PERFORMANCE AND MUSCULAR CREATINE OF BROILER CHICKENS FED LOW PROTEIN DIETS CONTAINING VARYING GLYCINE EQUIVALENT AND THREONINE LEVELS

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ABSTRACT
This study was conceived and aimed to investigate the interactive effects of a glycine equivalent (Gly_{equi}) and standardized ileal digestible threonine (SID Thr) levels in low crude protein diets on performance and muscular creatine content in broiler chickens from 21 to 42 days. A total of 1500, 21-day-old Cobb-Vantress® male broiler chickens were distributed in a completely randomized 5 × 3 factorial arrangement of Gly_{equi} × SID Thr with five replicates of 20 birds each. Fifteen dietary treatments of 16.5% CP were formulated to contain five levels of total Gly_{equi} (1.16, 1.26, 1.36, 1.46 and 1.56%) and three levels of SID Thr (0.58; 0.68 and 0.78%). Interactive effects ($p<0.05$) of Gly_{equi} and SID Thr levels were observed for weight gain, carcass yield, and muscular creatine content. Higher levels of Gly_{equi} increased ($p = 0.040$) weight gain in 0.58 and 0.68% SID Thr diets compare to the 0.78% SID Thr diet. Levels of Gly_{equi} equal to or above 1.26% in diets with 0.78% SID Thr resulted in birds with higher ($p=0.033$) muscular creatine content. There was a quadratic effect of Gly_{equi} levels for muscular creatine content ($p = 0.008$) with an estimated optimal levels of 1.47% Gly_{equi}. Therefore, the minimum dietary level of Gly_{equi} needed to improve performance in low crude protein diets is 1.26% with adequate SID Thr levels for broiler chickens.
Keywords: Broiler; Glycine equivalent; Threonine; Muscle creatine; Performance

INTRODUCTION

Reducing the crude protein (CP) content in diets of broiler chickens has created a mechanism capable of decreasing feed costs and nitrogen emissions from the production of meat products (Hilliar *et al*., 2020; Liu *et al*., 2021). However, low-protein diets tend to impair performance by decreasing weight gain, worsening feed conversion and increasing carcass fat content (Hejdysz *et al*., 2022). Meeting the nutritional requirements of essential amino acids (AAs) makes it possible to carefully reduce the crude protein in the diet without harming the performance of the birds (Dean *et al*., 2006). The concern with meeting only the needs of essential AAs increase the chance of a non-essential AAs becoming limiting in the diet, due to the amount of precursors for a given AA being insufficient or due to the delay of metabolic processes (Saleh *et al*., 2021). Addition of glycine (Gly), a non-essential AA, has proven to enhance the productive performance of broilers fed low protein diets during the starter and finisher phases (Dean *et al*., 2006; Siegert *et al*., 2016; Hofmann *et al*., 2019). Even with the ability of birds to synthesize Gly endogenously, this synthesis may not be metabolically efficient to meet the AA requirements of broilers in the starter phase (Ospina-Rojas *et al*., 2013a). Since Gly can be reversibly metabolized to serine (Ser) and, on a molar basis, Ser plays the same functions as Gly in chicken, it becomes necessary that the requirements of these AAs be considered jointly as Gly equivalents (Gly_{equi} (g/kg) = Gly + 0.7143 × Ser) in diet formulations (Dean *et al*., 2006). Moreover, threonine (Thr) being considered as the third limiting AA corn-soybean meal-based diets of broilers, serves as a possible metabolic precursor of Gly (Baker *et al*., 1972), which is potentially necessary in fulfilling the Gly requirement for poultry (Dean *et al*., 2006; Ospina-Rojas *et al*., 2013b). Several studies have shown that diets containing marginal levels of Gly_{equi}, a situation often seen in low-CP plant based diets, can be effective for broilers by ensuring they have adequate amounts of Thr (Corzo *et al*., 2009; Ospina-Rojas *et al*., 2013b; Aguihe *et al*., 2022). Thus, the present study was conducted to evaluate the Gly_{equi} requirements of broilers from 21 to 42 days of age, offered reduced CP diets

with varying standardized ileal digestible threonine (SID Thr) levels on performance and pectoral muscular creatine.

MATERIALS AND METHODS

Animals, facilities and management: All procedures adopted were approved by the Ethics Committee on the Use of Animals (CEUA) of Maringá State University (CEUA/PROTOCOL-7501230419/2019). The experiment was conducted in the poultry section of the Experimental Farm of Iguatemi, State University of Maringá-Brazil, using 1500, 21-day-old male Cobb-Vantress® broilers. The birds were housed in a conventional air-conditioned shed, with negative pressure ventilation and an evaporative plate, in 1.0 x 2.0-meter boxes, using new rice husk bedding. Water and feed were provided *ad libitum* throughout the experimental period through nipple drinkers and tube feeders, respectively.

Experimental design and diets: The broilers (average initial body weights of 1083 ± 5.1; p = 0.82) were distributed in a completely randomized design of 5 × 3 factorial arrangement (Gly_{equi} × SID Thr), with five replications of 20 birds per experimental pen. The birds received a conventional diet in the initial phase (1 to 21 days of age) and the experimental diets in the finishing phase, from 21 to 42 days of age. The treatments consisted of five levels of Gly_{equi} (1.16, 1.26, 1.36, 1.46 and 1.56%) and three levels of SID Thr (0.58, 0.68 and 0. 78%, corresponding to 85, 100 and 115% of the recommended SID Thr by Rostagno *et al.* (2011), respectively). A basal diet with 16.5% CP, 3.125kcal/kg ME, 1.04% SID Lys and 0.76% SID Met+Cys was formulated to meet the nutritional recommendations for male broilers of average performance as suggested by Rostagno *et al* (2011), except for the concentrations of Gly_{equi} and SID Thr (Table 1). The other experimental diets containing different concentrations of dietary Gly_{equi} and SID Thr were obtained by supplementing L-Gly and L-Thr in the basal diet mixture at the expense of the inert (Kaolin).

Data collection: Birds and experimental leftover feed were weighed at 21, 28, 35 and 42 days of age for performance evaluation (weight gain, feed intake and feed conversion ratio). At 42nd day of age, one bird was selected from each experimental pen and slaughtered. Breast muscles were removed and weighed on a precision scale to obtain the relative weight (%) in relation to live weight of the bird. Thereafter, samples of the pectoral muscle of the selected birds were collected, weighed and ground in a mixer type mill (Maxmac ZJB750, Maxmac Ltda, São Paulo, Brazil). The creatine concentration in the pectoral muscle was determined following the methodology of Chamruspollert *et al.* (2002).

Statistical analysis: The data obtained were subjected to 2-factor analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the SAS®. When the analysis of variance indicated significant treatment effects, means were separated using Tukey's Multiple Range Tests by LSMEANS procedure of SAS programme. Statistical differences were considered to be significant at $P < 0.05$. A polynomial regression model was applied and where quadratic responses ($P < 0.05$) were detected, the optimal Gly_{equi} level was calculated by taking the first derivative of the quadratic equation.

RESULTS AND DISCUSSION

At 42 days of age, the interaction effect of Gly_{equi} and SID Thr levels was significant for weight gain (p = 0.040) and pectoral muscles creatine (p = 0.033) but feed intake and feed:gain were not affected (Table 1). Results showed that higher (p = 0.040) weight gain was recorded for diets containing 0.58% and 0.68% SID Thr with increasing levels of Gly_{equi}. Thus, birds fed with the adequate (0.68%) SID Thr diets with more than 1.26% Gly_{equi} and deficient (0.58%) SID Thr diets containing Gly_{equi} levels equal to or greater than 1.36% showed higher (p = 0.040) weight gain. Among diets with 0.78% SID Thr, birds on 1.16% Gly_{equi} had increased (p = 0.040) weight gain compared to those on 1.26 to 1.56% Gly_{equi}. The results obtained for weight gain revealed that in low-protein diets, Thr supplementation at levels lower than and/or equal to require SID Thr could increase the Gly_{equi} requirements. However, supplementing Thr to provide excess dietary SID Thr levels reduces the requirement of Gly_{equi}, thus confirming the result of previous studies (Ospina-Rojas *et al*., 2013a,b; Aguihe *et al*., 2022). This shows that the decline in weight gain of birds fed with low-protein diets could be prevented when excess SID Thr is provided with marginal Gly_{equi} levels. However, some studies have shown that, excess Thr supplementation reduces the need for Gly_{equi}, since the enzymes Thr aldolase and Thr dehydrogenase are able to degrade excess Thr into Gly (Corzo *et al*., 2009; Ospina-Rojas *et al*.,

2013a). Therefore, increased Thr supplementation is unnecessary in diets containing adequate or high Gly_{equi} concentrations; because excess Thr compromises the productive performance of birds, increasing the elimination of uric acid, as this process requires high consumption of metabolic energy (Ospina-Rojas *et al*., 2013b). The results of the present study revealed that broilers fed with 0.58 and 0.68% SID Thr diets showed higher (p = 0.033) pectoral muscular creatine values at Gly_{equi} concentrations of 1.36 and 1.46% respectively. However, pectoral muscular creatine levels were lower (p = 0.033) in birds fed with 0.58% Thr diets at 1.16 and 1.56% Gly_{equi} levels and in the 0.68% Thr dietary group at 1.26% Gly_{equi}. In diets with 0.78% SID Thr, pectoral muscular creatine content was lower (p = 0.033) in diets containing 1.26% Gly_{equi} compared to other Gly_{equi} levels. An increase in the requirement of Gly_{equi} resulted in increased pectoral muscular creatine in birds fed with varying dietary levels of SID Thr with reduced CP concentration during grower stage. The present study, suggests that Thr supplementation to provide dietary SID Thr at levels lower or greater than the required (0.68%) showed an increment in the Gly_{equi} requirement for muscle creatine, thus confirming the report of Ospina-Rojas *et al*. (2013a) and Aguihe *et al* (2022). Therefore, increasing Gly_{equi} up to 1.56% in the diet through Gly supplementation may be favorable as a source of creatine, thus; promoting improved utilization of nutrients and energy for proper muscle growth in this tissue (Lemme *et al*., 2007).

Table 1. Performance of broilers fed diets with reduced protein and levels of glycine equivalent (Gly_{equi}) and standardized ileal digestible threonine (SID Thr) from days 21 to 42

Gly_{equi} (%)	SID Thr (%)	Weight gain (g)	Feed intake (g)	Feed:gain (g/g)	Pectoral muscular creatine (mg/g)
1.16	0.58	1905[b]	3326	1.75	3.84[b]
1.26	0.58	1927[b]	3333	1.73	4.15[ab]
1.36	0.58	1972[a]	3371	1.71	4.72[a]
1.46	0.58	1987[a]	3377	1.70	4.22[ab]
1.56	0.58	1977[a]	3424	1.73	4.19[b]
1.16	0.68	1903[b]	3212	1.69	4.31[ab]
1.26	0.68	2022[a]	3471	1.72	3.94[a]
1.36	0.68	2020[a]	3402	1.69	4.86[a]
1.46	0.68	2018[a]	3367	1.67	5.31[a]
1.56	0.68	1988[ab]	3408	1.72	4.85[a]
1.16	0.78	1987[a]	3352	1.69	3.33[b]
1.26	0.78	1930[b]	3362	1.75	4.52[a]
1.36	0.78	1928[b]	3342	1.73	5.00[a]
1.46	0.78	1921[b]	3381	1.76	5.24[a]
1.56	0.78	1924[b]	3318	1.73	5.26[a]
SEM		32.84	62.64	0.02	0.32
ANOVA			p-values		
Gly_{equi}		0.19	0.17	0.76	Q[1] (0.008)
SID Thr		0.04	0.86	0.03	0.048
Gly_{equi} × SID Thr		0.04	0.43	0.34	0.033

[a-b]Means in columns followed by different superscript are statistically different (p < 0.05).
Q: Quadratic effect
[1] $Y = -20.714 + 34.851x - 11.857x^2$ ($R^2 = 0.95$); Maximum point = 1.47% Gly_{equi}

CONCLUSION

The Gly_{equi} needed for improved performance in low protein diets with adequate levels of SID Thr (0.68%) for broilers (21 to 42 days old) is 1.26% to 1.36% with marginal levels of SID Thr. For deficient (0.58%) and excessive (0.78%) SID Thr diets, increasing Gly_{equi} levels is necessary in order to enhance pectoral muscular creatine.

REFERENCES

Aguihe, P.C., Hirata, K.A., Ospina-Rojas, C.I., dos Santos, T.C., Pozza, P.C., Iyayi, E.A. and Murakami, A.E. (2022). Effect of glycine equivalent levels in low protein diet containing

different SID threonine concentrations on performance, serum metabolites and pectoral muscular creatineof broiler chickens. *Ital. J. Anim. Sci*., 21:1000-09.

Baker, D.H., Hill, T.M., Kleiss, J.M. (1972). Nutritional evidence concerning formation of glycine from threonine in the chick. *J. Anim. Sci*., 34:82-86.

Chamruspollert, M., Pesti, G.M., Bakalli, R.I. (2002). Dietary interrelationships among arginine, methionine, and lysine in young broiler chicks. *Brit. J. Nutr*., 88:655-60.

Corzo, A., Kidd, M.T., Dozier, W.A., Kerr, B.J. (2009). Dietary glycine and threonine interactive effects in broilers. *J. Appl. Poult. Res*., 18:79 – 84.

Dean, D., Bidner, T.D., Southern, L.L. (2006). Glycine supplementation to low protein, amino acid-supplemented diets supports optimal performance of broiler chicks. *Poult Sci*., 85:288-96.

Hejdysz, M., Bogucka, J., Ziółkowska, E., Perz K., Jarosz L., Ciszewsi, A., Nowaczewski, S., Slosarz P., Kaczmarek, S.A. (2022). Effects of low crude protein content and glycine supplementation on broiler chicken performance, carcass traits, and litter quality. *Livestock Science*, 261:104930.

Hilliar, M., Hargreave, G., Girish, C.K., Barekatain, R., Wu, S.B., Swick, R.A. (2020).Using crystalline amino acids to supplement broiler chicken requirements in reduced protein diets. *Poult. Sci*., 99:1551-63.

Hofmann P., Siegert W., Kenéz Á., Naranjo V.D., Rodehutscord M. (2019). Very Low crude protein and varying glycine concentrations in the diet affect growth performance, characteristics of nitrogen excretion, and the blood metabolome of broiler chickens. *Journal of Nutrition*, 149: 1122–1132.

Lemme, A., Ringel, J., Rostagno, H.S., Redshaw, M.S. (2007). Supplemental guanidino acetic acid improved feed conversion, weight gain, and breast meat yield in male and female broilers. Presented at 16th European Symposium on Poultry Nutrition, France, 335-338.

Liu, S.Y., Macelline, S.P., Chrystal, P.V., Selle, P.H. (2021). Progress towards reduced-crude protein diets for broiler chickens and sustainable chicken meat production. *J. Anim. Sci. and Biotech*., 12:20-33.

Ospina-Rojas, I.C., Murakami, A.E., Moreira, I., Picoli, K.P., Rodrigueiro, R.J.B., Furlan, A.C. (2013a). Dietary glycine+serine responses of male broilers given low-protein diets with different concentrations of threonine. *British* Poult. Sci*.,* 54:486-93.

Ospina-Rojas, I.C., Murakami, A.E., Oliveira, C.A., Guerra, A.F.Q.G. (2013b). Supplemental glycine and threonine effects on performance, intestinal mucosa development, and nutrient utilization of growing broiler chickens. *Poult. Sci.,* 92:2724-31.

Rostagno, H.S., Albino, L.F.T., Donzele, J.L., Gomes, P.C., de Oliveira, R.F., Lopes, D.C., Ferreira, A.S., Barreto, S.L.T., Euclides, R.F. (2011). Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. Universidade Federal de Viçosa-UFV, Viçosa; p.186.

Saleh, A.A., Amber, K.A., Soliman, M.M., Morsy, W.A., Shukry, M., Alzawqari, M.H. (2021). Effect of low protein diets with amino acids supplementation on growth performance, carcass traits, blood parameters and muscle amino acids profile in broiler chickens under high` ambient temperature. *Agric.,* 11:185-197.

Siegert, W., Wild, K.J., Schollenberger, M., Helmbrecht, A., Rodehutscord, M. (2016). Effect of glycine supplementation in low protein diets with amino acids from soy protein isolate or free amino acids on broiler growth and nitrogen utilization. *Brit. Poult. Sci.,* 57:424-34.