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

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Effects of Dietary Amino Acid Density and Exogenous Protease Inclusion on Growth Performance and Apparent Ileal Amino Acid Digestibility in Turkeys[1]

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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. The objective of this experiment was to evaluate the effects of dietary amino acid density and exogenous protease inclusion on growth performance and amino acid digestibility in turkey poults. Hybrid turkey poults (n = 780) were fed diets formulated to provide low (LAA) or adequate (AAA) amino acid density (approximately 91 and 100% of the NRC[4] recommended requirement for digestible Lys, respectively) with each diet being fed with or without an exogenous protease. Poults received experimental diets from d 1 to 42 of age. Growth performance metrics were calculated from pen weights and feed consumption was recorded throughout each experiment, and digestibility data were obtained from analysis of ileal contents. Data were analyzed using SAS 9.4 with pen as the experimental unit and pen location as the blocking factor. For the overall experiment (d 0 to 42), poults fed AAA diets had improved ($P < 0.01$) ADG, ADFI, and feed conversion ratio (FCR) compared to those fed LAA diets. There was no difference ($P > 0.14$) in poult performance due to protease inclusion. There was an amino acid density × protease interaction ($P = 0.01$) for apparent ileal amino acid digestibility (AIAAD) of Trp. There was no difference in AIAAD coefficients of Trp in the LAA diets with or without protease. However, in the AAA diets, poults not receiving protease had greater AIAAD of Trp than those consuming protease. There was no difference ($P > 0.09$) in AIAAD coefficients of Arg, Met, Cys, Thr, Ile, Leu, Lys, or Val due to dietary amino acid density or protease inclusion. Greater amino acid density improved growth performance in poults up to 42 d of age. No improvement in growth performance was observed when poults were fed an exogenous protease in the starter phase, although protease inclusion increased ADG by 5.3% and ADFI by 4.1% during the grower phase. There was no benefit of increased dietary amino acid density or protease inclusion on AIAAD in poults.

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[4] National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/13298.

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 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. Furthermore, ileal digestibilities of the individual AAs in soybean meal range from 75 to 88% for poultry.[5]

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 digestion. Combining the effects of both exogenous and endogenous 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 turkey diets.

Materials and Methods

The Institutional Animal Care and Use Committee at Kansas State University (Manhattan, KS) reviewed and approved the protocols used in this study. A 42-d experiment was conducted at the Kansas State University Tom Avery Poultry Research Farm to evaluate the effects of dietary amino acid density and exogenous protease inclusion on growth performance and AIAAD in turkeys. A total of 780 1-d-old female poults (Hybrid Genetics, Ag Forte LLC, Aurora, MO) were weighed and randomly assigned to 1 of 32 floor pens with 24 or 25 poults per pen. Treatments were arranged in a 2 × 2 factorial of amino acid density (low [LAA] or adequate [AAA]) and commercial protease (with or without). Treatments were randomly assigned to floor pens within location block for a total of 8 replications per treatment. Poults were fed a starter diet from d 1 to 28 and a grower diet from d 29 to 42 (Table 1). Starter diets were formulated to provide 1.52% and 1.62% digestible Lys for the LAA and AAA treatments, respectively. Grower diets were formulated to provide 1.39% and 1.49% digestible Lys, respectively. A commercial enzyme complex with 3 unique proteases was added to the protease diets at 125 g/ton, and the same inclusion of sand was added to the diets without protease. Diets were balanced by energy and had equal amino acid ratios. Titanium dioxide was included in the grower diets at 0.5% as an indigestible marker for determination of AIAAD.

Diets were formulated using NRC[4] values for corn and soybean meal. Whole corn was ground to approximately 700 µm in the starter phase and 900 µm in the grower phase using a hammermill (Bliss Industries, Model 22115). Experimental mash diets were mixed in a 1-ton horizontal counterpoise mixer (Hayes and Stolz, Fort Worth, TX)

[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.

at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. To ensure uniform diet composition across low digestible Lys treatments, a single batch of the LAA starter phase formulation was mixed and split in half. Half of the batch was mixed with the exogenous protease and half was mixed with sand to form the LAA with protease and LAA without protease treatments, respectively. For the protease diets, exogenous protease was hand-mixed with 5 lb of ground corn for 60 s before mixing with all other ingredients to facilitate uniform dispersion of the protease throughout the batch. The same method was followed for the addition of sand in diets not containing protease. These methods were repeated to form the AAA treatments with and without protease for the starter phase, as well as the treatments for the grower phase.

Poults were maintained on a lighting schedule with 21 h of light and 3 h of dark in an environmentally-controlled room with *ad libitum* access to feed and water. Poults were housed in 5 × 14 ft floor pens with new pine shavings. Each pen was fitted with a single hanging metal feeder and a Lubing EasyLine 4-cup drinker system. Feeders were shaken twice a day to ensure adequate feed flow to the feeder pan. In the case of mortality, poult weight, treatment, pen number, and date of mortality were recorded. Pen weights and feed consumption were measured on d 14, 28, and 42 for calculation of body weight gain, feed intake, and feed efficiency. Diet samples were collected from each pen during the grower phase and composited by treatment for analysis of titanium dioxide and amino acid profile. On d 42, ileal contents from 2 poults per pen were collected, composited by pen, and immediately frozen. Ileal samples were subsequently freeze-dried and ground for analysis of titanium dioxide, gross energy, crude protein, and amino acid profile for calculation of AIAAD.[6]

Statistical analysis

Data were analyzed as a 2 × 2 factorial using the GLIMMIX procedure in SAS 9.4 with pen as the experimental unit and pen location as the blocking factor. Main effects included amino acid density and protease inclusion. Results were considered significant if $P \leq 0.05$.

Results and Discussion

There was not an amino acid density × protease interaction ($P > 0.12$) for BW, ADG, ADFI, or FCR (Table 2). On d 28 and 42, poults fed AAA had greater ($P < 0.01$) BW than those fed LAA diets. From d 1 to 28, poults fed AAA diets had improved ($P < 0.01$) ADG, ADFI, and FCR compared to those fed LAA diets. From d 1 to 28, there was no difference ($P > 0.43$) in poult performance due to protease inclusion. From d 29 to 42, poults fed AAA diets had improved (P < 0.04) ADG, ADFI, and FCR compared to those fed LAA diets. Poults had increased ($P < 0.03$) ADG and ADFI when fed an exogenous protease compared to those fed diets without added protease. There was no difference in final BW at d 42 ($P = 0.06$) or FCR in the grower phase ($P = 0.53$) due to protease inclusion. For the overall experiment (d 0 to 42), poults fed AAA diets had improved ($P < 0.01$) ADG, ADFI, and FCR compared to those fed LAA diets. There was no difference ($P > 0.14$) in poult performance due to protease inclusion.

[6] Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández, and C. F. M. de Lange. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest. Sci. 109:282-285.

There was an amino acid density × protease interaction ($P = 0.01$) for AIAAD of Trp (Table 3). There was no difference in AIAAD coefficients of Trp in the LAA diets with or without protease. However, in the AAA diets, poults not receiving protease had greater AIAAD of Trp than those consuming protease. There was no difference ($P > 0.09$) in AIAAD coefficients of Arg, Met, Cys, Thr, Ile, Leu, Lys, or Val due to dietary amino acid density or protease inclusion.

This turkey experiment was conducted from May to July, and outdoor ambient temperatures averaged 93°F during the 42-d experimental period with average temperatures rising to 99°F during the last 2 weeks of the study. Thus, despite attempts to provide adequate ventilation and airflow in the barn, it is possible poults experienced heat stress over the course of the study, especially during the grower phase. Depressions in feed intake are known to occur at temperatures exceeding the poult's thermoneutral zone, which is above approximately 75°F. The potential impacts that these environmental factors may have had on the response to protease treatment is not entirely known. Nonetheless, at 28 and 42 d of age, poults' BW and feed intake were below breed estimates. Theoretically, a decrease in feed intake, and thus protein intake, would perhaps work in the favor of protease supplementation.

In conclusion, greater amino acid density improved growth performance in poults up to 42 d of age. However, there was no improvement in growth performance observed when poults were fed an exogenous protease in the starter phase, although protease inclusion increased ADG by 5.3% and ADFI by 4.1% during the grower phase. There was no benefit of increased dietary amino acid density or protease inclusion on AIAAD in poults.

Table 1. Composition (as-fed basis) of turkey mash diets with low or adequate dietary amino acid densities with and without added protease[1]

Diet phase:	Starter		Grower	
Digestible Lys:	**LAA**	**AAA**	**LAA**	**AAA**
Ingredient, %				
Ground corn	44.15	39.25	49.96	45.31
Soybean meal	45.00	49.20	39.00	43.00
Soybean oil	4.00	4.70	4.00	4.65
Monocalcium phosphate	3.15	3.15	3.00	3.00
Limestone	2.05	2.02	1.85	1.82
Salt	0.36	0.36	0.30	0.30
L-Lysine HCl	0.27	0.27	0.30	0.30
DL-Methionine	0.34	0.37	0.28	0.31
L-Threonine	0.08	0.08	0.08	0.08
Vitamin premix[2]	0.20	0.20	0.20	0.20
Mineral premix	0.20	0.20	0.20	0.20
Choline chloride	0.20	0.20	0.20	0.20
Sodium bicarbonate	0.00	0.00	0.13	0.13
Titanium dioxide[3]	0.00	0.00	0.50	0.50
Calculated nutrients				
ME, kcal/lb	1,346	1,346	1,346	1,346
Crude protein, %	26.0	27.7	25.5	27.0
Arginine, %	1.55	1.67	1.53	1.63
Isoleucine, %	0.99	1.06	0.98	1.04
Leucine, %	1.89	1.98	1.87	1.96
Lysine, %	1.52	1.62	1.39	1.49
Methionine, %	0.68	0.72	0.63	0.65
Threonine, %	0.90	0.96	0.89	0.91
Tryptophan, %	0.28	0.30	0.27	0.29
Valine, %	1.06	1.13	1.05	1.11
Calcium, %	1.45	1.45	1.33	1.34
Phosphorus, %	1.09	1.10	1.05	1.07

[1]Starter diets were formulated to provide 1.52% and 1.62% digestible Lys for the low (LAA) and adequate (AAA) amino acid density treatments, respectively, and grower diets were formulated to provide 1.39% and 1.49% digestible Lys, respectively. Starter diets were fed from d 0 to 28, and grower diets were fed from d 29 to 42.

[2]Provided per kg of premix: vitamin A, 13,200,000 IU; vitamin D_3, 3,960,000 IU; vitamin E, 66,000 IU; vitamin B_{12}, 39.6 mg; menadione, 3,960 mg; riboflavin, 13,200 mg; D-pantothenic acid, 22,000 mg; thiamine, 3,960 mg; niacin, 110,000 mg; vitamin B_6, 7,920 mg; folic acid, 2,200 mg; biotin, 253 mg.

[3]Titanium dioxide was added to grower diets 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 turkey growth performance[1]

	No protease[2]		Protease			Probability, $P <$		
Dietary Lys:[3]	LAA	AAA	LAA	AAA	SEM[4]	Lys	Protease	Lys × protease
BW, lb								
d 1	0.139	0.139	0.139	0.139	0.0002	0.637	0.957	0.573
d 28	2.25	2.36	2.27	2.38	0.022	0.001	0.177	0.971
d 42	4.85	5.09	4.92	5.18	0.045	0.001	0.062	0.793
d 0 to 28								
ADG, lb	0.075	0.079	0.075	0.080	0.0008	0.001	0.426	0.617
ADFI, lb	0.107	0.110	0.107	0.110	0.0014	0.005	0.585	0.940
FCR	1.43	1.40	1.43	1.39	0.008	0.001	0.576	0.136
d 29 to 42								
ADG, lb	0.186	0.195	0.189	0.199	0.0017	0.001	0.028	0.625
ADFI, lb	0.307	0.317	0.312	0.325	0.0036	0.001	0.021	0.538
FCR	1.66	1.63	1.66	1.64	0.011	0.043	0.525	0.772
d 0 to 42								
ADG, lb	0.112	0.118	0.113	0.120	0.011	0.001	0.172	0.475
ADFI, lb	0.173	0.179	0.175	0.182	0.0020	0.001	0.137	0.582
FCR	1.55	1.52	1.56	1.52	0.009	0.001	0.755	0.799

[1]A total of 780 female poults were used in a 42-d study with 24 or 25 birds per pen and 8 pens per treatment.
[2]An exogenous protease was added to protease treatments at 125 g/ton, and sand replaced protease at the same inclusion level in treatments not containing protease.
[3]Low amino acid density (LAA) diets were formulated to provide 1.52% digestible Lys from d 0 to 28 and 1.39% digestible Lys from d 29 to 42. Adequate amino acid density (AAA) diets were formulated to provide 1.62% digestible Lys from d 0 to 28 and 1.49% digestible Lys from d 29 to 42.
[4]Pooled standard error of least squares means (n = 8).
FCR = feed conversion ratio.

Table 3. Effect of dietary amino acid density and exogenous protease inclusion on apparent ileal amino acid digestibility (AIAAD) coefficients in turkeys[1]

	No protease[2]		Protease			Probability, *P* <		
Dietary Lys:[3]	LAA	AAA	LAA	AAA	SEM[4]	Lys	Protease	Lys × protease
Arginine	91.3	92.0	91.0	91.6	0.006	0.24	0.59	0.97
Cysteine	77.4	79.7	77.4	75.8	0.016	0.85	0.22	0.23
Isoleucine	86.2	87.6	86.1	86.6	0.008	0.29	0.50	0.62
Leucine	87.1	88.3	86.8	87.0	0.008	0.37	0.33	0.54
Lysine	90.5	91.3	90.2	90.3	0.006	0.49	0.29	0.51
Methionine	94.4	95.2	94.8	94.4	0.003	0.59	0.54	0.09
Threonine	83.1	85.5	83.3	82.5	0.010	0.45	0.19	0.12
Tryptophan	89.3[b]	91.7[a]	89.8[ab]	88.6[b]	0.007	0.41	0.06	0.01
Valine	83.4	85.7	83.1	83.8	0.010	0.16	0.30	0.45

[1]A total of 64 poults were used to determine AIAAD on d 42 with 8 replicate pens per treatment. Ileal contents from 2 poults per pen were composited and AIAAD was calculated according to Stein et al. (2007).
[2]An exogenous protease was added to protease treatments at 125 g/ton, and sand replaced protease at the same inclusion level in treatments not containing protease.
[3]Low amino acid density (LAA) diets were formulated to provide 1.39% digestible Lys in the grower phase, and adequate amino acid density (AAA) diets were formulated to provide 1.49% digestible Lys in the grower phase.
[4]Pooled standard error of least squares means (n = 8).
[abc]Means within a row without a common superscript differ ($P < 0.05$).