The usefulness of a hydrolysed fish and rice starch elimination diet for the diagnosis of adverse food reactions in cats: an open clinical trial

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Background – Diagnosis of adverse food reaction (AFR) is based on an eight week elimination diet (ED) and is confirmed by relapse upon re-challenge with the previously fed diet. Hydrolysed EDs are commonly used for this purpose.

Objective – To evaluate the commercially available hydrolysed fish protein and rice starch ED Farmina UltraHypo (FUH) for the diagnosis of feline AFR.

Animals – Thirty-two nonseasonally pruritic cats.

Methods and materials – Pruritus was assessed with a new dual Visual Analog Scale, lesions with the Scoring Feline Allergic Dermatitis scale and Quality of Life with a validated questionnaire on days 0 and 56. Short-acting corticosteroids or oclacitinib were permitted during the first six weeks. Cats showing 50% pruritus and/or lesional improvement were separately challenged with their prior diet, fish and rice. Cats not responding to the study diet were fed another hydrolysed diet for two months.

Results – Twenty-five cats completed the ED: four dropped out due to vomiting and/or diarrhoea, one owing to low palatability and two were lost to follow-up. In 17 cats, pruritus improved by >50% and these underwent dietary challenges. Of these, nine reacted to their prior diet and/or fish and/or rice and were diagnosed with AFR, while eight did not relapse (and a diagnosis of AFR was considered to be doubtful). Of the eight cats in which pruritus did not improve, four underwent a second ED with no improvement.

Conclusion and clinical importance – FUH may be a useful ED for the diagnosis of feline AFR, even in cats reacting to fish or rice.

Introduction

Adverse food reaction (AFR) is defined as any unexpected reaction to food due to immunological and nonimmunological mechanisms, and thus encompasses both food allergy and food intolerance.1 Prevalence of feline AFR is estimated to be approximately 5% of all skin diseases, and ≤21% of all feline pruritic skin conditions.2

The most common dermatological signs of feline AFR are nonseasonal pruritus, leading to self-induced alopecia and excoriations, miliary dermatitis and eosinophilic reactions, albeit not yet determined in cats.3 Extensive protein hydrolysation is an enzymatic proteolytic process that cleaves large proteins into small peptides, thereby reducing the allergenicity of food components. Extensive protein hydrolysis (<10 kD) is important to prevent allergen recognition.4 Proteins present in vegetables also can be recognised by the immune system, and starch rather than flour preferably should be contained in these foods.5–7

Proteins in foods and Pet Food

Hydrolysed EDs are currently considered a valuable tool for the diagnosis of AFR, able to overcome the above-mentioned drawbacks of limited antigen diets. However, only a few investigations on undeclared protein content in these diets have been conducted.8–10 Hydrolysis is an enzymatic proteolytic process that cleaves large proteins into small peptides, thereby reducing the allergenicity of food components. Extensive protein hydrolysis (<10 kD) is important to prevent allergen recognition.11

Proteins present in vegetables also can be recognised by the immune system, and starch rather than flour preferably should be contained in these foods.12

Interestingly, one small in vitro study suggests that even hydrolysed diets could elicit an adverse response in cats comparable

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to that of their basal diet containing the same intact proteins.13 Two studies report the use of hydrolysed diets for chronic vomiting and diarrhea, inflammatory bowel disease and AFR with gastrointestinal signs, yet, to the authors’ knowledge, there are no studies reporting the use of hydrolysed diets for the diagnosis of AFR in cats with dermatological signs.14,15 Recently, a hydrolysed fish protein (herring) and rice starch diet (Vet Life Canine UltraHypo, Farmina Pet Food; Nola, NA, Italy) claiming the absence of peptides >6 kDa in size, was considered suitable for the diagnosis of AFR in dogs.16 A feline formulation of the same diet is commercially available. The aim of this study was to evaluate the performance of Vet Life Feline UltraHypo (FUH) diet for the diagnosis of AFR in cats with nonseasonal pruritic dermatitis. We hypothesised that this hydrolysed fish and rice starch diet would be well-tolerated by cats, including those sensitised to fish and/or rice.

Methods and materials

Animals

Inclusion criteria
Nonseasonally pruritic cats were recruited by four veterinarians in eight referral clinics and included whenever they showed clinical signs compatible with feline allergic dermatitis such as self-induced alopecia, self-inflicted excoriations, mililiary dermatitis and lesions of the eosinophilic granuloma complex. Owing to the fact that some of the included cats could have suffered flea bite allergy, whenever no preventive measures effective against fleas or other parasites had been adopted previously, a furalaner-moxidectin topical solution (Bravecto Plus, MSD; Milan, Italy) was administered one month before inclusion, in order to exclude flea bite allergy as a confounder of the study results.

Exclusion criteria
Cats were not enrolled whenever they showed seasonal pruritic flares or concurrent systemic diseases at the time of inclusion, or if they had been treated with short-acting glucocorticoids or oclacitinib (off-label) in the previous two weeks or with long-lasting glucocorticoids or ciclosporin within the previous two months. Presence of a skin or ear canal infection was not an exclusion criterion.

Evaluation of cats
At the time of inclusion (Visit 1, V1) all cats underwent a dermatological examination, including (when necessary) a cytological evaluation and AFR with gastrointestinal signs, yet, to the authors’ knowledge, there are no studies reporting the use of hydrolysed diets for the diagnosis of AFR in cats with dermatological signs.14,15 Recently, a hydrolysed fish protein (herring) and rice starch diet (Vet Life Canine UltraHypo, Farmina Pet Food; Nola, NA, Italy) claiming the absence of peptides >6 kDa in size, was considered suitable for the diagnosis of AFR in dogs.16 A feline formulation of the same diet is commercially available. The aim of this study was to evaluate the performance of Vet Life Feline UltraHypo (FUH) diet for the diagnosis of AFR in cats with nonseasonal pruritic dermatitis. We hypothesised that this hydrolysed fish and rice starch diet would be well-tolerated by cats, including those sensitised to fish and/or rice.

Informed consent and animal use
In order for their cats to be included in the study, owners needed to give oral informed consent. The food was marketed and labelled for the purpose of AFR diagnosis at the time the study was conducted. Additionally, the procedure of feeding a hypoallergenic diet for eight weeks, followed by challenge periods with the original diet or with single ingredients, was deemed to be the standard of care for the diagnosis of AFR.

Statistical analysis
With the aim of identifying possible clinical parameters that could predict response to ED and/or to provocation tests, several pre-study variables were compared between cats with confirmed AFR, those with doubtful diagnosis and those not responding to the diet. ANOVA was used for age, SCORFAD, pVAS and QoL scores, while Fisher’s test was used for sex and reproductive state. Changes in SCORFAD, pVAS and QoL between V1 and V2 in each group, were expressed as means. All statistical analyses were performed using SAS 9.2 (SAS Institute Inc.; Cary, NC, USA). Significance was set at P < 0.05.

Results
Thirty-two cats were included in the study: 24 European shorthair, two Devon rex, two British shorthair, and one each of European longhair, Persian, Chartreux and Maine coon. The mean age was 5.2 years (range five months–14 years). Ten were male (all castrated) and 22 were female (of which four were intact). Further historical data are given in Supporting information Tables S1 and S2.

Twenty-five cats completed the eight week trial with FUH (Figure 1 and Table S3). Of these, eight did not improve, while 17 (70.8%) were considered to have improved. All 17 cats which improved underwent the provocation test. Upon provocation, nine demonstrated a relapse of clinical signs and a relief of pruritus after reintroduction of the ED and were thus diagnosed with AFR. Among the nine cats with confirmed AFR, four reacted to rice and two to fish. Eight of the 17 cats that improved with the ED relapsed neither with the old diet, nor with fish or with rice, and a diagnosis of AFR was considered to be doubtful.
Eight cats did not improve with the ED (Figure 1 and Table S3). Of these, four underwent the second week hydrolysed ED period, and none of these improved.

Seven cats did not complete the elimination trial period (Figure 1 and Table S3). One refused to eat the diet owing to low palatability, one was euthanised as a consequence of a malignant tumour, one was lost to follow-up, and four had signs of vomiting and/or diarrhoea. Of these four cats, one improved on another hydrolysed ED (Royal Canin Anallergenic), while the others went back to their original diet and were treated symptomatically.

For humane reasons, concurrent antipruritic and/or antibiotic treatments were administered to 15 of the 25 cats that completed the diet (Table S4). Of these, five were diagnosed with AFR, in three AFR was excluded and seven had a doubtful diagnosis. Administered molecules comprised methylprednisolone 1 mg/kg once daily, betamethasone (0.1 mg/kg once daily), topical hydrocortisone aceponate, maropitant (2 mg/kg once daily), oclacitinib (1 mg/kg twice daily), cephalexin (20 mg/kg twice daily) and amoxicillin/clavulanic acid (15 mg/kg twice daily). In every case, with exception of one cat (Case 26 with a doubtful AFR diagnosis), all treatments were withdrawn (≥14 days before the final visit). Details on concomitant treatments in cats which completed the diet are reported in Table S4.

No statistically significant differences in age, sex, reproductive state or double pVAS scores were observed at V1 between cats that were ultimately confirmed with AFR, those that failed to respond to the diet, and those that failed to respond to provocative challenges.

Mean pruritus, lesional and quality of life scores are given in Table 1. In the nine cats with confirmed AFR, pVAS scores improved by a mean of 68.6%, SCORFAD scores by a mean of 87.5% and QoL by a mean of 48.6% at V2 (Table 2). The percentage improvements in other study animals are reported in Table 2. Improvements of all parameters in cats with confirmed or doubtful AFR were statistically significant (Table 1).

### Discussion

There are only two studies evaluating the efficacy of a particular diet in cats with food allergy. One study evaluated the efficacy of two commercial limited antigen diets in maintaining the remission obtained with a home-cooked diet in cats with dermatological manifestations of AFR. The other study investigated the efficacy of a hydrolysed diet in cats with gastroenteric signs of AFR. To the best of the authors’ knowledge, this is the first study to evaluate a hydrolysed ED for the diagnosis of AFR in cats with dermatological signs. Similar to the canine study with the same product, this study suggests that FUH is suitable for use as an ED for the diagnosis of feline AFR.

A decrease of pruritus and clinical signs was observed in 17 of 25 (68%) cats that completed the ED, in line with what was observed in dogs. However, only nine of 17 (53%) cats that improved on the ED and completed provocative challenges reacted to other foods. This resulted in an AFR prevalence of approximately 36% in cats with signs of cutaneous allergy (nine of 25 cats that completed the ED, similar to the percentage described in dogs, and higher than that reported previously in cats. This discrepancy could depend on the low number of studies and of cats analysed, as well as on different diagnostic procedures, including diets used for obtaining the diagnosis.

Two cats improving on the ED did not relapse on their old food, and recurred when fed whole rice. The reason for this is not completely understood: they may have been exposed to more than one diet in the past and not have been fed the culprit diet during the provocation test. This also could be a reason for improving during the ED and not deteriorating during the provocation phase in “doubtful” cases. In fact, similar to the canine study, several cats improved on the diet and did not relapse upon provocation, maybe as a result of a wrong choice of provocation diet, or administration of concomitant treatments (see discussion below) during the first month of ED, or thanks to the good-quality balanced formula of the ED. Those cats with a doubtful diagnosis of AFR were nearly as numerous as those with confirmed AFR, and this finding underpins the importance of performing several provocation tests at the end of the ED trial, for the
confirmation of the diagnosis of AFR. A false positive diagnosis of AFR could possibly imply pointless keeping a cat on an hypoallergenic diet for the rest of its life.

Fifteen of the 25 cats that completed the diet were treated with concomitant antipruritic and/or antibiotic therapy in the first period of the ED. Ideally, in order to precisely evaluate the efficacy of an ED, no treatment should be allowed during its administration; however, it would not have been ethical to leave the patients with pruritus and infections for such a long period of time. Short-acting topical and systemic antipruritic therapies and antibiotics thus were allowed, as generally prescribed in everyday practice, with a withdrawal of at least two weeks before the final evaluation visit. A washout period of two weeks was considered sufficient for the observation of re-occurrence of pruritus and/or lesions in nonresponding cats, similar to what recently has been suggested in dogs.25 Pruritus was considered to relapse earlier than skin lesions upon drug withdrawal, and its improvement at the end of the dietary trial was taken as major outcome of ED success. In just two AFR confirmed cases (cases 3 and 17) pruritus scores did not improve by >50%, and lesional improvement was used to define ED efficacy instead. The first cat (Case 3) had received no treatment during the ED and in the second case (Case 17) topical acepionate spray was withdrawn >28 d before the final visit, so that the lesional improvement observed cannot be considered a consequence of concomitant therapy in either case. Interestingly, seven of eight doubtful AFR cases (versus five of nine cats with AFR and three of eight cats without AFR) had been treated with concomitant therapy in the first month on the ED, which could have been responsible for their improvement during the ED trial period.

Four cats of nine (44%) with confirmed AFR relapsed with rice and two of nine (22%) to fish. These prevalences are much higher than what has been reported previously in dogs fed FUH and other hydrolysed diets.16,23 Gastrointestinal signs associated with this diet were represented by vomiting and diarrhoea in three cases and just diarrhoea in a fourth one, for a total of 12.5% of cats, in line with a previous canine report in which constipation, soft faeces or diarrhoea were observed in 10% of dogs fed hydrolysed diets.24

Cats not improving on the first ED trial were prescribed a second diet trial with a different hydrolysed protein and starch food, in order to minimise false negative diagnoses of AFR. Of four nonresponsive cats that underwent a second ED trial, none improved. However, the numbers are too low to draw a definitive conclusion on the sensitivity of FUH for the diagnosis of AFR in cats. In a previous canine study, 10% of dogs needed a second ED trial for the confirmation of AFR, yet no such data are available for the feline species.21

Similar to the study on dogs fed FUH or other studies on the efficacy of therapeutical interventions for pruritus in allergic cats, it is interesting to note that pruritus and lesions decreased by >60% to approximately 90%, while QoL did not reach 50% improvement in cats with confirmed or doubtful AFR.16,18,19 This observation confirms the need to measure QoL together with clinical parameters when evaluating therapeutical interventions in animals with allergic dermatoses: clinical improvement may not reflect a better quality of life, owing to the burden of the treatment on the pet and/or the owner.

There are several limitations in this study, besides the low number of included cats and the fact that the study was not controlled. One of these is that some of the animals were indoor–outdoor cats and may not have improved owing to uncontrolled access to nonpermitted food sources. Furthermore, in cats that were not on routine parasite control, a systemic parasiticide (fluralaner) was chosen, which could not have provided adequate flea control in flea allergic cats living under high environmental parasite pressure. In these cases pruritus due to flea bite allergy could have negatively influenced the evaluation of the efficacy of the diet. Last, and not least, the dual pVAS (licking and scratching) for the evaluation of feline pruritus used in this study is not yet validated, and may thus not provide a precise quantification of pruritus in cats. To the best of the authors’ knowledge, currently there are no validated feline pruritus scales. Among those used in other studies, the dual pVAS was considered the most reliable one, as it is based on the common observation that cats scratch and/or lick when feeling pruritus, and on the assumption that the two different behaviours should be evaluated separately. Even though the dual pVAS has not

### Table 2. Mean percentage improvement of pruritus score, lesional score and quality of life score after a two month hydrolysed fish and rice starch elimination diet in 25 cats with signs of cutaneous allergy

| Mean percentage improvement          | Number of cats | pVAS       | SCORFAD   | QoL       |
|--------------------------------------|----------------|------------|-----------|-----------|
| Confirmed AFR                        | 9              | 68.6       | 87.5      | 48.6      |
| Improved, no relapse upon provocation| 8              | 61.3       | 67.3      | 47.3      |
| Nonresponders                        | 8              | –3.3       | 21.4      | –4.6      |

AFR, adverse food reaction; pVAS, double pruritus Visual Analog Scale score (range 0–10); SCORFAD, Scoring Feline Allergic Dermatitis lesion index (range 0–16); QoL, quality of life score (range 0–45).
yet undergone complete validation procedures, its performance was substantiated by correlation analyses in a previous study.\(^\text{18}\)

In conclusion, the low prevalence of gastrointestinal adverse effects (12.5%) and the excellent palatability (97%) make FUH a useful option for the diagnosis of AFR in cats, if fed exclusively for at least eight weeks. In the case of failure, it is advisable to undertake a second diet trial with different protein and carbohydrate sources. Provocation tests with several diets or ingredients are always necessary to confirm the diagnosis of AFR.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Table S1.** Onset of pruritus, history of otitis, gastrointestinal signs and anal pruritus in 25 cats with AFR and in cats where this diagnosis was excluded or was doubtful.

**Table S2.** Signalment and historical data of 32 cats included in the study.

**Table S3.** Pruritus scores, lesional scores and quality of life scores of 32 cats with signs of cutaneous allergy that underwent a two month elimination diet with a hydrolysed fish and rice starch hypoallergenic diet.

**Table S4.** Molecules, doses, duration and withdrawal time of concomitant treatments in 25 cats with signs of cutaneous allergy undergoing an elimination diet.
Hydrolysed fish and rice starch diet for the diagnosis of adverse food reaction

Résumé
Contexte – Le diagnostic de réaction alimentaire indésirable (AFR) est basée sur un régime d’éviction de huit semaines (IED) et est confirmé par un test de proc-vocation avec l’alimentation précédente. Les EDs hydrolysées sont fréquemment utilisées à cet effet.

Objectif – Évaluer l’aliment FUH (Farmina UltraHypo) composé de protéines de poisson hydrolysées et d’amidon de riz en tant que ED pour le diagnostic de AFR chez le chat.

Sujets – Trente deux chats présentant un prurit non saisonnier.

Méthodes – Le prurit a été évalué avec une nouvelle échelle visuelle analogue, les lésions avec le score de dermatite allergique féline et la qualité de vie avec un questionnaire validé à jours 0 et 56. Les corticoïdes courte action ou l’oclactininib ont été autorisés au cours des six premières semaines. Les chats montrant une amélioration de 50% de prurit et/ou des lésions ont été provoqués séparément avec leur alimentation initiale, poisson et riz. Les chats ne répondant pas au régime d’éviction ont été nourris avec un autre aliment hydrolysé pendant deux mois.

Résultats – Vingt cinq chats ont complété le régime d’éviction : quatre ont été exclus en raison de vomissements et/ou diarrhée, un en raison de manque d’appétence et deux ont été perdus de vue. Pour 17 chats, le prurit s’est amélioré de plus de 50% et ont été provoqués. Parmi eux, neufs ont réagi à leur alimentation initiale et/ou poisson et/ou riz et ont été diagnostiqués AFR, pendant que huit n’ont pas rechuté (et un diagnostic d’AFR a été considéré comme douteux). Sur les huit cas pour lesquels le prurit ne s’est pas amélioré, quatre ont suivi un nouveau ED sans amélioration.

Conclusion et importance clinique – FUH peut être un ED utile pour le diagnostic d’AFR, même chez les chats réagissant au poisson ou au riz.

Resumen
Introducción – el diagnóstico de reacción adversa a los alimentos (AFR) se basa en una dieta de eliminación (ED) de ocho semanas y se confirma mediante una recaida al volver a exponer a la dieta previamente alimentada. Los ED hidrolizados se utilizan comúnmente para este propósito.

Objetivo – Evaluar la proteína de pescado hidrolizada disponible comercialmente y el almíndon de arroz ED Farmina UltraHypo (FUH) para el diagnóstico de AFR felina.

Animales – treinta y dos gatos con prurito no estacional.

Métodos – Se evaluó el prurito con una nueva Escala Visual Análoga dual, las lesiones con la escala Scoring Feline Allergic Dermatitis y la Calidad de Vida con un cuestionario validado en los días 0 y 56. Se permitió el uso corticosteroides de acción corta u oclactininib durante las primeras seis semanas. Los gatos que mostraban mejoría de un 50% de prurito y/o mejoría de las lesiones fueron expuestos por separado con su dieta anterior, pescado y arroz. Los gatos que no respondieron a la dieta del estudio fueron alimentados con otra dieta hidrolizada durante dos meses.

Resultados – Veinticinco gatos completaron la ED: cuatro abandonaron debido a vómitos y/o diarrea, uno debido a baja palatabilidad y dos se perdieron durante el seguimiento. En 17 gatos, el prurito mejoró en 50% y estos se sometieron a exposiciones dietéticas. De estos, nueve reaccionaron a su dieta previa y/o pescado y/o arroz y fueron diagnosticados con AFR, mientras que ocho no recayeron (y se consideró dudoso el diagnóstico de AFR). De los ocho gatos en los que el prurito no mejoró, cuatro se sometieron a una segunda ED sin mejoría.

Conclusión e importancia clínica – la FUH puede ser una ED útil para el diagnóstico de AFR felina, incluso en gatos que reaccionan al pescado o al arroz.

Zusammenfassung
Hintergrund – Die Diagnose einer Futtermittelnebenwirkung (AFR) basiert auf einer acht-wöchigen Eliminationsdiät (ED) und wird durch ein Wiederauftreten nach einer Provokation mit dem zuvor getüfterten Futter bewiesen. Hydrolysierter EDs werden häufig zu diesem Zweck eingesetzt.

Ziel – Die Evaluator einer kommerziell erhältlichen hydrolysierten Fischprotein und Reisstärke ED Farmina UltraHypo (FUH) zur Diagnose einer felinen AFR.

Tiere – Zweiunddreißig nicht saisonal juckende Katzen.

Methoden – Der Juckreiz wurde an den Tagen 0 und 56 mittels neuer dualer Visual Analog Scale erfasst, die Veränderungen mittels Scoring Feline Allergic Dermatitis Scale und die Lebensqualität mit einem validierten Fragebogen. Kurzwirksame Cortikosteroide oder Oclactinib waren während der ersten sechs Wochen erlaubt. Katzen, die eine 50%ige Verbesserung des Juckreizes und/oder eine Verbesserung der Veränderungen zeigten, wurden individuell mit ihrem früheren Futter, Fisch und Reis, provoziert. Katzen, die sich mit dem Futter der Studie nicht verbesserten, wurde zwei Monate lang eine andere hydrolysierte Diät getüftert.

Ergebnisse – Fünfundzwanzig Katzen beendeten die Studie; vier schieden aufgrund von Vomitus und/oder Durchfall, eine wegen schlechter Akzeptanz des Futters und zwei Katzen, weil es keinen Follow-Up gab, aus. Bei 17 Katzen verbesserte sich der Juckreiz um > 50% und diese wurden Futterprovokationen ausge- setzt. Von diesen reagierten neun auf ihre frühere Diät und/oder auf Fisch und/oder auf Reis und wurden...
**Resumo**

O diagnóstico de reação adversa a alimentos (AFR) é baseado em uma dieta de eliminação (DE) de oito semanas e é confirmado por recidiva após nova provocação com a dieta fornecida anteriormente. DEs hidrolisados são comumente usados para esse propósito.

**Objetivo**

Avaliar a DE à base de proteína hidrolisada de peixe e o amido de arroz disponível comercialmente Farmina UltraHypo (FUH) para o diagnóstico de AFR felina.

**Animais**

Trinta e dois gatos pruriginosos não sabor债券.

**Métodos**

O prurido foi avaliado com uma nova escala visual analógica dupla, as lesões com a escala Scoring Feline Allergic Dermatitis e Quality of Life com questionário validado nos dias 0 e 56. Corticosteroides de curta duração ou oclacinib foram permitidos durante as primeiras seis semanas. Gatos com melhora de prurido de 50% e/ou melhora das lesões foram desafiados separadamente com sua dieta anterior, peixe e arroz. Os gatos que não responderam à dieta do estudo foram alimentados com outra dieta hidrolisada por dois meses.

**Resultados**

Vinte e cinco gatos completaram a DE: quatro desistiram por vômito e/ou diarreia, um por paralatibilidade e dois não retornaram e perdeu-se o contato com os proprietários. Em 17 gatos, o prurido melhorou em > 50% e estes foram submetidos a desafios dietéticos. Destes, nove reagiram à dieta anterior e/ou peixe e/ou arroz e foram diagnosticados com AFR, enquanto oito não tiveram recidiva (o diagnóstico de AFR foi considerado duvidoso). Dos oito gatos em que o prurido não melhorou, quatro foram submetidos a uma segunda DE sem melhora.

**Conclusão e importância clínica**

FUH pode ser uma DE útil para o diagnóstico de AFR felina, mesmo em gatos que resgaram a peixe ou arroz.