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Maximal growth occurs at a broad range of essential amino acids to total nitrogen ratios in kittens*

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Summary. Kittens fed diets containing 2.0 and 3.0 times (×) the NRC (1986) essential amino acid (EAA) requirement (EAArq) and 210 to 560 g crude protein (CP)/kg diet had growth rates and plasma amino acid patterns that were not significantly different than kittens fed a control diet (CD) containing 1.5× EAArq and 350 g CP/kg diet. Growth rates of kittens fed diets containing only EAA (with nontoxic levels of arginine and methionine) and 280 to 460 g CP/kg diet were equivalent to those of kittens fed CD. Kittens fed only EAA and 140 and 210 g CP/kg diet had growth rates that were significantly lower than kittens fed CD. Since the growth rate of kittens fed 1.5× EAArq and 210 g CP/kg diet in a previous experiment was equivalent to kittens fed CD (Taylor et al., 1997), it is suggested that the requirement for CP is higher (up to 280 g CP/kg diet) when only EAA are fed. The higher crude protein requirement appears to be primarily a consequence of the high obligatory nitrogen loss as urea (especially from arginine) incurred in the conversion of nitrogen from EAA to dispensable amino acids in the liver and secondarily because of a slow rate of catabolism of the EAA. A 3-dimensional plot of weight gains vs. CP levels and EAA to total nitrogen (E:T) ratios of kittens shows a broad range of CP levels and E:T ratios that support optimal growth in the kitten. It is suggested that similar patterns would occur in the chick, rat and other species if adverse effects caused by excesses of specific amino acids are avoided.

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Abbreviations: CD, control diet; CP, crude protein; DAA, dispensable amino acids; EAA, essential amino acids; EAArq, essential amino acid requirement; E:T, essential amino acid nitrogen to total nitrogen ratio.

Introduction

Previous work in rats (Heger, 1990; Stucki and Harper, 1962), chicks (Stucki and Harper, 1961; Bedford and Summers, 1985), turkeys (Bedford and Summers, 1988), and pigs (Wang and Fuller, 1989) has suggested that growth rates are optimal when the dietary essential amino acid (EAA) to total nitrogen (E:T) ratio is between 0.4 to 0.65 and that diets containing only EAA (E:T ratio = 1.0) support poor growth rates. However, we have shown that kittens given diets that contain only EAA (E:T ratio = 1.0) can support near-maximal growth, if methionine and arginine are provided at concentrations within the tolerance of the kitten (Taylor et al., 1996). These results suggest that the poor growth rates reported in other species fed only EAA diets may be the result of one or more intolerances of EAAs rather than a metabolic inability of the animal to synthesize the dispensable amino acids (DAA) quickly enough to support rapid growth, as previously suggested (Adkins et al., 1966; Harper, 1974).

Furthermore, while the dietary crude protein (CP, nitrogen × 6.25) requirement of other species such as rats and chicks (NRC, 1978; NRC, 1984) has been well established, the CP requirement of the kitten is not firmly established. Different studies have suggested the requirement may range from 160 to 300 g CP/kg diet (Anderson et al., 1980; NRC, 1986; Smalley et al., 1985) or even higher (Hammer et al., 1996). Therefore, three experiments were conducted in which kittens were fed diets containing varying levels of CP and EAA at 2.0 or 3.0 times (×) the NRC (1986) EAA requirements (EAArq), or only EAA, in order to determine the effect of crude protein level and high E:T ratios on growth rates of kittens. Diets containing only EAA were formulated to avoid potential toxicities of arginine and methionine. A 3-dimensional plot of weight gains vs. CP level and E:T ratios of kittens fed diets from these and a previous set of similar experiments (Taylor et al., 1997) was constructed. This plot demonstrates that a wide range of CP levels and E:T ratios support optimal growth in the kitten.

Materials and methods

Animals

Fifteen female and fifteen male 8 to 12 wk old specific-pathogen-free kittens [mean bodyweight 941 ± 37 (SE) g] from the Nutrition and Pet Care Center, University of California, Davis, were adapted to a purified amino acid control diet and individually housed in stainless steel metabolism cages (0.61 × 0.61 × 0.66 m, l × w × h). The experimental protocol was approved by the University of California, Davis Animal Use

and Care Administrative Advisory Committee and was carried out in accordance with standards of the NIH *Guide for the Care and Use of Laboratory Animals* (NRC, 1985) and the Animal Welfare Act.

Diets

All diets used in the experiments contained crystalline amino acids as the sole source of nitrogen (Table 1). A control purified diet (CD) was prepared containing 106 g/kg diet of a 12-amino acid mixture, designated 12 EAA (consisting of the 10 EAA and cystine and tyrosine) which provided 1.5× the accepted EAArq for each EAA and tyrosine and cystine (NRC, 1986) and 247 g/kg diet of a DAA mixture such that it contained 350 g CP/kg diet (E:T ratio = 0.27). CD also contained 6.8 g sodium acetate/kg diet, 100 g starch/kg diet and 226 g dextrose/kg diet.

Three additional diets were prepared for experiment 1, each containing 2.0× EAArq (141 g 12-EAA mixture/kg diet) and 79, 149 and 429 g DAA mixture/kg diet such that the diets contained 210, 280 and 560 g CP/kg diet, respectively. Four additional diets were prepared for experiment 2 each containing 3.0× EAArq (212 g 12-EAA mixture/kg diet) and 86, 156, 226 and 366 g DAA mixture/kg diet such that the diets contained 280, 350, 420 and 560 g CP/kg diet, respectively.

Five additional diets were prepared for experiment 3, each containing EAA as the only source of dietary nitrogen. The first diet contained 2.1× EAArq (21 g arginine/kg

Table 1. Diet composition[1]

Ingredients	Exp. 1 diets	Exp. 2 diets	Exp. 3 diets
		g/kg diet	
12-Essential amino acid mixture (Exp. 1 & 2)[2,3]	141[4]	212[5]	–
10-Essential amino acid mixture (Exp. 3)[6]	–	–	117–529
L-methionine[3]	–	–	9–10
L-arginine[3]	–	–	21–30
Dispensable amino acid mixture[3,7]	79–429	86–366	0
Sodium acetate[8]	9.0	13.5	9.6–43.7
Dextrose[9]	7–356	0–274	0–425
Starch[10]	100	94–100	53–100
Crude protein[11]	210–560	280–560	140–460
E:T ratio[12]	0.23–0.61	0.34–0.69	1.0

[1] All diets contained (g/kg diet): animal tallow (Florin Tallow, Dixon, CA), 200; hydrogenated beef tallow (Bunge edible oils, Fort Worth, TX), 50; vitamin mixture (Williams et al., 1987), 10; mineral mixture (Williams et al., 1987), 50; choline chloride (Du Pont, Highland, IL), 3; taurine (Taisho Pharmaceutical, Torrance, CA), 1.5. [2] 12-Essential amino acid mixture composition (g/kg mixture): L-arg, 142; L-met, 57; L-his, 43; L-ile, 71; L-leu, 170; L-lys-HCl, 142; L-cystine, 50; L-phe, 57; L-tyr, 64; L-thr, 99; L-trp, 21; L-val, 85. [3] Ajinomoto USA Inc., Teaneck, NJ. [4] 2.0× the accepted essential amino acid requirement for kittens (NRC, 1986). [5] 3.0× the accepted essential amino acid requirement. [6] 10-Essential amino acid mixture composition (g/kg mixture): L-his, 53; L-ile, 89; L-leu, 212; L-lys-HCl, 177; L-cystine, 62; L-phe, 71; L-tyr, 80; L-thr, 124; L-trp, 27; L-val, 106. [7] Dispensable amino acid mixture composition (g/kg mixture): L-ala, 175; gly, 175; L-gln, 175; L-glu, 75; L-asn, 150; L-asp, 100; L-pro, 150. [8] Fisher Scientific, Santa Clara, CA. [9] Cerelose (dextrose), 2001 Corn Products, Englewood Cliffs, NJ. [10] Melojel, National Food Starch and Chemical, Bridgewater, NJ. [11] Calculated N × 6.25. [12] Essential amino acid to total nitrogen ratio.

diet, 9 g methionine/kg diet and 117 g/kg diet of a 10-EAA mixture consisting of all of the EAA and cystine and tyrosine except arginine and methionine) such that it contained 140 g CP/kg diet. The four other diets each contained 3.0× the requirement for arg (30 g arginine/kg diet) and 2.5× the requirement for met (10 g methionine/kg diet). They also contained 3.5×, 5.2×, 6.9× and 9.7× the EAArq for all of the other EAA (including tyrosine and cystine) or 188, 282, 377 and 529 g 10-EAA mixture/kg diet such that the diets contained 210, 280, 350 and 460 g CP/kg diet, respectively. The composition of the 12-EAA, 10-EAA and DAA mixtures are summarized in Table 1.

In all diets, sodium acetate was added on an equimolar basis to balance the hydrochloride associated with lysine in the 12-EAA and 10-EAA mixtures. Adjustments in amino acids and sodium acetate were made at the expense of dextrose. With the exception of amino acids, sodium acetate, dextrose and starch, all other diet ingredients were present in the same concentrations in all diets (table 1, footnote 1). The E:T ratio was calculated based on the ratio of the sum of the nitrogen contained in the essential amino acids plus cystine and tyrosine to that of the sum of the nitrogen contained in all of the amino acids in the diet. The crude protein was calculated from the total nitrogen in the diet (N × 6.25).

Design

All experiments used two latin squares, one square for male and the other square for female kittens. Kittens were allocated to rows and dietary periods of 10 days as columns. In experiment 1,4 × 4 latin squares were used. The kittens had a mean bodyweight of 989 ± 28 (SE) g. and the diets were CD and 2.0× EAArq and 210, 280 and 560 g CP/kg diet as treatments. In experiment 2,5 × 5 latin squares were used. The kittens had a mean bodyweight of 743 ± 30 (SE) g and the diets were CD and 3.0× EAArq with 280, 350, 420 and 560 g CP/kg diet. In experiment 3,6 × 6 latin squares were used. The kittens had a mean body weight 1075 ± 14 (SE) g and the diets were CD and only EAA with 140, 210, 280, 350, and 460 g CP/kg diet.

In all experiments, food intake and bodyweight were recorded daily. Blood was taken in heparinized syringes from the jugular vein of the unanesthetized kitten on d 8, 9 or 10 of each period. Plasma was treated with an equal volume of 0.28 mol/L sulfosalicylic acid and then prepared and analyzed for free amino acids using an amino acid analyzer (Model 7300, Beckman Instruments, Palo Alto, CA). During the last 7 d of each period, urine was collected and stored in containers with hydrochloric acid and feces were collected and stored at −20°C pending analysis. Nitrogen in diets, urine and feces was determined as previously described (Biourge et al., 1994) with an automatic nitrogen analyzer (Leco FP-248 model 601–700, Nitrogen determinator; Leco, St. Joseph, MI) for calculation of daily nitrogen retention.

Statistics

Analysis of variance (ANOVA) of the means of the slopes of the weight gain, nitrogen retention and food intake results from each period were used to detect any significant effects of treatment. When ANOVA revealed significant effects ($P < 0.05$), Tukey's method was used to determine which means were significantly different. Statistical analysis was performed using the SAS statistical package (PC-SAS, version 6.04, SAS Institute, Cary, NC). All results are expressed as means ± SEM.

Results

Mean daily weight gains of male and female kittens and both sexes combined and results of statistical analyses are presented in Table 2. The latin square designs used in these experiments do not allow for the determination of

Table 2. Mean weight gains for males and females and mean nitrogen retention and food intakes for both sexes combined for kittens from experiments 1, 2 and 3[1]

Diets	Weight gain (males)	(females)	(both sexes)	Nitrogen retention (both sexes)	Food intake (both sexes)
			g/d		
Exp. 1					
CD [350 g CP/kg (1.5× EAArq)]	29.1 ± 2.7[a]	29.2 ± 3.7[a]	29.1 ± 2.1[a]	0.99 ± 0.08[a]	76.9 ± 3.9[a]
210 g CP/kg (2.0× EAArq)	22.7 ± 6.9[a]	22.9 ± 3.5[a]	22.8 ± 3.6[a]	0.83 ± 0.16[a]	64.3 ± 7.4[a]
280 g CP/kg (2.0× EAArq)	38.8 ± 10.0[a]	31.3 ± 6.2[a]	35.0 ± 5.6[a]	1.55 ± 0.27[a]	76.1 ± 6.2[a]
560 g CP/kg (2.0× EAArq)	25.9 ± 1.9[a]	17.4 ± 1.6[a]	21.7 ± 2.0[a]	1.09 ± 0.57[a]	68.2 ± 3.9[a]
Exp. 2					
CD [350 g CP/kg (1.5× EAArq)]	27.2 ± 4.0[a]	20.4 ± 3.8[a]	23.8 ± 2.9[ab]	0.82 ± 0.12[a]	61.2 ± 5.5[ab]
280 g CP/kg (3.0× EAArq)	29.5 ± 2.8[a]	21.9 ± 4.9[a]	25.7 ± 2.9[a]	0.70 ± 0.07[a]	54.8 ± 5.7[ab]
350 g CP/kg (3.0× EAArq)	30.2 ± 3.1[a]	26.5 ± 2.0[a]	28.3 ± 1.8[a]	1.00 ± 0.12[a]	63.1 ± 5.9[a]
420 g CP/kg (3.0× EAArq)	17.8 ± 1.1[a]	17.5 ± 1.0[a]	17.7 ± 0.7[b]	1.05 ± 0.07[a]	53.8 ± 3.3[b]
560 g CP/kg (3.0× EAArq)	23.5 ± 3.0[a]	23.8 ± 2.9[a]	23.6 ± 2.0[ab]	0.93 ± 0.15[a]	60.9 ± 3.6[ab]
Exp. 3					
CD [350 g CP/kg (1.5× EAArq)]	19.5 ± 1.8[a]	19.8 ± 1.5[a]	19.6 ± 1.1[a]	0.68 ± 0.09[a]	53.5 ± 2.2[a]
140 g CP/kg (only EAA)	−3.2 ± 1.5[b]	−4.3 ± 3.0[b]	−3.8 ± 1.6[c]	0.07 ± 0.04[c]	36.8 ± 1.5[c]
210 g CP/kg (only EAA)	7.5 ± 2.3[ab]	9.9 ± 3.3[a]	8.7 ± 2.0[b]	0.41 ± 0.06[b]	45.3 ± 3.8[b]
280 g CP/kg (only EAA)	15.2 ± 3.5[a]	18.5 ± 4.0[a]	16.9 ± 2.6[ab]	0.71 ± 0.07[a]	50.8 ± 3.0[ab]
350 g CP/kg (only EAA)	18.4 ± 4.2[a]	16.3 ± 3.0[a]	17.3 ± 2.5[ab]	0.68 ± 0.09[ab]	54.0 ± 4.3[a]
460 g CP/kg (only EAA)	18.3 ± 6.4[a]	17.2 ± 4.1[a]	17.8 ± 3.6[ab]	0.73 ± 0.08[a]	47.9 ± 3.6[ab]

[1] Weight gain (derived from linear regressions) and food intake data represent daily means of the 10 d experimental periods and nitrogen retention data represent daily means of the last 7 d of the 10 d experimental periods. n = 6 for male and female weight gain means and n = 12 for nitrogen retention and food intake means unless otherwise noted. Values with different superscripts are significantly different for data within each colum of each experiment ($p < 0.05$).

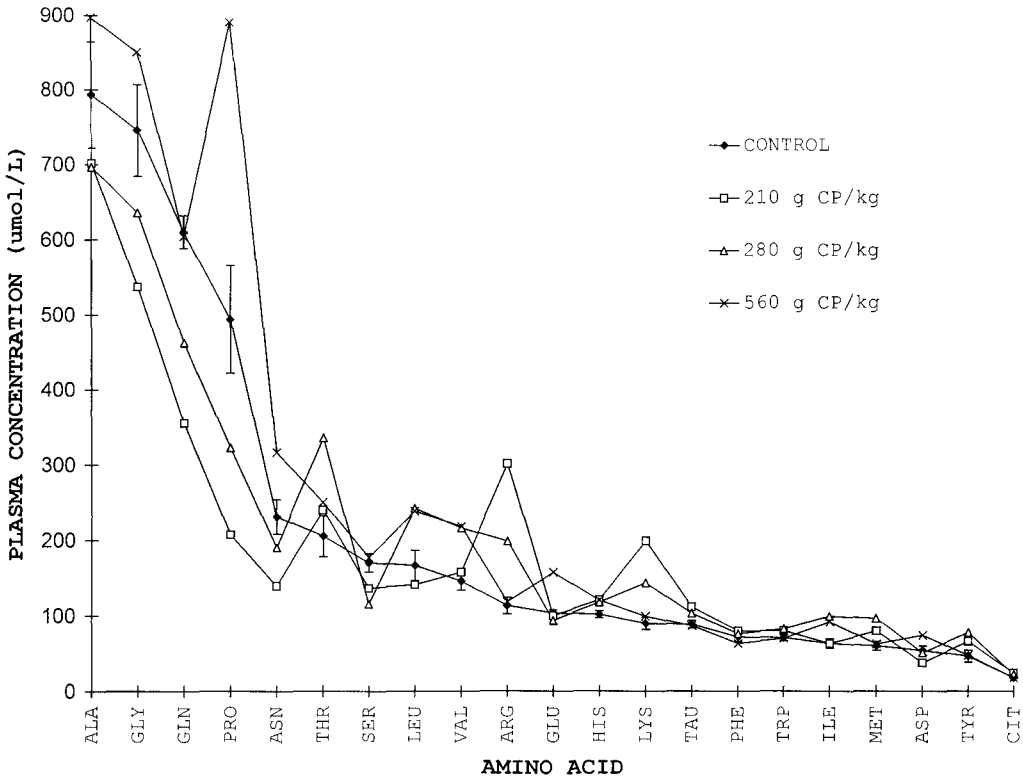

Fig. 1. Concentrations of plasma amino acids of kittens (Exp. 1) fed a control diet containing 1.5× EAArq and 350 g CP/kg and diets containing 2.0× EAArq and 210 to 560 g CP/kg. Each point represents the mean of 8 values. ±SEM bars are given for the control diet means

gender effects and their interactions with other factors when data for both sexes are combined. However, graphical comparisons of sex-specific weight gains across different dietary treatments (i.e. increasing levels of CP) showed that for all three experiments the lines were approximately parallel. Although absolute weight gains are greater for males than females, the effect of dietary treatment on weight gain in both sexes was similar. Therefore, mean weight gains, food intakes and nitrogen retentions in Table 2, plasma amino acid concentrations in Figs. 1, 2 and 3 and statistical analysis of these results are thus presented as combined results.

Experiment 1

Effects of feeding kittens CD versus diets containing 2.0× EAArq and 210, 280 and 560 g CP/kg on mean daily weight gain, food intake and nitrogen retention are summarized in table 2. Mean weight gains, nitrogen retentions and food intakes of kittens fed 210 and 560 g CP/kg diet were not significantly lower than those of kittens fed CD and 280 g CP/kg diet.

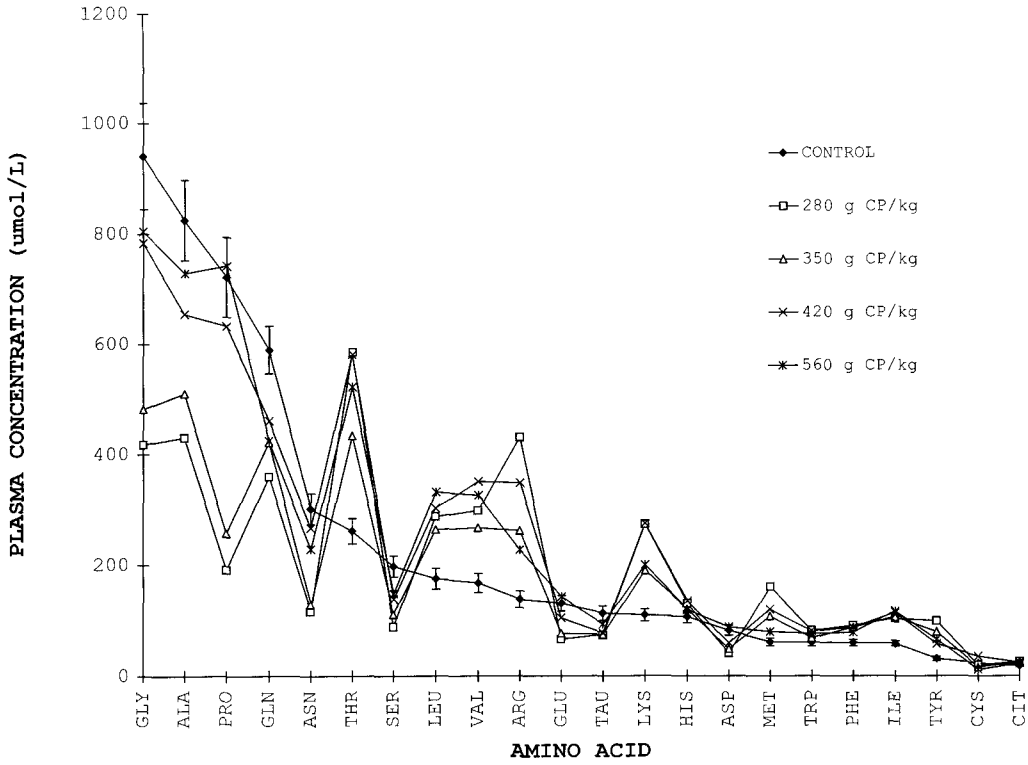

Fig. 2. Concentrations of plasma amino acids of kittens (Exp. 2) fed a control diet containing 1.5x EAArq and 350 g CP/kg and diets containing 3.0× EAArq and 280 to 560 g CP/kg. Each point represents the mean of 10 values. ±SEM bars are given for the control diet means

Mean plasma amino acid concentrations from kittens fed the diets in Exp. 1 are summarized in Fig. 1. Kittens fed diets containing 2.0× EAArq and 210, 280 and 560 g CP/kg had individual mean plasma EAA and DAA concentrations that were 0.85 to 2.6 and 0.4 to 1.8 times the mean EAA and DAA concentrations of kittens fed CD, respectively. Plasma concentrations of DAA from kittens fed 210 and 280 g CP/kg diets were lower than kittens fed CD or 560 g CP/kg diet. Plasma lysine and arginine concentrations were highest in kittens fed 210 g CP/kg diet (2.2 and 2.6 times that of CD-fed kittens, respectively). Plasma proline concentrations were 0.4 to 1.8 times that of CD-fed kittens (mean concentrations of plasma proline from kittens fed CD and 210, 280 and 560 g CP/kg diet were 494, 207, 323 and 890 μmol/L, respectively). Plasma glutamine concentrations were 0.6 to 1.0 times that of CD-fed kittens (mean concentrations of plasma glutamine from kittens fed CD and 210, 280 and 560 g CP/kg diet were 610, 360, 460 and 600 μmol/L, respectively).

Experiment 2

Effects of feeding kittens CD versus diets containing 3.0× EAArq and 280, 350, 420 and 560 g CP/kg on mean daily weight gain, nitrogen retention and

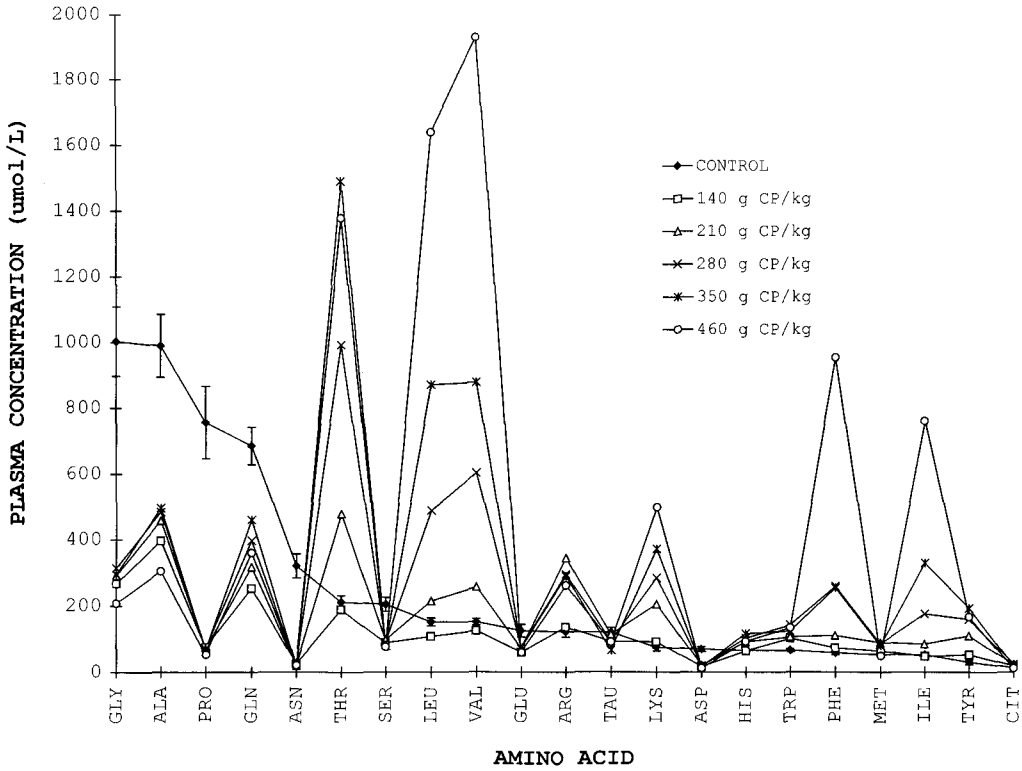

Fig. 3. Concentrations of plasma amino acids of kittens (Exp. 3) fed a control diet containing 1.5× EAArq and 350 g CP/kg and diets containing only EAA and 140 to 460 g CP/kg. Each point represents the mean of 12 values. ±SEM bars are given for the control diet means

food intake are summarized in Table 2. Mean weight gains, nitrogen retentions and food intakes of kittens fed 280 to 560 g CP/kg diet were not significantly different than those of kittens fed CD. However, weight gains of kittens fed 280 and 350 g CP/kg diet and food intakes of kittens fed 350 g CP/kg diet were significantly higher than those of kittens fed 420 g CP/kg diet.

Mean plasma amino acid concentrations from kittens fed the diets in Exp. 2 are summarized in Fig. 2. Kittens fed diets containing 3.0× EAArq and 280 to 560 g CP/kg had individual mean plasma EAA and DAA concentrations that were 1.1 to 3.1 and 0.3 to 1.1 times, respectively, that of the mean EAA and DAA concentrations of kittens fed CD. All plasma DAA concentrations in kittens fed 280 to 560 g CP/kg diet were lower than concentrations of CD fed kittens, with the exception of aspartic acid, glutamic acid and proline, in kittens fed 560 g CP/kg diet, which were slightly higher than kittens fed CD.

Experiment 3

Effects of feeding kittens CD versus diets containing only EAA and 140, 210, 280, 350 and 460 g CP/kg on mean daily weight gain, nitrogen retention and

food intake are summarized in Table 2. Mean weight gains, nitrogen retentions and food intakes of kittens fed 280, 350 and 460 g CP/kg diet were not significantly different than kittens fed CD. However, weight gains, nitrogen retentions and food intakes of kittens fed 210 g CP/kg diet were significantly lower than kittens fed CD and weight gains, nitrogen retentions and food intakes of kittens fed 140 g CP/kg diet were significantly lower than kittens fed all diets.

Mean plasma amino acid concentrations from kittens fed the diets in Exp. 3 are summarized in Fig. 3. Kittens fed diets containing only EAA and 140 to 460 g CP/kg had individual mean plasma EAA and DAA concentrations that were 0.7 to 16 and 0.06 to 0.7 times, respectively, that of the mean EAA and DAA concentrations of kittens fed CD. Plasma EAA concentrations in kittens fed 140 to 460 g CP/kg diet were higher than concentrations of kittens fed CD with the exception of isoleucine, histidine, valine, leucine and threonine in kittens fed 140 g CP/kg diet which were lower than kittens fed CD. Plasma EAA were very high in kittens fed 460 g CP/kg diet such that leucine, valine, phenylalanine, and isoleucine exceeded 10 times and lysine 6 times that of the concentrations of CD-fed kittens. All plasma DAA concentrations in kittens fed 140 to 460 g CP/kg diet were lower than concentrations of CD-fed kittens, especially asparagine and proline which were 6% to 8% and 7% to 10%, respectively, of CD fed kittens.

Discussion

There were no significant differences in growth rates and food intakes among kitten groups in experiment 1 fed diets containing 2.0× EAArq and 210, 280 and 560 g CP/kg diet, although kittens fed 2.0× EAArq and 280 g CP/kg diet and CD had slightly higher growth rates and food intakes. Plasma proline concentrations of kittens fed 2.0× EAArq and 560 g CP/kg diet were greatly elevated (1.8 times that of CD-fed kittens). Taylor et al. (1997) reported that kittens fed 1.5× EAArq and 560 g CP/kg diet also showed decreased weight gain and elevated plasma proline concentrations as compared to CD-fed kittens and it was hypothesized that, an intolerance of proline may have caused the decreased weight gain. Harper et al. (1970) have suggested that an excess of certain amino acids may cause a mild suppression of food intake with no other clinical signs or adverse metabolic effects.

Results from Exp. 3 were similar to our earlier results in that kittens fed only EAA and 280, 350 and 460 g CP/kg diets had growth rates that were not significantly different than kittens fed CD, even though mean weight gains were slightly lower. As in the previous study, the diets containing only EAA and 210 to 460 g CP/kg were modified such that arginine and methionine concentrations did not exceed the tolerance of kittens for these amino acids. Because arginine provided a significant amount of nitrogen to an EAA mixture, decreasing its level resulted in other EAA being increased to very high levels to achieve a CP concentration of 460 g/kg diet. It is important to note that the diet containing only EAA and 460 g CP/kg actually contained 569 g/kg diet of EAA (since the EAA mixture contained only about 12.9% nitrogen).

Except for arginine and methionine, all the EAA (plus tyrosine and cystine), were given at 9.7× their requirement and near-maximal growth was achieved.

We have shown that when the diet contains DAA, tryptophan, tyrosine, isoleucine and valine can be fed at 40×, 18×, 20× and 17× their requirement, respectively, with no adverse effects (Hargrove et al., 1988; Herwill, 1990). Few studies have been conducted to determine the tolerance of histidine, threonine, phenylalanine and lysine but in Exp. 3 they were present at 9.7× their requirement in the all EAA-460 g CP/kg diet and near-maximal growth was achieved in these kittens. This suggests that, with the exception of methionine and arginine, kittens have a very high tolerance for excess concentrations of EAA in the diet (Rogers and Morris, 1991).

Kittens fed the diet containing only EAA and 140 g CP/kg diet had growth rates that were zero or slightly negative. However, in a previous study (Taylor et al., 1997), kittens fed a diet containing 1.0× EAArq and 140 g CP/kg (E:T ratio = 0.47) had mean weight gains that were about three-fifths that of kittens fed the same CD (15 vs. 25 g/d, respectively). Furthermore, kittens given only EAA and 210 g CP/kg diet had growth rates that were about half that of kittens fed the diets containing only EAA and 280 to 460 g CP/kg (E:T ratio = 1.0) and CD. In our previous study, kittens fed a diet containing 1.5× EAArq and 210 g CP/kg (E:T ratio = 0.47) had weight gains that were equivalent to kittens fed CD (28 vs 24 g/d, respectively). Therefore, it appears that the CP requirement of kittens increases at high dietary concentrations of EAA, i.e. when E:T ratios are higher than that present in good quality meat proteins. These findings suggest that 210 g CP/kg diet can meet the kitten's requirement for nitrogen if the diet also contains 1.5× EAArq or E:T ratios around 0.5 while as much as 280 g CP/kg diet is required if the diet contains a high proportion of EAA (E:T ratios that approach 1.0).

Both EAA and DAA are required at the cellular level for animal growth. Some of the EAA in diets containing only EAA have to be catabolized (largely in the liver and kidney) to provide nitrogen for the synthesis of DAA. Kittens fed only EAA and 140 and 210 g CP/kg diets had weight gains that were significantly lower than kittens fed CD, and food intakes were about two-thirds and nine-tenths, respectively, of kittens fed CD. Similarly, kittens fed 1.0× EAArq and 140 g CP/kg diet (E:T ratio = 0.47) and 1.5× EAArq and 210 g CP/kg diet (E:T ratio = 0.47) in the previous study had mean food intakes that were, respectively, about nine-tenths and equivalent to that of kittens fed CD. Therefore, nitrogen intake for kittens fed each different diet with the same CP level was similar. This suggests that the conversion of EAA to DAA is inefficient in kitten liver probably because there is a lack of down regulation of hepatic urea cycle enzymes in the feline (Rogers et al., 1977) and therefore, a high obligatory nitrogen loss as urea occurs. Much of this obligatory loss of nitrogen may occur as urea directly from arginine. Arginine provided 31% and 29% of the dietary nitrogen in the diets containing only EAA and 140 and 210 g CP/kg diets, respectively. Only one-half of the dietary nitrogen provided by arginine is readily available for synthesis of DAA, the remainder of the nitrogen is obligatorily converted to urea which is excreted in the urine (Heger, 1990; Stein et al., 1986; Taylor et al., 1996).

Experiments in intact rats have shown that the time for two-thirds of an injected amino acid to be catabolized is much longer for the EAA than the DAA (1.2 to 6 hours, respectively) (Coulson and Hernandez, 1968). Branched chain amino acids are unique in that they are catabolized in muscle, instead of the liver. After absorption, these amino acids must be transported to the periphery, taken up by and catabolized by muscle, and then the nitrogen transported through the blood in the form of glutamine and alanine to other tissues such as the liver and kidneys for synthesis of other DAA. The kittens in Exp. 3 fed diets containing only EAA had plasma concentrations of the branched chain amino acids that were greatly elevated, while concentrations of glutamine and alanine were low (Fig. 3) suggesting a delay in the conversion of nitrogen from the branched chain amino acids to DAA.

In earlier experiments in kittens when high levels of DAA were fed, glutamate (and possibly proline) appeared to reach levels in the plasma associated with adverse effects (Deady et al., 1981; Taylor et al., 1997). To show the relationship among dietary CP, dietary EAA and plasma glutamate in the kitten, plasma glutamate and weight gain data from Exp. 1, 2 and 3 and from three previous experiments (Taylor et al., 1997) have been combined to create Fig. 4. The combined results in this figure indicates that increasing dietary DAA by increasing CP and/or decreasing EAA results in an increase

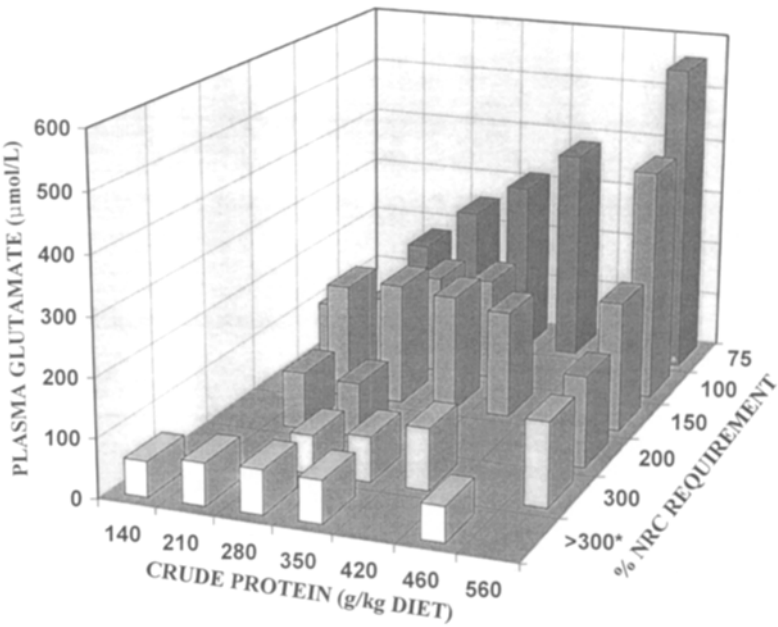

Fig. 4. Three-dimensional plot of mean plasma glutamate concentrations of kittens versus crude protein concentration versus % NRC essential amino acid requirement. This figure represents a compilation of data from experiments 1, 2 and 3 and three previous experiments (Taylor et al., 1997). Each column represents the mean of 8 to 12 values. *Diets represented in this row have greater than 300% of the essential amino acid requirement (except for 140 g CP/kg diet) and only essential amino acids

in plasma concentrations of glutamate to levels associated with toxicity in kittens. Plasma glutamate is normally less than 100 μmol/L and concentrations have not been shown to rise above 200 μmol/L unless dietary glutamate exceeds 60 g/kg diet (Deady et al., 1981). All the diets contained no more than 38 g glutamate/kg diet in the experiments shown in Fig. 5. Therefore, it is clear that high dietary concentrations of DAA other than glutamate results in the accumulation of glutamate in the body, undoubtedly contributing to the adverse effect of low E:T ratios on growth of kittens. This effect may be the result of high circulating levels of alanine, inhibiting the conversion of glutamate to alanine during absorption in the small intestine (Wiseman, 1964).

To show the extent to which dietary CP and E:T ratios can vary and still provide optimal growth in the kitten, weight gain data from Exp. 1, 2 and 3 and weight gain data from three previous experiments (Taylor et al., 1997) have been combined to create a 3-dimensional surface graph (Fig. 5). Because all six experiments used the same control diet, the mean weight gains of kittens fed each experimental diet were "normalized" relative to the mean weight gain of kittens fed CD within each experiment. The mean weight gain of kittens fed the CD (24.4 g/d) in the previous experiment in which diets containing 1.5× EAArq and 210 to 560 g CP/kg diet were fed was used as the "normal" value. Best fit lines were then drawn across the 27 weight gain points

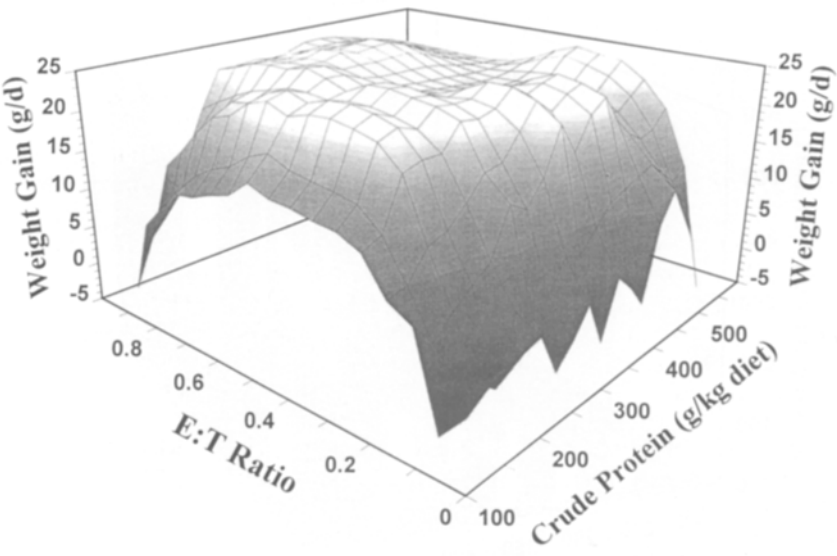

Fig. 5. Three-dimensional plot of weight gain of kittens versus crude protein concentration versus essential amino acid to total nitrogen (E:T) ratio of the diet. This figure represents a compilation of data from experiments 1, 2 and 3 and three previous experiments (Taylor et al., 1997). Because all six experiments used the same control diet, the mean weight gains of kittens fed each experimental diet were "normalized' relative to the mean weight gain of kittens fed CD within each experiment. The mean weight gain of kittens fed the CD (24.4 g/d) in the previous experiment in which diets containing 1.5× EAArq and 210 to 560 g CP/kg diet were fed was used as the "normal" value

positioned in space to create the surface graph shown. This graph illustrates that kittens grow maximally at a much broader E:T ratio and higher crude protein levels than chicks or rats. Stucki and Harper (1962) compiled a 3-dimensional surface graph that showed the optimal CP levels and E:T ratios that supported optimal growth of the chick were much narrower. The surface graph for the kittens, similar to that of the chicks demonstrates poor weight gains when diets containing low E:T ratios or low CP levels were fed, because the requirements for EAA and CP were not met. However, results from the chick showed decreased growth with diets containing high E:T ratios, whereas growth rates of kittens were optimal at much higher E:T ratios when the requirement for CP was met. We postulate that the difference between our observations and the chick study was a result of the dietary modifications we made to avoid EAA toxicities in the diets containing high E:T ratios. These modifications were not known to be necessary when the chick experiments were conducted. The surface graph for the kitten demonstrates a broad range of E:T ratios (i.e. 0.3 to 1.0) and CP levels (CP $\geq$ 280 g/kg diet) at which optimal growth can be achieved. Furthermore, it is likely that the area would be even broader if dietary modifications of DAA are made to avoid toxicities of specific DAA. In conclusion, experiments using wide E:T ratios at various CP levels in the diet of kittens show maximal growth at a broad range of E:T ratios and CP levels $\geq$280 g/kg diet.

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