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Anti-Fel d1 immunoglobulin Y antibody-containing egg ingredient lowers allergen levels in cat saliva

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Abstract

Objectives Fel d1 is the major cat allergen, causing IgE reactions in up to 90% of cat-allergic adults. Fel d1 secreted in saliva is spread to the haircoat during grooming. Current management includes attempts to reduce or eliminate exposure to Fel d1. A novel approach to reducing immunologically active Fel d1 (aFel d1) exposure, which involves binding the Fel d1 with an anti-Fel d1-specific polyclonal egg IgY antibody (sIgY), was evaluated. The hypothesis was that saliva from cats fed diets containing this sIgY would show a significant reduction in aFel d1.

Methods Two trials in cats were completed. In trial 1, saliva was collected 0, 1, 3 and 5 h post-feeding during a 2 week baseline and subsequent 6 week treatment period. Trial 2 included a control and treatment group, and saliva was collected once daily. Trial 2 cats were fed the control diet during a 1 week baseline period, and then fed either control or sIgY diet during the 4 week treatment period. Fel d1-specific ELISA was used to measure salivary aFel d1. Data were analysed using repeated-measures ANOVA and a linear mixed-model analysis.

Results Salivary aFel d1 decreased post-treatment in both trials. There were no differences in aFel d1 based on time of collection relative to feeding in trial 1. In trial 2, 82% of treatment group cats showed a decrease in aFel d1 of at least 20% from baseline vs just 38% of control cats. Only one (9%) treatment cat showed an increase in aFel d1 vs 63% of control cats.

Conclusions and relevance Feeding sIgY significantly reduced aFel d1 in the saliva of cats within 3 weeks. Although additional research is needed, these findings show promise for an alternative approach to the management of allergies to cats.

Keywords: Human allergy; Fel d1; diet; chicken IgY; allergen; allergies to cats

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Introduction

Human allergy to cats is a significant public health issue. These allergies, which typically cause nasal and ocular symptoms, are among the more common IgE-mediated allergic diseases in humans.[1] The prevalence of cat allergies varies by geography; however, based on skin-prick testing, allergies to cats range between 16.8% and 49.3%, with an average of 26.3% of people testing positive for cat allergens in Europe.[2] Data from the USA suggest similar prevalence rates, based on ELISA testing in subjects with self-reported allergies.[3] This problem and any possible resolutions should be of concern to veterinarians and other pet professionals because allergy to cats is a commonly declared reason for cats to be surrendered to a shelter.[4,5]

While cats produce several potential allergens, Fel d1 is the major allergen, causing IgE reactions in sera from 83.7% of children and 88–95% of adults with allergies to cats.[2,6–9] Approximately 90% of cat-allergic individuals have IgE directed against Fel d1, and 60–88% of all IgE produced in response to cat dander is specific to Fel d1.[1,7,10]

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Fel d1 is a small glycoprotein, approximately 35–39 kDa in size, produced primarily by the salivary and sebaceous glands of cats, with high concentrations found in saliva.[1] As cats groom, Fel d1 in the saliva is distributed within the haircoat and can then be shed with hair and dander. Prior research by our group showed a strong correlation between Fel d1 levels in saliva and on the hair (B Bastien, unpublished observations). In addition, its small size and structure allows Fel d1 to be easily and continuously airborne for long periods of time, making it one of the easiest allergens to inhale.[11,12] Its molecular structure also allows it to adhere to fabrics, carpet and upholstered furniture.[10,12–15] These factors can make it difficult to remove Fel d1 from homes, and allow it to travel on clothing and other items from cat-owning households to places where no cat is present. Fel d1 can be found in homes and buildings without cats, especially in communities where cat owners live.[16]

Treatments for IgE-mediated allergies in humans include the use of nasal decongestants, antihistamines and other medical options. As with all allergies, simple avoidance of the allergen is the most effective means of preventing symptoms. However, as noted above, the characteristics of Fel d1 make it difficult to avoid and nearly impossible to eliminate from the environment, and many cat-allergic cat owners find removal of a pet cat to be unacceptable.[17]

Another approach to reducing allergic symptoms is to block Fel d1's binding sites (epitopes), leaving it unable to bind with IgE and therefore unable to trigger an allergic response. This is one of the many mechanisms involved in allergen-specific immunotherapy (ASIT) used to stimulate production of anti-Fel d1 IgG antibodies within the affected person; these selective IgG antibodies bind to Fel d1, preventing its binding to IgE and thus the subsequent allergic response.[18–21] While the mechanism of action of ASIT involves various processes and ASIT has been used in allergy therapy, it has a number of drawbacks, including cost, time for response and risk of treatment-associated adverse effects.[1,21] A novel, alternative approach to achieve this blocking effect is based on feeding an anti-Fel d1-specific polyclonal antibody directly to the cat.

Anti-Fel d1-specific polyclonal immunoglobulin Y (sIgY) in chicken egg yolks has been shown to neutralise the allergenic functions of Fel d1 using both in vitro and ex vivo mast cell culture models (E Satyaraj, unpublished data). Immunoglobulin Y is an avian immunoglobulin equivalent to mammalian IgG. These antibodies are naturally produced by chickens in response to antigen exposure, and transferred and concentrated into egg yolks to provide passive immunity for offspring. As a result, large quantities accumulate in chicken egg yolks, which can be extracted, purified and delivered in food. These antibodies attach to active binding sites on targeted proteins, effectively reducing their antigenicity/allergenicity. Multiple studies have proven the safety and efficacy of oral administration of chicken egg-origin antibodies for reducing diarrhoea in domestic animals.[22,23] Chickens naturally produce Fel d1-specific IgY when in a shared environment with cats (E Satyaraj, unpublished data).

For our research, chickens were inoculated with Fel d1 to induce the formation of sIgY. The sIgY was then isolated and concentrated from the eggs and incorporated into cat food. The goal of the research presented here was to test the efficacy of the sIgY for reducing salivary levels of immunologically active Fel d1 (aFel d1) when fed to cats. aFel d1 refers to Fel d1 that is capable of binding human IgE on human mast cells and eliciting an allergic response. sIgY binding to Fel d1 not only blocks its ability to bind to human IgE, but also renders it unable to bind the capture antibody in an Fel d1-specific ELISA. Prior research by the authors and others showed that Fel d 1 levels vary widely among cats;[24,25] therefore, a protocol was designed wherein each cat served as its own control before and after receiving the sIgY-supplemented food.

Two studies are reported here. First, a pilot study was conducted in order to determine the appropriate time to collect saliva relative to meal feeding, and to determine the number of weeks necessary to see a change in salivary levels of aFel d1. The results of this pilot allowed the design of a controlled trial to compare within-cat effects and to compare the sIgY-fed cats with those fed a placebo control diet.

Materials and methods

Animals and diets

The study protocols were reviewed and approved by the Institution's Animal Care and Use Committee and complied with all regulations set forth in the United States Department of Agriculture Animal Welfare Act (Animal Welfare Act is Federal law in the USA that regulates the treatment of animals in research, exhibition, transport and by dealers).[26]

All cats were individually housed in accommodation that met or exceeded the requirements set forth in the Animal Welfare Act. Rooms were maintained between 50°F and 85°F (10°C and 29°C) and were set to a 12 h light/dark cycle. Cats were individually fed to maintain body weight, with food available up to 22 h daily, and water was available ad libitum. Body weight was monitored and the amount of food provided was adjusted as needed to maintain ideal body weight.

Cats were evaluated twice daily by trained personnel to ensure their good health and wellbeing, and veterinary care was provided as needed. All cats received a veterinary physical examination prior to the start and again at the conclusion of the studies. Following completion of each study, all cats were returned to the facility's general cat population.

The control and test diets were formulated and manufactured by Nestlé Purina PetCare Company. Both extruded dry diets provided complete and balanced nutrition according to the guidelines of the Association of American Feed Control Officials,[27] and Nestlé Purina PetCare Company's standards. The control and test diets were identical except that the test diet was supplemented with a dried egg product calculated to provide approximately 8 ppm (dry matter basis) anti-Fel d1 IgY (sIgY). A single production batch of each diet was using throughout this study. The control diet was fed to all cats during the baseline periods and was fed during the test period only to the control group, while cats in the test group received the sIgY-supplemented test diet.

Experimental design

Two trials were conducted using a 'before and after treatment' study design, such that each cat served as its own control. This is important due to the high cat-to-cat variability in aFel d1.[24,25]

Trial 1 Six adult domestic shorthair cats (n = 6, five neutered males, one spayed female; average age 8.5 years [range 1–13 years]) were enrolled after screening to ensure their salivary aFel d1 fell within detectable limits of the assay (>0.8 ng/ml). All cats were fed the control diet for 2 weeks, and during this period saliva was collected four times daily: once before fresh food was offered each morning, then at 1, 3 and 5 h after food was placed. Cats continued to have access to their food throughout this time. Saliva was collected for each cat daily for five consecutive days each week for the duration of the trial. Following the baseline period, all cats were fed the sIgY diet for 6 weeks, while saliva collections were continued on the same schedule.

Trial 2 Twenty (control n = 9, four neutered males, five spayed females [average age 5.5 years; range 1–13 years]; test n = 11, 10 neutered males, one spayed female [average age 7.4 years; range 2–14 years]) adult domestic shorthair cats were enrolled after screening to ensure their salivary aFel d1 fell within detectable limits of the assay. All cats were fed the control diet for a 1 week baseline period; there followed a 4 week test period during which the control group continued to receive the control diet and the test group received the sIgY diet. Saliva samples were collected once daily for five consecutive days each week, approximately 5 h after fresh food was provided each morning throughout the study.

Sample collection and Fel d1 analysis

To collect saliva, a Salivette (Sarstedt) was placed in the cat's mouth, and the cat was allowed to chew on the Salivette for 10–15 s. It was then removed, transferred to a collection tube and stored at 4°C. The tube was spun at 1000 *g* for 2 mins, and the saliva transferred to a microtube, frozen at –20°C and kept frozen until ready for analysis. Each saliva sample was quantitatively (µg/ml) analysed for aFel d1 reactivity using an ELISA kit (6F9/3E4: Indoor Biotechnologies) according to the manufacturer's instructions. sIgY-bound Fel d1 is not able to bind the capture antibody in this ELISA and is not detected by this assay.

Data processing and statistical analysis

For trial 1, aFel d1 and change in aFel d1 were determined for each cat first by calculating the means of all samples from the baseline period, and every consecutive 2 weeks during the 6 week treatment period. Two week data were pooled for this trial owing to the small number of cats and the anticipated large day-to-day variation in salivary aFel d1.[25] One-way repeated measures ANOVA (rANOVA) was performed to evaluate the treatment effect over time, with Wilcoxon signed rank testing used to further differentiate times within the treatment period. Subsequently, the data were pooled and separated based on time (h) relative to feeding and rANOVA was performed to determine any differences based on time of sample collection. Finally, the data were reanalysed using rANOVA and just the 5 h post-feeding samples for each cat.

For trial 2, aFel d1 for each cat was determined by calculating the mean of the five daily samples collected 5 h post-feeding from baseline (week 1), and each week during the 4 week treatment period. Changes in aFel d1 from baseline were calculated for each cat. A linear mixed-effect model was used to fit the data, with changes in aFel d1 level as the dependent variable, treatment, time (days) and treatment by time interaction as fixed effects, and baseline aFel d1 as a covariate. Time variable was considered continuous in this analysis.

Results

Trial 1

When samples for all hourly and daily time points were included, there was a significant ($P = 0.023$) decrease in mean salivary aFel d1, beginning within 2 weeks of starting the treatment diet (Table 1). The average decrease over the 6 week treatment period, relative to baseline, was 29.6%.

When data were pooled within the baseline period and analysed for differences based on sampling time relative to feeding, there were no differences ($P = 0.30$). Likewise, when the data were pooled during the treatment period and analysed based on hours post-feeding, there were no differences ($P = 0.56$). Based on these results (Table 2), all subsequent analyses were performed on the samples taken at 5 h post-feeding.

When the rANOVA was repeated using just the 5 h post-feeding data, the mean aFel d1 during the

Table 1 Salivary active Fel d1 (aFel d1) (μg/ml) from trial 1

Study period*	Wisteria	Ferris	Laddie	Rooney	Louie	Phils	Group mean	% reduction†
Baseline	8.92 ± 2.81	6.50 ± 2.34	5.00 ± 2.57	5.04 ± 2.56	26.56 ± 14.10	8.43 ± 5.50	10.08[a] ± 9.94	
Weeks 1 and 2	7.53 ± 1.86	5.23 ± 1.82	4.73 ± 1.39	5.02 ± 6.47	16.53 ± 8.17	7.88 ± 3.58	7.82[b] ± 6.16	22.39
Weeks 3 and 4	6.97 ± 3.44	3.96 ± 2.03	3.54 ± 1.82	2.81 ± 1.98	14.96 ± 7.33	7.06 ± 3.29	6.55[c] ± 5.59	34.99
Weeks 5 and 6	6.21 ± 2.50	3.03 ± 1.21	4.03 ± 1.22	3.53 ± 2.51	18.37 ± 6.29	6.56 ± 3.03	6.95[b,c] ± 6.20	30.97

Salivary aFel d 1 measured in four samples per cat daily, before and after treatment with specific polyclonal immunoglobulin (sIgY)-supplemented diet. Results presented as mean ± SD
*Baseline: 2 week period during which all cats received the control diet; weeks 1–6: 6 week treatment period during which all cats received the sIgY-supplemented diet
†Decrease from baseline concentrations determined as the mean of individual percentage change from baseline calculated for each cat
[a,b,c]Means with different superscripts differ significantly ($P < 0.05$)

Table 2 Mean salivary active Fel d1 (aFel d1) (μg/ml) from trial 1 based on time of sample collection relative to feeding, before and during treatment with specific polyclonal immunoglobulin (sIgY)-supplemented diet

	Pre-feeding	1 h post-feeding	3 h post-feeding	5 h post-feeding	rANOVA
Baseline period					
Wisteria	9.13 ± 2.79	8.68 ± 2.78	9.68 ± 3.18	8.20 ± 2.67	
Ferris	7.50 ± 1.90	4.93 ± 2.23	7.25 ± 1.81	6.17 ± 2.75	
Laddie	5.77 ± 0.85	3.86 ± 1.69	4.46 ± 1.92	5.91 ± 4.26	
Rooney	5.33 ± 3.04	4.05 ± 1.93	5.36 ± 2.57	5.42 ± 2.69	
Louie	25.39 ± 11.17	19.30 ± 5.88	25.15 ± 9.23	36.39 ± 21.16	
Phils	9.74 ± 5.77	4.99 ± 2.28	10.21 ± 6.33	8.78 ± 5.79	
Mean	10.48 ± 8.67	7.63 ± 6.30	10.35 ± 8.46	11.81 ± 14.28	$P = 0.30$
Treatment period					
Wisteria	8.04 ± 2.76	6.48 ± 2.74	5.77 ± 2.27	7.32 ± 2.62	
Ferris	4.80 ± 2.08	4.04 ± 2.10	3.82 ± 1.87	3.62 ± 1.51	
Laddie	4.32 ± 1.49	4.19 ± 1.32	4.04 ± 1.85	3.86 ± 1.60	
Rooney	3.86 ± 3.91	4.92 ± 6.68	3.42 ± 2.68	2.95 ± 2.05	
Louie	15.66 ± 5.89	16.72 ± 9.62	17.74 ± 7.19	16.37 ± 6.49	
Phils	7.39 ± 2.82	5.86 ± 2.51	7.46 ± 3.54	7.94 ± 4.01	
Mean	7.34 ± 5.29	7.04 ± 6.71	7.04 ± 6.20	7.01 ± 5.76	$P = 0.56$

Results presented as mean ± SD
rANOVA = repeated measures ANOVA

treatment period decreased by 40% from baseline, but this did not achieve statistical significance ($P = 0.11$), probably as a result of the reduction in number of samples and small number of cats.

Trial 2

Salivary aFel d1 decreased from baseline in both the control and treatment group cats, but achieved significance only in those on the sIgY diet (Figure 1). A significant reduction was achieved by week 3 of the treatment period. Comparing the mean aFel d1 from baseline with that from weeks 3 and 4 of the treatment period showed a 24% decrease from baseline in the treatment group compared with a 4% decrease for the same periods in the control group cats. Nine of the 11 cats (81.8%) in the treatment group showed a reduction in salivary aFel d1 of at least 20% vs baseline, with only one cat showing an increase over baseline (Figure 2). This contrasts to 3/8 cats (37.5%) in the control group showing a reduction in salivary aFel d1, with increases in five cats (62.5%) in the control group.

Discussion

This study demonstrated that it is feasible to reduce the immunologically active Fel d1 allergen from cats by feeding them a diet containing anti-Fel d1 polyclonal IgY from chicken eggs. Previous research from our laboratory documented the efficacy of sIgY in vitro, but this

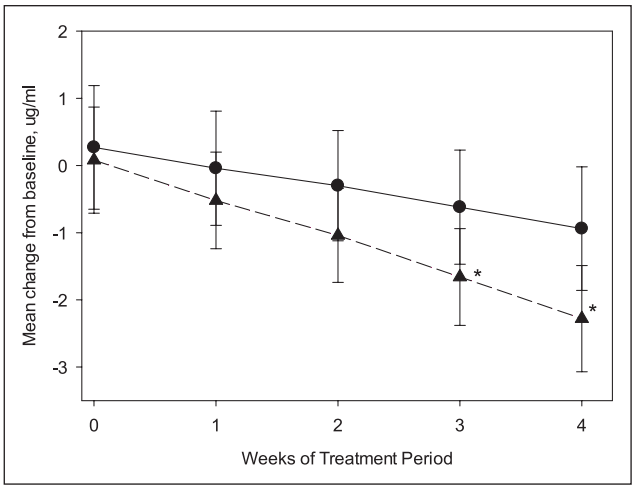


Figure 1 Mean change from baseline in salivary active Fel d1 in control cats (circles) and those fed the specific polyclonal immunoglobulin (sIgY)-supplemented diet (triangles), based on linear mixed-model assay. Asterisks indicate measurements significantly different from baseline ($P < 0.05$)

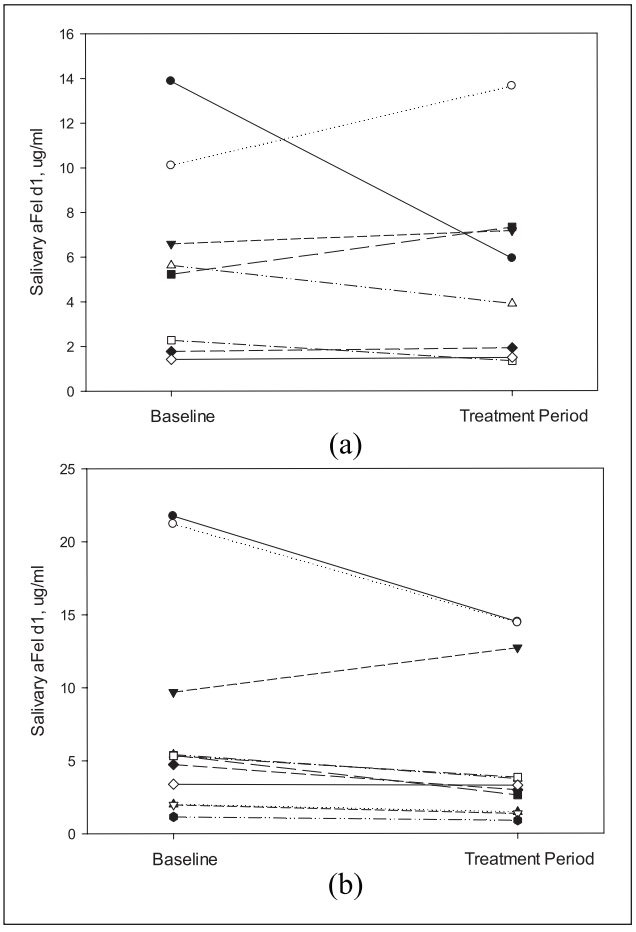


Figure 2 Salivary active Fel d1 (aFel d1) from cats before and after being fed (a) a control diet or (b) a diet containing specific polyclonal immunoglobulin (sIgY). Treatment period data includes weeks 3 and 4

is the first study to evaluate the effects of sIgY on salivary aFel d1 in vivo.

Trial 1 was designed to confirm if sIgY would have a detectable impact and to help determine appropriate protocols for additional testing. As such, we were able to document an approximately 30% reduction in salivary aFel d1 simply by feeding the food containing sIgY. Further, we identified that a single saliva sample taken 5 h after feeding was adequate to assess the aFel d1 levels within our study protocol.

Trial 2 built upon this learning and showed a significant reduction in salivary aFel d1 in test cats in comparison with control cats. Performing a controlled trial was important as salivary aFel d1 can vary considerably both among cats as well as within cats over time.[25] This natural variation was confirmed here as shown by the variability of aFel d1 among cats within the baseline period, and among the control cats over the duration of the study.

In trial 2, over 80% of cats fed the sIgY diet showed a reduction in aFel d1 of at least 20%, vs only 38% of control cats, and the overall reduction in aFel d1 averaged 24% in treated cats vs only 4% in control cats. Salivary Fel d1 is distributed to cats' haircoats during grooming and subsequently spread to the environment on shed hair and dander.[28] Although the magnitude of improvement observed in this study meets the World Allergy Organisation standards for a clinically relevant effect,[29] additional research will be needed to determine if the reduction in salivary aFel d1 will have a clinically important impact on aFel d1 in hair or in the environment.

Given that Fel d1 is the major cat allergen, causing IgE reactions in up to 95% of adults with allergies to cats,[2,6–8] a reduction in aFel d1 may contribute to a reduction in symptoms associated with this allergy. If this is ultimately shown, this sIgY dietary approach may not only benefit cat-allergic individuals with cats, but also non-cat owners who are allergic to cats. One study showed a 34% prevalence of cat allergy in people who had never kept cats in their homes.[6] Fel d1 can be relatively high in environments where cats have never been kept and the clothes of cat owners are the main source for allergen dispersal into cat-free environments.[12] A reduction in salivary aFel d1 may ultimately reduce environmental exposure.

Removal of cats from the home is usually considered the 'first-line measure' for controlling cat allergies from a medical perspective; however, there are no documented reports on the efficacy of cat removal in reducing clinical symptoms in highly sensitised individuals.[12] In addition, many cat owners are unwilling to give up their cats.[17,30,31] Despite this, allergies to cats are a commonly declared reason for cats to be surrendered to a shelter.[4,5] Among those cats entering shelters, only about 37% are adopted and 41% are euthanased.[32] Therefore, if the reduction in salivary aFel d1 afforded using this sIgY dietary approach proves to be clinically meaningful, it may also

benefit cats by providing an alternative to relinquishment and by allowing more quality interactions between cats and their allergic owners.

While chicken eggs and egg yolks – all of which naturally contain IgY – have long been consumed by humans and their pets, the use of hen yolk IgY as a functional food is a newer phenomenon within the past three decades.[33] Initially shown to be safe and effective for providing passive immunity against rotavirus, chicken IgY has since shown safe and effective benefits for various prophylactic and therapeutic applications addressing diseases of the skin, oral cavity and gastrointestinal tract in humans and animals.[22,23,33] The use of anti-Fel d1-specific IgY in eggs as a safe and effective means of reducing immunologically active Fel d1 without compromising total Fel d1 in cats is a new application with potential benefits for both cats and people.

Conclusions

Research in our laboratory had previously documented efficacy using in vitro and ex vivo models (unpublished), setting the stage for these in vivo studies in cats. Although the studies reported here are small, statistically significant reductions in salivary aFel d1 were documented. Additional research will be needed to determine if this reduction is sufficient to reduce environmental allergen load or to reduce clinical symptoms of allergies to cats in sensitive individuals.

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Conflict of interest The authors are employees of Nestlé Purina PetCare.

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Ethical approval The study protocols were reviewed and approved by the Institution's Animal Care and Use Committee and complied with all regulations set forth in the USDA Animal Welfare Act (Animal Welfare Act is Federal law in the United States that regulates the treatment of animals in research, exhibition, transport, and by dealers).[26]

Informed consent Not applicable, no client-owned cats participated in the study.

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