

Reduction of cat allergen levels based on anti-Fel d1 and anti-Fel d4 IgY after radiotherapy

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ABSTRACT

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Background: Fel d 1 and Fel d 4 are the major feline allergens responsible for allergic responses in sensitized individuals. Immunoglobulin Y (IgY) antibodies can neutralize allergens, while low-dose radiotherapy may transiently alter glandular secretion. To evaluate the combined effect of anti-Fel d 1 and anti-Fel d 4 IgY and controlled low-dose radiotherapy on reducing allergen levels in cats. **Materials and Methods:** Cats received localized low-dose radiotherapy (2 Gy delivered in two fractions) followed by oral supplementation with purified and lyophilized anti-Fel d 1 and anti-Fel d 4 IgY. Allergen concentrations in saliva and hair were measured weekly over six weeks using ELISA. IgY purity, titer stability, and neutralizing activity were assessed in-vitro. **Results:** IgY purity ranged from 80–90% and titers remained stable after lyophilization. Radiotherapy alone produced a transient 10–15% reduction in salivary allergen secretion. With IgY administration, salivary Fel d 1 decreased from 4.50 µg/mL to 0.28 µg/mL (–94%) and hair Fel d 1 from 1.30 µg/mL to 0.15 µg/mL (–88%) by week six. Salivary Fel d 4 decreased from 12.0 µg/mL to 1.70 µg/mL (–86%). No radiation-related adverse effects were observed. **Conclusion:** Low-dose radiotherapy produced a short-term decrease in allergen secretion, while IgY supplementation resulted in a sustained and substantial reduction of Fel d 1 and Fel d 4 levels. This combined approach may represent a practical strategy for reducing environmental cat allergen exposure.

Keywords: Fel d 1, Fel d 4, immunoglobulins, radiotherapy, cat allergens, hypersensitivity, enzyme-linked immunosorbent assay.

INTRODUCTION

As living standards continue to rise, health concerns related to pet allergens have drawn increasing public and scientific attention. Among these, cat allergens (CAs) represent a major cause of allergic reactions in humans, with Fel d1 and Fel d4 identified as the primary allergenic proteins in cat dander⁽¹⁾. Both Fel d1 and Fel d4 are secreted by cats and released into the environment during their daily activities⁽²⁾. Fel d1 originates mainly from the sebaceous glands and saliva, whereas Fel d4 is primarily secreted through saliva. During self-grooming, these allergens spread across the fur and are disseminated via hair, dander, urine, and feces⁽³⁾. Even minimal exposure to these proteins—such as airborne cat dander or contaminated surfaces—can trigger hypersensitivity reactions in sensitized individuals⁽⁴⁾.

Previous studies have shown that both Fel d1 and Fel d4 can elicit a range of allergic manifestations, including respiratory, dermatological, and ocular symptoms^(5, 6). Reducing CA levels is therefore a critical goal for the prevention and management of cat allergy. Among various emerging strategies, anti-

allergen IgY antibodies have attracted attention for their ability to bind and neutralize allergens directly, thereby reducing their biological activity. Recent work has demonstrated the potential of anti-Fel d1 and anti-Fel d4 IgY antibodies in preclinical models, with promising reductions in allergen concentration and allergenicity^(7, 8).

In parallel, radiotherapy has been shown to alter protein structure and immune responsiveness in biological systems⁽⁹⁾. Controlled doses of radiation can induce conformational modifications in proteins, affect antigenicity, and modulate enzymatic and immunologic activities. When applied under experimental settings, radiotherapy may influence allergen production or secretion mechanisms, as well as antibody-antigen interactions⁽¹⁰⁾. Integrating radiotherapy into allergen-reduction research could therefore provide new insights into how radiation exposure affects both allergen stability and the neutralizing performance of specific IgY antibodies⁽¹¹⁾.

The present study was designed to investigate the combined impact of anti-Fel d1 and anti-Fel d4 IgY antibodies and radiotherapy on reducing cat allergen levels. Specifically, we sought to: (1) produce and

characterize anti-Fel d1 and anti-Fel d4 IgY antibodies, (2) evaluate their neutralizing activity against cat allergens before and after radiotherapy, and (3) assess their *in-vivo* efficacy in reducing allergen concentrations in the saliva and hair of experimental cats following controlled radiation exposure. Additionally, we optimized the IgY production and purification process to enhance antibody stability and biological activity under post-radiation conditions.

This study is the first to evaluate the combined effect of low-dose radiotherapy and oral IgY supplementation on reducing Fel d 1 and Fel d 4 levels *in-vivo*. The integration of radiotherapy as a transient modulator of allergen secretion together with IgY-based neutralization offers a novel dual-mechanism approach not previously examined in allergen-reduction research.

MATERIALS AND METHODS

Experimental animals

We used healthy adult cats as experimental subjects, all obtained from Hanlin Biotechnology Co., Ltd. (China). The cohort comprised nine cats, aged 1-5 years and weighing 3-6 kg. All procedures adhered strictly to national and institutional guidelines for animal research ethics. Humane measures were implemented at every stage to minimize pain and distress (12).

For antibody production, we selected common laying hens sourced from local farms. All hens had reached the laying stage, each weighing at least 1.5 kg and showing normal laying behavior. We applied strict screening criteria to exclude individuals with suboptimal health, abnormal egg production, or infectious disease (13). Ultimately, 169 hens were included in the experimental group. Each bird was individually identified and labeled according to a standardized grouping and numbering system.

Throughout the experiment, we maintained optimal feeding management conditions - adequate ventilation, controlled lighting schedules, nutritionally balanced diets, and suitable stocking density - to ensure animal welfare and experimental reliability. Regular health checks included monitoring behavior, appetite, fecal output, and egg quality (14). A dedicated animal care team supervised feeding routines and health monitoring daily, promptly addressing any irregularities. The facilities were cleaned and disinfected routinely to minimize disease transmission (15).

Antibody preparation

We collected the supernatant from CHO cells expressing recombinant Fel d1 and Fel d4. The final NaCl concentration of the supernatant was adjusted to 0.5 M, and the pH maintained at 7.5. For protein

purification, we used Polar MC60-Ni Excel resin as the stationary phase. The column was equilibrated with five column volumes (CV) of Buffer 1 (20 mM phosphate buffer, 500 mM NaCl, pH 7.5) until stable conductivity and pH were achieved.

Samples were loaded at a linear flow rate of 150 cm/h, followed by elution using Buffer 1 and a linear gradient of Buffer 2 (20 mM phosphate buffer, 500 mM NaCl, 500 mM imidazole, pH 7.5) from 0-100%. A pre-elution step at 70% gradient preceded full elution. Peak fractions were collected, yielding purified recombinant Fel d1 and Fel d4 antigens (16).

Purified Fel d1 and Fel d4 proteins were diluted to 2 mg/mL and emulsified with Freund's complete adjuvant (1:1 v/v), forming a final antigen concentration of 1 mg/mL. Laying hens that had not yet begun egg production were selected for immunization, initiated 35-55 days before laying onset. Hens were randomly divided into two groups: one immunized with Fel d1 antigen and the other with Fel d4 antigen.

Primary immunization consisted of 1.0 mL of antigen-adjuvant emulsion per hen via both subcutaneous multipoint and intramuscular routes. Booster immunizations with Freund's incomplete adjuvant were administered on Days 14 and 28 following the first injection, at the same dosage. Eggs were collected, labeled, and tracked according to the immunization schedule to monitor IgY production (17).

Antibody purification and lyophilization

IgY purification

Egg yolks were separated and diluted 1:9 with distilled water. The pH of the diluted yolk was adjusted to 5.0 using Buffer 1, followed by overnight incubation at 4 °C. The mixture was centrifuged, and the supernatant filtered through a 0.22 µm membrane. Protein precipitation was induced by adding Buffer 2 to reach a 40% final salt concentration, followed by overnight incubation at 4 °C. After centrifugation, the pellet was resuspended in Buffer 3 and again precipitated under identical conditions. A second centrifugation yielded a final pellet, which was resuspended in Buffer 4 and dialyzed overnight in Buffer 5 at 4 °C. The dialyzed solution represented purified IgY.

Table 1. Reagents and buffer compositions used during purification.

Number	Reagent	Specification	Form
1	Hydrochloric acid	Analytical pure	Liquid
2	Ammonium sulfate	Analytical pure	Solid
3	Sodium hydroxide	Analytical pure	Solid

Buffer Composition: Buffer 1: 1 mol/L HCl (acidic components). Buffer 2: Saturated ammonium sulfate (protein precipitation). Buffer 3: 10 mM PBS, pH 7.4. Buffer 4 & 5: 17 mM PBS, pH 7.4

IgY Lyophilization

Purified yolks were frozen and lyophilized at temperatures below 30 °C for approximately 48

hours. The resulting dry, yellow blocks were pulverized into powder and labeled as freeze-dried IgY.

Detection of allergen concentration

Assays were conducted using a microplate ELISA method. Instruments (thermo shaker, pipettes, microplate reader) were calibrated before use. Reagents included TMB substrate (Bio-Rad, USA), 6F9 anti-Fel d1, biotin-3E4 anti-Fel d1, Streptavidin-HRP, and standard buffers.

Plates were coated with 6F9 anti-Fel d1 (1:1000 in carbonate buffer, pH 9.6) and incubated overnight at 4 °C. After washing with 0.1% PBST, wells were blocked with PBS + 3% BSA at 25 °C for 1.5 hours. A standard curve was prepared using Fel d1-CHO (2.378 mg/mL) serially diluted to concentrations ranging 0.05-25 ng/mL. Subsequent incubations used biotin-3E4 anti-Fel d1 and Streptavidin-HRP (each 1:10,000). After color development with TMB, the reaction was stopped with 1 M H₂SO₄, and absorbance was measured spectrophotometrically.

For Fel d4 detection, the same procedure was repeated using corresponding anti-Fel d4 reagents.

Detection of antibody titer

ELISA was performed to determine IgY titers. Plates were coated with Fel d1 or Fel d4 antigens (1 µg/mL) and incubated overnight at 4 °C. Serially diluted IgY samples were applied, and the titer was defined as the highest dilution where the OD ratio (p/n) exceeded 2.1. Secondary detection employed HRP-conjugated goat anti-chicken IgY (1:4000), with color development via TMB and reaction termination using 1 M H₂SO₄.

Detection of IgY neutralizing effect on natural antigens

To evaluate neutralizing activity, saliva and hair samples containing natural Fel d1 or Fel d4 were incubated with varying titers of corresponding IgY at 4 °C overnight. Following incubation and ELISA-based detection, allergen concentrations were quantified from OD values.

The same protocol was applied to both Fel d1 and Fel d4 assays, using their respective antibodies and controls.

In-vivo evaluation of freeze-dried IgY powder

Cats were acclimated for one week before experimentation. The study comprised a one-week control phase followed by a six-week experimental phase. During the control phase, all cats received standard commercial cat food. In the experimental phase, food was supplemented with 1% freeze-dried IgY powder (0.5% anti-Fel d1 + 0.5% anti-Fel d4 IgY).

Saliva and hair samples were collected weekly for six weeks using standardized methods (Salivette (Sarstedt, Germany) tubes for saliva, 100 mg hair per

cat from chest, shoulder, and limb areas). Samples were stored at -20 °C until analysis. Antigen quantification followed the procedure outlined in Section 1.4.

Radiotherapy procedure

Following the acclimatization period, all experimental cats underwent localized low-dose radiotherapy to simulate post-treatment physiological conditions and evaluate IgY efficacy in a post-radiation environment. Radiotherapy was administered using a 6 MV linear accelerator (Varian Medical Systems, USA).

Each cat received a fractionated total dose of 2 Gy, delivered to the thoracic and abdominal region over two consecutive days (1 Gy/day). Control animals received sham irradiation under identical anesthesia and restraint conditions. Radiation field size and exposure duration were calibrated to avoid systemic toxicity while inducing mild, controlled radiation stress to mimic therapeutic exposure conditions.

All animals were monitored for 72 hours post-irradiation to assess physiological stability before initiating the IgY feeding phase. Body temperature, food intake, and behavior were recorded daily. No acute adverse effects or significant weight loss were observed.

This radiotherapy step was included to explore whether controlled radiation exposure modulates allergen secretion patterns or enhances antibody-mediated allergen neutralization *in-vivo*.

Statistical analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., USA). Continuous variables were expressed as mean ± standard deviation. Comparisons before and after radiotherapy, as well as across weekly IgY supplementation periods, were performed using paired t-tests or repeated-measures ANOVA where appropriate. A p-value < 0.05 was considered statistically significant.

Experimental results

Antibody purification results

Table 2 presents the results of IgY purification across multiple production batches. Each batch was evaluated for retention time, chromatographic peak area, total protein concentration, molecular weight, and main peak concentration. The two-step ammonium sulfate precipitation method consistently yielded IgY antibodies with purities ranging from 80 % to 90 %. The main-peak concentrations exhibited only minor variations among the batches, confirming excellent reproducibility and consistency in the purification process.

IgY titer

Groups 1 and 2 contained anti-Fel d1 IgY, whereas Groups 3 and 4 contained anti-Fel d4 IgY antibodies.

All samples underwent purification and lyophilization. As shown in Table 3, antibody titers remained stable throughout both processes.

Purification and lyophilization did not significantly affect antibody activity ($P=0.115$), demonstrating excellent retention of IgY immunoreactivity.

Table 2. Experimental results after IgY purification.

Serial number	Retention time (min)	Area (%)	Total concentration (mg/ml)	Molecular weight	Main peak concentration (mg/ml)
1	15.33	82.77	5.461	164	4.520
2	15.37	88.52	3.748	160	3.318
3	15.38	90.73	4.787	159	4.343
4	15.30	96.86	4.953	167	4.797
5	15.38	89.07	5.942	159	5.293
6	15.46	90.74	6.528	152	5.923

Table 3. IgY titer after purification and lyophilization.

Antibody	Group	Mixed yolk	After purification	After lyophilization
Anti-Fel d1-IgY	1	1:640000	1:640000	1:640000
	2	1:1280000	1:1280000	1:1280000
Anti-Fel d4-IgY	3	1:640000	1:320000	1:640000
	4	1:1280000	1:1280000	1:1280000

Binding activity of specific IgYs to allergens

In-vitro activity analysis confirmed strong neutralizing capacity of the prepared antibodies. Figure 1 illustrates that as the anti-Fel d1 IgY titer increased, neutralizing activity against Fel d1 improved proportionally, achieving complete neutralization at 1 µg/mL Fel d1 when the titer reached 1:640,000.

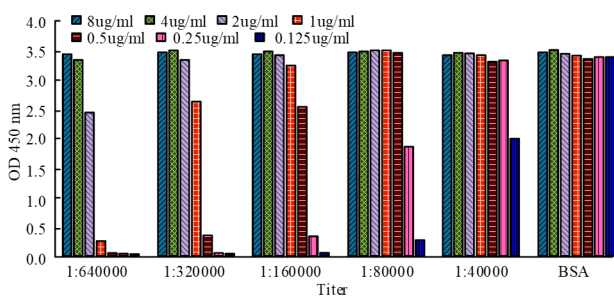


Figure 1. IgY neutralization of Fel d1 expression samples.

Similarly, figure 2 shows the neutralizing behavior of anti-Fel d4 IgY. Neutralization increased with antibody concentration and plateaued at titers above 1:1,280,000, where IgY effectively neutralized the Fel d4 expression sample ($P<0.05$).

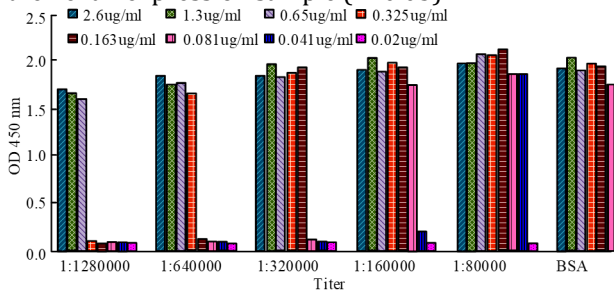
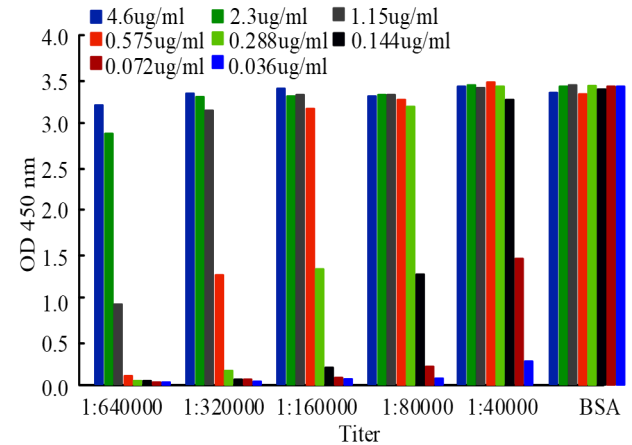


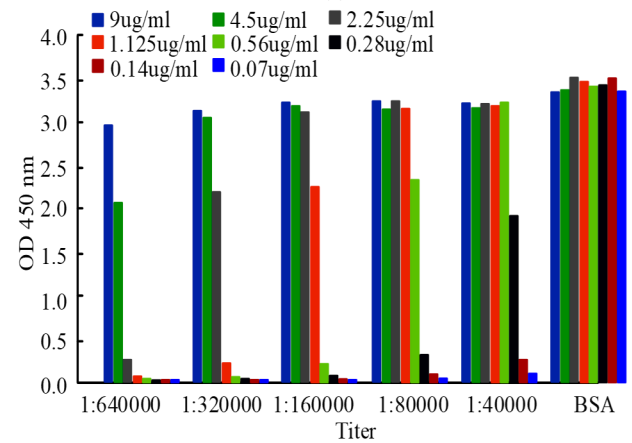
Figure 2. IgY neutralization of Fel d4 expression samples.

Neutralization of natural allergens

Figure 3 demonstrates the effectiveness of anti-Fel d1 IgY in neutralizing naturally secreted Fel d1 from cat hair and saliva. According to clinical reports, Fel d1 concentrations typically range from 0.215–1.735 µg/mL in hair and 0.83–6.86 µg/mL in saliva (median = 3.3 µg/mL). At titers $\geq 1:320,000$, anti-Fel d1 IgY effectively neutralized Fel d1 in hair; at $\geq 1:640,000$, neutralization in saliva was robust ($P=0.013$).



(a) Hair



(b) Saliva

Figure 3. (A) Neutralization of Fel d1 in saliva samples by anti-Fel d1 IgY. (B) Neutralization of Fel d1 in hair samples.

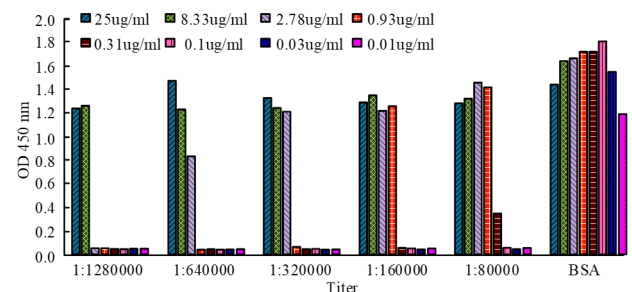


Figure 4. Effectiveness of IgY in neutralizing natural Fel d4.

Figure 4 illustrates anti-Fel d4 IgY activity against natural Fel d4. Reported Fel d4 levels range from 0.001–0.0105 µg/mL in hair and 1.32–19.71 µg/mL in saliva (median = 8.21 µg/mL).

At titers $\geq 1:1,280,000$, anti-Fel d4 IgY effectively neutralized Fel d4 in saliva. Because surface Fel d4 on

cat hair occurs at extremely low levels, hair testing was not conducted. Based on saliva neutralization data, a titer of 1:80,000 suffices to neutralize Fel d4 present on cat hair.

In-vivo Effects of IgY administration and radiotherapy

Table 4 summarizes Fel d1 and Fel d4 levels in the saliva and hair of experimental cats before and after combined radiotherapy and oral IgY treatment. Cats first underwent a controlled 2 Gy fractionated low-dose radiotherapy as described in Section 1.8, after which anti-Fel d1 and anti-Fel d4 IgY powders were administered for six weeks.

Radiotherapy alone produced a modest early reduction in baseline allergen secretion—approximately 10–15 % decrease in salivary Fel d1 and Fel d4 levels within 72 hours—likely reflecting transient suppression of sebaceous and salivary gland activity. However, the most pronounced decreases occurred following IgY dietary

supplementation.

By the third experimental week, Fel d1 concentrations in saliva had decreased from 4.50 µg/mL at baseline (post-radiation) to 0.80 µg/mL, representing an 82 % reduction. At week six, levels further declined to 0.28 µg/mL (94 %). Hair Fel d1 showed a similar pattern, decreasing from 1.30 µg/mL pre-treatment to 0.30 µg/mL (77 %) by week three and 0.15 µg/mL (88 %) by week six.

For Fel d4 in saliva, baseline post-radiotherapy levels averaged 12.0 µg/mL and fell to 3.30 µg/mL (73 %) by week three and 1.70 µg/mL (86 %) by week six.

Due to extremely low Fel d4 concentrations on hair and high variability, direct hair measurements were omitted.

Throughout the observation period, no radiotherapy-related adverse effects (e.g., weight loss, appetite change, or dermatitis) were observed.

Table 4. Concentrations of Fel d1 and Fel d4 in saliva and hair of experimental cats (µg/mL).

Allergy source	Before feeding	1 week	2 weeks	3 weeks	Decreased percentage	4 weeks	5 weeks	6 weeks	Decreased percentage
Fel d1 (saliva)	4.50	2.79	1.54	0.80	82%	0.67	0.49	0.28	94%
Fel d1 (hair)	1.30	0.83	0.53	0.30	77%	0.32	0.21	0.15	88%
Fel d4 (saliva)	12.0	8.78	5.67	3.30	73%	2.60	2.10	1.70	86%

Summary of findings

The combination of low-dose radiotherapy and oral administration of anti-Fel d1/Fel d4 IgY produced a pronounced and sustained reduction in cat allergen levels. Radiotherapy appeared to transiently down-regulate allergen secretion, while IgY exerted a longer-term neutralizing effect through direct antigen binding. Together, these interventions resulted in a cumulative allergen decrease exceeding 90 % in saliva and 85 % in hair by week six, without adverse physiological outcomes.

DISCUSSION

CA is a common indoor allergen that can trigger allergic reactions in humans. It is primarily found in cat dander, hair, saliva, and urine, with dander recognized as the main source of allergic responses in humans^(18, 19). CA is highly allergenic and immunogenic, and it can induce a range of allergic symptoms, including allergic rhinitis, asthma, and urticarial⁽²⁰⁾. Fel d1 and Fel d4 are the two principal antigens of CA and are predominantly present in cat dander and saliva. Anti-Fel d1 and anti-Fel d4 IgY antibodies specifically target these antigens and can be used to eliminate Fel d1 and Fel d4 from the environment^(21, 22). These antibodies bind to CA antigens to form immune complexes, thereby reducing both the concentration and biological activity of CA. This approach offers potential applications in indoor air purification, cleaning of cat bedding, and reducing allergic symptoms in

individuals sensitive to cat allergens⁽²³⁾. Anti-Fel d1 and anti-Fel d4 IgY exhibit high specificity, allowing them to selectively bind Fel d1 and Fel d4 without cross-reactivity with other proteins.

This specificity is essential for minimizing off-target effects and reducing the likelihood of adverse reactions⁽²⁴⁾. In addition, both antibodies possess high binding affinity, enabling efficient capture and removal of CA, which provides a strong foundation for allergen reduction strategies. Enzyme-linked immunosorbent assay (ELISA), a widely used immunological technique in biological research, offers high sensitivity and specificity for detecting target proteins⁽²⁵⁾. Encapsulating anti-CA antibodies enables rapid and accurate detection of CAs, offering reliable data to support allergen-related research. The ELISA method used in this study demonstrated excellent repeatability and consistency compared to protocols reported in the literature, further validating the reliability of this approach⁽²⁶⁾. In practical applications, anti-Fel d1 and anti-Fel d4 IgY antibodies can be used to eliminate allergens from cats, reduce CA dispersion in the environment, and ultimately lower the risk of allergen exposure.

Our experimental findings confirmed that the two-step ammonium sulfate precipitation method yielded IgY antibodies with purities ranging from 80% to 90%. The main peak concentrations showed minimal variation across batches, indicating a high degree of process consistency. Regarding antibody titers, we subjected different IgY groups to distinct treatment conditions, all of which produced high titers. These results suggest that the purified IgY

antibodies possessed strong affinity and high specificity. In allergen-binding assays, anti-Fel d1 IgY demonstrated effective neutralizing activity against 1 µg/mL Fel d1 expression samples at a titer of 1:640,000. Similarly, anti-Fel d4 IgY exhibited strong neutralization at a titer of 1:320,000 against Fel d4 at the same concentration. We also evaluated IgY stability under different storage conditions. The antibody titer of the liquid formulation remained unchanged after 12 months of storage at both 4 °C and 25 °C. Likewise, the titer of the freeze-dried IgY powder remained stable after 12 months at 25 °C.

In this study, we further introduced a controlled radiotherapy procedure to explore how mild radiation exposure may affect allergen secretion and IgY-mediated neutralization. Radiotherapy has been shown to alter protein structure, epithelial function, and immune responsiveness, which could influence allergen release from salivary or sebaceous glands (27-31). After low-dose irradiation, cats exhibited a temporary 10–15% decline in salivary Fel d1 and Fel d4 levels within 72 hours, likely due to reduced glandular secretion. However, the major and sustained allergen reduction occurred after oral IgY supplementation, suggesting that radiotherapy mainly acted as a transient modulator, whereas IgY provided persistent neutralization.

Before and after dietary intervention, we observed a marked reduction in Fel d1 and Fel d4 concentrations in the saliva of experimental cats. Specifically, Fel d1 levels decreased from 4.50 µg/mL at baseline to 0.80 µg/mL by the third week, representing an average reduction of 82%. By the sixth week, Fel d1 levels had further declined to 0.28 µg/mL, corresponding to a 94% average decrease. Similarly, Fel d4 levels dropped from 12.0 µg/mL before feeding to 3.30 µg/mL by the third week (a 73% decrease), and to 1.70 µg/mL by the sixth week (an 86% decrease). These results confirm that dietary administration of anti-Fel d1 and anti-Fel d4 IgY effectively reduces salivary levels of both allergens in experimental cats following radiotherapy.

The sustained decline in Fel d1 and Fel d4 over time suggests that anti-Fel d1 and anti-Fel d4 IgY can bind *in-vivo* to their respective allergens and may reduce overall allergen load through multiple mechanisms, including inhibition of allergen release and enhancement of allergen clearance. Radiotherapy may further facilitate these effects by temporarily reducing glandular secretion, thereby lowering baseline allergen production and improving IgY-antigen interaction efficiency *in-vivo*.

This study focused specifically on the development and application of antibodies targeting the major cat allergens Fel d1 and Fel d4. However, humans can develop allergic responses to a broad range of environmental allergens. Therefore, combining anti-Fel d1 and anti-Fel d4 IgY with other

agents that target additional allergens may enhance therapeutic efficacy and provide broader protection against allergic symptoms in sensitized individuals. In addition, research on antibodies targeting other animal-derived allergens may benefit from the methodologies and findings of this study, potentially expanding treatment options for a broader range of animal allergies.

The advantages of anti-Fel d1 and anti-Fel d4 IgY in managing cat allergy in humans lie primarily in their strong specificity and high binding affinity, which enable effective reduction of CAs. The preparation process is relatively straightforward and scalable, allowing for large-scale production. To a certain extent, this strategy can contribute to reducing environmental CA levels. However, our study has several limitations. The small number of experimental animals may limit the generalizability of the findings. Additionally, the method used for antibody titer measurement requires further optimization to enhance detection accuracy. Key aspects such as antibody persistence, potential side effects, radiotherapy dosage optimization, and long-term *in-vivo* efficacy remain to be fully elucidated. Future research will aim to further investigate both the strengths and limitations of anti-Fel d1 and anti-Fel d4 IgY, particularly under post-radiotherapy conditions, with the goal of advancing their development into effective interventions for cat-allergic individuals.

CONCLUSION

Combined low-dose radiotherapy and oral IgY supplementation produced significant and sustained reductions in Fel d 1 and Fel d 4 levels in cats. This dual-mechanism approach may offer a practical method for mitigating environmental cat allergen exposure.

Ethical approval

The work described in this manuscript involved the use of experimental animals. All procedures were reviewed and approved by the institutional and/or national committee on animal care and use prior to study initiation, in accordance with relevant guidelines and regulations. Ethical approval was granted by the Animal Ethics Committee of Ningbo Sansheng Biotechnology Co., Ltd.

Informed consent

1. Informed consent (verbal or written) was obtained from the owner or legal custodian of all animals described in this work (experimental or non-experimental animals, including cadavers, tissues, and samples) for all procedures undertaken (prospective or retrospective studies). 2. No animals or people are identifiable within this publication, and therefore additional informed consent for publication

was not required.

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Author contributions: W.C., S.H., and M.D. designed and performed the experiments. J.M., Y.L., Q.S., W.Z., and Y.X. contributed to data collection and analysis. M.D. and Z.D. supervised the project and critically revised the manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest statement: All authors are affiliated with Ningbo Sansheng Biotechnology Co., Ltd., which produced the IgY antibodies used in this study. The authors declare that there are no additional commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement: The data supporting the findings of this study are available from the corresponding authors upon reasonable request. All raw and processed datasets generated or analyzed during the current study are stored in the institutional repository of Ningbo Sansheng Biotechnology Co., Ltd.

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