect live weight and relative weights of heart, gizzard, proventriculus, liver and pancreas, as well as ambient temperature did not affect these variables, which differs from what was observed by OLIVEIRA NETO et al. (2000), who found a reduction in size of these organs because of heat stress in broilers. Temperature increase reduced only the relative liver weight (P <0.05). Different authors (OLIVEIRA et al., 1997, in pigs and ZANUSSO et al., 1999, in birds) also observed a reduction of the relative weights of organs, due to high temperature.

There was no significant difference or interaction for glutamine supplementation and ambient temperature for weight and length of the small and large intestines (Table 6).

Table 6. Mean values of live body weight (g) and organ weights* (%) and length of the intestines (cm) according to the addition of glutamine (G) to the diet and ambient temperature (T)

Variabels	L-Glutamine, %			Temperature, °C			G x T	CV
	0.0	1.0	P	Thermoneutral	Hot	P	P	(%)
Body weight, g	1351	1393	0.546	1420	1323	0.172	0.334	12.25
Heart, %	0.41	0.42	0.818	0.41	0.42	0.860	0.966	10.18
Gizzard, %	1.52	1.47	0.634	1.49	1.49	0.984	0.984	16.74
Proventriculus, %	0.37	0.38	0.531	0.38	0.37	0.459	0.790	11.97
Liver, %	1.85	1.91	0.434	1.99A	1.77B	0.007	0.925	9.41
Pancreas, %	0.22	0.23	0.749	0.22	0.23	0.810	0.462	10.64
Small intestine, %	2.57	2.51	0.659	2.53	2.54	0.879	0.921	13.18
Large intestine, %	0.91	0.99	0.143	0.95	0.96	0.985	0.931	13.61
Small intestine, cm	115.9	122.3	0.234	121.7	116.6	0.342	0.862	10.74
Large intestine, cm	31.5	32.5	0.382	32.0	32.0	1.000	0.382	8.56

* Relative weight of organ (%) = (weight ÷ live weight) x 100.

CONCLUSIONS

Heat stress and glutamine supplementation do not promote significant morphological changes in the intestinal mucosa of light commercial laying hens in laying phase. The heat stress decreases the production and quality of eggs and glutamine supplementation improves the egg quality and feed conversion.

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Submitted on: November, 28, 2007. Accepted on: April, 27, 2011.