



The Effect of Keratinase-psyllium Husk Formulated as Feed Additive for Trichobezoar Prevention in Feline

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ABSTRACT

Background: Trichobezoars represent a frequent digestive issue in cats, often resulting from excessive hair ingestion during grooming. This study evaluated the effects of keratinase-psyllium husk as a feed additive to prevent trichobezoar formation.

Methods: The trial included 20 healthy adult cats, randomly divided into three groups: KP-L (low-dose keratinase-psyllium husk), KP-H (high-dose keratinase-psyllium husk) and Control (standard diet without additives). Each group received their respective diet for pre-trial 3 days and a formal trial 14 days. Parameters analyzed included keratin degradation, fecal trichobezoar excretion and blood indicators to assess overall health status.

Result: The findings demonstrated that cats in the KP-H group exhibited significantly higher keratin degradation and increased fecal trichobezoar excretion compared to the KP-L and Control groups. Both KP-L and KP-H groups showed improved hair elimination, though the effects were dose-dependent. No adverse effects on hematological or biochemical parameters were observed, indicating the safety of the additive. The study suggests that dietary supplementation with keratinase-psyllium husk effectively enhances hair degradation and trichobezoar excretion in cats, with higher doses showing greater efficacy. This additive presents a promising preventive approach for managing hairball formation in feline diets.

Key words: Feed additive, Feline, Keratinase, Psyllium husk, Trichobezoar.

INTRODUCTION

Trichobezoars, commonly known as hairballs, are among the most frequent digestive disorders in cats, resulting from the ingestion of significant amounts of hair during grooming. Although most ingested hair is excreted through feces, accumulation within the digestive system may lead to trichobezoar formation. This condition can cause clinical symptoms such as vomiting, hair-laden feces and other digestive disturbances (Milteneburg *et al.*, 2019; Loureiro *et al.*, 2014). In severe cases, trichobezoars can lead to intestinal obstructions, significantly impacting feline health and welfare (Yin *et al.*, 2022).

The etiology of trichobezoar formation is multifactorial, involving excessive grooming, gastrointestinal motility disorders, food allergies and indigestion. Additional factors include fiber-deficient diets, physiological conditions impairing hair expulsion and stressful environments that increase grooming frequency (Cannon, 2013). Treatment strategies range from dietary modifications and pharmacological interventions to surgical removal in severe cases. High-fiber diets are often recommended to enhance gastrointestinal motility and reduce hair accumulation, while lubricants may aid in hair passage. Although surgery effectively removes obstructions, it carries inherent risks and does not address underlying causes. Preventive strategies emphasize balanced diets, adequate hydration and routine hairball remedies (Pawensk *et al.*, 2023). By managing grooming behaviors and optimizing dietary interventions, feline welfare and overall health can be improved (Gibb *et al.*, 2015).

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Dietary interventions are a promising approach to reducing hairball formation. Loureiro *et al.* (2014, 2017) demonstrated that incorporating sugarcane fiber or beet pulp into cat food significantly reduced hairball formation. Additionally, Milteneburg *et al.* (2019) found that keratinase, when combined with sugarcane fiber, promoted hairball excretion via fecal elimination.

Keratinase is an enzyme capable of degrading keratin, the fibrous structural protein in hair. Its enzymatic action produces sulfur-containing amino acids and peptide fragments, which intestinal microbes can convert into short-chain fatty acids (SCFAs) for cellular utilization (Koh *et al.*, 2016). Thus, keratinase degradation contributes to amino acid recovery and reuse. Recent studies indicate that keratinase can degrade hair by up to 62% (Singh and Kushwaha, 2015) and chicken feathers by up to 72% (Wang *et al.*, 2009). While widely used in

veterinary medicine, further research is required to confirm its safety.

Psyllium husk powder is derived from the outer shells of *Plantago psyllium* seeds and is commonly used in traditional herbal medicine. Plant based supplementation has widely applied to broilers and farm animals (Kale *et al.*, 2015; Sachan *et al.*, 2014). As an indigestible fiber, psyllium husk absorbs water, stimulates gastrointestinal motility and increases bowel movement frequency. When hydrated, psyllium forms a gel-like mucilage property utilized in nutritional food processing. Studies suggest that psyllium husk can lower cholesterol and low-density lipoprotein levels, reduce glucose absorption in type 2 diabetes patients and, depending on dosage and timing, inhibit vitamin and mineral absorption (Darooghegi *et al.*, 2020; Gibb *et al.*, 2015). Psyllium husk consists of nearly 85% carbohydrates, with 90% total dietary fiber (TDF), predominantly insoluble (80%) and rich in hemicellulose arabinoxylan (45-60% of the husk) (Cheryl *et al.*, 2006; Weber *et al.*, 2015). Its gelatinous mucus facilitates hair binding to food particles, increasing hair excretion in feces, making it a potential treatment for hairball disease.

This study aims to evaluate the efficacy of keratinase-psyllium-formulated cat food in preventing and treating trichobezoars. By assessing the impact of different psyllium husk powder concentrations on hair degradation in the gastrointestinal tract, this research seeks to provide valuable data for developing effective dietary solutions for hairball management in cats.

MATERIALS AND METHODS

All animal experiments conducted in this research adhered to the ethical principles outlined by the National Research Council Guidelines and received approval from the Institutional Animal Care and Use Committee of Nanjing Agricultural University. The study was carried out at the Laboratory of Clinical Veterinary Science at Nanjing Agricultural University from July 2023 to July 2024.

A total of twenty healthy adult experimental cats, aged 3±1 years and weighing 4±0.5 kg, with a body condition score of 4-5, were selected, ensuring an equal distribution of males and females. Each cat was individually housed in a single cage under controlled environmental conditions: temperature maintained at 20±3°C, relative humidity at 50-60%, a 12-hour light-dark cycle, good ventilation and ad libitum access to water. Body weight was recorded weekly. The cats were randomly divided into three groups, ensuring an equal distribution of sexes: KP-L (low-dose keratinase-psyllium husk), KP-H (high-dose keratinase-psyllium husk) and Control (standard diet without additives). The KP-L group received a diet supplemented with 25 mg psyllium husk and 6 mg keratinase, the KP-H group received 100 mg psyllium husk and 6 mg keratinase, while the Control group received a standard diet without supplementation. KP-L and KP-H groups were administered their respective

doses in the form of gastro-resistant capsules before evening feeding. The Control group received no additives.

The experimental period consisted of a pre-trial period lasting 3 days and a formal trial period lasting 14 days. The pre-trial period was intended to help the cats acclimate to the new diet and stabilize metabolic parameters. Before, during and after the experiment, all cats underwent health examinations to ensure overall well-being. Food was divided into two equal portions and fed twice daily (at 10:00 a.m. and 6:00 p.m.), with daily food intake measured. Each cat was housed in an individual stainless-steel metabolic cage (0.5 × 0.5 × 0.6 m). The diet was sourced from PARTNER, formulated according to the European Pet Food Industry Federation Nutritional Guidelines (FEDIAF, 2012) and analyzed for ingredient composition by CTI (Table 1). Additional materials and reagents included Tris-HCl solution from Sigma-Aldrich, keratinase from NOVUS, casein from Bidepharm, TCA from JandK, Na₂CO₃ from CNW and psyllium husk from NOW. Gastro-resistant empty capsules were obtained from DR.caps and various chemicals such as methanol, 1-propanol, pyridine, hexane and ethyl acetate were sourced from ANPEL and Sinopharm. Ultra-pure water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

A buffer solution mimicking the pH of the feline gastrointestinal tract was prepared and maintained at 38-39°C in a water bath, with pH adjusted accordingly. The selected keratinase was mixed with an appropriate substrate and its enzymatic activity was assessed under different pH conditions using spectrophotometry. A 1% Tris-HCl (pH 8.0) solution was used as the casein substrate. One mL of 200 U/mL keratinase solution was added to 1 mL of the casein substrate at 40°C for 10 minutes, followed by the addition of 2 mL of 10% TCA to terminate the reaction. A blank control was prepared by adding TCA before introducing the substrate. The mixture was centrifuged at 4000 rpm and 1 mL of the supernatant was transferred to a glass tube, followed by the addition of 5 mL of 0.4 mol/L Na₂CO₃ solution and 1 mL of Folin-phenol reagent. After incubation at 40°C for 15 minutes, absorbance was measured at 680 nm.

Table 1: Ingredients and chemical composition of control, KP-L and KP-H diets.

Ingredient	Content
Crude protein (%)	32.95
Ash (%)	8.1
Crude fat by acid hydrolysis (%)	15.20
Calcium (%)	1.25
Crude fibre (%)	2.90
Moisture (%)	6
Water-soluble chloride (%)	0.63
Phosphate (%)	1.04
Taurine (%)	0.47
Gross energy (kcal/kg)	4003.95
ME (kcal/kg)	3,690.00

Hair samples collected from the experimental cats were subjected to keratinase-substrate incubation for 24 hours to assess hair degradation. The extent of degradation was evaluated through visual examination, assessment of hair breakage and microscopic observation. pH adjustments were made using HCl or NaOH. A standard tyrosine curve was generated using standard solutions of different concentrations (0-100 µg/mL), with absorbance measured at 275 nm and a linear regression equation of $y=0.0119x+0.0885$ (Fig 1).

On days 0 and 14 of the formal trial period, 2 mL of fasting blood samples were collected from the cephalic vein of each cat. Samples were left to stand for 30 minutes before centrifugation at 3500 rpm for 5 minutes. The serum was stored at -20°C for subsequent analysis. A fully automated blood cell analyzer was used to conduct routine blood tests, assessing the impact of different keratinase doses on feline health. Hematological parameters included hemoglobin (HGB), red blood cell count (RBC), white blood cell count (WBC), neutrophils (NEU), eosinophils (EOS), basophils (BAS) and platelet count (PLT).

Fecal samples were collected from each cat on day 14, stored on dry ice and transferred to a -80°C freezer within one hour. Short-chain fatty acid (SCFA) content was determined using gas chromatography-mass spectrometry (GC-MS). To prepare samples, 50 µL of 30% phosphoric acid solution and 300 µL of acetone were added, followed by homogenization for 3 minutes at 12000 rpm and centrifugation for 10 minutes. The supernatant was appropriately diluted for analysis using an Agilent 7820 gas chromatography system (Agilent Technologies, USA). The DB-FFAP (30 m × 0.25 mm × 0.25 µm) gas chromatographic column was employed, with an injection volume of 1 µL, a split ratio of 10:1, helium as the carrier gas at a 1.0 mL/min flow rate and column oven temperature programming from an initial 70°C for 5 minutes, increasing to 100°C at 6°C/min.

The Coomassie Brilliant Blue method was used to quantify keratin content in fecal samples. Approximately 100 mg of feces was homogenized with 1 mL of cold PBS or

distilled water, supplemented with a protease inhibitor cocktail and centrifuged at 12000 rpm for 10 minutes at 4°C. The supernatant was mixed with SDS-PAGE sample buffer in a 1:1 ratio, vortexed and boiled at 95°C for 5 minutes. Samples were then centrifuged and 20 µL was loaded onto an SDS-PAGE gel for electrophoresis at 200V for 60 minutes. Coomassie Brilliant Blue G-250 staining solution was prepared by dissolving 0.25 g of dye in 45 mL methanol, 45 mL distilled water and 10 mL glacial acetic acid, filtering before use.

Statistical analysis was performed using SPSS version 23.0, with results expressed as mean±standard deviation. Statistical significance was set at $P < 0.05$. Normality and homogeneity of variance were assessed before applying one-way analysis of variance (ANOVA) for between-group and within-group comparisons. If assumptions were unmet, non-parametric tests such as the Kruskal-Wallis test were used. Fecal characteristics, defecation frequency, microbial content and degradation product levels were analyzed using ANOVA. If significant differences were detected, Tukey's test was conducted at a 5% significance level.

RESULTS AND DISCUSSION

In vitro keratinase activity analysis

The variation of keratinase activity at different pH levels and different temperatures during the experimental period is presented in Table 2. The results indicate that enzyme activity is significantly inhibited at pH 2.5, suggesting a temporary suppression of enzyme activity in the gastric environment. Keratinase activity substantially increases at pH 6.8, simulating the intestinal environment. The enzyme activity is most significant at 39°C, while it significantly decreases at the high temperature of 90°C.

Short-term *in vivo* safety analysis

Throughout the entire administration period, no adverse reactions or severe adverse reactions related to the

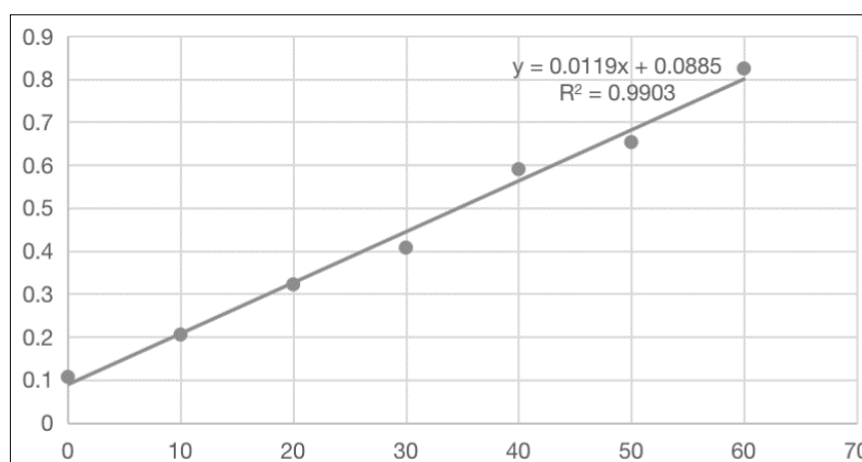


Fig 1: Standard curve of tyrosine.

consumption of keratinase and psyllium husk powder were observed based on weekly vital signs and physical examinations. The results of blood routine examinations and various laboratory indicators related to liver and kidney functions are presented in Table 3 to 4.

Blood routine examination results

Blood routine examinations were conducted on days 0 and 14 of the experiment. The indicators and normal reference ranges are shown in Table 3-4. The changes in the experimental groups and the blank control group at Days 0, 14.

Throughout the experiment, no significant differences ($P>0.05$) were observed across all measured parameters, including inflammatory cell counts, red blood cell-related indices, platelet-related markers and indicators of liver and renal injury. The counts of WBC, NEUT and LYM remained stable within normal ranges, showing no notable variation between experimental and control groups. Similarly, red blood cell parameters such as RBC, HGB, HCT, MCV and MCH exhibited minor fluctuations over time but remained within physiological limits, with no significant differences

among experimental groups or in comparison to the control. Platelet-related parameters, including PLT, MPV, PCT and PDW, followed the same trend, showing only slight variations while maintaining stability. Additionally, markers related to liver and renal function fluctuated within normal limits without statistically significant changes. Overall, all measured indicators demonstrated consistency throughout the study, indicating that the experimental conditions did not induce any adverse physiological effects.

Efficacy analysis

Incidence of trichobezoars in the faeces

As shown in (Fig 2A), we collected trichobezoars of various sizes in the feces. In day 14, cats in the KP-H group produced more feces compared with Control group, with a significant increase in trichobezoars in the feces (Fig 2B-C). At the same time, the number of small hairballs in the feces of the KP-H group increased, while the number of large hairballs decreased (Table 5).

Changes in short-chain fatty acid indicators

The changes in short-chain fatty acid indicators are shown in (Fig 2). As shown in (Fig 3A), total intestinal SCFA increased in a dose-dependent manner with KP supplementation. Compared with the Control group, the KP-H group significantly increased the contents of acetic acid, propionic acid, butyric acid and isobutyric acid and the effect of KP-L was weaker than that of the KP-H group (Fig 3B-E). In addition, KP supplementation did not affect the contents of valeric acid, isovaleric acid and hexanoic acid (Fig 3F-G).

Table 2: Changes of enzyme activity under different pH (%) and temperature (U/mL).

pH	Enzyme activity%
2.5	39.36
6.8	87.25
Temperature(°C)	Enzyme activity U/mL
39	68750
90	35250

Table 3: Changes in blood routine test in each group during the test period.

Subject	Unit	Reference value	Control D0	Control D14	KP-LD0	KP-LD14	KP-HD0	KP-HD14
WBC	$\times 10^9/L$	5.5~19.5	14.60 $\pm$ 11.09 ^a	15.61 $\pm$ 9.04 ^a	17.27 $\pm$ 4.53 ^a	17.98 $\pm$ 3.32 ^a	15.45 $\pm$ 7.00 ^a	14.65 $\pm$ 6.34 ^a
NEUT	$\times 10^9/L$	3.12~12.58	7.89 $\pm$ 1.04 ^a	8.60 $\pm$ 1.42 ^a	10.15 $\pm$ 2.97 ^a	9.27 $\pm$ 1.66 ^a	9.54 $\pm$ 1.67 ^a	8.29 $\pm$ 2.03 ^a
LYM	$\times 10^9/L$	0.73~7.86	3.68 $\pm$ 0.58 ^a	3.56 $\pm$ 0.25 ^a	3.45 $\pm$ 0.53 ^a	3.06 $\pm$ 0.48 ^a	3.17 $\pm$ 1.20 ^a	3.28 $\pm$ 0.91 ^a
RBC	$\times 10^{12}/L$	4.6~10.20	6.79 $\pm$ 0.26 ^a	6.47 $\pm$ 0.34 ^a	7.09 $\pm$ 0.40 ^a	6.87 $\pm$ 0.08 ^a	6.51 $\pm$ 0.27 ^a	7.15 $\pm$ 0.38 ^a
HGB	g/L	85~153	121.30 $\pm$ 2.75 ^a	119.05 $\pm$ 5.16 ^a	109.65 $\pm$ 2.25 ^a	114.75 $\pm$ 2.95 ^a	104.58 $\pm$ 11.20 ^a	117.75 $\pm$ 13.71 ^a
HCT	%	26.0~47.0	37.48 $\pm$ 4.61 ^a	26.90 $\pm$ 0.85 ^a	28.54 $\pm$ 1.09 ^a	28.15 $\pm$ 0.99 ^a	27.80 $\pm$ 0.49 ^a	29.06 $\pm$ 1.35 ^a
MCV	fL	38.0~54.0	41.14 $\pm$ 0.85 ^a	41.98 $\pm$ 0.88 ^a	40.45 $\pm$ 0.78 ^a	42.17 $\pm$ 1.35 ^a	40.95 $\pm$ 1.77 ^a	43.12 $\pm$ 1.79 ^a
MCH	pg	11.8~18.0	14.72 $\pm$ 0.53 ^a	15.16 $\pm$ 0.59 ^a	13.96 $\pm$ 0.30 ^a	14.74 $\pm$ 0.29 ^a	15.49 $\pm$ 0.34 ^a	15.04 $\pm$ 0.32 ^a
PLT	$\times 10^9/L$	100~518	266.80 $\pm$ 24.88 ^a	267.50 $\pm$ 34.84 ^a	273.80 $\pm$ 34.58 ^a	285.16 $\pm$ 27.11 ^a	308.75 $\pm$ 17.53 ^a	302.18 $\pm$ 13.80 ^a
MPV	fL	9.9~16.3	12.46 $\pm$ 0.52 ^a	12.33 $\pm$ 0.45 ^a	12.68 $\pm$ 0.42 ^a	13.06 $\pm$ 0.28 ^a	13.69 $\pm$ 0.76 ^a	13.28 $\pm$ 0.91 ^a
PCT	%	0.09~0.7	0.30 $\pm$ 0.07 ^a	0.25 $\pm$ 0.05 ^a	0.37 $\pm$ 0.03 ^a	0.32 $\pm$ 0.02 ^a	0.41 $\pm$ 0.03 ^a	0.29 $\pm$ 0.01 ^a
PDW	%	12.0~17.5	14.82 $\pm$ 0.29 ^a	13.83 $\pm$ 0.15 ^b	15.28 $\pm$ 0.13 ^a	14.32 $\pm$ 0.08 ^a	14.46 $\pm$ 0.27 ^a	15.08 $\pm$ 0.28 ^{**a}

Note: Results are expressed as mean $\pm$ standard deviation. Different letters (a,b,c) within the same column indicate significant differences ($P<0.05$), while the same letters indicate no significant difference ($P>0.05$).

Table 4: Changes of Serum biochemical indexes in each group during the test period (U/L).

Subject	Unit	Control D0	Control D14	KP-LD0	KP-LD14	KP-HD0	KP-HD14
ALT	U/L	53.56 $\pm$ 4.47 ^a	54.26 $\pm$ 10.6 ^a	58.79 $\pm$ 14.19 ^a	61.53 $\pm$ 9.79 ^a	48.71 $\pm$ 3.72 ^a	57.58 $\pm$ 9.31 ^a
AST	U/L	29.25 $\pm$ 5.73 ^a	31.32 $\pm$ 8.15 ^a	27.56 $\pm$ 3.63 ^a	32.03 $\pm$ 3.71 ^a	27.06 $\pm$ 5.65 ^a	32.15 $\pm$ 8.24 ^a
CREA	μ mol/L	87.31 $\pm$ 3.37 ^a	90.17 $\pm$ 4.25 ^a	86.27 $\pm$ 7.90 ^a	83.78 $\pm$ 5.62 ^a	88.68 $\pm$ 6.42 ^a	92.26 $\pm$ 4.27 ^a
UREA	mmol/L	6.99 $\pm$ 0.35 ^a	7.30 $\pm$ 0.53 ^a	6.19 $\pm$ 0.44 ^a	7.26 $\pm$ 0.35 ^a	8.02 $\pm$ 0.47 ^a	7.94 $\pm$ 0.44 ^a

Coomassie brilliant blue method

The keratin content in feces during the experiment is shown in (Fig 4). Samples from KP-H group showed a higher keratin content compared to the control group.

Table 5: Average distribution of trichobezoars sizes in each treatment group (number/cat/day).

Time	KP-L	KP-H	Control
Small	2.1	5.7	2.7
Medium	4.3	2.2	1.4
Large	1.2	0	1.9

During the *in vitro* experiment, the biological activity of certain keratinases used was tested under pH 2.5 and pH 6.8, the enzyme activity showed more rapid movement under pH 6.8 compared to pH 2.5. While higher enzyme activity was spotted at the temperature of 39°C.

A short-term *in vivo* safety assessment of various dosages of keratinase-psyllium husk showed no significant negative impact on the hepatorenal function of the tested subjects. The combination of keratinase and psyllium husk as a feed additive is rare, so the effect of this combination on overall health was carefully observed during the initial phase of our experiment.

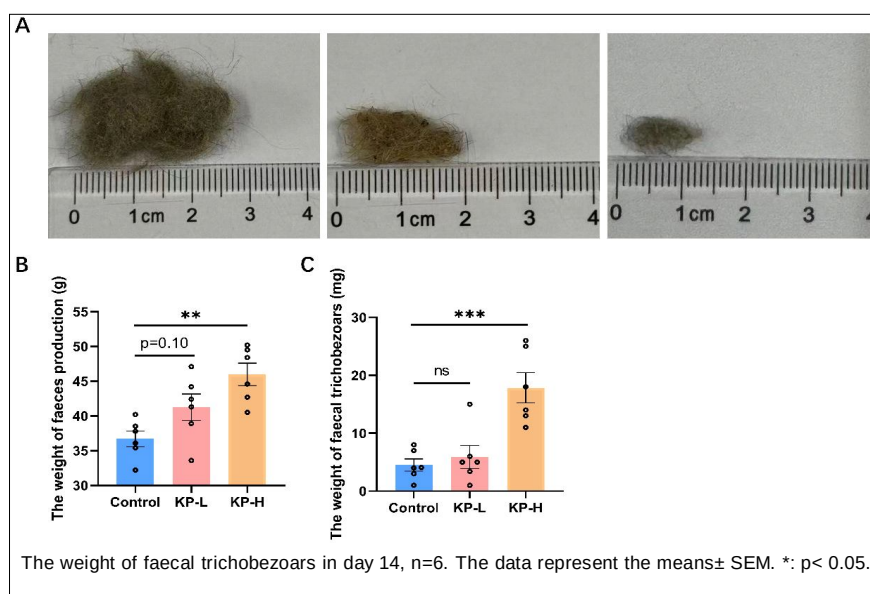


Fig 2: (A) Observed different size trichobezoars in the faeces (from large to small). (B) The weight of faeces production in day 14, n=6. (C).

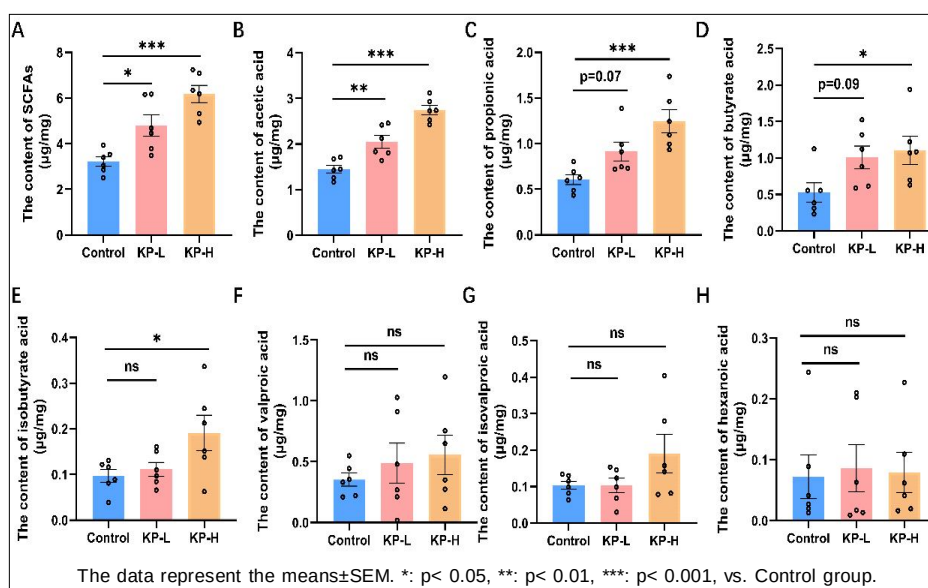


Fig 3: The content of SCFAs (A), Acetic acid (B), propionic acid (C), butyric acid (D), isobutyric acid (E), valeric acid (F), isovaleric acid (G) and hexanoic acid (H) in all groups, n=6.

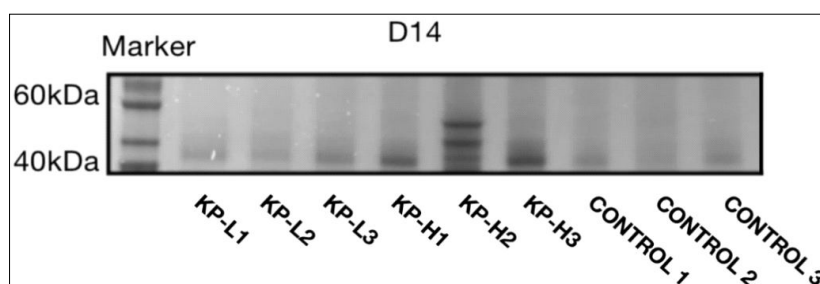


Fig 4: Keratin content in feces on day 14 (D14), Target protein (40kDa–60kDa).

Our results indicated that supplementation with keratinase-psyllium husk did not significantly affect the blood routine or serological indices of the animals. While keratinase is widely used as a feed additive, its thermal stability remains a subject of ongoing discussion (Singh and Kushwaha, 2015). The inclusion of psyllium husk in human diets has been shown to be beneficial in lowering serum cholesterol and other studies have demonstrated that psyllium-enriched extruded dry diets can positively impact feline constipation (Wolever *et al.*, 1994; Freiche *et al.*, 2011). Similar to the observed variability in growth performance among lambs fed sprouted barley (Alharthi *et al.*, 2024), the impact of keratinase-psyllium husk supplementation may depend on factors such as dosage, dietary composition and individual differences.

Considering individual differences in the experiment, in trial KP-H group, there was a significant increase in keratin content compared to the control group in the Coomassie Brilliant Blue test, indicating a certain effect of keratinase on hair degradation and hairball prevention. These results suggest that the administration of keratinase in combination with psyllium husk powder leads to an increased degradation of keratin in the feline gastrointestinal tract, resulting in higher keratin content in feces. The increase in keratin content in the feces on D14 indicates effective breakdown and subsequent excretion of keratin, particularly in KP-H Group, where the increase was most pronounced. This demonstrates the efficacy of keratinase in degrading keratin under the experimental conditions.

In the KP-H trial group, there was a significant increase ($P < 0.05$) in the levels of acetic acid, isovaleric acid and isobutyric acid in feces, indicating that the formula had a positive effect on degrading keratin in the gastrointestinal tract, as expected. Carbohydrates, meanwhile, pass through the upper digestive system and are fermented by gut bacteria in the cecum and large intestine (Roy *et al.*, 2006). Furthermore, keratin degradation by keratinase begins with disulfide bond reduction by disulfide reductase, followed by keratinolytic protease K1 unwinding and cleaving the keratin fibers. Finally, keratinolytic protease K2 breaks down the resulting peptides into amino acids (Nnolim *et al.*, 2020). Additionally, recent studies have shown that Astragalus polysaccharides enhance the intestinal barrier by increasing the levels of isobutyrate in the gut. Isobutyrate

is a short-chain fatty acid that promotes the health of intestinal epithelial cells, maintaining the integrity of the intestinal barrier and reducing the risk of harmful substances entering the bloodstream through the gut wall (Yang *et al.*, 2024). Under normal conditions, fiber fermentation produces short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate, which are crucial for gut health. However, SCFA production decreases when microbes switch to amino acids or fats due to a shortage of fermentable fibers (Koh *et al.*, 2016). This fermentation process produces various metabolites, with SCFAs being the most prominent. Importantly, SCFAs such as acetate, propionate and butyrate account for about 95% of these metabolites and play a vital role in maintaining microbial balance in the gut's anaerobic environment. Around 95% of SCFAs are absorbed in the intestines, while the remaining 5% are excreted in feces (Cook and Sellin, 1998). Moreover, the relative proportions of each SCFA depend on several factors, including the substrate, the composition of gut microbiota and the transit time through the digestive system. Approximately 500-600 mM of SCFAs are produced daily in the large intestine, with the amount being largely influenced by dietary fiber intake. However, psyllium and gums do not contribute to this process (Canfora *et al.*, 2015; Macfarlane and Macfarlane, 2003).

The increase in stool quantity associated with higher dietary fiber intake in the large intestine may result from changes in microbial populations or alterations in the transit time through the large bowel (Hall *et al.*, 2013). In fact, studies have shown that dietary fiber can influence transit time (Lewis *et al.*, 1994). Gut transit time affects the balance between saccharolytic and proteolytic fermentation, while gut microbiota can stimulate gut motility, thereby impacting transit time and SCFA production. This study highlights the direct relationship between gut transit time and the formation of SCFAs (Procházková *et al.*, 2023). Furthermore, increasing evidence suggests that SCFAs offer various health benefits, such as enhancing the gut barrier (Chen *et al.*, 2018), regulating brain functions by promoting intestinal gluconeogenesis and helping to control appetite, glucose and lipid metabolism (De Vadder *et al.*, 2014; Frost, 2014).

CONCLUSION

In this study, a randomized, parallel-controlled clinical trial method was employed. The results indicate that the formula containing keratinase had no adverse effects on blood

indicators or health functions of the organisms and no adverse reactions related to its consumption were observed. Overall, the ratios in the trial KP-H group positively excretion hairballs and degraded cat hair.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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