Valine Requirement of the Growing Kitten[1]

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ABSTRACT The valine requirement of the kitten was studied using a crossover design with two groups of three weanling kittens. The kittens were fed a semipurified diet containing only free amino acids as a source of nitrogen containing 0.0%, 0.6%, or 1.8% valine. The mean ± SE of the linear components of growth (g/day) of the six kittens were respectively: −13.8 ± 1.7, 17.3 ± 4.8, and 15.3 ± 2.6. Corresponding food intakes were 26.0 ± 3.9, 50.4 ± 2.5, and 44.0 ± 1.5. Nitrogen balance was positive with the same amount retained for the two valine-containing diets, while nitrogen balance was negative for the kittens fed the valine-free diet. Plasma valine dropped markedly when the dietary valine was decreased from 1.8% to 0.6% (363 ± 59 versus 66 ± 13 nmole/ml), and was further decreased (33 ± 13) when the valine-free diet was fed. It does not appear that the valine requirement of the kitten exceeds 0.6% of a diet containing 25% fat. These results support the hypothesis that the high protein requirement of the kitten is not a result of a high requirement for all the essential amino acids, but a result of a high "nitrogen" requirement. J. Nutr. *107:* 1308–1312, 1977.

INDEXING KEY WORDS feline valine requirement · cat nutrition · kitten valine requirement · amino acid requirement

The minimum protein requirement of the weanling kitten and the adult cat appears to be about 29% and 19% of the energy respectively (1–4). All species decrease their intake of food when their dietary protein is deficient, but the cat appears to be particularly sensitive to the lack of dietary protein. Thus, several groups of workers have been unsuccessful in their attempts to get cats to eat a protein-free diet (1, 2, 4), while others have achieved limited success (3). In the scanty literature available on the amino acid requirements of the cat, there is considerable disagreement with regard even to the essentiality of the various amino acids. Thus, Miller and co-workers [2–4] suggest that the cat can synthesize sufficient methionine to meet its needs, whereas Ritter and Owens [5] have indicated that the growing kitten requires about 0.9% methionine in a diet that contains 70.06% cystine. There is also disagreement as to the nutritional value of wheat gluten for the growing kitten (3, 5). The results of supplementation studies in which lysine was added to wheat gluten (6) would support the idea that lysine is an essential amino acid. The cat does ap-

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[2] Rambaut, P. & Miller, S. (1967) Studies in feline sulfur amino acid metabolism. In: Proc. Seventh International Congress of Nutrition, Hamburg, 1966, pp. 164, Pergamon Press, New York.

[3] Dymsza, H. A. & Miller, S. A. (1964) Dietary methionine requirement of the cat. Federation Proc. *23*, 186, Abs. No. 512.

[4] Rambaut, P. C. & Miller, S. A. (1965) Studies of Sulfur amino acid nutrition in the adult cat. Federation Proc. *24*, 373, Abs. No. 1348.

[5] Ritter, S. M. & Owens, F. N. (1974) Methionine requirements of the growing cat. J. Anim. Sci. *39*, 981, Abs. No. 68, and Owens, F. N. Personal communication.

pear to be peculiarly low in the efficiency of utilization of protein, since the NPU of several typical proteins was found to be 30% to 50% (4). The same proteins result in NPU of about twice these figures when each is fed to rats.

The high protein requirement of the cat could be a result of a high essential amino acid requirement or a high nitrogen requirement (independent of any single amino acid need). In order to test the hypothesis that the high protein requirement is a consequence of a high requirement of all the essential amino acids, and at the same time get an idea of the requirement of one amino acid for the kitten, we have devised an amino acid diet for kittens which will provide normal growth, and then tested the weight gain, food intake, nitrogen balance, and plasma amino acid pattern of kittens given three levels of valine.

METHODS

Six kittens from our cat colony which had been fed a semipurified diet from weaning and vaccinated against panleukopenia, were adapted to the basal amino acid diet. The kittens were housed in individual cat metabolism cages and averaged 1.6 kg at the beginning of the experiment. The kittens were divided randomly into two groups and were given the diets as shown below:

9 Day periods	Group A (3 kittens)	Group B (3 kittens)
1	Basal	0.6% valine
2	0.6% valine	Basal
3	Valine-free	Basal
4	Basal	Valine-free

The kittens were given food and water once a day at 16:00 hours. Body weight and food intakes were measured daily. Feces were collected daily, and 9 day collections were pooled and frozen. Urine was collected daily in sulfuric acid to maintain the pH less than 1, frozen, and pooled in 3 day collection periods. Jugular blood samples were taken from each kitten at 10:00 to 11:00 hours on the 5th day of each experimental period.

Diets. The composition of the basal diet is shown in tables 1 and 2. The turkey fat was found to be more acceptable to kittens than diets based only on partially hydrogenated vegetable fat. Presumably this was the result of flavor components present in the turkey fat; however the possible benefit of the presence in the turkey fat of other, unknown essential nutrients has not been excluded. Two other diets were also used: A 0.6% valine diet and a valine-free diet. These two experimental diets were made isonitrogenous by adding an appropriate quantity of extra alanine to the experimental diets. Since little was known concerning the amino acid requirements

TABLE 1

Composition of basal semi-purified amino acid diet for kittens

Dietary component	% of diet
Amino acid mixture[1]	34.7
Turkey fat[2]	25.0
Starch[3]	19.27
Sucrose[4]	15.7
Salt mixture[5]	4.0
Vitamins[6]	1.0
Choline chloride	0.33
Total	100.00

[1] See table 2 for Composition. [2] Heat rendered by cooking in large open pans +0.02% BHT. [3] Pearl Starch (raw cornstarch) A. E. Staley Mfg. Co., Decatur, Illinois. [4] California and Hawaii Sugar Co., San Francisco. [5] Hegsted's Salt Mixture IV (7). [6] Ref. (8) plus biotin, folic acid and vitamin B_{12}, see Reference (9) for complete composition of Vitamin Mixture.

TABLE 2

Amino acid mixture used for complete amino acid diet for kittens

Amino acid	% of diet	Amino acid	% of diet
L—His·HCl·H_2O	1.20[1]	L—Ser	1.00
L—Ile	1.80	L—Pro	2.00
L—Leu	2.40	Gly	2.00
L—Lys·HCl	2.80[2]	L—Glu	6.00
L—Met	1.10	L—Ala	1.00
L—Cys·Cys	0.80	Na-acetate[4]	2.50
L—Phe	1.50	% of diet total	34.70%
L—Tyr	1.00	Amino acids only	32.35%
L—Thr	1.40	Protein equivalent[5]	28.01%
L—Trp	0.40	Nitrogen × 6.25	27.24%
L—Val	1.80	Amino acids as % of calories in basal diet	23.0%
L—Arg·HCl	2.00[3]		
L—Asn	2.00		

[1] 0.98 free base. [2] 2.26 free base. [3] 1.66 free base. [4] Added to balance the hydrochlorides. [5] Weight of amino acids less one mole of water per mole of amino acid.

of the kitten, we used the formulation normally used for rats in our laboratory, doubling the total quantity. This was approximately 2.5 to 3 times the rat requirement for the essential amino acids. The valine provided by an equivalent quantity of nitrogen from beef, soybean protein, casein or fish protein would be 1.38% to 1.89% of the diet (10). It would therefore appear that the valine content of our control amino acid diet (1.8% of the diet) should be adequate.

Analysis. The feces were lyophilized, milled, and samples taken for the determination of nitrogen. The assays (Kjeldahl) were also done on aliquots of the urine samples and on samples of each of the diets used. The blood samples were taken in heparinized syringes and were immediately placed on ice, centrifuged and the plasma was 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.[6]

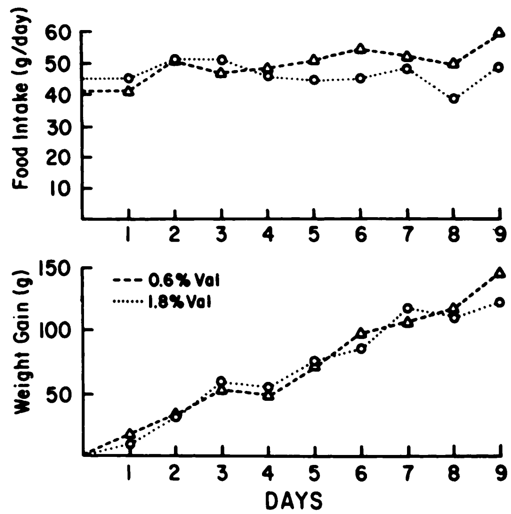

Fig. 2 The effect of dietary valine concentrations on the growth and food intake of growing kittens. (n = 6).

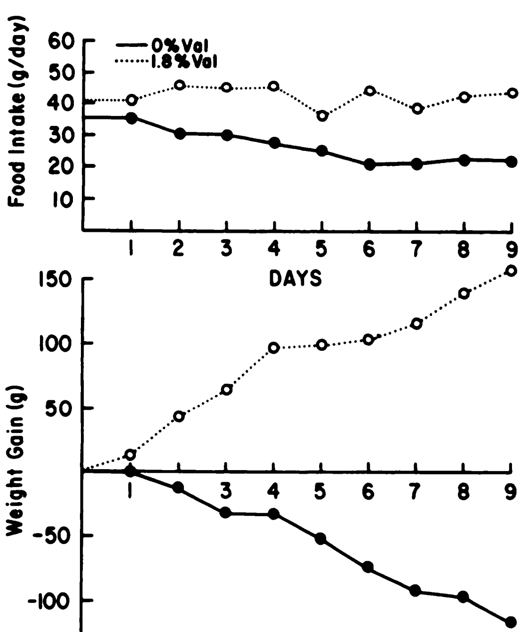

Fig. 1 The effect of a lack of dietary valine on the growth and food intake of growing kittens. (n = 6).

RESULTS

A comparison in periods 3 and 4, of the valine-free and a 1.8% valine diet on mean weight gain and food intake of the kittens is shown in figure 1. The mean ± SE of the linear components of body weight gains (g/day) of the six kittens receiving the basal diet as compared to the same kittens receiving the valine-free diet were respectively, 17 ± 5.0 and −13.8 ± 1.7. The corresponding food intakes were 42.5 ± 6.4 and 26.1 ± 3.9. The mean daily food intakes and weight gains of the kittens given the basal diet (1.8% valine) compared to the same kittens receiving the 0.6% valine diet in periods 1 and 2, are shown in figure 2. The mean ± SE of the linear components of weight gains (g/day) of the six kittens receiving the 0.6% and 1.8% valine diets were 17.3 ± 4.8 and 13.6 ± 2.6 respectively. Corresponding food intakes were 50.4 ± 2.5 g and 46.3 ± 1.8 g. A summary of the nitrogen balance of the kittens fed the three diets from both experiments is shown in figure 3. These results confirmed the weight gain data in that when the kittens were given

[6] 121 M Beckman Amino Acid Analyser.

either 0.6% or 1.8% valine, they were in positive nitrogen balance and retained the same amount of nitrogen; whereas, when the kittens were given the valine-free diet, the kittens were in negative nitrogen balance during all the collection periods.

The effect of the various levels of dietary valine on plasma valine concentrations is shown in figure 4. There was a marked drop in plasma valine when the kittens were given the 0.6% valine diet as compared to that found after they received the basal diet (1.8% dietary valine): 66 ± 13 versus 363 ± 59 nmole/ml respectively. There was, however, a further drop to 33 ± 13 nmole/ml after the kittens were given the valine-free diet. There were much less differences for the other amino acids as effected by diet, although there were a few dispensable amino acids which dropped somewhat when the valine-free diet was given.

DISCUSSION

From the results of these experiments, it seems clear that valine is an essential amino acid for the cat. This is expected since valine has been shown to be an essential amino acid in all species that have been tested (11). The unexpected finding was that 0.6% of valine in the diet was sufficient for good growth. This represents about 0.5% of the energy as valine and is about the same or even lower than the requirement of the rat and other omnivores for valine (8). At 0.6%, valine in the diet is considerably lower than would ever be present in the diet if a sufficient quantity of protein were present in the diet (see Introduction) to meet the minimum protein requirement of the kitten. If the requirements of the other essential amino acids are as low proportionally to that found for valine, it does not appear that the hypothesis that the protein requirement of the cat is unusually high because of a high requirement of all the essential amino acids, is tenable. Why does the cat have such a high minimal protein requirement compared to that of other mammalian species? For example, the rat, which grows faster than the cat per unit of body size, requires considerably less protein in the diet (12% versus 29% of energy). There is a greater difference in the minimal protein requirement between the adult rat and the young growing rat (4% versus 12% of energy; a threefold difference) than there is between the adult cat and the young growing kitten (19% versus 29% of energy; a 50% increase) (1–5, 8).

Most mammals have the capacity to adapt to various levels of protein intake (12–14), whereas it has been reported that the cat does not have the adaptive capability to decrease its urea cycle enzymes and thus conserve nitrogen.[7] The low NPU reported for the cat fed good proteins (4) is consistent with the idea that the cat has

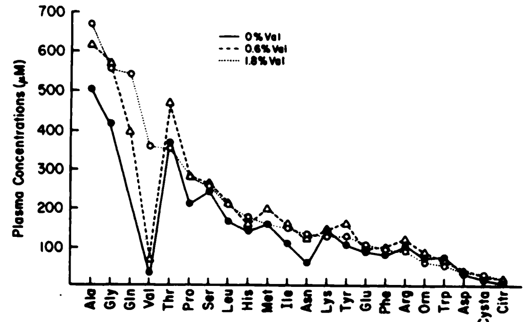

Fig. 4 The effect of dietary valine concentrations on plasma amino acid patterns of growing kittens. (n = 6).

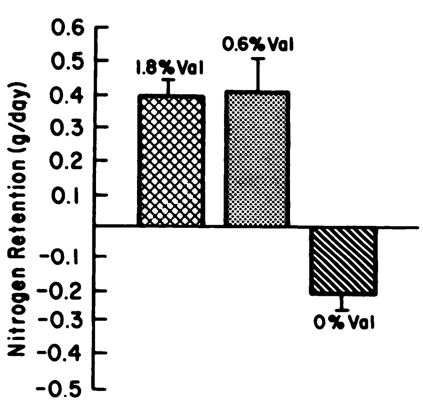

Fig. 3 The effect of dietary valine concentrations on nitrogen balance of growing kittens. (n = 6).

[7] Rogers, Q. R., Morris, J. G. & Freedland, R. A. (1976) The influence of the level of dietary protein and starvation on the activities of several hepatic enzymes involved in amino acid, carbohydrate and lipid metabolism in the adult cat. Federation Proc. *35*, 257, Abs. No. 285.

a high nitrogen requirement, but can utilize essential amino acids as efficiently as other mammals. All these results tend to support the hypothesis that the cat can conserve and efficiently utilize any one amino acid, but that the cat cannot efficiently conserve nitrogen and, therefore, has a high protein requirement. The present results indicate that the cat can utilize valine as efficiently as other mammals (omnivores or herbivores). This is consistent with the idea that when protein synthesis is stimulated (postprandial phase), the essential amino acids can be efficiently and effectively removed from the blood stream because of the low Km of the amino acid activating enzymes for their respective amino acids as compared to the higher Km for each respective catabolic enzyme (15). Nevertheless, if nitrogen per se cannot be conserved, growth becomes limiting because of a lack of key metabolites (perhaps dispensable amino acids, purines, and/or heme, etc.) for growth or maintenance. The results of the plasma valine samples in the kittens fed the three levels of dietary valine support the above idea. The valine concentration dropped markedly in the plasma (from 363 to 66 nmole/ml) without any drop in growth rate; and yet when no valine was added to the diet, a relatively small further drop in plasma (66 to 33 nmole/ml) resulted in the inability to remove sufficient valine from the blood plasma to even support maintenance. Similar experiments with other amino acids (unpublished observations), support the idea that the essential amino acid requirements (as a % of energy), are not higher for the kitten than for the rat and other herbivores and omnivores. It will be interesting to see whether these findings are unique to the cat or whether they are generally true for other strict carnivores.

ACKNOWLEDGMENTS

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ERRATA

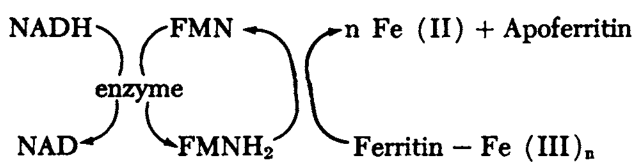
Low, R. B. & Cerauski, P. W. (1977) Biosynthesis of muscle proteins in fasted rats. J. Nutr. *107*, 1244–1254.

Reference No. 49 is incorrect: it should be as follows:

49. Vidrich, A., Airhart, J., Bruno, M. K. & Khairallah, E. A. (1977) Compartmentation of free amino acids for protein biosynthesis: Influence of diurnal changes in hepatic amino acid concentrations on the composition of the precursor pool charging aminoacyl-transfer ribonucleic acid. *Biochem. J. 162*, 257–266.

Sirivech, S., Driskell, J. & Frieden, E. (1977) NADH-FMN oxidoreductase activity and iron content of organs from riboflavin and iron-deficient rats, *107*, 739–745.

The reaction sequence shown at the top of page 740 should read as follows:



Reiser, S. & Hallfrisch, J. (1977) Stimulation of neutral amino acid transport by fructose in epithelial cells isolated from rat intestine. J. Nutr. *107*, 767–774.

Units in tables 1–5 should read mM not M.

Hardy, A. J., Morris, J. G. & Rogers, Q. R. (1977) Valine requirement of the growing kitten. J. Nutr. *107*, 1308–1312.

Page 1308 should read:

"Thus, Muller and co-workers (2–4) suggest that the cat can synthesize sufficient methionine to meet its needs, whereas Ritter and Owens (5) have indicated that the growing kitten requires about 0.9% methionine in a diet that contains 0.06% cystine, not 70.06% as reported therein or 0.60% as reported in Ritter, S. M. & Owens, F. N. (1974) Methionine requirements of the growing cat. J. Anim. Sci. 39, 981 abstract No. 68, based on a personal communication with Owens, F.N.

Proceedings of the 1977 Annual Meeting of the American Institute of Nutrition as published in the August 1977 issue of the Journal.

The date of death for Dr. John W. Bratzler was incorrectly listed; Dr. Bratzler died on March 8, 1975. Also, listed incorrectly in the list of committees, was Dr. R. V. Swift as a member of the Ad Hoc Committee on the History of Nutrition; please delete name from the list.