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Dietary Interactions Between Threonine and Crude Protein in Diets for Growing Tom Turkeys 8 to 12 Weeks of Age¹

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Abstract: An experiment was conducted to estimate the Thr needs of male Large White turkeys from 8 to 12 week of age and to evaluate the effects of dietary CP on Thr needs. The dietary treatments consisted of a 2 x 6 factorial arrangement with two levels of CP (16.0 and 19.8%), each containing 0.50% total Thr by analysis. Aliquots of each CP basal diet were supplemented with L-Thr to provide total Thr levels of 0.50, 0.57, 0.64, 0.71, 0.78, and 0.85%. Initial and final bird weights were taken to determine BW gain; feed conversion ratios were calculated with adjustment for weight of birds that died during the study. Nonlinear and quadratic regression analyses were used to estimate a Thr requirement to optimize BW gain and feed conversion. For both BW gain and feed conversion, significant interactions were observed between dietary CP and Thr levels. Performance was superior for poult fed the low CP diet series, noted primarily at the lower levels of Thr. As levels of Thr reached a surfeit, no significant differences in performance were noted between poult fed the two CP levels. Using nonlinear regression analysis, dietary Thr levels of 0.68 (16.0% CP) and 0.76% (19.8%) were adequate for maximum BW gain while Thr levels of 0.61 (16.0% CP) and 0.70% (19.8%) were adequate for optimum feed conversion. However, when estimates were based on fitting a quadratic regression, dietary Thr levels of 0.83 (16.0% CP) and 0.98% (19.8%) were adequate for maximum BW gain, while Thr levels of 0.77 (16.0% CP) and 0.82% (19.8%) were adequate for optimum feed conversion. While the interactions between dietary CP and Thr may be interpreted to suggest that dietary CP levels influence amino acid requirements, the two basal diets were composed of protein sources that differ in digestibility of Thr. It is possible that the reduced BW gains and impaired feed conversion observed at the lowest level of Thr supplementation on the high CP diets were the result of a lower level of digestible Thr, rather than a direct influence of CP level per se.

Key Words: Turkeys, threonine, crude protein, requirements

Introduction

It is common practice to formulate turkey diets with minimum nutrient values for dietary CP and the most limiting essential amino acids. Little research exists, however, concerning needs for amino acids other than Lys and TSAA for growing and finishing turkeys. In addition, satisfying amino acid needs while maintaining a minimum CP may frequently increase diet cost. An understanding of how CP affects individual amino acid requirements will allow nutritionists to formulate nutritionally adequate diets at least cost.

Grau (1948) suggested that amino acids are used less efficiently at high dietary CP levels. Similarly, research with chicks has demonstrated that requirements for Met and Lys increase as CP increases (Morris *et al.*, 1987; Abebe and Morris, 1990; Morris *et al.*, 1992). However, Boomgaardt and Baker (1973) demonstrated that the chick's Lys requirement remained constant in diets differing in CP from deficient to adequate. Thus, published reports evaluating the interrelationship of CP and amino acid requirements vary.

The Thr needs of poultry may be increased when they are fed diets high in CP. Robbins (1987) demonstrated that the Thr requirement of a chick increases as dietary CP increases. No research exists evaluating the effect of CP on Thr needs of turkeys. Therefore, this experiment was conducted to determine if dietary CP affects the Thr needs of male turkeys 8 to 12 week of age and to provide estimates of the Thr requirements for this age period for male Large White turkeys.

Materials and Methods

Day-old male poults of a commercial Large White strain² were

randomly allocated into floor pens in a commercial steel-truss type house with 16 poults placed in each of 96 pens (0.34 m²) per bird). Each pen contained one bell water fountain and two tube feeders. New softwood shavings served as litter. Infrared brooding lamps provided heat while thermostatically controlled fans and sidewall curtains controlled ventilation. The initial brooding temperature was 32 °C, decreasing 2 °C per week to a final temperature of 24 °C.

All poults were fed nutritionally adequate diets (NRC, 1994) from 0 to 8 week of age. From 8 to 12 week, turkeys received one of 12 dietary treatments in a 2 x 6 factorial arrangement with eight replicate pens per treatment. The experimental treatments consisted of two basal test diets differing in CP, each with six levels of Thr. The test diets (Table 1) were composed primarily of corn, soybean meal, peanut meal, and wheat middlings. Lysine, Met, and TSAA were formulated to meet a minimum of 110% of NRC (1994) recommendations. Other essential amino acids were formulated to provide a minimum of 105% of NRC (1994) recommendations. The low-CP (16.0%) and high-CP (19.8%) diets each contained 0.50% Thr by analysis. Aliquots of each CP diet were supplemented with L-Thr in five increments of 0.07% up to 0.85% total dietary Thr. The L-Thr was added in place of an isonitrogenous-isocaloric mixture composed of 61.32% L-Glu, 37.76% cornstarch, and 0.92% cellulose. The two basal CP diets were analyzed in duplicate for CP, dry matter (AOAC, 1984) and total amino acids (Llames and Fontaine, 1994). Composite samples of the twelve dietary treatments were analyzed in duplicate for CP, dry matter, and for added Thr (NFIA, 1991).

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²British United Turkeys of America, Lewisburg WV 24901)

Table 1: Ingredient composition (g/kg) and nutrient analysis of test diets

Ingredient	Low CP	High CP		
Yellow corn	754.88	550.30		
Soybean meal	140.00	0.00		
Poultry oil	17.00	65.00		
Peanut meal	9.00	277.00		
Wheat middlings	8.00	41.00		
Defluorinated phosphate	18.96	18.09		
Ground limestone	11.26	12.21		
Sodium bicarbonate	6.00	6.00		
Feed grade salt	0.44	0.38		
BMD-50 ¹	0.50	0.50		
Inert filler ²	3.60	3.60		
Vitamin premix ³	5.00	5.00		
Trace mineral mix ⁴	1.00	1.00		
L-Lysine HCl (98%)	10.16	10.80		
DL-Methionine (98%)	4.06	3.80		
L-Arginine	3.33	0.00		
L-Valine	3.16	2.08		
L-Isoleucine	3.07	2.59		
L-Tryptophan	0.58	0.65		
	1000.00	1000.00		
Nutrient composition ⁵	C	A	C	A
ME, kcal/kg	3145.66		3149.36	
Crude protein, %	16.01	16.61	19.80	19.89
Thr, %	0.50	0.50	0.50	0.50
Lys, %	1.43	1.62	1.43	1.43
Met, %	0.64	0.63	0.61	0.56
Ile, %	0.84	0.85	0.84	0.75
Val, %	0.95	1.01	0.95	0.91
Arg, %	1.16	1.22	1.65	1.57
Trp, %	0.21	0.22	0.21	0.22
Met+Cys, %	0.88	0.91	0.88	0.87
Gly+Ser, %	1.21	1.17	1.65	1.63
Phe+Tyr, %	1.16	1.12	1.46	1.42
Ca, %	0.90		0.90	
Nonphytate P, %	0.45		0.45	

¹Alpharma, Inc., Ft. Lee, NJ. Each kg contains 110 g bacitracin activity.

²Aliquots of L-Thr were added in place of an isonitrogenous-isocaloric filler.

³Provides per kg of diet: Vitamin A (from retinyl acetate), 9,900 IU; cholecalciferol, 3,300 IU; vitamin E (from dl-alpha tocopheryl acetate), 13 IU; vitamin B₁₂, 0.013 mg; riboflavin, 6.6 mg; niacin, 66 mg; d-pantothenic acid, 16.5 mg; choline, 660 mg; menadione (from menadione dimethylpyrimidinol), 1.1 mg; folacin, 1.1 mg; thiamin (from thiamin mononitrate), 1.1 mg; pyridoxine (from pyridoxine hydrochloride), 3.3 mg; d-biotin, 0.11 mg; Se, 0.20 mg; ethoxyquin, 125 mg.

⁴Provides per kg of diet: Mn (from MnSO₄·H₂O) 100 mg; Zn (from ZnSO₄·7H₂O) 100 mg; Fe (from FeSO₄·7H₂O) 50 mg; Cu (from CuSO₄·5H₂O) 10 mg; I (from Ca(IO₃)₂·H₂O) 1 mg.

⁵C = calculated; A = analyzed.

Body weight of individual poult was obtained at 8 and 12 week of age and BW gain during the period determined. Feed consumption was determined for the test period. Mortality was recorded throughout the 8 to 12 week test period; weight of dead birds was used to adjust feed conversion data. Feed conversion (FCR) was calculated from these data (FCR = grams feed consumed ÷ (weight gain of live birds + weight gain of dead birds)).

Table 2: Body weight gain (g) from 8 to 12 weeks by male Large White turkeys fed diets with two crude protein levels and various levels of threonine

% Thr	16.0% CP	19.8% CP	Mean
0.50	3023 ^f	2490 ^g	2756
0.57	3490 ^e	2899 ^f	3195
0.64	3827 ^d	3372 ^e	3600
0.71	4098 ^{bc}	3372 ^e	4054
0.78	4163 ^{bc}	4009 ^c	4211
0.85	4243 ^{ab}	4259 ^{ab}	4295
Mean	3807	3563	
Factor	P diff		
Crude protein	0.001		
Threonine level	0.001		
Protein x threonine	0.001		
Coefficient of variation	3.57		

^{abcdegh}Means with common superscript do not differ significantly (P < 0.05).

Table 3: Feed conversion (grams feed per gram gain) from 8 to 12 weeks by male Large White turkeys fed diets with two crude protein levels and various levels of threonine

% Thr	16.0% CP	19.8% CP	Mean
0.50	3.468 ^d	4.411 ^c	3.939
0.57	2.950 ^c	3.758 ^d	3.354
0.64	2.745 ^{abc}	2.927 ^{bc}	2.836
0.71	2.563 ^{ab}	2.683 ^{abc}	2.623
0.78	2.674 ^{abc}	2.407 ^a	2.540
0.85	2.569 ^{ab}	2.403 ^a	2.540
Mean	2.828	3.098	
Factor	P diff		
Crude protein	0.001		
Threonine level	0.001		
Protein x threonine	0.001		
Coefficient of variation	5.64		

^{abcdegh}Means with common superscript do not differ significantly (P < 0.05).

Pen means were the experimental unit for all analyses. All data were first subjected to the analysis of variance using the General Linear Models procedure of the SAS Institute (1991). Initial BW was used as a covariate for analyzing BW gain. The treatments were analyzed as a factorial design with CP levels and supplemental Thr levels as the main effects and the interaction of CP and supplemental Thr. When significant differences (P < 0.05) among or between treatments were observed, means were separated by repeated t-tests using the lsmeans option of SAS.

Estimation of a Thr concentration supporting the maximum dependent variable response (BW gain or feed conversion) was estimated in two ways. First, broken-line response curves to graded levels of Thr were analyzed by a one-slope model using the PROC NLIN procedure of the SAS Institute (1991) as suggested by Robbins (1986). Secondly, the data were fitted to a quadratic regression equation (PROC REG; SAS Institute, 1991), $y = a + bx + cx^2$ where x = Thr concentration; y = dependent variable; a = intercept; b = coefficient for the linear slope; c = coefficient for the quadratic slope. The Thr concentration supporting maximum performance was then estimated by identifying the point at which the slope of the best-fit curve was equal to zero (Neter and Wasserman, 1974). The equation was: (max) = $-b/2c$

Results and Discussion

Results of the analysis for total amino acids in the basal diets indicated that analyzed values were in close agreement with calculated values (Table 1). In addition, analysis of supplemental amino acids indicated that values were in close agreement with calculated values (data not shown).

Body weight gain of male Large White turkeys during the period of 8 to 12 week was significantly influenced by dietary CP, Thr level, and the interaction of CP and Thr level (Table 2). Overall, poult fed the low CP (16%) diet had significantly greater BW gain than did those fed the diet with high CP (19.8%). This was most prominent at lower levels of Thr supplementation, resulting in the significant interaction. At higher levels of Thr, where BW gain was at a maximum, no significant difference in BW gain was observed between poult fed the two CP levels.

A similar response was observed in regard to feed conversion by male Large White turkeys (Table 3). The FCR was significantly influenced by both the level of Thr and the level of dietary CP, with a significant interaction between Thr and dietary CP. Overall, FCR of poult fed the low CP diets was significantly less than that of poult fed the diets with high CP; this difference was predominantly at the lower levels of Thr with no significant difference between CP levels in FCR at the higher levels of Thr. While these interactions between dietary CP and Thr may be interpreted to support the suggestions by several authors that dietary CP levels influence amino acid requirements, it should be noted that the two basal diets were composed of protein sources that differ in their amino acid digestibility (NRC, 1994). The low CP diet attained most of its crude protein from dehulled soybean meal, with minimum quantities of peanut meal, while the high CP diet contained a major share of its protein from peanut meal and wheat middlings with no soybean meal. Although based on small sample numbers, the true digestibility coefficients of Thr for peanut meal and wheat byproducts by NRC (1994) are considerably less than suggested for dehulled soybean meal. Because the diets in this experiment were formulated on the basis of total amino acids, it is possible that the reduced BW gains and higher FCR observed at the lowest level of Thr supplementation on the high CP diets were the result of a lower level of digestible Thr for these diets compared to the low CP diets. At the higher levels of Thr supplementation, exceeding the Thr requirement, no significant differences in BW gain were noted between poult fed the two CP diets. Although similar differences in digestibility between these two major protein sources existed for other amino acids, the diets were formulated to provide a surfeit of these amino acids while Thr was at a deficit.

Estimation of a Thr requirement from the data resulted in considerable differences depending upon the method of assay. The lowest Thr estimates were obtained by using the one-slope nonlinear regression model. For poult fed the low CP diet, the inflection point of the one-slope model for BW was at $0.68 \pm 0.008\%$ Thr, while for poult fed the high CP diet the inflection point was $0.76 \pm 0.01\%$ Thr. Both are lower than the level of 0.80% Thr suggested by NRC (1994), which is based upon mathematical modeling (Hurwitz *et al.*, 1983) and not verified by feeding trials. For FCR, the one-slope nonlinear regression model indicated an inflection point of $0.61 \pm 0.02\%$ Thr for poult fed the low CP diet and $0.70 \pm 0.02\%$ for poult fed the high CP diet. These are both less than the level of 0.80% Thr suggested by NRC (1994).

Results of fitting quadratic equations to the BW gain and FCR are shown in Fig. 1 and 2, respectively. The Thr concentration supporting maximum performance was estimated by identifying the point at which the slope of the best-fit curve was equal to zero (Neter and Wasserman, 1974). Results of these estimates

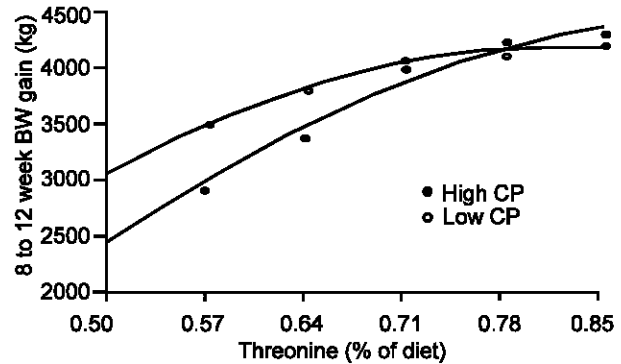


Fig. 1: Main effect means of 8 to 12 week BW gain responses in turkeys receiving graded levels of threonine in diets containing Low CP (16.01% of diet) or High CP (19.08% of diet). Probability values for linear and quadratic responses in both Low and High CP curves were ($P < 0.001$) and ($P < 0.001$) respectively. Quadratic response curves for the Low CP and High CP responses were $Y = -3363 + 18266X - 11010X^2$ and $Y = -4309 + 17994X - 9097X^2$, respectively, where Y represents the BW gain response and X represents total dietary threonine.

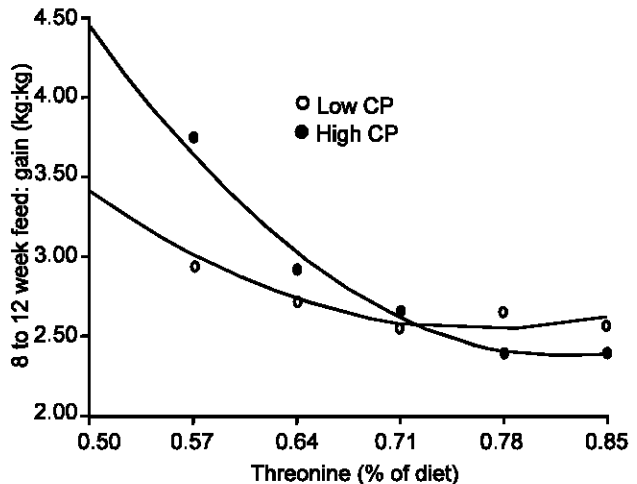


Fig. 2: Main effect means of 8 to 12 week feed conversion responses in turkeys receiving graded levels of threonine in diets containing Low CP (16.01% of diet) or High CP (19.08% of diet). Probability values for linear and quadratic responses in both Low and High CP curves were ($P < 0.001$) and ($P < 0.001$) respectively. Quadratic response curves for the Low CP and High CP responses were $Y = 9.745 - 18.754X + 12.213X^2$ and $Y = 15.944 - 33.034X^2$, respectively, where Y represents the feed conversion response and X represents total dietary threonine.

indicated that dietary Thr levels of 0.83 (16.0% CP) and 0.98% (19.8% CP) were adequate for maximum BW gain, while Thr levels of 0.77 (16.0% CP) and 0.82% (19.8% CP) were adequate for optimum feed conversion. These levels are in closer agreement to NRC (1994) estimates than those obtained from the one-slope nonlinear regression model.

Considerable controversy exists regarding means of estimating nutrient requirements from biological data (Robbins *et al.*, 1979; Morris, 1989; Schutte and Pack, 1995). Remmenga *et al.* (1997)

conducted a simulation study to compare several procedures for estimating the maximum effective dose in a quantitative dose-response model. The simulation indicated that the estimate of the maximum effective dose is influenced more by the choice of model than by the method of estimation. They concluded that studies should be designed with a maximum number of data points to allow for more sensitive estimates of maximum response. Results of the present experiment confirm observations from previous studies (Waldroup *et al.*, 1998; Kidd *et al.*, 1998) in that NRC (1994) Thr recommendations for growing turkeys are safe estimates. Lehmann *et al.* (1997) evaluated Thr responses in 8 to 12 week old toms and found that a Thr level of 0.69% of the diet was adequate to support optimum BW gain and FCR. The Thr levels found to be adequate for gain and FCR by turkeys fed the high CP diet in the present study were $0.76\% \pm 0.01$ and $0.70\% \pm 0.02$, respectively, using the one-slope nonlinear regression and 0.98 and 0.82% by quadratic regression. For the poult fed the low CP diets, Thr levels of $0.68\% \pm 0.01$ and $0.61\% \pm 0.02$ provided optimum BW gain and FCR, respectively, when estimated by the one-slope nonlinear regression and 0.83 and 0.82%, respectively, by quadratic regression. Thus the one-slope model estimates are in close agreement with the estimates of Lehmann *et al.* (1997) who used an exponential response function (Schutte and Pack, 1995) to estimate the Thr requirement. Lilburn and Barbour (1996) evaluated Thr responses in turkeys from 1 to 14, 21 to 35, 55 to 69, 77 to 92, and 99 to 114 days of age and found that Thr needs at the two oldest time periods are higher than NRC (1994) estimates in terms of BW gain and carcass development, but suggested that the NRC recommendations overestimate the Thr needs of the younger turkeys. The results of Lilburn and Barbour (1996) in turkeys 77 to 92 d, which overlaps our test period of 8 to 12 week, indicated improvements in BW gain, FCR, and carcass development up to a dietary level of 0.81% Thr.

Caution must be expressed in suggesting from the results of this study that the Thr requirement of the turkey increases as dietary CP increases, as reported by previous authors for broilers (Grau, 1948; Morris *et al.*, 1987; Robbins, 1987; Abebe and Morris, 1990; Morris *et al.*, 1992). Although significant interactions existed between dietary CP and Thr levels for both BW gain and FCR in the present study, the diets were composed of primary protein sources differing in Thr digestibility; thus, although total Thr values were identical for the two unsupplemented CP diets, differences in digestible Thr undoubtedly existed. Because estimates for amino acid digestibility coefficients for peanut meal and wheat byproducts are based upon rather sparse numbers as compared to corn and soybean meal (NRC, 1994), we hesitate to estimate possible differences in digestible Thr content of the two different diets. The fact that performance was similar between poult fed the two CP diets at surfeit levels of Thr suggests that this explanation is plausible. Robbins (1987) points out the pitfalls of using different protein sources to evaluate effects of protein level on amino acid requirements. Results of the present study suggest that use of dietary Thr levels at or near those suggested for the growing turkey by NRC (1994) recommendations should be adequate to support maximum BW gains and feed conversion and will not be affected by moderate fluctuations in dietary CP.

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