ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 4 (5): 250-257, 2005
ISSN 1682-8356

Effects of Glycine and Threonine Supplementation on Performance of Broiler Chicks Fed Diets Low in Crude Protein

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Abstract: A study was conducted to evaluate effects of dietary crude protein (CP) level and Gly and Thr supplementation on performance of male broiler chicks during the period of 0 to 21 days. Diets were formulated based on NRC (1994) recommendations (Lysine at 110% of suggested level). All diets were formulated to contain 3,200 ME kcal/kg. A minimum dietary electrolyte balance of 250 meq/kg was stipulated with a minimum of 0.25% sodium and 0.20% chloride. Five primary diets were formulated to contain 16%, 18%, 20%, 22%, and 24% CP. The diets were then supplemented with additional 0, 0.2%, 0.4% Gly or 0, 0.2%, 0.4% Thr in all possible combinations resulting in a 5x3x3 factorial arrangement. Each of the 45 treatments was fed to six replicate pens of five male commercial broilers. The test diets and tap water were provided for the *ad libitum* consumption from 1 to 21 d of age. At 21 d of age body weight and feed consumption were determined. Reducing dietary protein below 22% significantly reduced 21 d BW and depressed feed conversion. Addition of 0.2 or 0.4% Gly significantly improved BW and numerically improved feed conversion at lower levels of protein but at protein levels of 20% or more the basal diets appeared to provide sufficient Gly. These data suggest that requirements for Gly suggested by NRC (1994) are inadequate in diets with low CP. Thr supplementation was ineffective in improving performance at low CP levels suggesting that the present recommendations of NRC (1994) are adequate. Mortality did not vary significantly among chicks receiving all experimental diets.

Key words: Broilers, amino acids, glycine, threonine, crude protein

Introduction

Supplementation of poultry diets with synthetic amino acids may improve the overall amino acid balance and enable a reduction in the crude protein level. It has been consistently demonstrated that addition of Lys and Met to broiler diets successfully reduces the crude protein level in diets to a point without adversely affecting the broiler performance (Lipstein and Bornstein, 1975; Lipstein *et al*., 1975; Waldroup *et al*., 1976; Uzu, 1982; Uzu, 1983). When formulating such low protein diets, the balance among the essential amino acids (EAA) are assumed to be of great importance since the reduction of dietary protein content can only be effective when the EAA requirements of chicks are met. The minimum level to which it is theoretically possible to decrease the dietary protein content is related in part to the balance required by chicks. However, performance declines as the crude protein level is further reduced even if all known amino acid requirements are satisfied (Edmonds *et al*., 1985; Fancher and Jensen, 1989a,b,c; Bregendahl *et al*., 2002; Si *et al*., 2004a; Jiang *et al*., 2005).

Threonine was discovered over 60 years ago and is considered to be the third limiting amino acid for broilers fed corn-soybean meal diets (Fernandez *et al*., 1994). Davis and Austic (1982) demonstrated that adding individual amino acids other than Thr or groups of amino acids to a diet that was marginally adequate in Thr resulted in growth depression and this effect could be prevented by supplementation of additional Thr to the diet. Kidd *et al*. (2000) pointed out that crude protein levels of broiler diets fed by commercial companies did not result in a Thr deficiency. However, marginal reductions in the dietary CP might render the diets deficient in Thr. Rangel-Lugo *et al*. (1994) stated that raising the amino acid content of the diet increased the Thr requirement expressed as the Thr concentration in the diet. However, when Fancher and Jensen (1989c) added Thr to a 16% CP diet at 10% over NRC (1994) recommendations there was no significant effect on body weight gain.

Glycine was first shown to be needed in the diet of growing chickens by Almquist *et al*. (1940) and Almquist (1942). Gly affects protein synthesis not only as a building block for protein itself, but is required for the formation of DNA, RNA, creatine, and uric acid (Ngo *et al*., 1977). Gly is considered as a semi-essential amino acid in young broiler chicks (Graber and Baker, 1973) since chicks can synthesize Gly, but there is still a considerable requirement for Gly to be supplied via the diet. With uric acid being the primary end product of nitrogen excretion in broilers, this increases the metabolic requirement for Gly since each molecule of uric acid formed represents a loss of one molecule of Gly for chicks (Sonne *et al*., 1946). Almquist and Grau

(1944) stated that Gly was an indispensable amino acid for maximal chick growth. Douglas *et al*. (1958) reported that Gly was a limiting amino acid in their corn-soybean meal diet as additional Gly resulted in an improved growth performance of chicks. Waterhouse and Scott (1961) found that the effect of Gly was more pronounced at the lower levels of protein and decreased as the protein increased until it had almost no effect when the CP level reached 36%. Featherston (1975) and Ngo and Coon (1976) also demonstrated that the Gly supplementation had a positive effect on the performance of chicks. However, Cave (1978) reported that the high-level inclusion of Gly depressed the growth performance of chicks. Baker and Sugahara (1970) indicated that Thr might reduce the dietary need for Gly. Previous studies from our laboratory have indicated that reducing the dietary protein below 20% results in a significant reduction in the live performance of chicks up to 21 d, even though indispensable amino acids were greater than the 110% of NRC (1994) recommendations (Si 2004 a,b,c). In a study by Jiang *et al.* (2005) the addition of 0.2% Gly but not 0.2% Pro, 0.2% Arg, or 0.4% Glu gave significant improvement in low CP diets but did not support performance equal to that of diets with 22% CP. The purpose of the present study was to investigate the influence of diets with various CP levels supplemented with different levels of Gly and Thr on the performance of male broilers from 0 to 21 d of age.

Materials and Methods

Diet formulation: Diets were formulated to provide a minimum of 100% of NRC (1994) amino acid recommendations for the 0 to 21 d old chick with Lys at a minimum of 110%. Corn and soybean meal served as the primary ingredients. The corn and soybean meal were analyzed for crude protein prior to diet formulation using Association of Official Agricultural Chemists (AOAC, 1970) procedures. Five primary diets were formulated to contain 16%, 18%, 20%, 22%, and 24% CP. The crude protein and ME equivalency values of the amino acid supplements (NRC, 1994) were considered in the formulation. Requirements for the amino acids were met when necessary by adding crystalline amino acids to isocaloric diets. Needs for Met were provided by supplementation with DL Met; additional needs for total sulfur amino acids (TSAA) were provided by L-cystine to avoid possible imbalance of Met and Cys in diets low in CP. Diets were formulated to be isocaloric with 3,200 ME kcal/kg. A minimum dietary electrolyte balance (DEB) of 250 Meq/kg was stipulated with a minimum of 0.25% sodium and 0.2% chloride. Sodium chloride, sodium bicarbonate, and potassium bicarbonate were used to adjust the DEB. Diets were supplemented with a complete vitamin mix obtained from a commercial integrator. Choline chloride was used to provide 1040 mg/kg supplemental choline. A complete trace mineral mix with highly available sources of minerals was used. The composition of diets is shown in Table 1.

The experimental treatments consisted of a complete factorial arrangement with five levels of crude protein (16%, 18%, 20%, 22%, and 24%), three additional levels of Gly (0, 0.2%, and 0.4%) and three additional levels of Thr (0, 0.2%, and 0.4%) in all possible combinations for a total of 45 dietary treatments. Supplementation of the amino acids was made at the expense of corn starch using aliquots of a common mix of each of the five crude protein levels. Each of these 45 treatments was fed to six replicate pens of five male chicks. Diets were provided in mash form.

Chicks and housing: Male chicks of a commercial broiler strain[2] were obtained from a local hatchery where they had been vaccinated in ovo for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. They were randomly assigned to compartments in electrically heated brooders with raised wire floors. Five chicks were placed in each of 270 compartments. The test diets and tap water were provided for *ad libitum* consumption from 1 to 21 d of age. Continuous 24 hr fluorescent lighting was provided. Care and management of the chicks followed recommended guidelines (FASS, 1999).

Measurements: Birds were group weighed by pen at 21 d. Feed consumption during the period was determined by weighing the feed container at the start and the end of the study. Mortality was checked twice daily; birds that died were weighed with the weight used to adjust the feed conversion [FCR = total feed consumed ÷ (weight of live birds + weight of dead birds)]. Mixed diets were analyzed for the crude protein, total amino acids and supplemental amino acids.

Data analysis: Pen means served as the experimental unit. Data were subjected to the analysis of variance (SAS Institute, 1991) as a complete factorial arrangement using the General Linear Models procedure. Mortality data were transformed $\sqrt{n+1}$ to prior to analysis; data are presented as natural numbers. Main effects of crude protein levels, levels of added Gly, and levels of added Thr were considered along with all possible two way and three way interactions. The means for treatments showing significant difference in the analysis of variance were separated using repeated t-test using probabilities generated by the LS means option of SAS. All statements of significance are based on the 5% level of probability.

Results and Discussion

Analyzed CP values for diets with 22%, 20%, 18% and

Table 1: Composition (g/kg) of starter diets with reduced crude protein levels

Ingredient	CP (%)				
	24	22	20	18	16
Yellow corn	453.20	515.58	578.51	641.39	720.87
Dehulled soybean meal	418.04	364.99	309.44	250.44	171.54
Poultry oil	77.23	66.94	56.56	46.31	31.58
Dicalcium phosphate	17.35	17.71	18.10	18.53	19.12
Ground limestone	12.11	12.16	12.22	12.27	12.33
Sodium chloride	3.56	3.49	1.98	1.26	0.30
Sodium bicarbonate	2.00	2.00	4.09	5.04	6.32
Potassium carbonate	0.00	0.00	0.13	1.95	4.42
DL-Methionine	1.15	1.45	1.76	2.10	2.57
L-Cystine	0.36	0.68	1.01	1.37	1.85
L-Lysine Hcl	0.00	0.00	1.20	3.41	6.37
L-Threonine	0.00	0.00	0.00	0.93	2.26
L-Arginine	0.00	0.00	0.00	0.00	2.41
L-Tryptophan	0.00	0.00	0.00	0.00	0.36
L-Isoleucine	0.00	0.00	0.00	0.00	1.02
L-Phenylalanine	0.00	0.00	0.00	0.00	0.19
L-Valine	0.00	0.00	0.00	0.00	1.49
Vitamin premix[1]	2.00	2.00	2.00	2.00	2.00
Trace mineral premix[2]	1.00	1.00	1.00	1.00	1.00
Choline chloride (60%)[3]	2.00	2.00	2.00	2.00	2.00
Variable[4]	10.00	10.00	10.00	10.00	10.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00

[1]Provides per kg of diet: vitamin A 7714 IU; cholecalciferol 2204 IU; vitamin E 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione 1.5 mg; folic acid 0.9 mg; thiamin 1.54 mg; pyridoxine 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg; Se 0.1 mg. [2]Provides per kg of diet: Mn (from $MnSO_4 \cdot H_2O$) 100 mg; Zn (from $ZnSO_4 \cdot 7H_2O$) 100 mg; Fe (from $FeSO_4 \cdot 7H_2O$) 50 mg; Cu (from $CuSO_4 \cdot 5H_2O$) 10mg; I (from $Ca(IO_3)_2 \cdot H_2O$) 1 mg. [3]Provides 1040 mg/kg supplemental choline. [4]Variable amounts of Gly, Thr, and corn starch.

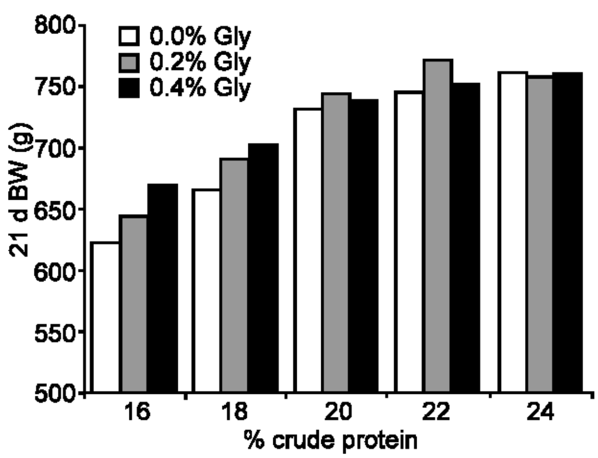

Fig. 1: Influence of dietary crude protein level and supplemental Gly on 21 d body weight of male broilers fed diets calculated to provide recommended levels of essential amino acids (NRC, 1994)

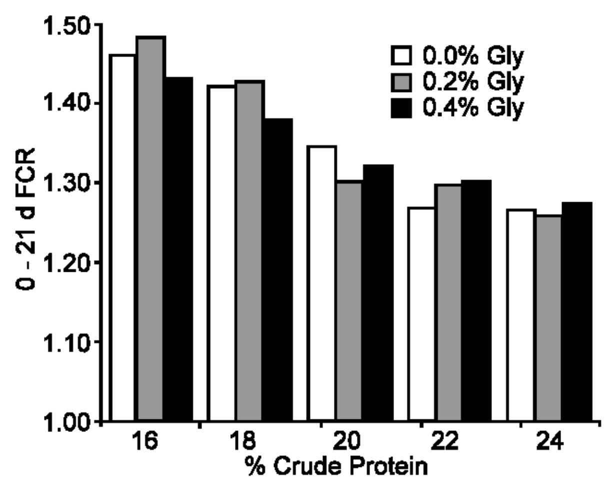

Fig. 2: Influence of dietary crude protein level and supplemental Gly on 0-21 d feed conversion ratio of male broilers fed diets calculated to provide recommended levels of essential amino acids (NRC, 1994)

16% CP were in close agreement with calculated values (Table 2). Analyzed levels of Ile, Val, and Phe plus Tyr were below the calculated values. The level of Thr, Gly and all other amino acids in all diets were in good agreement with calculated values.

The 21 d BW of the birds was significantly influenced by

Table 2: Nutrient analysis of diets with reduced crude protein levels

Nutrient1	Dietary CP (%)				
	24	22	20	18	16
Crude protein (C)[2]	24.00	22.00	20.00	18.00	16.00
Crude protein (A)[3]	24.28	22.69	20.72	18.57	16.40
Met (C)	0.50	0.50	0.50	0.50	0.50
Met (A)	0.51	0.50	0.51	0.51	0.50
Cys (C)	0.40	0.40	0.40	0.40	0.40
Cys (A)	0.46	0.46	0.46	0.45	0.45
TSAA (C)	0.90	0.90	0.90	0.90	0.90
TSAA (A)	0.97	0.96	0.97	0.96	0.95
Lys (C)	1.42	1.27	1.20	1.20	1.20
Lys (A)	1.32	1.20	1.19	1.18	1.19
Trp (C)	0.29	0.26	0.24	0.21	0.20
Trp (A)	0.27	0.24	0.21	0.18	0.17
Thr (C)	0.98	0.89	0.81	0.80	0.80
Thr (A)	0.97	0.86	0.78	0.77	0.76
Ile (C)	1.24	1.13	1.00	0.88	0.80
Ile (A)	1.08	0.93	0.84	0.71	0.68
His (C)	0.62	0.57	0.51	0.44	0.35
His (A)	0.69	0.60	0.55	0.49	0.41
Val (C)	1.31	1.19	1.06	0.93	0.90
Val (A)	1.20	1.02	0.95	0.83	0.80
Leu (C)	2.02	1.88	1.73	1.57	1.34
Leu (A)	2.07	1.81	1.70	1.53	1.34
Arg (C)	1.81	1.64	1.46	1.27	1.25
Arg (A)	1.86	1.49	1.41	1.15	1.17
Gly (C)	1.10	1.00	0.89	0.78	0.62
Gly (A)	1.05	0.92	0.83	0.73	0.62
Ser (C)	1.19	1.08	0.97	0.84	0.68
Ser (A)	1.28	1.10	1.02	0.90	0.76
Gly+Ser (C)	2.28	2.08	1.86	1.62	1.30
Gly+Ser (A)	2.33	2.02	1.85	1.63	1.38
Phe (C)	1.31	1.20	1.08	0.94	0.79
Phe (A)	1.24	1.07	1.00	0.89	0.73
Tyr (C)	0.96	0.87	0.78	0.69	0.56
Tyr (A)	0.83	0.70	0.67	0.55	0.47
Phe+Tyr (C)	2.27	2.07	1.86	1.63	1.34
Phe+Tyr (A)	2.07	1.77	1.67	1.44	1.20
Na+K-Cl, meq/kg	271.04	251.36	250.10	250.00	250.00

[1]All values expressed as % of diet unless otherwise stated. All diets calculated to contain 3200 ME kcal/kg.
[2]Calculated values. [3]Analyzed values.

dietary protein level and supplemental Gly, with a significant interaction between protein level and supplemental Gly (Table 3). Birds fed diets with less than 22% CP had significantly reduced BW, even though the diets met the minimum suggested amino acid requirements (NRC, 1994). Addition of 0.2 or 0.4% Gly significantly improved overall performance, but the improvement was greatest at the lower levels of CP, resulting in the significant interaction (Fig. 1). When Gly was added to diets with 16 or 18% CP, the 21 d BW was significantly improved, although not completely restoring performance equal to that of diets with 22 or 24 % CP. Addition of 0.2 or 0.4% Gly to diets with 20, 22, or 24% CP had little or no benefit. This is in agreement with a previous report from our laboratory (Jiang *et al.*, 2005). There was no significant improvement in BW related to the addition of Thr to the diet, nor were there any interactions between or among Thr, Gly, and dietary CP. Feed conversion was also significantly influenced by dietary CP level (Table 4). Feed conversion worsened as CP level reduced. Chicks fed diets with 20% CP had inferior conversion as compared to those fed 24% CP, with chicks fed 22% CP intermediate between these two groups. Feeding less than 20% CP resulted in

Table 3: Effect of additional Thr and Gly to amino acid supplemented low protein diets on 21 d body weight (g) of male broilers

Crude Protein %	Added Thr (%)	Added Gly (%)			Mean
		0.00	0.20	0.40	
16	0.00	633	626	702	654
	0.20	618	647	650	638
	0.40	613	657	651	640
	Mean	621[h]	643[gh]	668[f]	644[m]
18	0.00	667	690	695	684
	0.20	668	696	708	691
	0.40	663	684	700	683
	Mean	666[fg]	690[ef]	701[e]	686l
20	0.00	730	732	720	728
	0.20	726	744	758	743
	0.40	733	749	732	738
	Mean	730[d]	742[bcd]	737[cd]	736[k]
22	0.00	714	762	740	739
	0.20	763	768	742	757
	0.40	755	785	763	768
	Mean	744[bcd]	772[a]	748[abcd]	755[j]
24	0.00	751	775	763	763
	0.20	769	744	749	754
	0.40	764	750	765	760
	Mean	761[ab]	756[abc]	759[abc]	759[j]
Mean	0.00	699	717	724	713
	0.20	709	720	721	716
	0.40	706	725	722	718
	Mean	704[y]	721[x]	723[x]	

xyzMeans with no common superscripts differ significantly (P<0.05). jkllmMeans with no common superscripts differ significantly (P<0.05). abcdefghMeans with no common superscripts differ significantly (P<0.05)

Source of variance	P diff
Crude protein (CP)	0.01
Glycine (Gly)	0.01
CP x Gly	0.04
Threonine (Thr)	0.71
CP x Thr	0.34
Gly x Thr	0.87
CP x Gly x Thr	0.40
Coefficient of Variation	5.13

conversion that was significantly poorer than that of birds fed 20% or more CP. Addition of Gly had no significant effect on feed conversion, and no significant interaction of dietary CP and Gly was observed. However, there was a trend to improved feed conversion when 0.4% additional Gly was added to diets with 16, 18, and 20% CP (Fig. 2). Addition of Thr had no significant effect on feed conversion with no interaction among or between Thr, Gly, and dietary CP. Mortality did not vary significantly among chicks receiving all experimental diets (data not shown).

Although all the essential amino acids levels in diets used in this study are above NRC (1994) recommended levels most of them tend to decline towards their minimum requirements as dietary CP levels decreases. It is possible that some essential amino acids whose requirements are based on limited studies, such as Gly and Ser, may become limiting. In comparison to the 1.25% level of Gly + Ser suggested by NRC (1994), Heger and Pack (1996) reported that Gly and Ser needs ranged from 1.5 to 1.6% at 17% CP up to 1.7to 1.8% at 23% CP. Schutte et al. (1997) recommended 1.9% of total Gly and Ser when birds were fed low CP diets fortified with amino acids. No response to Gly was observed in the present study in diets up to 18% CP at which point the diet contained 1.62% Gly+Ser. No response was noted when Gly was added to diets containing 20% CP at which point the diets contained 1.85% Gly+Ser. Therefore, our results are in general agreement with the work of Heger and Pack (1996) and Schutte *et al.* (1997)

Table 4: Effect of additional Thr and Gly to amino acid supplemented low protein diets on 0 to 21 d feed conversion (g feed per g gain) of male broilers

Crude Protein %	Added Thr %	Added Gly (%) 0.00	0.20	0.40	Mean
16	0.00	1.407	1.531	1.377	1.438
	0.20	1.454	1.432	1.461	1.449
	0.40	1.510	1.471	1.437	1.473
	Mean	1.457	1.478	1.425	1.453[a]
18	0.00	1.458	1.479	1.342	1.427
	0.20	1.395	1.416	1.371	1.394
	0.40	1.414	1.377	1.423	1.405
	Mean	1.422	1.424	1.379	1.408[b]
20	0.00	1.322	1.302	1.315	1.313
	0.20	1.387	1.255	1.297	1.313
	0.40	1.359	1.336	1.349	1.348
	Mean	1.356	1.298	1.320	1.325[c]
22	0.00	1.297	1.280	1.281	1.286
	0.20	1.237	1.313	1.284	1.278
	0.40	1.263	1.302	1.339	1.301
	Mean	1.266	1.298	1.301	1.289[cd]
24	0.00	1.290	1.252	1.258	1.267
	0.20	1.254	1.261	1.206	1.240
	0.40	1.251	1.262	1.355	1.289
	Mean	1.265	1.258	1.273	1.265[d]
Mean	0.00	1.355	1.369	1.315	1.346
	0.20	1.345	1.336	1.324	1.335
	0.40	1.360	1.349	1.381	1.363
	Mean	1.353	1.351	1.340	

Source of variance	P diff
Crude protein (CP)	0.01
Glycine (Gly)	0.61
CP x Gly	0.34
Threonine (Thr)	0.15
CP x Thr	0.95
Gly x Thr	0.18
CP x Gly x Thr	0.37
Coefficient of Variation	7.21

in regard to minimum levels of Gly+Ser. This is also in agreement with Almquist and Grau (1944), Douglas *et al*. (1958), Waterhouse and Scott (1961) and Ngo and Coon (1976). The significant effect of Gly in low CP diets may be due to its role in uric acid formation. More research needs to be done on needs for individual amino acids in diets low in CP, especially for amino acids other than Lys and Met.

High levels of crystalline amino acids were added to the low CP diets in order to maintain minimum recommended levels. There is always controversy with respect to the biological efficiency of free crystalline amino acids (Batterham, 1974; Sibbald and Wolynetz, 1985; Izquierdo *et al*. 1988). However, Batterham (1974) and Batterham and O'Neill (1978) pointed out that the optimum utilization of crystalline amino acids could be achieved by the ad libitum feeding regime which would provide a constant flow of digesta to the gastrointestinal tract. Another influencing factor might be the composition of the free amino acids. A balanced mixture of several crystalline amino acids may be more effective compared with supplying a single EAA to the diet low in CP. Further research needs to be conducted to investigate if a balanced mixture of EAAs added to low CP diets could support optimal broiler performance.

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