The nitrogen requirement of the weanling kitten

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1. The nitrogen requirement of the weanling kitten was determined in a series of three experiments. In each experiment, diets were formulated to provide the growing kitten with the essential amino acids at or above the level of requirement. Expt 1 utilized a 4 × 4 balanced Latin square design with two groups of kittens (four male and four female). The crystalline L-amino acid diets were presented at four levels of dietary crude protein (N × 6·25) of 140, 160, 180 and 200 g/kg diet. The design for Expts 2 and 3 was a 6 × 6 balanced Latin square. For each of these experiments, groups of six male and six female kittens were assigned to diets. The six levels of dietary crude protein were 120, 140, 160, 180, 200 and 220 g/kg diet; dietary N was supplied by crystalline L-amino acids for Expt 2 and casein plus a supplementary amino acid mix for Expt 3. Food intake, weight gain and N retention were determined in each experiment.

2. A sigmoidal model $y = P1 + P2/[1 + e^{(P3+P4 \cdot x)}]$ was fitted to the response of weight gain and N retention to dietary N. The calculated requirement (95% of the upper asymptote, P1 + P2) for these experiments varied from 170 to 230 g protein/kg diet with the majority of these values falling between 180 and 200 g protein/kg.

3. On the basis of these three experiments, the kitten's requirement for dietary crude protein is between 180 and 200 g/kg diet (28·8–32·0 gN/kg) for purified diets which provide a calculated 21 MJ metabolizable energy/kg diet.

The dietary requirement for protein is based on two metabolic demands: (1) the necessity for sufficient quantities of essential amino acids in the proper proportions, (2) the necessity for adequate amounts of nitrogen to provide for the synthesis of non-essential amino acids and other nitrogenous compounds.

Within the last 5 years the essential amino acid requirements for maximal kitten growth have been determined with crystalline L-amino acid diets. Protein requirements of the kitten determined before this information was available may have been inflated due to insufficient amounts of a particular amino acid. In addition, protein requirements determined using animal protein sources may be confounded by the association of the level of protein and palatability of the diet. Based on studies in which either casein or a mixture of fish and liver were used as the protein source (Dickinson & Scott, 1956; Miller & Allison, 1958; Jansen *et al.* 1975), the (US) National Research Council (1978) recommended that 'a protein of a quality equivalent to that derived from unprocessed mammalian, avian or fish muscle should be presented at a level of 28% of metabolizable energy (ME) in the diet of a growing kitten. This is equivalent to 35% protein in a diet (dry basis) providing 21 kJ (5 kcal) ME/g dry matter'. To define the N requirement more precisely we have completed three experiments in which all essential amino acids were supplied in adequate amounts and N was supplied as individual amino acids or casein. Food intake, weight gain and N retention have been determined in all three studies with a total of six different dietary protein levels from 120 to 220 g crude protein (N × 6·25)/kg diet.

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METHODS

Diets

Diets for the three experiments were formulated to provide adequate amounts of the essential amino acids and a mixture of non-essential amino acids at the lowest crude protein level tested in each experiment (Rogers & Morris, 1982). Growth rate of rats is greater when the non-essential amino acids have been provided by a combination of amino acids (Rogers *et al.* 1970) and it has been noted that asparagine may be necessary for maximal growth of kittens (E. M. Kamakawa, J. G. Morris and Q. R. Rogers, unpublished results).

Compositions of the diets at the lowest protein level are given in Tables 1 and 2. Crystalline L-amino acids were the only source of dietary N in Expts 1 and 2 (Table 3). Four levels of dietary crude protein were used in Expt 1 (140, 160, 180 and 200 g/kg diet) and two additional levels (120 and 220 g/kg diet) in Expt 2. The 20 g/kg increments of dietary crude protein in both experiments were attained by increasing the proportion of amino acid mix and decreasing starch by an equivalent proportion.

The previously mentioned six levels of dietary protein (120–220 g/kg) were used in Expt 3, but as a combination of casein plus supplemental essential amino acids (Table 3). The amount of casein was increased to achieve the 20 g/kg increments of dietary protein; the supplemental amino acid mix remained constant (56·4 g protein/kg diet) for all six levels of dietary protein. Food and water were available *ad lib.* throughout the study.

Animals

Specific pathogen-free kittens were weaned and given a 240 g amino acid/kg diet (equivalent to 231·2 g crude protein/kg diet) for 3 weeks before the beginning of each study (Smalley *et al.* 1983). Following this adjustment period, kittens were selected for the experiments based on a body-weight gain of at least 10 g/d and an initial weight of approximately 1 kg. The mean initial weights for Expt 1 were 890 (SE 41) g for female kittens and 850 (SE 21) g for males. For Expts 2 and 3, mean initial weights were somewhat higher: 1322 (SE 51) g and 1249 (SE 47) g for females, and 1084 (SE 65) g and 1237 (SE 21) g for males respectively.

Kittens were randomly assigned to diets for the first period; in subsequent periods, diet assignments were in accordance with a balanced Latin square design (Cochran & Cox, 1957). Each experiment was composed of two Latin squares, one of female and the other of male kittens. Each period was 10 d in length. Body-weight and food intake were measured daily throughout each period. Mean daily weight gains for each period were calculated from a least-squares regression of body-weight on time. Urine and faeces of each kitten were collected daily and grouped for analysis into two 5-d portions for each period. N determinations were performed on diet, urine and faecal samples by the Kjeldahl procedure (Association of Official Agricultural Chemists, 1975). N retention was calculated by subtracting urinary and faecal N from dietary N intake. Hair loss was ignored in calculating N retention due to the difficulty of obtaining complete collections. Blood samples for plasma amino acid analysis were obtained from the jugular vein at 10.00–12.00 hours on the 7th day of each period for Expts 2 and 3 and stored at −81° until analysed. For analysis, samples were thawed, deproteinized with equivalent volumes of a solution of 60 g sulphosalicylic acid/l, brought to a pH of 2·2 with lithium hydroxide solution and analysed on a Beckman Model 121-MB amino acid analyser (Beckman Instruments, Palo Alto). Samples for Expt 3 were analysed for each individual animal. For Expt 2, plasma samples were pooled according to diet for each sex.

Table 1. *Expt 1. Diet composition (g/kg diet)*

Amino acid mix*	150·0 (143·1)
Sodium acetate†	14·9
Chicken fat	250·0
Sucrose	200·0
Starch	318·8
Mineral mix‡	40·0
Potassium chloride	10·0
Vitamin mix§	10·0
Choline bitartrate	6·3
Cellulose‖	10·0

* For details, see Table 3. Value in parentheses is amount of free base amino acid mix.

† Sodium acetate added in amounts equimolar to the hydrochlorides in the amino acid mix.

‡ For details of composition, see Smalley *et al.* (1983).

§ Contained (mg/kg diet): retinyl palmitate 11, cholecalciferol 0·05, DL-α-tocopheryl acetate 160, menadione 15, thiamin hydrochloride 25, riboflavin 10, pyridoxine 10, nicotinic acid 100, calcium pantothenate 20, *myo*-inositol 200, folic acid 10, cobalamin 0·05, biotin 1, ascorbic acid 200, taurine 200.

‖ Solkafloc (non-nutritive fibre); Brown & Company, Berlin, New Hampshire. Added at 10 g/kg completed diet.

Table 2. *Expts 2 and 3. Diet composition (g/kg diet)*

	Expt 2	Expt 3
Amino acid mix*	124·5 (117·8)	56·7 (52·2)
Casein†	—	78·8
Sodium acetate‡	14·9	10·0
Turkey fat§	250·0	250·0
Sucrose	200·0	200·0
Starch‖	344·3	338·2
Mineral mix¶	50·0	50·0
Vitamin mix**	10·0	10·0
Choline bitartrate	6·3	6·3
Cellulose††	10·0	10·0

* Values in parentheses are free base amino acid mix.

† Vita-free casein; US Biochemical Corporation, Cleveland, Ohio.

‡ Sodium acetate added in amounts equimolar to the hydrochlorides in the amino acid mix.

§ Rendered turkey fat; distributed by Valley Fresh, Turlock, California.

‖ Melojel, food grade maize starch; National Starch and Chemical Co., Bridgewater, New Jersey.

¶ Contained (g/kg mix): $CaHPO_4$ 390·0, K_2HPO_4 90·0, $CaCO_3$ 110·0, $MgSO_4$ 45·0, KCl 200·0, NaCl 140·0, trace mineral mix 25·0. Trace mineral mix contained (g/kg mineral mix): $MnSO_4 . H_2O$ 3·84, $ZnSO_4 . 7H_2O$ 4·45, $CuSO_4 . 5H_2O$ 0·80, $FeC_6H_5O_7 . 3H_2O$ 10·0, $Ca_5(IO_6)_2$ 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 the vitamin mix is the same as previously reported in Table 1 with the exception that taurine was 375 mg/kg diet.

†† Solkafloc (non-nutritive fibre); Brown & Company, Berlin, New Hampshire. Added as 10 g/kg completed diet.

Statistical treatment

The balanced Latin square design used for these experiments provides measures of the direct effects that have been corrected for residual effects of the preceding period (Cochran & Cox, 1957). In each experiment an analysis of variance was performed on male and female kittens both separately and combined for food intake, weight gain and N retention. Contingent on the indication of a significant direct effect by ANOVA, differences between individual

Table 3. *Amino acid composition of diets (g/kg diet)*

Expt no. ...	1*	2*	3†	
Arginine‡	13·2	13·2	13·7	(10·8)
Histidine‡	4·9	4·9	5·3	(3·0)
Isoleucine	7·0	7·0	7·6	(3·6)
Leucine	12·0	12·0	16·6	(6·0)
Lysine‡	11·2	11·2	12·4	(6·2)
Methionine	5·0	5·0	5·3	(3·2)
Cysteine	4·5	4·5	4·8	(4·3)
Phenylalanine	6·0	6·0	6·7	(2·7)
Tyrosine	5·0	5·0	5·9	(1·3)
Threonine	9·0	9·0	9·0	(6·0)
Tryptophan	2·0	2·0	2·4	(1·4)
Valine	7·0	7·0	9·1	(3·7)
Alanine	10·0	5·0	2·4	—
Glycine	7·0	7·0	1·4	—
Proline	7·0	4·0	8·7	—
Asparagine	7·0	7·0	—	—
Glutamine	13·0	8·0	—	—
Aspartic acid	5·0	—	5·2	—
Serine	—	—	3·6	—
Glutamic acid	7·0	—	28·9	—
Total	142·8	117·8	149·0	(52·2)
Nitrogen × 6·25	140·4	119·9	120·0	—

* Composed of crystalline L-amino acids; Ajinomoto USA Inc., Raleigh, North Carolina.

† Composed of 78·8 g casein/kg diet ('Vita-free' casein; US Biochemicals Corporation, Cleveland, Ohio) plus a supplementary L-amino acid mix. Amino acid composition of casein was determined by acid-hydrolysis under N_2 and subsequent analysis on an amino acid analyser (Model 121MB; Beckman Instruments, Palo Alto, California). Amounts contributed by the amino acid mix are given in parentheses.

‡ In crystalline L-amino acid mixtures, these amino acids were added as arginine hydrochloride, histidine hydrochloride monohydrate and lysine hydrochloride but were calculated here as free base to facilitate comparison between diets.

means were determined with the Student–Newman–Keuls' (SNK) multiple range test (Steel & Torrie, 1980). The criterion of significance was $P < 0{\cdot}05$ for all statistical analysis.

A sigmoidal response function was fitted to weight gain and N retention of the following groups: Expt 2, male and combined sexes; Expt 3, male, female and combined sexes. For the function used,

$$y = \mathrm{P1} + \mathrm{P2}/[1 + e^{(\mathrm{P3}+\mathrm{P4}\,.\,x)}],$$

y is the response (weight gain or N retention) and x is the level of dietary crude protein (Robbins *et al.* 1979). The four parameters (P1, P2, P3, P4) were calculated by use of BMDP statistical software package program (PAR) which utilizes a least-squares procedure to estimate parameters of a specified non-linear regression (Ralston, 1981). The adequacy of each model was determined by testing for lack of fit, which entails subdividing the residual sum of squares into pure error SS, and lack of fit SS (Draper & Smith, 1981). When adequacy was indicated, the overall regression was subsequently tested. The requirement was calculated as 95% of the upper asymptote (P1 + P2; Table 7, p. 508).

Table 4. *Effect of dietary protein level on food intake (g/d) for three experiments**

(Values are means of average food intake of each animal over a 10 d period; no. of kittens given in parentheses)

Expt no....	1			2			3		
Dietary protein equivalent (g/kg diet)	♂ (4)	♀ (4)	♂+♀ (8)	♂ (6)	♀ (6)	♂+♀ (12)	♂ (6)	♀ (6)	♂+♀ (12)
120	—	—	—	53	39	46	50	32[a]	41[a]
140	45	41	43	54	40	47	55	31[a]	43[a]
160	48	39	44	52	40	46	55	41[b]	48[ab]
180	45	38	42	58	41	50	62	46[b]	54[b]
200	44	41	42	54	42	48	56	44[b]	50[b]
220	—	—	—	54	45	49	60	44[b]	52[b]
Pooled SE	4·0	0·9	1·5	1·9	1·8	1·3	2·5	3·1	2·0

[a, b] Means within a column with a different superscript letter were significantly different ($P < 0·05$).

* For details of experiments, see p. 502.

Table 5. *Effect of dietary protein level on weight gain (g/d) for three experiments**

(Values are means of least squares regression of daily body-weights for each animal over a 10 d period; no. of kittens given in parentheses)

Expt no....	1			2			3		
Dietary protein equivalent (g/kg diet)	♂ (4)	♀ (4)	♂+♀ (8)	♂ (6)	♀ (6)	♂+♀ (12)	♂ (6)	♀ (6)	♂+♀ (12)
120	—	—	—	10[a]	7[a]	9[a]	8[a]	−1[a]	4[a]
140	12	8	10[a]	13[a]	8[a]	10[a]	14[ab]	0·2[a]	7[a]
160	18	11	14[b]	14[a]	11[ab]	12[a]	17[b]	8[b]	12[b]
180	18	12	15[b]	24[b]	14[ab]	19[b]	27[c]	16[c]	22[c]
200	19	16	18[b]	21[b]	17[ab]	19[b]	28[c]	19[c]	24[c]
220	—	—	—	21[b]	20[b]	21[b]	32[c]	16[c]	24[c]
Pooled SE	2·8	1·7	1·2	1·6	2·5	1·5	2·4	1·6	1·4

[a, b, c] Means within a column with a different superscript letter were significantly different ($P < 0·05$).

* For details of experiments, see p. 502.

RESULTS

In both Expts 1 and 2, food intake did not differ in relation to dietary protein level. In Expt 3, food intakes of female and combined sexes were significantly lower on the two lowest dietary protein levels (Table 4).

Analysis of variance indicated a significant effect ($P < 0·05$) of dietary protein level on weight gain for the sexes treated individually and combined for Expts 2 and 3 and for the combined sexes of Expt 1 (Table 5). SNK multiple-range test on values for the combined sexes in Expt 1 indicated that weight gain was significantly lower on 140 g protein/kg diet compared with the three higher protein levels. For the male and combined sexes in Expt 2, and male, female and combined sexes in Expt 3, weight gains were significantly greater at the three highest levels of dietary protein (180–220 g/kg diet) compared with the three lowest levels. Female weight gain in Expt 2 was significantly greater at 220 g

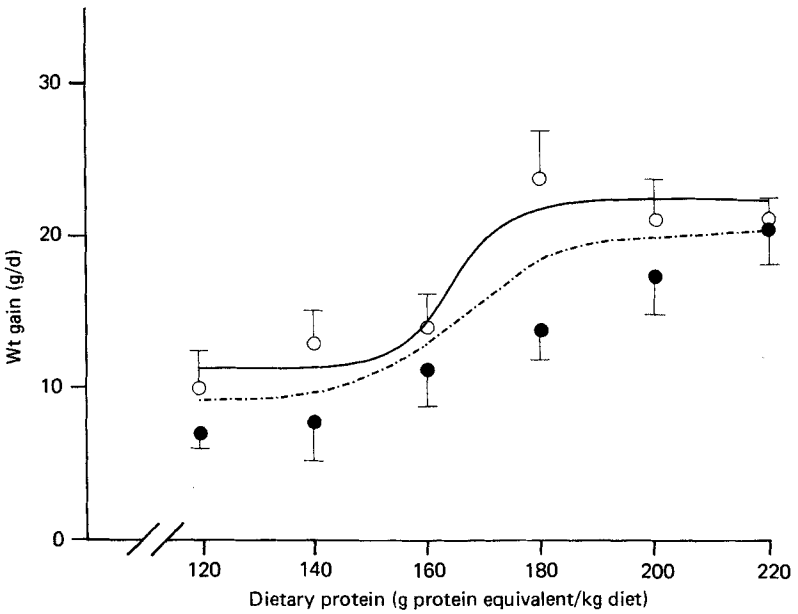

Fig. 1. Expt 2 (amino acid diets). Mean daily weight gain of male (○) and female (●) kittens. Vertical bars represent the standard error of the individual means. Curves represent calculated non-linear regressions (♂, ——; ♂+♀, – · –) whose parameters are given in Table 7. For details of diets, see p. 502 and Table 2.

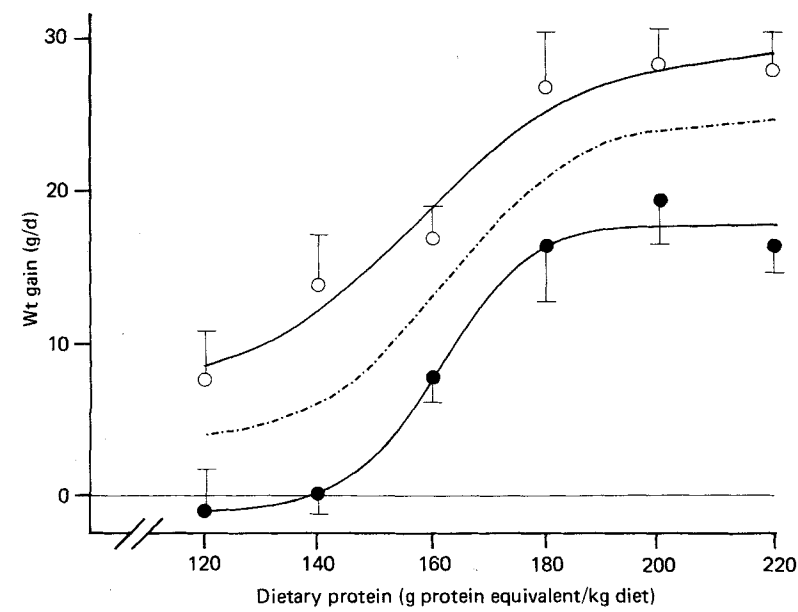

Fig. 2. Expt 3 (casein diets). Mean daily weight gain of male (○) and female (●) kittens. Vertical bars represent the standard error of the individual means. Curves represent calculated non-linear regressions (♂ and ♀ individually, ——; ♂+♀, – · –) whose parameters are given in Table 7. For details of diets, see p. 502 and Table 2.

Table 6. *Effect of dietary protein level on nitrogen retention (g N/d) for three experiments**

(Values are means of average N retention of each animal over a 10 d period; no. of kittens given in parentheses)

Expt no....	1			2			3		
Dietary protein equivalent (g/kg diet)	♂ (4)	♀ (4)	♂+♀ (8)	♂ (6)	♀ (6)	♂+♀ (12)	♂ (6)	♀ (6)	♂+♀ (12)
120	—	—	—	0·37[a]	0·26[a]	0·32[a]	0·36[a]	0·13[a]	0·25[a]
140	0·39	0·28	0·34[a]	0·47[a]	0·32[a]	0·40[ab]	0·55[b]	0·19[a]	0·37[a]
160	0·55	0·34	0·44[ab]	0·52[a]	0·39[a]	0·46[b]	0·61[b]	0·45[b]	0·53[b]
180	0·58	0·47	0·52[b]	0·77[b]	0·53[b]	0·65[c]	0·92[c]	0·61[b]	0·77[c]
200	0·55	0·53	0·54[b]	0·82[b]	0·61[b]	0·72[c]	0·91[c]	0·66[b]	0·78[c]
220	—	—	—	0·87[b]	0·78[c]	0·83[d]	1·03[c]	0·67[b]	0·85[c]
Pooled SE	0·10	0·01	0·05	0·06	0·04	0·04	0·06	0·07	0·05

[a, b, c, d] Means within a column with a different superscript letter were significantly different ($P < 0·05$).

* For details of experiments, see p. 502.

protein/kg diet compared with the two lowest levels of dietary protein given in this experiment (120 and 140 g/kg diet). Weight gains on the four highest levels of dietary protein were not significantly different for this group.

The sigmoidal model was fitted to weight gains of male and combined sexes in Expt 2, and male, female and combined sexes in Expt 3. The estimated parameters are found in Table 7 (p. 508) and the resulting regressions are shown in Figs. 1 and 2. The weight gain of one male kitten on the 220 g/kg diet in Expt 3 was not included in calculating parameters for the non-linear regression. During the previous period this animal had been on the lowest protein diet (120 g/kg) and had gained a total of 26 g during the 10 d period. When given the 220 g/kg diet this kitten gained close to 500 g for the 10 d period, almost twice the gain of the other male kittens on this diet. This observation was rejected for the male weight gain and N-retention group for Expt 3, in accordance with the Anscombe & Tukey (1963) rule for the Latin-square classification as explained in Snedecor & Cochran (1967).

For all five regressions the F values of the lack of fit mean square divided by the pure error mean square was less than one, indicating that the model provided an adequate description of the data. Tests of the overall regressions were significant ($P < 0·05$) in each case. The requirement, calculated as 95% of the upper asymptote, was 170 g/kg and 190 g/kg for male and combined sexes in Expt 2, and 200, 180 and 190 g protein/kg diet for male, female and combined sexes in Expt 3.

Analysis of variance indicated a significant effect of dietary protein level on N retention for the female and combined sexes in Expt 1, and for the male, female and combined sexes in Expts 2 and 3 (Table 6). For the combined sexes in Expt 1, the range test indicated that N retention was significantly decreased on the 120 g protein/kg diet compared with that on the 180 and 200 g protein/kg diet. Male kittens in Expt 2 and the male and combined sexes in Expt 3 had a significantly higher N retention on the three highest levels of dietary protein (180–220 g/kg) compared with the three lowest levels. For female kittens in Expt 3, N retention on the four highest levels of dietary protein (160–220 g/kg) was significantly greater than retention on the two lowest levels. N retention for the female and combined sexes in Expt 2 was significantly greater at the highest level of dietary protein (220 g/kg) compared with the lower five levels.

The sigmoidal model was fitted to N retention *v.* dietary crude protein level for the same

Table 7. *Parameters for the sigmoidal curves**

Parameter... / Expt no.†	P1 Mean	P1 SE	P2 Mean	P2 SE	P3 Mean	P3 SE	P4 Mean	P4 SE	Calculated requirement‡ (g/kg diet)
				Wt gain					
2 ♂	11·55	0·64	10·42	0·83	49·08	0·53	−300·0	0	170
♂+♀	9·05	0·47	11·17	0·69	18·01	3·5	−108·86	20·9	190
3 ♂	7·23	2·26	22·13	3·05	11·04	3·35	−70·03	20·4	200
♀	−0·95	0·94	18·82	1·32	22·21	7·16	−137·99	44·5	180
♂+♀	3·54	0·93	21·11	1·31	14·54	2·52	−89·79	15·4	190
				N retention					
2 ♂	0·39	0·03	0·47	0·04	15·03	4·05	−89·53	24·0	190
♂+♀	0·28	0·04	0·61	0·08	7·88	1·67	−45·14	9·8	230
3 ♂	0·37	0·05	0·56	0·07	12·83	3·81	−81·56	23·5	190
♀	0·12	0·04	0·55	0·05	16·80	5·05	−107·26	31·6	180
♂+♀	0·22	0·04	0·63	0·05	11·42	2·15	−72·15	13·1	190

* The non-linear model used for these calculations was $y = P1 + P2/[1 + e^{(P3+P4 \cdot x)}]$, obtained from Robbins *et al.* (1979).

† For details of experiments, see p. 502.

‡ Requirement calculated as levels of x (dietary protein) at 95% of the upper asymptote (P1+P2). For calculating parameters, dietary protein levels were expressed as g/g diet, rather than as g/kg diet, due to computer exponent limitations.

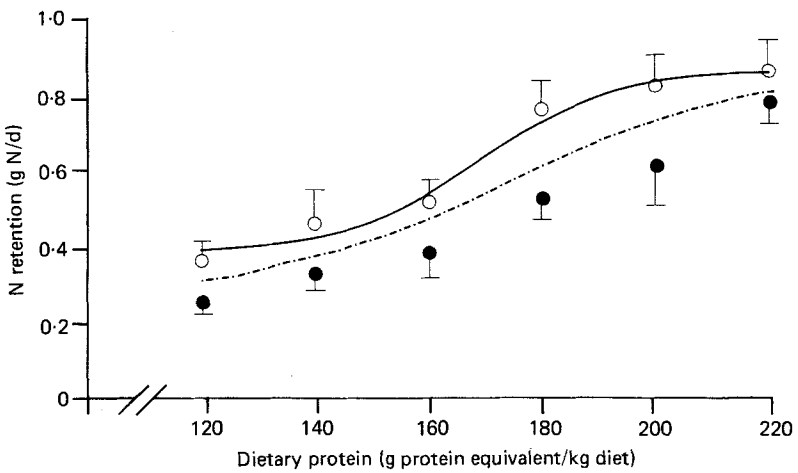

Fig. 3. Expt 2 (amino acid diets). Mean daily N retention of male (○) and female (●) kittens. Vertical bars represent the standard error of the individual means. Curves represent calculated non-linear regressions (♂, ——; ♂+♀, –·–) whose parameters are given in Table 7. For details of diets, see p. 502 and Table 2.

five groups which had been fitted for weight gain (Table 7 and Figs. 3 and 4). The F values for lack of fit for each of the five regressions were less than one, and the regressions were significant. The calculated protein requirements (95% of the upper asymptote) for male and combined sexes in Expt 2 were 190 and 230 g/kg respectively; and for male, female and combined sexes in Expt 3, 190, 180 and 190 g/kg diet respectively (Table 7).

Plasma amino acid values for Expts 2 and 3 are given in Table 8.

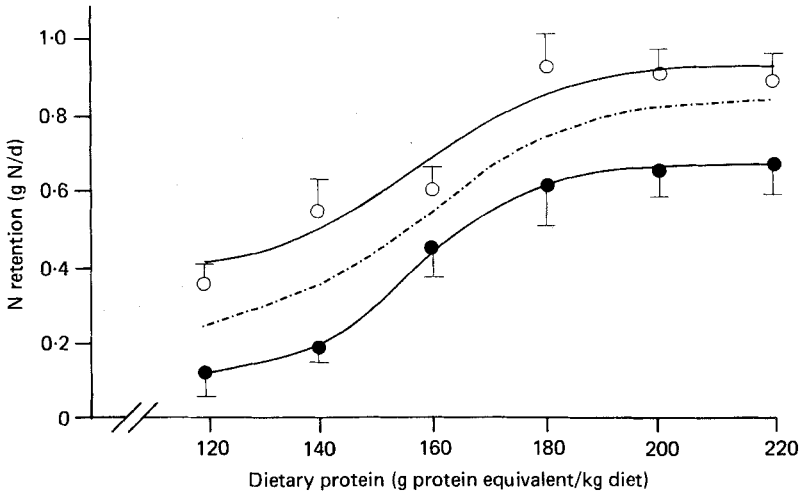

Fig. 4. Expt 3 (casein diets). Mean daily N retention of male (○) and female (●) kittens. Vertical bars represent the standard error of the individual means. Curves represent calculated non-linear regressions (♂ and ♀ individually, ——; ♂+♀, –·–) whose parameters are given in Table 7. For details of diets, see p. 502 and Table 2.

Table 8. *Plasma amino acid levels (μmol/l)*

*Expt no.**...	2		3			
Dietary protein equivalent (g/kg diet)...	120 (pooled values)	220 (pooled values)	120		220	
			Mean	SE	Mean	SE
Alanine	493	534	482	6	659	77
Arginine	201	384	166	16	181	20
Asparagine	135	216	48	14	119	12
Aspartic acid	40	51	28	2	44	3
Citrulline	21	26	14	2	22	2
Cystathionine	55	88	31	7	53	10
Glutamic acid	79	85	77	16	90	7
Glutamine	395	402	354	37	562	32
Glycine	1644	1839	502	17	604	101
Histidine	146	289	129	11	162	48
Hydroxyproline	61	58	54	4	48	4
Isoleucine	48	65	45	3	93	8
Leucine	91	124	80	6	166	14
Lysine	178	241	129	18	258	29
Methionine	82	148	50	8	116	15
Ornithine	134	119	49	5	56	30
Phenylalanine	89	86	88	5	116	10
Proline	129	144	117	12	292	46
Serine	228	212	196	16	274	22
Threonine	192	336	125	13	251	25
Tryptophan	67	68	58	5	78	4
Tyrosine	48	70	61	4	114	8
Valine	92	167	131	11	280	25

* For details of experiments, see p. 502.

DISCUSSION

The majority of information on weight gain and N retention obtained in the present study indicates that the crude protein requirement of kittens on a purified diet providing 21 MJ ME/kg diet is between 180 and 200 g/kg diet. In Expt 2, female kittens, in contrast to male, appeared to reach maximum weight gain and N retention at 220 g/kg. This may indicate that either female kittens have a greater dietary protein requirement than male kittens or that utilization of an amino acid is less efficient in the female in comparison with the male kitten. The weight gain and N retention of the female kittens on Expt 3 do not support the former supposition; weight gain and N retention were maximized at the same dietary protein level for both sexes. On the other hand, the differences between female growth rates and N retention in Expt 2 *v.* Expt 3 may be due to differences in the non-essential amino acid compositon of the diets. Previous work from this laboratory has indicated the importance of asparagine for maximal growth of kittens (E. M. Kamakawa, J. G. Morris and Q. R. Rogers, unpublished results); perhaps other non-essential amino acids are also necessary to support maximal growth, and this need may differ between the two sexes. Plasma amino acid values were not useful in determining the requirement. Most of the essential and non-essential amino acids in the dietary mixture increased in plasma with each increment of dietary protein; non-essential amino acids not in the dietary mixture remained relatively constant for all protein levels.

Previous investigators, utilizing diets based on unsupplemented natural proteins, have estimated the N requirement to be somewhat greater than our results suggest. Krehl & Welt (1948) gave semi-purified diets modelled after the solids in cow's milk or bitch's milk to kittens and adult cats. Those diets modelled after cow's milk were found to be inadequate for kitten growth but sufficient to maintain adult cats. Bitch's milk, conversely, was able to maintain a moderate rate of growth in the kittens. This was most likely due to the higher protein content of the bitch's milk in comparison with the cow's milk (25 *v.* 20% of total energy; Bernhart, 1961).

Dickinson & Scott (1955) found that a diet in which protein provided 240 g/kg dry diet, composed of herring and liver, was insufficient for growth of kittens. In a subsequent study (Dickinson & Scott, 1956) these investigators examined growth of kittens at four levels of dietary protein: 230, 300, 370 and 430 g/kg dry diet. Protein in these diets was supplied by white fish, herring and liver. They concluded that growth was satisfactory only at the 370 or 430 g/kg level of protein. The decrease in palatability of the lower-protein diets may have resulted in decreased food intake and thus confounded the results.

Casein has been utilized as the protein source in many N-requirement studies. It has the advantage of being relatively constant in composition and readily available. A disadvantage of casein is that arginine and total sulphur amino acids become limiting for growing kittens when the diet contains less than 200–240 g casein crude protein/kg diet.

Scott *et al.* (1957) found that 220 g casein/kg was insufficient to maintain growth in kittens but that 330 g casein/kg was adequate. Likewise, Miller & Allison (1958) found that kitten's growth rate was highest on a diet in which casein composed 250–300 g/kg of a dry diet with approximately 290 g fat/kg diet.

More recently, Jansen *et al.* (1975) examined growth and carcass N content on four different levels of dietary protein as casein: 260, 310, 360 and 400 g/kg dry diet. Neither weight gain nor carcass N composition differed significantly with dietary protein content, but weight gain did plateau at 360–400 g protein/kg diet. These workers concluded that 350 g protein/kg was a reasonable requirement to provide for maximal weight gain of kittens.

The requirement range we propose (180–200 g protein/kg diet) is higher than that estimated by Anderson *et al.* (1980) utilizing crystalline amino acid diets. In one of their

experiments, three levels of dietary protein equivalent were tested: 134, 158 and 182 g/kg diet. These levels were formulated by keeping the essential amino acids constant and increasing the proportion of non-essential amino acids. There was no significant change in weight gain, weight gain:feed value, weight gain:N intake value or ammonia-N excretion between treatments; in addition, weight gains were poor (9–10 g/d) on all diets. In another experiment, the 158 g protein/kg diet was compared with a commercial cat chow; weight gain over a 32 d period was somewhat under 20 g/d on the 157·5 g protein equivalent/kg purified diet. The estimate by Anderson *et al.* (1980) of the requirement is based on the equivalent response to the commercial cat chow.

The kitten's protein requirement for growth is greater than that of many other mammals. Direct comparison of protein requirements between species is complicated by several factors, i.e. the energy concentration in the diet, the amino acid composition of the diet in relation to the essential amino acid requirements of the species, the digestibility of the dietary protein and the age of the animals used in the study. In addition, requirements of several species (e.g. pig, mink and fox) are typically determined with mixed-protein diets, involving vegetable or meat by-product proteins, and cannot be considered indicative of the minimal amount of N required to attain maximal N retention by these species. To avoid some of these difficulties, only requirements determined with either high quality, semi-purified protein sources or crystalline amino acids were examined for comparison.

For maximal weight gain in growing rats given lactalbumen- or casein-based diets, crude protein levels of 115–120 g/kg diet are required in diets containing 120–150 g fat/kg (Breuer *et al.* 1964; Bunce & King, 1969; Burns *et al.* 1982). The growing mouse has a similar requirement of 125 g protein/kg diet, determined with a casein plus amino acid diet with 100 g fat/kg diet (John & Bell, 1976).

The dog has been shown to have a somewhat higher N requirement than either the rat or mouse. Milner (1981) was able to attain maximal weight gain on diets providing 140 g protein equivalent/kg, composed of crystalline L-amino acids with 50 g fat/kg. In the same study, N balance increased with each increment of dietary protein. Using lactalbumen-based diets with 120 g fat/kg, Burns *et al.* (1982) found no change in weight gain of young beagles on diets with between 150 and 200 g protein/kg diet.

The requirement range as determined in the present study is based on diets containing 250 g fat/kg diet and having an apparent crude protein digestibility of 0·90 and 0·95 for the amino acid and casein diets respectively (determined on 200 g protein/kg diet). Commercial cat foods are less digestible, with apparent crude protein digestibility for semi-moist and dry foods of 0·75 and for canned food of 0·80 (Kendall *et al.* 1982). Application of the requirement determined in the present study to commercial foods must take into account this lower digestibility and also the differences in energy density.

Burger *et al.* (1985) suggest that the minimal protein requirement of the adult cat is 125 g/kg diet. This indicates that approximately 0·66 of the kitten's requirement for N is utilized for maintenance. For omnivorous mammals, the proportion of the N requirement needed for maintenance is much lower: 0·35 for the rat and 0·33 for the dog. The kitten's high N requirement for maintenance is consistent with the finding that unlike omnivorous mammals, such as the rat (Schimke, 1962; Anderson *et al.* 1968; Das & Waterlow, 1974), the activities of enzymes involved in N catabolism in the kitten are unaffected by dietary protein level (Rogers *et al.* 1977). In omnivorous mammals, the ability to adapt to variations in dietary protein intake with changes in N catabolic enzyme activity is beneficial in that it contributes to the conservation of amino acids on low-protein intakes and, conversely, provides a mechanism to catabolize excess amino acids on a high-protein regimen. Although the relative affinities for amino acids by the N catabolic enzymes and the tRNA synthetases are unknown in the kitten, this inability to adapt enzymically to variations in dietary protein content may account in part for the kitten's high N requirement for maintenance.

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