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Increasing dispensable amino acids in diets of kittens fed essential amino acids at or below their requirement increases the requirement for arginine*

T. P. Taylor[1], **J. G. Morris**[1], **P. H. Kass**[2], and **Q. R. Rogers**[1]

[1] Department of Molecular Biosciences and [2] Department of Population Health and Reproduction, University of California, Davis, California, U. S. A.

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Summary. Kittens fed diets containing 0.75 × the NRC (1986) essential amino acid requirement (EAArq) and 210 to 560 g crude protein(CP)/kg diet exhibited, with increasing CP: 1) decreasing weight gain, 2) decreasing plasma arginine concentrations, 3) increasing urinary orotic acid excretion, 4) increasing plasma glutamic acid concentrations, and 5) plasma isoleucine concentrations at levels that suggest a marginal isoleucine deficiency. Kittens fed a control diet (CD) containing 1.5 × EAArq and 350 g CP/kg diet had maximal weight gains and no orotic aciduria. It was concluded that the decreased weight gain and adverse metabolic effects were caused by arginine deficiency and possibly glutamic acid toxicity induced by high dietary dispensable amino acids. Kittens fed the diets containing 1.0 × EAArq and 350 and 560 g CP/kg diet had depressed plasma arginine and elevated glutamic acid concentrations and orotic aciduria. These results indicate that 10 g arg/kg diet is not adequate at CP concentrations above 280 g/kg and the calculated requirement of arginine is (0.02 g arginine/g CP) × (Y g CP/kg diet) + (4.0 g arginine/kg diet) where Y is the dietary CP level.

Keywords: Essential amino acids – Dispensable amino acids – Crude protein – Orotic acid – Feline

Abbreviations: CD, control diet; CP, crude protein (g CP/kg diet = g nitrogen/kg diet × 6.25); DAA, dispensable amino acids; EAA, essential amino acids; EAArq, essential amino acid requirement.

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Introduction

Previous work in rats (Heger, 1990; Stucki and Harper, 1962), chicks (Bedford and Summers, 1985; Stucki and Harper, 1961), turkeys (Bedford and Summers, 1988), and pigs (Wang and Fuller, 1989) has shown that growth rates are maximal when essential amino acids (EAA) and dispensable amino acids (DAA) are fed in equivalent portions. Diets that contain low levels of EAA and high levels of DAA support poor growth rates. The cause of the poor growth rates found in studies with these animals has not been established but was suggested to be caused by a deficiency of one or more EAA or a toxicity of one or more DAA (Harper et al., 1970; Taylor, 1995).

We have shown that, if methionine and arginine are provided at dietary concentrations that are below toxic levels, diets that contain mostly or only EAA can support growth rates in kittens that are nearly equivalent (not significantly different) to those of Kittens fed a control diet that contains equivalent portions of EAA and DAA (Taylor et al., 1996). However, it is unknown what the effect of feeding diets that contain low levels of EAA (i.e. EAA at or near their requirement) and high levels of DAA would have on growth rates of kittens. Furthermore, while the dietary crude protein (CP, nitrogen $\times$ 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, particularly when kittens are fed the EAA near the requirement. Different studies have suggested the CP requirement to be anywhere from 160 to 300 g CP/kg diet (Anderson et al., 1980; NRC, 1986; Smalley et al., 1985) or possibly even higher (Hammer et al., 1996). Therefore, a series of three experiments was conducted in which kittens were fed diets containing varying levels of CP and EAA at 0.75, 1.0 or 1.5 $\times$ the accepted NRC (1986) EAA requirements (EAArq) in order to determine the CP requirement and the range of dietary amino acid patterns that support optimal growth of the kitten.

Materials and methods

Animals

Seventeen female and seventeen male 8 to 12 wk old specific-pathogen-free kittens [mean body weight 1209 $\pm$ 30 (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 $\times$ 0.61 $\times$ 0.66 m, l $\times$ w $\times$ 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

The composition of all diets is summarized in Table 1. All diets used in the experiments contained crystalline amino acids as the sole source of nitrogen. A purified control diet (CD) used in each experiment was prepared containing 103 g/kg diet of an EAA mixture which provided 1.5 $\times$ the accepted EAArq for each EAA including tyrosine

Table 1. Diet composition[1]

Diets[2]	Essential amino acid mixture[3,4]	Dispensable amino acid mixture[4,5]	Dextrose[6]	Sodium acetate[7]	E:T ratio[8]
			g/kg diet		
Exp. 1					
210 g CP/kg (0.75 × EAArq)	55[9]	159	359	13.1	0.23
280 g CP/kg (0.75 × EAArq)	55	229	289	13.1	0.17
350 g CP/kg (0.75 × EAArq)	55	299	219	13.1	0.14
420 g CP/kg (0.75 × EAArq)	55	369	149	13.1	0.11
560 g CP/kg (0.75 × EAArq)	55	509	9	13.1	0.08
Exp. 2					
140 g CP/kg (1.0 × EAArq)	74[10]	72	423	17.5	0.47
210 g CP/kg (1.0 × EAArq)	74	142	353	17.5	0.31
280 g CP/kg (1.0 × EAArq)	74	212	283	17.5	0.23
350 g CP/kg (1.0 × EAArq)	74	282	213	17.5	0.18
560 g CP/kg (1.0 × EAArq)	74	492	3	17.5	0.11
Exp. 3					
210 g CP/kg (1.5 × EAArq)	110[11]	107	342	26.3	0.47
280 g CP/kg (1.5 × EAArq)	110	177	272	26.3	0.35
CD[12][350 g CP/kg (1.5 × EAArq)]	110	247	202	26.3	0.27
420 g CP/kg (1.5 × EAArq)	110	317	132	26.3	0.23
560 g CP/kg (1.5 × EAArq)	110	457	9	26.3	0.17

[1] All diets contained (g/kg diet): starch (Melojel, National Food Starch and Chemical, Bridgewater, NJ), 100; 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] Diets are listed as concentration of crude protein (CP, nitrogen × 6.25) and the factor times (×) the essential amino acid requirement for kittens (EAArq; NRC 1986). [3] Essential amino acid mixture composition (g/kg mixture): L-arg-HCL, 163; L-met, 54; L-his-HCl-H_2O, 55; L-ile, 68; L-leu, 163; L-lys-HCl, 136; L-cys, 48, L-phe, 54; L-tyr, 61; L-thr, 95; L-trp, 20; L-val, 82. [4] Ajinomoto USA Inc., Teaneck, NJ. [5] 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. [6] Cerelose (dextrose), 2001 Corn Products, Englewood Cliffs, NJ. [7] Sodium acetate-3H_2O, Fisher Scientific, Santa Clara, CA. [8] Essential amino acid to total nitrogen ratio. [9] 0.75 × the essential amino acid requirement. [10] 1.0 × the essential amino acid requirement. [11] 1.5 × the essential amino acid requirement. [12] CD, control diet, fed in all experiments.

and cystine (NRC 1986) and 247 g/kg diet of a DAA mixture such that it contained 350 g CP/kg diet.

Five additional diets were prepared for experiment 1, containing 0.75 × EAArq (55 g EAA mixture/kg diet) and 159, 229, 299, 369 and 509 g DAA mixture/kg diet such that the diets provided 210, 280, 350, 420 and 560 g CP/kg diet, respectively. Five additional diets were prepared for experiment 2, containing 1.0 × EAArq (74 g EAA mixture/kg diet) and 72, 142, 212, 282 and 492 g DAA mixture/kg diet such that the diets provided 140, 210, 280, 350 and 560 g CP/kg diet, respectively. Four additional diets were prepared for experiment 3, containing 1.5 × EAArq (110 g EAA mixture/kg diet) and 107, 177, 317 and 457 g DAA mixture/kg diet such that the diets provided 210, 280, 420 and 560 g CP/kg diet, respectively. Sodium acetate was added on an equimolar basis to balance the hydrochloride associated with arginine, lysine and histidine in the EAA mixture. Adjustments in amino acids and sodium acetate were made at the expense of dextrose. With the exception of the amino acid mixtures, sodium acetate and dextrose, all other diet ingredients were present in the same concentration (Table 1).

Design

Exp. 1: Two 6 × 6 balanced latin square designs were constructed, one for six female and one for six male kittens [mean bodyweight 1275 ± 40 (SE) g], with kittens as rows, periods (10 d each) as columns and CD and diets containing 0.75 × EAArq and 210, 280, 350, 420 and 560 g CP/kg diet as treatments. A feeding error was made in the CD fed kittens during d 4, 5 and 6 of period 4 so data obtained from these two kittens during this period were excluded from final analysis and presentation of results. *Exp. 2*: Two 6 × 6 balanced latin square designs were constructed, one for six female and one for six male kittens [mean bodyweight 1301 ± 34 (SE) g], with kittens as rows, periods (10 d each) as columns and CD and diets containing 1.0 × EAArq and 140, 210, 280, 350 and 560 g CP/kg diet as treatments. A feeding error was made in the CD fed kittens during d 1, 2 and 3 of period 3 so data obtained from these two kittens during this period were excluded from final analysis and presentation of results. *Exp. 3*: Two 5 × 5 latin square designs were constructed, one for five female and one for five male kittens [mean bodyweight 1018 ± 36 [SE] g] with kittens as rows, periods (10 d each) as columns and CD and diets containing 1.5 × EAArq and 210, 280, 420 and 560 g CP/kg diet as treatments.

In all experiments, food intake and bodyweight were recorded daily. Blood was taken from each kitten in heparinized syringes from the jugular vein of the unanesthetized kittens on d 8, 9 or 10 of each period. Plasma was treated with an equal volume of 0.28 mol/L sulfosalicylic acid 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. Due to the fact that plasma arginine concentrations were low enough to indicate a possible arginine deficiency (Zicker and Rogers, 1990), orotic acid in urine (an indicator of arginine deficiency) was determined using strong anion exchange HPLC (Radial Pak SAX #87752, Waters, Millipore Division, Milford, MA) as previously described (Biourge et al., 1994).

In experiments 1 and 3, feces were collected during the last 7 d of each period and stored at −20°C until analyzed. 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. Four nitrogen retention values in exp. 1 and three in exp. 3 were excluded because of collection problems attributable to vomiting, diarrhea and other difficulties.

Statistics

Analysis of variance (ANOVA) of the means of the slopes of the weight gain, nitrogen retention, urinary orotic acid and food intake data from each period was used to detect

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 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 essentially parallel. This demonstrates that although absolute weight gains may differ between males and females, the effect of dietary treatment on weight gain in both sexes was essentially identical. Therefore, mean weight gains, food intakes and nitrogen retentions in table 2, mean weight gains and urinary orotic acid excretion in Fig. 1 and 3 and mean plasma amino acid concentrations in Fig. 2, 4 and 5 and statistical analyses of these results are thus presented as the combined results to demonstrate the overall effects of feeding the various dietary amino acid compositions for kittens of both sexes.

Experiment 1

Effects of feeding kittens CD versus diets containing 0.75 × EAArq and 210, 280, 350, 420 and 560 g CP/kg on mean daily weight gain, food intake, nitrogen retention and urinary orotic acid excretion are summarized in Table 2 and Fig. 1. Mean weight gain, nitrogen retention and food intake for both sexes combined for kittens fed CD were significantly higher than those of kittens fed all other diets ($P < 0.0001$). Orotic acid was not detected in the urine from kittens fed CD. Kittens fed diets containing 0.75 × EAArq and 210 to 560 g CP/kg exhibited decreasing weight gains and food intakes with increasing CP such that kittens fed the highest CP diet (560 g/kg diet) had weight loss (−3.8 g/d) and had the lowest nitrogen retention and food intake. Mean daily urinary orotic acid excretion increased with increasing levels of CP. Orotic acid excretion was highest in the kittens fed the 560 g CP/kg diet (74.3 ± 8.8 μmol/d). Kittens fed the 420 and 560 g CP/kg diets exhibited emesis and diarrhea and occasionally plasma was icteric. The most severe cases of these clinical signs occurred in the latter half of the experiment (i.e. periods 4, 5 and 6) and in kittens fed the 560 g CP/kg diet.

Mean plasma amino acid concentrations of blood from kittens fed the diets in Exp. 1 are summarized in Fig. 2. Kittens fed diets containing 0.75 × EAArq and 210 to 560 g CP/kg had mean individual plasma EAA concentrations that were 33% to 74% of the mean EAA concentrations of kittens fed CD. However, most mean concentrations of EAA in all dietary groups were above mean concentrations that were previously shown to be present when

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)	*Weight gain* (females)	*Weight gain* (both sexes)	*Nitrogen retention* (both sexes)	*Food intake* (both sexes)
			g/d		
Exp. 1					
CD[350 g CP/kg (1.5 × EAArq)][2]	34.8 ± 4.2[a]	31.6 ± 6.0[a]	33.2 ± 3.5[a]	1.18 ± 0.11[a]	72.3 ± 6.9[a]
210 g CP/kg (0.75 × EAArq)	23.0 ± 2.2[ab]	15.0 ± 3.4[b]	19.0 ± 2.3[b]	0.49 ± 0.05[b]	66.4 ± 5.0[b]
280 g CP/kg (0.75 × EAArq)	16.2 ± 2.3[bc]	12.0 ± 2.5[bc]	14.1 ± 1.8[b]	0.56 ± 0.06[b]	66.2 ± 4.3[b]
350 g CP/kg (0.75 × EAArq)	13.5 ± 3.5[bc]	8.3 ± 2.9[bc]	10.9 ± 2.3[bc]	0.54 ± 0.08[b3]	64.7 ± 4.5[bc]
420 g CP/kg (0.75 × EAArq)	4.9 ± 1.1[cd]	1.0 ± 1.4[c]	2.9 ± 1.1[cd]	0.27 ± 0.10[bc3]	56.0 ± 3.9[c]
560 g CP/kg (0.75 × EAArq)	−6.1 ± 2.6[d]	−1.5 ± 2.2[c]	−3.8 ± 1.7[d]	0.09 ± 0.14[c]	42.6 ± 2.3[c]
Exp. 2					
CD[350 g CP/kg (1.5 × EAArq)][2]	26.9 ± 1.9[a]	22.5 ± 4.5[a]	24.7 ± 2.4[a]	nd	81.6 ± 4.5[a]
140 g CP/kg (1.0 × EAArq)	15.2 ± 4.2[a]	14.5 ± 1.5[ab]	14.9 ± 2.1[b]	nd	70.6 ± 5.4[b]
210 g CP/kg (1.0 × EAArq)	17.4 ± 4.6[a]	16.8 ± 3.9[ab]	17.1 ± 2.9[ab]	nd	73.5 ± 4.3[ab]
280 g CP/kg (1.0 × EAArq)	14.6 ± 2.7[a]	16.2 ± 2.2[ab]	15.4 ± 1.7[b]	nd	73.9 ± 4.9[ab]
350 g CP/kg (1.0 × EAArq)	13.4 ± 1.2[a]	13.5 ± 2.5[ab]	13.5 ± 1.3[b]	nd	68.3 ± 4.5[b]
560 g CP/kg (1.0 × EAArq)	−0.8 ± 2.1[b]	3.5 ± 2.9[b]	1.3 ± 1.8[c]	nd	53.4 ± 4.1[c]
Exp. 3					
210 g CP/kg (1.5 × EAArq)	31.2 ± 2.9[ab]	24.9 ± 2.4[a]	28.1 ± 2.1[a]	1.05 ± 0.13[a]	88.7 ± 7.7[a]
280 g CP/kg (1.5 × EAArq)	30.9 ± 2.5[ab]	25.0 ± 2.7[a]	28.0 ± 1.9[a]	1.35 ± 0.13[a]	90.6 ± 4.5[a]
CD[350 g CP/kg (1.5 × EAArq)	29.9 ± 3.3[ab]	19.0 ± 3.8[ab]	24.4 ± 2.9[a]	1.18 ± 0.12[a4]	89.1 ± 6.6[a]
420 g CP/kg (1.5 × EAArq)	33.7 ± 1.5[a]	23.8 ± 1.3[a]	28.8 ± 1.9[a]	1.36 ± 0.21[a]	88.2 ± 4.0[a]
560 g CP/kg (1.5 × EAArq)	21.8 ± 2.2[b]	11.2 ± 3.3[b]	16.5 ± 2.6[b]	1.15 ± 0.24[a5]	76.7 ± 4.6[b]

[1] Weight gains (derived from linear regressions of daily cat weights) and food intakes represent daily means of the 10 d experimental periods and nitrogen retentions 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 within each column of each experiment with different superscripts are significantly different ($p < 0.05$). *nd* = not determined. [2] n = 5 for male and female weight gains and n = 10 for nitrogen retention and food intakes. [3] n = 10. [4] n = 9. [5] n = 8.

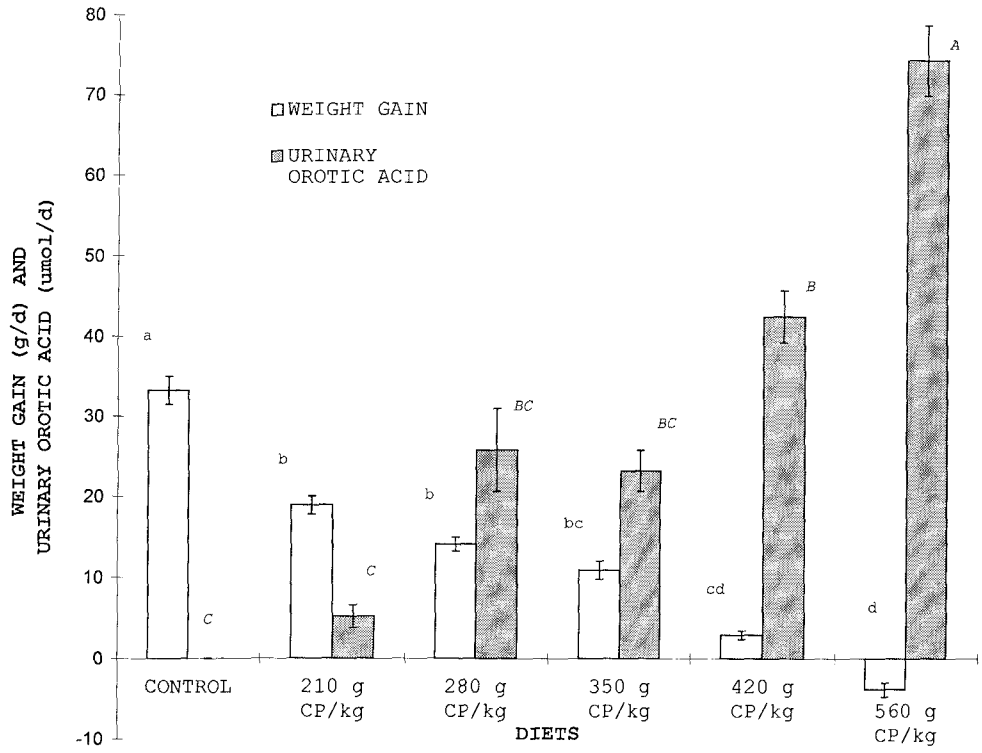

Fig. 1. Weight gain (g/d) and urinary orotic acid excretion (μmol/d) of kittens (Exp. 1) fed a control diet containing 1.5 × EAArq and 350 g CP/kg or diets containing 0.75 × EAArq and 210 to 560 g CP/kg (orotic acid was detected in 0, 4, 8, 12, 12 and 12 of 12 samples from kittens fed the control diet and diets containing 0.75 × EAArq and 210, 280, 350, 420 and 560 g CP/kg diet, respectively). Each bar represents the mean ±SEM of 12 values except for the control diet which is for 10 values. Weight gains labeled with different letters are significantly different, $p \leq 0.05$

each of the EAA was individually fed at its requirement (Zicker and Rogers, 1990). Exceptions were the concentrations of arginine in kittens fed 420 and 560 g CP/kg diets (72 and 66 μmol/L, respectively, as compared to 75 μmol/L previously reported by Zicker and Rogers (1990), plasma methionine concentration in kittens fed 210 g CP/kg diet (26 μmol/L, compared to 30 μmol/L) and isoleucine in kittens fed all except CD (21 to 29 μmol/L, compared to 30 μmol/L). Kittens fed 280 and 350 g CP/kg diets had arginine concentrations that were only slightly higher than those previously reported (81 μmol/L, for both) and those fed 280 g CP/kg diet had methionine concentrations that were only slightly higher (33 μmol/L).

Kittens fed diets containing 0.75 × EAArq and 210 to 560 g CP/kg had mean individual plasma DAA concentrations that were 85% to 304% of the mean DAA concentrations of kittens fed CD. Plasma glutamic acid concentrations were 96% to 304% of CD fed kittens (mean concentrations of plasma glutamic acid from kittens fed CD and 210, 280, 350, 420 and 560 g CP/kg diet were 184, 177, 252, 311, 382 and 559 μmol/L, respectively). Plasma proline concentrations were 92% to 231% of CD fed kittens (mean concentrations of

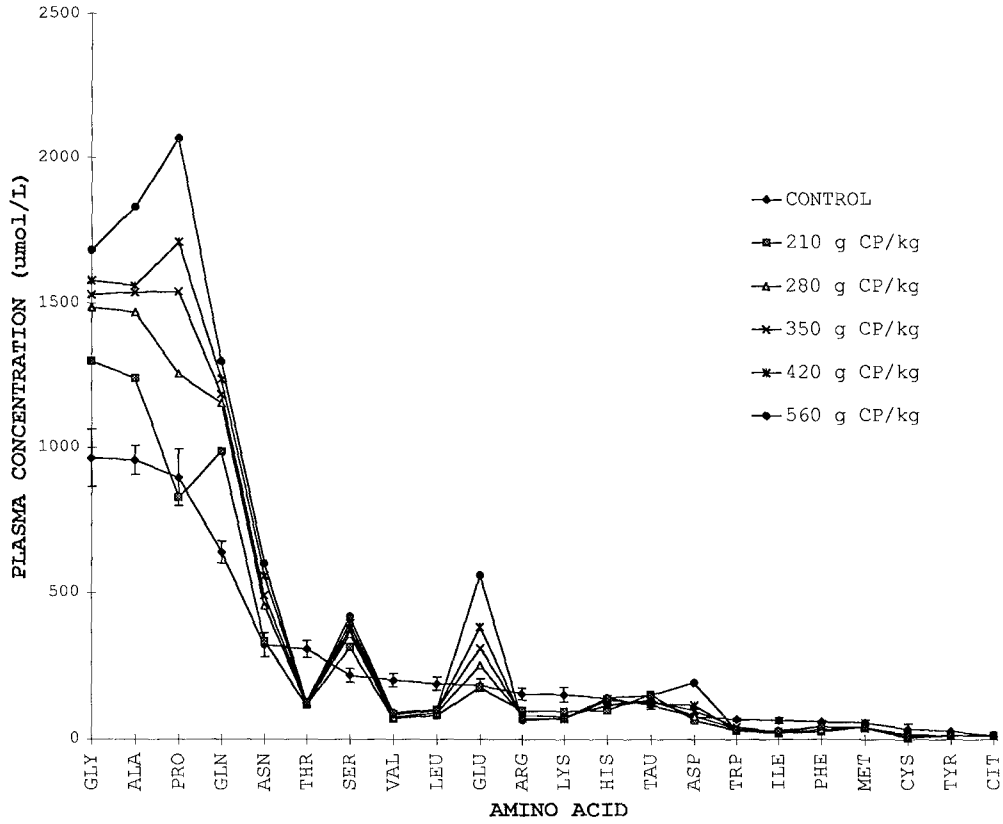

Fig. 2. Concentrations of plasma amino acids of kittens (Exp. 1) fed a control diet containing 1.5 × EAArq and 350 g CP/kg or diets containing 0.75 × EAArq and 210 to 560 g CP/kg. Each point represents the mean of 12 values except for the control diet which is for 10 values. ±SEM bars are given for the control diet means

plasma proline from kittens fed CD and 210, 280, 350, 420 and 560 g CP/kg diet were 896, 828, 1254, 1539, 1709 and 2066 μmol/L, respectively).

Experiment 2

Effects of feeding kittens CD versus diets containing 1.0 × EAArq and 140, 210, 280, 350 and 560 g CP/kg on mean daily weight gain, food intake and urinary orotic acid excretion are summarized in Table 2 and Fig. 3. Kittens fed CD had the highest mean weight gain for both sexes combined such that it was significantly higher ($P < 0.0001$) than weight gains of kittens fed all other diets except for those fed the 210 g CP/kg diet. Kittens fed the 560 g CP/kg diet had the lowest weight gain (1.3 g/d). Kittens fed this diet exhibited sporadic emesis and diarrhea although clinical signs were not as severe or as frequent as those seen in Exp. 1 when kittens were fed the 0.75 × EAArq and 420 and 560 g CP/kg diet. Orotic acid was not detected in the urine from the kittens fed CD or diets containing 1.0 × EAArq and 140, 210 and 280 g CP/kg but was detected in the urine of kittens fed the 350 and 560 g CP/kg diets

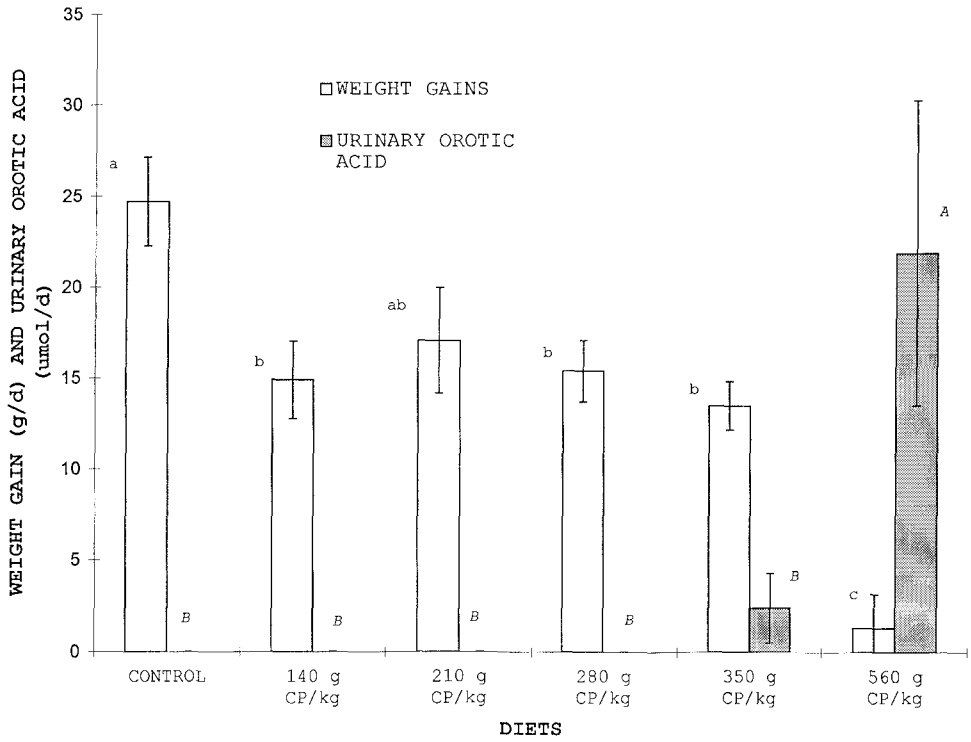

Fig. 3. Weight gain (g/d) and urinary orotic acid excretion (μmol/d) of kittens (Exp. 2) fed a control diet containing 1.5 × EAArq and 350 g CP/kg or diets containing 1.0 × EAArq and 140 to 560 g CP/kg (orotic acid was detected in 0, 0, 0, 0, 2 and 9 of 12 samples from kittens fed the control diet and diets containing 1.0 × EAArq and 140, 210, 280, 350 and 560 g CP/kg diet, respectively). Each bar represents the mean ± SEM of 12 values except for the control diet which is for 10 values. Weight gains labeled with different letters are significantly different, $p \leq 0.05$

(mean daily urinary orotic acid excretion of 2.4 ± 1.9 and 21.9 ± 8.4 μmol/d, respectively).

Mean plasma amino acid concentrations of blood from kittens fed the diets in Exp. 2 are summarized in Fig. 4. Kittens fed diets containing 1.0 × EAArq and 140 to 560 g CP/kg had mean individual plasma EAA concentrations that were 40% to 94% of the mean EAA concentrations of kittens fed CD. However, mean concentrations of EAA in all dietary groups were above mean concentrations that have previously been shown to be present when each of the EAA was fed at the requirement (Zicker and Rogers, 1990) except for concentrations of arginine in kittens fed the 560 g CP/kg diet (68 μmol/L) and isoleucine in kittens fed 140 and 280 g CP/kg diets (29 and 30 μmol/L, respectively). Kittens fed 350 g CP/kg diet had arginine concentrations that were only slightly higher than those previously reported (78 μmol/L).

Kittens fed diets containing 1.0 × EAArq and 140 to 560 g CP/kg had mean individual plasma DAA concentrations that were 19% to 275% of the mean DAA concentrations of kittens fed CD. Plasma glutamic acid concentrations were 68% to 275% of kittens fed CD (mean concentrations of plasma

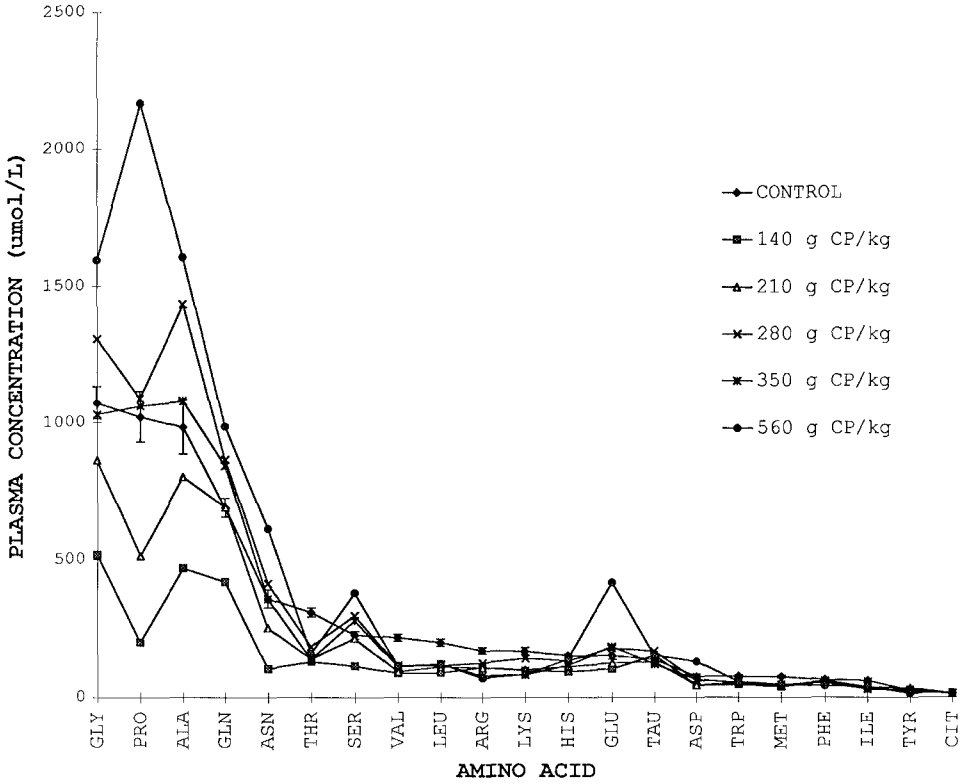

Fig. 4. Concentrations of plasma amino acids of kittens (Exp. 2) fed a control diet containing 1.5 × EAArq and 350 g CP/kg or diets containing 1.0 × EAArq and 140 to 560 g CP/kg. Each point represents the mean of 12 values except for the control diet which is for 10 values. ±SEM bars are given for the control diet means

glutamic acid from kittens fed CD and 140, 210, 280, 350 and 560 g CP/kg diet were 150, 102, 124, 176, 182 and 413 μmol/L, respectively). Plasma proline concentrations were 19% to 212% of CD fed kittens (mean concentrations of plasma proline from kittens fed CD and 140, 210, 280, 350 and 560 g CP/kg diet were 1021, 199, 513, 1087, 1062 and 2165 μmol/L, respectively).

Experiment 3

Effects of feeding kittens CD versus diets containing 1.5 × EAArq and 210, 280, 420 and 560 g CP/kg on mean daily weight gain, food intake and nitrogen retention are summarized in Table 2. There were no significant differences in mean weight gains, nitrogen retentions and food intakes for both sexes combined for kittens fed CD and diets containing 1.5 × EAArq and 210, 280 and 420 g CP/kg. However, weight gain and food intake of kittens fed the 560 g CP/kg diet were significantly lower than those for all other treatment groups. Orotic acid was not detected in any of the urine from kittens in this experiment.

Mean plasma amino acid concentrations of blood from kittens fed the diets in Exp. 3 are summarized in Fig. 5. Kittens fed diets containing 1.5 ×

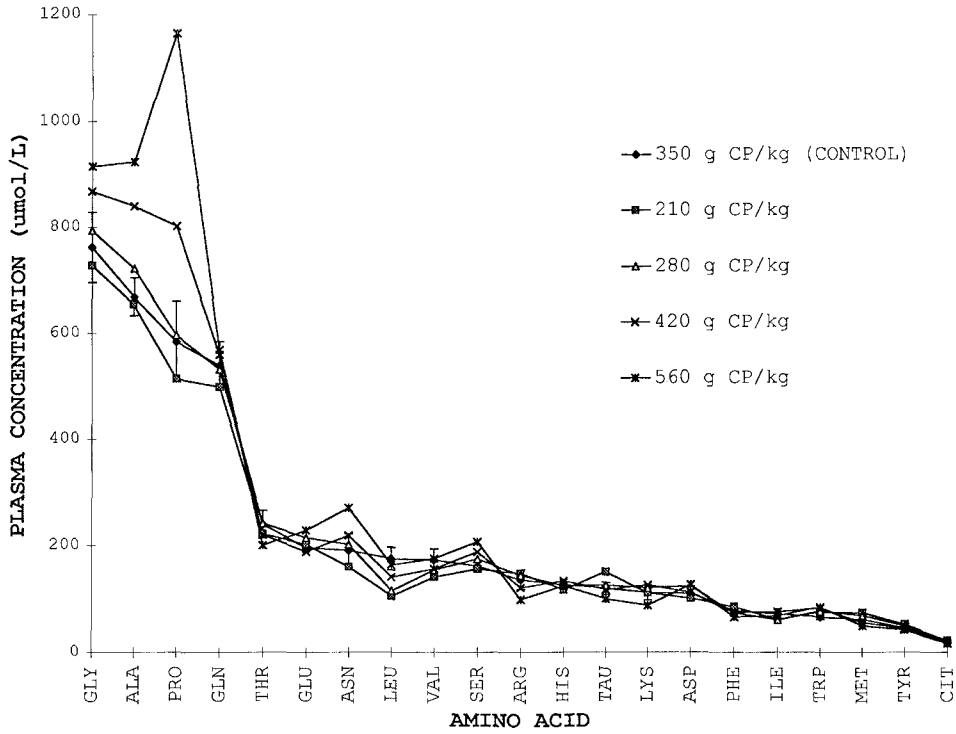

Fig. 5. Concentrations of plasma amino acids of kittens (Exp. 3) fed a control diet containing 1.5 × EAArq and 350 g CP/kg or diets containing 1.5 × EAArq and 210 to 560 g CP/kg. Each point represents the mean of 10 values. ±SEM bars are given for the control diet means

EAArq and 210, 280, 420 and 560 g CP/kg had mean individual plasma EAA and DAA concentrations that were 60% to 129% and 88% to 200%, respectively, of the mean EAA and DAA concentrations of kittens fed CD. Plasma glutamic acid concentrations were 96% to 117% of CD fed kittens (mean concentrations of plasma glutamic acid from kittens fed CD and 210, 280, 420 and 560 g CP/kg diet were 196, 201, 214, 188 and 228 μmol/L, respectively). Plasma proline concentrations were 88% to 200% of CD fed kittens (mean concentrations of plasma proline from kittens fed CD and 210, 280, 420 and 560 g CP/kg diet were 584, 513, 596, 801 and 1165 μmol/L, respectively).

Discussion

Kittens fed diets containing 0.75 × EAArq and 210 to 560 g CP/kg and 1.0 × EAArq and 350 and 560 g CP/kg developed orotic aciduria and had substantially lower plasma arginine concentrations. This suggests that the decreased weight gains, nitrogen retentions and food intakes and exhibited emesis and diarrhea that occurred in kittens fed these diets were caused by hyperammonemia resulting from arginine deficiency. Acute experiments (Morris and Rogers, 1978a) have shown that within 1–4 hours after a single meal of an arginine-deficient diet, cats exhibit emesis, hypersalivation, ataxia

and some become comatose and die. These results indicate that, at least for arginine deficiency, an adverse metabolic response is responsible for the decreased food intake which then results in the decreased growth rate. It was also shown* (Morris, 1985) that when there is insufficient dietary arginine to maintain normal urea cycle function that orotic aciduria occurs and is the most sensitive metabolic index of arginine insufficiency. Thus, the feline has an absolute requirement for arginine. Studies have shown that feeding a diet containing less than 8 g arginine/kg to growing kittens with 272 g CP/kg results in the same adverse effects – decreased weight gain, emesis, diarrhea and orotic aciduria (Costello et al., 1980; Morris and Rogers, 1978b). As evidenced by the increasing urinary orotic acid excretion, decreasing plasma arginine concentrations and decreasing weight gains that occurred when increasing CP levels and 0.75 × and 1.0 × EAArq (7.5 and 10 g arginine/kg diet, respectively) were fed, it appears that the kittens' requirement for arginine is a function of the level of dietary CP such that the requirement increases with increases in CP.

The mean daily urinary orotic acid excretion and dietary CP level of kittens from Exp. 1 were fit with a least squares linear regression trend line ($y = 0.19x - 34.3$, $r^2 = 0.95$). The regression suggests that at 182 g CP/kg diet orotic aciduria would just begin to occur in kittens fed 7.5 g arginine/kg diet. A similar regression trend line for data from kittens from Exp. 2 ($y = 0.08x - 24.3$, $r^2 = 0.98$) suggests that at 298 g CP/kg diet orotic aciduria would just begin to occur in kittens fed 10 g arginine/kg diet. When kittens were fed the diet containing 1.5 × EAArq and 560 g CP/kg no orotic aciduria occurred. Assuming that 7.5, 10 and 15 g arginine/kg diet just meet the kittens' requirement for arginine when 182, 298 and 560 g CP/kg diet, respectively, are fed, a linear regression of these three points ($y = 0.02x + 4.0$, $r^2 = 0.99$) suggests that the requirement for arginine is linearly related to dietary CP level. Based on these results, the requirement for arginine is calculated to be (0.02 g arginine/g CP) × (y g CP/kg diet) + (4.0 g arginine/kg diet) where y is the dietary CP level. It is important to note that the formula incorrectly suggests that kittens have a requirement of 4 g arginine/kg diet even when fed a diet containing no protein. We have fed kittens protein-free diets without observing any clinical signs of hyperammonemia (unpublished results). More experiments need to be conducted to establish a more precise quantitative relationship of arginine requirement and CP.

Plasma concentrations of isoleucine in kittens fed diets containing 0.75 × EAArq and 210 to 560 g CP/kg and 1.0 × EAArq and 140 and 280 g CP/kg and plasma concentrations of methionine in kittens fed diets containing 0.75 × EAArq and 210 and 280 g CP/kg suggest that these two EAA are limiting in kittens fed diets containing the EAA at or below the NRC requirements. Isoleucine deficiency is a possible explanation for the decreased weight gain found in Kittens fed 1.0 × EAArq and 280 g CP/kg diet even though, from

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* See Proceedings of the Symposium on Metabolic Role of Urea Cycle Intermediates: Nutritional and clinical aspects (1985) J Nutr 115: 505–541

previous work, kittens fed this level of isoleucine with an excess of other EAA had weight gains that were at or near maximum (Hargrove et al., 1984). Weight gains and plasma isoleucine concentrations of these kittens were actually lower than those of kittens fed 1.0 × EAArq and 210 g CP/kg diet suggesting that a leucine-isoleucine and valine antagonism or an amino acid imbalance response may have occurred (Hargrove et al., 1988; Harper et al., 1970). However, this is not consistent with earlier findings in the cat that increasing CP (using both EAA and DAA as the source of CP) in a diet limiting in isoleucine actually results in increased weight gains (Rogers et al., 1990). One important difference in the current results vs. the previous results is that in the earlier study the imbalanced diets contained more than 1.0 × EAArq for all but the limiting EAA. The present results are also inconsistent with the recommendation by the NRC (1986) that higher concentrations of CP are needed to get maximal growth if EAA are not provided in excess of their requirement. It would appear that the addition of a complete amino acid mixture lacking isoleucine is beneficial whereas adding only DAA (under certain conditions) is detrimental (Rogers et al. 1990; Strieker, 1991; Hammer et al., 1996).

The elevated plasma concentrations of glutamic acid in kittens fed 0.75 × EAArq and 280, 350, 420 and 560 g CP/kg diet and 1.0 × EAArq and 560 g CP/kg diet were probably contributing to the decreased weight gain and vomiting that occurred in kittens fed these diets containing the highest levels of CP. Rats and chicks are quite tolerant of high dietary levels of glutamic acid with no adverse effects occurring when concentrations of up to 150 g glutamic acid/kg diet are fed (Harper et al., 1970; Klain et al., 1958, Swendseid et al., 1962). In contrast, the kitten has been shown to be more sensitive to an excess of this DAA, with adverse effects occurring at dietary concentrations above 60 g glutamic acid/kg, leading to plasma concentrations of 250 to 300 μmol/L (Deady et al., 1981). However, dietary glutamic acid concentrations in the diets containing 0.75 × EAArq and 280, 350, 420 and 560 g CP/kg and 1.0 × EAArq and 560 g CP/kg were only 17 to 38 g glutamic acid/kg and 37 g glutamic acid/kg, respectively. Nevertheless, the resulting plasma glutamic acid concentrations in some kitten groups in the present work were far higher (311 to 559 μmol/L) than those reported by Deady et al. (1981) to cause adverse effects when 90 or 120 g glutamic acid/kg diet were fed.

It should be noted that glutamine and alanine were both present at concentrations of 89 g/kg in the 0.75 × EAArq and 560 g CP/kg diet and it is possible that elevated levels of these DAA contributed to the elevated plasma glutamic acid. In the enterocyte, glutamine is readily converted to glutamic acid and an alanine amino transferase converts glutamic acid and pyruvate to alanine and α-ketoglutarate so that normally alanine enters the bloodstream rather than glutamic acid (Souba, 1992; Wiseman, 1964). It is possible that high levels of glutamine and alanine may decrease the tolerance of glutamic acid by inhibiting this mechanism. More experiments need to be conducted to confirm this suggestion.

Unexpectedly, kittens fed the diet that contained 1.5 × EAArq and 560 g CP/kg (34 g glutamic acid/kg, 80 g alanine/kg and 80 g glutamine/kg) did

not develop hyperglutamatemia or exhibit any of the signs of glutamic acid toxicity such as weight loss or vomiting even though dietary DAA concentrations in this diet were not much lower than those in the diet that contained 1.0 × EAArq and 560 g CP/kg diet. Since weight gain was higher in this group of kittens, an increase in the anabolic utilization of amino acids may have increased the clearance of the alanine and glutamine which were postulated to increase plasma glutamic acid. Furthermore, the additional arginine in this diet may have increased the efficiency of nitrogen removal via urea synthesis and, also, ultimately contributed to a more rapid removal of excess alanine, glutamine and glutamic acid. Therefore, it appears that the tolerance of dietary glutamic acid is lower when high protein diets, low in EAA and deficient in arginine, are fed to kittens.

Kittens fed 1.5 × EAArq and 560 g CP/kg diet had significantly lower weight gains than kittens fed CD and 1.5 × EAArq and 210, 280 and 420 g CP/kg diets. Plasma concentrations of proline were greatly elevated in kittens fed the highest levels of CP in all three experiments. With no other obvious explanation, it is possible that an intolerance of proline may have caused the lower weight gains of kittens fed the 560 g CP/kg diet in Exp. 3 and also contributed to the decreased growth rates of kittens fed the high CP diets in Exp. 1 and 2. The rat and the chick can tolerate proline at concentrations as high as 40 to 50 g proline/kg diet in low protein diets with only a slight growth depression (Harper et al., 1970; Sauberlich, 1961), but the tolerance of the kitten for proline has not been determined. Further work in the kitten on the level of tolerance of proline and other DAA is necessary before a complete explanation of the lower weight gains of kittens fed 1.5 × EAArq and 560 g CP/kg diet can be given.

A variety of factors have been reported that result in depressed weight gains when disproportionate quantities of amino acids are present in the diet of the kitten, i.e. excess glutamic acid, inadequate arginine for a high levels of CP and excess methionine that is ameliorated by glycine (Deady et al., 1981; Fau et al., 1987; Taylor et al., 1996). We conclude that the increased requirement for arginine with increasing CP level, and specific dispensable amino acid toxicities (glutamic acid and possibly others) are the primary cause of the adverse effects and depressed growth that occur at low essential amino acid to total nitrogen ratios. Moreover, future experiments need to be conducted in kittens to ascertain other possible amino acid interactions that may influence growth and metabolism as dietary EAA are increased.

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Authors' address: Q.R. Rogers, Vet Med: Molecular Biosciences, University of California, Davis, CA 95616, U.S.A.

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