Maintenance threonine requirement and efficiency of its use for accretion of whole-body threonine and protein in young chicks

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Broiler chicks were fed on chemically-defined crystalline amino acid diets containing graded levels of L-threonine (Thr) during the period 10–20 d post-hatching. Doses of Thr represented 5, 10, 15, 40, 55, 70 and 95 % of its ideal level for maximal weight gain and feed efficiency. Other amino acids were maintained at minimized excess levels that were 15 % (of ideal) above the various doses of Thr. Following 10 d of feeding and a 24 h fast, chicks were killed for whole-body protein and amino acid analysis. Using pen accretion means, weight gain (r^2 0·98), protein accretion (r^2 0·99), and Thr accretion (r^2 0·99) were linear ($P < 0·01$) functions of Thr intake. Slope of the Thr accretion regression line indicated that 82 % of the Thr intake was recovered in whole-body protein. At zero Thr intake, chicks lost 11·9 mg Thr/d. The Thr maintenance requirement was 45·7 mg/d per kg body weight$^{0·75}$. Increasing doses of Thr resulted in increased ($P < 0·05$) concentrations of methionine, isoleucine, histidine and lysine in whole-body protein. Other indispensable amino acids, including Thr, also tended to increase. Whole-body glycine, proline, serine and cystine concentrations decreased ($P < 0·05$) as Thr was increased in the diet. The maintenance need for Thr represented 5·5 % of the total need for Thr. The data suggest that efficiency of Thr utilization is constant at all levels of Thr intake between 5 and 95 % of the level required for maximal weight gain and feed efficiency.

Threonine: Amino acids: Broiler chicks

Knowledge of maintenance requirements and efficiencies of utilizing indispensable amino acids for whole-body protein accretion is essential to developing accurate models of amino acid requirements. Most investigators who have studied protein or amino acid accretion over a wide array of deficient dosing levels have concluded that accretion of both protein and the limiting amino acid is a linear function of intake of either protein or the limiting amino acid (Hegsted & Neff, 1970; Batterham *et al.* 1990; Baker, 1991; Chung & Baker, 1992*a*; Bikker, 1994; Adeola, 1995; Baker *et al.* 1996). This implies that utilization of a limiting amino acid is constant over a wide range of intakes. Where comparisons have been made among amino acids, however, efficiencies of retaining certain amino acids, e.g. lysine, have been higher than those of retaining amino acids such as threonine (Thr), isoleucine, tryptophan or methionine (Baker, 1991; Batterham, 1994; Adeola, 1995; Gahl *et al.* 1997).

Maintenance amino acid requirement estimates for poultry are limited, and those that exist have been obtained using N balance collection assays in adult cockerels (Leveille &

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Fisher, 1959, 1960; Leveille *et al.* 1960; Burnham & Gous, 1992). The study reported here utilized broiler-strain chickens, and efficiencies of utilization and maintenance Thr requirements were determined using whole-body N and amino acid analysis of chicks fed on crystalline amino acid diets in which absorption efficiencies of amino acids could be assumed to be 100 % (Chung & Baker, 1992b). Thr was selected for intense study because it is an important limiting amino acid for poultry and pigs, and it is also an amino acid whose maintenance requirement has been found to be high (relative to lysine) for swine (Baker *et al.* 1966; Fuller, 1994).

MATERIALS AND METHODS

General procedures

Housing, handling, feeding and killing procedures were in accord with policies of the University of Illinois Committee on Laboratory Care. Male 1-d-old broiler chicks (Avian x Avian strain; Pine Manor Inc., Goshen, IN, USA) were fed on a standard 230 g protein/kg maize–soyabean-meal diet until 16.00 hours on day 9 post-hatching when diet and water were removed. On day 10, following a 16 h fast, chicks were individually weighed and wing-banded, after which they were assigned four per pen to battery pens (Petersime Incubator Co., Gettysberg, OH, USA) in an environmentally-controlled room with 24 h fluorescent lighting. Diets were then randomly assigned to battery pens. Four pens of four chicks were assigned to each dietary treatment. Feed and water were provided *ad libitum* until day 20, at which time they were removed for 24 h (to remove gut fill). Chicks were then killed by CO_2 asphyxiation for whole-body analysis of protein and amino acids. After final fasted body weights were recorded, chicks were immediately frozen at $-4°$. Initial body composition data were obtained from sixteen randomly selected chicks at day 10. Average weight of these chicks was 157 g, the same as the average initial weight of chicks assigned to the experimental diets. Following the 16 h fast, these chicks were killed using CO_2 gas, and they were immediately frozen at $-4°$.

Diets

Six diets were fed, each containing a different dose (5, 10, 15, 40, 55, 70, 95 % of the Thr required for maximal weight gain; Baker & Han, 1994), with amino acids other than Thr maintained at 15 % above the Thr level, expressed in terms of ideal levels. For example, when Thr was fed at 55 % of its ideal level, all other amino acids were fed at 70 % of their ideal levels. The variable ingredients were incorporated into the chemically-defined diet at the expense of maize starch. Table 1 shows the ideal levels of amino acids and ideal ratios (g/kg lysine) of the essential amino acids (Baker & Han, 1994). True digestibility of free amino acids was assumed to be 100 % (Chung & Baker, 1992b). The basal diet contained 15·8 MJ ME/kg, and it was assumed that as Thr and other amino acids were increased at the expense of maize starch, dietary ME remained essentially constant.

Analytical procedures

Frozen chicks from each pen (four chicks per pen) were combined and chopped into small pieces, after which the pieces were ground three times. The first two grindings utilized a 6 mm die, and the third a 3 mm die. The grinder (Model 4812; Hobart, Troy, OH, USA) was prechilled with solid CO_2 before each pass through the die to minimize water loss in

Table 1. *Required level and ideal ratio of essential amino acids for young broiler chicks*

Amino Acid*	Required (ideal) level† (g/kg)	Ideal ratio‡ (g/kg lysine)
Lysine	9·00	1000
Arginine	9·45	1050
Histidine	3·30	370
Methionine	3·24	360
Cystine	3·24	360
Phenylalanine	5·20	580
Tyrosine	4·25	470
Threonine	6·03	670
Leucine	9·81	1090
Isoleucine	6·03	670
Valine	6·93	770
Tryptophan	1·44	160
Glycine	5·85	650
Proline	3·96	440
Glutamic acid	120·0	NA

NA, not applicable.

* All amino acids were provided as L-isomers and were obtained from Nutri-Quest, Chesterfield, MO, USA. Lysine was provided as L-lysine hydrochloride and histidine was provided as L-histidine hydrochloride.H_2O. The non-amino acid portion of diets consisted of (g/kg diet): soyabean oil 100, solka floc 30, mineral mixture (Oduho *et al.* 1994) 53·7, $NaHCO_3$ 15, choline chloride 2, vitamin mixture (Oduho *et al.* 1994) 2, and maize starch, to 1 kg. Ethoxyquin (125 mg/kg) and DL-α-tocopheryl acetate (20 mg/kg) were also contained in the diet.

† These required amino acid levels apply to chicks 1–3 weeks of age fed on purified diets containing 147 g protein equivalent and 15·8 MJ ME/kg.

‡ Ideal ratios were based on previous research in our laboratory (Baker & Han, 1994).

samples. After grinding, a subsample weighing about 350 g was obtained. Subsamples were placed in freezer bags, and then frozen at $-4°$ overnight, after which they were freeze-dried. After freeze-drying, samples were weighed to determine DM. Freeze-dried samples were subsequently ground in a food processor (Model MM-2M; Cuisinart Inc., Norwich, CT 06360, USA). Whole-body amino acid composition was determined on duplicate samples following 24 h hydrolysis with 6 M-HCl. Amino acids were quantified by ion-exchange chromatography (Model 119CL Amino Acid Analyzer; Beckman Instruments, Palo Alto, CA, USA). Total N was determined by the macro-Kjeldahl procedure (Association of Official Analytical Chemists, 1990) on duplicate freeze-dried samples.

Statistical analysis

Results were analysed using pen means as the experimental unit. Analysis of variance and linear-regression equations were computed using the general linear models procedures of Statistical Analysis Systems (1990). Three linear-regression equations were determined: Thr and protein (N x 6·25) accretion *v.* Thr intake, and Thr accretion *v.* protein accretion; the latter used to determine whether the protein accreted contained a constant amount of Thr and whether zero protein accretion resulted in zero Thr accretion. Standard errors were computed for each regression coefficient. Durbin–Watson values were computed for each of these regression equations to prove lack of autocorrelation among data points (Durbin & Watson, 1951; Draper & Smith, 1981). Having established that use of all dosing data points (5, 10, 15, 40, 55, 70, 95 % of the ideal Thr level) produced an excellent description of the linear regression of Thr (r^2 0·99) and protein (r^2 0·99) accretion *v.* Thr intake, the Thr requirement for maintenance (zero Thr or protein accretion) was calculated by determining

Thr intake at zero accretion. Estimates of Thr maintenance requirements (mg/d) were converted to units of intake per kg body weight$^{0.75}$ by assuming that body weight was the average weight (initial weight + final weight) divided by 2 of chicks fed on Thr at 95 % of its ideal level.

<div align="center">RESULTS</div>

Accretion of body weight, whole-body protein, and whole-body Thr increased linearly ($P < 0.01$) as dietary Thr levels increased from 5 to 95 % of ideal levels (Table 2). Thr intake increased linearly ($P < 0.01$), but diet intake increased ($P < 0.01$) only up to 55 % of the ideal Thr level, after which it tended to plateau. Thus, diet intakes of chicks fed on Thr levels representing 55, 70 and 95 % of the ideal level did not differ significantly ($P > 0.10$).

The regression of accretion (Y; mg/d) *v.* Thr intake (X; mg/d) was described well (r^2 0.99) by the straight line: $Y = -13.75 + (0.82$ (SE 0.012))X (Fig. 1(a)). The slope value from the best-fit linear-regression equation indicated that 82 (SE 1.2) % of the Thr intake was recovered as Thr in the whole-body protein accreted. Protein accretion (Y; g/d) as a function of Thr intake (X; mg/d) was also described well (r^2 0.99) by a straight-line fit: $Y = -0.267 + (0.0186$ (SE 0.001))X (Fig. 1(b)). Extrapolating the linear-regression equations for Thr and protein accretion to the Y intercept indicated that zero Thr intake resulted in a net daily loss of 13.75 mg whole-body Thr and 267 mg whole-body protein. Daily Thr intake required for daily zero Thr accretion was calculated to be 16.7 mg/d and for zero protein accretion 14.3 mg/d. Converting these values to mg/d per kg body weight$^{0.75}$ (assuming an average body weight of 261.5 g) gave maintenance Thr requirement estimates of 45.7 mg/d per kg body weight$^{0.75}$ based on zero Thr accretion and 39.2 mg/d per kg body weight$^{0.75}$ based on zero protein accretion.

Fig. 2 shows that Thr accretion (Y; mg/d) was a straight-line function of protein accretion (X; g/d). The linear-regression equation, $Y = -1.88 + (44.15$ (SE 0.5))X (r^2 0.99), indicated that for each 1 g increase in protein accretion Thr accretion was increased by 44.1 mg. This suggests that Thr concentration in the whole-body protein accreted was a

Table 2. *Accretion of body weight, protein, and threonine in young broiler chicks fed on graded levels of threonine (Thr)**

Dietary Thr		Intake		Accretion		
Level (g/kg)	% of ideal†	Diet‡ (g/d)	Thr (mg/d)§	Body wt§ (g/d)	Protein§ (g/d)	Thr§ (mg/d)
0.30	5	15.4	4.6	−1.20	−0.23	−11.9
0.60	10	16.5	9.9	−0.83	−0.11	−6.4
0.90	15	16.7	15.1	−0.38	0.00	−2.3
2.41	40	25.8	62.3	5.88	0.97	40.0
3.32	55	34.7	115.2	11.70	1.94	83.2
4.22	70	36.7	154.7	15.25	2.67	116.9
5.73	95	37.3	213.6	20.92	3.62	158.37
Pooled SEM		1.0	4.5	0.5	0.1	4.2

* Data are means of four pens of four Avian x Avian chicks during a 10 d feeding period (10–20 d post-hatching); average initial weight was 157 g. For details of procedures, see pp. 112–114.

† Ideal ratio (g/100 g lysine; based on Baker & Han, 1994). All amino acids other than Thr (Table 1) were maintained at a 15 % excess level relative to Thr, e.g. when Thr was fed at 40 % of its ideal level, all other amino acids were fed at 55 % of their ideal levels.

‡Quadratic ($P < 0.01$) response.

§ Linear ($P < 0.01$) response.
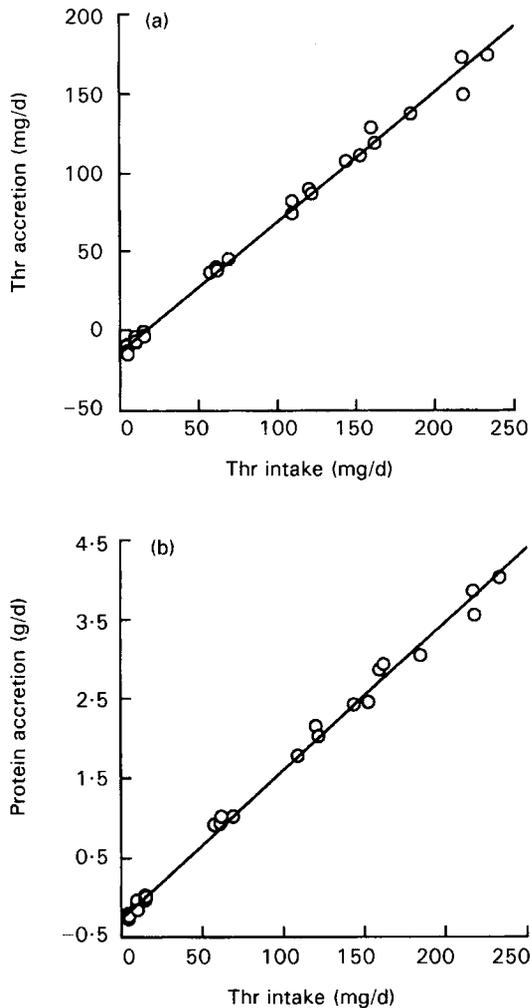
Fig. 1. Best-fit straight-line plots of (a) whole-body threonine (Thr) accretion (Y) as a function of Thr intake (X; $Y = -13.75 + (0.82$ (SE 0.012))X, r^2 0.99) and (b) whole-body protein accretion (Y) as a function of Thr intake (X; $Y = -0.267 + (0.0186$ (SE 0.001))X, r^2 0.99) for chicks fed on graded increments of L-Thr. Each data point represents the mean value of four male chicks during the period 10–20 d post-hatching. For details of procedures, see pp. 112–114.

constant 4·41 (SE 0·05)% at all levels of Thr intake. Indispensable amino acids tended to increase in whole-body protein, whereas glycine, proline, serine and cystine concentrations decreased ($P < 0.05$) as dietary Thr was increased from 5 to 95 % of its ideal level (Table 3). Among indispensable amino acids, only lysine, methionine, isoleucine and histidine increased significantly ($P < 0.05$) as Thr was incremented.

DISCUSSION

The efficiency of recovering absorbed Thr in whole-body protein was 82 (SE 1·2)% and was constant at all levels of Thr intake between 5 % and 95 % of its requirement for
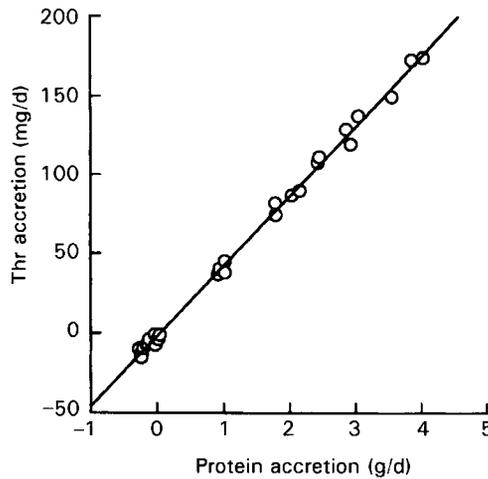
Fig. 2. Best-fit straight-line plot of whole-body threonine (Thr) accretion (Y) as a function of protein accretion (X) for chicks fed on graded increments of L-Thr ($Y = -1.88 + (44.15$ (SE $0.5))X$, r^2 0.99). Each data point represents the mean value of four male chicks during the period 10–20 d post-hatching. For details of procedures, see pp. 112–114.

maximal growth. Thus, Thr utilization showed no evidence of diminishing returns (i.e. declining efficiency) as its level in the diet was increased. This finding of constant efficiency agrees with previous chick work from our laboratory (Baker, 1991; Baker *et al.* 1996) in which constant utilization was observed for valine (73 %), lysine (80 %) and isoleucine (61 %). Likewise, similar methodology involving graded dosing in pigs has resulted in efficiency estimates of 74 % (Bikker, 1994) to 86 % (Batterham *et al.* 1990; Adeola, 1995) for lysine and 72 % for methionine (Chung & Baker, 1992a). These pig studies also resulted in efficiency estimates that were constant at all levels of intake of the limiting amino acid. Batterham (1994) evaluated efficiencies of retaining ileal digestible amino acids from soyabean meal in growing pigs and reported efficiencies (including maintenance costs) of 75 % for lysine, 64 % for Thr, 45 % for methionine and 38 % for tryptophan. It seems obvious from the studies that have been reported that individual indispensable amino acids differ in the efficiency with which absorbed amino acids are retained in whole-body protein. Our estimate of Thr utilization in chicks is similar to that observed for lysine, but work in pigs and rats has suggested that lysine is retained more efficiently than Thr (Adeola, 1995; Gahl *et al.* 1997).

Models that are employed to estimate amino acid requirements from estimates of maintenance needs plus estimates of whole-body protein accretion rates must consider not only differences in true digestibility of amino acids in feed ingredients and diets, but also differences in the efficiencies with which absorbed amino acids are utilized for whole-body protein accretion. With our estimate of the maintenance requirement for absorbed Thr of 45.7 mg/d per kg body weight$^{0.75}$ together with our recent estimates of the total requirement for digestible Thr of 0.61 % of diet (615 mg/d per kg body weight$^{0.75}$) for 3–6-week-old broilers and 0.52 % of diet (484 mg/d per kg body weight$^{0.75}$) for 6–8-week-old broilers (Webel *et al.* 1996), one can calculate that the maintenance requirement represents 7.4 % of the total digestible Thr need during the 3–6-week growth period and 9.4 % during the 6–8-weeks growth period. Assuming the digestible Thr requirement of chicks from hatching to 3 weeks post-hatching (National Research Council, 1994) is 0.70 % of diet (833 mg/d per kg body weight$^{0.75}$), the maintenance requirement calculates to be 5.5 % of

Table 3. *Amino acid composition of whole-body protein (g/16 g nitrogen) in young broiler chicks fed on graded levels of threonine (Thr)†*

Dietary Thr (% of ideal§)	Asp	Thr	Ser*	Glu	Pro*	Gly*	Ala	Cys*	Val	Met†	Ile†	Leu	Tyr	Phe	His†	Lys†	Arg
5	9·07	4·23	5·22	13·55	6·91	9·62	6·52	1·38	4·27	1·97	3·37	7·25	2·96	4·14	2·12	6·30	6·81
10	9·08	4·24	5·15	13·60	6·82	9·42	6·53	1·30	4·33	2·01	3·41	7·33	3·04	4·16	2·16	6·42	6·79
15	9·03	4·21	5·13	13·49	6·72	9·31	6·43	1·29	4·24	1·99	3·39	7·22	2·96	4·10	2·14	6·41	6·74
40	9·07	4·25	5·03	13·64	6·51	8·93	6·39	1·23	4·30	2·07	3·45	7·31	3·13	4·10	2·27	6·56	6·70
55	9·13	4·30	5·04	13·75	6·35	8·51	6·35	1·24	4·25	2·07	3·42	7·31	3·03	4·05	2·38	6·68	6·56
70	9·32	4·35	4·91	14·00	6·43	8·78	6·52	1·19	4·34	2·11	3·55	7·37	3·07	4·07	2·50	6·82	6·69
95	9·23	4·35	4·87	13·89	6·15	8·24	6·34	1·09	4·32	2·12	3·54	7·42	3·02	4·10	2·58	6·88	6·56
SEM	0·13	0·05	0·08	0·18	0·12	0·22	0·11	0·07	0·05	0·05	0·04	0·09	0·06	0·05	0·03	0·09	0·10

* Significant ($P < 0.05$) linear decrease with increasing dietary Thr level.
† Significant ($P < 0.05$) linear increase with increasing dietary Thr level.
‡ For details of birds and procedures, see pp. 112–114.
§ Ideal ratio (g/100 g lysine; based on Baker & Han, 1994).

the total Thr requirement during this early growth period. (The National Research Council (1994) lists the total Thr requirement as 0·80 % of a maize–soyabean-meal diet. Assuming true digestibility of Thr is 87 % (National Research Council, 1994), the digestible Thr requirement is calculated to be 0·70 % of diet.)

With Thr at 70 % of its ideal level, 75·5 % (116·9 mg/d) of the Thr intake (154·7 mg/d) was recovered in whole-body protein (Table 2). Of the 24·5 % not recovered, 5·5 % can be assigned to the maintenance need, and the remaining 19 % can be assigned to oxidation, primarily via the Thr dehydrogenase (*EC* 1.1.1.103) pathway (Davis & Austic, 1994). This 19 % oxidative loss agrees well with the 18 % loss (100 minus 82 %) estimated from regression (Fig. 1). This contrasts with the 27 % oxidative loss observed for valine (Baker *et al.* 1996). Calculated oxidative loss estimates of 39 % for isoleucine and 20 % for lysine (Baker, 1991) lend credence to the argument that indispensable amino acids have different efficiencies of utilization, probably because of different turnover rates for the amino acids in body tissue pools.

An attempt was made to partition the absorbed dietary Thr into various categories so that the inefficiencies of Thr retention might be better understood (Table 4). After subtracting both obligatory losses associated with maintenance, and oxidation losses associated with Thr ingestion (18 % of intake), values were obtained for the residual Thr remaining. Regression of the values for actual Thr accreted (Y; mg/d) *v.* the residual Thr available for accretion (X; mg/d) gave the equation $Y = 3·0 + 1·00X$ (r^2 0·99). The slope value of 1·00 indicates that, after accounting for maintenance losses and oxidation losses associated with Thr ingestion, predicted residual Thr retention agrees well with the actual values measured for Thr accretion. Also interesting to note is that with the first three doses of Thr, all of which were below the estimated maintenance requirement of 16·7 mg/d, actual Thr accreted was less negative than that predicted by subtracting obligatory losses and oxidation losses from Thr intake. This implies that utilization of absorbed Thr below the Thr level required for maintenance (i.e., zero Thr accretion) is very efficient.

Concentrations of most indispensable amino acids increased in whole-body protein whereas glycine, serine, and proline decreased as dietary Thr increased from 5 to 95 % of its ideal level for maximal growth rate. This probably reflects that contractile proteins are increasing and collagen protein is decreasing as Thr is increased from deficiency to near adequacy. Other workers have noted a similar increase in indispensable amino acids and a decrease in glycine and proline as the dietary level of an essential amino acid is increased (Batterham *et al.* 1990; Chung & Baker, 1992*a*; Bikker, 1994; Baker *et al.* 1996; Gahl *et al.*

Table 4. *Estimated distribution of absorbed threonine at different levels of threonine (Thr) intake in young broiler chicks*

Dietary Thr absorbed (mg/d)	Obligatory loss* (mg/d)	Oxidation loss† (mg/d)	Residual Thr‡ (mg/d)	Thr accreted (mg/d)
4·6	16·7	0·8	−12·9	−11·9
9·9	16·7	1·8	−8·6	−6·4
15·1	16·7	2·7	−4·3	−2·3
62·3	16·7	11·2	34·4	40·0
115·2	16·7	20·7	77·8	83·2
154·7	16·7	27·8	110·2	116·9
213·6	16·7	38·4	158·5	158·4

* Maintenance requirement, consisting primarily of endogenous gut losses and obligatory Thr oxidation; calculated from data shown in Table 2.
† 18 % of absorbed Thr of dietary origin; calculated from data shown in Table 2 and Fig. 1 (a).
‡ Thr remaining after subtracting obligatory losses and oxidation losses associated with Thr ingestion. A negative value indicates a deficit.

1997). That cystine, arginine, and phenylalanine tended to decrease rather than increase in whole-body chick protein may be related to changes in feather protein contributions to whole-body protein.

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