Excess Dietary Lysine Does Not Cause Lysine-Arginine Antagonism in Adult Cats1,2

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EXPANDED ABSTRACT

KEY WORDS: • lysine • arginine • feline • cats • herpesvirus

Feline herpesvirus type 1 (FHV-1) is one of the most common feline pathogens in the world (1). Primary infection with FHV-1 in susceptible kittens causes severe upper respiratory and ocular disease with ~100% morbidity particularly in multi-cat environments (2). After primary exposure, FHV-1 establishes lifelong neural latency with periodic reactivation in ~80% of cats (2). Presently no medications or vaccines have demonstrated reduced establishment of latency or frequency of reactivation. Oral lysine administration to cats infected with FHV-1 is associated with a significant reduction in the severity of clinical signs and basal viral shedding (3,4). The mechanism of action is hypothesized to be reduced viral replication due to antagonism of arginine by excess lysine (5). The most logical means of controlling viral shedding in multi-cat environments is to reduce viral shedding in latently infected cats. Nutritional control of viral shedding would provide a simple and efficient means of implementing such a management technique.

Determination of the safety of lysine supplementation is imperative due to cats’ exquisite sensitivity to arginine deficiency (6). Although previous studies demonstrated that exogenous supplementation with lysine increases plasma lysine without antagonizing plasma arginine concentrations (3,4), the effect was short lived (3 h) in the one study where it was evaluated (3). It is hypothesized that if the lysine content of a commercial diet was increased, cats consuming that diet ad libitum would have and maintain elevated plasma lysine concentrations throughout the day and forgo the variable concentrations observed with exogenous supplementation. This consistent elevation in plasma lysine concentrations may be beneficial in controlling FHV-1. However, to the authors’ knowledge, the safety of lysine administration >19 g/kg of diet (7) has never been evaluated. The objective of this study was to determine the safety of excess lysine supplementation to a commercial-type, expanded diet fed to healthy adult cats.

MATERIALS AND METHODS

The experimental protocol was approved by the Animal Care and Use Administrative Advisory Committee of the University of California, Davis.

Animals and their management

Healthy intact adult female, domestic short-hair cats (n = 36) from the Feline Nutrition and Pet Care Center of the University of California, Davis were used. Cats were individually housed in metabolic cages (60 × 60 × 60 cm) in rooms with controlled temperature (21 ± 2°C) and a 14:10-h light:dark cycle. The animals had free access to water throughout the study.

Diets

The basal diet was a commercially prepared, experimental, dry expanded diet manufactured by Nestlé Purina PetCare Company. The proximate composition of the diet (in g/kg of diet) was 320 protein, 150 fat, and 13 crude fiber.9 The caloric density of the diet was 409 kcal metabolizable energy (ME)/100 g of diet. The calculated lysine content was 13 g/kg of diet. The analyzed lysine content was 10.7 ± 0.2 g/kg of diet (mean ± SEM, n = 9 samples). The calculated arginine

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2042S
content was 13 g/kg of diet. The analyzed arginine content was 12.9 ± 0.2 g/kg of diet (mean ± SEM, n = 9 samples).

**Design**

All of the cats were adapted to the basal diet for 1 wk. Individual food-intake and lysine-intake amounts were determined daily throughout the study. Cats were fed once daily at 1100 h. Based on food-intake determinations during wk 1, each cat was fed 120 g of food/d, which was an amount greater than any cat consumed daily. All cats had free access to their food. During wk 2, 12.7 g of deionized water was added daily to each cat’s portion before feeding. On wk 3, the cats were assigned to six dietary treatment groups. Cats in the control group (n = 6 cats) were fed 120 g of the basal diet (11 g of lysine/kg of diet) with 12.7 g of added water. Cats in the other five treatment groups (n = 6 cats/group) were fed the basal diet but with lysine added as lysine acetate dissolved in deionized water to yield a total dietary concentration (diet plus supplemental lysine) of 36, 61, 86, 111, or 131 g of lysine/kg of diet. The lysine acetate solution was prepared and applied to the diets on a daily basis. Dissolution of the lysine acetate in water permitted a thorough and even application of the amino acid throughout the basal diet. Cats in the control group and the dietary treatment groups that consumed 36, 61, or 86 g of lysine/kg of diet received a total of 12.7 g of deionized water in their diet daily. The treatment groups with 111 and 131 g of lysine/kg of diet were added after no significant changes were observed in cats that received the diets containing lower lysine concentrations. To get sufficient lysine acetate into solution to provide 111 or 131 g of lysine/kg of diet, it was necessary to add 18.7 or 22.7 g, respectively, of deionized water to these diets. In all aspects of the study other than this, cats in these two dietary treatment groups were treated identically to those in the previous groups. Therefore, the results from all six treatment groups are reported simultaneously. Cats were observed daily and weighed weekly throughout the study. Blood was collected on d 2 (2 d after beginning the lysine-supplemented or control diets), d 7, and d 14 (end of the project) by routine venipuncture at 1500 h. Blood was collected into heparinized syringes and separated into two tubes (whole blood and plasma). Plasma was obtained by centrifugation of the heparinized blood sample at 10,000 × g for 15 min, immediate deproteinization with an equal volume of 0.24 mol/L sulfosalicylic acid, and centrifugation again at 10,000 × g for 15 min at 4°C. All samples were stored at −80°C until analysis. Sample preparation of the basal diet for analysis was previously described (8). Amino acid analysis of plasma and basal diet samples was performed using an amino acid analyzer (model 6300, Beckman Instruments, Palo Alto, CA).

The effect on food intake of the increased amount of water needed to dissolve the lysine acetate into solution for the treatment groups with 111 and 131 g of lysine/kg of diet was determined in a separate study. Individual food-intake amounts were determined for six cats fed the basal diet with the same amounts of deionized water added as in the initial experiment. Each cat was fed 120 g of basal diet for 1 wk and subsequently received the same amount of diet supplemented with 12.7, 18.7, or 22.7 g of deionized water for 1 wk at each level of water supplementation. Dietary dry-matter intake amounts were determined daily for each cat.

**Statistical analysis**

All results are expressed as means ± SEM. One-way ANOVA was used to test for differences in means among groups. Repeated-measures ANOVA with two grouping variables (time and treatment) and four repeated measures (amino acid concentration, food intake, lysine intake, or body weight) was used to test for variable interactions and variance of plasma amino acid concentration, food intake, lysine intake, and body weight. Specific differences were determined using a Tukey-Kramer post-hoc test. (Systat 10.2, SPSS, Chicago, IL). For all analyses, differences were considered significant at P ≤ 0.05.

**RESULTS**

Mean plasma lysine concentrations were greater in all dietary treatment groups compared with cats that consumed the basal (11 g of lysine/kg of diet) diet (P ≤ 0.05) and tended to increase with increasing lysine intake. There was a significant effect of treatment on mean plasma lysine concentrations (P ≤ 0.05, Table 1) but not mean plasma arginine concentrations (P ≥ 1.0). There was no significant time-by-treatment interaction (arginine, P ≥ 0.7; lysine, P ≥ 0.8) nor a significant main effect of time on either mean plasma arginine (P ≥ 0.06) or mean plasma lysine (P ≥ 0.7) concentrations. Other mean plasma amino acid concentrations did not differ significantly among dietary treatment groups (data not shown).

One cat from the control group was removed from the study during wk 2 for reasons unrelated to dietary treatment. There were no significant changes (P ≥ 1.0) in the mean body weights of cats in any group throughout the study. There were significant main effects of both time and treatment on food-intake values in this study (Fig. 1, P ≤ 0.05); however, there was no significant time-by-treatment interaction on food intake (P ≥ 0.3). Food-intake amounts in the groups that consumed the diets with 111 or 131 g of lysine/kg of diet began to decrease compared with the other dietary treatments on d 2. The mean food-intake values for cats that consumed 11, 36, 61, 86, 111, or 131 g of lysine/kg of diet on d 2 were 86.0 ± 7.8, 79.3 ± 6.3,

### Table 1

<table>
<thead>
<tr>
<th>Dietary lysine, g/kg of diet</th>
<th>Plasma lysine, nmol/mL of plasma</th>
<th>Plasma arginine, nmol/mL of plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d 2</td>
<td>d 7</td>
</tr>
<tr>
<td>111a,b</td>
<td>96 ± 16</td>
<td>81 ± 15</td>
</tr>
<tr>
<td>361b</td>
<td>151 ± 21</td>
<td>202 ± 31</td>
</tr>
<tr>
<td>611c</td>
<td>296 ± 55</td>
<td>331 ± 74</td>
</tr>
<tr>
<td>861d</td>
<td>232 ± 53</td>
<td>402 ± 65</td>
</tr>
<tr>
<td>111e</td>
<td>481 ± 84</td>
<td>502 ± 55</td>
</tr>
<tr>
<td>131f</td>
<td>474 ± 74</td>
<td>396 ± 76</td>
</tr>
</tbody>
</table>

1 All concentrations are expressed as means ± SEM (n = 5 or 6 cats).
2 For plasma arginine concentrations, there was no significant time-by-treatment interaction (P ≥ 0.7) and no significant time (P ≥ 0.06) or treatment (P ≥ 1.0) effects.
3 For plasma lysine concentrations, there was no significant time-by-treatment interaction (P ≥ 0.8) nor a significant time effect (P ≥ 0.7), but there was a significant main effect of treatment (P ≤ 0.05). Based on the effect of treatment, groups not sharing a common (non-numeric) superscript (a, b, c, or d) were significantly different (P ≤ 0.05) with respect to plasma lysine concentration using the Tukey-Kramer post-hoc test.
4 Basal diet.
DISCUSSION

Recent studies show that supplementation with exogenous L-lysine reduces FHV-1 shedding and clinical signs of FHV-1–associated disease in cats (3,4). Owing to cats’ exquisite sensitivity to arginine deficiency, it was necessary to investigate the safety of this approach before other mechanisms of lysine delivery were explored. The results of this study indicate that 131 g of lysine/kg of diet may not antagonize arginine in healthy adult cats, and that as much as 86 g of lysine/kg of diet may be fed without any recognized adverse effects.

In this study, plasma lysine values increased with dietary lysine intake. Studies in other species show similar responses to increased dietary lysine concentrations (9,10). The slight variations in mean plasma lysine concentrations within a dietary treatment group over the course of the study are most likely the result of differences in each individual cat’s food intake before blood collection. Every cat was offered fresh food 4 h before blood collection. Some cats ate the fresh food immediately, whereas others did not. However, food-intake amount was measured only once daily before fresh food was offered.

Plasma arginine concentrations did not decrease nor were clinical signs of arginine deficiency observed with increasing dietary lysine concentrations. This was also observed in kittens, young pigs, and growing dogs that were fed excess lysine in diets that contained arginine concentrations at or above the recommended minimal requirements (9–11). Furthermore, unlike plasma lysine concentrations, plasma arginine concentrations were not affected by food intake. This is most likely because all dietary treatments contained arginine somewhat in excess of the minimal recommended arginine requirement for adult cats.

The mean body weights of the cats did not differ significantly among or change within the dietary treatment groups throughout the study. However, there was a reduction in food-intake amount in the cats that consumed 111 or 131 g of lysine/kg of diet, and by d 14, this difference approached significance. A longer study period may have yielded a statistically significant difference. Two likely causes for this reduction in food-intake amount are the increased quantities of water needed to incorporate the higher amounts of lysine into solution in the 111 and 131 g of lysine/kg of diet concentrations, or toxic effects of high concentrations of lysine in these two diets. There was no change in dietary dry-matter intake when equivalent amounts of water only were added to the basal diet. Therefore, the reduction in food-intake level in the cats that consumed 111 or 131 g of lysine/kg of diet was most likely the result of the excess dietary lysine and not the water used to incorporate it into solution.

It is hypothesized that the reduction in food intake in this study was the result of an amino acid toxicity. A reduction in food intake can be the result of amino acid imbalance, toxicity, or antagonism (12). Excess dietary lysine was shown to antagonize arginine in chicks (13), rats (14), guinea pigs (15), and growing dogs (9). An amino acid imbalance resulting in a reduction in both food-intake and weight gain was reported for young pigs that consumed diets that contained 34.5 or 46.5 g of lysine/kg of diet and 5.3 g of arginine/kg of diet (11). A reduction in food intake coupled with the absence of reduced plasma amino acid concentrations and clinical signs of arginine deficiency support an amino acid toxicity at the two highest dietary lysine concentrations fed in this study.

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LITERATURE CITED