Skin Lesions Associated with Lysine Deficiency in Kittens are Characterized by Inflammation

Jennifer A. Larsen, DVM, PhD, DACVN

Catherine A. Outerbridge, DVM, MVSc, DACVIM, DACVD

Andrea J. Fascetti, VMD, PhD, DACVN, DACVIM

Verena K. Affolter, DVM, PhD, DEVCP

*From the Departments of Molecular Biosciences (Dr. Larsen and Dr. Fascetti),
Medicine and Epidemiology (Dr. Outerbridge), and
Pathology, Microbiology, and Immunology (Dr. Affolter),
School of Veterinary Medicine, University of California, Davis, CA 95616* .

Address correspondence to Dr. Larsen (jalarsen@vmth.ucdavis.edu).

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ABSTRACT

Processing substantially reduces the bioavailability of lysine in pet foods, and certain populations of cats may be at risk for deficiency. A previous study reported that kittens consuming lysine deficient diets developed facial skin lesions; however these were not characterized with histopathology. The hypothesis of this study was that kittens with lysine deficiency develop histopathologically distinct skin lesions. Twelve, male, 7-to-9 week old, healthy kittens were fed diets either replete (16 g/kg as fed) or deficient in lysine (4 g/kg as fed) in a prospective, controlled feeding trial for 1 week. Standard skin biopsies were examined in a blinded fashion. Plasma was analyzed for amino acid concentrations. The median average daily gain of the control and test group kittens was 37.8 g (range 23.8 to 40.3) and 0.8 g (range -12.7 to 7; $p < 0.05$), respectively. Five kittens in the test group (5/8; 62.5%) and one in the control group (1/4; 25%) developed facial lesions with dark adherent crusting near the dorsal nasal planum, chin, and/or adjacent to the philtrum.

Histopathologic examination revealed superficial and deep perivascular pleocellular dermatitis with mild acanthosis, hyperkeratosis, intra-epidermal pustules, superficial folliculitis, and furuncles. There was no difference in plasma lysine concentrations between groups ($p = 0.064$). Histopathologic characterization of facial skin lesions suspected to be associated with lysine deficiency in kittens were not consistent with other dermatological diseases, and were not associated with decreased plasma lysine concentrations.

INTRODUCTION

Lysine is an essential amino acid with a specific susceptibility to the conditions of moist heat processing, which can result in

Table 1. Composition of kitten diets replete or deficient in lysine

Ingredient (g/kg diet)	Control Diet	Test Diet
Lysine (as HCl)	16	4
Amino acid mix[a]	291.5	291.5
Chicken fat	250	250
Cornstarch	100	100
Mineral mix[b]	50	50
Vitamin mix[b]	10	10
Dextrose	254	277
Sodium acetate[c]	21	14
Choline chloride	3	3

[a]Supplied by Ajinomoto USA Inc., Teaneck, NJ (g/kg diet): arginine HCl, 24.8; methionine, 8.9; histidine HCl, 8.4; isoleucine, 11.4; leucine, 25.4; cysteine, 8.9; phenylalanine, 9.4; tyrosine, 10.4; threonine, 15.4; tryptophan, 4.4; valine, 13.4; taurine 1.5; alanine, 26.1; glycine, 26.1; glutamine, 26.1; glutamate, 11.2; asparagine, 22.4; aspartate, 14.9; proline, 22.4.
[b]Composition of mineral and vitamin mixes provided in: Williams JM, Morris JG, Rogers QR. Phenylalanine requirement of kittens and the sparing effect of tyrosine. J Nutr 1987;117:1102-7.
[c]Sodium acetate (Fisher Scientific, Santa Clara, CA) was added at equimolar concentrations to that of the hydrochlorides (arginine, histidine, and lysine) to replace an equal weight of dextrose.

decreased bioavailability relative to other amino acids.[1] In addition, collagen-rich protein sources contain cross-linked lysine that is resistant to digestive enzymes.[2-3] Further, cereal grains, which provide low concentrations of lysine, are often used to provide dietary protein for cats.

Of 20 cat foods tested for bioavailable lysine content, 45% were below the Association of American Feed Control Officials minimum allowance for growth and reproduction, while 15% were below the minimum allowance for adult maintenance.[4] The effect of processing together with the use of collagen-rich or cereal-based protein sources may result in clinical lysine deficiency if foods are not adequately supplemented. This is particularly a concern for cats consuming lower protein diets and for those with a low energy requirement.

Historically, weight loss was recognized as the sole clinical sign of lysine deficiency in kittens.[5] Adverse effects of lysine deficiency on the integument of turkeys and rats have been described; these studies report only texture differences and a lack of pigmentation, without effects on skin per se.[6-7] Crusted facial skin lesions in kittens consuming lysine deficient diets were noted in one study; however, biopsies were not performed.[8]

The objective of this study was to document the clinical appearance and histopathological features of skin lesions associated with lysine deficiency in kittens, and to compare these findings with dermatological changes seen with other disease entities.

MATERIALS AND METHODS

Twelve male 7- to 9-week old specific-pathogen-free domestic short-hair kittens were weaned onto a complete purified diet (control diet; Table 1) for a 2-week adaptation period. Kittens were housed in groups of 4, and had free access to food and water. Food intake was not measured. Their care was in compliance with the Guide for the Use and Care of Laboratory Animals and the Animal Welfare Act. The experimental protocol was approved by the University of California, Davis's Institutional Animal Care and Use Committee.

Body weights were measured daily during the adaptation and study periods. At the start of the study period (day 0), four kittens continued on the control diet, and

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Table 2. *Average daily weight gain and presence of gross dermatological lesions kittens fed diets replete or deficient in lysine for 1 week*

	Average daily gain (g)	Gross lesions present
Control		
Kitten 1	23.8	yes
Kitten 2	38.7	no
Kitten 3	40.3	no
Kitten 4	37.0	no
Test		
Kitten 5	0.7	yes
Kitten 6	6	no
Kitten 7	7	no
Kitten 8	-4.7	yes
Kitten 9	-3.8	yes
Kitten 10	-12.7	yes
Kitten 11	1.0	yes
Kitten 12	5.7	no

eight kittens were transitioned to the test diet (Table 1). All diets provided amino acids in crystalline form as the sole source of protein and in excess of requirements by a factor of approximately 2 (except the test diet which provided lysine at 25% of the concentration in the control diet; 4 and 16 g/kg as fed, respectively).[9] Analysis of the diets confirmed the amino acid concentrations (4.4 and 16.7 g/kg as fed for test and control diets respectively). Diets were isocaloric, but not isonitrogenous.

On day 7, 3 mL of venous blood was collected from each kitten following a 3-hour fast. Plasma was separated, processed, and maintained at –80°C until analysis. An automated amino acid analyzer (Biochrom 30 amino acid analyzer, Biochrom Ltd, Cambridge, England) was used to measure plasma amino acid concentrations.

Also on day 7, buprenorphine (0.03 mg/kg) was administered via the oral mucosa 1 hour prior to biopsy. Kittens were anesthetized with intravenous injection of ketamine (5 mg/kg) and diazepam (0.25 mg/kg). Biopsy samples were collected with a 4 mm punch at two sites from each kitten (rostral muzzle on either side of philtrum and/or chin). Fixed samples were embedded in paraffin, and slices of each sample were stained and prepared for histopathologic examination. The pathologist (VKA) was blinded to dietary treatment.

Data Analysis

Spreadsheet software was used to calculate descriptive statistics (Microsoft Excel, Redmond, WA, USA). Student's t-test was performed to identify statistically significant differences in average daily gain (ADG) and lysine plasma amino acid concentrations between the test and control groups, with p set at 0.05. Data are reported as median (range).

RESULTS

Median body weight of the kittens at the beginning of the study was 1,343 g (1,150 to 1,556). The median ADG of the control and test group kittens was 37.8 g (23.8 to 40.3), and 0.8 g (-12.7 to 7; $p < 0.05$), respectively. Individual ADG values for each kitten are given in Table 2.

Three control kittens (3/4; 75%) had no gross skin lesions. One control kitten (1/4; 25%) had small focal areas of mild, dark

Figure 1. Very mild crusting on the skin of a kitten fed a purified diet replete in lysine for one week. This kitten showed the lowest growth rate in the control group and was the only one to develop lesions.

adherent crusting adjacent to the philtrum on the rostral muzzle and on the chin near the mucocutaneous junction (Figure 1). This kitten had the lowest ADG in the control group, which was 61.5% of the mean ADG of the other 3 control kittens.

Three test kittens (3/8; 37.5%) had no gross skin lesions; these all had the highest ADG in the test group (Table 2). The five test kittens (5/8; 62.5%) with skin lesions had focal areas of dark adherent crusting adjacent to the philtrum, on the dorsal muzzle above the nasal planum, or on the rostral chin (Figure 2). One affected test kitten had a pigmented macule on the chin with no evidence of crusting. All affected kittens had noticeable lesions by day 3.The lesions progressed with slight increases in size and amount of crusting until biopsies were taken on day 7. Unaffected kittens showed no lesions at any time during the study.

The biopsy samples of 5/8 test group kittens were characterized by mild to moderate superficial and deep perivascular lymphocytic, mastocytic, and histiocytic dermatitis. The epidermis was slightly acanthotic, and there was mild to moderate compact or parakeratotic hyperkeratosis. Further findings included exocytosis of neutrophils (2/5 kittens), intraepidermal small pustules (2/5 kittens), crusts (3/5 kittens), luminal folliculitis (4/5 kittens), a furuncle (1/5 kittens), and hydradenitis (3/5 kittens). Cocci were observed on the surface of 3/5 cats, and in an intraepidermal pustule in 1/5 kittens.

Biopsies of the control kitten with mild gross lesions revealed crusting, mild epidermal acanthosis and exocytosis, intraepidermal pustules, luminal folliculitis intra- infundibular bacteria, and a furuncle. Solitary furuncles (2/4 kittens) and hydradenitis (1/4 kittens) were seen in biopsy samples of control kittens that clinically appeared normal.

Plasma lysine concentrations were 75.6 (47.1 to 93.5) and 94.2 (73.7 to 112.8) nmol/mL for kittens in the test and control groups, respectively (reference value $108 + 6$ nmol/mL; $p = 0.064$). Concentrations of other plasma amino acids remained normal and did not differ between groups.

Following uneventful recovery from anesthesia, kittens were offered the standard colony dry extruded diet. By day 7 after the end of the study, growth rates normalized for all kittens, with ADG of 32.9 g (30.6 to 60.3) and 40.7 g (27.6 to 42.7; $p = 0.74$) for test and control groups, respectively. Lesions on all affected kittens resolved within 5-7 days.

Figure 2. Moderate crusting on the skin of a kitten fed a purified diet deficient in lysine for one week.

DISCUSSION

Dermatologic manifestations of dietary deficiencies are well described for several nutrients in dogs and cats, including linoleic acid, vitamin A, zinc, niacin, and several amino acids. Effects of amino acid deficiencies are less well described, with the exception of foot pad and perioral skin lesions induced by methionine deficiency in kittens.[10] Twenty-three days of consumption of a methionine deficient diet resulted in crusted, erosive lesions at the commissure of the mouth at day. 15 and on the footpads within 23 days.[10] Histopathologic features were compatible with a keratinization defect, in addition to evidence of bacterial infection (parakeratosis, acanthosis, neutrophilic exocytosis, and furunculosis). Lesions resolved within 7 days of consumption of a diet providing adequate methionine.[10]

Similar plasma lysine concentrations in the test and control groups in the present study may reflect the high mean growth rate of the control group compared to that reported in previous studies using similar diets (35 vs. 24-26 g/d).[5,8] Circulating essential amino acids used for tissue accretion during the period of rapid growth may result in decreased plasma concentrations in the post-prandial period. Further, although the plasma concentrations of a limiting dietary amino acid typically decrease, consumption of half of the required amount has been reported to result in normal growth and intermediate plasma concentrations.[5] As such, it is possible that the difference in the dietary lysine concentrations were not large enough to result in statistically significant changes in plasma lysine concentrations despite clear effects on ADG as well as potentially skin lesion development.

Facial skin lesions can be associated with feline herpes virus (FHV),[11] and these infections are sometimes treated with pharmacological doses of oral lysine that may reduce viral shedding and the severity of conjunctivitis.[12-13] The lesions of FHV are focal vesicular, crusted, and ulcerated areas that typically develop on the dorsal muzzle, an anatomic location close to where lesions developed in some kittens in the present study. However, he facility used in this study was FHV-free. In addition, the clinical appearance of the lesions differ. Also, histopathologically, the lesions of FHV are necrotizing, with severe, often eosinophilic, inflammation, which is in contrast to our findings.[11] Thus, it seems unlikely that lysine deficiency plays a role in the development of skin lesions associated with FHV.

The skin lesions observed in 5/8 test group kittens cannot be directly correlated to low plasma lysine concentrations. Daily food intake was not measured, and absolute intake of lysine could not be calculated. This may have enabled a dose response curve for development of skin lesions. This relationship might have demonstrated that low lysine intake was associated with the development of skin lesions in the control kitten, although the documented poor growth also supports this hypothesis. However, this would not clarify the lack of lesion development in 3/8 test group kittens that remains unexplained. However, interestingly, these three kittens had higher ADG compared to the other test group kittens.

Alternatively, nutritional stress secondary to deficiency may predispose to other disease mechanisms that impact the superficial skin barrier and are manifested clinically as dermatitis with bacterial infection. Moreover, insufficient diet may result in decreased general well being of the kittens, and hence, less careful self-grooming. Conversely, it is also possible that mechanical irritation to the hair follicles resulted from the grooming of sticky diet with the consistency of cookie dough. This effect may have also been exacerbated by lysine deficiency.

Since lysine is an essential amino acid likely to be limiting in some feline diets, certain cat populations may be at risk for lysine deficiency. This study provides clinical descriptions and histopathological features of lesions suspected to be associated with lysine deficiency. Kitten numbers as well as the constraint on length of the feeding trial

due to effects of lysine deficiency limited this study. Measuring food intake as well as lysine concentrations in the skin may have helped to further support a direct relationship between lysine deficiency and the development of skin lesions.

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