The tryptophan requirement of the kitten

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1. To estimate the tryptophan requirement of the kitten, six male and six female kittens were presented diets containing 0·7, 0·9, 1·1, 1·3, 1·5 and 3·0 g tryptophan/kg diet for six experimental periods lasting 10 d in accordance with a 6 × 6 balanced Latin-square design.

2. Mean daily weight gain and nitrogen retention (N intake minus urinary and faecal N) plateaued at 1·1 g tryptophan/kg diet indicating that the minimal tryptophan requirement of the kitten was 1·1 g/kg diet.

3. Plasma tryptophan concentration exhibited a marked increase at dietary tryptophan concentrations above 1·3 g/kg diet.

The essentiality of tryptophan in the diet of growing kittens was shown by Rogers & Morris (1979) who reported weight loss and depressed food intake in kittens whose diet was changed from one containing 4·0 g tryptophan/kg diet to one devoid of tryptophan. They further reported that weight gain was not affected when kittens were given diets containing 4·0 *v.*2·0 g tryptophan/kg diet. Anderson *et al.* (1980) suggested that 1·5 g/kg diet was the minimal tryptophan requirement for growth in kittens. This estimate was based on a 4 × 4 Latin-square-design experiment using four kittens given 0, 1·0, 2·5 or 4·0 g tryptophan/kg diet and a randomized block design with groups of five kittens allotted to diets containing 0·5, 1·5 or 2·5 g tryptophan/kg diet. In both experiments weight gain was the only factor measured. In the 4 × 4 Latin-square-design experiment maximal growth was attained at 2·5 g tryptophan/kg diet which was significantly higher than the growth at 1·0 g tryptophan/kg diet. In their second experiment maximal growth was attained at 1·5 g tryptophan/kg diet, which they assumed was the tryptophan requirement.

The purpose of this report was to define more precisely the tryptophan requirement of the growing kitten based on weight gain and nitrogen balance and to examine whether plasma tryptophan concentrations could be used to estimate the tryptophan requirement.

METHODS

Animals

Specific-pathogen-free domestic short-hair kittens vaccinated against panleukopenia were given a semi-purified diet from weaning. A preliminary period of 10 d before the feeding of the experimental diets was used to adapt the kittens to the purified amino acid diet (Table 1). Six males and six females were selected on the basis of body-weights in the range of 900–1200 g and weight gains of at least 10 g/d during the preliminary period. At the beginning of the feeding of the experimental diets the mean (and SD) body-weights were 1087(91) g for the males and 1105(45) g for the females. The kittens were housed individually in stainless-steel metabolism cages with food and water available *ad lib.*

Diets

Six isoenergetic and isonitrogenous diets containing 240 g crystalline amino acid mix/kg were used. The energy contents of the diets per g dry matter (by analysis) were 22·7 kJ gross energy, 19·3 kJ apparent digestible energy and 18·4 kJ apparent metabolizable energy. The

Table 1. *Composition of basal diet (g/kg diet)*

Amino acid mix*	240·0
Sodium acetate	16·4
Beef tallow	250·0
Sucrose	150·0
Starch†	280·0
Mineral mix‡	50·0
Vitamin mix‖	10·0
Cellulose§	10·0
Choline chloride	3·46
Taurine	0·75

* Composition of amino acid mix (g/kg diet): Arg hydrochloride 20·0, Cys 6·0, Met 9·0, His HCl . H_2O 6·0, Ile 9·0, Leu 12·0, Lys HCl 14·0, Phe 7·0, Tyr 6·0, Thr 10·0, Trp 3·0, Val 9·0, Ala 19·0, Asp 10·0, Asn 20·0, Glu 20·0, Gln 30·0, Gly 20·0, Pro 10·0.

† Melojel, food grade maize starch; National Starch and Chemical Company, Bridewater, New Jersey.

‡ Composition of mineral mix (g/kg mix): $CaHPO_4$ 390·0, $CaCO_3$ 110·0, $MgSO_4$ 45·0, K_2HPO_4 90·0, KCl 100·0, $KHCO_3$ 100·0, $NaHCO_3$ 140·0, trace minerals 25·0. Individual trace elements (g/kg mineral mix): $MnSO_4 . H_2O$ 3·84, $ZnSO_4 . 7H_2O$ 4·45, $CuSO_4 . 5H_2O$ 0·80, ferric citrate . $3H_2O$ 10·0, pentacalcium orthoperiodate 0·15, $SnCl_2 . 2H_2O$ 0·10, Na_2SeO_3 0·03, $(NH_4)_6Mo_7O_4 . 4H_2O$ 0·04, $CrCl_3 . 6H_2O$ 0·26, $NiCl_2 . 6H_2O$ 0·30, NaF 0·14, $NH_4VO_3 . 4H_2O$ 0·02, carrier (NaCl) 4·87.

‖ Composition of vitamin mix (mg/kg diet): retinyl palmitate 80, cholecalciferol 5, DL-α-tocopherol 640, menadione 15, thiamine hydrochloride 25, riboflavin 10, pyridoxine 10, nicotinic acid 100, calcium pantothenate 20, *myo*-inositol 200, folic acid 10, cobalamin 50, biotin 1, ascorbic acid 400, sucrose 8434.

§ Solka-Floc, wood cellulose; Brown & Co. Berlin, New Hampshire, USA. Added to complete diet to facilitate faecal collection.

experimental diets were the same as the basal diet (Table 1) except that tryptophan was varied and alanine and starch were adjusted to make the diets isonitrogenous. All diets contained adequate levels of the essential amino acids (except tryptophan) to support normal growth (Rogers & Morris, 1979). The tryptophan levels of the six experimental diets were 0·7, 0·9, 1·1, 1·3, 1·5 and 3·0 g tryptophan/kg diet.

Design

Six pairs of kittens consisting of one female and one male were assigned experimental diets for sequential 10 d periods in accordance with 6 × 6 balanced Latin square design (Cochran & Cox, 1957) which allows for analysis of residual, period, animal and treatment effects.

Food intake and body-weight were recorded daily. Mean daily weight gain over the 10 d periods was determined by a least-squares regression analysis of daily body-weights. Urine acidified with sulphuric acid was collected daily and pooled in two separate 5 d collection periods to determine if there were any carry-over effects between experimental periods. Faeces were collected daily and pooled over the 10 d period. The N contents of food, urine and faeces were measured by the Kjeldahl procedure (Association of Official Agricultural Chemists, 1975). In N balance determinations, hair loss was disregarded.

Plasma amino acid analysis

On the 9th day of each experimental period, at 13.00–15.00 hours, 3 ml blood samples were drawn with heparinized syringes from the jugular vein of unanaesthetized kittens and immediately placed on ice. Plasma was separated by centrifugation and frozen (−80°) until analysis. Samples were prepared for amino acid analysis by the addition of equal volumes of sulphosalicylic acid (60 g/l), centrifuged to remove the protein precipitate, and adjustment of the filtrate to pH 2·2 with lithium hydroxide. An equivalent of 40 μl plasma was applied to the amino acid analyzer (Model 121MB; Beckman Instrument, Palo Alto, CA).

Table 2. *Effect of dietary tryptophan level on weight gain, food intake and nitrogen retention*

(Mean values with their standard errors for six kittens in the single sex groupings and for twelve kittens in the combined sex group)

Group	Dietary tryptophan (g/kg)	Wt gain (g/d)		Food intake (g/d)		N retention (g/d)	
		Mean	SE	Mean	SE	Mean	SE
Males	0·7	10·7^{a}	2·9	58·9^{a}	8·2	0·33^{a}	0·10
	0·9	20·2^{ab}	2·5	66·4^{a}	3·7	0·56^{ab}	0·08
	1·1	32·0^{b}	5·6	75·1^{a}	11·5	0·85^{b}	0·17
	1·3	27·5^{b}	4·6	70·6^{a}	4·8	0·84^{b}	0·11
	1·5	30·1^{b}	3·3	71·9^{a}	6·2	0·83^{b}	0·12
	3·0	30·1^{b}	3·1	69·5^{a}	4·2	0·79^{b}	0·07
Females	0·7	9·2^{a}	0·4	59·5^{a}	4·8	0·32^{a}	0·05
	0·9	15·9^{ab}	3·0	62·4^{a}	2·7	0·49^{ab}	0·09
	1·1	21·9^{b}	1·2	67·2^{a}	3·1	0·55^{ab}	0·03
	1·3	18·6^{ab}	3·8	62·1^{a}	5·4	0·63^{b}	0·06
	1·5	22·0^{b}	3·1	61·5^{a}	4·8	0·54^{ab}	0·08
	3·0	25·4^{b}	3·2	61·7^{a}	5·3	0·62^{b}	0·10
Sexes combined	0·7	10·0	1·4	59·2^{a}	4·5	0·32^{a}	0·06
	0·9	18·0^{a}	2·0	64·4^{a}	2·3	0·52^{b}	0·06
	1·1	27·0^{a}	3·2	71·2^{a}	5·8	0·70^{c}	0·09
	1·3	23·1^{a}	3·1	66·3^{a}	3·7	0·73^{c}	0·07
	1·5	26·0^{a}	2·5	66·7^{a}	4·1	0·69^{c}	0·08
	3·0	27·8^{a}	2·2	65·6^{a}	3·4	0·70^{c}	0·06

^{a,b,c} Values within the same column and sex grouping not sharing a common superscript letter were significantly different: $P < 0·05$.

Statistical analysis

Values for weight gain, N retention, food intake and plasma tryptophan were subjected to analysis of variance. When analysis of variance indicated significant differences between treatments ($P < 0·05$) the SNK multiple-range test (Steele & Torrie, 1960) was used to determine which means were significantly different. Values for males and females were analysed separately and combined because previous results from this laboratory have shown significant differences between male and female kittens in daily weight gain, food intake and N retention (Schaeffer *et al.* 1982; Smalley *et al.* 1983). To determine the response relationships a least-squares linear regression was fitted to the increasing points of response to dietary tryptophan concentration. A straight line parallel to the x axis was fitted to the mean of all the points after which the response had plateaued. The intersection of the two lines was used to indicate the minimal dietary requirement for maximal response.

RESULTS

The effects of dietary tryptophan on weight gain, N retention, food intake and plasma tryptophan are shown in Table 2 and Figs. 1–3. There were no significant residual effects in any of the factors measured. Differences in N retention between the first and second halves of each experimental period were not significant, hence the results are presented for each 10 d period.

Weight gain

Mean daily weight gain for the males given the 0·7 g tryptophan/kg diet was less ($P < 0·05$) than at all other levels except 0·9 g tryptophan/kg diet. At 0·9 g tryptophan/kg diet the mean daily weight gain for the males was not significantly different ($P > 0·05$) than at any

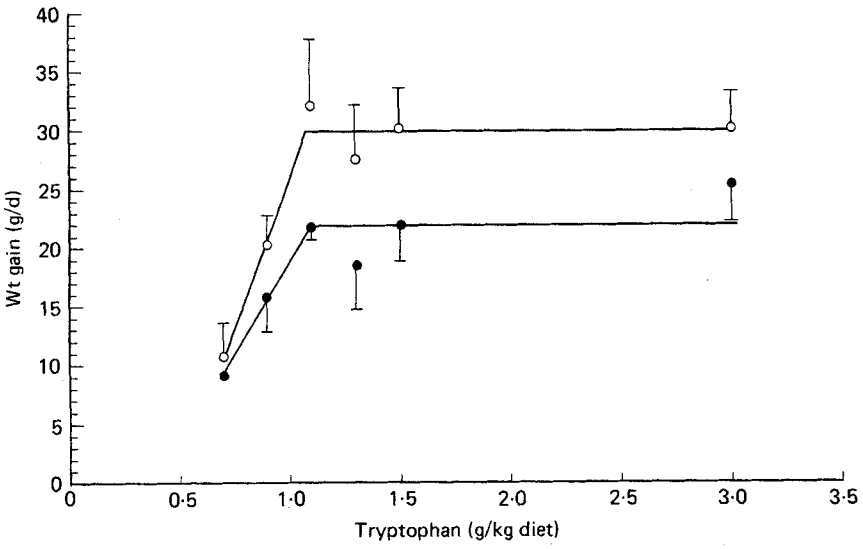

Fig. 1. The effect of dietary tryptophan on weight gain. Points represent mean values with their standard errors for six kittens: (○——○), males; (●——●), females.

other level. The female kittens showed a slightly different response. Their mean daily weight gain at 0·7 g tryptophan/kg diet was significantly lower ($P < 0·05$) than at 1·1, 1·5 and 3·0 g tryptophan/kg diet but was not different ($P > 0·05$) from the weight gains at 0·9 and 1·3 g tryptophan/kg diet. Analysis of the mean daily weight gains for the sexes combined showed growth at 0·7 g tryptophan/kg diet was significantly less ($P < 0·05$) than at all other levels of dietary tryptophan.

A plot of dietary tryptophan *v.* weight gain (Fig. 1) showed a linear increase in weight gain until 1·1 g tryptophan/kg diet. At 1·1 g tryptophan/kg diet the weight gain plateaued at (mean and SD) 30(2) g/d for males and 22(3) g/d for females (values for weight gains from 1·1 to 3·0 g tryptophan/kg diet). The break-point in the growth response curve indicated a tryptophan requirement of 1·1 g tryptophan/kg diet.

N balance

N balance results were similar to those for growth. For males, the N retained at 0·7 g tryptophan/kg diet was lower ($P < 0·05$) than all other levels except at 0·9 g tryptophan/kg diet. N retention for males at 0·9 g tryptophan/kg diet was not significantly different ($P > 0·05$) from the N retention at any other level of dietary tryptophan. For the females the N retention at 0·7 g tryptophan/kg diet was less ($P < 0·05$) than at 1·3 and 3·0 g tryptophan/kg diet while the N retentions at 0·9, 1·1 and 1·5 g tryptophan/kg diet were not significantly different from any of the other treatments. For the males and females combined, N retention at both 0·7 and 0·9 g tryptophan/kg diet was significantly less than for the other treatment levels ($P < 0·05$).

The male and female N retention response curves (Fig. 2) showed a linear increase in N retention until 1·1 g tryptophan/kg diet, after which the N retention plateaued at (mean and SD) 0·83(0·03) g N/d for males and 0·59(0·05) g N/d for females (values for N retentions from 1·1 to 3·0 g tryptophan/kg diet). Although the females showed maximal N retention at 1·3 g tryptophan/kg diet, there were no statistical differences in N retention for the dietary tryptophan range of 0·7–3·0 g/kg diet ($P > 0·05$). The over-all N retention

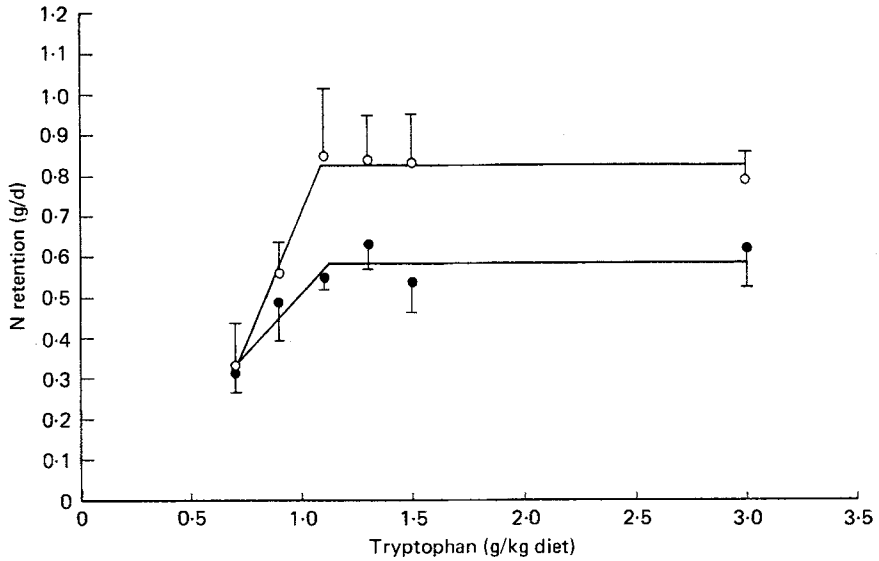

Fig. 2. The effect of dietary tryptophan on nitrogen retention. Points represent mean values with their standard errors for six kittens: (○——○), males; (●——●), females.

results supported the growth results indicating that 1·1 g/kg diet was the tryptophan requirement.

Food intake

The level of dietary tryptophan had no significant effect ($P > 0{\cdot}05$) on food intake. Females showed no significant period effects in food-intake. Male food intake was significantly higher ($P < 0{\cdot}05$) in the last experimental period compared with all the other periods and food intake in the fifth period (second to last period) was greater than in the first period. Male food intake was greater than female food intake ($P < 0{\cdot}01$).

Plasma tryptophan

As there were no significant differences in the tryptophan concentration of the plasma between males and females ($P > 0{\cdot}05$) a combined response curve was plotted. The combined values for tryptophan concentrations of plasma over the range of 0·7–1·3 g tryptophan/kg diet were not significantly different ($P > 0{\cdot}05$). These values were significantly less than the tryptophan concentrations of plasma from kittens given the diets containing 1·5 and 3·0 g tryptophan/kg diet ($P < 0{\cdot}05$). The response of plasma tryptophan to dietary tryptophan (Fig. 3) showed a break-point occurring between 1·3 and 1·5 g tryptophan/kg diet. Maximal growth and N retention were attained before the break-point in plasma tryptophan.

DISCUSSION

Results for both N retention and growth indicated that 1·1 g tryptophan/kg diet was the minimal requirement for the growing kitten. Weight gain for both males and females and the N retention for males and the sexes combined increased linearly with increasing dietary tryptophan until 1·1 g tryptophan/kg diet, after which the responses plateaued. The N retention values for the sexes combined gave a clear break-point both graphically and statistically at 1·1 g tryptophan/kg diet with N retentions at 0·7 and 0·9 g tryptophan/kg diet significantly lower ($P < 0{\cdot}05$) than at the other treatment levels.

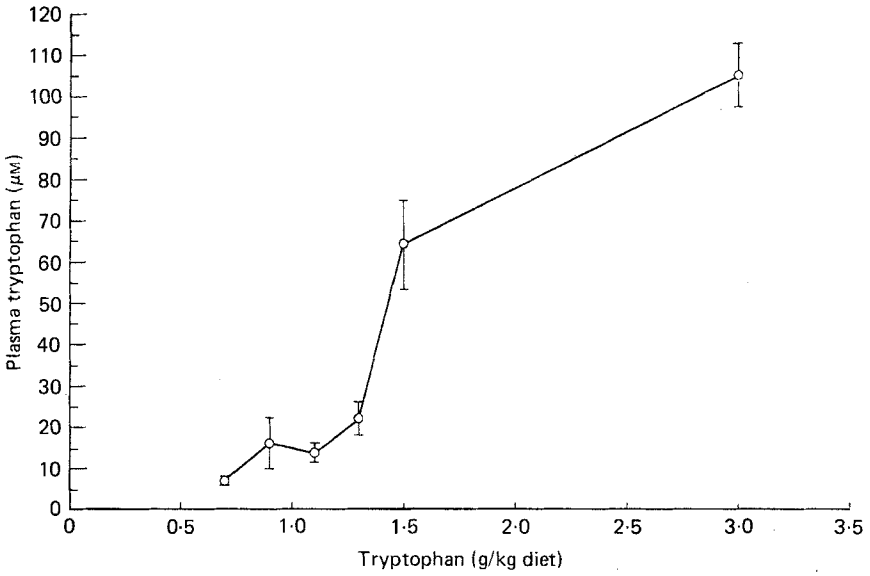

Fig. 3. The effect of dietary tryptophan on plasma tryptophan concentration. Points represent mean values with their standard errors for twelve kittens.

The results reported here showed a considerably lower tryptophan requirement for the kitten than the value of 1·5 g tryptophan/kg diet reported by Anderson *et al.* (1980) using greater increments of tryptophan in the diet. These authors did not state the sex of the kittens used in their experiments; growth was the only factor measured and the maximal growth of their kittens was suboptimal, being only 10–12 g/d compared with 25–30 g/d reported here.

The tryptophan requirements reported for other species are comparable with our value for the kitten. Tryptophan requirements reported for 35–60 kg swine ((US) National Research Council, 1979) and for rats (Rao *et al.* 1959) are the same as that reported here for the kitten. However, in the rat, reported values for the tryptophan requirement vary widely. Rao *et al.* (1959) attained maximal rat growth at 1·1 g tryptophan/kg diet. Young & Munro (1973) reported maximal rat growth occurring between 1·1 and 1·65 g tryptophan/kg diet and graphical interpolation of their results indicated maximal growth at 1·4 g tryptophan/kg diet. Using plasma tryptophan, McLaughlan & Illman (1967) indicated 1·3 g tryptophan/kg diet as the requirement for the young rat. The (US) National Research Council (1978) suggested 1·5 g tryptophan/kg diet as the requirement for the growing rat.

In the present study the break-point in plasma tryptophan occurred at a dietary tryptophan level higher than that required for maximal growth and N retention. In a study using young rats, Young & Munro (1973) showed maximal growth and the break-point in plasma tryptophan concentration occurred between 1·1 and 1·65 g tryptophan/kg diet. Although these authors concluded that the plasma tryptophan break-point occurred before maximal growth, based on the values presented it could not be established whether the plasma break-point occurred before or after maximal growth because no levels of tryptophan between 1·1 and 1·65 g tryptophan/kg diet were tested.

Feline tryptophan metabolism differs from that of most other mammalian species as there is very little conversion of tryptophan to niacin. Carvalho da Silva *et al.* (1952) showed that

cats stopped growing, lost weight and eventually died when given a niacin-free diet containing excess tryptophan. Jackson (1939) showed that no kynurenic acid could be isolated from the urine of cats kept on a diet providing excess tryptophan. Work by Ikeda *et al.* (1965) indicated that cats have the enzymic capacity to synthesize niacin from tryptophan, but that the high activity of hepatic picolinic carboxylase (thirty to fifty times that of the rat) is responsible for the cat's inability to convert appreciable amounts of tryptophan to niacin.

The results reported here strongly suggest 1·1 g tryptophan/kg diet as the minimal requirement of the kitten for maximal growth and N retention.

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