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Improving the Utilization of Diets Low in Crude Protein for Broiler Chicken 1. Evaluation of Special Amino Acid Supplementation to Diets Low in Crude Protein[1]

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Abstract: Reduction of the dietary crude protein (CP) by amino acid supplementation is effective to a point but performance eventually declines. This study was conducted to determine if supplementation with certain amino acids that serve as precursors to metabolites involved in the urea cycle or the formation of essential amino acids might improve the performance at low protein levels. Diets were formulated to meet 100% or 110% of NRC recommendations for essential amino acids with Lys at 110% or 120% of recommendations. The CP levels were 16, 18, 20, 22 or 24%. The CP and ME equivalency values of amino acids were considered in the formulation. A minimum dietary electrolyte balance [(Na+K)-Cl)] of 200 meq/kg was maintained. Amino acids added to the low CP diets included: 1) Gly; 2) Gly + Arg; 3) Gly + Pro; 4) Arg + Pro; 5) Gly + Arg + Pro; 6) Gly + Arg + Pro + Glu. The Gly, Arg, and Pro were added at 0.2% while Glu was added at 0.4%. Each dietary treatment was fed to six pens of five male chicks from 1 to 21 d. Feeding diets with less than 22% CP resulted in loss in the body weight (BW) and impaired the feed conversion ratio (FCR) regardless of the amino acid status. Supplementation of low CP diets with Gly significantly improved the performance of broilers but did not reach that obtained on diets with 22 or 24% CP. None of the other amino acid supplements consistently influenced the performance of chicks in the absence of Gly. These data suggest that Gly may be a limiting factor in diets low in CP. It is likely that some of the requirements for lesser researched essential amino acids may be insufficient to support performance at lower levels of crude protein.

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

Introduction

Since the 1950's supplements of methionine and lysine have been successfully used in poultry diets allowing for a reduction in crude protein levels. With the development of additional feed-grade amino acids such as threonine and tryptophan the potential exists to reduce the CP level in diets even further since it is commonly agreed that greater performance in chicks can be achieved if the essential amino acids (EAA) in low CP diets are equivalent to those needed in the higher CP diets (Pinchasov *et al.*, 1990). However, broilers do not seem to perform as well when the intact protein content in diets is reduced to a greater extent and replaced with several EAAs (Edmonds *et al.*, 1985; Fancher and Jensen, 1989 a, b; Bregendahl *et al.*, 2002; Si *et al.*, 2004a). The reasons for the failure to obtain the performance with low protein amino acid fortified diets equal to that of higher protein diets are not clearly understood. In previous studies carried out in our laboratory, we examined the influence of electrolyte balance, ratio of Met to Cys, ratio of Lys to other EAA, ratio of Trp to large neutral amino acids, and ratio of EAA to non-essential amino acid (NEAA) on the performance of chicks fed low CP diets (Waldroup *et al.*, 2002; Si *et al.*, 2001, 2004a, 2004b, 2004c, 2004d). None of these approaches improved the performance of broilers fed low crude protein diets comparable to that of positive control diets with typical CP levels.

Gly has long been noted to improve the performance of broilers. Fisher *et al.* (1955) reported that additional Gly was essential to support optimal growth and feed efficiency for fast growing chicks. Greene *et al.* (1960) also stated that growth, feed efficiency and nitrogen retention improved with the addition of Gly to the diet. Waterhouse and Scott (1961) demonstrated that the Gly requirement decreased as the CP levels increased in diets.

Arg is usually involved in the protein synthesis, the formation of ornithine and urea. The production of guanidoacetic acid is also dependent on Gly (Smith and Lewis, 1963). In addition, broilers are unable to synthesize Arg due to the lack of certain enzymes. Snetsinger and Scott (1961) postulated that Gly and Arg function in overcoming amino acid toxicities by enhancing the excretion of excess nitrogen via the uric acid and the urea cycle respectively. Jensen and Mendonca (1988) noticed that the increased fat deposition of chicks fed low CP diets could be prevented by including glutamic acid in diets to make the nitrogen level equivalent to the higher CP level diets. Deschepper and DeGroote (1995) also considered glutamic acid as the precursor for some NEAAs and non-protein sources in the diet. The function of Pro in low CP diets has not been well investigated.

The purpose of the present study was to investigate possible increased needs for certain amino acids for which the needs might be increased at lower CP levels due to their roles in the urea cycle, the limitation of synthesis of EAAs or other similar functions.

Materials and Methods

Diet formulation: A series of experimental diets were comprised primarily of corn and soybean meal of known crude protein content and were formulated to contain 3,200 kcal/kg ME. A minimum dietary electrolyte balance [(Na + K) - Cl] of 200 meq/kg was stipulated with 0.25% sodium and a minimum of 0.2% sodium bicarbonate. The corn and soybean meal used were analyzed for crude protein (N x 6.25) prior to diet formulation using Association of Official Agricultural Chemists (AOAC, 1970) procedures. Essential amino acids other than Lys were stipulated at either 100 (Series 1) or 110% (Series 2) of NRC (1994) recommendation levels. Lys was stipulated at 110% (Series 1) or 120% (Series 2) of NRC (1994) recommendation level. Five primary diets were formulated within each series to contain 24, 22, 20, 18, and 16% CP. The crude protein and ME equivalent values of the amino acid supplements (NRC, 1994) were considered in the diet formulation. Crystalline amino acids were utilized as needed to meet the minimum needs for amino acids within the specified level of CP.

Diets with 24 and 22% CP were fed without additional amino acid supplementation. Diets with 20, 18 and 16% CP were fed with the addition of various combinations of amino acids that might be lacking or limiting in the low CP diets. Amino acids or combinations of amino acids that were added included: 1) Gly; 2) Gly + Arg; 3) Gly + Pro; 4) Arg + Pro; 5) Gly + Arg + Pro; 6) Gly + Arg + Pro + Glu. This resulted in a total of 40 experimental diets. Gly, Arg, and Pro were added at 0.2% each, alone or in combination, while Glu was added at 0.4%; each replaced an equivalent level of corn starch in the diet. Arginine serves in the production of ornithine, creatine, and urea, proline may not be adequately synthesized at low protein levels, glycine is involved in urea cycle formation, and glutamic acid is a precursor for some of the dispensible amino acids and is often used in amino acid studies as a nonspecific nitrogen source. Diets were supplemented with vitamin and trace mineral mixes obtained from a commercial integrator. The composition of the basal diets is shown in Table 1 with the calculated nutrient values provided in Table 2. Diets were fed in mash form.

Chicks and Housing: Male chicks of a commercial broiler strain (Cobb 500[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 placed in compartments in electrically heated brooders with raised wire floors. Five male chicks were placed in each of 240 pens. Each of the experimental diets was fed to six replicate pens of five male chicks of a commercial broiler strain, stratified across tiers in the batteries. 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.

Measurements: Birds were group weighted 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)]. All mixed feeds were analyzed for crude protein (AOAC, 1970) and supplemental amino acids (Fontaine and Eudaimon, 2000).

Data analysis: Pen means served as the experimental unit. Data were first subjected to the analysis of variance (SAS Institute. 1991) as a single factor arrangement using the General Linear Models procedure. The means for treatments showing significant difference in the analysis of variance were compared using the least significant difference procedure. Following this, various comparisons were made among the dietary treatments to identify significant relationships among crude protein levels, level of dietary amino acids, and supplemental amino acids. All statements of significance are based on a 5% level of probability.

Results and Discussion

Results of the assays for crude protein and supplemental amino acids indicated good agreement with calculated values (Table 2). Reduction of the crude protein content, while maintaining minimum recommended (NRC, 1994) levels of nutritionally essential amino acids, resulted in a significant decrease in 21 d BW (Table 3). When all 40 treatments were compared, it is obvious that as dietary crude protein is reduced below 22%, performance declined. Increasing the minimum level of nutritionally essential amino acids from 100 to 110% of NRC (1994) with Lys at 110 or 120% failed to overcome the reduction and in fact appeared to exacerbate the problem, especially at lower levels of crude protein. Similar response to increased amino acid levels at low levels of CP has been seen previously in trials in our laboratory (Si *et al.*, 2004c). Although addition of supplemental amino acids

Table 1: Composition (g/kg) of diets with different levels of crude protein and amino acids

Ingredient	% Crude protein/% of NRC amino acid recommendations[1]									
	24/100	24/110	22/100	22/110	20/100	20/110	18/100	18/110	16/100	16/110
Yellow corn	455.29	455.89	518.68	520.58	584.13	588.81	650.54	663.81	727.08	767.59
Soybean meal (47%)	424.80	423.55	370.99	368.05	314.48	307.53	254.21	233.53	175.11	116.13
Dicalcium phosphate	17.30	17.32	17.67	17.70	18.06	18.12	18.49	18.68	19.10	19.62
Poultry oil	78.43	78.13	67.88	67.21	56.75	55.28	45.49	42.25	32.21	20.47
Ground limestone	12.10	12.10	12.16	12.16	12.22	12.22	12.27	12.27	12.32	12.33
Sodium chloride	3.57	3.57	3.50	3.50	3.42	3.42	3.34	3.33	3.26	3.22
Sodium bicarbonate	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Potassium sulfate	0.00	0.00	0.00	0.00	0.00	0.00	0.88	3.01	5.55	10.05
L-Lysine Hcl	0.00	0.00	0.00	0.73	1.17	2.97	3.40	5.73	6.36	10.14
DL-Methionine	1.51	2.44	2.12	3.07	2.77	3.77	3.46	4.64	4.41	6.07
L-Arginine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.80	2.43	5.74
L-Threonine	0.00	0.00	0.00	0.00	0.00	0.88	0.92	2.09	2.24	4.08
L-Valine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.08	1.47	3.79
L-Phenylalanine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	3.91
L-Isoleucine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.52	1.00	3.20
Glycine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.23
L-Leucine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.57
L-Histidine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99
L-Tryptophan	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.35	0.87
Vitamin premix[2]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Trace mineral mix[3]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00

[1]All essential amino acids at 100 or 110% of NRC (1994) recommendations with Lys at 110 or 120%. [2]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; choline 1040 mg; thiamin 1.54 mg; pyridoxine 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg; Se 0.1 mg. [3]Provides per kg of diet: Mn 100 mg; Zn 100 mg; Fe 50 mg; Cu 10 mg; I 1 mg.

Gly, Arg, Pro, and Glu aided in improving BW of chicks fed diets low in CP, they seldom brought the performance up to that of chicks fed diets with 24% CP. In the second comparison, the influence of specific amino acid supplementation was evaluated while excluding performance of birds fed 22 or 24% CP (Table 3). Performance of birds fed the different levels of supplemental amino acids was compared to that of birds fed the same diet without any supplemental amino acids. Values in parentheses indicate the probability of difference between the two means. It is apparent that addition of Gly, alone or in combination with Arg or Pro, significantly improved performance of birds fed the low CP diets. When Gly was not supplemented, performance was not significantly improved by addition of Arg+Pro. There was a significant interaction between minimum level of nutritionally essential amino acids and supplementation with the other amino acids; the response to the supplemental amino acids was less when the diets contained a minimum of 110% of recommended amino acids (120% for Lys) even though the diets with 100 and 110% contained the same level of CP.

In the third comparison, performance was compared over all levels of CP and minimum levels of essential amino acids (Table 3). This comparison clearly shows the response to supplemental Gly, alone or in combination with Arg, Pro, or Glu. When Gly was not included in the supplement, BW was not significantly improved by addition of the other amino acids. The addition of Arg, Pro, or Glu did not appear to improve performance any greater than did Gly

Table 2. Nutrient analysis of diets with different levels of crude protein and amino acids

Nutrient (%)[2]	% Crude protein/% of NRC amino acid recommendations[1]										
	Basis[3]	24/100	24/110	22/100	22/110	20/100	20/110	18/100	18/110	16/100	16/110
Crude protein	C	24.00	24.00	22.00	22.00	20.00	20.00	18.00	18.00	16.00	16.00
Crude protein	A	23.40	23.81	22.34	22.31	20.36	19.94	18.32	18.12	17.13	16.32
Met	C	0.54	0.63	0.57	0.66	0.60	0.69	0.63	0.74	0.68	0.81
Lys	C	1.43	1.42	1.27	1.32	1.20	1.32	1.20	1.32	1.20	1.32
Trp	C	0.29	0.29	0.26	0.26	0.24	0.23	0.21	0.22	0.20	0.22
Thr	C	0.98	0.98	0.90	0.89	0.81	0.88	0.80	0.88	0.80	0.88
Ile	C	1.24	1.24	1.13	1.13	1.01	1.00	0.88	0.88	0.80	0.88
His	C	0.62	0.62	0.57	0.56	0.51	0.50	0.45	0.42	0.36	0.39
Val	C	1.31	1.30	1.19	1.19	1.07	1.06	0.94	0.99	0.90	0.99
Leu	C	2.01	2.01	1.88	1.87	1.74	1.72	1.58	1.52	1.36	1.32
Arg	C	1.81	1.80	1.64	1.63	1.47	1.45	1.28	1.38	1.25	1.38
Pro	C	1.39	1.39	1.31	1.30	1.22	1.20	1.12	1.08	0.98	0.87
TSAA	C	0.90	0.99	0.90	0.99	0.90	0.99	0.90	0.99	0.90	0.99
Phe+Tyr	C	2.28	2.28	2.08	2.08	1.88	1.86	1.65	1.57	1.35	1.47
Gly+Ser	C	2.29	2.28	2.08	2.08	1.87	1.84	1.64	1.56	1.32	1.38
Total EAA[4]	C	15.16	15.22	13.89	13.94	12.61	12.74	11.42	11.62	10.34	11.10
EAA % of CP	C	63.17	63.42	63.13	63.36	63.05	63.70	63.44	64.55	64.63	69.38
Added Met	C	0.15	0.24	0.31	0.31	0.28	0.37	0.35	0.46	0.44	0.60
Added Met	A	0.15	0.28	0.21	0.38	0.32	0.42	0.33	0.53	0.55	0.62
Added Lys	C	0.00	0.00	0.00	0.07	0.09	0.23	0.26	0.45	0.49	0.79
Added Lys	A	0.00	0.00	0.00	0.06	0.11	0.23	0.28	0.47	0.50	0.86

[1]All essential amino acids at 100 or 110% of NRC (1994) recommendations with Lys at 110 or 120%. [2]All diets calculated to contain 3,200 ME kcal/kg. [3]C = calculated from NRC (1994); A = Analyzed. [4]Sum of all essential amino acids including Cys and Tyr.

alone. Basically the same pattern of response was observed in comparison 4, where the response to supplemental amino acids was evaluated in diets with 16, 18, or 20% CP over both levels of essential amino acids (Table 3). The effects of dietary crude protein, levels of essential amino acids, and supplementation with additional amino acids on feed conversion followed much the same pattern as observed with BW (Table 4). In the overall comparison of treatments, it was again observed that a decrease in the dietary CP resulted in a significant deterioration in performance that was not improved by increasing the minimum level of essential amino acids. Addition of the supplemental amino acids improved performance but did not totally overcome the negative effects of reduction in dietary CP.

In the second comparison, where the addition of supplemental amino acids was evaluated in diets with 100 or 110% of minimum NRC (1994) recommended levels of essential amino acids (110 and 120% for Lys) it can be seen that any combination of supplemental amino acids involving Gly improved performance when diets contained a minimum of 100% of recommended essential amino acids but was much less effective when added to diets with 110% of recommended levels. In the third comparison, when supplemental amino acids were evaluated in diets with 16, 18, and 20% CP including both levels of minimum amino acids, it is strongly clear that addition of Gly significantly improved feed conversion; some improvement was seen when Arg, Pro, or Glu was included but not to the extent observed with Gly. As seen in comparison 4, the response to Gly was relatively similar over all three levels of CP.

Table 3: Effects of dietary crude protein and amino acid levels and supplementation with various amino acids on 21 d body weight of male broilers (means of six pens of five chicks)

Crude Protein	AminoAcid % of NRC[2]	Additional amino acids[1]						
		None	Gly	Gly+Arg	Gly+Pro	Arg+Pro	Gly+Arg+Pro	Gly+Arg +Pro+Glu
1. Comparison of all 40 experimental treatments[3]								
24	100	791^{a}	---	---	---	---	---	---
24	110	773^{ab}	---	---	---	---	---	---
22	100	755^{abc}	---	---	---	---	---	---
22	110	723^{b-g}	---	---	---	---	---	---
20	100	700^{g-n}	730^{bcd}	718^{c-h}	676^{e-m}	722^{b-g}	696^{d-k}	717^{c-h}
20	110	668^{h-o}	705^{c-j}	724^{b-f}	730^{bcde}	715^{c-i}	737^{bcd}	723^{b-g}
18	100	630^{m-q}	666^{h-o}	674^{f-m}	702^{c-j}	632^{l-q}	684^{d-l}	663^{i-p}
18	110	621^{n-r}	654^{j-p}	718^{c-h}	646^{k-p}	643^{k-p}	702^{c-j}	646^{k-p}
16	100	531^{stu}	611^{pqr}	615^{o-r}	584^{qrs}	576^{rs}	630^{m-q}	582^{st}
16	110	495^{tu}	524^{tu}	522^{tu}	545^{st}	483^{u}	537^{st}	547^{tu}
2. Comparison of supplemental amino acids at different dietary amino acid levels[4]								
-------	100	610	669 (0.02)[5]	669 (0.01)	654 (0.07)	643 (0.17)	670 (0.01)	654 (0.07)
-------	110	595	628 (0.31)	655 (0.06)	640 (0.16)	614 (0.56)	659 (0.05)	639 (0.18)
3. Comparison of supplemental amino acids in diets with 16, 18, and 20% crude protein								
20-18-16	All	608	649 (0.02)	662 (0.01)	647 (0.01)	629 (0.18)	664 (0.01)	647 (0.02)
4. Comparison of supplemental amino acids in diets with different levels of crude protein and amino acids								
20	All	684	718 (0.03)	721 (0.02)	703 (0.12)	719 (0.02)	717 (0.03)	720 (0.02)
18	All	626	660 (0.12)	696 (0.03)	674 (0.03)	638 (0.58)	693 (0.01)	655 (0.19)
16	All	513	568 (0.02)	569 (0.01)	565 (0.02)	530(0.47)	584 (0.01)	565 (0.02)

[1]Addition of 0.2% Gly, Pro, Arg, or 0.4% Glu in various combinations to low protein diets. These supplements calculated to provide equivalent of 0.23, 0.15, 0.40, or 0.24% CP, respectively (NRC, 1994). [2]All essential amino acids at 100 or 110% of NRC (1994) recommendations with Lys at 110 or 120%. [3]Means with common superscripts do not differ significantly ($P<0.05$). [4]Does not include diets with 22 or 24% CP. [5]Value in parenthesis indicate probability of difference from unsupplemented control diet in series.

Although the total level of essential amino acids in the diets declined as crude protein level was reduced, the ratio of essential to nonessential amino acids remained relatively the same (Table 2) Stucki and Harper (1961) found the optimum EAA/NEAA ratio was near 2:1 for diets with a wide range of dietary nitrogen level; namely, about 33% of the dietary nitrogen had to be provided from NEAA in order to obtain maximum growth of broiler chickens. According to their calculation, Gly was considered as NEAA besides Lys, Met, Cys, Thr, Ile, Val, Leu, Phe, Tyr, His, and Trp. However, Sugahara and Ariyoshi (1968) pointed out that Stucki and Harper (1961) neglected the difference of DL-form of amino acids in their control diet. By using only the L-isomer of all the racemic amino acids, they narrowed down the EAA/NEAA ratio to 1:1 in respect to the lowest feed conversion and 1.5:1 when growth rate was the highest. This means that optimum performance of broilers could be achieved when dietary NEAA ranged from 40 to 50% of the dietary nitrogen. Bedford and Summers (1985) confirmed that in all cases, apart from the proportion of carcass protein, optima were achieved at the 55:45 EAA/NEAA ratio. The ratio of EAA/NEAA has also been studied with rats (Heger, 1990), pigs (Wang and Fuller, 1989), turkeys (Bedford and Summers, 1988), and kittens (Rodgers *et al.*, 1998). Except for kittens, results of the other three species have shown optimal growth rates when the dietary EAA/NEAA ranging from 40:60 to 65:35. Values in the present study lie within these ranges.

Although all the essential amino acids levels in diets used in this study are

Table 4: Effects of dietary crude protein and amino acid levels and supplementation with various amino acids on 0 to 21 d feed conversion by male broilers (means of six pens of five chicks)

Crude Protein%	AminoAcid % of NRC[2]	Additional amino acids[1] None	Gly	Gly+Arg	Gly+Pro	Arg+Pro	Gly+Arg+Pro	Gly+Arg+Pro+Glu
1. Comparison of all 40 experimental treatments[3]								
24	100	1.259[s]	---	---	---	---	---	---
24	110	1.305[rs]	---	---	---	---	---	---
22	100	1.326[qr]	---	---	---	---	---	---
22	110	1.354[n-r]	---	---	---	---	---	---
20	100	1.437[e-k]	1.359[m-r]	1.350[n-r]	1.363[k-r]	1.384[j-q]	1.357[m-r]	1.346[o-r]
20	110	1.415[g-n]	1.376[k-q]	1.348[o-r]	1.355[n-r]	1.375[k-q]	1.363[l-r]	1.338[pqr]
18	100	1.444[e-j]	1.446[e-j]	1.426[f-l]	1.411[h-o]	1.453[e-i]	1.421[f-m]	1.428[f-l]
18	110	1.499[b-e]	1.502[b-e]	1.403[h-p]	1.447[e-j]	1.450[e-j]	1.375[k-q]	1.391[i-q]
16	100	1.634[a]	1.478[c-g]	1.525[bcd]	1.548[b]	1.533[bc]	1.494[b-f]	1.465[d-h]
16	110	1.655[a]	1.546[b]	1.547[b]	1.540[bc]	1.636[a]	1.542[bc]	1.499[b-e]
2. Comparison of supplemental amino acids at different dietary amino acid levels[4]								
-------	100	1.500	1.427 (0.01)[5]	1.433 (0.02)	1.440 (0.06)	1.456 (0.11)	1.424 (0.007)	1.413 (0.001)
-------	110	1.515	1.474 (0.28)	1.432 (0.03)	1.447 (0.07)	1.487 (0.45)	1.426 (0.02)	1.409 (0.001)
3. Comparison of supplemental amino acids in diets with 16, 18. and 20% crude protein								
20-18-16	All	1.514	1.451 (0.01)	1.433 (0.001)	1.444 (0.007)	1.472 (0.09)	1.425 (0.002)	1.411 (0.001)
4. Comparison of supplemental amino acids in diets with different levels of crude protein and amino acids								
20	All	1.426	1.368 (0.03)	1.349 (0.005)	1.359 (0.02)	1.380 (0.09)	1.360 (0.02)	1.342 (0.002)
18	All	1.472	1.474 (0.92)	1.415 (0.04)	1.429 (0.12)	1.452 (0.45)	1.398 (0.007)	1.410 (0.02)
16	All	1.645	1.512 (0.001)	1.536 (0.001)	1.544 (0.004)	1.586 (0.03)	1.518 (0.001)	1.482 (0.001)

[1]Addition of 0.2% Gly, Pro, Arg, or 0.4% Glu in various combinations to low protein diets. These supplements calculated to provide equivalent of 0.23, 0.15, 0.40, or 0.24% CP, respectively (NRC, 1994). [2]All essential amino acids at 100 or 110% of NRC (1994) recommendations with Lys at 110 or 120%. [3]Means with common superscripts do not differ significantly (P<0.05). [4]Does not include diets with 22 or 24% CP. [5]Value in parenthesis indicate probability of difference from unsupplemented control diet in series.

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 1.7 to 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. Our results indicate that it is preferable to maintain a higher level of Gly than recommended by NRC (1994) as the dietary CP level is reduced. This is 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 the 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.

In conclusion, the performance of chicks fed low CP diets supplemented with certain amino acids (Gly, Arg, Pro and Glu) is inferior to that of chicks fed higher CP diets composed of the intact protein even though minimum recommended levels of essential amino acids are maintained. The reasons for this are not totally clear. Gly and Arg are more important in maintaining the performance of chicks on the lower CP levels. The effect of Pro in low CP diets needs further investigation. The amounts of crystalline amino acids added to low CP diets may affect the feed intake of chicks and thus depress their performance. Further investigations on the feed intake of chicks fed low CP diets are suggested.

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