A randomized, double-blinded crossover trial testing the benefit of two hydrolysed poultry-based commercial diets for dogs with spontaneous pruritic chicken allergy

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Background – Hydrolysed protein diets are used to diagnose and treat dogs with cutaneous adverse food reactions (CAFR). Little is known about what proportion of dogs hypersensitive to the native protein would react to its hydrolysed form.

Objectives – To determine the clinical allergenicity of hydrolysed poultry feather (RCU) and chicken liver diets (HZD) in dogs with chicken induced CAFR.

Methods – In this randomized, double-blinded, crossover trial, ten dogs with chicken induced CAFR were selected after a positive oral challenge to chicken meat and a negative one to corn. Test diets were fed for 14 days separated by a 14 day wash-out period. Owners rated pruritus daily with a Visual Analog Scale (PVAS). The challenge was ended if a flare in pruritus occurred (i.e. PVAS ≥5/10).

Results – The median PVAS scores before feeding RCU and HZD were 0.9 and 1.7, respectively (Wilcoxon signed rank test, P = 0.46). Pruritus scores increased significantly after feeding HZD (Friedman’s test, P < 0.001) but not after feeding RCU (P = 0.895). None of the dogs fed RCU, but four dogs fed HZD (40%), were withdrawn after a flare in pruritus developed (Fisher’s test, P = 0.04). The maximal PVAS score was significantly higher after HZD (median: 4.7) compared to RCU (2.5) (Wilcoxon signed rank test, P = 0.01). One dog in each group was withdrawn due to diarrhoea.

Conclusions – The hydrolysed poultry feather diet did not induce pruritus flares in dogs allergic to chicken in contrast to the hydrolysed chicken liver diet that led to pruritus flares in 40% of these dogs.

Introduction

Cutaneous adverse food reactions (CAFR) in dogs manifest with a variety of clinical signs that include nonseasonal pruritus with or without concurrent skin lesions. The diagnosis of CAFR is contingent upon demonstrating both clinical improvement during an elimination diet trial with a novel or hydrolysed protein and by the subsequent recurrence of signs after challenge with the original diet.

Identifying a novel protein suitable for a diet trial has become a challenge due to the increased over-the-counter availability of diets containing ingredients included in commercial veterinary diets designed for elimination trials. Moreover, the widespread habit of feeding table scraps and the potential for immunological cross-reactivity between proteins further reduce the number of available proteins for testing.

For almost two decades, veterinarians have been using hydrolysed protein diets for the diagnosis and management of dogs with CAFR as an alternative to feeding novel protein sources. In these diets, the enzymatic hydrolysis of native proteins from chicken liver (Hill’s z/d ultra), poultry feathers (Royal Canin Ultamino/Anallerogenic) or soybeans (Royal Canin HP, Purina HA) is used to reduce their antigenicity following cleavage of peptide bonds. A similar approach has been employed in the management of milk-allergic infants using amino acid-based, extensively hydrolysed (peptide size <3 kDa) or partially hydrolysed (peptide size <5 kDa) diets. The rationale for such interventions is based on the knowledge that, in human patients with IgE-mediated CAFR, allergenic proteins that are able to cross-link IgE antibodies on the surface of mast cells, and thus trigger their activation, are usually larger than 10 kDa. Although the molecular weight of partially and extensively hydrolysed infant formulas is normally below 5 kDa and 3 kDa, respectively, up to 25% of milk allergic children will still react to those diets. Similarly, between 20% and 50% of dogs ingesting partial hydrolysates derived from foods to which they were spontaneously hypersensitive exhibit a worsening of their clinical signs (reviewed in ). The most likely reason for the observed allergenicity could be the presence of large incompletely hydrolysed peptides. Indeed, some partially hydrolysed infant milk formulas

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might contain up to 18% of peptides larger than 6 kDa, whereas some extensively hydrolysed formulas might contain up to 5% of peptides larger than 3.5 kDa, as reviewed.7 Additionally, peptides as small as 3–5 kDa could cross-link IgE antibodies in the form of haptens, or as small as 0.5 kDa could activate lymphocytes in non-IgE-mediated CAFR.8,12–16

Ultamino (RCU), also known as “Anallergenic” (Royal Canin; Gueilph, ON, Canada), is a commercially available hydrolysed poultry feather protein-based diet with purified cornstarch. The company provided analysis reports that more than 99% of peptides found in the final product are below 6 kDa, with larger peptides originating mostly from chickory root fibre. Ninety five percent of the peptides originating from poultry feathers are below 1 kDa in weight and 88% are single amino acids.

Hill’s Prescription Diet z/d Ultra (HZD), recently renamed as Hill’s Prescription Diet z/d Canine (Hill’s Pet Nutrition; Topeka, KS, USA) is a commercially available hydrolysed chicken liver and cornstarch containing diet. The company provided analysis stating that the average size of the animal derived peptides is below 1 kDa; published analysis of the chicken liver hydrolysate reported 78% of peptides to be below 1 kDa, with approximately 7% exceeding 5 kDa.8,17

Our objective was to determine the clinical allergenicity of RCU and HZD diets in dogs with proven chicken induced CAFR.

Material and methods

Study design

This was a prospective, double-blinded, randomized, crossover study comparing the allergenicity of a hydrolysed poultry feather diet (RCU) to that of a hydrolysed chicken liver diet (HZD). The randomization of diets into group A (the diet to be fed first) or B (the diet to be fed next) was done for each individual case by one of the authors (PB) using a computer-generated number. The randomization code was used by the sponsoring company to repackage each diet into identical, sealed, white bags labelled only with a letter A or B. These bags were then delivered to the investigators’ clinic. Although the identical, fully sealed packaging of the two diets guaranteed that all investigators remained blinded, it is still possible that the owner, if interested, could have identified the origin of the diets based on the differences in the colour and shape of the kibbles. The randomization code was kept by a third person employed at North Carolina State University until the last dog finished both phases. This person was then responsible for assigning the interventions based on the randomization sequence for each dog. The assessment of pruritus was performed by the owner before enrolment (Day 0) and on every day of the study (for testing and wash-out phases) using the validated Pruritus Visual Analog Scale (PVAS).18

Selection of study subjects

Ten dogs were estimated to be sufficient to achieve a >95% power of detecting an average difference of 2.5/10 pruritus units with a standard deviation of one unit at P = 0.05 (http://powerandsamplesize.com/Calculators/). Client-owned dogs suffering from chicken induced, pruritic CAFR were selected for the study based on following inclusion criteria: (i) a history of nonseasonal pruritic skin condition with any type of lesional phenotype (atopic dermatitis, urticaria, angioedema, folliculitis, otitis, etc.) currently managed with a nonchicken-based diet (i.e. a “nonreactive diet”); (ii) PVAS ≤2.5 prior to feeding RCU and HZD;19 (iii) a positive challenge to chicken meat in the 2 month period preceding the enrolment; this was defined as a flare of at least moderate pruritus (PVAS ≥5) within 2 weeks of beginning the ingestion of chicken meat; (iv) a negative challenge to corn in the 2 month period preceding enrolment (i.e. no flare of pruritus within 2 weeks of ingesting corn); and (v) no history of gastrointestinal AFRs.

At the time of enrolment, dogs should have been receiving neither oral antihistamines for 7 days, short-acting systemic or topical glucocorticoids for at least 3 weeks nor long-acting injectable glucocorticoids for 6 weeks. The use of cyclosporin was acceptable as this medication had been shown previously not to abrogate the acute clinical response to repeated oral challenges to corn in dogs with spontaneously developing corn-induced CAFR.20 Based on this observation, a dog that had been on cyclosporin for at least 8 weeks could still be included in this study if: (i) it had a positive challenge after eating chicken meat while receiving cyclosporin and (ii) cyclosporin treatment was continued at the same dosage at which the positive challenge occurred for the duration of this trial.

Interventions

Each enrolled dog was fed either RCU or HZD for a maximum of 2 weeks, separated by a 2 week (or until the PVAS remained ≤2.5 for at least one full week)19 wash-out phase, and then the diets were reversed. Dogs received their “nonreactive diet” during the wash-out phase. Daily PVAS scores were recorded by the owner into printed PVAS sheets, which were collected at the end of the study. Phases ended early if the dog developed a flare of pruritus or if other unacceptable adverse events (e.g. persistent vomiting, diarrhoea) developed. In addition, at the end of both phases, owners were asked to comment on the palatability of each diet via a questionnaire (Data S1) and to assess the stool quality using the Nestle Purina faecal scoring system.

Outcome measures

The primary outcome measure was the development of a pruritus flare defined as a PVAS ≥5 within 2 weeks of feeding the RCU or HZD. Secondary outcome measures included the maximum, the average and the final (i.e. at the last day) PVAS values recorded during each 2 week intervention. In addition, owners were asked to assess the stool quality of their dogs while fed the RCU and HZD diets, as well as their perceived palatability of these diets.

Statistics

Because of the relatively small number of dogs in this study, analyses done were nonparametric and results were reported as medians. Within each diet group, the PVAS values were compared using repeated measures ANOVA (Friedman test) with missing values after early withdrawal being replaced using the “last value carry forward” rule. Pruritus values were also compared between RCU and HZD groups using paired t-tests (Wilcoxon signed rank tests), whereas categorical values were compared using Fisher’s test. All analyses were two-tailed and the level of significance was set at 5% (P < 0.05). Analyses were done using Prism 5.0 (Graphpad; San Diego, CA, USA).

Results

Subjects

Ten client owned dogs with chicken induced CAFR characterized by pruritus and skin lesions compatible with atopic dermatitis were enrolled in this randomized, double blinded, crossover trial. None of these dogs exhibited CAFR to corn and none suffered from gastrointestinal AFR. The median age of recruited dogs was 4 years (range: 2–10 years) with a male to female ratio of 3:7; all dogs were spayed or neutered. There were three boxers, two Labrador retrievers, two American Staffordshire terriers, one Doberman, one basenji-crossbred and one Chinese shar-pei.
Seven dogs were maintained only on a limited ingredient or hydrolysed diet, whereas the other three, in addition to a strict diet, had been receiving daily ciclosporin (median dosage: 2.5 mg/kg) and ketoconazole (median dosage: 5 mg/kg) for more than 3 months prior to inclusion in this study (median time: 5 months).

**Assessment of pruritus**

The median PVAS values before feeding RCU and HZD were 0.9 (range: 0–2.5) and 1.7 (range: 0–2.5), respectively (Wilcoxon signed rank test, \( P = 0.46 \)).

Altogether, the PVAS values were not significantly different after feeding RCU compared to those at baseline (Friedman test; \( P = 0.895 \)). Conversely, pruritus scores were significantly different after dogs were fed HZD (\( P < 0.001 \)).

Four of the 10 dogs (40%) had to end the HZD phase early (median: 5.5 days; range: 4–8 days) due to a flare of pruritus, whereas none of the 10 dogs ended the RCU phase early (Fisher’s test, \( P = 0.04 \)) (Figure 1).

Secondary outcome measures are presented in Table 1 and Figures 2–4. Maximal and average PVAS values were significantly higher after feeding HZD than RCU.

**Assessment of diet palatability**

Eight dogs ate both diets immediately and they continued to eat with a full appetite throughout each phase of the study. The other two dogs ate the food immediately (RCU: one dog, HZD: one dog), but their appetite appeared reduced throughout their respective phases.

**Assessment of stool quality**

The median of the faecal consistency scores before feeding RCU and HZD was three for both diets

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**Table 1. Secondary outcome measures**

<table>
<thead>
<tr>
<th></th>
<th>RCU</th>
<th>HZD</th>
<th>RCU versus HZD</th>
<th>( P ) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum PVAS</td>
<td>2.5</td>
<td>0.0–4.3</td>
<td>4.7</td>
<td>0.5–7.4</td>
</tr>
<tr>
<td>Average PVAS</td>
<td>1.3</td>
<td>0.0–3.5</td>
<td>3.0</td>
<td>0.4–4.3</td>
</tr>
<tr>
<td>Final PVAS</td>
<td>1.9</td>
<td>0.0–3.6</td>
<td>4.1</td>
<td>0.2–7.4</td>
</tr>
</tbody>
</table>

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(Wilcoxon signed rank test, \( P = 0.024 \)). One dog fed RCU and another dog fed HZD had to end the corresponding phases early due to diarrhoea (RCU: faecal score 7, Day 5; HZD: faecal score 6, Day 6). The latter dog also concurrently exhibited a flare of pruritus.

**Discussion**

In this randomized, double-blinded, crossover trial, the hydrolysed poultry feather diet did not induce pruritus flares in any of the ten dogs with chicken-induced pruritic CAFR, whereas the chicken liver-based hydrolysed diet caused a moderate flare of pruritus in 4 of 10 of these dogs (40%).

This difference in the reaction rate could be due to a difference in allergenicity associated with a different degree of protein hydrolysis between RCU and HZD. The different protein composition of feathers used as the source of the hydrolysate in RCU compared to HZD represents another possible explanation of their different allergenicity.

In this RCT, 40% of dogs with chicken-induced CAFR exhibited a moderate flare of pruritus after feeding the HZD diet. This finding is compatible with previously reported data in which 20–50% of dogs reacted to hydrolysates derived from ingredients to which these dogs were allergic. One of the reasons for the observed reactivity to HZD diet could be the presence of residual peptides of larger sizes, as 3% of poultry-based peptides were reported to be larger than 10 kDa.

This situation is similar to that existing in human medicine, as extensively hydrolysed formulas developed for children with cow’s milk allergy have been shown to contain up to 2.5% of peptides larger than 6 kDa with the highest detectable size ranging from 7 up to 14 kDa, as reviewed. A review of studies evaluating the efficacy of various extensively hydrolysed formulas showed up to 60% reactivity in children with cow’s milk allergy. Among these formulas, those with the lowest percentage of peptides exceeding 6 kDa appeared to be better tolerated. Unfortunately, there are no available studies that would confirm the suitable threshold of allergenic proteins in extensively hydrolysed diets.

There is a growing body of evidence that non-IgE, lymphocyte-mediated immune pathways could be involved in humans and dogs with delayed type CAFR, such as food-induced atopic dermatitis. Although the exact pathomechanism remains unknown, it is believed that the presentation of food allergens to food allergen-specific T cells plays an important role in disease initiation and perpetuation. Such presentation depends, in the case of CD4+ T cells, on MHC class II molecules presenting peptides ideally 14–20 amino acid (1.6–2.3 kDa) long. Furthermore, very high concentrations of smaller peptides (less than 0.5 kDa) have been shown to be capable of activating T cells in vitro. Considering the peptide size requirements for active stimulation of food allergen-specific T cells, it is not surprising that extensively hydrolysed and, more often, amino acid-based diets have been recommended for management of non-IgE-mediated milk allergies in children. The benefit of an extensively hydrolysed diet in which the majority of the protein is in the form of amino acids in dogs with lymphocyte-mediated CAFR remains to be studied.

Both RCU and HZD contain cornstarch. The allergenicity of this component was not assessed in this study because all participating dogs had been shown not to react to corn in their pre-enrolment challenges. Because cornstarch might still contain traces of higher molecular weight protein, it is possible that this ingredient could trigger an allergic response in corn allergic dogs. Indeed, oral challenge with cornstarch resulted in an adverse reaction in 30% of dogs allergic to corn. Further studies are needed to determine the allergenicity of these diets in corn allergic dogs.

Even though protein hydrolysis tends to increase the bitter taste of the digested product, hydrolysed diets usually appear appetent to dogs; see reviews. Similarly, both RCU and HZD diets were well accepted by all 10 dogs included in this study.

One dog from each diet group had to be withdrawn due to severe diarrhoea. As none of these dogs suffered with gastrointestinal AFRs, one can only speculate on the origin of this diarrhoea. In people, changes in digestibility and osmolarity of the hydrolysates are known occasionally to cause diarrhoea. As the osmolarity is usually directly associated with the degree of hydrolysis, one could hypothesize that the RCU diet would have higher osmolarity than HZD and, therefore, it should cause softer stool and diarrhoea more frequently; surprisingly, this was not observed in this study. Nevertheless, the occurrence of diarrhoea in dogs fed hydrolysed diets is infrequent, and it is currently estimated to be about 10%. This percentage is similar to that seen in our dogs.

In conclusion, the extensively hydrolysed poultry feather diet RCU induced pruritus flares in none of ten dogs allergic to chicken meat. As such, this diet presents a valuable diagnostic and/or treatment tool for dogs suffering with CAFR. Further studies are necessary to address the efficacy of this diet in dogs with corn associated and in those with non-IgE-mediated CAFR.

**Acknowledgements**

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**References**


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

Data S1. Insert questionnaire.
Métodos – en este estudio al azar, doble ciego y cruzado, 10 perros con CAFR inducida al pollo se seleccionaron tras una exposición oral positiva a carne de pollo y negativa a maíz. Las dietas probadas fueron administradas durante 14 días separadas por un periodo de lavado de 14 días. Los propietarios valoraron el prurito diariamente con una escala visual análoga (PVAS). La reexposición se terminó si ocurría un incremento intenso del prurito (PVAS> 5/10).

Resultados – los valores medios de PVAS antes de administrar RCU y HZD fueron de 0.9 y 1.7 respectivamente (prueba de rangos determinados de Wilcoxon, \( P = 0.46 \)). Los valores de prurito aumentaron significativamente tras la administración de HZD (prueba de Friedman, \( P < 0.001 \)) pero no tras la administración de RCU (\( P = 0.895 \)). Ninguno de los perros administrado RCU, pero cuatro perros administrado HZD 40% fueron eliminados tras desarrollar un incremento intenso del prurito (prueba de Fisher, \( P = 0.04 \)). El valor máximo de PVAS fue significativamente mayor tras HZD (media: 4.7) comparado con RCU (2.5) (prueba de rangos determinados de Wilcoxon, \( P = 0.01 \)). Un perro de cada grupo fue eliminado debido al desarrollo de diarrea.

Conclusión – la dieta hidrolizada de plumas de aves domésticas no indujo incremento del prurito en perros alérgicos a pollo, en contraste con la dieta hidrolizada del hígado de pollo que produjo incremento intenso del prurito en un 40% de los perros.

Zusammenfassung

Hintergrund – Diäten mit hydrolysierten Proteinen werden zur Diagnose und zur Behandlung von Hunden mit kutanen Futterunverträglichkeiten (CAFR) verwendet. Es ist wenig bekannt über den Anteil der Hunde, die auf die hydrolysierte Form reagieren würden, wenn sie auf das natürliche Protein hypersensibel sind.

Ziele – Das Ziel dieser Studie war es die klinische Allergenität auf hydrolysierte Putenfeder- (RCU) und Hühnerleberdiäten (HZD) bei Hunden, die eine durch Huhn induzierte CAFR haben, festzustellen.


Ergebnisse – Die medianen PVAS Werte vor der Fütterung von RCU bzw HZD betrugen 0.9 bzw 1.7 (Wilcoxon-Vorzeichen-Rang Test, \( P = 0.46 \)). Die Juckreizwerte nahmen nach der Fütterung der HZD signifikant zu (Friedman’s Test, \( P < 0.001 \)), aber nicht nach der Fütterung von RCU (\( P = 0.895 \)). Keiner der Hunde, die die RCU bekamen, musste aus der Studie genommen werden. Vier Hunde, denen HZD (40%) gefüttert wurde, wurden nach einem Juckreizschub aus der Studie genommen (Fisher’s Test, \( P = 0.04 \)). Der maximale PVAS Wert war nach HZD Fütterung signifikant höher (median: 4.7) im Vergleich zu RCU Fütterung (2.5)(Wilcoxon -Vorzeichen-Rang Test, \( P = 0.01 \)). Ein Hund aus jeder Gruppe wurde wegen Durchfall aus der Studie genommen.

Schlussfolgerungen – Die hydrolysierte Proteinfutterdiäten induzierte keine Juckreizschübe bei Hunden, die auf Huhn allergisch waren, im Gegensatz zur hydrolysierten Hühnerleberdiäten, die bei 40% dieser Hunde zu Juckreizschüben führten.

要約

背景 – 加水分解タンパク食は、皮膚食物有害応答(CAFR)のイスを診断および治療するのに使用されている。本来のタンパクに過敏反応を示すすべての加水分解後のタンパクに反応する割合はほとんど知られていない。

目的 – 鳥肉誘発性CAFRのイスにおいて、加水分解家禽羽毛(RCU)および家禽肝臓(HZD)の臨床的アレルゲン性を調査すること。

方法 – このランダム化二重盲検交差試験では、鳥肉への経口暴露試験に陽性で、トウモロコシに陰性であった10頭の鳥肉誘発性CAFRを選出した。試験を行った給与を14日間ウォッシュアウト期間で分けて、14日間給与した。また、ビジュアルアナログスケール(PVAS)を使って毎日どう評価について記録した。

結果 – RCUおよびHZDを給与前の平均PVASスコアはそれぞれ0.9と1.7であった。サイコロの符号付き検定により、RCUおよびHZDを給与した後有意に増加したが(フィッシャーの検定, \( P < 0.001 \))、RCU給与後は増加しなかった(\( P = 0.895 \)). HZDを給与したイスは脱落しなかったが、RCUを給与したイスは4頭(40%)が、脱落が見られた。脱落したイスの検定(フィッシャーの検定, \( P = 0.04 \)). 最大PVASスコアはHZD(平均:4.7)を給与後、RCUの給与後(2.5)と比較し、有意に高かったサイコロの符号付き検定(\( P = 0.01 \)), それぞれの豚の頭のイスが下痢を理由に脱落した。

結論 – 加水分解家禽羽毛給与は、鳥肉にアレルギーを示したイスにおいてその再燃を誘発しなかった。一方、加水分解家禽肝臓給与はそれらのイスの40%にその再燃を起こした。

要約

背景 – 水解蛋白采用用于诊断和治疗犬食物副反应(CAFR), 但对导致犬过敏的天然蛋白的水解形式,对其过敏比例研究甚少。

目的 – 确定鸡肉过敏犬对水解家禽羽毛日粮(RCU)和家禽肝日粮(HZD)的临床反应原性。
方法 — 设计随机、双盲、交叉实验，选择十只犬，激发试验发现对鸡肉反应阳性、谷物反应阴性，从而确诊为鸡肉过敏。分别饲喂实验日粮14日，隔14日歇日，主人每日使用直观类比标度(PVAS)评估瘙痒。若爆发瘙痒则实验终止(如PVAS ≥5/10)。

结果 — 饲喂RCU和HZD前，PVAS分数中值分别为0.9和1.7(威氏符号秩次检验，P = 0.46)。饲喂HZD后瘙痒指数明显增长(弗氏检验，P < 0.001)，但是饲喂RCU后未出现(P = 0.895)。零只饲喂RCU患犬，四只饲喂HZD患犬(40%)因爆发瘙痒退出实验(费希尔检验，P = 0.04)。HZD组(中值：4.7)最高PVAS分数明显高于RCU组(2.5)(威氏符号秩次检验，P = 0.01)，每组中各有一只犬因腹泻退出实验。

总结与临床意义 — 水解家禽羽毛日粮不会引起鸡肉过敏患犬瘙痒，而水解鸡肝日粮会导致40%鸡肉过敏患犬瘙痒。