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Low Crude Protein Corn and Soybean Meal Diets with Amino Acid Supplementation for Broilers in the Starter Period. 2. Effects of Feeding 13% Crude Protein

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Abstract: Two experiments were conducted with the objective of testing the effects of feeding 13% CP diets with crystalline amino acid supplementation and various protein equivalents on the performance of broilers in the starter growth period. In each experiment, commercial broilers were fed a diet formulated to meet NRC requirements for the first seven days. The diet contained 23% CP and 3200 kcal/kg ME and also served as the Positive Control Diet (PC). On day 7, birds were sorted by weight into battery pens with 5 birds per pen. In the first experiment, six dietary treatments were utilized with eight replicates per treatment for a total of 48 pens. For the remaining dietary treatments, 13% CP diets were formulated and various levels of crystalline amino acids were added back to meet either digestible amino acid levels from a 22% CP diet from previous experiments from our lab at the University of Missouri (Guaiume, 2007) or digestible amino acid requirements set by Baker and coworkers (1993) using the ideal protein concept. One treatment using the University of Missouri values contained no glutamic acid and a low protein equivalent of 15.5% (MLPE), while others contained varying levels of glutamic acid to achieve a high protein equivalent of 20% (MHPE) or a mid-level protein equivalent of 18% (MMPE). Similarly, two treatments were developed using Baker *et al.* (1993) amino acid values and glutamic acid to achieve a 20% high protein equivalent (BHPE) or an 18% mid-level equivalent (BMPE). In Experiment 2, four dietary treatments with 12 replicates were utilized for a total of 48 pens. The same 23% CP diet used as the PC in Experiment 1 was utilized in Experiment 2. The remaining treatments in Experiment 2 consisted of 13% crude protein diets with crystalline amino acids added back to meet control levels and either no glutamic acid to yield a protein equivalent of 17.5% (PE-17.5), or glutamic acid added to meet an 18.75% (PE-18.75) or 20% (PE-20) protein equivalent. All diets were formulated on a digestible basis and were designed to be isocaloric. Birds received feed and water *ad libitum*. At the conclusion of each experiment, Body Weight Gain (BWG), Feed Intake (FI) and Feed:Gain (F:G) were measured. In Experiment 1, birds consuming the PC treatment achieved significantly greater ($p<0.05$) BWG than birds in any other treatment. A significant difference ($p<0.05$) in intake was seen between the BMPE treatment and all others. A significantly improved F:G ($p<0.05$) was observed in the PC treatment. Additionally, the BMPE treatment resulted in impaired F:G ($p<0.05$) when compared to the MMPE and MHPE treatments. In Experiment 2, birds receiving the PE-17.5 treatment gained significantly less weight ($p<0.05$) than those consuming other dietary treatments. There were no significant differences ($p>0.05$) in feed intake. Birds in the PC groups displayed significantly improved F:G over all other treatments ($p<0.05$).

Key words: Low crude protein, broilers, amino acids, digestible feed formulation, performance

INTRODUCTION

Two previous trials conducted in this laboratory indicated that a 15% CP diet with crystalline amino acid supplementation can yield similar performance in broilers from 0-3 weeks of age. In the current trials, two additional experiments were conducted with the objective of testing the effects of feeding a 13% CP diet with crystalline amino acid supplementation on the performance of broilers in the same growth period.

Use of the ideal protein concept can allow for determination of digestible amino acid requirements for birds at any age period (Baker, 2003) and the formulation of diets on a digestible basis, which can help reduce the level of excess protein in the diet. Using this method, the requirements of all indispensable amino acids are related to lysine and can be easily and quickly modified as the requirement for lysine changes. One of the practical uses of the ideal protein concept is to formulate low protein diets with crystalline amino acids added back. Furthermore, it has been shown to be useful in tracking the order of limitation of amino acids as they might change in diets in which the level of protein or the ingredient profile and therefore the amino acid profile, changes (Han *et al.*, 1992; Baker *et al.*, 1993; Wang *et al.*, 1997). It is possible to use the ideal protein concept to feed animals more precisely and

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avoid the use of excessive levels of crude protein to meet amino acid requirements. However, in order to truly establish exact amino acid requirements, it is necessary to significantly reduce crude protein and add back large amounts of crystalline amino acids in order to make it possible to titrate individual amino acids. These experiments were conducted in an attempt to reduce the level of crude protein that can be fed with various levels of amino acid supplementation. Additionally, these experiments examined the importance of the level of protein equivalence in low crude protein rations. When birds are fed standard levels of crude protein, nonessential amino acids can be formed in the body from excess essential amino acids. However, when low crude protein diets are fed, this excess is reduced, leaving less essential amino acids available for conversion to the nonessentials. To prevent a deficiency of essential amino acids and the resultant poor performance, many researchers add supplemental non-essential amino acids, a non-specific nitrogen source such as glutamic acid, or both to low crude protein diets. While a number of researchers have reported the importance of supplementing nonessential amino acids to low crude protein diets for improving live performance (Han *et al*., 1992; Aletor *et al*., 2000; Dean *et al*., 2006), others have suggested that glutamic acid or other non-specific nitrogen sources result in little or no advantage when added to low protein broiler rations (Kerr and Kidd, 1999) or swine rations (Kephart and Sherritt, 1990). For this reason, a variety of protein equivalencies, achieved by the addition of glutamic acid, were used in these trials and performance was measured. The overall objective of this research is to eventually determine the lowest possible level of crude protein that may be fed to broilers that will achieve similar growth to those consuming standard diets, allowing the establishment of precise digestible amino acid requirements.

MATERIALS AND METHODS

Bird husbandry: Day-old straight run broiler chicks were obtained from a commercial hatchery and fed a NRC-type corn and soybean meal diet until seven days of age. On day seven, birds were wing-banded and weighed. Birds were computer sorted by weight into battery pens with 5 birds per pen to obtain similar starting pen weights and bird weight distribution. Experiment 1 utilized 240 birds to provide eight replications of six treatments, while Experiment 2 utilized 240 birds to provide twelve replicates of four treatments. Chicks were provided access to experimental diets and water *ad libitum* for fourteen days and trials were terminated on day 21. Chicks were housed in stainless steel batteries with 24 hrs of fluorescent lighting. The room was thermostatically controlled with temperatures maintained near 90 degrees F for the first week post-hatch and a two degree reduction in temperature every four days thereafter. Birds were cared for using husbandry guidelines derived from University of Missouri standard operating procedures.

Dietary treatments: All diets were formulated on a digestible basis utilizing least-cost formulation software. The amino acid digestibility values used in these experiments for the corn and SBM were obtained previously by precision feeding a known sample of each ingredient to cecectomized roosters that had been removed from feed for 24 hrs to clear the gut. Excreta were collected for 48 hrs after precision feeding and were then dried in a forced air oven and ground. Samples were sent to the University of Missouri Agricultural Experiment Station Chemical Laboratory for a complete amino acid analysis and digestibility values were then determined.

In each experiment, a 23% CP, 3200 kcal/kg ME NRC-type diet was utilized as the Positive Control (PC). In Experiment 1, the remaining dietary treatments consisted of 13% CP diets to which varying levels of crystalline amino acids were added back to meet either digestible amino acid levels from a 22% CP diet used in previous experiments from our lab at the University of Missouri (Guaiume, 2007) or digestible amino acid requirements established by Baker *et al*. (1993) using the ideal protein concept. The total amino acid levels of the dietary treatments for Experiment 1 are provided in Table 1. One treatment using the University of Missouri values contained no glutamic acid and a low protein equivalent of 15.5% (MLPE), while others contained varying levels of glutamic acid to achieve a high protein equivalent of 20% (MHPE) or a mid-level protein equivalent of 18% (MMPE). Similarly, two treatments were developed using Baker *et al*. (1993) amino acid values and glutamic acid to achieve a 20% high protein equivalent (BHPE) or an 18% mid-level equivalent (BMPE). The ingredient and nutrient compositions of the experimental diets in Experiment 1 are provided in Table 2.

Experiment 2 used the same Positive Control Diet (PC), as well as three additional treatments. These consisted of 13% crude protein diets with crystalline amino acids added back to meet control levels and either no glutamic acid to yield a protein equivalent of 17.5% (PE-17.5), or glutamic acid added to meet an 18.75% (PE-18.75) or 20% (PE-20) protein equivalent. The ingredient and nutrient composition of dietary treatments for Experiment 2 are provided in Table 3.

Measurements: Dietary treatments were evaluated from 7-21 days of age by measuring feed intake, body weight gain and feed:gain. Feed:gain was adjusted for mortality by adding each mortality weight to the appropriate pen gain then dividing feed consumed by gain.

Table 1: Amino acid levels[1] of experimental diets for broilers fed 13% crude protein (Experiment 1)

	Experimental diets (%)					
Treatment	PC[1]	MLPE	MHPE	MMPE	BHPE	BMPE
Crude protein	23.000	13.000	13.000	13.000	13.000	13.000
Protein equivalent	23.000	15.500	20.000	18.000	20.000	18.000
Amino acid						
Lysine	1.360	1.090	1.090	1.090	1.120	1.120
TSAA	0.866	0.810	0.810	0.810	0.810	0.810
Threonine	0.851	0.728	0.728	0.728	0.750	0.750
Valine	1.143	0.905	0.905	0.905	0.860	0.860
Arginine	1.543	1.342	1.342	1.342	1.180	1.180
Leucine	2.020	1.758	1.758	1.758	1.240	1.240
Histidine	0.625	0.514	0.514	0.514	0.350	0.350
Isoleucine	1.054	0.817	0.817	0.817	0.750	0.750
Phenylalanine	1.168	0.956	0.956	0.956		
Tryptophan	0.241	0.220	0.220	0.220	0.180	0.180
Glycine	0.636	0.437	0.437	0.437		
Glutamic acid	0.000	0.000	4.524	2.503	5.491	3.471

[1]All values are expressed an a digestible basis

Table 2: Composition of experimental diets for broilers fed 13% crude protein (Experiment 1)

Treatment	PC	MLPE	MHPE	MMPE	BHPE	BMPE
Corn	51.624	69.285	69.285	69.285	69.285	69.285
Soybean meal	39.822	16.151	16.151	16.151	16.151	16.151
Lard	4.648	1.755	1.755	1.755	1.755	1.755
Dicalcium phosphate	1.699	1.900	1.900	1.900	1.900	1.900
Limestone	1.174	1.206	1.206	1.206	1.206	1.206
Sodium bicarbonate	0.300	1.000	1.000	1.000	1.000	1.000
Salt	0.256	0.260	0.260	0.260	0.260	0.260
Sucrose[3]		5.280	0.756	2.777	0.797	2.817
Coban	0.075	0.075	0.075	0.075	0.075	0.075
Vitamin premix[1]	0.075	0.075	0.075	0.075	0.075	0.075
Choline chloride	0.050	0.149	0.149	0.149	0.149	0.149
Calcium trace mineral[2]	0.100	0.100	0.100	0.100	0.100	0.100
Selenium premix[2]	0.030	0.030	0.030	0.030	0.030	0.030
Copper sulfate	0.013	0.013	0.013	0.013	0.013	0.013
DL Methionine	0.116	0.227	0.227	0.227	0.132	0.132
Arginine		0.477	0.477	0.477	0.317	0.317
Glycine		0.039	0.039	0.039	0.150	0.150
Histidine		0.165	0.165	0.165		
Isoleucine		0.215	0.215	0.215	0.174	0.174
Leucine		0.383	0.383	0.383		
Lysine		0.444	0.444	0.444	0.482	0.482
Phenylalanine		0.250	0.250	0.250		
Threonine		0.209	0.209	0.209	0.231	0.231
Tryptophan		0.098	0.098	0.098	0.058	0.058
Valine		0.214	0.214	0.214	0.169	0.169
Cystine						
Glutamic acid			4.524	2.503	5.491	3.471
Calculated to contain						
Crude protein, %[4]	23.000	13.000	13.000	13.000	13.000	13.000
Protein equivalent, %[5]	23.000	15.500	20.000	18.000	20.000	18.000
ME, kcal/kg	3200.000	3200.000	3200.000	3200.000	3200.000	3200.000
Calcium, %	1.000	1.000	1.000	1.000	1.000	1.000
Available Phosphorus, %	0.450	0.450	0.450	0.450	0.450	0.450

[1]Vitamin premix provided the following amounts per kilogram of diet: vitamin D3, 200 IU; vitamin A, 1,500 IU; vitamin E, 101 IU; niacin, 35 mg; D-Pantothenic acid, 14 mg; riboflavin, 4.5 mg; pyridoxine, 3.5 mg; menadione, 2 mg; folic acid, 0.55 mg; thiamine, 1.8 mg.

[2]Mineral premix provided the following amounts per pound of premix per ton of feed: Mn, 11.0%; Zn, 11.0%; Fe, 6.0%; I, 2,000 ppm; Mg, 2.68%; Se, 600 ppm.

[3]Synthetic amino acids and glutamic acid added at expense of sucrose.

[4]Crude protein values calculated from protein provided from corn and soybean meal.

[5]Protein equivalent calculated from protein provided from corn and soybean meal plus protein from synthetic amino acids

Table 3: Composition of experimental diets for broilers fed 13% crude protein (Experiment 2)

Treatment	PC	PE-17.5	PE-18.75	PE-20
Corn	51.642	68.231	68.231	68.231
Soybean meal	39.822	16.322	16.322	16.322
Lard	4.648	2.138	2.138	2.138
Dicalcium phosphate	1.699	1.902	1.902	1.902
Limestone	1.174	1.204	1.204	1.204
Sodium bicarbonate	0.300	1.500	1.500	1.500
Salt	0.256	0.261	0.261	0.261
Sucrose[3]	0.000	3.085	1.860	0.598
Coban	0.075	0.075	0.075	0.075
Vitamin premix[1]	0.075	0.075	0.075	0.075
Choline chloride	0.050	0.149	0.149	0.149
Calcium trace mineral[2]	0.100	0.100	0.100	0.100
Selenium premix[2]	0.030	0.030	0.030	0.030
Copper sulfate	0.013	0.013	0.013	0.013
DL methionine	0.116	0.229	0.229	0.229
Arginine		0.680	0.680	0.680
Glycine		0.239	0.239	0.239
Histidine		0.307	0.307	0.307
Isoleucine		0.533	0.533	0.533
Leucine		0.651	0.651	0.651
Lysine		0.786	0.786	0.786
Phenylalanine		0.463	0.463	0.463
Threonine		0.333	0.333	0.333
Tryptophan		0.119	0.119	0.119
Valine		0.453	0.453	0.453
Cystine		0.122	0.122	0.122
Glutamic acid			1.225	2.487
Calculated to contain				
Crude protein, %[4]	23.000	13.000	13.000	13.000
Protein equivalent, %[5]	23.000	17.500	18.750	20.000
ME, kcal/kg	3200.000	3200.000	3200.000	3200.000
Calcium, %	1.000	1.000	1.000	1.000
Available Phosphorus, %	0.450	0.450	0.450	0.450

[1]Vitamin premix provided the following amounts per kilogram of diet: vitamin D3, 200 IU; vitamin A, 1,500 IU; vitamin E, 101 IU; niacin, 35 mg; D-Pantothenic acid, 14 mg; riboflavin, 4.5 mg; pyridoxine, 3.5 mg; menadione, 2 mg; folic acid, 0.55 mg; thiamine, 1.8 mg.

[2]Mineral premix provided the following amounts per pound of premix per ton of feed: Mn, 11.0%; Zn, 11.0%; Fe, 6.0%; I, 2,000 ppm; Mg, 2.68%; Se, 600 ppm.

[3]Synthetic amino acids and glutamic acid added at expense of sucrose.

[4]Crude protein values calculated from protein provided from corn and soybean meal.

[5]Protein equivalent calculated from protein provided from corn and soybean meal plus protein from synthetic amino acids

Statistical analysis: Data were analyzed with pen gain as the experimental unit using the JMP® statistical analysis software package (SAS Institute; Cary, NC). Analysis of Variance (ANOVA) with a one-way design using the general linear model was performed and the level of significance was established at $p<0.05$. Mean comparisons for all pairs were conducted using the Least Significant Difference test.

RESULTS AND DISCUSSION

In these experiments, body weight gain, feed intake and feed:gain were measured in order to determine whether low crude protein rations with various protein equivalents and crystalline amino acid supplementation can support similar performance to that achieved with a NRC-type diet.

In Experiment 1, birds consuming the PC treatment had significantly greater ($p<0.05$) BWG than birds in any other treatment. A significant difference ($p<0.05$) in intake was seen between the BHPE treatment, which displayed the lowest FI and the BMPE treatment, which displayed the highest feed intake. A significantly improved F:G ($p<0.05$) was observed in the PC treatment. Additionally, the BMPE treatment resulted in impaired F:G ($p<0.05$) when compared to the MMPE and MHPE treatments. The results from Experiment 1 are shown in Table 4.

In Experiment 2, birds receiving the PE-17.5 treatment gained significantly less body weight ($p<0.05$) than those consuming the PC treatment. There were no significant differences ($p>0.05$) in feed intake among any of the treatments. Birds in the PC group displayed significantly improved F:G ($p<0.05$) over all other treatments. These results are displayed in Table 5.

The results of these experiments indicate that a 13% CP diet with crystalline amino acid supplementation did not

Table 4: Performance of broiler chicks fed a 13% crude protein diets from 7-21 days of age (Experiment 1)

Treatment	Weight gain (g)	Feed intake (g)	Feed: Gain
PC	752[a]	988[ab]	1.31[a]
MLPE	643[b]	982[ab]	1.52[bc]
MHPE	667[b]	973[ab]	1.47[b]
MMPE	660[b]	988[ab]	1.48[b]
BHPE	639[b]	961[b]	1.50[bc]
BMPE	651[b]	999[a]	1.53[c]
Pooled SEM	65.78	8.5	14.3

[ab]Values with differing letters are significantly ($p<0.05$) different

Table 5: Performance of broiler chicks fed a 13% crude protein diets from 7-21 days of age (Experiment 2)

Treatment	Weight gain (g)	Feed intake (g)	Feed: Gain
PC	655[a]	815[a]	1.25[a]
PE-17.5	606[b]	803[a]	1.31[b]
PE-18.75	634[ab]	813[a]	1.29[b]
PE-20	630[ab]	825[a]	1.30[b]
Pooled SEM	8.5	11.4	9.5

[ab]Values with differing letters are significantly ($p<0.05$) different

support similar performance to a 23% CP industry-type diet. In Experiment 1, the addition of glutamic acid to achieve various protein equivalencies failed to bring performance up to control levels. However, the diets with the lowest protein equivalencies in both those formulated with amino acid levels from the University of Missouri and Baker's ideal ratios resulted in numerically worse feed conversions, although statistically these treatments were similar to others. In Experiment 2, the birds receiving the PE-17.5 treatment, which contained amino acids added to meet levels found in the control but no glutamic acid, gained significantly less weight than birds in the PC group. This is in disagreement with some of the previously mentioned literature concerning the importance of adding a non-specific nitrogen source to diets with low levels of CP; however, the 13% crude protein used in these experiments is significantly lower than those reported in the literature, which ranged from 15.3% CP (Aletor *et al.*, 2000) up to just a two or three percent reduction in CP and may have resulted in a deficiency in one or more essential amino acid that was not observed when higher levels of crude protein were fed.

It is unknown why a 13% crude protein diet with amino acid supplementation is unable to provide similar performance in broilers as a 23% crude protein diet, especially as trials conducted at the University of Missouri using turkeys indicate that protein can be reduced from 28% to 10% with the addition of essential amino acids and achieve adequate performance (Moore *et al.*, 2001). A variety of explanations have been hypothesized. One of these includes an insufficient amount of nitrogen for synthesis of nonessential amino acids, leading to a deficiency of essential amino acids. Another hypothesis involves an unfavorable dietary Electrolyte Balance (dEB). In these experiments, the diets low in crude protein replaced soybean meal with a range of crystalline amino acids. This can result in a reduction in dietary K and an increase in the levels of Cl- supplied by those amino acids, lowering the dEB in these types of diets, which may cause the depression in performance (Patience, 1990; Aftab *et al.*, 2006). However, it has been reported that maintaining dEB in low protein diets did not restore performance to the level of control diets (Han *et al.*, 1992; Si *et al.*, 2004), indicating that a disruption in dEB is not the cause of depressed performance in birds consuming low crude protein rations. As a precaution, 1.5% sodium bicarbonate was added to the experimental diets in Experiment 2 (as opposed to 1.0% to the diets in Experiment 1) to prevent a disruption in the metabolic acid-base balance due to high levels of added amino acids.

Another hypothesis is that the requirement for glycine is actually higher in low CP diets than typical high CP diets and a glycine deficiency is the main cause behind the depressed performance observed with the use of diets significantly low in CP. Increased performance has been observed in birds consuming low protein diets when the level of glycine was increased, suggesting that glycine may have a more specific role than previously believed and that the NRC-suggested requirements may be too low (Corzo *et al.*, 2004; Waldroup *et al.*, 2005; Aftab *et al.*, 2006; Dean *et al.*, 2006; Namroud *et al.*, 2008). Namroud and collaborators (2008) suggest that adding significant amounts of crystalline amino acids to low intact CP diets increases blood and excretory ammonia concentrations, which may cause a reduction in growth and appetite due to negative effects on tissue metabolism. They state that in birds, the conversion of ammonia to uric acid requires 1 glycine molecule, resulting in a greater than expected glycine requirement. This may be one explanation why researchers have seen improved performance when birds have been fed diets with increased glycine supplementation. The formulation of low crude protein diets involves a decrease in intact protein sources such as soybean meal that contain relatively high levels of glycine compared to other ingredients, so it may be important to consider glycine levels specifically, rather than just total NEAA levels, when formulating low crude protein diets. While an amino acid deficiency might play a role in the decreased performance observed in birds consuming the 13% CP rations, the ratio between Nonessential Amino Acids (NEAA) and Essential Amino Acids (EAA) may also be an important factor. A ratio near 50:50 NEAA:EAA is often suggested, although a 5% variation in either direction is not uncommon (Aftab *et al.*, 2006). In Experiment 1, the NEAA:EAA ratio ranged from approximately 17:83 for the MLPE treatment to approximately 42:58 for the MHPE diets, while the BHPE treatment had a ratio near 49:51 and the BMPE treatment ratio was near 41:59. Interestingly, the wide variation of the NEAA:EAA ratios in the dietary treatments in this experiment did not seem to cause any clear discrepancies in performance.

Some researchers propose that decreased feed intake, which might occur for a variety of reasons, may be to blame for decreased performance. However, there is little agreement in the literature as to why this might occur; indeed, some researchers have found decreased feed intake in birds consuming low CP diets while others have not. In these experiments, the PC treatment did not result in significantly increased ($p<0.05$) feed intake than other treatments.

Conclusion: Previous research from our laboratory indicates that a diet with 15% CP may support similar performance to that achieved with a 23% CP ration. It is still unclear at this time why a 13% CP ration with amino acid supplementation cannot yield similar results to a 23% CP ration. It may be that the minimum amount of intact protein that is required in the diet to achieve performance similar to that from a 23% CP diet is above 13%. It does not appear that using a non-specific nitrogen source such as glutamic acid to increase the protein equivalent of a 13% CP ration can alleviate the resulting depression in performance. The lowest level to which crude protein can be reduced with amino acid supplementation in broiler diets without reducing bird performance remains unknown. Further research on the subject could potentially allow researchers to determine the digestible requirements for the essential amino acids more precisely by using such diets to study each amino acid through titration experiments and eventually allow formulation of economically efficient diets that precisely meet birds' requirements with little or no excess, leading to significant cost savings in the future.

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