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Therapeutic Efficacy of a Nutritional Combination of N-3 PUFAS and *Arctium lappa* Extract on Canine Atopic Dermatitis

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Abstract: The purpose of this study was to evaluate the therapeutic efficacy of a diet containing Polyunsaturated Fatty Acids (n-3 PUFAs) supplemented with the *Arctium lappa* (AL) plant (Fito Progres® ADULT Sensitive, Blaufelden Wiesenbach, Germany) on Canine Atopic Dermatitis (CAD). Twenty atopic dogs were fed a specifically prepared diet for 90 days and were then returned to their usual diet for another 90 days. A physiological and complete dermatological examination was conducted every 30 days. The examination of the dogs' skin included an assessment of the Canine Atopic Dermatitis Extent and Severity Index (CADESI) and the Visual Analogue Scale (VAS) for pruritus based on owner-derived data. The results show a significant reduction ($p<0.01$) in the total CADESI score for the 1st 90 days of the trial (V90) compared to day 0 (V0; selection visit) and the same trend is also evident during the following 90 days. Therefore, the positive effect of the feed persisted for 90 days after the studied diet was suspended. Furthermore, the research shows a highly significant reduction ($p<0.01$) in pruritus during the period the dogs received the studied diet compared to V0 while the pruritus increased significantly in the second period of the trial ($p<0.05$) compared to V90. The number of dogs with pruritus decreased after 90 days of feeding with the formulated diet while it increased after the studied diet was suspended. The results of this study suggest that the studied diet could be a powerful tool in the management of CAD.

Key words: Atopia, dogs, PUFAs, *Arctium lappa*, fatty acids

INTRODUCTION

Canine Atopic Dermatitis (CAD) is a chronic, pruritic and inflammatory skin disease that occurs in 10-15% of the canine population (Singh *et al*., 2009). CAD therefore constitutes a serious medical problem in veterinary medicine and has been reported to be one of the most common causes of canine pruritus (Saridomichelakis *et al*., 1999). The development and severity of the disease are related to a set of interactions among genetic, immunological, environmental, pharmacological and physiological factors as well as to the functional state of skin barriers (Griffin and DeBoer, 2001).

In most cases, the clinical symptoms of CAD begin with pruritus and erythema (Griffin and DeBoer, 2001). There are often secondary infections that contribute to the clinical signs and chronic inflammation along with self-injury. CAD mainly affects young dogs (1-3 years) and can at least initially have a seasonal trend but often tends to become chronic over time. Some breeds (Labrador Retriever, Boxer, German Shepherd, West Highland White Terrier and Cocker Spaniel) are more predisposed to this type of problem (Griffin and DeBoer, 2001). Atopy is caused by an immunologically mediated reaction to allergens which in 80% of allergy-based clinical diseases is mediated by IgE antibodies (Milgrom, 2002). Exposure to an antigen sets off an immune-mediated cascade of inflammatory events that are mediated by some substances such as cytokines and lipid mediators (eicosanoids). Recent studies have shown that a disorder in the metabolism of fatty acids in particular a deficiency of the enzymes delta 5 and 6 desaturases may also be considered as a predisposing cause or an aggravating factor of CAD (Fuhrmann *et al*., 2006). Clinical trials have been performed since 1980 to test the efficacy of pharmacological interventions for the treatment of this disease and the severity of skin lesions

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and pruritus can be reduced with a combination of anti-inflammatory treatments (McDonough *et al*., 2008; Mueller *et al*., 2004 a, b; Olivry and Sousa, 2001; Paterson, 1994; Steffan *et al*., 2006). Anecdotal data suggest that some atopic dogs have clinically improved during diet trials (DeBoer and Griffin, 2001; Hillier and Griffin, 2001). Recently, many articles have been published regarding the nutritional treatment of CAD through, the use of nutraceuticals and phytocomplexes alone or in combinations (Bond and Lloyd, 1992; Logas and Kunkle, 1994; Paterson, 1995; Scott *et al*., 1992).

As far as the use of nutraceuticals is concerned, some studies have demonstrated that oral n-3 Polyunsatured Fatty Acids (n-3 PUFAs) decrease inflammation and pruritus in atopic dogs and feline dermatosis (Scott *et al*., 1992). Other studies have shown that the use of fatty acids can reduce the amount of glucocorticoids or act synergistically with antihistamines (Paterson, 1995). The therapeutic effects of n-3 fatty acids occur either through the action of the fatty acids themselves or through their activated derivatives (eicosanoids). As for phytotherapy, several studies have been performed to screen plants for medicinal activities and especially for skin problems (Mkrtchian *et al*., 1998; Suzuki *et al*., 2002). *Arctium lappa* (AL), a plant member of the Compositae family is used as a traditional medicine in Brazil and throughout Asia and is known to have an anti-inflammatory effect.

Arctiin and its metabolite Arctigenin, Fructooligosaccharides and Fructofuranan are biologically active components that are isolated from parts of the AL plant (Kardosova *et al*., 2003). Some clinical studies have evaluated n-3 PUFAs dietary supplementation for the treatment of CAD; other studies show the phytotherapic effect of the AL contained in the diet on skin problems however, to the researchers' knowledge, there are no studies that describe the effects of a combination of n-3 PUFAs and AL on the dietary management of CAD as an alternative to a pharmacological treatment. The aim of this study was to evaluate the efficacy of a diet rich in fish derived n-3 PUFAs, supplemented with AL (Fito Progres® ADULT Sensitive, Blaufelden Wiesenbach, Germany) for the dietary management of CAD.

MATERIALS AND METHODS

This study was designed as a prospective controlled study. All the visits and food administration were performed with the owners' consent and under good clinical practices (ethical guidance published in No. 289 of the national Gazzetta Ufficiale (GU), 10 December, 1996, 47-53).

Animals and inclusion criteria: Total of 20 privately owned dogs (5 males and 15 females) aged between 1 and 10 years were selected for the study. All the dogs had been suffering from CAD for at least 6 months they were diagnosed according to the diagnostic criteria proposed by Willemse (1986) and resulted positive to the intradermal test. Complete anamnesis, physiological and dermatological examinations were conducted. The initial diagnostic investigation included the absence of skin infections or parasitic infestations, flea and tick control and the absence of food hypersensitivity. The dogs had not been treated with antihistamines, antibiotics, antifungal or topical therapies and no other food or drug had been administered in the 15 days preceding the trial or during the study period.

The experimental trial lasted 180 days. The atopic dogs were fed a diet rich in n-3 PUFAs and supplemented with *Arctium lappa* (Fito Progres® ADULT Sensitive, Blaufelden Wiesenbach, Germany) for 90 days after which the animals were returned to their usual diets for a further period of 90 days. The dogs were evaluated on days 0 (V0, selection visit), 30 (V30), 60 (V60), 90 (V90), 120 (V120), 150 (V150) and 180 (V180). The skin examination of the dogs included the assessment of the Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) (Olivry and Mueller, 2003) and the Visual Analogue Scale (VAS) for pruritus on the basis of owner-derived data (Singh *et al*., 2009). The number of dogs with or without pruritus was also verified.

Statistics: A statistical analysis was performed in two steps for all the CADESI lesions and for pruritus: the first step was descriptive and the second one inferential.

The mean and standard deviation were estimated for each visit for the descriptive statistics. Since, the assumption of normality was not satisfied for all of the visits, a non-parametric analysis was conducted. The Wilcoxon test was then applied to compare each visit with the V0 and to compare V120, V150, V180 with V90.

Finally, Cuzick's non-parametric test for the trend across ordered groups was calculated separately for the period of treatment (i.e., V0, V30, V60 and V90) and for the following period (i.e., V120, V150 and V180).

RESULTS AND DISCUSSION

The nutritive composition and the as fed analytical percentage values of the tested diet, the breed distribution, the weight and sex of the dogs enrolled in the study are shown in Table 1-3, respectively. Table 4 shows the mean scores of the data on the individually tested

lesions (erythema, lichenification, excoriation) and the total assessment of the CADESI score during the selection visit (V0) at 30 (V30), 60 (V60), 90 (V90) days in which the dogs were fed the studied diet and after 30 (V120), 60 (V150) and 90 (V180) days from the return to the usual diet. A highly significant ($p<0.01$) reduction in erythema can be shown in Table 4 for the 90 days of administration of the studied diet, compared to V0. The significant reduction, compared to V0, remains in the period when the dogs began to eat their usual diet (V120, $p<0.05$; V150, $p<0.01$; V180 $p<0.05$). Moreover, researchers have verified that the erythema values at V120, V150 and V180 were not significantly different from V90 which confirms the prolonged efficacy of the studied diet. The excoriation data shows a continuous and highly significant ($p<0.01$) reduction in the severity of the symptoms for the period in which the studied diet was administered, compared to V0. The significant reduction in excoriation, compared to V0, remained in the period when the dogs were fed their usual diets (V120, $p<0.01$; V150, $p<0.01$; V180, $p<0.01$); also in this case, the values at V 120, V150, V180 are not significantly different from V90. Lichenification shows a significant reduction at V30 and V60 ($p<0.05$) and a highly significant reduction at V90 ($p<0.01$), compared to V0.

A significant reduction in lichenification ($p<0.01$) was observed at V120 and at V150 ($p<0.05$), compared to V0. Furthermore, no significant differences were observed between the V120, V180, V150 and the V90 data. This suggests that the therapeutic efficacy of the diet in reducing lichenification is prolonged and persists until 180 days. Finally, Table 4 shows the total CADESI score, it considers all the lesions and represents a summary of the data.Table 4 shows a significant reduction in the total CADESI (erythema, lichenification and excoriation) value at V30, V60 and V90 ($p<0.01$) compared to V0. This reduction is also, evident during the following 90 days (V120, V150, V180, $p<0,01$), compared to V0. Moreover, in the latter period (V120, V150, V180), the values are not significantly different from V90 and this shows that the effect of the feed persists until 180 days from the beginning of the study.

Figure 1 shows the VAS of the pruritus on a 1-10 scale in relation to its intensity. The results show a highly significant reduction in pruritus ($p<0.01$) during the period the dogs were fed the studied diet V30, V60 and V90 compared to V0. The reduction of the pruritus during the suspension period of the studied diet (V120, V150 and V180) is not significant with respect to V0. A significant increase in the pruritus values can be observed comparing the V120, V150, V180 data with the V90 data. This indicates a gradual return of the pruritus due to the return to the usual diet. Figure 2 shows the number of animals without (negative) or with (positive) pruritus. It can be seen that all the atopic dogs except 1 had pruritus at V0 (19 positive and 1 negative), after 30 and 60 days, the number of positive dogs decreased while the negative dogs increased at 90 days, the number of positive

Table 1: Nutrient composition of the test diet the analitycal percentage values are as fed

Nutrient composition	Values
Protein (%)	27.00
Fat (%)	17.00
Total n-6 PUFAs (%)	1.68
Total n-3 PUFAs (%)	0.40
Fibre (%)	2.50
Arctium lappa (%)	0.01
Moisture (%)	10.00
Ash (%)	6.00
Calcium (%)	0.90
Phosphorus (%)	0.75
Vit. A kg^{-1} (UI)	12000.00
Vit. D3 kg^{-1} (UI)	1200.00
Vit. E kg^{-1} (mg)	250.00
Zn kg^{-1} (mg)	75.00
Cu kg^{-1} (mg)	10.00

Ingredients: cereal, fish and fish by-products, vegetable by-products, oils and fats

Table 2: Breed distribution of the dogs enrolled in the study

Breeds	Number
Boxer	1
Dalmata	1
Dogue de bordeaux	1
Labrador	2
Labrador retriever	1
Crossbreed	7
German shepherd	3
Sharpei	1
West highland white terrier	3
Total	20

Table 3: Weight and sexes of the dogs enrolled in the study (mean and standard deviation)

Sex	n	Weight
Female	15	22.7±10.1 (range 5.5-35)
Male	5	36.1±16.3 (range 22-63)

Table 4: Data on the individually tested erythema, lichenification and excoriation and the total CADESI scores (mean and standard deviation)

Lesions	V0	V30	V60	V90	V120	V150	V180
Erythema	15.19±12.38	9.19±12.28**	8.43±12.51**	9.38±15.57**	11.72±13.14*	11.2±12.96**	10.25±13.73*
Excoriation	2.86±5.670	0.66±1.830**	0.38±1.070**	0.34±0.970**	0.09±0.300**	0.2±0.690**	0.50±2.010**
Lichenification	4.09±7.480	3.05±7.170*	1.86±3.800*	1.05±2.750**	0.71±2.300**	1.2±2.780*	1.30±3.060
Total CADESI	22.14±22.20	12.90±19.56**	10.66±15.85**	10.76±17.65**	12.52±13.44**	12.6±13.27**	12.05±14.45**

*V30, V60, V90, V120, V150, V180 vs. V0 ($p<0.05$); **V30, V60, V90, V120, V150, V180 vs. V0 ($p<0,01$); V120, V 150, V180 vs. V90 ($p<0.05$); V120, V150, V180 vs. V90 ($p<0.01$)

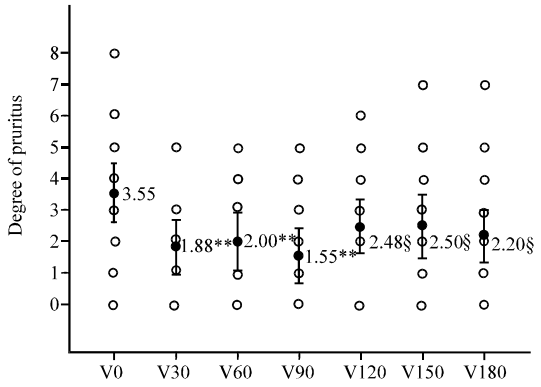

Fig. 1: Changes (mean and standard deviation) in pruritus in VAS (Visual Analogue Score) at V0 (Selection visit), V30, V60, V90 (tested diet from days 0-90), V120, V150 and V180 (standard diet from days 90-180) (n = 20); *V30, V60, V90,V120, V150, V180 vs V0 ($p<0.05$); **V30, V60, V90, V120, V150, V180 vs. V0 ($p<0.01$); §V120, V150, V180 vs. V90 ($p<0.05$); §§V120, V150, V180 vs. V90 ($p<0.01$)

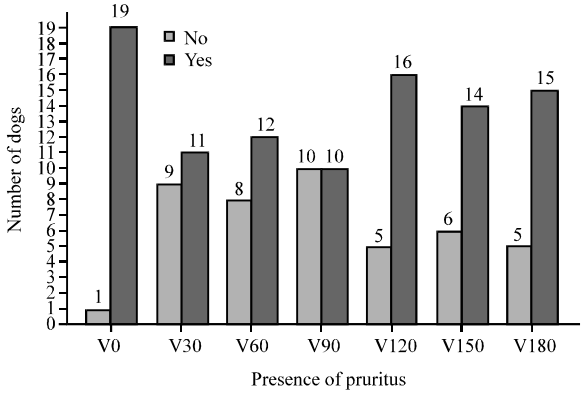

Fig. 2: Changes in the number of dogs with or without pruritus at V0 (Selection visit) and at V30, V60, V90 (tested diet from days 0-90), V120, V150 and V180 (standard diet from days 90-180) (n = 20)

and negative dogs was equal (10 negative and 10 positive). This means that the pruritus disappeared in 9 dogs after 90 days. Finally, the number of dogs with pruritus increased at V180 (15 positive dogs and 5 negative dogs).

CAD is a common and complex inflammatory skin disease (Olivry and Mueller, 2003). The results obtained indicate that the studied diet (Fito Progres® ADULT Sensitive, Blaufelden Wiesenbach, Germany) was effective in significantly reducing the typical lesions of CAD and the positive effect of the feed persisted for 90 days after the studied diet was suspended.

This reduction is due to the effect of the high content of fish derived n-3 PUFAs and the AL supplementation of the studied diet. Some studies explain the action mechanism of PUFAs and AL against CAD; both these substances act on the modulation of the inflammation and immune processes that are characteristic of CAD (Ring *et al*., 2001). The pathogenesis of CAD is complex and allergies can play an important role in the development of this skin disease (Blank and Rivera, 2004; Knipping *et al*., 2008; Pearlman, 1999). Over the last two decades, many studies have investigated the role of the metabolism of PUFAs in the pathogenesis of inflammatory and atopic diseases. Both epidemiologic studies and intervention trials have shown preventive and therapeutic effects of dietary PUFAs in particular omega-3 derived from fish, on the development of CAD (Kitz *et al*., 2006). Long chain n-3 PUFAs give rise to anti-inflammatory eicosanoids and inhibit the pro-inflammatory eicosanoids derived from arachidonic acid and consequently have positive effects in allergic inflammation (Kitz *et al*., 2006). Besides being precursors of anti-inflammatory mediators, n-3 PUFAs also directly influence immune interactions; they in fact play a crucial role in the immune system (Stehle *et al*., 2007). In a recent study, it has been shown that n-3 PUFAs inhibit T-cell proliferation in dogs and have a direct influence on T-helper cell differentiation (Hwang, 2000). In addition, n-3 PUFAs can influence the immune system by acting as second messengers, regulators of signal transducing molecules or transcription factors (Endres *et al*., 1993). N-3 fatty acids have been reported to suppress interleukin-2 production and mononuclear cell proliferation and the decreased production of IL-1 and TNF-alpha is accompanied by an increase in Eicosapentaenoic Acid (EPA) in the membrane phospholipids of the mononuclear membrane. Another action mechanism of PUFAs towards CAD is an increase in the epidermal barrier function. Abnormalities of the stratum corneum of the skin and percutaneous penetration of allergens in dogs with atopic dermatitis have been demonstrated (Campbell and Dorn, 1992; Marsella *et al*., 2006). Increased concentrations of n-3 fatty acids in the diet may change the cutaneous fatty acids profile, improve the epidermal barrier function of atopic dogs and improve the clinical signs of CAD (Campbell and Dorn, 1992). For these reasons, the diets recommended for dogs with atopic dermatitis usually have a higher content of PUFAs derived from fish. The discovery of some phytocomplexes that can be used for the treatment of allergic disease opens the way to important perspectives in human and animal health. The action of *Arctium lappa* is due to the properties of the

active components present in its immune phytocomplex (Actiin and Actigenin). Knipping *et al*. (2008) have been studied the *in vitro* and *in vivo* anti-allergic effects of *Arctium lappa* and have indicated that it is very promising for use in anti-allergic treatments. These studies show that the AL extract significantly *in vitro* reduced the degranulation and biosynthesis of cysleukotrienes in Peripheral Blood Mononuclear Cells (PBMCs) and that the *in vivo* active AL component was able to inhibit an acute skin response due to allergens in mice.

Lin *et al*. (1996) demonstrated that the crude root extract of AL has anti-inflammatory effects as it inhibits carrageenan-induced rat paw oedema. This might be due to its free radical-scavenging activity. Iwakami *et al*. (1992) found that the aqueous extract of AL had an inhibitory effect on the binding of the platelet-activating factor, a compound released from activated basophils, that causes platelet aggregation which is relevant for inflammatory processes such as those observed in allergies. The results of this prospective study show a significant decrease of the clinical signs of CAD in dogs under this diet trial correlable to efficacy of AL. Zhao *et al*. (2009) have investigated the anti-inflammatory mechanism of the Arctigenin contained in AL in this study it was demonstrated that arctigenin suppressed Lipopoly-Saccharide (LPS)-stimulated Nitric Oxide (NO) production and pro-inflammatory cytokine secretion including TNF-alpha and IL-6 in a dose-dependent manner. Arctigenin also greatly inhibited the expression of iNOS and iNOS enzymatic activity. These results indicate that a potent inhibition on NO, TNF-alpha and IL-6 might be responsible for the anti-inflammatory mechanism of Arctigenin may induce the decrease of the skin lesions in CAD. What has been described shows that the effects of n-3 PUFA and AL are based on the anti-inflammatory and immune actions of these substances.

CONCLUSION

In this study, AD is proving to be one of the most complex diseases and correcting one abnormality at a time may lead to only partial success. It is possible that a combination of early modulation of the immune system with an early intervention to improve the barrier function of the skin may lead to the most rewarding results (Marsella and Girolomoni, 2009). The International Task Force for Canine AD currently recommends a multifaceted approach to treat dogs with AD (Olivry *et al*., 2010). It would be interesting to perform haematological control in dogs under diet trial to asses the anti-inflammatory effects (iNOS, TNF-alpha, etc.) in future trials. This prospective study shows that a combination of fish derived n-3 PUFAs and *Arctium lappa* extract, contained in this diet could be used effectively as a nutritional adjuvant in the therapy of CAD.

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REFERENCES

Blank, U. and J. Rivera, 2004. The ins and outs of IgE-dependent mast-cell exocytosis. Trends Immunol., 25: 266-273.

Bond, R. and D.H. Lloyd, 1992. Randomised single-bind comparison of an evening primrose oil and fish oil combination and concentrations of these oils in the management of canine atopy. Vet. Dermatol., 3: 215-219.

Campbell, K.L. and G.P. Dorn, 1992. Effects of oral sunflower oil and olive oil on serum and cutaneous fatty acid concentrations in dogs. Res. Vet. Sci., 53: 172-178.

DeBoer, D.J. and C.E. Griffin, 2001. The ACVD taskforce on canine atopic dermatitis (XXI): Antihistamine pharmacotherapy. Vet. Immunol. Immunop., 81: 323-329.

Endres, S., S.N. Meydani and R. Ghorbani, 1993. Dietary supplementation with n-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. J. Leukocyte Biol., 54: 599-603.

Fuhrmann, H., A. Zimmermann and T. Guck, 2006. Erythrocyte and plasma fatty acid patterns in dogs with atopic dermatitis and healthy dogs in the same household. Can. J. Vet. Res., 70: 191-196.

Griffin, C.E. and D.J. DeBoer, 2001. The ACVD task force on canine atopic dermatitis (XIV): Clinical manifestations of canine atopic dermatitis. Vet. Immunol. Immunop., 81: 255-269.

Hillier, A. and C.E. Griffin, 2001. The ACVD task force on canine atopic dermatitis (X): Is there a relationship between canine atopic dermatitis and cutaneous adverse food reactions?. Vet. Immunol. Immunopathol., 81: 227-231.

Hwang, D., 2000. Fatty acids and immune responses-a new perspective in searching for clues to mechanism. Annu. Rev. Nutr., 20: 431-456.

Iwakami, S., J.B. Wu and Y. Ebizuka, 1992. Platelet Activating Factor (PAF) antagonists contained in medicinal plants: Lignans and sesquiterpenes. Chem. Pharm. Bull., 40: 1196-1198.

Kardosova, A., A. Ebringerova and J. Alfoldi, 2003. A biologically active fructan from the roots of *Arctium lappa* L., var. Herkules. Int. J. Biol. Macromol., 33: 135-140.

Kitz, R., M.A. Rose and H. Schonborn, 2006. Impact of early dietary gamma-linolenic acid supplementation on atopic eczema in infancy. Pediatr. Allergy Immu., 17: 112-117.

Knipping, K., E. Esch and S. Wijering, 2008. *In vitro* and *in vivo* anti allergic effects of *Arctium lappa* L. Exp. Biol. Med., 233: 1469-1477.

Lin, C.C., J.M. Lu and J.J. Yang, 1996. Anti-inflammatory and radical scavenge effects of *Arctium lappa*. Am. J. Chin. Med., 24: 127-137.

Logas, D. and G.A. Kunkle, 1994. Double-blinded cross-over study with marine oil supplementation containing high-dose eicosapentaenoic acid for the treatment of canine pruritic skin disease. Vet. Dermatol., 5: 99-104.

Marsella, R. and G. Girolomoni, 2009. Canine models of atopic dermatitis: A useful tool with untapped potential. J. Invest. Dermatol., 129: 2351-2357.

Marsella, R., C. Nicklin and J. Lopez, 2006. Studies on the role of routes of allergen exposure in high IgE-producing beagle dogs sensitized to house dust mites. Vet. Dermatol., 17: 306-312.

McDonough, A.K., R.J. Curtis and G.K. Saag, 2008. The epidemiology of glucocorticoid-associated adverse events. Curr. Opin. Rheumatol., 20: 131-137.

Milgrom, H., 2002. Attainments in atop: Special aspects of allergy and IgE. Adv. Pediatr., 49: 273-277.

Mkrtchian, T.A., G.G. Snapian and G.A. Nikogosian, 1998. Pectin production from burdock (*Arctium* sp.). Ukr. Biokhim. Zh., 70: 98-105.

Mueller, R.S., K.V. Fieseler and M.J. Fettman, 2004b. Effect of ù-3 fatty acids on canine atopic dermatitis. J. Small Anim. Pract., 45: 293-297.

Mueller, R.S., K.V. Fieseler and S. Zabel, 2004a. Conventional and rush immunotherapy in canine atopic dermatitis. Vet. Dermatol., 15: 1-19.

Olivry, T. and C.A. Sousa, 2001. The ACVD task force on canine atopic dermatitis (XIX): General principles of therapy. Vet. Immunol. Immunopathol., 81: 311-316.

Olivry, T. and R.S. Mueller, 2003. Evidence-based veterinary dermatology: A systematic review of the pharmacotherapy of canine atopic dermatitis. Vet. Dermatol., 14: 121-146.

Olivry, T., D.J. DeBoer and C. Favrot, 2010. Treatment of canine atopic dermatitis: Clinical practice guidelines from the international task force on canine atopic dermatitis. Vet. Dermatol., 21: 233-248.

Paterson, S., 1994. Use of antihistamines to control pruritus in atopic dogs. J. Small Anim. Pract., 35: 415-419.

Paterson, S., 1995. Additive benefits of EFAs in dogs with atopic dermatitis after partial response to anti-histamine therapy. J. Small Anim. Pract., 36: 389-394.

Pearlman, D.S., 1999. Pathophysiology of the inflammatory response. J. Allergy Clin. Immun., 104: S132-137.

Ring, J., U. Kramer and T. Schafer, 2001. Why are allergies increasing?. Curr. Opin. Immunol., 13: 701-708.

Saridomichelakis, M.N., A.F. Koutinas, D. Gioulekas and L. Leontidis, 1999. Canine atopic dermatitis in Greece: clinical observations and the prevalence of positive intradermal test reactions in 91 spontaneous cases. Vet. Immunol. Immunopathol., 69: 61-73.

Scott, D.W., W.J. Miller and G.A. Decker, 1992. Comparison of the clinical efficacy of two commercial fatty acid supplements (EfaVet and DVM Derm Caps), evening primrose oil, and cold water marine fish oil in the management of allergic pruritus in dogs: A double-blinded study. Cornell. Vet., 82: 319-329.

Singh, S.K., U. Dimri and K.S. Saxena, 2009. Therapeutic management of canine atopic dermatitis by combination of pentoxifylline and PUFAs. J. Vet. Pharmacol. Ther., 33: 495-498.

Steffan, J., C. Favrot and R.S. Mueller, 2006. A systematic review and meta-analysis of the efficacy and safety of cyclosporine for the treatment of canine atopic dermatitis in dogs. Vet. Dermatol., 17: 3-16.

Stehle, M.E., T. Goebel and M. Hancaruk, 2007. The influence of polyunsaturated fatty acids on the T cell response in dogs with atopic dermatitis. Vet. Dermatol., 18: 194-194.

Suzuki, S., T. Umezawa and M. Shimada, 2002. Stereochemical diversity in lignan biosynthesis of *Arctium lappa* L. Biosci. Biotech. Bioch., 66: 1262-1269.

Willemse, A., 1986. Atopic skin disease: A review and re-consideration of diagnostic criteria. J. Small Anim. Pract., 27: 771-778.

Zhao, F., L. Wang and K. Liu, 2009. *In vitro* anti-inflammatory effects of arctigenin, a lignan from *Arctium lappa* L., through inhibition on iNOS pathway. J. Ethnopharmacol., 122: 457-462.