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Effects of Dietary L-Glutamine or L-Glutamine Plus L-Glutamic Acid Supplementation Programs on the Performance and Breast Meat Yield Uniformity of 42-d-Old Broilers

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■**Keywords**

Breast, growth, non-essential amino acids, uniformity.

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ABSTRACT

This study aimed at evaluating four dietary L-Glutamine (L-Gln) or L-Gln plus L-Glutamate (L-Glu) supplementation programs on the performance, breast yield, and uniformity of broilers. A total of 2,112 one-d-old male Cobb 500® broilers were distributed according to a randomized block design in a 2 × 4 factorial arrangement (L-Gln or L-Gln plus L-Glu × 4 supplementation programs), totaling eight treatments with 12 replicates of 22 broilers each. The supplementation programs consisted of the dietary inclusion or not of 0.4% of L-Gln or L-Gln plus L-Glu for four different periods: 0 days (negative control), 9d, 21d, and 42d. Feed intake (FI, g), body weight gain (BWG, g), feed conversion ratio (FCR, kg/kg), coefficient of variation of body weight (CV, %), body weight uniformity (UNIF, %), breast weight (BW, g), breast yield (BY, %), coefficient of variation of breast weight (CV_B), breast uniformity ($UNIF_B$), coefficient of variation of breast yield (CV_{BY}), and breast yield uniformity ($UNIF_{BY}$) were evaluated. Birds fed the diets treatments supplemented with L-Gln or L-Gln plus L-Glu for 9d presented 3% higher BWG ($p<0.05$) compared with the controls. The L-Gln or L-Gln plus L-Glu supplementation until broilers were 21 days old resulted in 14, 10, 16, and 12% improvements ($p<0.05$) in CV, UNIF, CV_{BY}, $UNIF_{BY}$, respectively. The supplementation of 0.4% L-Gln (L-Gln 99%) or L-Gln plus L-Glu (minimum 95%) to pre-starter and starter broiler diets is recommended to improve body weight gain and uniformity.

INTRODUCTION

L-glutamine (L-Gln) is considered a non-essential amino acid (AA) because most animal cells are able to synthesize them (Murakami *et al*., 2007). It is the most prevalent AA in the bloodstream, accounting for around 35% of nitrogen (N) in the plasma and in the free AA pool in the body (Newsholme *et al*., 1986). Additionally, the ability to receive and donate N makes of L-Gln the main vehicle for nitrogen transfer between tissues (Bartell and Batal, 2007). L-Gln is the main metabolic fuel for small intestine enterocytes, lymphocytes, macrophages, and fibroblasts (Cynober, 1999; Andrews and Griffiths, 2002), and it is considered an essential amino acid in some species under inflammatory conditions, such as infection and injury (Newsholme, 2001). Also, it may stimulate feed intake through the production of orexigenic and anorexigenic neuropeptides in mammalian (Zeni *et al*., 2000) and chicken brain (Khondowe *et al*., 2012)

However, in certain pathological circumstances such as trauma and infection, specific tissues need a greater amount of L-Glutamine than muscle catabolism is capable of producing (Calder, 1994). Also, L-Glutamine may be converted to L-Glutamic acid (L-Glu) to produce immune response factors to be used against pathogen in the body (Newsholme *et al*., 2003).

It is known that L-Glutamine influences intestinal development (Zaravize *et al*., 2011) and that the greatest growth rate of the gastrointestinal tract of broilers occurs during the first week of life (Uni & Ferket, 2004). Also, it was reported that L-Gln supplementation may increase intestinal villus height in turkey poults (Yi *et al*., 2005) and that it may stimulate gut cell proliferation (Inoue *et al*., 1993, Sakamoto *et al*., 2011) that aid maintaining gut integrity, intestinal barrier function, and gut mucosal regeneration (Sanz *et al*., 2004), which is important for preventing bacterial infections. Adjei *et al*. (1994) reported that L-Gln has been shown to prevent intestinal hyperpermeability and bacterial translocation in mice submitted to immune challenge.

Moreover, there are few studies on L-Gln or L-Gln plus L-Glu supplementation in broiler diets according to rearing phase.

Currently, the nutritional broiler requirements are evaluated at different phases of life in order to formulate diets that meet their needs with greater precision (Rostagno *et al*., 2011).

Thus, the aim of this study was to evaluate the effect of dietary supplementation programs of L-Glutamine or L-Glutamine plus L-Glutamic acid on the performance, breast meat yield, and broiler uniformity.

MATERIAL AND METHODS

The study was carried out according to the recommendations of the Ethics Committee for Animal Use of the Department of Animal Science (DZO/ UFV), under protocol n. 38/2013.

The experiment was carried out at the Poultry Section of the Department of Animal Science, Federal University of Viçosa. The birds were distributed in a randomized block design using a 2 × 4 factorial arrangement (L-Gln or L-Gln plus L-Glu × 4 periods, totaling eight treatments with 12 replicates of 22 birds per experimental unit. The experimental blocks were determined by positions interfering with ambient light.

In order to increase stress, birds were subjected to feed and water fasting for 24 hours upon their arrival at the experimental farm. Reused litter was used as challenge the broilers' health status.

Three basal diets were formulated (Table 1) to meet broiler nutritional requirements according to Rostagno *et al.*(2011), as a function of rearing phase.

The eight treatments consisted of inclusion of L-Gln or L-Gln plus L-Glu at 0.4% in the basal diets in different dietary supplementation programs: negative control (without supplemental L-Gln or L-Gln plus L-Glu in any of the experimental phases), and supplementation from d 1 to 9, d 1 to 21, or d 1 to 42.

Table 1 – Ingredients and nutrient composition of the experimental diets (g/kg diet, on as-fed basis).

Ingredients (g/kg)	Pre-starter (1 to 9d)	Starter (9 to 21d)	Grower/finisher (21 to 42d)
Corn	357.5	422.0	488.3
Soybean meal 45%	371.2	324.0	287.2
Sorghum	150.0	150.0	150.0
Corngluten meal 60%	50.0	40.0	0.0
Soybean oil	21.8	19.5	37.8
Limestone	9.2	9.1	7.6
Bi-calcium phosphate	19.1	15.6	10.8
Salt	5.1	4.8	4.5
L-lysine HCl, 79%	2.8	2.5	2.0
DL-methionine, 99%	2.7	2.3	2.4
L-threonine, 98%	0.4	0.3	0.4
Choline chloride, 60%	1.0	1.0	1.0
Mineral mixture[1]	1.3	1.1	0.8
Vitamin mixture[2]	1.3	1.1	0.8
Anticoccidial (salinomycin 12%)	0.5	0.5	0.5
Avilamycin	0.1	0.1	0.1
BHT[3]	0.1	0.1	0.1
Starch[4]	6.0	6.0	6.0
Calculated values			
Crude protein, g/kg	244.3	221.4	185.1
Metabolizable energy, kcal/kg	2,950	3,000	3,150
Calcium, g/kg	9.2	8.2	6.4
Available phosphorus, g/kg	4.7	4.0	3.0
Digestible phosphorus, g/kg	4.0	3.4	2.7
Sodium g/kg	2.2	2.1	2.0
Potassium g/kg	8.4	7.7	7.2
Digestible lysine, g/kg	13.1	11.7	10.1
Digestible Met+Cys, g/kg	9.4	8.5	7.4
Digestible threonine, g/kg	8.5	7.6	6.6

[1] Amount per kg of feed: Broilers: Pre-starter: Cu, 12.5 mg; Fe, 6.5 mg; I, 1,25 mg; Mn, 88 mg; Se, 0.375 mg; Zn, 81.3 mg. Starter: Cu, 11 mg; Fe, 55 mg; I, 1.10 mg; Mn, 77 mg; Se, 0.330 mg; Zn, 71.5 mg. GrowerII (34 – 42 days): Cu, 7.5 mg; Fe, 37.5 mg; I, 0.75 mg; Mn, 53 mg; Se, 0.225 mg; Zn, 48.8 mg.

[2] Amount per kg of feed: Pre-starter: Vit A, 9375 IU; Vit D_3, 2375 IU, Vit E, 35 IU; Vit K_3, 1.88 mg; Vit B_1, 2.50; Vit B_2, 6.25 mg; Nicotinic acid, 37.5 mg; Pantothenic acid, 12.5 mg; Vit B_6, 3.5 mg; Vit B_{12}, 0.015 mg; Folic acid, 0.875 mg; Biotin, 0.088 mg; Starter: Vit A, 8250 IU; Vit D_3, 2090 IU, Vit E, 31 IU; Vit K_3, 1.65 mg; Vit B_1, 2.20; Vit B_2, 5.5 mg; Nicotinic acid, 33 mg; Pantothenic acid, 11 mg; Vit B_6, 3.08 mg; Vit B_{12}, 0.013 mg; Folic acid, 0.77 mg; Biotin, 0.077 mg; Choline, 330 mg. Grower II (34 – 42 days): Vit A, 5625 IU; Vit D_3, 1425 IU, Vit E, 21 IU; Vit K_3, 1.13 mg; Vit B_1, 1.50; Vit B_2, 3.75 mg; Nicotinic acid, 22.5 mg; Pantothenic acid, 7.5 mg; Vit B_6, 2.10 mg; Vit B_{12}, 0.009 mg; Folic acid, 0,525 mg; Biotin, 0.053 mg;

[3] Butyl hydroxy toluene

[4] L-Glutamine and AminoGut® were added at the expense of starch in the experimental diets.

The evaluated products consisted of a commercial L-Glutamine (99% of L-Glutamine) and AminoGut® as source of L-Glutamine plus L-Glutamic acid, which is a commercial dietary supplement containing a mixture of L-Glutamine and L-Glutamic acid (minimum 95%).

The following parameters were evaluated: feed intake (FI, g); body weight gain (BWG, g); feed conversion ratio (FCR, kg/ kg); breast weight (BW, g); breast yield (BY, %); coefficients of variation of body weight (CV, %), breast weight (CV_B, %), and breast yield (CV_{BY}, %); and uniformity of body weight (UNIF, %), breast weight ($UNIF_B$, %), and breast yield ($UNIF_{BY}$, %).

On d42, 10 birds per pen were sacrificed to evaluate breast traits, according to Sakomura & Rostagno (2007).

Data were analyzed using the MIXED procedure of SAS (SAS Institute, 2010). Data were submitted to analysis of variance (ANOVA) and means were compared using Tukey's test at 5% probability level. In order to statistically analyze uniformity and coefficient of variation, all data were by applying the arcsine of the square root of the percentage value / 100. Then, this value was multiplied by (180 / π) to obtain the data on degrees, and processed as shown by the following equation: UNIF or CV (degrees) = [Arc sen √% / 100 x (180 / π)] (Carvalho, 2009).

The mixed model included the fixed effects of treatments, random effects of block, and residual error, as follows: The statistical model applied was: $Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \delta_k + \varepsilon_{ijk}$, where Y_{ijk} is the observation "k" of AA"i" in phase "j"; μ is the overall mean; τ_i is the effect of AA"i'; β_j is the effect of phase "j"; $(\tau\beta)_{ij}$ is the interaction of AA"i" × phase "j"; δ_k is the effect of block "k"; and ε_{ijk} is the residual random error.

RESULTS AND DISCUSSION

No interaction (p>0.05) was observed between L-Gln or L-Gln plus L-Glu supplementation and the dietary supplementation programs for any of the parameters evaluated during the experimental period. Similarly, there was no influence (p>0.05) of the dietary supplementation of L-Gln or L-Gln plus L-Gluon any of the evaluated parameters (Table 2).

The treatments did not influence FI, FCR, BW, BY, CV_B, or $UNIF_B$ (p>0.05). Some studies did not show any influence of dietary L-Gln or L-Glu supplementation on broiler FI and FCR during different production phases (Bartell & Batal, 2007; Fasina *et al.*, 2010; Ayazi, 2014). Nevertheless, recent studies have been shown that glutamine and glutamate may induce the production

Table 2 – Experimental results obtained for broiler performance, breast characteristics, coefficient of variation and uniformity.

Treatments		Parameters[1]										
		FI	BWG	FCR	CV	UNIF	BW	BY	CV_B	$UNIF_B$	CV_{BY}	$UNIF_{BY}$
L-Gln	0d	4159.8	2446.2	1.702	10.4	68.4	625.2	30.8	13.4	60.7	7.18	81.12
	9d	4241.8	2541.3	1.669	10.0	70.7	634.6	31.2	12.6	56.5	6.43	83.47
	21d	4219.5	2519.1	1.675	8.8	76.6	631.9	31.0	11.1	64.1	6.38	86.89
	42d	4238.5	2555.9	1.659	8.5	78.9	647.6	31.5	11.0	64.6	5.58	94.63
L-Gln+L-Glu	0d	4186.3	2472.1	1.695	10.0	69.1	625.5	31.1	12.7	59.8	7.41	80.55
	9d	4185.8	2504.8	1.671	9.3	71.7	625.4	30.9	12.0	63.0	6.66	83.71
	21d	4184.2	2503.0	1.672	8.5	76.9	630.1	31.2	11.7	61.0	6.15	85.92
	42d	4211.5	2501.1	1.685	8.5	76.0	626.3	31.2	11.2	62.7	5.73	89.72
p-value		0.56	0.29	0.49	0.87	0.91	0.62	0.42	0.80	0.74	0.54	0.87
SEM		39.5	24.1	0.012	0.44	3.03	9.75	0.25	0.78	4.83	0.32	3.34
0d		4173.1	2459.1^{b}	1.698	10.2^{b}	68.8^{b}	625.4	30.9	13.0	60.3	7.30^{b}	80.84^{b}
9d		4213.8	2523.0^{a}	1.670	9.6^{b}	71.2^{ab}	630.0	31.0	12.3	59.8	6.55^{ab}	83.59^{b}
21d		4201.8	2511.1^{a}	1.673	8.7^{a}	76.8^{a}	631.0	31.1	11.4	62.6	6.26^{a}	86.41^{ab}
42d		4225.0	2528.5^{a}	1.672	8.5^{a}	77.4^{a}	636.9	31.3	11.1	63.6	5.66^{a}	92.17^{a}
p-value		0.34	<0.01	0.06	<0.01	0.01	0.64	0.45	0.08	0.83	<0.01	0.02
SEM		33.5	18.6	0.008	0.31	2.14	7.39	0.18	0.55	3.43	0.28	2.40
L-Gln		4214.9	2515.6	1.676	9.4	73.7	634.9	31.1	12.0	61.5	6.39	86.53
L-Gln+L-Glu		4191.9	2495.2	1.681	9.1	73.4	626.8	31.1	11.9	61.6	6.49	84.97
p-value		0.27	0.18	0.59	0.24	0.91	0.21	0.89	0.81	0.96	0.29	0.30
SEM		29.9	15.1	0.006	0.22	1.51	5.87	0.13	0.39	2.45	0.14	2.15

[a,b] Means followed by different letters in the same column are significantly different by Tukey test at 5% probability level (p<0.05). SEM, pooled standard error of the means;

[1]Feed intake (FI, g), body weight gain (BWG, g), feed conversion ratio (FCR, kg/kg), coefficient of variation of body weight(CV, %), body weight uniformity (UNIF, %), breast weight (BW, g), breast yield (BY, %), coefficient of variation of breast weight (CV_B, %), breast uniformity ($UNIF_B$, %), coefficient of variation of breast yield (CV_{BY}, %), breast yield uniformity ($UNIF_{BY}$, %).

of orexigenic and anorexigenic neuropeptides, such as hypothalamic NPY, AgRP, POMC, MC4R, and CRF, in the brain, affecting mammalian (Zeni *et al*., 2000) and chicken (Khondowe *et al*., 2012) feed intake. Wang *et al*. (2012) also reported that administration of L-glutamate (1.6 µmol) decreased feed intake and increased hypothalamic CRF and MC4R mRNA expression levels. However, in those studies, the AA were injected directly in the central nervous system (ICV), and therefore, further studies to evaluate the influence of glutamine and glutamate on broiler feed intake are needed.

The BWG, CV, UNIF, CV_{BY}, and $UNIF_{BY}$ were influenced ($p<0.05$) by the evaluated treatments. The supplementation of L-Gln has been shown significant influence on broiler weight gain (Yi *et al*., 2005; Bartell & Batal, 2007; Ayazi *et al*., 2014; Fathi *et al*., 2014; Shakeri *et al*., 2014). This suggests that glutamine supplementation may be indicated to reduce flock variation, which aids broiler carcass processing. These effects are possibly explained by the importance of glutamine as the most abundant free amino acid in the bloodstream, accounting for approximately 25-35 % of the total free AA pool in the body and for over 60% of the total free AAs in the skeletal muscle (Zaravize *et al*., 2010). Glutamic acid also is recognized as substrate for the synthesis of non-essential amino acid, having crucial importance in modern low crude protein diets (Berres *et al*., 2010). In the first studies on glutamine, McKeehan (1982) gathered information showing the influence of glutaminolysis on perfect non-carcinogenic cell proliferation. Additionally, glutamine may improve intestinal mucosa development and gut health because it is used as energy substrate for high-speed replication tissues, such as the intestinal mucosa (enterocytes and colonocytes), presenting protective and restorative roles in humans (Sanz *et al*., 2004). Sifa *et al*. (2005) corroborated this influence on broiler gut. They reported evidences that glutamine dietary supplementation increases jejunum villus height and the thickness of the muscle layer of mucosa, and prevent the reduction of villus width and crypt depth. Other authors confirmed this concept, reporting that the jejunum was the first segment influenced by dietary Gln, followed by the ileum, while the duodenum was less affected (Yanfen *et al*., 2006). On the other hand, Murakami *et al*. (2006) observed greater development of the duodenum (segment with high enzyme activity), followed by the jejunum and the ileum. Additionally, the use of glutamine as substrate for gut cells may improve the production of some enzymes, such as maltase and sucrase, which improve nutrient hydrolysis and consequently absorption, and may enhance broiler growth rate (Sakamoto *et al*., 2011).

Birds fed diets with 0.4% L-Gln or L-Gln plus L-Glu until day 9 presented 3% higher BWG ($p<0.05$) compared with those fed the control diet. In addition, the dietary supplementation of L-Gln or L-Gln plus L-Glu from days 9-21 improved ($p<0.05$) CV, UNIF, CV_{BY}, and $UNIF_{BY}$ in 14, 10, 16, and 12%, respectively. This phase is critical for the development of the small intestine and for the stimulation of the production of mucosal enzymes, on which dietary L-Gln has considerable proven effects (Sifa *et al*., 2005, Yanfen *et al*., 2006; and Sakamoto *et al*., 2011). The nutrition of broilers during the first week after hatch has been extensively studied due to the strong correlation between body weight on day 7 and body weight at slaughter. Moreover, this phase represents about 20% of the life of a broiler, when the fastest growth rate of the gut occurs (Uni and Ferket, 2004).

These results suggest that the supplementing broiler diets with L-Gln or L-Gln plus L-Glu during the first 21 days of live is sufficient to obtain benefits during the entire production period. These effects may be explained by the importance of the gastrointestinal development of broilers during the starter rearing phase. Manvailer (2013) observed that the best L-Gln supplementation strategy was 1.0% of L-Glutamine until 14 days of age, 0.5% of L-Glutamine until 21 days, and 0.0% between 22 to 42 days of age, resulting in greater body weight, body weight gain, carcass weight, breast weight, and breast yield. Similarly, Zavarize *et al*. (2011) observed that the inclusion of 1.0% L-Gln in the diet of broilers from 1 to 21 days reared under non-stressful conditions improved broiler performance, and suggested that AA supplementation during the finisher phase was not necessary. However, the different criteria used for this evaluation among studies may yield different results. Sakamoto *et al*. (2011) observed a linear increase in maltase activity in 14-d-ol broilers and in sucrase activity in 42-d-old broilers fed increasing levels of L-Gln plus L-Glu (AminoGut®). Those authors concluded that dietary supplementation of these AA is important in broiler nutrition, and recommend the use 2.8% of L-Gln plus L-Glu until 42 days of age. Therefore, it seems that beneficial results are achieved with L-Gln or L-Gln plus L-Glu supplementation during the entire production cycle of broilers. However, the impact of this supplementation is more evident during the starter phase, probably due to the development of the gut in these animals (Maiorka *et al*., 2002; Sakamoto *et al*., 2006; Murakami *et al*., 2007).

One of the main consequences of the lack of body uniformity in commercial broiler production systems is the incidence of clinical and subclinical diseases (Russel, 2003). Therefore, considering that L-Gln and L-Gln may promote intestinal mucosa health, some diseases may be prevente. Yi *et al*. (2005) reported that diets containing 1% L-Gln fed during the first 48 hours of the broilers' life improved growth, stimulated IFN-γ (ng/mL) production, and decreased the mortality of animals contaminated with *E. maxima* at 7 and 14 days of age. Also, lymphoid organs can be improved by L-Gln dietary supplementation. Huang *et al*. (2007) observed higher BWG and growth enhancement of the immune organs (thoracic gland, spleen and bursa of Fabricius) of broilers fed glutamine. In addition of enhancing the immune response, dietary L-Gln supplementation may aid the body to fight against microbes that invade the body by enhancing the protection against secondary adverse effects on the immune system, such as peroxide production. Jing Ge *et al*. (2009) showed a significant increase in superoxide dismutase and GSH-Px levels, improving the antioxidant capacity of broilers fed diets with 0.5% L-Gln. Testing high stocking densities under tropical conditions, Shakeri *et al*. (2014) verified reduced mortality rates in broilers fed diets with L-Gln plus L-Glu.

Among the possible explanations for these results are the better intestinal integrity, enhanced immune response, and better antioxidant protection provided by glutamine and glutamate, ultimately leading to overall better health status of the birds. Therefore, the results of the present study corroborate several previous research studies of the role of those AA in broiler nutrition and suggests that glutamine or glutamine combined with acid glutamic can be supplemented in pre-starter and starter diet to improve broiler performance and reduce body weight variation in broiler flocks.

CONCLUSION

The dietary supplementation of pre-starter and starter diets with 0.4% L-Gln (L-Gln 99%) or L-Gln plus L-Glu (minimum 95%) is recommended to improve the performance, breast weight, and uniformity of broilers.

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