Essentiality of Amino Acids for the Growing Kitten[1]

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ABSTRACT The effect of deleting each of the amino acids known to be essential for the young rat was determined in post weanling kittens fed a purified diet containing only L-amino acids as the source of dietary nitrogen. When any one of the 10 amino acids (arginine, lysine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, valine) were deleted from the diet, food intake decreased, the kittens lost weight, and there was a dramatic drop in each corresponding amino acid in the blood plasma; indicating that each of the above amino acids is essential for the kitten. Deletion of all the amino acids except the 10 essential amino acids plus alanine resulted in a decreased weight gain to about ⅓ normal; indicating that although all the other amino acids could be synthesized, one or more of the dispensable amino acids may be required for maximal growth. When any one of the essential amino acids was decreased to one-half that present in the basal diet, there was no decrease in weight gain, indicating that the high protein requirement of the kitten is not the result of an unusually high requirement for the essential amino acids. J. Nutr. *109:* 718–723, 1979.

INDEXING KEY WORDS feline amino acid requirements · kitten amino acid requirements · cat amino acid requirements · essentiality of amino acids in the kitten · amino acid requirements of the kitten

There has been a dearth of information on the amino acid requirements of the cat as compared to that of man, dog, rat, and other domesticated animals. It is surprising that in the late 1970's, over 40 years after Rose and co-workers had discovered threonine and thereby completed the groundwork for adequate methods to experimentally establish the essentiality and requirements of amino acids (1), that there has been so little work published on the amino acid requirements of the cat. In the meager literature available on amino acid nutrition in the cat, uncertainties have developed, even with regard to the essentiality of certain amino acids. Using adult cats, Miller and co-workers [2-4] have suggested that the cat could synthesize sufficient methionine to meet its needs; whereas Ritter and Owens,[5] and more recently, Teeter et al., have presented evidence that the growing kitten (2) and the adult cat (3) require dietary methionine. Although there is not complete agreement as to the nutritional value of wheat gluten for the kitten (4, 5), the improvement of growth found by supplementing wheat gluten with lysine (6) supports the idea that lysine is an essential amino

Received for publication September 13, 1978.

[1] This work was supported in part by a gift from the Quaker Oats Company, Research Foundation, Barrington, Illinois 60010 and in part by funds provided through a National Institutes of Health, Public Health Services Biomedical Research support Grant #5501RR05457. The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

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acid. Hardy et al. (7) have reported that valine is essential and we have recently reported (8, 9) the strikingly adverse effect of an arginine deficiency (8, 9) in the cat.

The minimal protein requirement of the weanling kitten and adult cat is reported to be 29 and 19%, respectively, of the energy requirement (4, 10–12). These levels are considerably higher than that known to be required by many other mammalian species (13).

The objectives of this report were: 1) to establish which amino acids are essential for the growing kitten, 2) to seek an explanation for the high dietary protein requirement of the cat, and 3) to obtain a first approximation of the essential amino acid requirement of the growing kitten.

METHODS

Sixty-four post weanling conventional kittens which had been fed a purified diet from weaning vaccinated against panleukopenia, were adapted to the basal amino acid diet [6] (7). The kittens were housed in separate stainless steel cages in an air-conditioned room (23 ± 2°) in conformance with the American Association for Accreditation of Laboratory Animal Care Standards. The lighting was constant during each experiment, but between experiments varied as seasonal lighting changed. The kittens were assigned as they came available to individual groups, each from 3 to 12 kittens (as indicated in the results) over a 2-year period until completion of the studies. Feed and water were provided ad libitum. Kittens were first fed the basal amino acid diet [6] (7) then a diet devoid of one of the amino acids. Following the diet lacking one amino acid, kittens were then either fed the basal diet or a diet containing the amino acid at one-half the basal level. In some experiments the diet containing the one-half level of a given amino acid was fed following the basal amino acid diet. All diets were kept isonitrogenous by adjusting the level of alanine and carbohydrate. Food intake and weight gains were measured daily for 7 to 14 days (normally 9 to 10 days) and jugular blood samples were taken on day 5 or 6 at 1000 to 1200 hours (without fasting). The blood samples were taken from the jugular vein of unanesthetized kittens in heparinized syringes and were immediately placed on ice. They were then centrifuged and the plasma frozen until the amino acid analyses could be performed. Before analysis, the plasma samples were thawed, an equal volume of 6% sulfosalicylic acid was added, the protein precipitate was removed via centrifugation, an aliquot of the supernatant was neutralized to pH 2.2 with lithium hydroxide and the equivalent of 40 μl of plasma was placed on the amino acid analyzer.[7]

RESULTS

The effects of deleting each of the amino acids known to be essential for the rat and of deleting one of several dispensable amino acids on weight gains are shown in table 1. The mean weight gain of each group of kittens fed the initial basal diet varied from 5.5 to 24 g/day. The two basal groups which gained less than 10 g/day and some of the other groups, had depressed gains due to post weanlng respiratory infections. When each of the 10 amino acids that are essential for the growing rat (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) were separately deleted from the diet, every single group and every single kitten within each group lost weight; providing strong evidence that these amino acids are also essential for the kitten. Results of statistical analysis for all groups are not presented because of the small numbers per group. Except for the minus arginine group, the mean weight losses were between 4.5 and 19.5 g/day; starting the first day with minimal gain or no loss followed by consistent linear weight losses after the third day (see graph for minus valine diet (7) for a typical weight loss curve). After feeding the minus arginine diet the kittens lost 93 ± 7 g the first day and 136 ± 31 g by the end of the second day. The mean

[6] The basal amino acid diet contained (in % of diet): amino acid mixture, 34.7; turkey fat, 25.0; starch, 19.27; sucrose, 15.7; salt mixture, 4.0, vitamin mixture 1.0; and choline chloride, 0.33. The amino acid mixture contained (in % of the diet): L-His·HCl·H_2O, 1.2; L-Ile, 1.8; L-Leu, 2.4; L-Lys·HCl, 2.8; L-Met, 1.1; L-Cys-Cys, 0.80; L-Phe, 1.5; L-Tyr, 1.0; L-Thr, 1.4; L-Trp, 0.4; L-Val, 1.8; L-Arg·HCl, 2.0; L-Asn, 2.0; L-Ser, 1.0; L-Pro, 2.0; Gly, 2.0; L-Glu, 6.0; L-Ala, 1.0; and sodium acetate, 2.5

[7] 121M Beckman Amino Acid Analyzer.

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TABLE 1

Effect of deletion from or halving the level of individual amino acids in the basal diet on the weight gain of kittens[1]

Amino acid	Basal		Devoid		Basal	Basal		Half		Basal	
					g/day						
Alanine	13.4±1.5	(3)	14.6±1.1	(3)	13.1±1.5 (3)	—		—		—	
Arginine*	18.5±2.0	(5)	−47.6±3.0[2]	(5)	—‖	7.1±5.4[3]	(10)	12.2±7.2[3]	(10)	—	
Asparagine	7.5±0.5	(3)	6.8±2.3	(3)	—	—		—		—	
Cystine*	19.6±2.6	(12)	8.3±3.1	(12)	→	→		15.2±2.1[4]	(12)	19.3±2.0	(12)
Histidine	13.6±4.0	(3)	− 4.5±1.1	(3)	17.1±2.7 (3)	→		13.1±2.1	(3)	—	
Isoleucine	13.4±2.4	(2)	−10.3±4.5	(3)	→	→		11.2±2.7	(3)	—	
	—		—		—	12.8±1.1	(4)	10.3±1.8	(4)	14.9±2.3	(4)
Leucine	13.1±2.1[5]	(3)	− 7.3±1.3	(3)	→	→		11.9±1.9	(3)	13.4±2.0	(2)
Lysine	11.5±1.7	(3)	−11.5±1.0	(3)	22.7±4.1 (3)‖	14.9±2.1	(3)	13.5±2.9	(3)	18.6±1.6	(3)
Methionine	5.5±1.7	(3)	−19.5±5.8	(3)	→	→		24.5±5.5	(3)	24.0±6.4	(3)
	—		—		—	10.9±3.4	(4)	15.4±4.8	(4)	19.2±4.6	(4)
Phenylalanine*	23.0±2.7	(5)	−10.1±1.9	(5)	→	→		31.1±2.0	(5)	22.5±0.7	(5)
Proline	10.6±1.0	(3)	11.1±1.1	(3)	—	—		—		—	
Threonine	17.4±4.1	(3)	−14.6±2.6	(3)	→	→		28.4±6.7	(3)	16.7±5.4	(3)
Tryptophan*	13.1±0.9	(5)	−13.2±1.8	(5)	23.0±2.7 (5)‖	6.1±1.0	(4)	11.2±2.4	(4)	9.4±1.1	(4)
Tyrosine*	23.8±5.1	(6)	18.4±4.4	(6)	15.6±2.9 (3)	—		—		—	
Valine*,[6]	17.0±5.0	(6)	−13.8±1.7	(6)	→	→		17.3±4.8	(6)	13.6±2.6	(6)
Alanine only for Disp AA*,[7]	16.0±1.8	(6)	6.5±1.1	(6)	10.0±2.2 (6)	—		—		—	

The values in this table are mean ±SEM. [1] Kittens were fed each diet for 7 to 14 days in sequence left to right except when there is an asterisk, in which case a crossover design was used. One-half of the kittens were fed each of the two diets during the first period and the diets switched during the second period. A double line indicates a separate group of kittens. Number of kittens per group given in parenthesis beside weight gain values. [2] Five-day mean weight loss. Mean weight loss was 136±31 for the first 2 days the diet was fed. [3] Costello, M. J. (1978) The Arginine Requirement of the Growing Cat, MS Thesis, University of California, Davis. [4] Cystine-free diet to which additional methionine (equimolar to cystine deleted) was added. [5] This group of kittens was fed the half histidine diet prior to the leucine-free diet. [6] Taken from Hardy et al. (7). [7] The basal dispensable amino acids were deleted and an isonitrogenous quantity of arginine (4.0%) and alanine (8.72%) were added to this diet.

weight loss over a 5-day period was −47.6 ± 3.0 g/day. This rate of body weight loss is dramatically different from that following the deletion of any of the other essential amino acids and led us to more extensive experiments with the minus arginine diet which have been published separately (8, 9).

There was no depression in weight gain when alanine, asparagine, proline, and tyrosine were deleted from the diet. When cystine was deleted from the diet, there was a decrease in weight gain but the decrease was not significant (14). The reduction of each of the separate essential amino acids to half that present in the basal diet resulted in weight gains similar to that of the kittens fed the basal diet (table 1). The deletion of all the dispensable amino acids (at the same time) except alanine [8] resulted in a lower weight gain (6.5 versus 16.0 g/day).

The measurements of food intake of the kittens fed the various diets are reported in table 2. With the exception of the kittens fed the arginine-free diet, the mean food intake of the diets devoid of each of the essential amino acids was only 54 to 70% of the intake recorded for the basal diet. When arginine was deleted, the mean (5 day) food intakes dropped to 20% of that eaten by the basal group.

The effect of deletion or halving each amino acid on the concentration of the free-amino acid in blood plasma is shown in table 3. Each amino acid decreased in plasma when it was deleted from the diet. Among the essential amino acids, histidine decreased the most (to 5% of normal) and lysine decreased the least (to 30% of normal). Among the dispensable amino acids deleted, plasma-free asparagine decreased the most (to 17% of basal), whereas tyrosine decreased the least (to 43% of basal). When each essential amino acid was decreased to one-half that present in the basal diet, threonine decreased the most (to 24%

[8] Note that cystine and tyrosine were included in the essential amino acid mixture.

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of basal) and phenylalanine decreased the least (to 57% of basal). When all the dispensable amino acids except alanine [8] were omitted from the diet, except for asparagine, there was only a minor decrease in the concentration of each dispensable free amino acid. Asparagine exhibited a major decrease from 187 ± 20 for kittens given the dispensable amino acid mixture to 10.5 ± 6 μM for kittens given alanine as the only dispensable amino acid.

DISCUSSION

Our results show that the 10 amino acids which are essential for most animals (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) are essential for the growing kitten. Except for the uniqueness of the response to an arginine-free diet, which is discussed in detail elsewhere, (8, 9) the response of kittens to the lack of any one of the essential amino acids is typical of that of other species. That is, a 30 to 50% decrease in food intake, a loss of body weight, and a decline in the concentration of the deleted amino acid in the plasma. It is unlikely that the cat can synthesize any of the above essential amino acids. Whereas, it is obvious from the result of the last group in table 1 (alanine only for dispensable amino acids) that the kitten can synthesize some of each of the dispensable amino acids (i.e., the kitten did grow although at a lower rate). It would appear, however that one or more of the dispensable amino acids may be necessary for maximal growth. However, it is not clear from these results, whether one of the dispensable amino acids may be required for maximal growth or whether the cat, similar to the rat, simply cannot synthesize all the dispensable amino acids from a single nitrogen source at a rate commensurate with maximal growth. Asparagine dropped the most in the plasma from kittens fed the diet devoid of the dispensable amino acids so the possible growth response to dietary asparagine should be reexamined in young post-weaning specific pathogen-free kittens. Although it may appear at first glance that there was a response to cystine,

TABLE 2

Effect of deletion from or halving the level of individual amino acids in the basal diet on food intake of kittens

Amino acid	Basal	Devoid	Basal	Basal	Half	Basal
			g/day			
Alanine	39.9 ± 1.9 (3)	43.7 ± 1.7 (3)	40.5 ± 6.0 (3)	—	—	—
Arginine*	48.6 ± 2.6 (5)	9.6 ± 2.0[2] (5)	—‖	40.8 ± 10.2[3] (10)	43.3 ± 10.4[3] (10)	—
Asparagine	51.4 ± 6.6 (3)	46.3 ± 4.2 (3)	—	—	—	—
Cystine*	43.7 ± 3.2 (12)	37.4 ± 3.5 (12)	→	→	43.4 ± 3.7[4] (12)	53.3 ± 3.5 (12)
Histidine	40.1 ± 5.2 (3)	23.4 ± 3.6 (3)	39.1 ± 5.6 (3)	→	41.3 ± 6.5 (3)	—
Isoleucine	47.2 ± 11.2 (2)	23.5 ± 1.6 (3)	→	→	39.4 ± 5.9 (3)	—
	—	—	—	41.9 ± 3.2 (4)	46.9 ± 4.1 (4)	45.3 ± 3.4 (4)
Leucine	41.3 ± 6.5[5] (3)	28.9 ± 4.0 (3)	→	→	38.9 ± 6.6 (3)	39.5 ± 7.5 (3)
Lysine	35.0 ± 3.4 (3)	23.9 ± 1.9 (3)	44.2 ± 2.2 (3)	44.5 ± 1.0 (3)	53.5 ± 3.6 (3)	62.3 ± 11.4 (3)
Methionine	47.0 ± 6.9 (3)	25.3 ± 4.6 (3)	→	→	49.5 ± 12.5 (3)	69.3 ± 4.2 (3)
	—	—	—	38.6 ± 8.0 (4)	49.9 ± 8.0 (4)	53.7 ± 11.2 (4)
Phenylalanine*	44.7 ± 1.3 (5)	26.0 ± 0.6 (5)	→	→	51.5 ± 2.8 (5)	56.4 ± 2.3 (5)
Proline	45.6 ± 5.4 (3)	39.1 ± 5.0 (3)	—	—	—	—
Threonine	46.1 ± 9.8 (3)	25.0 ± 1.3 (3)	→	→	48.4 ± 7.5 (3)	51.3 ± 8.5 (3)
Tryptophan*	37.0 ± 2.4 (5)	25.4 ± 2.5 (5)	44.7 ± 1.3 (5)‖	35.8 ± 3.8 (4)	43.3 ± 7.0 (4)	48.1 ± 5.3 (4)
Tyrosine*	50.7 ± 5.4 (6)	55.9 ± 8.9 (6)	53.2 ± 11.4 (3)	—	—	—
Valine*,[6]	42.5 ± 6.4 (6)	26.1 ± 3.9 (6)	→	→	49.7 ± 7.4 (6)	45.7 ± 4.8 (6)
Alanine only for Disp AA*,[7]	37.9 ± 3.6 (6)	31.1 ± 4.7 (6)	41.6 ± 5.9 (6)	—	—	—

[1] Kittens were fed each diet for 7 to 14 days in sequence left to right except when there is an asterisk, in which case the kitten were crossed over, ½ fed basal and ½ fed experimental diet. A double line indicates a separate group of kittens. Intakes reported as mean ±SEM, number of kittens per group given in parenthesis beside food intake values. [2] Five-day mean food intake. [3] Costello, M. J. (1978) The Arginine Requirement of the Growing Cat, MS Thesis, University of California, Davis. [4] Cystine-free diet to which additional methionine (equimolar to cystine deleted) was added. [5] This group of kittens was fed the half histidine diet prior to the leucine-free diet. [6] Taken from Hardy et al. (7). [7] The basal dispensable amino acids were deleted and an isonitrogenous quantity of arginine (4.0%) and alanine (8.72%) were added to this diet.

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TABLE 3

Effect of deletion from or halving the level of amino acids in the basal diet on the concentration of each respective amino acid in the blood plasma of kittens[1]

Amino acid	Basal[1]	−AA	½ level
	μM		
Arginine[2]	110± 8	28 ± 3.0	72 ± 7.9
Citrulline[2]	13± 1	6.7± 1.2	6.1± 2.0
Ornithine[2]	48± 4	8.7± 2.0	10.8± 1.3
Asparagine	104± 9	18 ± 3.7	
Histidine[3]	180± 9	9.4± 4.0	108 ±14
Isoleucine	134±10	8.1± 3.1	45 ± 6.4
Leucine	174±13	25 ±12	65 ±15
Lysine	149±11	45 ± 6.6	70 ± 1.0
Methionine	177±12	11 ± 4.7	68 ±25
Phenylalanine[4]	93± 7	11 ± 2.7	53 ± 7.7
Tyrosine	118± 7	34 ± 2.9[5]	55 ± 7.2[5]
Proline[6]	276±23	81 ± 9.9	
Threonine	327±25	60 ± 3.3	79 ±20
Tryptophan[4]	71± 4	8.9± 2.3	32 ± 3.3
Tyrosine	118± 7	50 ± 4.8	
Valine[7]	337±23	33 ±13	66 ±13

[1] Mean±SEM. n = 38 for basal group, see Table 1 for n for each experimental group. Kittens were bled without fasting from the jugular vein on the 5th or 6th day of the experiment at 1000 to 1200 hours. [2] Four hours after feeding 12 g of arginine-free diet, citrulline and ornithine values from the same kittens. [3] These values for the experimental diets were for the second day. The 14th-day values for the histidine deficient and ½ level were, respectively, 17±9.1 and 73±9.9. [4] Some of the kittens fed the phenylalanine experimental diets and all of the kittens fed the tryptophan-free diet were also fed hydroxyproline for proline. [5] Tyrosine value is from plasma taken from kittens fed the phenylalanine-free diet and diet containing ½-level of phenylalanine. [6] Another group of four kittens fed hydroxyproline for proline for 3 to 4 weeks had a plasma proline level of 60±5.2. [7] Group labeled ½ level was fed ½ level present in the basal diet. Taken from Hardy et al. (7)

a paired t-test (14) resulted in no statistical difference between the control and the cystine-free group. Teeter et al. (2) have recently reported that cystine is dispensable.

The most surprising finding (apart from the cats' response to an arginine-free diet) was that the kittens grew just as well when any one of the essential amino acids was independently reduced to one-half that present in the basal diet. A diet containing about 16% of a high quality protein would provide about one-half the level of essential amino acids present in our basal diet. In long-term experiments (6 months), using conventional kittens, a 16.5% soy protein-methionine supplemented diet was found to be insufficient to maintain body weight and general good health.[9] It is generally accepted that the protein requirement of the kitten is about 30% of the diet (4, 10–12). These results support the hypothesis that the cat requires a high protein diet for its nitrogen (13, 14) rather than requiring a high level of essential amino acids in the diet. The high nitrogen requirement appears to be the result of a lack of adaptation in nitrogen catabolic enzymes in the cat (15) which results in excessive wastage of nitrogen when cats consume a low protein diet.

The results indicate that the cat can conserve and efficiently utilize any one amino acid, (including all the essential amino acids), but that the cat cannot efficiently conserve nitrogen. In order to confirm this hypothesis, it will be important to establish the minimal requirement of each of the essential amino acids.

ACKNOWLEDGMENTS

The authors would like to thank Jane Bishop, Arthur Aguirre, A. J. Hardy, and Saundra E. Willis for technical assistance during the course of these experiments.

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