Dietary Crude Protein Increases Slightly the Requirement for Threonine in Kittens\textsuperscript{1,2,3}

VICTORIA A. HAMMER,\textsuperscript{4} QUINTON R. ROGERS\textsuperscript{5} AND JAMES G. MORRIS

Department of Molecular Biosciences, University of California-Davis, Davis, CA 95616

ABSTRACT Previous work indicates that the essential amino acid requirements for kittens are not positively correlated with the concentration of dietary nitrogen as they are in other species. In the studies presented here, the interaction between graded levels of threonine and dietary crude protein was investigated. Dose-response curves were generated using six 4 × 4 Latin squares. Each square represented one concentration of threonine (4.0, 5.0, 6.0, 7.0, 9.0 or 12.0 g/kg diet) and four concentrations of crude protein (150, 200, 300 and 500 g/kg diet). Food intake, weight gain, nitrogen retention and plasma amino acids were measured. There was no strong positive relationship between the threonine requirement of kittens and dietary crude protein. Increasing crude protein when threonine was limiting in the diet increased growth and food intake under some conditions, whereas under other conditions food intake and growth were decreased in a manner consistent with an amino acid imbalance response. An additional experiment was done to verify some of these findings. The requirement for threonine was found to increase from 5.0 g/kg diet at 150 and 200 g crude protein/kg diet to 6.0 g/kg diet when crude protein was 300 or 500 g/kg diet. The requirement for crude protein in the kitten appears to be between 200 and 300 g/kg diet. J. Nutr. 126: 1496–1504, 1996.

INDEXING KEY WORDS:
- feline • protein • threonine • requirement
- amino acid imbalance

The domestic cat (Felis domesticus), the most thoroughly studied mammalian carnivore, has some nutritional peculiarities related to protein and amino acid metabolism [MacDonald et al. 1984]. For example, the protein requirement for cats is substantially higher than that of omnivores and is primarily an increased requirement for maintenance rather than for growth (Rogers and Morris 1982). This high protein requirement is the result of highly active nitrogen catabolic enzymes in cat liver, which are nonadaptive [Rogers et al. 1977]. Thus, obligatory nitrogen loss is high even when cats are fed low protein diets. Cats have also been shown to lose a greater amount of nitrogen during food deprivation than do rats and humans [Bourge et al. 1994]. In addition to their unusual regulation of protein metabolism, cats also have a very limited ability to synthesize arginine for growth [Morris and Rogers 1978], have a high requirement for sulphur amino acids [Teeter et al. 1978] and are unable to synthesize substantial amounts of taurine, qualifying it as an essential amino acid [Hayes et al. 1975].

The relationship between the dietary requirements for essential amino acids and those for dietary crude protein (CP) appears to be unusual in cats when compared with other domestic species. The effect of CP on the requirement for essential amino acids in kittens has been examined in this laboratory. Work by Strieker (1991) indicated that when either threonine or isoleucine were fed below the NRC requirement (80 and 90% of the requirement, respectively) increasing dietary nitrogen from 200 to 300 g/kg diet resulted in increased weight gain. There was a similar pattern when either leucine or methionine was the limiting amino acid. In another experiment it was determined that the requirement for methionine at 150, 200 and 300 g CP/kg diet

\textsuperscript{1} Part of the results from Experiment 1 were presented at FASEB 1991, Atlanta, GA [Hammer, V. A., Rogers, Q. R. & Morris, J. G. (1991) The effect of dietary nitrogen in kittens fed graded levels of threonine. FASEB J. 3: A595 [abs.]].

\textsuperscript{2} Support for this research was provided by the WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, UK, and by the California Foundation for Biomedical Research, La Jolla, CA.

\textsuperscript{3} The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

\textsuperscript{4} Present address: Nutrition Department, Pennsylvania State University, University Park, PA 16802.

\textsuperscript{5} To whom reprint requests should be addressed.
was 3.5 g/kg diet, whereas the methionine requirement decreased to 2.5 g methionine/kg diet at 500 g CP/kg diet (Strieker 1991). The increase in nitrogen retention with increasing CP was accompanied by an increase in food intake. This response is different from that observed in other domestic species when CP is increased in a diet without increasing the limiting essential amino acid.

In contrast to what has been observed in cats, other species, e.g., chickens (Boomgaardt and Baker 1971; Grau and Kamel 1950), dogs (Milner 1981), pigs (Becker et al. 1957), rats (Salmon 1954, Sauberlich 1956) and turkeys (Kratzer et al. 1950), exhibit a decrease in growth and food intake when CP is increased in a diet without increasing the limiting essential amino acid. The depression in growth and food intake seen when CP is increased in a diet limiting in an essential amino acid has been described as an amino acid imbalance response (Harper et al. 1970). As a consequence of an amino acid imbalance response, more of the limiting amino acid is necessary to achieve optimal growth at higher levels of dietary CP. In other words, as the level of dietary CP increases, the requirement for an essential amino acid increases.

In the experiments by Strieker (1991) described above, the growth response seen in kittens when CP is increased in diets deficient in either isoleucine, leucine, methionine or threonine suggests that cats are not susceptible to amino acid imbalances as are other species. Yet, an amino acid imbalance response has been previously observed in kittens fed a diet limiting in threonine. When Titchenal et al. (1980) fed kittens diets with 4.0 g threonine/kg diet (57% of NRC requirement) and dietary CP was doubled from 175 to 350 g/kg diet, decreased food intake and a depression in growth resulted. Thus, in these two studies where threonine was limiting in the diet of kittens (Strieker 1991, Titchenal et al. 1980), increasing CP increased growth and food intake under one circumstance and decreased growth and food intake under another. From these results it is unclear how dietary CP might affect the requirement for threonine in kittens.

In these studies we investigated the effect of increasing CP in the diets of kittens when threonine was added at either 4.0, 5.0, 6.0, 7.0, 9.0 or 12.0 g/kg diet. We found that increasing dietary CP does increase the requirement for threonine somewhat in the cat, although the strong positive relationship between CP and the requirement for an essential amino acid that is commonly seen in other species is not apparent in kittens. Cats appear to be less susceptible than other species to a typical nutritional amino acid imbalance response, which may account for their differential response to increased dietary CP.

**MATERIALS AND METHODS**

**Experiment 1.** Forty-eight male kittens were used. At the start of the experiment the average weight of the kittens ± SEM was 1430 ± 30 g, and the average age ± SEM was 13.3 ± 0.3 wk. Food intake, weight gain and nitrogen retention were measured at six concentrations of dietary L-threonine (4.0, 5.0, 6.0, 7.0, 9.0 and 12.0 g/kg diet) and four concentrations of dietary CP (150, 200, 300 and 500 g/kg diet, Table 1). The experiment was completed in two identical replicates, with each replicate consisting of six 4 × 4 Latin squares. Each of the six squares in a replicate represented one level of dietary threonine, and the treatment within each square was four levels of dietary CP. The sources of variation controlled within each square were the kitten and the time period. A given kitten received each treatment for 14 d. Therefore, in the completed experiment, each dietary treatment was given to eight kittens for a period of 2 wk, and each kitten received four different levels of dietary CP but one level of dietary threonine. The two replicates of the experiment were completed within 4 wk of each other. Nitrogen retention was measured for the last 7 d of each treatment period, and blood was collected 3 h after presentation of food on d 11 or 12 of each treatment period.

**Experiment 2.** To verify and study the growth effect seen at 6.0 g threonine/kg diet in Experiment 1, kittens were fed 6.0 g threonine/kg diet with either 200, 300 or 500 g CP/kg diet. Twenty-four kittens were used, 12 males and 12 females. Because there was no evidence that dietary CP would affect the growth of male and female kittens differently, both sexes were included in this experiment. At the start of the experiment the average weight of the kittens ± SEM was 1180 ± 58 g, and the average age ± SEM was 11.8 ± 0.3 wk. All kittens were fed a diet with 6.0 g threonine and 200 g CP/kg diet for 7 d. Kittens were then assigned to one of three groups using a randomized complete block design, with the kittens being blocked on the basis of sex and body weight. During the experimental period, kittens received one of three diets for 12 d. During the last 8 d of the experimental period, after the animals had a chance to adapt to their new diets, urine and feces were collected for determination of nitrogen balance. Although food intake and body weight were measured daily throughout the experiment, only data from the 8 d corresponding to the measurement of nitrogen balance were used for statistical analysis. Blood was collected 5 h after presentation of food on the 1st d of the 12-d treatment period and 3 h after presentation of their food on d 11 of the treatment period.

**General.** Specific pathogen-free domestic short-hair kittens from the Nutrition and Pet Care Center at the University of California-Davis were used. These studies adhered to the Guide for the Care and Use of Laboratory Animals developed by the Institute of Laboratory Animal Resources of the National Research Council and were approved by the University of California Animal Use and Care Administrative Advisory Committee.

Before the first treatment period, the kittens were adapted for several weeks to a complete purified diet.
for which the protein source was a combination of soy, casein and lactalbumin. This adaptation period allowed the kittens to become accustomed to eating an experimental diet so that a smooth transition could be made to the test diets. The kittens were housed in individual stainless steel metabolism cages and allowed free access to food and water. Food intake (corrected for spillage) and body weight were measured daily. Urine and feces were collected during each treatment period to determine nitrogen retention. Urine was collected daily into a container containing hydrochloric acid to acidify the urine and prevent bacterial degradation and volatilization of nitrogenous compounds. Feces were collected daily and frozen. Urine, feces and diets were analyzed for nitrogen in Experiment 1 using the macro Kjeldahl method [Kane 1984]. In Experiment 2, free nitrogen was measured with a thermal conductivity detection system after high temperature combustion of the sample [Model FP-428 Nitrogen Determinator, LECO, St. Joseph, MI].

For determination of plasma amino acids, jugular blood samples were taken in heparinized syringes from unanesthetized kittens 2–4 h after presentation of fresh food (Model 7300 amino acid analyzer, Beckman Instruments, Palo Alto, CA). Plasma was prepared immediately for amino acid analysis. An equal volume of sulfosalicylic acid (275 mmol/L) was added to precipitate plasma proteins, and the deproteinized plasma was stored at −80°C until analysis.

**Diets.** Each diet contained 100 g/kg diet of soy protein and 60 g/kg diet of an essential amino acid mixture (Table 1). These two sources of essential amino acids, when combined, provided 1.5 times the National Research Council [1986] requirement of every essential amino acid except threonine. l-cystine and l-tyrosine were included in the essential amino acid mixture to make up half of the requirement for methionine and phenylalanine, respectively. The diets were formulated in such a way that all diets contained a basal level of threonine at 4 g/kg diet. A mixture of threonine, alanine and starch was then added to each diet to bring the total amount of threonine up to the appropriate level, i.e., 5.0, 6.0, 7.0, 9.0 and 12.0 g/kg diet. When the level of threonine was increased in a diet, l-alanine was de-

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>150 g/kg</th>
<th>200 g/kg</th>
<th>300 g/kg</th>
<th>500 g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy protein grade II&lt;sup&gt;1&lt;/sup&gt;</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Essential amino acid mixture&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>l-Threonine&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0–8</td>
<td>0–8</td>
<td>0–8</td>
<td>0–8</td>
</tr>
<tr>
<td>l-Alanine&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0–6</td>
<td>0–6</td>
<td>0–6</td>
<td>0–6</td>
</tr>
<tr>
<td>Amino acid mixture&lt;sup&gt;3,4&lt;/sup&gt;</td>
<td>0</td>
<td>50</td>
<td>150</td>
<td>350</td>
</tr>
<tr>
<td>Sodium acetate&lt;sup&gt;5&lt;/sup&gt;</td>
<td>15.6</td>
<td>22.2</td>
<td>35.3</td>
<td>61.5</td>
</tr>
<tr>
<td>Starch&lt;sup&gt;6&lt;/sup&gt;</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cerealose&lt;sup&gt;7&lt;/sup&gt;</td>
<td>400–404</td>
<td>345–348</td>
<td>232–235</td>
<td>6–8</td>
</tr>
<tr>
<td>Animal tallow&lt;sup&gt;8&lt;/sup&gt;</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Hydrogenated beef tallow&lt;sup&gt;9&lt;/sup&gt;</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mixture&lt;sup&gt;10&lt;/sup&gt;</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mixture&lt;sup&gt;11&lt;/sup&gt;</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline chloride&lt;sup&gt;12&lt;/sup&gt;</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Taurine&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

<sup>1</sup> United States Biochemical, Cleveland, OH.

<sup>2</sup> Essential amino acid composition (g/kg mixture): l-Arginine·HCl, 180.5; l-Histidine·H₂O·HCl, 53.0; l-Isoleucine, 59.6; l-Leucine, 190.4; l-Lysine·HCl, 144.0; l-Cystine, 109.0; l-Methionine, 81.1; l-Tyrosine, 62.9; l-Phenylalanine, 38.1; l-Threonine, 87.5; l-Tryptophan, 19.9; l-Valine, 82.8.

<sup>3</sup> Ajinomoto USA, Raleigh, NC.

<sup>4</sup> Amino acid composition (g/kg mixture): l-Arginine·HCl, 88.2; l-Histidine·HCl·H₂O, 30.1; l-Isoleucine, 36.8; l-Leucine, 87.2; l-Lysine, 72.7; l-Cystine, 25.2; l-Methionine, 29.1; l-Tyrosine, 33.0; l-Phenylalanine, 29.1; l-Tryptophan, 10.7; l-Valine, 43.6; l-Asparagine·H₂O, 72.7; l-Glutamine, 193.8; glycine, 71.7; l-Proline, 79.5; l-Alanine, 96.9.

<sup>5</sup> Sodium acetate·3H₂O, Fisher Scientific, Santa Clara, CA.

<sup>6</sup> Com Starch, Meloijel, Bridgewater, NJ.

<sup>7</sup> A. E. Staley, Decatur, IL.

<sup>8</sup> Florin Tallow, Dixon, CA.

<sup>9</sup> Bunge Edible Oil, Fort Worth, TX.

<sup>10</sup> See Williams et al. 1987.

<sup>11</sup> See Williams et al. 1987.

<sup>12</sup> International Mineral and Chemical, Terre Haute, IN.
creased isonitrogenously. A third amino acid mixture containing essential [minus threonine] and dispensable amino acids was used to increase the CP level to either 200, 300 or 500 g/kg diet. Essential amino acids made up 44% of this mixture, and the NRC requirements (with the exception of threonine) were used to determine the relative amount of each amino acid. The other 56% of the amino acid mixture was composed of the dispensable amino acids, asparagine, glutamine, glycine, proline and alanine. In total, essential amino acids were approximately 150, 187, 260 and 410% of the NRC in the 150, 200, 300 and 500 g CP/kg diets, respectively. Sodium acetate was added on an equimolar basis to balance the hydrochloride associated with arginine, histidine and lysine in the amino acid mixtures. Adjustments in amino acids and sodium acetate were made at the expense of cellose. Diets were formed into pellets using a meat grinder without the cutting blade.

**Statistics.** Statistical analysis was performed using PC-SAS, Version 6.03 [SAS, Cary, NC]. In both experiments, the plasma amino acid data underwent logarithmic transformation before analysis. Probability levels < 0.05 were considered significant. In Experiment 1, standard Latin square assumptions were made that there were no interactions among CP levels, cats and the sequence of treatments. An initial analysis was run to determine if threonine or CP effects were the same in the two replicates of Latin squares. When no significant interactions were found, subsequent analyses did not include these effects in the model. Thus, analysis of the full data set treated the two replicates of Latin squares as blocks and included no interactions between the block and the treatment effects. Finally, a series of stratified analyses were run to assess the effect of CP within threonine concentrations. Multiple comparisons among CP levels were calculated using Tukey's studentized range test [Steel and Torrie 1980]. Broken-line analysis [Robbins 1986] of the nitrogen retention data was used to estimate the dietary threonine requirement at different levels of dietary CP.

In Experiment 2, the data were analyzed as a randomized complete block, with sex and body weight as the blocking factors. An initial analysis was conducted to determine if the CP effects were the same in the two replicates of the randomized complete block. When no significant interaction was found, the data from the two replicates were combined. Thus, dependent variables were analyzed as a function of diet, body weight, sex and diet by sex interaction. Comparisons of means were made with Duncan's new multiple-range test [Steel and Torrie 1980].

**RESULTS**

**Experiment 1.** The effect of dietary CP on food intake was variable depending on the level of dietary threonine. At the two lowest levels of dietary threonine, 4.0 and 5.0 g/kg diet, there were no significant differences in food consumed when different amounts of CP were added to the diet (Fig. 1A). The feeding pattern observed at threonine levels ≥ 6.0 g/kg diet was somewhat different. At the higher levels of dietary threon-
nine, stepwise increases in dietary CP up to 300 g/kg diet resulted in large stepwise increases in food intake (see Fig. 1A for significant differences). Additionally, the kittens ate almost the same amount of the 500 g CP/kg diet as they had the 300 g CP/kg diet, clearly indicating that the additional CP had neither a positive nor a negative effect on food intake at these levels of dietary threonine.

The overall pattern of weight gain was comparable with that of food intake [Fig. 1B]. At dietary threonine levels of 4.0 and 5.0 g/kg diet, kittens fed 300 g CP/kg diet had the greatest average daily weight gain, which was significantly greater than the 150 g CP group but not the 200 or 500 g CP/kg diet groups. On the other hand, at threonine levels ≥ 6.0 g/kg diet, weight gain increased with increasing dietary CP (see Fig. 1B for significant differences). Considering all treatments, except the highest CP level [500 g/kg diet] at 4.0 and 5.0 g threonine/kg diet, stepwise increases in CP caused stepwise increases in weight gain.

Although the nitrogen retention had a similar pattern to the food intake and weight gain data, there were fewer significant differences among treatments. At 4.0, 5.0, 7.0 and 1.2 g threonine/kg diet, there were no significant differences in nitrogen retention at any level of dietary CP [Fig. 1C]. When threonine concentration was 6.0 g/kg diet, nitrogen retention increased as dietary CP increased above 200 g CP/kg diet. In general, increases in CP up to 300 g/kg diet led to increased nitrogen retention.

Broken-line analysis of the nitrogen retention data indicated that the dietary threonine requirement was 4.9 g/kg diet at both 150 and 200 g CP/kg diet (se = 0.01 and 0.01, respectively) and 6.0 g/kg diet (se = 0.91 and 0.60, respectively) at both 300 and 500 g CP/kg diet.

To determine the nitrogen requirement of kittens as a function of body weight, nitrogen retention [g/(d·kg body weight)] was expressed in relation to total nitrogen intake [g/(d·kg body weight)]. Only the data from kittens fed levels of dietary threonine well above the requirement (0.7, 0.9 and 1.2 g threonine/kg diet) were examined. Broken-line analysis failed to reveal a clear break point that would indicate the nitrogen requirement per kg body weight [Fig. 2].

Plasma amino acids exhibited several different patterns of change as dietary CP increased [see Hammer 1993 for tabulated results]. For descriptive purposes, the amino acids have been grouped according to the pattern of change observed when dietary CP was increased [across all levels of dietary threonine], starting with those amino acids least affected. Plasma concentrations of aspartate and citrulline generally were not affected by the level of dietary CP, and levels of phenylalanine, tyrosine, tryptophan and histidine tended only to be higher in the 300 and 500 g CP diet groups compared with the 150 g CP diet group. In general, plasma concentrations of methionine, lysine, alanine, glutamine, half-cystine, asparagine and glycine increased with increasing CP up to 300 g/kg diet but not with a further increase to 500 g/kg diet. Levels of ornithine, proline, valine, isoleucine and leucine were the most responsive to dietary CP levels and increased in plasma as dietary CP increased up to 500 g/kg diet.

The concentrations of plasma threonine and serine exhibited much different patterns than those of the other amino acids. Plasma threonine increased linearly with increasing dietary threonine, but dietary CP did not have a significant effect on plasma threonine concentrations [Fig. 3A]. The plasma serine concentrations were affected by both dietary threonine and dietary CP [Fig. 3B]. At levels of dietary CP above 150 g/kg diet, there appeared to be an inverse relationship between plasma serine and the amount of threonine in the diet, although with this experimental design it is not statistically valid to compare the data between levels of dietary threonine. When dietary threonine was > 7.0 g/kg diet, this relationship was no longer apparent.

Evidence of a threonine deficiency was demonstrated in kittens fed the lowest level of dietary threonine, 4.0 g/kg diet. The kittens developed evidence of vestibular dysfunction manifested by disequilibrium, abnormal righting reflex, head tremor, impaired physiological nystagmus and placing reflexes. These symptoms of threonine deficiency in kittens are consistent with what has been previously observed [Titchenal et al. 1980], although forelimb lameness was not evident in this experiment. All clinical signs disappeared within 2 wk after refedding a commercial diet.

Experiment 2. In this trial, there were no significant differences among any of the diet conditions in food intake or weight gain (Table 2). Nitrogen retention was significantly greater in the 500 g CP/kg diet group compared with the 200 g CP/kg diet group, but there was no difference between the 300 and 500 and 200 g CP/kg diet groups. There was no evidence of a diet by sex interaction.
Blood, for determination of plasma amino acids, was drawn after the kittens had been exposed to the experimental diets for only 5 h and on d 11 of the treatment period (see Hammer 1993 for tabulated results). At 5 h, there were no significant elevations in plasma essential amino acids in the 300 g CP/kg diet group compared with the 200 g CP/kg diet group. In kittens fed 500 g CP/kg diet, plasma concentrations of the essential amino acids, valine, methionine, lysine, histidine and arginine were greater than in the 200 g CP/kg diet group. Plasma threonine was not significantly affected by the level of CP (48, 49 and 52 μmol/L with the 200, 300 and 500 g CP/kg diets, respectively). The dispensable amino acids asparagine, glutamine, proline and glycine all increased in plasma as dietary CP increased.

By d 11 of the experimental period, all of the essential amino acids were significantly elevated in the plasma of the kittens fed CP at 500 g/kg diet when compared with the 200 g CP group, and many of them were elevated in the 300 g CP group. Plasma concentrations of threonine were not significantly different among groups (Table 2). The dispensable amino acids that were elevated on d 1 were also elevated on d 11.

Plasma serine was significantly elevated in the 300 g CP/kg diet when compared with the 200 g CP/kg diet group but not when compared with the 500 g CP/kg diet group (73, 102 and 78 μmol/L in the 200, 300 and 500 g CP/kg diet groups, respectively).

**DISCUSSION**

The pattern of increased growth, seen when CP was increased from 200 to 300 g/kg diet at all levels of dietary threonine, provides evidence that the nitrogen requirement in kittens is above the 180–200 g/kg diet suggested by Smalley et al. [1985]. These findings are consistent with the results of Strieker [1991], who completed a study similar to Experiment 1 described here but used methionine as the limiting amino acid. Hargrove et al. [1988] also saw increased growth when 10% leucine was added to a diet with 200 g CP/kg. Together, with the results of Strieker and Hargrove, these two experiments indicate that 200 g CP/kg diet is below the requirement for nitrogen in kittens.

**TABLE 2**

**Food intake, weight gain, nitrogen retention and plasma threonine in kittens fed 6.0 g threonine/kg diet and either 200, 300 or 500 g crude protein/kg diet (Experiment 2)**

<table>
<thead>
<tr>
<th>Dietary crude protein</th>
<th>Food intake1</th>
<th>Weight gain1</th>
<th>Nitrogen retained1</th>
<th>Plasma threonine3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/day</td>
<td></td>
<td></td>
<td>μmol/L</td>
</tr>
<tr>
<td>200 g/kg diet</td>
<td>53</td>
<td>17</td>
<td>0.68b</td>
<td>60</td>
</tr>
<tr>
<td>300 g/kg diet</td>
<td>55</td>
<td>24</td>
<td>0.84ab</td>
<td>52</td>
</tr>
<tr>
<td>500 g/kg diet</td>
<td>63</td>
<td>25</td>
<td>1.07*</td>
<td>57</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>3</td>
<td>2</td>
<td>0.05</td>
<td>4</td>
</tr>
</tbody>
</table>

1 Represent daily means of the last 8 d of the 12-d experimental period.
2 Samples collected on d 11 of the experimental period; n = 8/group. Different letters represent differences between diet groups, P ≤ 0.05.
In addition to examining the nitrogen requirement as a function of dietary concentration, we expressed nitrogen retention in relation to total nitrogen intake and attempted to determine the nitrogen requirement of kittens as a function of body weight. Broken-line analysis failed to determine a nitrogen requirement on this basis. These results suggest that cats do not have tight control on the efficiency of utilization of protein, which is probably secondary to nonadaptive protein catabolic enzymes in this species (Rogers et al. 1977).

In Experiment 1, there was some indication that, under some circumstances, increasing dietary CP above 300 g/kg diet might improve growth, i.e., the nitrogen requirement might be > 300 g/kg diet. When dietary threonine was held constant at 6.0 g/kg, kittens ate the most and retained the most nitrogen on 500 g CP/kg diet [Fig. 1A–C]. Therefore, in Experiment 2, CP levels of 200, 300 and 500 g/kg were retested with dietary threonine held constant at 6.0 g/kg. In this experiment, the group of kittens fed 500 g CP/kg diet did not grow significantly faster than the kittens fed 300 g CP/kg diet [Table 2]. These results do not support a dietary nitrogen requirement > 300 g/kg diet in kittens, although the trend toward greater nitrogen retention in the group fed the higher level of CP suggests that a larger number of kittens might have been able to reveal a subtle response difference.

Broken-line analysis of the nitrogen retention data indicated that at 150 and 200 g CP/kg diet [Fig. 1C], the requirement for threonine was 4.9 g/kg diet. When the level of dietary CP was increased to 300 or 500 g/kg diet, the requirement was 6.0 g/kg diet. Thus, there was a slight positive relationship between the requirement for threonine and the level of CP in the diet. Although these results are somewhat different from those of Strieker [1991], who did not find a positive relationship between the requirement for methionine and dietary CP, they clearly differ from what would be expected in other species.

In many other species, such as chickens (Grau and Kamel 1950) and rats (Salmon 1954), there is a strong positive relationship between the requirement for an essential amino acid and the level of CP in the diet. That is, when one essential amino acid is limiting in the diet in these species, greater weight gain is achieved with lower levels of CP. The requirement for tryptophan in the chicken is a good example. When dietary tryptophan is 0.5 g/kg diet, optimal weight gain in the chick is achieved at 87 g CP/kg diet, but food intake decreases and growth slows when CP is increased to 116 g/kg diet (Boomgaardt and Baker 1971). To achieve equal or better weight gain at the higher level of dietary nitrogen, the level of dietary tryptophan must be increased. This effect has been described as an amino acid imbalance response (Harper et al. 1970).

Amino acid imbalance is generally characterized by a growth depression that is a direct result of a decrease in food intake. There is no apparent impairment in the utilization of CP or of the limiting amino acid from the imbalanced ration (Fisher and Shapiro 1961, Harper et al. 1970). The feeding depression caused by the addition of dietary CP can be prevented or reversed by addition of the limiting amino acid to the diet (Harper et al. 1970). Absence of a strong positive relationship between the requirement for threonine in this study or for methionine (Strieker 1991) suggests that cats are not susceptible to amino acid imbalance so that, under most conditions, the addition of dietary CP when an essential amino acid is limiting does not cause a growth depression.

Although, overall, the results of the threonine and methionine studies suggest that cats are not susceptible to an amino acid imbalance, in Experiment 1 there was some evidence of an amino acid imbalance response. When kittens were fed 4.0 or 5.0 g threonine, increasing CP from 300 g/kg diet to 500 g/kg diet tended to decrease food intake, with a subsequent depression in weight gain [Figs. 1A and B]. The nutritional effect of an amino acid imbalance has been investigated previously in cats. Titchenal et al. (1980), using threonine as the limiting amino acid (4.0 g threonine/kg diet), observed a feeding depression in cats when the amino acid mixture was doubled from 175 to 350 g/kg diet without increasing threonine. However, when Strieker [1991] fed very low levels of dietary methionine and high levels of dietary CP, she saw no evidence of an amino acid imbalance response. In other species, amino acid imbalance was demonstrated with both threonine and methionine as the limiting amino acid, and the imbalance affected the requirement for both amino acids (Fisher and Shapiro 1961, Grau and Kamel 1950, Sauberlich 1956).

The most consistently observed biochemical changes in other species fed amino acid-imbalanced diets are the marked alterations in the plasma amino acid pattern shortly after the ingestion (3 h) of the imbalanced diet. The growth-limiting amino acid in the blood plasma falls rapidly, and the concentrations of most of the amino acids added to create the imbalance increase. Work by Tews et al. [1979 and 1980] stressed that competition for brain transport of the limiting amino acid in the plasma may play a major role in induction of the imbalanced response in rats. In Experiment 1, there were no changes in plasma threonine as the level of dietary CP changed [Fig. 3A]. Even those diets that appeared to cause a feeding depression, 4.0 or 5.0 g threonine and 500 g CP/kg diet, did not cause a depression in plasma threonine. Thus, there was no biochemical evidence of an amino acid imbalance in conjunction with the feeding depression.

The plasma threonine results from Experiment 1 [Fig. 3A] are consistent with studies that showed that plasma threonine becomes elevated when high CP diets are fed [Tews et al. 1984], and overall threonine catabolism in the cat does not increase more than two- to threefold in response to high dietary CP [Hammer
This is very different from what has been shown to occur when rats are fed high CP diets. In rats, the concentration of plasma threonine decreases when a high CP diet is consumed freely (Anderson et al. 1968), and threonine catabolism increases as much as 30-fold (Bird and Nunn 1983).

Plasma serine, on the other hand, appeared to be affected by both the levels of dietary threonine and dietary CP, although dietary serine was not added to any of the diets (Fig. 3B). None of the other plasma amino acids showed this unusual pattern. The plasma serine concentrations in Experiment 2 were consistent with these observations. It is unlikely that the synthesis of serine from threonine was greater when kittens were fed low levels of dietary threonine. Serine would be made from threonine via pathways initiated by L-threonine 3-dehydrogenase and threonine aldolase (Bird et al. 1984; Malkin and Greenberg 1964), and recent work in our laboratory has shown no differences in the activity of these enzymes in kittens fed either 4.0 or 8.0 g/kg dietary threonine (Hammer 1993). One might speculate that both dietary threonine and CP are required in adequate amounts for high activity of serine catabolic enzymes, but no reasonable conclusion can be drawn from these results without further study because plasma serine concentrations could be affected by both the rates of synthesis and degradation, as well as rates of protein turnover.

The differential effect of CP on the requirements for threonine and methionine in kittens may be accounted for by metabolism (Meister 1965) and transport differences (Tews et al. 1979 and 1980) between the two amino acids, e.g., the first step in threonine metabolism is a committed one, whereas methionine can be resynthesized from its keto acid or from homocysteine (Meister 1965). These factors may determine whether the concentration of the limiting amino acid becomes low enough in the brain to cause the feeding depression associated with an amino acid imbalance. Future studies need to examine the effects of threonine- and methionine-deficient, high CP diets on either brain or cerebrospinal fluid amino acids in kittens to determine if amino acid patterns in those tissues support this hypothesis.

In conclusion, results from these studies indicated that the requirement for nitrogen in the kitten was between 200 and 300 g/kg diet, which is higher than previous results would indicate (Smalley et al. 1985). The requirement for threonine showed a weak positive relationship with the level of dietary CP, so that the requirement was 4.9 g/kg diet at both 150 and 200 g CP/kg diet and 6.0 g/kg diet at both 300 and 500 g CP/kg diet. Cats appeared to be less susceptible to a typical nutritional amino acid imbalance response than other species, which may explain the lack of a stronger relationship between dietary CP and the requirement for dietary threonine. In general, when the amino acid disproportion was not extreme, an increase in dietary CP led to an increase in food intake, which provided more of the limiting amino acid for growth. But, when the amino acid disproportion was extreme, with threonine as the limiting amino acid, the extra CP caused an amino acid imbalance.

ACKNOWLEDGMENTS

We thank Vicki Medeiros, Ken Lindley, Dan Wong and Tim Taylor for their assistance with the animal work and with sample analysis. We also thank Brian Hrupka for his assistance with statistical analysis and Grant Gilford for his clinical evaluation of the kittens. Some of the amino acids were generously donated by Ajinomoto USA, Raleigh, NC, the hydrogenated beef tallow was a generous gift from Kal Kan Food, Vernon, CA and the vitamin mixture was a generous gift from Hoffman-La Roche, Nutley, NJ.

LITERATURE CITED


Kane, P. F. (1984) Comparison of HgO and CuSo, as digestive cata-


Robbins, K. R. (1986) A method, SAS program, and example for fitting the broken-line to growth data. In: The University of Tennessee Agricultural Experiment Station Research Report 86–09, Knoxville, TN.


