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# Non-controlled, open-label trial to assess clinical and immunological parameters in atopic dogs feeding monoprotein grain free diet versus a standard grain diet

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**ABSTRACT**

Canine atopic dermatitis (cAD) is a common inflammatory skin disease that is treated with medicines or allergen-specific immunotherapy. An improvement diet can help treatment of cAD. The purpose of this study was compare two diets on clinical and immunological parameters in atopic dogs without food hypersensitivity. Diet A, a commercial based on rice, was offered to 22 atopic dogs during 30 days and Diet B (grain free, rich in salmon) was given to 8 atopic dogs. Clinical scores were assessed by CADESI-4 and PVAS at the beginning (T0) and at the end of the study (T30). $CD4^+$ and $CD8^+$ were measured in PBMCs, and serum cytokines (TNF-α, IL-10, IL-31 and IL-34) were determined. Both diets decreased CADESI-4 score and Diet A decreased PVAS score ($p < 0.05$). There were no statistical significant differences between diets at T30 for $CD4^+$ and $CD8^+$. A decrease in the IL-31 concentrations and increase in IL-10 levels ($p < 0.05$) was observed with Diet A at T30. There were no differences between any of the two diets when the other results at T0 and T30 were compared for any of the parameters analysed. In conclusion, the results indicate that dietary intervention had not influence on cellular component of the immune system, but a positive effect was observed on IL-31, IL-10 serum levels for Diet A. Further studies are needed to enrich dietary components of the food for atopic dogs without food hypersensibility to help improvement the management of the cAD.

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## 1. Introduction

Canine atopic dermatitis (cAD) is a very frequent disease in canine dermatology currently considered as a multifactorial and complex inflammatory syndrome with an immunological basis (Marsella 2021). The pathogenesis of the disease is complex which includes numerous immunitary mediators, such as cytokines and different T cell subsets (Berker et al. 2017). It has been described changes in the lymphocytes subsets mainly Treg (Hauck et al. 2016; Lee et al. 2020) and different concentrations in the serum levels of regulatory cytokines (Pucheu-Haston et al. 2015a,b; Martins et al. 2018).

Systemic treatment with corticosteroid or ciclosporin (Marsella 2021), oclacitinib (Gonzales et al. 2014), lokivetmab (Michels et al. 2016), or immunotherapy (Keppel et al. 2008), allows cAD to be controlled. Lipid metabolism is crucial in the maintenance of the epidermal barrier, and there are studies that found lower serum levels of polyunsaturated fatty acids (PUFAs) in atopic than healthy dogs (Marsella 2021). However, remains unclear whether these differences are due altered absorption, absolute decrease intake or altered fatty acid metabolism (Pucheu-Haston et al. 2015b).

In dogs, studies have been carried out to see if certain diets or functional foods can help in the treatment of chronic diseases such as cAD, and in this sense, an important effort is being made to try to improve the evolution of cAD through the diet. Some components of the diet, such as PUFAs have influence in T cell subsets in peripheral blood mononuclear cells (PBMC) and in the improvement of the clinical dermatological scoring of atopic dogs (Witzel-Rollins et al. 2019). Recently, the focus has been on

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how the diet can have beneficial effects on the health of pets, with some nutrients in the diet being considered as nutraceuticals (Di Cerbo et al. 2017). A correct diet is a guarantee of health for dogs and can help treating chronic diseases such as cAD.

The aim of this study was to evaluate whether there were significant differences in clinical score (CADESI-4 and PVAS) and immunological parameters (CD4, CD8 T cells, IL-31, IL-34, IL-10, and TNF-α serum concentration) in dogs suffering atopic dermatitis without food hypersensitivity when they were fed with a salmon monoprotein free grain diet vs. a grain standard diet.

## 2. Materials and methods

### 2.1. Ethical approval

This study was included under Project License PI54/17 approved by the Ethic Committee for Animal Experiments for the University of Zaragoza. The care and use of animals were performed according to the Spanish Policy for Animal Protection RD53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes. All clients were informed of potential risks and benefits of participation. Written consent forms were obtained from the owners of the animals evaluated in this study.

### 2.2. Animals

A total of 46 client-owned dogs with cAD were initially included in this study but finished the study with 30 dogs. Dogs were diagnosed with cAD at the Veterinary Hospital, University of Zaragoza, Spain. The diagnosis of cAD was based on clinical history, dermatological examination and a protocol for the exclusion of causes of pruritus that includes strict antiparasitic control, hydrolysed or monoproteic elimination diet for a minimum period of eight weeks, and compliance with five Favrot et al. (2010) criteria. Atopic dogs with concurrent systemic disease (endocrine, metabolic, chronic renal failure, leishmaniosis, and so on) were excluded from the study. Oclacitinib, at a dose of 0.3–0.5 mg/kg every 24h, was administrated to all dogs because this treatment is considered standard of care, and dietary intervention was considered as an adjuvant of the treatment protocol.

This study was a non-controlled, open-label, 30 days long feeding survey. Clinical outcomes classified according to the Canine Atopic Dermatitis Extent and Severity Index (CADESI-4) (Olivry et al. 2014) and Pruritus Visual Analog Scale (PVAS) (Rybnícek et al. 2009) were recorded during the veterinary consultation by the same veterinarian: in the enrolment (T0) and 30 days after (T30) in which blood samples were collected. The dogs were eating a hypoallergenic diet until the time the study began. When animals were in the study, no changes in the diet, supplements or others systemic or topical medications were allowed for the 30 days duration of the study.

Dogs fed Diet A had between 7 and 138 months old (median = 65.5), with 16 males and 6 females and body weight varied between 6 and 64 kg (median = 25). The age interval of dogs feeding Diet B were into 14–120 months old (median = 63.5), there were six males and two females and their body weight varied between 12 and 38.5 kg (median = 17.8). Between two groups, there were 20 breeds of dogs and none predominated over the other.

### 2.3. Diets

All dogs entered into the study were previously on anti-parasitic control and on a trial with hypoallergenic diets. Dogs with a history of previously eating hypoallergenic monoprotein diets received a hydrolyzed diet and those previously fed with hydrolyzed diets received a hypoallergenic monoprotein diet for neither more than 8 weeks. In no case improvement in the CADESI-4 score or the PVAS score were observed. Dogs were offered two types of diet and the distribution was done randomly before the start of the study. Diet A was a commercial available kibbles diet based on rice, salmon and hydrolysed chicken (Compy Supreme fresh salmon and rice, Bynsa-United Petfood Spain). Diet B, was a grain free diet, contained mainly pea starch and fresh salmon (made especially for this research, Bynsa-United Petfood Spain). Details of the diets are shown in Table 1. The two diets reported similar analytical composition in nutrients and the qualitative differences were: Diet A contained grains and salmon 20%; and Diet B was a grain-free diet (pea starch) with salmon monoprotein 40%. Both diets were rich in polyunsaturated essential fatty acids (PUFA) from salmon with a mean concentration of 1.64 gr/100 gr fish for omega-3 fatty acids, and 0.242 gr/100 gr fish for omega-6 fatty acids.

Dogs owners received the diet al.ong with instructions regarding recommended daily feeding quantities. Each dog that participated in the study was randomly assigned the diet it was going to consume.

**Table 1.** Composition of the diets used in the experiment.

| Diet A | Percentage | Diet B | Percentage |
|---|---|---|---|
| Rice + tapioca starch | 36 | Fresh salmon | 40 |
| Fresh salmon | 20 | Pea starches | 29 |
| Dehydrated poultry proteins | 18 | Salmon meal | 12 |
| Corn vegetable proteins | 13 | Pea vegetable proteins | 10 |
| Beer yeast | 1.5 | Vegetables and fruits | 0.12 |
| Aloe vera | 0.03 | Aromatic herbs (*Thymus vulgaris*, *Ocimum basilicium*, *Zingiber officinalis*, St. William's wort, *Rosmarinus officinalis*) | 0.05 |
| Crude protein | 26 | Crude protein | 27 |
| Crude fat | 16 | Crude fat | 17 |
| Crude fibre | 2.5 | Crude fibre | 3 |
| Raw ash | 7.5 | Raw ash | 7 |

Dogs were removed during the course of the study if they had an adverse reaction as diarrhoea or vomiting, or errors in the compliance protocol. Diet A was consumed at the beginning of the experiment by 26 dogs, but at the end of the trial, only 22 did come for follow-up dermatological examinations and blood sampling. Diet B was given to 20 dogs and only 8 of them completed the trial and came for follow-up visits. Twelve dogs fed Diet B did not finish (five showed very soft faeces, four did not want to eat the diet, and three did not attend all controls).

### 2.4. Blood samples

Five milliliters of blood from the jugular veins was obtained for two tubes: 1 mL with EDTA as anticoagulant in one tube and the other 4 mL in a tube without anticoagulant to obtain serum. The tubes were kept at 4 °C for $CD4^+$ and $CD8^+$ T cells analysis by imaging flow cytometry. Serum was obtained by centrifugation (3000 rpm, 4 °C for 15 min) and then maintained at −20 °C until analysis of cytokines by ELISA.

### 2.5. Measurement of $CD4^+$ and $CD8^+$ T cells by imaging flow cytometry

The levels of $CD4^+$ and $CD8^+$ T cells were evaluated in PBMCs. Briefly, 1 mL of blood with EDTA was lysed with 10 mL of erythrocyte lysis buffer (150 mM $NH_4Cl$, 8 mM $KHCO_3$, 2 mM EDTA, pH 7). The lysed blood was centrifuged at 300 × g for 5 min at 4 °C to separate the debris. The sample was then washed three times with PBS. A vial with $1 \times 10^6$ cells in PBS was labeled with 10 µL of rat anti-dog CD4:FITC/CD8:RPE (clone YKIX302.9/YCATE55.9; Biorad, Hercules, CA, USA) at 4 °C for 45 min. The stained cells were fixed with 20 µL of paraformaldehyde before cytometric analysis. Image flow cytometry was conducted using an ImageStream®X cytometer (Amnis®, Seattle, WA, USA). Cell sample acquisition (10,000 events) was performed with the INSPIRE® software and analyzed using the manufacturer's software (IDEAS v6.2). The removal of clustered cells was performed among the focused cells with the biparametric histogram between aspect ratio and area. With this technique it was possible to visualize the separated cells (without doublets) and with the aspect ratio at the same time the round cells were selected. The results are expressed as the percentage of positive cells within the selected gates for cell surface markers.

### 2.6. Serum concentrations of cytokines

The serum levels of the cytokines TNF-α, IL-10, IL-31, and IL-34 were determined using ELISA kits. Canine TNF-α, and IL-10 kits were obtained from DuoSet® ELISA Development Systems (R&D Systems, Inc., MN, USA). The concentrations of serum IL-31 and IL-34 were determined using the canine IL-31 and IL-34 ELISA kits (MyBioSource, San Diego, CA, USA). All samples and controls were analyzed in duplicate, in accordance with the manufacturer's instructions. Absorbance values were read at 450 nm (reference wavelength) using an automatic microELISA reader (Microplate Photometer, HiPo MPP-96; Riga, Latvia). Cytokine levels were calculated by interpolation from a standard curve obtained with the controls provided in the kits.

### 2.7. Statistical analysis

IBM SPSS 26 for Windows (IBM Corp., Armonk, NY, USA) was used for statistical analysis. A Shapiro-Wilk's test was performed to determine if the data were normally distributed. Differences between diets (Diet A, Diet B) at each time were performed with non-parametric unpaired Mann-Whitney U-test and unpaired Student's t-test when the data assumed the normality. Comparison for each diet and at T0 and T30 were performed utilizing Wilcoxon signed rank test and paired Student's t-test when the data assumed the normality. Correlation analysis between cytokine levels and clinical parameters (CADESI-4 and PVAS) was performed using Spearman´s rank correlation coefficient. General Linear Models (GLM) were analysed to assess the influence of 'Group' (Diet A, Diet B) and 'Time' (T0, T30) and their paired interactions on the T cell subset, cytokines concentrations. Statistical significance was set at $p < 0.05$.

## 3. Results

### 3.1. Effect of the diets on CADESI-4 and PVAS clinical scores

Both diets decreased clinical scores of the CADESI-4 index detected at T30 ($p < 0.05$, Table 2), but the reduction percentage of Diet B (64.3%) was greater than that of Diet A (41%). Diet A lowered the PVAS score ($p < 0.001$), but Diet B had no effect on pruritus ($p > 0.05$). No effect of the interaction of diet x time ($p > 0.05$) was observed on clinical scores when GLM analysis was performed, neither when comparison between Diet A and Diet B were made for each time.

### 3.2. Effect of the diets on leukocytes and $CD4^+$ and $CD8^+$ concentration in peripheral blood

Table 3 shows the results obtained for lymphocyte and $CD4^+$ and $CD8^+$ T cells subset for the two diets assayed. There were no statistical significant differences ($p > 0.05$) in the comparison between diets A and B at the beginning (T0) and at the end of the study (T30) for none of the cellular populations analysed. There were also no statistically significant differences ($p > 0.05$) when data for each diet was analysed over time. No effect of the interaction of diet x time was observed ($p > 0.05$) when GLM analysis was performed.

**Table 2.** Effect of two diets consuming over time on CADESI-4 and PVAS in dogs with atopic dermatitis.

| | | T0 | T30 | Percentage reduction (%) | *p* Value[§] |
|---|---|---|---|---|---|
| CADESI-4 | Diet A | 39 ± 42 | 23 ± 28 | 41 | 0.009 |
| | Diet B | 35 ± 43 | 12.5 ± 12 | 64.3 | 0.040 |
| | *p* Value* | 0.653 | 0.319 | | |
| PVAS | Diet A | 5.8 ± 2.7 | 3.3 ± 1.8 | 43 | 0.000 |
| | Diet B | 4.7 ± 2.7 | 4.5 ± 2.4 | 4.2 | 0.811 |
| | *p* Value | 0.349 | 0.131 | | |

[§]Comparison over time for each diet is shown in the rows; *Comparison between groups (Diet A, Diet B) for each time is shown in the columns. Data are means ± SD.

**Table 3.** Percentage (%) and concentration (µl) of T cells subsets analysed by imaging flow cytometry in the peripheral blood from dogs with atopic dermatitis consuming different diets over time.

| | | T0 | T30 | *p* Value[§] |
|---|---|---|---|---|
| Lymphocytes (µl) | Diet A | 1659 ± 1032 | 1621 ± 1295 | 0.359 |
| | Diet B | 1575 ± 1394 | 1522 ± 615 | 0.397 |
| | *p* Value | 0.095 | 0.501 | |
| $CD4^+$ (%) | Diet A | 47.8 ± 11.1 | 53.1 ± 9.5 | 0.074 |
| | Diet B | 45.2 ± 15.9 | 51.7 ± 10.4 | 0.411 |
| | *p* Value | 0.736 | 0.691 | |
| $CD8^+$ (%) | Diet A | 24.4 ± 16.6 | 25.1 ± 14.1 | 0.373 |
| | Diet B | 24.3 ± 14.4 | 31.7 ± 19.4 | 0.423 |
| | *p* Value | 0.819 | 0.368 | |
| $CD4^+$ (µl) | Diet A | 736 ± 348 | 943 ± 856 | 0.644 |
| | Diet B | 577 ± 266 | 774 ± 325 | 0.233 |
| | *p* Value | 0.322 | 0.917 | |
| $CD8^+$ (µl) | Diet A | 342 ± 267 | 419 ± 318 | 0.706 |
| | Diet B | 294 ± 200 | 509 ± 436 | 0.332 |
| | *p* Value | 0.332 | 0.431 | |
| Ratio $CD4^+/CD8^+$ | Diet A | 2.95 ± 1.88 | 2.83 ± 1.36 | 0.878 |
| | Diet B | 2.50 ± 0.98 | 2.41 ± 1.4 | 0.611 |
| | *p* Value | 0.812 | 0.233 | |

[§]Comparison over time is shown in the rows for each diet; *Comparison between groups (Diet A, Diet B) for each time are shown in the columns. Data are means ± SD.

**Table 4.** Serum changes in the concentrations of cytokines analysed by ELISA tests in dogs with atopic dermatitis consuming different diets over time.

| | | T0 | T30 | *p* Value[§] |
|---|---|---|---|---|
| IL-31 (pg/ml) | Diet A | 249 ± 123 | 179 ± 133 | 0.002 |
| | Diet B | 200 ± 102 | 153 ± 76 | 0.567 |
| | *p* Value* | 0.351 | 0.794 | |
| IL-34 (pg/ml) | Diet A | 145 ± 72 | 143 ± 84 | 0.635 |
| | Diet B | 127 ± 55 | 132 ± 54 | 0.911 |
| | *p* Value | 0.531 | 0.95 | |
| TNF-α (pg/ml) | Diet A | 15.7 ± 12.3 | 13.6 ± 10.2 | 0.305 |
| | Diet B | 10.5 ± 7.3 | 10.3 ± 4.8 | 0.732 |
| | *p* Value | 0.223 | 0.905 | |
| IL-10 (pg/ml) | Diet A | 63.6 ± 3.7 | 94.7 ± 36.5 | 0.024 |
| | Diet B | 69 ± 10.2 | 77.7 ± 31.5 | 0.999 |
| | *p* Value | 0.429 | 0.398 | |

[§]Comparison over time for each diet is shown in the rows; *Comparison between groups (Diet A, Diet B) for each time is shown in the columns. Data are means ± SD.

### *3.3. Effect of the diets on serum cytokine levels*

The higher effect of the diet on cytokine levels was observed with Diet A (Table 4). IL-31 ($p < 0.01$) was lower at T30 and increase in the IL-10 level ($p < 0.05$) was detected at the end of the study. However, no statistical significant differences ($p > 0.05$) was observed in the Diet B group, when concentration of cytokines were evaluated at T0 and T30. No effect of the interaction of diet x time ($p > 0.05$) was observed on serum cytokine concentrations when GLM analysis was performed. No differences were found ($p > 0.05$) when comparison between Diet A and Diet B were made for each. IL-31 showed a positive and significant correlation with the CADESI-4 score for dogs eating Diet A ($p = 0.003$) but not for PVAS ($p = 0.0652$). No significant correlation ($p > 0.05$) were found for dogs eating Diet B between IL-31 and CADESI-4 and PVAS. No correlation ($p > 0.05$) were found between IL-34, IL-10 and clinical parameters for none of the diets analysed.

## 4. Discussion

Most of the papers that evaluate the effect of diet on cAD focus mainly on the improvement of clinical parameters, evaluated in the follow-up of the condition of the patients by a decrease in CADESI-4 index and PVAS. Throughout the study, a greater effect of Diet A over Diet B was observed on clinical parameters when data were compared with the basal results. Diet A is based on dehydrated chicken and salmon protein and with grain. Diet B is salmon monoprotein and grain-free. Both diets rich in fish have a large amount of PUFAs with biological effects (Stoeckel et al. 2011). Also, Diet B is rich in antioxidants and botanical ingredients can be considered such as functional foods (Di Cerbo et al. 2017). There were no differences between both diets in decrease CADESI-4 scores at the end of the assay indicating that both were adequate in decrease clinical signs, such as De Santiago et al. (2021) found with diets enriched with antioxidants and polyphenols. However, in our study, a greater effect of Diet B on clinical score was expected, because was richer in salmon and PUFAs than Diet A. Probably, the low statistical power by the few dogs consuming Diet B contributed to the results not reaching statistically significant differences. The percentage of reduction observed on clinical scores with Diet B, was higher than Diet A for CADESI-4, but not for PVAS, so neither diet really helped reduce PVAS. Evaluation of CADESI-4 includes different clinical signs and lesions in addition to pruritus (erythema, lichenification and alopecia/excoriation) and the pruritus score was diluted among the other clinical signs. PUFA-rich diets for dogs have been shown to be suitable for treating and reducing medication needs in atopic dogs (Bensignor et al. 2008; De Santiago et al. 2021; Watson et al. 2022; Szczepanik et al. 2022). However, intervention of the dog diet with essential fatty acid-enriched diets (Bensignor et al. 2008; Watson et al. 2022), only a modest itch improvement was observed following one month such as in our study.

The benefits of the tested diets on clinical parameters were observed at 30 days. In other papers, the time needed to observe positive effects is variable, ranging from two weeks (Szczepanik et al. 2022), one month (Bensignor et al. 2008; Witzel-Rollins et al. 2019; De Santiago et al. 2021) to the three months needed by Watson et al. (2022). The differences observed are probably due to the different composition of the diet, the inclusion of additives such as antioxidants and polyphenols, the number of animals and the use or not of medications to treat the condition. Since cAD is a chronic disease in dogs, it is necessary for the animals to be on the therapeutic diet for a long time to maintain the beneficial effects of the food.

Canine atopic dermatitis is an immunological base disease in which $CD4^+$ and $CD8^+$ T cells and cytokines play an important role to explain the pathogenesis of the disease (Pucheu-Haston et al. 2015a). Other articles have analysed other T cell populations (Majewska et al. 2016; Herrmann et al. 2023) in atopic dogs, but in the study by Jasiecka-Mikołajczyk et al. (2018) demonstrated that oclacitinib had no influence on the expression of CD25 and Foxp3 on $CD4^+$ and $CD8^+$ T cells, but a dramatic loss of $CD4^+$ and $CD8^+$ T cells was caused by oclacitinib when the drug was evaluated *in vitro*. In this study, no effect of the diet on the cells of the immune system was found. An increased of percentage of $CD8^+$ T cells have been described in cAD (Majewska et al. 2016; Verde et al. 2022), suggesting the cellular suppressive mechanisms of the immune response in the pathogenesis of the cAD. The lack of effect of the diet on the lymphocyte population may be due to the fact that the components of the diets were not capable of modifying the percentage of T cells. Also, another possibility was that time necessary to observe the effects of the diet was small, and it was not enough to express the effect of diet on cell populations. In general, more time is required to observe any effect of feeding on immunological parameters. An increase of the $CD4^+$ T cells in the peripheral blood of the atopic dogs has been found (Hauck et al. 2016), but Rostaher et al. (2018) observed an elevated circulating T regulatory cells in an experimental model of cAD that no correlated with the clinical scores. These differences observed in the results could be due to experimental or natural cases of cAD or that the process was acute or chronic.

In this study, all dogs were treated with oclacitinib, because pruritus was the most common clinical sign. Jasiecka-Mikołajczyk et al. (2018) observed a dramatic decreased of both $CD4^+$ and $CD8^+$ T cells evaluated *in vitro* from leukocyte obtained of peripheral blood from atopic dogs attributing to the fact that oclacitinib exerts a proapoptotic action on canine T cells. In a recent study, De Caro Martins et al. (2022) only found a slight increase in $CD4^+$ levels without changes in $CD8^+$ percentages and in the $CD4^+/CD8^+$ ratio in peripheral blood of atopic dogs treated with oclacitinib for one year. Instead, Herrmann et al. (2023) did not find any effect of oclacitinib on the percentage of regulatory T cells ($CD4^+CD25^+FoxP3^+$), indicating the variation of results found depending on whether the studies are done with peripheral lymphocytes *in vivo* or after stimulation with antigens with *in vitro* studies. This observation did not found in our atopic dogs, and no effect of the oclacitinib treatment on lymphocyte population *in vivo* was detected, probably because the dose was low. In this study, Diet B had a high percentage of fresh salmon and salmon meal (52%) with an omega-6/omega-3 ratio of 0.4. Diets rich in salmon are high in fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Stoeckel et al. 2011) which have an important effect on biological functions. The main reason to justify the variation of the results found in the literature review are in the protocols used that differ in age, sex, type of diet, source of the diets, antioxidants and other components of the food, and duration of feeding. We think that it is necessary to carry out more studies to standardize the conditions of the experiment in order to be able to draw conclusions about the effect of diets on the percentage of circulating T cells in atopic dogs.

As a consequence of the atopic condition, elevation of serum IL-31 and IL-34 have been reported with a strong correlation between clinical scores and serum concentration (Gonzales et al. 2014; Gow et al. 2020; Verde et al. 2022) that can be used to diagnosis or follow-up of the disease. In our study, a significant decrease in the serum levels of IL-31 was observed in dogs feeding Diet A for 30 days. We have not found another article in which the effect of diet on serum levels of IL-31 in atopic dogs is evaluated. We also found a positive correlation between the decrease of IL-31 and the CADESI-4 score in dogs eating Diet A, which confirms usefulness of IL-31 as a biomarker for the diagnosis and monitoring of cAD (Verde et al. 2022). IL-34 is a cytokine that has received increasing interest due to its involvement in the pathogenesis of cAD (Gow et al. 2020; Verde et al. 2022). No effect of both diets on serum levels of this cytokine was found. Gow et al. (2020) found no variation in IL-34 serum levels in atopic dogs treated with corticosteroids or oclacitinib. Our atopic dogs were treated with oclacitinib, and no effect on IL-34 serum levels was observed, confirming the results contributed by Gow et al. (2020). TNF-α is secreted by Th1 cells and is implicated in the pathogenesis of the cAD (Berker et al. 2017). We have not found scientific articles of the effect of the diet on this cytokine in atopic dogs to compare our results. TNF-α is a pro-inflammatory cytokine, and the minimum variation observed in this study, indicates no effect of the pharmacological or dietary treatment on serum levels. In recent studies, no differences in the TNF-α serum levels were detected between healthy and atopic dogs (Koury et al. 2019; Verde et al. 2022), suggesting the few variations of TNF-α during the development of the atopic disease in dogs.

An increase in IL-10 serum levels was observed from dogs eating Diet A. IL-10 is an anti-inflammatory cytokine produced mainly by Treg cells that play an important role in the induction and maintenance of the immune response, but interpretation of its role can be complicated (Pucheu-Haston et al. 2015a). The effect of the cAD treatment on this cytokine is variable and higher or lower level compared to healthy dogs or at basal levels have been described (Keppel et al. 2008; Majewska et al. 2016; Koury et al. 2019), and for Mazrier et al. (2022) these findings support that Treg is impaired during cAD. However, serum IL-10 levels in dogs with cAD are often lower than those in healthy animals or basal levels (Majewska et al. 2016; Verde et al. 2022). In our study, both diets increased serum IL-10 levels at the end of the study, especially Diet A, indicating the positive effect of feeding on serum levels of this cytokine. This increase in serum IL-10 levels did not correlate with clinical improvement expressed in CADESI-4 and PVAS scores, as previously reported by Verde et al. (2022) in atopic dogs.

## 5. Conclusions

In conclusion, the main influence on the serum immunological parameters of both diets tested was a decrease of the IL-31 serum levels, a known pruritic cytokine, mainly for Diet A. No influence on cellular component of the immune system assessed by $CD4^+$ and $CD8^+$ T cells in peripheral blood was observed. However, a decrease in CADESI-4 scores at 30 days post-treatment, were observed with both diets; but only Diet B achieved a clinical improvement greater than 50%. Further studies are needed to improve and standardise the components of the diets to control cAD and reduce the clinical signs.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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