Threonine Requirement of the Grower Turkey Tom

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Two experiments were conducted to determine threonine requirement for maximum performance and breast and drumstick major muscle relative weights of turkey toms raised from 8-10 wk and 11-13 wk of age. Also plasma free amino acid concentration in response to feeding a diet with graded levels of threonine was measured. A corn-peanut meal diet was formulated to contain 0.59, 0.72, 0.85, 0.98 and 1.11% threonine and fed to Large White turkey males (British United Turkeys, BUT) in both trials. Each treatment was replicated among 5 and 8 individual cages in experiments 1 and 2, respectively. Two days prior to the end of each trial, blood was removed from the brachial vein of five toms per treatment and analyzed for plasma amino acid concentration. At the end of each experiment, final body weight and feed consumption were recorded and feed efficiency was computed. Birds were then sacrificed and pectoralis major and gastrocnemius muscles were weighed. The 0.85% dietary threonine resulted in the highest weight gain and feed efficiency ratio in the first trial. In the second experiment an increase ($P \le 0.05$) in weight gain was also obtained by the 0.85% dietary threenine with no further significant improvement beyond that level. Therefore the current results indicate that the threonine requirement for maximum weight gain of turkey toms raised from 8 to 10 and 11 to 13 wk of age is 0.85%. The changes in blood threonine concentration further supported the estimated requirement of threonine obtained from the performance data. Increasing dietary threonine from 0.59 to 1.11% during both growing periods, however, had no effect on pectoralis major or gastrocnemius muscles yield. In conclusion threonine requirement for maximum performance of the grower turkey tom raised from 8 to 13 wk of age was 0.85% diet, a value slightly higher than that reported in the literature.

Key words: grower turkey, performance, plasma amino acid, threonine requirement

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Introduction

The documented crude protein (CP), metabolizable energy (ME), and threonine requirements were practically kept unchanged for turkey toms raised from 8-12 wk of age (NRC, 1977; NRC, 1994). A mathematical model based on nutrient needs for physical and analytical development of body maintenance and growth of turkeys fed nutritionally balanced diets (NRC, 1977) to determine CP, ME and amino acid requirements showed that the threonine requirement of a turkey tom raised from 8 to 13 wk of age was 2.39 g/Mcal or 0.72% (Hurwitz et al., 1983 a). The findings of that research as well as Hurwitz et al. (1983b) were used as basic data in documenting the updated standards for the requirement of threonine as well as other amino acids for maximum growth of turkeys (NRC, 1994). Reducing CP in turkey diets, while keeping methionine and lysine balanced might limit threonine as well as other essential amino acids such as valine, isoleucine, and arginine in supporting maximum growth especially when feed ingredients such as wheat, sorghum, milo, barley or peanut meal replace corn (Hurwitz *et al.*, 1983b; Kidd and Kerr, 1996; Kidd *et al.*, 1997; Kidd *et al.*, 1998).

In a study by Lehmann et al. (1997), weight gain and feed conversion were not affected by an increase in threonine level from 0.69 to 0.91% in a wheat-cornsoybean meal diet fed to turkey toms from 8 to 12 wk of age, but the requirement of threonine for maximum performance was estimated to be equal or less than 0.69%. The data in the same study also showed that the threonine level to support maximum whole breast and pectoralis major muscle yields of a 20 wk old turkey tom was 0.64% whereas thigh and drumstick maximum yields were achieved with 0.58% threonine. In subsequent research works only performance was measured to determine the requirement of threonine to support maximum growth of turkey toms raised from hatch to 9 wk of age (Waldroup et al., 1998; Kidd et al., 1998). Those researchers reported that maximum weight gain and feed efficiency can be achieved through feeding a practical diet containing

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0.86% (Waldroup *et al.*, 1998) or 0.77% threonine (Kidd *et al.*, 1998) in birds raised from 6 to 9 wk of age.

It has been established that some of the ingested threonine in broiler chicks is converted to glycine when a threonine deficient or glycine free diet was supplemented with threonine (Baker et al., 1972). Other reports suggested that the nutritional and metabolic status of glycine and serine in relation to that of threonine need to be understood, because the metabolic degradation of threonine produces glycine and serine whereas nitrogen excretion through the uric acid pathway requires the availability of glycine, glutamate, and aspartate (Baker et al., 1972; Lehmann et al., 1997; Kidd et al., 2001). A strong relationship was found to exist between plasma amino acid level and dietary amino acid adequacy in which plasma amino acid profile has been used as a response parameter to estimate amino acid requirement of the bird (Zimmerman and Scott, 1965). For it has been found that threonine plasma concentration stayed constant at dietary threonine levels lower than that required for maximum egg mass and feed efficiency of laying hens, but started to increase linearly at levels higher than the requirement (Ishibashi et al., 1998).

More research is required to further establish the requirement for maximum growth of the grower turkey tom as well as determining the threonine needs for maximum growth of commercially important cut-up parts such as breast muscle. It is also important to look into the changes in blood amino acid concentration, especially threonine, glycine and serine, in response to variations in dietary threonine, because of the important metabolic relationship that exists among those amino acids and threonine requirement. Therefore, the present work was conducted to estimate the threonine requirement for maximum body weight gain and efficiency of feed utilization and to study the effect of threonine supplementation on pectoralis major and gastrocnemius muscles yield of turkey toms raised from 8-13 wk of age. In addition the variations in plasma free amino acid profile of 10 and 13 wk old turkey toms in response to changes in dietary threonine were examined.

Materials and Methods

Day-old Large White turkey toms (British United Turkeys, BUT) were obtained from The Ohio State University, Ohio Agricultural Research and Development Center (OARDC) hatchery, wing banded and distributed among four floor pens covered with wood shaving. The birds were fed an OARDC nutritionally balanced turkey diet. At 8 and 11 wk of age a number of birds was randomly selected from the main flock and weighed for experiments 1 and 2, respectively.

The first experiment was conducted to estimate the threonine requirement for maximum performance and breast and drum major muscle growth of turkey toms raised from 8–10 wk of age. In addition changes in plasma amino acid concentration of 10 wk old tom in response to feeding graded levels of dietary threonine were measured.

At 8 wk of age, the main flock was subjected to an overnight fast and twenty-five birds were selected and distributed at random among twenty-five individual wood cages with 5 cages per treatment having a similar average body weight of $3233 \text{ g} \pm 15.7$ per treatment. Each cage was equipped with a feeder and drinker and wood shaving was used as litter. The cages were placed in an environmentally controlled room with an average room temperature of 17° C and 23 h of light per day. Birds were offered feed and water *ad libitum*.

A corn-peanut meal basal diet containing 22.8% CP, 3013 kcal ME/kg, and 0.59% threonine was prepared (Table 1). L-threonine was added to the basal diet at an increment of 0.13% replacing cellulose to make experimental diets containing 0.59, 0.72, 0.85, 0.98 and 1.11% threonine. Each dietary treatment was distributed at random among five cages in a complete randomized design. Two days before the end of the trial, blood samples were removed from all toms using heparin washed 5 ml syringes, centrifuged and plasma samples were stored at -20° C. Total free amino acid concentration in plasma was determined following the High Performance Liquid Chromatography method of 'Waters1' 'Pico.Tag' where sample was derivatized before separation on a C₁₈ reverse phase column $(3.9 \times 150 \text{ mm})$ and the internal standard was Norleucine Thiodiglycol.

At the end of the experiment, the overnight fasted birds were weighed along with the remaining feed and feed efficiency was computed. All birds were sacrificed by cervical dislocation and pectoralis major and gastrocnemius muscles of the left breast and drum, respectively, were dissected and weighed. Data were analyzed using the GLM procedure with dietary threonine level being the main variable and initial body weight included as a covariate. Comparison of treatment least square means was performed through running the repeated *t*-test matrix (SAS, 1992).

In the second experiment, threonine requirement of turkey toms raised from 11–13 wk of age was determined and the change in plasma amino acid profile was measured for 13 wk old toms fed graded levels of threonine. Management, statistical design, experimental procedures, and criteria measured in the second experiment were similar to those of the first experiment with the exception of using eight birds (replicate) per treatment, where the average initial body weight per treatment was $6124 \text{ g} \pm 97.2$. The individual cages were placed in two environmentally controlled rooms with average room temperature of 20° C. In the present experiment five toms per treatment were randomly selected for blood sampling.

Results and Discussion

Variation in initial body weight had no effect on weight gain and feed efficiency and the relative weights of pectoralis major and gastrocnemius muscles in both experi-

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Table 1. Composition of corn-peanut meal basal diet fed to turkey toms from 8-10 (Experiment 1) and 11-13 (Experiment 2) wk of age

Ingredients	(%)
Corn	57.5
Peanut meal ¹	29.0
Fish meal	2.00
Blended fat	3.40
Limestone	1.30
Dicalcium phosphate	1.25
Cellulose (Solka floc)	0.84
DL-methionine	0.33
L-lysine.HCl	0.97
L-leucine	0.18
L-tryptophan	0.05
L-isoleucine	0.28
L-valine	0.22
Potassium phosphate	0.07
Sodium phosphate mono bas	ic 0.61
Vitamin and Trace mineral p	premix ^{2,3} 2.00
Calculated nutrient composit	tion
Crude protein	22.8
ME (kcal/kg)	3013
Threonine (%)	0.59
Analyzed nutrient compositi	on
Crude protein	22.0

¹Crude protein composition of peanut meal was 50.9% (AOAC, 1984).

²Composition of main premix (g per 45.4 kg diet): corn (ground), 490; choline chloride, 54.5; amprolium (25%), 22.7; selenium premix (200 mg Se/kg), 45.4; bacitracin MD, 22.7; vitamin premix, 227; and mineral premix, 45.4 (Lilburn and Emmerson, 1993).

³Supplied unit per kg diet: vitamin A, 8,745 IU; cholecalciferol, 3,745 IU; vitamin E, 60 IU; vitamin K (menadione sodium bisulfite), 2.91 mg; thiamine HCl, 2.2 mg; riboflavin, 6.6 mg; niacin, 99 mg; pantothenic acid, 15.4 mg; folic acid, 1.2 mg; pyridoxine, 2.2 mg; biotin, 165 mg; zinc oxide (72% Zn), 147 mg; manganous oxide (55% Mn), 152 mg; copper sulfate (25.2% Cu), 35 mg; furrous sulfate monohydrate (31% Fe), 72 mg; and potassium iodide, 1.5 mg.

ments ($P \ge 0.05$). Performance and growth of breast and drumstick major muscles in response to feeding turkey toms a corn-peanut meal based diet containing 0.59, 0.72, 0.85, 0.98 and 1.11% threonine from 8 -10 and 11-13 wk of age are presented in Table 2. Increasing dietary threonine level from 0.59 to 1.11% did not affect weight gain and relative weights of the breast and drumstick major muscles of turkey toms raised from 8-10 wk of age (P >0.05). But the 0.85% dietary threonine resulted in the highest numerical weight gain. The basal diet resulted in the lowest ($P \le 0.09$) feed efficiency value among all other treatments that were comparable to each other with the 0.85% threonine level having the highest numerical feed efficiency ratio. The current results on body weight and feed efficiency ratio are in agreement with those obtained by Waldroup et al. (1998) on male turkeys raised from 6 to 9 wk of age. But in another similar study on male turkeys, the threonine requirement was estimated to be 0.77% (Kidd *et al.*, 1998). Both research teams indicated that the threonine requirement reported in NRC (1994) for poults between 8 and 12 wk of age is adequate.

In the second experiment there was a gradual increase in weight gain of turkey toms in response to an increase in dietary threonine from 0.59 to 0.85% ($P \le 0.05$). Beyond that level there was no significant change in weight gain although birds on the 1.11% threonine diet gained numerically more weight than those consuming the 0.85 and 0.98% dietary threonine. In addition, male turkeys showed a gradual improvement in efficiency of feed utilization in response to increasing the level of dietary threonine from 0.59 to 1.11% ($P \le 0.05$). The performance results indicated that the required threonine level supporting maximum weight gain and feed efficiency of 8 -10 wk old male turkeys was 0.85% diet. In addition, the performance data obtained from the birds that were raised from 11-13 wk of age further support our interpretation and showed a threonine requirement level of 0.85% (Table 2). It is well known that the requirement of birds for amino acids decreases with age. The fact that the threonine requirement of BUT turkey toms (0.85%) did not decrease as expected beyond 10 wk of age indicates that these birds were still undergoing a critical growth period till 13 wk of age, thus requiring the same level of threonine (0.85%). The reported threonine requirement herein was slightly higher than that reported for 8-12 wk old male turkeys (NRC, 1994), that could be attributed to the difference in turkey strains raised.

Similar to the 10 wk old birds, there was no change in the relative weights of the breast and drumstick major muscles of 13 wk old birds fed a diet with graded levels of thre- onine. A similar observation on breast, thigh, and drumstick muscles of 20 wk old male turkeys fed a diet containing threonine levels ranging from 0.49 to 0.64% were reported (Lehmann *et al.*, 1997). However, the best-fit statistical model showed that the threonine requirement for breast meat deposition in these birds was higher than that required for maximum performance and equal to 0.64%. Further studies are required to determine the threonine requirement that supports maximum growth in muscles of commercial importance in grower male turkeys.

The concentrations of threonine, serine, glycine, methionine, lysine and tyrosine in plasma of turkey toms fed a diet containing threonine levels between 0.59 and 1.11% from 8–10 and 11–13 wk of age are presented in Table 3. The 10 wk old toms showed no change in plasma threonine concentration when the level of dietary threonine was increased from 0.59 to 0.72%. However, increasing dietary threonine level to 0.85% and beyond resulted in a significant increase in plasma threonine levels (P < 0.01). A similar trend was observed for the 13 wk old toms, where no change in plasma threonine concentration occurred with dietary threonine levels between 0.59 and

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Age (wk)	Threonine level (%)	Weight gain (g)	Feed efficiency (g:g) ¹	PMJ ² (%)	GST ³ (%)
8-10	0.59	1293	0.35 ^b	7.17	0.49
	0.72	1503	0.41 ^a	7.34	0.51
	0.85	1563	0.45 ^a	6.76	0.48
	0.98	1443	0.41ª	7.40	0.47
	1.11	1495	0.42 ^a	6.92	0.48
	SEM^4	117	0.021	0.26	0.02
	$\mathbf{P} \! > \! \mathbf{F}^5$	0.45	0.09	0.33	0.85
11-13	0.59	1552°	0.27°	7.53	0.49
	0.72	1724 ^{bc}	0.32 ^b	7.05	0.52
	0.85	1946 ^{ab}	0.33 ^b	7.51	0.55
	0.98	1985 ^{ab}	0.35 ^{ab}	7.11	0.49
	1.11	2021 ^a	0.36 ^{ab}	7.66	0.50
	SEM	116	0.017	0.19	0.02
	$\mathbf{P} \! > \! \mathbf{F}$	0.02	0.0008	0.10	0.28

Table 2. Weight gain and feed efficiency and relative weights of the pectoralis major breast muscle and gastrocnemius muscle of turkey toms fed a diet with graded levels of threonine from 8-10 (Experiment 1) and 11-13 (Experiment 2) wk of age

^{a-c} Least square means in the same column of each age period with different superscripts are significantly different (P<0.05), except for 8-10 wk feed efficiency where P<0.09.

¹Least square means of five birds per treatment (8-10 wk) and eight birds per treatment (11-13 wk).

² Pectoralis major muscle.

³Gastrocnemius muscle.

⁴Pooled standard error of the least square means.

⁵ Probability values.

Table 3. Plasma threonine (Thr), serine (Ser), glycine (Gly), methionine (Met), lysine (Lys), and tyrosine (Tyr) of turkey toms fed a diet containing graded levels of threonine from 8-10 (Experiment 1) and 11-13 (Experiment 2) wk of age

		Amino Acid concentration (nmole/ml)						
Age (wk)	Threonine level (%)	Thr^1	Ser	Gly	Met	Lys	Tyr	
10	0.59	111 ^b	927ª	569	137	202	322	
	0.72	203 ^b	705 ^b	545	169	181	295	
	0.85	455ª	785 ^{ab}	617	214	195	326	
	0.98	529ª	751 ^b	546	174	183	330	
	1.11	603ª	631 ^b	518	168	174	287	
	SEM^2	49.7	59.2	48.5	18.2	22.1	32.5	
	$\mathbf{P} \! > \! \mathbf{F}^3$	0.0001	0.03	0.69	0.10	0.90	0.85	
13	0.59	111 ^d	893ª	543	138	173	294ª	
	0.72	236 ^{cd}	650 ^b	487	152	206	198 ^b	
	0.85	377 ^{bc}	614 ^b	437	148	166	187^{b}	
	0.98	510 ^b	620 ^b	483	150	167	215 ^b	
	1.11	747 ^a	704 ^b	592	165	170	242 ^{ab}	
	SEM	59.3	46.5	43.0	13.4	25.3	20.1	
	$\mathbf{P}\!>\!\mathbf{F}$	0.0001	0.004	0.14	0.74	0.78	0.02	

^{a-d} Least square means in the same column within an age period having different superscripts were significantly different (P < 0.05).

¹Least square means of five birds per treatment.

² Pooled standard error of least square means.

³ Probability value.

0.72% beyond which plasma threonine showed a gradual increase as the level of dietary threonine increased from 0.85 to 1.11% ($P \le 0.01$). The observed change in the pro-

file of plasma threonine concentration in response to a variation in dietary threonine content of 10 and 13 wk old male turkeys were similar to that observed for laying

hens (Ishibashi *et al.*, 1998). Both studies showed a slow change in plasma threonine at dietary levels equal or below requirement with a sharp linear increase in plasma threonine at dietary threonine levels higher than the requirement. Therefore plasma threonine concentration response further support our estimation of the threonine requirement. Similarly the threonine requirement of the laying hen was confirmed by the profile of plasma threonine concentration (Ishibashi *et al.*, 1998). The same response and trend in plasma amino acid concentration with its relationship to requirement has been reported for lysine, arginine and valine in broiler chicks (Zimmerman and Scott, 1965).

Plasma serine concentration dropped significantly when the level of threonine in the diets fed to turkey toms from 8-10 or 11-13 wk was increased from 0.59 to 0.72%, 0.85, 0.98 or 1.11% (P<0.05). Plasma serine concentration was intermediate, however, for the 10 wk old turkey toms on the 0.85% threonine. During the two age periods under investigation plasma concentrations of glycine, methionine, and lysine were not changed at all levels of supplementary dietary threonine. The profile observed for plasma serine and glycine concentrations agreed with that of the laying hen (Ishibashi et al., 1998). Plasma serine concentration in the laying hen was dropped as soon as the level of threonine was increased beyond deficient levels and continued to be low thereafter. However, glycine was practically unaffected by changes in dietary threonine level. The relationship observed among plasma threonine, serine, and glycine in response to changes in dietary threonine strongly suggest that an interaction among those amino acids do exist affecting their metabolism in grower male turkey toms. This interaction should be elucidated in future studies on chicken and turkey.

A similar relationship concerning the changes in serine and glycine in response to dietary threonine could not be detected in growing pigs (Defa et al., 1999). However, the relationship among plasma threonine, serine and glycine in grower turkey toms established in the current study further support the remarks mentioned by previous researchers regarding the establishment of threonine requirement in turkeys or broilers (Lehmann et al., 1997; Kidd et al., 1998; Kidd et al., 2001). Only plasma tyrosine of the 13 wk old tom was affected by dietary threonine when its plasma concentration was decreased as the level of dietary threonine was increased from 0.59 to 0.72, 0.85 or 0.98% (P < 0.02). As the level of dietary threonine went up to 1.11%, however, plasma tyrosine concentration increased to levels similar to that of birds consuming the diets with lower dietary threonine.

In conclusion, the threonine requirement for maximum weight gain and feed efficiency and plasma threonine accumulation of BUT male turkeys raised from 8-10 wk of age was 0.85%. However, it did not decrease till 13 wk of age as expected, indicating that the toms still undergoing a critical growth period requiring the same level of dietary threonine (0.85%). This value was slightly higher than

that reported by the NRC (1994) for threonine requirement (0.80% diet) of male turkeys raised from 8–12 wk of age.

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