Effects of dietary lysine supplementation on upper respiratory and ocular disease and detection of infectious organisms in cats within an animal shelter

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Objective—To determine within a cat shelter effects of dietary lysine supplementation on nasal and ocular disease and detection of nucleic acids of *Chlamyphila felis*, feline calicivirus (FCV), and feline herpesvirus (FHV-1).

Animals—261 adult cats.

Procedures—Cats were fed a diet containing 1.7% (basal diet; control cats) or 5.7% (supplemented diet; treated cats) lysine for 4 weeks. Plasma concentrations of lysine and arginine were assessed at the beginning (baseline) and end of the study. Three times a week, cats were assigned a clinical score based on evidence of nasal and ocular disease. Conjunctival and oropharyngeal swab specimens were tested for FHV-1, FCV, and *C. felis* nucleic acids once a week.

Results—Data were collected from 123, 74, 59, and 47 cats during study weeks 1, 2, 3, and 4, respectively. By study end, plasma lysine concentration in treated cats was greater than that in control cats and had increased from baseline. There was no difference between dietary groups in the proportion of cats developing mild disease. However, more treated cats than control cats developed moderate to severe disease during week 4. During week 2, FHV-1 DNA was detected more commonly in swab specimens from treated versus control cats.

Conclusions and Clinical Relevance—Dietary lysine supplementation in the amount used in our study was not a successful means of controlling infectious upper respiratory disease within a cat shelter. Rather, it led to increases in disease severity and the incidence of detection of FHV-1 DNA in oropharyngeal or conjunctival mucosal swab specimens at certain time points. (Am J Vet Res 2009;70:1391–1400)

Abbreviations

<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Description</th>
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<tbody>
<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>FCV</td>
<td>Feline calicivirus</td>
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<tr>
<td>FHV-1</td>
<td>Feline herpesvirus type 1</td>
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<tr>
<td>HRR</td>
<td>Hazard rate ratio</td>
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<tr>
<td>IQR</td>
<td>Interquartile range</td>
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<td>IURD</td>
<td>Infectious upper respiratory disease</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<td>VI</td>
<td>Virus isolation</td>
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Within multicat environments such as animal shelters, breeding catteries, cat shows, research colonies, and even multicat households, IURD (ie, rhinitis, pharyngitis, and keratoconjunctivitis caused by infectious organisms) is a common and major management problem. Although many infectious agents are likely involved, evidence gathered over many years on multiple continents suggests that the major pathogens are FHV-1, FCV, and *Chlamyphila felis* (previously *Chlamydia psittaci*). Of these, FHV-1 is believed to be the most common and to cause the most severe and recurrent disease. Infection of susceptible kittens with FHV-1 causes marked IURD, with approximately
100% morbidity. Illness may be fatal, particularly in young kittens. After primary infection, FHV-1 establishes lifelong neural latency in at least 80% of infected cats. Periodic viral reactivation and shedding take place throughout life in most cats and are associated with chronic rhinosinusitis and keratoconjunctivitis in many. Physiologic stressors such as intercurrent disease, pregnancy, lactation, and rehousing increase reactivation rates and ensure perpetuation of the virus in feline populations, particularly in cats housed in shelters. Therefore, latently infected cats represent the major source of infection for cats immunologically naive to FHV-1. To date, medications that reduce establishment of latency or frequency of reactivation of FHV-1 in cats have not been identified.

The impact of IURD is perhaps greatest within shelter environments. In shelters, there is sometimes rapid turnover of large numbers of cats with variable vaccination and immune statuses and which are kept in conditions known to exacerbate shedding of some infectious respiratory pathogens. Methods for controlling the spread of IURD include quarantine, which consumes time, resources, and space; systemic administration of antiviral agents to newly infected cats, which is expensive and sometimes associated with considerable toxic effects, and vaccination, which is of unknown efficacy in latently infected cats. As a result, IURD is one of the major reasons for euthanasia or difficulty in rehousing cats from shelters. Because latently infected cats are the most important natural reservoir for infection of susceptible cats, the most logical means of control in shelter environments is reduction of viral shedding by carrier cats.

Encouraging data exist regarding antiviral effects of lysine against FHV-1 in vitro, in cats undergoing experimental primary herpetic infection treated with 500 mg of lysine every 12 hours, and in latently infected cats treated orally with 400 mg of lysine every 24 hours. Both of these in vivo studies involved investigation of the effect of bolus administration of lysine to small numbers of experimentally infected cats, and outcomes of a recent study suggest these data may not be directly applicable to larger groups of naturally infected cats. In that study, 144 cats in a shelter received oral boluses of 230 mg (kittens) or 500 mg (adult cats) of lysine once daily for the duration of their stay at the shelter and outcomes were compared with those of an untreated control group. No significant treatment effect was detected on the incidence of IURD, the need for antimicrobial treatment for IURD, or the interval from admission to onset of IURD. Additionally, bolus administration of lysine to individual cats within multicat environments such as shelters, where FHV-1 is prevalent, may not be practical. It is also plausible that twice-daily handling of these cats may actually stimulate further viral reactivation through stress and cause transfer of pathogens between cats by shelter workers administering the lysine.

If bolus administration is not essential for efficacy of lysine in management of IURD, then dietary lysine supplementation would be an excellent means of providing additional lysine in multicat environments. We found that healthy cats fed a diet supplemented with up to 8.6% lysine developed no toxic effects and had significant increases in plasma lysine concentration, whereas plasma arginine concentration and food intake were unaffected. These results suggested that dietary lysine supplementation could be done safely and efficaciously in healthy cats. We then conducted a study in which 100 cats in a colony in which IURD was enzootic were rehoused to stimulate viral reactivation and fed a diet containing 1.1% or 5.1% lysine for 52 days. Compared with cats in the control group, cats fed the supplemented diet had a higher plasma lysine concentration but also had more severe disease and had FHV-1 DNA detected more often in swab specimens collected from the conjunctival fornix. However, increased disease severity and FHV-1 DNA detection were most noteworthy in a subpopulation of male cats that exhibited fighting behavior. Therefore, a definitive conclusion regarding the efficacy of dietary lysine supplementation for control of disease and shedding of FHV-1 in cats with enzootic IURD was not possible. In addition, we are unaware of any studies conducted to determine the effect of lysine on the replication, clinical severity, or shedding of other pathogens involved in feline IURD.

The purpose of the study reported here was to test the hypothesis that dietary lysine supplementation of cats housed in a shelter in which IURD was enzootic would reduce detection of specific microbial nucleic acids and clinical signs of IURD in the cats. Our objectives were to determine the effect of dietary lysine supplementation on the development and severity of clinical signs of IURD and on detection of ocular and oropharyngeal surfaces of nucleic acids from FHV-1 along with 2 other organisms commonly associated with IURD (C felis and FCV) in cats in a shelter environment in which IURD was enzootic.

Materials and Methods

Animals—The study was conducted between April and October 2007 at a local humane shelter in which approximately 11,000 dogs and cats were cared for annually. All cats admitted to the shelter received a subcutaneous microchip at entry, and their position within the shelter was recorded once a day by shelter staff. Cats were managed in accordance with standard shelter protocols as follows. For approximately 1 to 5 days after arrival, cats were individually housed in stainless steel cages (71 cm deep X 56 to 71 cm wide X 71 cm high) with solid walls and grilled front doors. In this initial sorting room, cats were examined, tested for FIV antibody and FeLV antigen, and assessed behaviorally by shelter staff. Cats that had negative results for circulating FIV antibody and FeLV antigen and that were judged by the shelter staff as healthy and potentially adoptable were moved to the adoption area. In the adoption area, most cats were housed individually in plastic-walled enclosures (79 cm deep X 84 cm wide X 183 cm high) with glass doors. Cats left the adoption area if they were euthanatized, adopted, or moved to the hospital ward in a separate building. Shelter staff decided, on the basis of shelter protocols, when cats were moved between adoption rooms and the hospital ward. In the hospital ward, cats were individually housed in cages identical to those in the sorting room.

Materials and Methods
Baseline clinical assessment—Cats that had negative results for circulating FIV antibody and FeLV antigen and that were judged by the shelter staff as healthy and potentially adoptable were eligible for inclusion in the study and were examined within 3 days of admission to the shelter by 1 of 2 investigators (TLD or HDW). Body weight was recorded and age was estimated on the basis of dental examination. Cats estimated to be > 6 months old were assigned a score for clinical signs of ocular or nasal disease by use of a published scoring system. Severity of conjunctivitis was assigned a score of 0 (none) through 3 (moderate to severe). Severity of blepharospasm was assigned a score of 0 (none) through 4 (eye completely closed). Severity of ocular discharge was assigned a score of 0 (none) through 3 (marked mucopurulent discharge). Sneezing was assigned a score of 0 (absent) or 1 (present). Severity of nasal discharge was assigned a score of 0 (none) through 3 (marked mucopurulent discharge). The total clinical disease score was defined as the sum of all ocular (conjunctivitis, blepharospasm, and ocular discharge) scores plus nonocular (sneezing and nasal discharge) scores. The minimum total clinical disease score possible was 0, and the maximum was 14. Only cats with a total clinical disease score ≤ 9 at initial examination were admitted to the study. Cats received medications only within the hospital, where they were treated according to shelter protocols, none of which included lysine administration.

Baseline sample collection and analysis—After baseline clinical assessment, a venous blood sample for measurement of baseline plasma concentrations of lysine and arginine was collected from each cat into a lithium heparin tube and immediately placed on ice. Within 1 to 3 hours after collection, blood samples were centrifuged at 453 × g for 10 minutes, and plasma was harvested and stored at −20°C until analysis was performed with an automated amino acid analyzer, as described. Reference ranges for plasma lysine and arginine concentrations as established by the Amino Acid Laboratory of the University of California-Davis School of Veterinary Medicine were 84 to 116 and 114 to 152 nmol/mL, respectively.

After blood sample collection, swab specimens were collected for baseline pathogen nucleic acid detection. One drop of 0.5% proparacaine was applied to each eye, and a dry polyester swab was vigorously rolled in the ventral conjunctival fornix of both eyes. This swab was immediately placed in a tube containing 0.5 mL of lysis buffer diluted 1:1 with purified water for subsequent analysis by means of PCR assay. Two similar polyester swabs were then simultaneously used to swab the oropharynx, with an attempt made to touch the tonsilar region. One of the swab specimens was placed in the same tube as the conjunctival swab specimen, and the second swab specimen was placed in 1 mL of viral transport medium (Dulbecco modified Eagle medium, 1% fetal calf serum, gentamicin [50 µg/mL], amphotericin B [2.5 µg/mL], and penicillin G [100 µg/mL]). Both tubes were immediately placed on ice and transported to the laboratory within 1 to 3 hours after preparation for storage until they were processed. Swab specimens for VI were stored at −20°C and swab specimens for the PCR assay were stored at −80°C until analyzed. Conjunctival swab specimens were not submitted for VI because FHV-1 has been isolated more consistently from oropharyngeal swab specimens than from conjunctival swab specimens.

Experimental protocol—Following collection of baseline data, stratified allocation of cats to 1 of 2 groups was performed according to sex (without consideration of neuter status) and clinical disease score at admission (≤ 4 or 5 to 9). Management of the 2 groups differed only by lysine content of the diet fed. Cats in the control group received the basal diet, and cats in the treatment group received the same basal diet supplemented with lysine. Other than amino acid content, the composition of both diets was almost identical (Appendix). Only 1 batch of each diet was prepared and used throughout the entire study. Food samples from each diet were collected approximately every other month throughout the study (total of 3 samples) and stored at −20°C until they were assessed with an automated amino acid analyzer, as described. The mean ± SD dietary lysine concentration (g/kg of diet) for the 3 dietary assessments made during the study was 16.77 ± 1.73 for the basal diet and 57.19 ± 0.97 for the lysine supplemented diet. The mean dietary arginine concentration (g/kg of diet) was 19.51 ± 2.06 for the basal diet and 19.22 ± 0.22 for the lysine supplemented diet.

Cats had free access to water and their respective diet throughout the study. Personnel performing dietary and plasma amino acid analyses were unaware of which diet the cats were receiving.

Sometimes, pairs of cats were housed together. In that situation, both cats were placed on the same study diet. In all locations within the shelter except the hospital, cats in the study received the assigned study diet and water only. To avoid errors in diet fed, study personnel placed plastic bags containing the appropriate diet on the door of each cat cage for the shelter staff to dispense daily. Within the shelter, diets were identified only by a letter (A or B). Cats in the shelter hospital were offered the study diet they had been assigned along with various commercial canned cat foods intended to stimulate appetite. Cats remained in the study for up to 4 weeks.

Throughout the study period, all cats were examined 3 times weekly by the same 2 investigators who made the baseline assessments of disease status and assigned a clinical disease score, as described for the baseline assessment. Once a week, conjunctival and oropharyngeal swab specimens were collected and stored for VI and PCR assay, as described for the baseline assessment. A venous blood sample for analysis of plasma amino acid concentration was collected during week 4 (days 21 to 28) of the study and processed as described for the baseline sample. Because cats could be adopted (or otherwise leave the study) at any time, blood samples at study end were not collected at identical time points for all cats.

Cats were weighed at baseline and then once weekly. Any cat in which the body weight decreased from the value recorded at baseline by ≥ 10% was removed from the study and treated in accordance with shelter protocols, regardless of the apparent cause of the weight loss.
In addition to loss of body weight, cats were removed from the study if they were adopted, euthanatized, or inadvertently fed anything but the assigned diet except while in the hospital. All data collected prior to removal of cats from the study were included in the analyses. Personnel making decisions regarding euthanasia, adoption, and movement of cats within the hospital were all unaware of which diet was fed.

**Microbiologic assessment**—For isolation of FHV-1 and FCV, Crandell-Rees feline kidney cells were grown to confluence in 24-well disposable tissue culture plates. Each well contained 1 mL of medium (1 part Leibowitz-15 medium and 1 part Eagle minimum essential medium with 10% heat-inactivated fetal bovine serum, penicillin-streptomycin [100 U/mL], and 10% fetal bovine serum). A 100-µL aliquot of the viral transport medium in which each oropharyngeal and conjunctival swab specimen was suspended was added to the culture medium and swirled for 20 minutes. Cultures were maintained at 38°C in 5% CO₂ in air. One investigator (MJB) examined the cultures each morning for evidence of a cytopathic effect typical of FHV or FCV. Samples that yielded no cytopathic effect within 5 days after culture preparation were considered negative.

For all PCR assays, nucleic acids were extracted from the swab specimens suspended in lysis buffer by use of an automated nucleic acid purification system according to the manufacturer's protocol, with an elution volume of 200 µL. For detection of *C. felis*, a conventional PCR assay was performed in accordance with a published protocol. For detection of FHV-1, a PCR assay was performed as described, except that 15 µL of extract was used for all analyses. The success of DNA extraction was assessed by use of a conventional PCR assay that detected feline glyceraldehyde dehydrogenase RNA, as described. The positive control sample for the FHV-1 PCR assay consisted of a commercial vaccine containing *C. felis*. Negative control samples for conventional PCR assays included water in place of extract.

To obtain cDNA from extracted FCV RNA, 20 µL of extracted nucleic acid sample was first treated with 10 units of DNase 1 at 37°C for 15 minutes, followed by inactivation of the enzyme at 85°C for 5 minutes. Complementary DNA was then synthesized by adding 100 units of reverse transcriptase, 600 ng of random hexamer primers, 10 units of RNase inhibitor, and 1 mM deoxynucleoside triphosphates in a final volume of 40 µL and incubated at 50°C for 2 hours. Afterward, sample volume was adjusted with water to 100 µL. Feline calcivirus cDNA was subjected to a real-time PCR assay that involved SYBR green techniques. A software package was used to design PCR primers based on a homologous region within the GenBank sequences M86379, L40021, AF109465, U13992, M32296, and D31836 of FCV. The SYBR green PCR mixture contained 2 µM of each primer, a 2X concentration of a commercial SYBR green PCR mastermix, and 5 µL of the diluted cDNA sample for a final volume of 12 µL, from which DNA was amplified in an automated fluorometer by use of the manufacturer's standard amplification conditions: 2 minutes at 50°C, 10 minutes at 95°C, 40 cycles of 15 seconds at 95°C, and 60 seconds at 60°C, followed immediately by the generation of a dissociation curve to confirm amplification specificity by raising the temperature from 60°C to 95°C. Serial dilutions of virus stock were used to determine the detection limit of this assay, which was 20 plaque-forming units/mL. The intra-assay and interassay coefficients of variation were 0.9% and 1.0%, respectively. The assay was able to detect 26 of 27 field isolates of FCV, whereas no product was detected when isolates of FHV-1, FeLV, feline coronavirus, feline panleukopenia virus, canine parvovirus type 2, canine herpesvirus, canine distemper virus, equine influenza virus A, and canine adenovirus type 2 were assessed. The positive control sample for the FCV reverse transcription PCR assay included RNA extracted from a commercially available feline vaccine. The negative control sample for the assay included water in place of extract. The success of the reverse transcription reaction was evaluated by use of a PCR assay to amplify feline glyceraldehyde phosphate dehydrogenase RNA, as described. All personnel performing microbiological assays were unaware of which diet cats received.

**Statistical analysis**—The following nonparametric tests were used to make bivariate comparisons between continuous or categorical variables: Mann-Whitney test to compare groups with respect to baseline clinical disease scores and plasma amino acid concentrations at baseline and week 4, Wilcoxon signed-rank test to compare amino acid concentrations over time within groups, and χ² test of homogeneity to compare sex and source distributions between dietary groups. For the comparison involving source, only cats with an identified source were included and the number of cats surrendered or returned by their owners was compared with the number of stray and feral cats within each dietary group. Comparison of ages between dietary groups was not possible because age was estimated.

Several longitudinal outcomes were also evaluated. For healthy cats (clinical disease score ≤ 1 at admission), the interval from start of diet feeding until development of clinical disease score of 2 to 5 (mild disease) and interval until score of > 5 (moderate to severe disease) were assessed, whereas for cats with a clinical disease score ≥ 2 at admission, the interval from beginning the diet until recovery (clinical score ≤ 1) was assessed. For all cats that were hospitalized, the interval from admission to the shelter until hospitalization was assessed. Cox proportional hazards regression models were constructed for both outcomes to evaluate the effects, if any, of diet while controlling for sex. Interactions between diet and sex were also evaluated. Results are reported as HRR and 95% CI.

The potential effects of diet (while controlling for sex) on the presence or absence of microbiological evidence of infection with any of the 3 pathogens at specific times after infection were evaluated by means of logistic regression. Interactions between diet and sex were also assessed. Results are reported as OR and 95% CI. Duration of hospitalization was compared between groups with the Student t test. Descriptive statistics are
reported as mean ± SD or median and IQR. For all analyses, values of P ≤ 0.05 were considered significant.

Results

Animals—During the study period, 1,293 cats that entered the shelter were identified as potential participants. However, only 261 (20.2%) cats met all study inclusion criteria. Of these cats, 200 (76.6%) were surrendered to the shelter by their owners (170 surrendered for the first time and 30 returned) and 55 (21.1%) were stray or feral. The source of 6 cats (3 from each dietary group) was not known. One hundred twenty-eight cats (60 female and 68 male; 95 owner-surrendered and 30 stray) were enrolled into the control group that received the basal diet, and 133 cats (81 female and 52 male; 105 owner-surrendered and 25 stray) were enrolled into the treatment group that received the same basal diet supplemented with lysine.

Sixty-seven cats from the control group and 71 cats from the treatment group remained in the study for < 1 week. By the end of week 2, an additional 26 cats from the control group and 23 cats from the treatment group had exited the study. An additional 7 cats from the control group and 8 cats from the treatment group exited by the end of week 3. By the end of week 4, an additional 6 cats from each group had exited. Therefore, there were 123, 74, 59, and 47 cats still in the study at the end of weeks 1, 2, 3, and 4, respectively. The 5 most common reasons for exiting the study were similar for both groups: adoption (28 control and 34 treatment cats), euthanasia (30 for both groups), weight loss (22 control and 15 treatment cats), accidental placement on regular shelter cat food (14 control and 13 treatment cats), and owner reclamation (2 control and 6 treatment cats). Less common reasons for exiting the study (affecting < 3 cats/group) included transfer of the cat to another shelter, failure to collect swab specimens from the cat as described, discovery that the cat did not meet all study inclusion criteria, and inability to locate the cat within the shelter.

At the beginning of the study, sex distribution differed between the control and treatment groups (P = 0.03). However, no difference in sex distribution was evident at the end of any of the following 3 weeks (P = 0.23 to 1.0). Distribution by source did not differ between the 2 dietary groups at any time point assessed during the study (P = 0.66 to 1.0). The distribution of baseline clinical disease scores for all 261 cats entering the study did not differ significantly (P = 0.38) between cats in the control (median; 1; IQR, 0 to 1) and treatment (median 1; IQR, 0 to 1) groups. There were also no significant differences between groups with respect to plasma arginine (P = 0.21) and lysine (P = 0.54) concentrations at baseline (Table 1).

Plasma amino acid concentrations—Analysis of results of plasma amino acid assays included data for only the 47 cats (22 control and 25 treatment cats) from which blood samples were collected at the beginning and within the final 8 days of the study. A final sample was obtained from 11 cats on day 21 (3 control and 8 treatment cats), 2 control cats on day 23, 1 control cat on day 24, 5 cats on day 25 (2 control and 3 treatment cats), and 28 cats on day 28 (13 control and 15 treatment cats). Of these, 3 control cats (1 each on days 21, 23, and 25) and 6 treatment cats (1 each on days 21 and 25 and 4 on day 28) were in the hospital and allowed to consume a commercial diet in addition to the study diet at the time the blood sample was collected. Within the control group, the median plasma lysine concentration did not vary significantly (P = 0.09) between samples collected at the beginning and end of the study (Table 1). However, within the treatment group, the median plasma lysine concentration was significantly (P < 0.001) greater in samples collected at the end of the study (246.7 nmol/mL) than in those collected at baseline (129.9 nmol/mL). There were no significant differences between baseline and study-end plasma arginine concentrations within the control group (P = 0.45) or the treatment group (P = 0.21). Median plasma arginine concentration did not differ significantly between dietary groups at baseline (P = 0.21) or study end (P = 0.44). However, at study end, the median plasma lysine concentration of treatment cats (246.7 nmol/mL) was significantly (P < 0.001) greater than that of control cats (104.0 nmol/mL).

Clinical disease scores—Of the 261 cats in the study, 231 (88.5%) had an initial clinical disease score of ≤ 1 and were considered healthy. Healthy cats in the treatment group were no more likely than healthy cats in the control group to develop mild disease (clinical disease score < 5) during the study (HRR, 0.87; 95% CI, 0.54 to 1.40; P = 0.56) nor was any effect of sex detected (HRR, 0.75; 95% CI, 0.47 to 1.21; P = 0.24). By contrast, healthy cats in the treatment group appeared more likely than healthy cats in the control group to develop moderate to severe disease (clinical disease score ≥ 5), and this difference approached significance (HRR, 8.24; 95% CI, 0.98 to 68.94; P = 0.052), with no evidence of a sex

Table 1—Median (IQR) plasma amino acid concentrations (nmol/mL) of lysine and arginine in shelter cats fed a diet containing 1.7% lysine (control group; n = 22) or 5.7% lysine (treatment group; 25).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>Treatment group</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline lysine concentration</td>
<td>140.6 (102.8–163.9)</td>
<td>129.9 (91.2–159.9)</td>
<td>0.54</td>
</tr>
<tr>
<td>Baseline arginine concentration</td>
<td>122.9 (100.1–136.8)</td>
<td>112.0 (101.4–126.1)</td>
<td>0.21</td>
</tr>
<tr>
<td>Week 4 lysine concentration</td>
<td>104.9 (74.4–126.0)</td>
<td>246.7 (152.4–307.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Week 4 arginine concentration</td>
<td>109.9 (93.1–126.0)</td>
<td>106.7 (87.1–123.2)</td>
<td>0.44</td>
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</table>

* A value of P < 0.05 was considered significant for differences in concentrations between the control and treatment groups.

Only cats for which data were available at the beginning (baseline) and end (week 4) of the study are included.
Table 2—Number of shelter cats fed a diet containing 1.7% lysine (control group) or 5.7% lysine (treatment group) in which Chlamydophila felis, FCV, or FHV-1 was detected by use of a PCR assay (C felis and FHV-1 DNA), a reverse transcription PCR (RT-PCR) assay (FCV RNA), or VI (FHV-1 and FCV) at various points during the dietary trial.

<table>
<thead>
<tr>
<th>Pathogen (assay)</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
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<tr>
<td></td>
<td>Control group</td>
<td>Treatment group</td>
<td>Control group</td>
<td>Treatment group</td>
</tr>
<tr>
<td>C felis (PCR)</td>
<td>1 (35)</td>
<td>0 (38)</td>
<td>1 (35)</td>
<td>0 (38)</td>
</tr>
<tr>
<td>FCV (RT-PCR)</td>
<td>6 (35)</td>
<td>3 (38)</td>
<td>4 (35)</td>
<td>5 (38)</td>
</tr>
<tr>
<td>FCV (VI)</td>
<td>7 (35)</td>
<td>3 (39)</td>
<td>1 (34)</td>
<td>2 (38)</td>
</tr>
<tr>
<td>FHV-1 (PCR)</td>
<td>7 (35)</td>
<td>2 (39)</td>
<td>20 (35)</td>
<td>31 (39)</td>
</tr>
<tr>
<td>FHV-1 (VI)</td>
<td>0 (35)*</td>
<td>0 (39)</td>
<td>3 (34)</td>
<td>6 (38)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate total number of cats tested. Differences between dietary groups were not significant (P > 0.05), except where indicated (1; P = 0.04).

Discussion
In the study reported here, we assessed the effect of a diet containing 5.7% lysine on clinical signs of IURD and oropharyngeal and conjunctival presence of nucleic acids of 3 pathogens commonly involved in IURD in cats: FCV-1, FCV, and C felis. Ingestion of the lysine-supplemented diet was associated with an increase in plasma lysine concentration by the fourth week of diet consumption. Despite this, more cats on the lysine-supplemented diet had moderate to severe signs of IURD during week 4 than did cats on the basal diet. Additionally, during week 2, FHV-1 DNA was more commonly detected in swab specimens from the conjunctival and oropharyngeal mucosa of cats that consumed the lysine-supplemented diet than from cats that consumed the unsupplemented diet. These findings suggested that dietary lysine supplementation in the amount used in our study was not a successful means of controlling IURD within a cat shelter and may have even increased clinical signs of disease and presence of FHV-1 DNA on oropharyngeal or conjunctival mucosa.

It is important to assess the likely clinical importance of the findings for which statistical significance was detected. With respect to differences in clinical disease scores during week 4, all cats in the control group except for 1 cat identified as an outlier had a clinical score of 0. By contrast, examination of the IQR for the treatment group revealed that approximately 25% of cats in that group had a score ≥ 5 during week 4. As an example, a score of 5 could be assigned to a cat that was sneezing and had unilateral mild blepharospasm and marked conjunctival hyperemia. When assessing the clinical importance of the data reported here, it should also be mentioned that significant differences were not detected when a clinical disease score cut point of 2 was used during week 4 or for clinical comparisons made between groups during any week other than week 4 by use of either cut point. A significant difference between groups with respect to detection of herpetic DNA was evident during week 2 only. At that time, the odds of vi-
Regardless of clinical importance, our findings are consistent with data from 2 studies in which the effect of lysine supplementation within cat populations with enzootic IURD was evaluated. In 1 study, 144 cats within a shelter received a daily dose of lysine (250 or 500 mg, depending on their age) in a small amount of canned food for the duration of their stay at the shelter. There was no significant difference in incidence of IURD, need for antimicrobial treatment for IURD, or interval from admission to onset of IURD between cats that received the lysine and those that did not. In the other study, FHV-1 was detected more commonly in swab specimens from the conjunctival fornix of cats fed a diet containing 5.1% lysine than in cats that were fed a basal diet containing 1.1% lysine. The cats that received the lysine-supplemented diet also had significantly higher disease scores, compared with cats that received the basal diet. However, those findings were attributed to a subset of male cats that received the lysine-supplemented diet but exhibited fighting behavior that likely contributed to additional stress within that group. In the present study, no sex-specific effects of dietary lysine supplementation on disease score or detection of FHV-1 DNA were evident. Several other important differences existed between the latter study and the one reported here. In the previous study, all cats were domestic shorthair cats bred within a closed colony; healthy when admitted to the study; on a uniform, stable, and adequate plane of nutrition before the test diet was administered; housed as a group; and simultaneously exposed to a major stressor (rehousing). Additionally, cats in the previous study were assessed for presence of only 2 organisms (FHV-1 in all cats and C felis in some cats) at only 1 anatomic site (the conjunctival fornix). By contrast, the present study involved cats within a shelter and was, therefore, prone to more inherent variability. However, we used a stratified approach to group assignment, and there were no differences between dietary groups with respect to attrition rate, reason for attrition, and number of cats completing the study. Consequently, the design of the present study was sufficiently robust to ensure that group composition and management were uniform and that diet fed was the major variable throughout the study. Despite attempts to control all variables other than the lysine content of the diet fed, at least 2 potential confounding issues existed in the present study. First, it is possible that some cats inadvertently received the incorrect diet. Despite the fact that large signs indicating dietary group and bags of the appropriate food were hung on the door of cat cages, and although cats were individually caged, there were rare instances in which cats were discovered to have received the incorrect diet. These cats were removed from the study. However, because an increase in median plasma lysine concentration was detected at study end in cats that received the supplemented diet but not in cats fed the basal diet, it appears unlikely that provision of the incorrect diet affected measured outcomes. Second, cats from both dietary groups admitted to the hospital were offered commercial food in addition to the test diets. Although similar numbers of cats from each dietary group were hospitalized, cats received various commercial canned foods in addition to their assigned diet while hospitalized. This dietary change likely had no impact on cats fed the basal diet. Although the lysine content of the commercial cat foods used within the hospital was not analyzed, the Association of American Feed Control Officials recommends a minimal lysine content to support adult maintenance, growth, and reproduction (8.3 to 12 g of lysine/kg of food dry matter). Therefore, the lysine content of any commercial food that cats consumed while in the hospital was likely more similar to that of the basal diet than the lysine-supplemented diet. However, assuming that cats assigned to the lysine-supplemented group also consumed these commercial canned diets in addition to the supplemented test diet while hospitalized, their lysine intake was likely not as high as when they were consuming the lysine-supplemented diet only. Whereas this may have contributed to the failure of the lysine-supplemented diet to produce a detectable reduction in IURD in the present study, it would not have affected the rate at which cats were admitted to the hospital or any data collected prior to their admission.

The association of dietary lysine supplementation with increased IURD severity and the detection of FHV-1 DNA in this and a previous study was intriguing given the success of once- or twice-daily bolus administration of lysine for control of conjunctivitis in cats undergoing experimental primary FHV-1 infection and reduction of FHV-1 DNA detection at the ocular surface of cats latently infected with FHV-1. These discrepancies may represent a difference in disease syndromes between experimentally infected, purpose-bred cats and naturally infected, heterogeneous populations of cats, as suggested by the lack of effect of bolus lysine administration in a shelter environment. Alternatively, the discrepancies may reflect a difference in the manner in which dietary lysine supplementation and bolus lysine administration increase the plasma lysine concentration in cats. Median plasma lysine concentration in the treated cats in the present study (247 nmol/mL) was somewhat lower than mean values reported in 2 studies in which pathogen or pathogen DNA detection or clinical disease was successfully controlled by bolus lysine administration (309 nmol/mL and 669 nmol/mL, respectively). By contrast, in another study in which dietary lysine supplementation failed to control IURD, mean plasma lysine concentration in cats fed the supplemented diet for 52 days was 164 nmol/mL at study end. This suggests that bolus administration of 400 or 500 mg of lysine may increase the plasma lysine concentration to a greater degree than does the feeding of diets containing 5.1% or 5.7% lysine. It should also be considered that 6 of 24 cats fed the lysine-supplemented diet in the pres-
ent study had blood collected for week 4 plasma amino acid analysis while they were hospitalized. Consequently, the lower mean plasma concentration in those cats versus cats in other studies may have been attributable to consumption of canned commercial food with lower lysine content during hospitalization.

Whereas discrepancies among study results may be attributable to study design, data from other studies indirectly suggest that plasma lysine concentration is highly labile and that comparisons of plasma lysine concentrations among studies may not be feasible. For example, the mean plasma lysine concentration of blood samples collected from cats 1 day after a 21-day course of twice-daily oral boluses of lysine (500 mg/bolus), which had resulted in a significant increase in plasma lysine concentration earlier in the study, was no longer significantly different from the initial value. In another study in cats, blood samples collected 3 and 24 hours after oral administration of a bolus of lysine (400 mg) yielded mean plasma lysine concentrations of 309 and 200 nmol/mL, respectively. The plasma lysine concentration in the 3-hour sample was significantly higher in lysine-treated cats than in untreated cats, whereas the concentration in the 24-hour sample was not. The same study revealed that 1 orally administered bolus of 100, 200, or 400 mg yields a peak plasma lysine concentration between 1 and 3 hours after administration. Finally, in a study in which 50 cats were fed a diet containing 5.1% lysine, the mean plasma lysine concentration was 307 nmol/mL after 17 days but decreased to 164 nmol/mL after 52 days of feeding. In the present study in which cats were fed a similar diet, a mean plasma lysine concentration of 247 nmol/mL was achieved 21 to 27 days after diet initiation. When the data from the 3 time points from these 2 studies are considered together, it appears possible that plasma lysine concentration decreases with time in cats fed a supplemented diet. However, given the lability of plasma lysine concentrations, accurate comparison of plasma amino acid data among studies is difficult because of the marked variation in dose, dose frequency, and sample timing for plasma lysine assessment relative to lysine administration used in each of these studies. Regardless, it remains possible that there is a critical plasma lysine concentration for control of IURD, as has been proposed for humans affected by herpes labialis caused by herpes simplex virus type 1. If so, then it is possible that bolus administration yields an increase in plasma lysine concentration that is higher than this critical concentration more reliably than does dietary supplementation.

Estimated lysine intake may be compared among studies more reliably than can comparison of plasma lysine concentrations. Because adult cats eating a commercial, dry, expanded diet ingest approximately 1 to 3 g of lysine/d, it is likely that cats receiving a 500-mg bolus of lysine twice a day while simultaneously eating to meet their energy needs consume approximately 2 to 4 g of lysine/d. Cats consuming diets containing 36 or 61 g of lysine/kg of diet reportedly have a mean lysine intake of 2.8 or 4.1 g/d, respectively. Therefore, the diet supplemented with 57 g of lysine/kg of diet in the present study approximated the amount of lysine that a cat eating a commercial diet and receiving a bolus of 500 mg of lysine twice daily would consume. However, dietary lysine supplementation presumably relies upon cats consistently consuming sufficient food each day to increase the plasma lysine concentration sufficiently to achieve a clinical effect. Cats can reduce their food and therefore their lysine intake when ill. Some cats in the present study may have had transient anorexia, but this cannot be confirmed because food intake was not measured and body weight was measured only once a week. One of the major advantages of bolus lysine administration is the ability to continue lysine administration during a period of anorexia. However, bolus administration of lysine also has several disadvantages, such as time and costs associated with administration, along with potential induction of stress and transmission of disease among animals by personnel administering the lysine. This may explain the finding that bolus administration of 230 or 500 mg of lysine once a day in shelter cats failed to reduce signs of IURD in those cats.

The mechanism by which lysine limits viral replication is not fully understood; however, results of in vitro studies suggest that this effect is exerted via antagonism of arginine, and some human clinical trials have involved reduction of arginine intake. This raises concern regarding lysine administration in cats because cats are particularly sensitive to arginine deficiency. Consequently, arginine intake was not restricted in the present or any other studies in which additional lysine was provided. Although arginine intake was not restricted in a study in which cats received a diet containing 5.1% lysine, there was a decrease in mean plasma arginine concentrations in control and treated cats by day 17 of diet administration, and concentrations continued to decrease until the study ended (day 52). By contrast, in the present study, median plasma arginine concentration did not differ between baseline and study end for either dietary group or between dietary groups at baseline or study end. Although the lysine content of the diet used in the present (5.7%) and previous (5.1%) studies were similar, the arginine content of the diet in the present study (1.9%) was slightly higher than that in the other study (1.1%). This higher arginine content may have contributed to the higher median plasma arginine concentrations reported here. Additionally, the diets used in the present and the previous studies were produced by different manufacturers and contained different ingredients. It is possible that some of the potential effects of diet on plasma arginine concentration such as a reduction in protein digestibility and consequent increases in ammonia production and arginine utilization in the urea cycle that were proposed for the other study were not relevant to the present study. It is also possible that a decrease in plasma arginine concentration may have been detected if the present study had continued for more than 28 days.

Arginine is apparently critical for healthy immune function. For example, T lymphocytes depend on arginine for proliferation, expression of T-cell receptor complexes, and development of immunologic memory. Also, arginine is the sole amino acid substrate for the production of nitric oxide. However, inflammatory stimuli induce expression of a specific isoform of nitric oxide synthetase and arginase I, both of which reduce
the amount of circulating arginine and thereby reduce the immune response. Therefore, if lysine administration was associated with reduced plasma arginine concentration, then this might explain the paradoxical increase in severity of IURD and detection of FHV-1 DNA in cats that received a lysine-supplemented versus unsupplemented diet in another study. In the present study, cats that consumed the lysine-supplemented diet did not have a significant reduction in median plasma arginine concentration by week 4, and yet they had an increase in clinical disease severity at week 4 and in FHV-1 DNA detection at week 2. Although cats fed an unsupplemented diet in the other study had a decrease in mean plasma arginine concentration, they did not have an increase in clinical disease severity. Consequently, there may be no correlation between plasma arginine concentration and clinical disease severity.

A secondary goal of the present study was to determine whether lysine supplementation had an effect on the detection of ocular and respiratory pathogens other than FHV-1 in shelter cats. During week 2 of the study, FHV-1 DNA was detected less commonly in cats that received the lysine-supplemented diet rather than the unsupplemented diet. However, a difference in detection of FCV or C felis nucleic acids could not be evaluated because of the low number of cats in which these nucleic acids were detected. Overall, regardless of the study week or diet received, nucleic acids of FHV-1 were detected in 5% to 80% of cats, those of FCV were detected in 8% to 29% of cats, and those of C felis were detected in 0% to 3% of cats. These findings are similar to those reported for cats in other shelters and confirm that FHV-1 is the most commonly encountered respiratory and ocular pathogen in shelters, whereas C felis is uncommonly detected. Our data also confirmed that the probability of detecting FHV-1 DNA increases with time following admission to a shelter; FHV-1 DNA was detected in 12% of cats on admission to the shelter and in 69% of cats 2 weeks later. This finding supported the hypothesis that cats undergo herpetic reactivation and begin viral shedding within approximately 1 week after entering a shelter.

Because VI yielded so few positive test results, differences in pathogen detection rates between dietary groups could only be evaluated by use of PCR assay results. Comparison of the 2 methods was not the focus of the present study; however, several possible explanations for the discrepancies in results exist. Cross-contamination may have resulted in false-positive results of the PCR assay, although this is unlikely because false-positive results were not detected when negative control samples were evaluated. Additionally, results of PCR assays for herpesviral DNA may not always reflect shedding of viable, infectious virus by test subjects. The greater fragility of FHV-1 relative to FCV may explain the greater difference between the results of the 2 detection methods for FHV-1, compared with the difference between results for FCV in the present study. Regardless of the cause of the discrepancies, results of our study confirmed that PCR assays are more sensitive than culture for the detection of herpesviruses.

Results of the present study suggested that cats fed the lysine-supplemented diet ate sufficient quantities and absorbed sufficient lysine to significantly increase their plasma lysine concentration to values similar to but somewhat lower than those yielded by bolus lysine administration. Despite the increase in plasma lysine concentration, there was no significant reduction in severity of IURD or FHV-1 DNA detection rate in the supplemented cats. Rather, at certain points throughout the 4-week study, more cats fed the lysine-supplemented diet had moderate to severe illness and FHV-1 DNA detected in swab specimens collected from their oropharyngeal and conjunctival mucosa than did cats fed the unsupplemented basal diet. When these results are considered together with data from 2 other studies, in which lysine supplementation was provided to cats in IURD-endemic populations, the data suggest that neither 5% to 6% dietary lysine supplementation nor once-daily bolus administration of 250 or 500 mg of lysine in cats within a shelter environment is effective in reducing severity of clinical signs of IURD or the probability of FHV-1 DNA detection. In fact, dietary lysine supplementation may exacerbate clinical signs of IURD and detection of FHV-1 DNA at some time points in a feline shelter.

References


Appendix

Proximate composition (g/kg of diet) of experimental diets as reported by the manufacturer.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Basal diet*</th>
<th>Lysine-supplemented diet†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>335</td>
<td>351</td>
</tr>
<tr>
<td>Crude fat</td>
<td>152</td>
<td>152</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Lysine</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>Arginine</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

*The basal diet was composed of the following ingredients (g/kg of diet): corn gluten meal (219.2), ground brown rice (200), poultry by-product meal (184.3), corn flour (174.2), porcine animal fat preserved with butylated hydroxyanisole (62.1), fish meal (58.1), soybean oil (50), dicalcium phosphate (15.6), potassium chloride (8.5), sodium chloride (5.8), phosphoric acid (4.7), calcium carbonate (3.1), choline chloride (2.5), taurine (1.9), l-arginine (1.8), pyridoxine hydrochloride (1.3), menadione dimethylpyrimidinol bisulfite (1.0), m-aminobenzoate (1.0), l-tryptophan (0.7), thiamin mononitrate (0.7), vitamin A acetate (0.6), zinc sulfate (0.5), cholecalciferol (0.4), α-tocopherol acetate (0.3), magnesium sulfate (0.3), biotin (0.2), folic acid (0.2), manganese sulfate (0.2), calcium pantothenate (0.1), riboflavin (0.1), cyanocobalamin (0.1), nicotinic acid (0.1), copper sulfate (0.1), calcium iodate (0.05), and sodium selenite (0.001).†The lysine-supplemented diet was composed of the following ingredients (g/kg of diet): corn gluten meal (230.0), ground brown rice (200.0), poultry by-product meal (154.3), corn flour (141.0), porcine animal fat preserved with butylated hydroxy anisole (68.9), l-lysine acetate (56.4), soybean oil (50.0), fish meal (33.5), calcium phosphate (19.4), potassium chloride (8.5), phosphoric acid (7.5), calcium carbonate (6.6), sodium chloride (5.8), l-arginine (3.7), calcium chloride (2.9), taurine (1.3), pyridoxine hydrochloride (1.3), magnesium potassium sulfate (1.1), menadione dimethylpyrimidinol bisulfite (1.1), m-aminobenzoate (1.1), l-tryptophan (1.1), thiamin mononitrate (0.7), vitamin A acetate (0.6), zinc sulfate (0.5), cholecalciferol (0.4), α-tocopherol acetate (0.4), biotin (0.2), folic acid (0.2), manganese sulfate (0.2), calcium pantothenate (0.1), riboflavin (0.1), cyanocobalamin (0.1), nicotinic acid (0.1), copper sulfate (0.1), calcium iodate (0.05), and sodium selenite (0.001).