

Effects of Dietary Amino Acid Density and Exogenous Protease Inclusion on Growth Performance and Apparent Ileal AA Digestibility in Broilers¹

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Summary

Protein is one of the most expensive nutrients in poultry diets. In an effort to minimize feed costs, protein digestion and utilization by the animal must be carried out as efficiently as possible. Therefore, the objective of this study was to evaluate the effects of dietary AA density and exogenous protease inclusion on growth performance and AA digestibility in broilers. Treatments consisted of a 2 × 4 factorial design with main effects of commercial protease (with or without) and digestible Lys (1.12, 1.15, 1.18, or 1.21%). Broiler chicks were housed in 4 Petersime batteries and treatments were randomly assigned to 80 cages within location block, resulting in 10 cages per treatment with 6 chicks per cage at placement. A commercial enzyme complex with 3 proteolytic activities was added to the protease diets at 0.25 lb/ton, and the same inclusion of sand was added to the diets without protease. Diets were balanced by energy and Lys:amino acid ratios. Titanium dioxide was included in the diets at 0.5% as an indigestible marker. On d 20, ileal contents from 2 chicks per cage were collected and composited by cage for calculation of apparent ileal AA digestibility. Growth performance metrics were calculated from cage weights and feed consumption was recorded throughout the experiment, and AA digestibility data were obtained from analysis of ileal contents. Data were analyzed using SAS 9.4 with cage as the experimental unit and cage location as the blocking factor. There was no evidence of an amino acid density × protease interaction ($P > 0.05$) for BW, ADG or ADFI. There was an amino acid density × protease interaction (quadratic, $P < 0.05$) for feed conversion ratio (FCR). Chicks fed 1.12 and 1.21% digestible Lys diets with added protease had a 2-point improvement in FCR compared to chicks fed these diets without protease. There was no difference in FCR between birds consuming diets with or without protease when fed 1.15 and 1.18% digestible Lys diets. There was no evidence of difference ($P > 0.10$) in ADG or ADFI

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due to dietary amino acid density throughout the feeding period. However, broiler FCR was improved (linear, $P < 0.01$) by increasing dietary amino acid density from 1.12 to 1.21% digestible Lys. There was no evidence ($P > 0.10$) of main effect of added protease on BW, ADG, ADFI, or FCR. There was not an amino acid density \times protease interaction ($P > 0.09$) or main effect of dietary amino acid density or protease inclusion ($P > 0.12$) on apparent ileal digestibility (AID) of Lys, Arg, Met, Cys, Thr, Ile, Leu, Val, or Trp. In conclusion, increasing dietary amino acid density improved FCR in broiler chicks, and the rate of improvement was dependent on the inclusion of an exogenous protease.

Introduction

Biological tissues and metabolic processes used for growth and maintenance require certain amino acids. If not provided in the correct ratio, body protein must be degraded to provide the necessary amino acids (AA) needed for such processes. Commonly in poultry diets, dietary protein is supplied by solvent-extracted soybean meal, not only because of its high protein content but also because of its AA profile. The ratios of essential AA within soybean meal make it one of the most desirable proteinaceous feed ingredients as it closely matches the balance of AAs needed by the growing chick. However, the apparent ileal digestibility of CP in soybean meal in broilers is approximately 82%, indicating 18% of the feedstuff remains undigested and is not used for growth. Furthermore, ileal digestibility of the individual AAs in soybean meal range from 75 to 88% for broilers.⁵ In diets using a byproduct protein source, such as meat and bone meal or feather meal, AA digestibility coefficients can be as low as 65%, suggesting an even greater opportunity for improvement of AA digestibility in byproduct diets.

Exogenous proteases are nutritional tools designed to enhance protein digestion in livestock and poultry diets. They are added to the diet to supplement the activity of endogenous proteases that are secreted by the animal and drive protein degradation. Combining the effects of both proteases results in the potential for greater peptide bond cleavage and, thus, more efficient proteolysis. Improvements in protein digestion through the use of an exogenous protease may allow the quantity of protein in the diet to be lowered without impairing animal performance. Such a dietary modification could ultimately lower production expenses by reducing feed costs. However, available research concerning exogenous protease use is inconsistent and limited. Thus, the following experiment was designed to determine the effect of exogenous protease inclusion on growth performance and AA digestibility in broiler diets.

Materials and Methods

A total of 480 1-d-old male Cobb 500 broilers (Cobb-Vantress, Siloam Springs, AR) were used in a 20-d study to determine the effects of adding protease to diets with varying concentrations of digestible Lys on growth performance and apparent ileal digestibility (AID). Treatments consisted of a 2×4 factorial design with main effects of commercial protease (with or without) and digestible Lys (1.12, 1.15, 1.18, or 1.21%). Chicks were housed in 4 Petersime batteries, and treatments were randomly assigned to 80 cages within location block, resulting in 10 cages per treatment with 6 chicks

⁵ Ravindran, V., L. I. Hew, G. Ravindran, and W. L. Bryden. 2005. Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. *J. Anim. Sci.* 81:85-97.

per cage at placement. A commercial enzyme complex with 3 proteolytic activities was added to the protease diets at 0.25 lb/ton, and the same inclusion of sand was added to the diets without protease. Diets were balanced by energy and Lys:AA ratios. Titanium dioxide was included in the diets at 0.5% as an indigestible marker.

Whole corn was ground to approximately 700 μm using a 3-high roller mill (RMS, Model 924). Experimental diets were mixed in a 1000-lb horizontal counterpoise mixer (Hayes and Stolz, Fort Worth, TX) at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS (Table 1). To ensure uniform diet composition across treatments, the 1.12% and 1.21% digestible Lys treatments were each mixed in a single batch. Feed from each treatment was then blended to create the intermediate (1.15% and 1.18%) treatments. Half of each treatment batch was mixed with the exogenous protease and half was mixed with sand to form the Lys treatments with protease and Lys treatments without protease, respectively. All diets were steam conditioned (Wenger twin shaft pre-conditioner, Model 150) for approximately 30 s at 185°F and subsequently pelleted using a 30-horsepower pellet mill (1012-2 HD Master Model, CPM) equipped with a $\frac{5}{32} \times 1 \frac{1}{4}$ in. pellet die. Pellets were cooled to room temperature in an experimental counterflow cooler and crumbled (CME, EcoRoll 7).

Chicks were maintained on a 24-h lighting schedule in an environmentally controlled room with *ad libitum* access to feed and water. Each cage measured approximately 39.4 \times 28.7 in. and was fitted with a single bulk feeder and waterer. In the case of mortality, chick weight, treatment, cage number, and date of mortality were recorded. Initial cage BW was measured at placement of chicks on d 1. Cage weights and feed consumption were measured on d 7, 14, and 20 for calculation of body weight gain, feed intake, and feed efficiency. Diet samples were collected for analysis of titanium dioxide and amino acid profile. On d 20, ileal contents from 2 chicks per cage were collected, composited by cage, and immediately frozen. Frozen ileal samples were subsequently freeze-dried and ground for analysis of titanium dioxide, crude protein, and AA profile for calculation of apparent ileal AA digestibility. Values for AID (%) were calculated as indicated below using the following equation.⁶

$$\text{AIDAA (\%)} = \left[1 - \left(\frac{\text{AA}_{\text{diet}}}{\text{AA}_{\text{digesta}}} \right) \times \left(\frac{\text{Ti}_{\text{digesta}}}{\text{Ti}_{\text{diet}}} \right) \right] \times 100$$

Statistical analysis

Data were analyzed as a randomized complete block design using the MIXED procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC) to evaluate interactive and main effects of Lys and protease. Increasing Lys level linear and quadratic polynomials were used for main effects of Lys. Results were considered significant at $P \leq 0.05$.

Results and Discussion

There was no evidence of a Lys \times protease interaction ($P > 0.05$) for BW, ADG, or ADFI (Table 3). There was a Lys \times protease interaction (quadratic, $P < 0.05$) for FCR from d 0 to 20. Chicks fed 1.12 and 1.21% digestible Lys diets with added protease had

⁶ H. H. Stein, B. Sève, M. F. Fuller, P. J. Moughan, C. F. M. de Lange, Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application, *Journal of Animal Science*, Volume 85, Issue 1, January 2007, Pages 172–180, <https://doi.org/10.2527/jas.2005-742>.

a 2-point improvement in FCR compared to chicks fed these diets without protease. There was no difference in FCR between birds consuming diets with or without protease when fed 1.15 and 1.18% digestible Lys diets. There was no evidence of difference ($P > 0.10$) in ADG or ADFI due to dietary Lys concentration throughout the feeding period. However, from d 0 to 20, broiler FCR was improved (linear, $P < 0.01$) by increasing the dietary digestible Lys from 1.12 to 1.21%. There was no evidence ($P > 0.10$) of main effect that added protease influenced BW, ADG, ADFI, or FCR. There was no evidence of a dietary Lys \times protease interaction ($P > 0.09$) or main effect of dietary digestible Lys concentration or protease inclusion ($P > 0.12$) on AID of Lys, Arg, Met, Cys, Thr, Ile, Leu, Val, or Trp (Table 4).

In conclusion, increasing dietary AA density improved FCR in broilers, and the rate of improvement was dependent on the inclusion of an exogenous protease. The results of this study would suggest an apparent improvement in FCR by way of increased nutrient availability; however, AID of AA was not affected by protease supplementation in broilers.

Table 1. Diet composition (as-fed)^{1,2}

| Item, % | 1.12 | 1.21 |
|-------------------------------|-------|-------|
| Ground corn | 60.26 | 56.16 |
| Soybean meal | 32.50 | 36.00 |
| Choice white grease | 2.00 | 2.60 |
| Monocalcium phosphate | 2.10 | 2.05 |
| Limestone | 1.40 | 1.40 |
| Salt | 0.23 | 0.23 |
| L-Lysine HCL | 0.15 | 0.15 |
| DL-Methionine | 0.23 | 0.26 |
| L-Threonine | 0.08 | 0.09 |
| L-Valine | 0.00 | 0.01 |
| Vitamin premix ³ | 0.25 | 0.25 |
| Choline chloride | 0.10 | 0.10 |
| Sodium bicarbonate | 0.20 | 0.20 |
| Titanium dioxide ⁴ | 0.50 | 0.50 |
| Total | 100 | 100 |

¹A total of 480 1-d-old male Cobb 500 broilers (Cobb-Vantress, Siloam Springs, AR) were used in a 20-d study to determine the effects of adding protease to diets with varying concentrations of digestible Lys on growth performance and ileal digestibility. Each diet was fed with and without an exogenous protease, which was added to the feed at 0.25 lb/ton.

²Diets formulated to provide 1.12 and 1.21% digestible Lys were blended to create the intermediate digestible Lys diets.

³Provided per lb of premix: vitamin A, 1,402,927 IU; vitamin D₃, 501,045.45 IU; vitamin E, 3,006 IU; vitamin B₁₂, 2 mg; menadione, 150 mg; riboflavin, 1,202 mg; D-pantothenic acid, 1,202 mg; thiamine, 200 mg; niacin, 5,010 mg; vitamin B₆, 250 mg; folic acid, 125 mg; biotin, 6 mg.

⁴Titanium dioxide was added as an indigestible marker for determination of apparent ileal amino acid digestibility.

Table 2. Effect of dietary amino acid density and exogenous protease inclusion on broiler growth performance¹

| Dietary Lys, ³ %: | | | | | | | | | | Probability, <i>P</i> < | | | | |
|------------------------------|-------------------|--------------------|-------------------|--------------------|-----------------------|--------------------|--------------------|-------------------|------------------|-------------------------|-----------|----------|----------------|-----------|
| | No protease | | | | Protease ² | | | | SEM ⁴ | Lys | | Protease | Lys × protease | |
| | 1.12 | 1.15 | 1.18 | 1.21 | 1.12 | 1.15 | 1.18 | 1.21 | | Linear | Quadratic | | Linear | Quadratic |
| BW, lb | | | | | | | | | | | | | | |
| d 0 | 0.089 | 0.090 | 0.089 | 0.089 | 0.089 | 0.090 | 0.089 | 0.089 | 0.0003 | 0.425 | 0.385 | 0.385 | 0.638 | 0.891 |
| d 20 | 1.77 | 1.76 | 1.77 | 1.81 | 1.79 | 1.77 | 1.79 | 1.81 | 0.022 | 0.198 | 0.146 | 0.380 | 0.779 | 0.976 |
| d 0 to 20 | | | | | | | | | | | | | | |
| BWG, lb | 1.67 | 1.67 | 1.68 | 1.68 | 1.69 | 1.68 | 1.70 | 1.70 | 0.023 | 0.456 | 0.796 | 0.350 | 0.939 | 0.742 |
| FI, lb | 2.09 | 2.07 | 2.06 | 2.05 | 2.08 | 2.07 | 2.09 | 2.05 | 0.025 | 0.179 | 0.779 | 0.650 | 0.897 | 0.532 |
| FCR | 1.25 ^a | 1.24 ^{ab} | 1.22 ^b | 1.22 ^{bc} | 1.23 ^{ab} | 1.24 ^{ab} | 1.23 ^{ab} | 1.20 ^c | 0.007 | 0.001 | 0.226 | 0.181 | 0.925 | 0.035 |

¹A total of 480 1-d-old Cobb 500 male broilers were used in a 20-d study with 10 replicate cages per treatment and 6 broilers per cage.

²An exogenous protease was added to protease treatments at 0.25 lb/ton, and sand replaced protease at the same inclusion level in treatments not containing protease.

³Diets were formulated to provide 1.12, 1.15, 1.18, or 1.21% digestible Lys, approximately 95, 97.5, 100, and 102.5% of the digestible Lys recommendation, respectively.

⁴Pooled standard error of least squares means (n = 10).

^{abc}Means within a row without a common superscript differ (*P* < 0.05).

FI = feed intake. FCR = feed conversion ratio.

Table 3. Effect of dietary amino acid density and exogenous protease inclusion on apparent ileal amino acid digestibility (AID) coefficients in broilers¹

| Dietary Lys, ³ %: | No protease | | Protease ² | | SEM ⁴ | Probability, <i>P</i> < | | |
|------------------------------|-------------|------|-----------------------|------|------------------|-------------------------|------|----------------|
| | 1.12 | 1.21 | 1.12 | 1.21 | | Protease | Lys | Lys × Protease |
| Arg | 88.3 | 89.6 | 88.9 | 87.9 | 0.006 | 0.39 | 0.88 | 0.09 |
| Cys | 70.7 | 72.6 | 70.6 | 72.8 | 0.013 | 0.95 | 0.12 | 0.88 |
| Ile | 81.8 | 82.9 | 81.6 | 80.7 | 0.009 | 0.19 | 0.95 | 0.28 |
| Leu | 82.7 | 83.4 | 82.5 | 81.5 | 0.009 | 0.25 | 0.89 | 0.35 |
| Lys | 85.6 | 86.8 | 85.6 | 84.5 | 0.009 | 0.21 | 1.00 | 0.21 |
| Met | 92.2 | 92.7 | 92.0 | 91.5 | 0.006 | 0.26 | 0.99 | 0.37 |
| Thr | 76.2 | 77.3 | 76.4 | 77.2 | 0.010 | 0.97 | 0.35 | 0.86 |
| Trp | 85.4 | 84.3 | 84.3 | 84.9 | 0.006 | 0.72 | 0.68 | 0.19 |
| Val | 78.4 | 79.4 | 78.4 | 78.0 | 0.010 | 0.48 | 0.75 | 0.51 |

¹A total of 64 broilers were used to determine AA AID on d 20 with 8 replicate cages per treatment. Ileal contents from 2 broilers per cage were composited and AA AID was calculated.

²An exogenous protease was added to protease treatments at 0.25 lb/ton, and sand replaced protease at the same inclusion level in treatments not containing protease.

³Diets were formulated to provide 1.12, 1.15, 1.18, or 1.21% digestible Lys, approximately 95, 97.5, 100, and 102.5% of the digestible Lys recommendation, respectively.

⁴Pooled standard error of least squares means (n = 8).