Chapter 16

Functional Pet Foods

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Additional information is available at the end of the chapter

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Abstract

Functional foods provide health benefits if they are consumed on regular basis. Some nutraceutical pet diets have been demonstrated to exert health benefits *in vitro* and *in vivo* while also exhibiting palatability to the animals. The aim of this chapter is to provide an overall update of commercially available pet diets with proven efficacy against pathologies with an inflammatory background. Research on pet food is still scarce and biased. The ultimate success of functional pet foods will depend on delivering bioactive components in a predictable and assured manner to effectively reduce the risk of disease and/or support the body. Our investigations outlined the improved health status of sick *dogs* by means of a commercially available nutraceutical pet diet approach. Therefore, additional investigations into the consumption of functional foods in domestic animal nutrition should be done in order to study dietary interventions for disease prevention and treatment.

Keywords: functional foods, nutraceutical pet diets, proven efficacy

1. Introduction

The interest in the efficacy and safety of pet food has been growing worldwide with vegetables, whole grains, fortified active principles, fruits, probiotics, prebiotics, and herbal extracts as the most effective substances available. In addition, the use of antibiotics in the agriculture and intensive farming has also become a relevant concern with consequent potential health risks derived by their entry/accumulation in human food and animal food supply chains.

2. Antibiotic residues in pet food and adverse food reactions

In the last 35 years, a surprising increase of skin and gastrointestinal (GI) diseases in both *cats* and *dogs* has been observed [1]. For instance, *in vivo* studies have widely demonstrated that the most commonly responsible ingredients for the onset of cutaneous and gastrointestinal adverse food reactions are beef, dairy products, wheat, and to some degree, lamb, soy, and fish [2–5]. However, only in a few cases were such adverse food reactions clearly ascribed to the presence of food additives such as dyes, preservatives, or even antibiotics [6–8].

One of the main symptoms of skin-related diseases is severe itching, which may lead to self-inflicted injuries caused by obsessive scratching, while frequent gastrointestinal symptoms are vomiting and diarrhea, which continue to persist after therapy. These phenomena suggest paying particular attention to the history of several examined cases in order to determine their primary and real cause.

We recently identified a specific compound, oxytetracycline (OTC), as the possible underlying cause of most inflammatory pathologies both *in vitro* [9, 10] and *in vivo* [11, 12]. OTC belongs to the class of tetracyclines, which are the most widely and legally used antibiotics in intensive farming, for example, poultry [10], livestock [13], and aquaculture [14], due to their low cost and efficacy [15]. Unfortunately, OTC also has a high affinity for calcium-rich tissues such as bone and teeth [16] and can remain fixed for extended periods in treated animals even respecting withdrawal time [10]. Moreover, pet food production relies on meat (mainly poultry) by-products, which are mechanically separated [17, 18]. This kind of separation, and the common use of important percentages of bone meal mixed with meat meal, generates a bone-based meal-bearing OTC residues that is present in commercially available diets (canned, semi-moist, and especially dry) in a percentage of 20–30% and can accumulate within pet's body.

Odore et al. and Di Cerbo et al. recently demonstrated the significant *in vitro* toxicity of milled bone from chickens treated with OTC, either alone or diluted 1:4, toward peripheral blood mononuclear cell (PBMC) culture (**$p < 0.01$ and ***$p < 0.001$, respectively) [9, 10]. Conversely, bone derived by chickens untreated with OTC did not show any cytotoxic effect (**Figure 1**).

Furthermore, Di Cerbo et al. have recently shown the *in vitro* ability of the OTC to induce a significant interferon (IFN)-γ release from human T lymphocytes and non-T cells [9].

Besides the ability to induce mortality of both the T lymphocytes and non-T cells after a 48-h co-incubation, OTC was also able to induce the release of pro-inflammatory cytokines in the first 10–12 h of challenging. More in detail, T lymphocytes increased their IFN-γ production once exposed to OTC or to the culture media conditioned with the bone derived by OTC-treated chickens in order to resemble the same conditions of intensive farming [15].

Both the innate immunity (non-T cells, mainly represented by natural killer (NK) lymphocytes) and the acquired immunity (T lymphocytes, $CD8^+$, and $CD4^+$) [19, 20] resulted to be influenced by the OTC toxicity (**Figure 2**).

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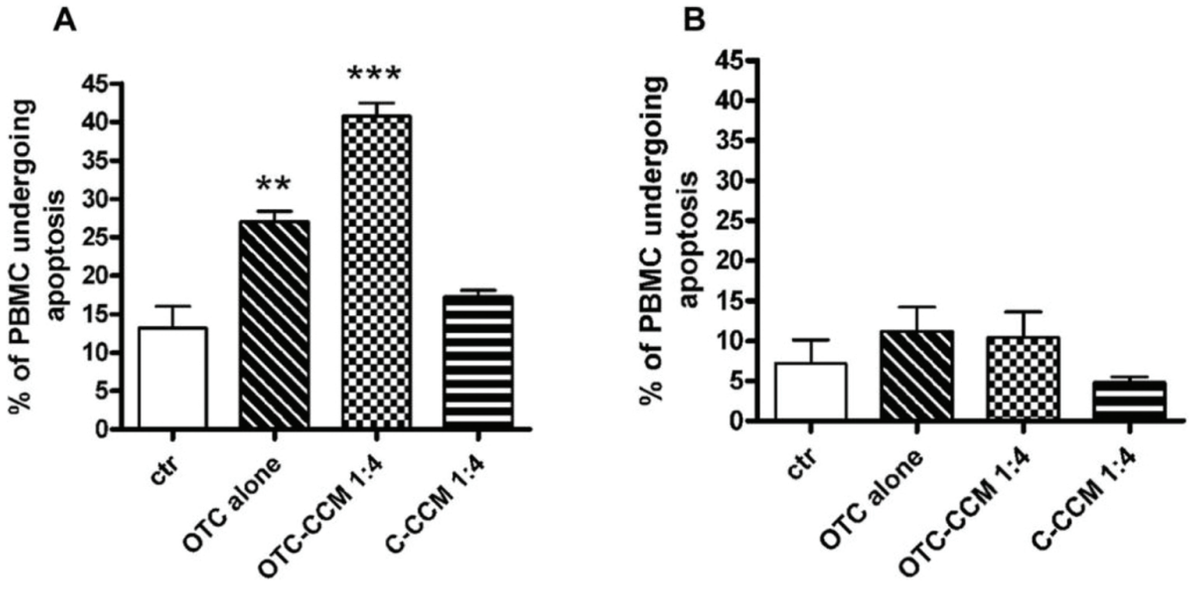

Figure 1. Percentage of PBMC undergoing apoptosis. On the *x*-axis, different cell incubations and conditioned cell culture medium dilutions, after 12h (B) and 24h (A) of incubation, are shown. OTC-CCM indicates the conditioned cell culture medium challenged with a ground bone of chickens treated with OTC; the C-CCM indicates the growth medium challenged with a ground bone of chickens treated without OTC, while OTC alone indicates a growth medium with the addition of 1 μg/ml of OTC. The "ctr" indicates the incubation in the growth medium with Annexin V staining, which has been used as a control of the apoptosis that occurs in the cells when in a culture without any other incubation is maintained; $^{**}p < 0.01$, and $^{***}p < 0.001$ (with the permission of John Wiley and Sons) [18].

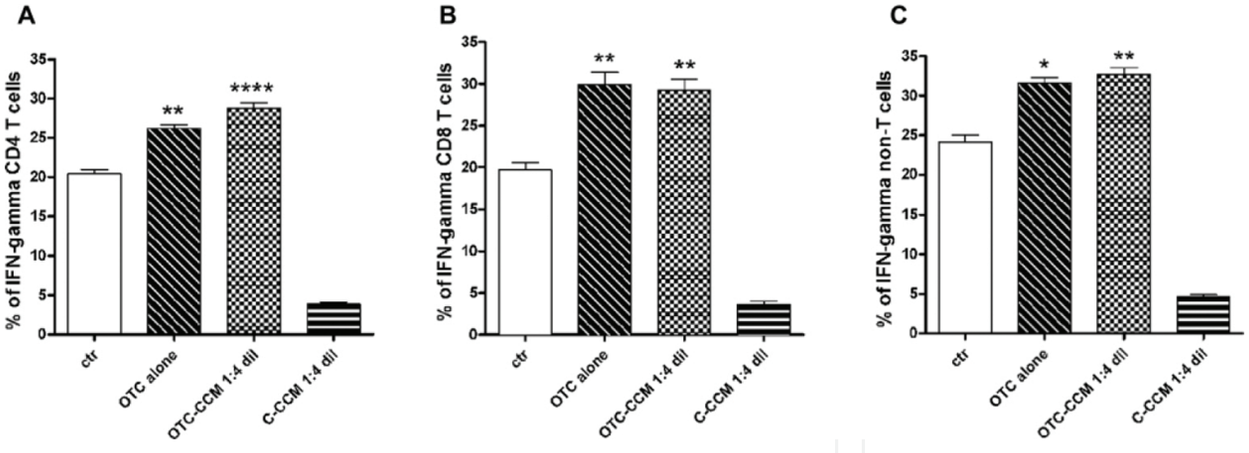

Figure 2. Percentage of IFN-γ production in CD4+ and CD8+ T lymphocytes and in non-T cells. The bar column graphs represent the mean values of the percentage of IFN-γ-producing cells. On the *x*-axis, different cell incubations and conditioned cell culture medium dilutions are shown. OTC-CCM indicates the conditioned cell culture medium challenged with a ground bone of chickens treated with OTC; the C-CCM indicates the growth medium challenged with a ground bone of chickens treated without OTC, while OTC alone indicates a growth medium with the addition of 1 μg/ml of OTC. The condition indicates as "ctr" refers to basal IFN-γ production. All the cell cultures (ctr, OTC alone, OTC-CCM, and C-CCM) were maintained in a growth medium added with PMA and ionomycin to induce cytokine production. Panels A, B, and C show IFN-γ production in CD4+ T lymphocytes, CD8+ T lymphocytes, and in non-T cells, respectively; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.001$ (with the permission of John Wiley and Sons) [18].

In this context, it is known that IFN-γ represents the main cytokine involved in the immune response [21], as well as a crucial element in the onset of impaired tissue homeostasis conditions, typically related to autoimmunity or chronic inflammation [22–30].

These observations clearly reinforce the potential toxicity associated with chronic consumption of poultry bones and derivatives by pets and pave the way for a new concept of food sensitization due to contaminant presence as main enhancers of inflammatory processes, which typically characterize skin and gastrointestinal diseases. Although the Food and Drug Administration [31] and World Health Organization [32] have recently established maximum residue limits in foods, antibiotic residues in foods may still be present [33] thus explaining the persistence of dermatological manifestations in many pets. Moreover, international laws do not impose an antibiotic concentration evaluation in bones and fat, which are considered inedible, thus making pet food dangerous for pet's health [34].

A wide number of scientific reports suggest the possible toxicity and harmfulness of OTC toward human and pet health as a consequence of the consumption of meat derived from intensive farming [15, 35–40].

All of these data may explain why chicken proteins, widely considered hypoallergenic and highly effective from a dietary point of view, play an important role in the etiology of several inflammatory pathologies. It is worth noting that the similarities between these phenomena and food allergies, atopy, and Flea allergy dermatitis have been observed. In spite of the limited evidence that canine food allergy is suggested to resemble a type I hypersensitivity reaction to allergens ingested by food, it cannot be excluded that non-IgE-mediated food allergies may also occur. Although literature reports have been evidenced that the prevalence of food allergies in *dogs* and *cats* is still unknown, the impressive number of cases is not justified merely on the basis of increased allergy spreading in civilized societies. Furthermore, it has been observed that 25% of the *cats* with both chronic GI and skin problems do not clinically express food allergies, while the remaining 75% had only gastrointestinal problems. On the other hand, there are no data regarding food allergies related to gastrointestinal problems in *dogs*. In addition, food allergies can be often confused with pyoderma, pruritic exudative dermatitis or "hot spots."

Based on our recent studies, we investigated the sera of 24 *dogs* with food-adverse reactions, that is, itching, diarrhea, otitis, dermatitis, conjunctivitis, overnight fasting, vomiting, flatulence, interdigital pyoderma, and anal sacs repletion for the presence of any haptens which might be responsible for such conditions by means of an enzyme-linked immunosorbent assay (ELISA) (FS0059, IDLABS™ Inc. Biotechnology, PO Box 1145, Station CSC, London ON N6A 5K2, Canada) according to the manufacturer's instructions [12].

Results indicated the presence of OTC and doxycycline in all animal sera. Although only eight out of 24 *dogs* (33%) showed antibiotic concentrations above the ELISA detection limit (7.5 ng/ml or ppb), all the remaining *dogs* presented serum levels of both antibiotics. OTC serum levels ranged from 2.61 to 56.04 ng/ml (6.30 ± 2.12; mean ± standard error of the mean), whereas doxycycline serum levels ranged from 1.28 to 22.84 ng/ml (5.20 ± 0.89; mean ± standard error of the mean). Our preliminary clinical investigation further confirmed the haptenic toxic-

sensitizing mechanism due to prolonged subliminal oral intake of OTC-enriched bone-meal-based feeds derived from animals grown under a chronic tetracycline administration regime.

3. Herbal extracts: possible pets' health allies

What differentiates common pet food from a functional pet food is the presence of a protein source free of any contaminants, for example, antibiotics and hormones (as happens in intensive farming) as well as the addition of antioxidants, minerals, trace elements, herbal extracts, and medical plants in order to, respectively, stabilize, preserve, and improve the whole nutritional profile of the food.

Many scientific studies clearly demonstrate the efficacy of functional herbal extracts or medical plants for disease prevention or treatment, to improve overall health status or even to delay aging [41].

Based on these observations, we recently studied the anti-inflammatory and antitoxic activity of a well-standardized mixed pool of herbal extracts, as part of a commercially available pet food diet, following an OTC challenge [42]. More in detail, the extracts within the pool were *Ascophyllum nodosum* (66.3%), *Cucumis melo* (1.5%), *Carica papaya* (3.1%), *Aloe vera* (3.1%), *Haematococcus pluvialis* (1.1%), *Curcuma longa* (2.3%), *Camellia sinensis* (1.5%), *Punica granatum* (1.5%), *Piper nigrum* (0.6%), *Polygonum cuspidatum* (1.5%), *Echinacea purpurea* (3.1%), *Grifola frondosa* (6.3%), and *Glycine max* (4.6%).

As previously explained, OTC is able to induce a significant IFN-γ release from human T lymphocyte and non-T cell [9] *in vitro*. Here, we demonstrated that this significant release was significantly reduced after a 24-h individual co-incubation of human T lymphocyte and non-T cells with all aforementioned extracts with the only exception of *A. vera* (**Figure 3**).

Further, we reported the same antitoxic and anti-inflammatory activity of these extracts, with the only exception of *C. papaya* and with *P. nigrum*, on canine T lymphocytes challenged with OTC (**Figure 4**).

The antitoxic and anti-inflammatory activity exerted by the extracts represents a further proof of the usefulness of the addition of selected and standardized herbal extracts within a pet food possibly free of any contaminant. In this way, it is possible to achieve a functional pet food able to support and enhance standard pharmacological treatments in the presence of infections or inflammatory diseases.

However, it is worth noting that enzyme deficiencies or different metabolic pathways make some plants, for example, onions, leeks, garlic, and chives, toxic for *dogs* and *cats* but not for humans [43]. For instance, one of the toxic effects of these plants is the oxidative hemolysis, which results from the inability of the antioxidant metabolic pathways to counteract an excess of oxidants in the erythrocytes. The toxicological mechanism is the following: (1) oxidation of the exposed β-93 cysteine residues present in the hemoglobin and consequent sulfhemoglobin formation, (2a) precipitation, aggregation, and binding of sulfhemoglobin to the cell membrane and formation of the Heinz bodies, and (2b) membrane cross-linking reactions occurring

and eccentrocyte formation, (3) erythrocyte fragility increase and consequent extravascular hemolysis, (4) decreased blood oxygen transportation capacity, and (5) impaired delivery of oxygen to the tissues [44–46].

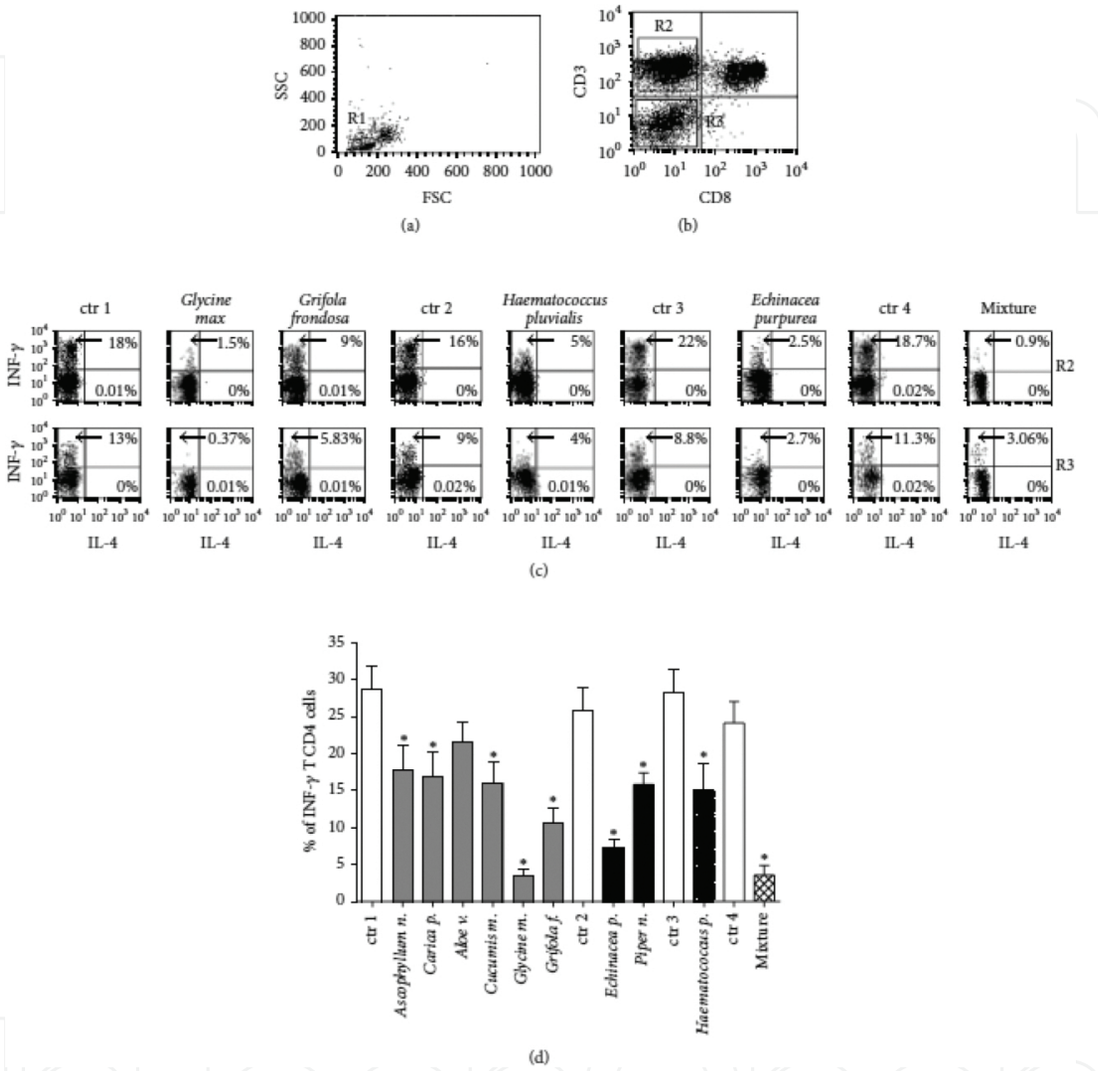

Figure 3. The effects of botanicals on cytokine production by human PBMCs. (a) shows the gating on viable lymphocytes (R1 in dot plot graph) based on FSC and SSC parameters (see Section 2); (b) represents the gating on TH lymphocytes ($CD3^+$ $CD8^-$ as R2 in the dot plot graph) and on non-T cells ($CD3^-$ $CD8^-$ cells as R3 in the dot plot graph); and (c) shows the IFN-γ and IL-4 production in human TH lymphocytes and non-T cells incubated with ad hoc medium derived from botanicals or from mixture (see Section 2). Cytokine production was evaluated as percentage of IFN-γ (*y*-axis) and IL-4 (*x*-axis)-producing cells. The percentage of IFN-γ (upper left quadrant inside the dot plots) and IL-4 (lower right quadrant inside the dot plots)-producing CD4 T (R2) and non-T (R3) cells is reported. The different cell incubations with ad hoc medium derived from botanicals or from mixture (see Section 2) are indicated on top of each graph. (d) reports the statistic representation of 10 experiments on human $CD4^+$ T lymphocytes evaluated as percentage of IFN-γ-producing cells, $^*p < 0.05$. The different cell incubations with ad hoc medium derived from botanicals or from mixture (see Section 2) are indicated on top of each column. The abbreviation "ctr" in (c) and (d) indicates the basal cytokine production by PMBCs stimulated by PMA and ionomycin and in the presence of the ad hoc medium based on the same solubilizing vehicle but free from the botanicals (see Section 2); specifically, ctr 1 (*Ascophyllum n.*, *Carica p.*, *Aloe v.*, *Cucumis m.*, *Glycine m.*, and *Grifola f.*), ctr 2 (*Echinacea p.*, *Piper n.*), ctr 3 (*Haematococcus p.*), and ctr 4 (the mixture of all the botanicals) (with the permission of Hindawi) [42].

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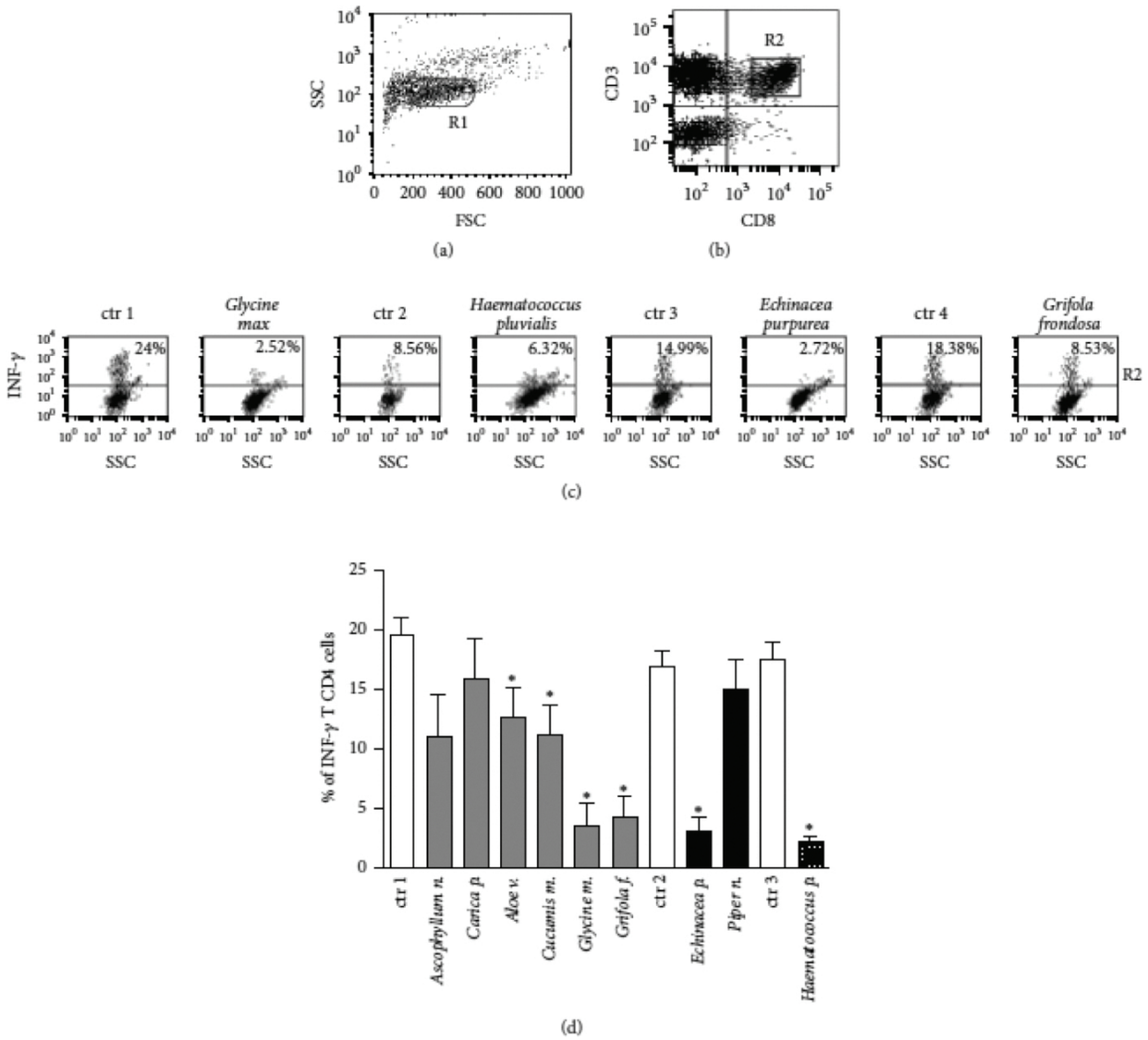

Figure 4. The effects of botanicals on IFN-γ production by canine PBMCs. (a) shows the gating on viable lymphocytes (R1 in dot plot graph) based on FSC and SSC parameters (see Section 2). (b) represents the gating on $CD4^+$ T lymphocytes ($CD3^+$ $CD8^-$ as R2 in the dot plot graph). (c) reports the results from one representative experiment showing the percentage (the number in upper quadrant) of IFN-γ-producing canine CD4+ T lymphocytes gated on R2 (*y*-axis); *x*-axis indicates the SSC parameter (see Section 2). The different co-incubations of cells with ad hoc medium or mixture (see Section 2) are indicated on the top. (d) shows the statistic representation of the IFN-γ production by canine $CD4^+$ T lymphocytes evaluated as percentage of IFN-γ-producing cells in 10 representative experiments, $^*p < 0.05$. The abbreviation "ctr" in (c) and (d) indicates the basal IFN-γ production by PMBCs stimulated by PMA and ionomycin and in the presence of the ad hoc medium based on the same solubilizing vehicle but free from the botanicals (see Section 2): specifically, ctr 1 (*Ascophyllum n., Carica p., Aloe v., Cucumis v., Glycine m.*, and *Grifola f.*), ctr 2 (*Echinacea p., Piper n.*), and ctr 3 (*Haematococcus p.*) (with the permission of Hindawi) [42].

This phenomenon can be obviously exacerbated in the presence of heritable high erythrocyte-reduced glutathione and potassium concentrations or glucose-6-phosphate dehydrogenase deficiency or zinc deficiency [47, 48].

Thus, dietary supplements, home-made or commercially available pet food containing some plants or herbal extracts, might transform a functional food into a poisoning food.

4. Pet diets and animal well-being

Some scientific evidence has pointed out the efficacy of selected ingredients, as part of a commercially available diet, in relieving inflammatory conditions in pets by means of an immune modulatory and antioxidant activity [49–57].

Pasquini et al. [53] demonstrated that *dogs* fed a specific diet (F10 Maxi Maintenance®) based on maize, fish meal (20%), maize oil, fish oil, brewer's yeast, beet pulp, minerals, MOS, FOS, *Elaeis guineensis*, *Yucca schidigera*, *C. papaya*, *Ananas* spp., *P. granatum*, *Panax ginseng*, and *Rosmarinus officinalis* was able to influence gender, age, and breed-derived lipid metabolism alterations in healthy *dogs* by significantly decreasing C-tot, C-high-density lipoprotein (HDL), and C-low-density lipoprotein (LDL) ($^{*}p < 0.05$) [57].

Further studies have then clearly demonstrated the synergic efficacy of selected ingredients in modulating several inflammatory conditions, which commonly affect pets, especially *dogs* [49–52, 56, 58].

An inflammatory condition can also occur during food allergy reactions, which usually takes place after the intake of a harmless dietary component [59]. Generally, food-allergic reactions in pets include cutaneous (flush, itching, dandruff, skin malodor, dry fur, and skin lesions) and gastrointestinal manifestations (dehydration, appetite loss, regurgitation, emesis, abdominal pain, flatulence, borborygma, diarrhea, weight loss, stool consistency, blood, and mucus presence in the stool) [56].

Based on these observations, we conducted two different clinical evaluations aimed to validate two different commercially available formulas for aforementioned dermatological and gastrointestinal issues. For instance, a mixture of fish, potato, *A. vera, Arctium lappa, Malva sylvestris,* and *Ribes nigrum* (FORZA10 Dermo Active™) resulted particularly effective in halving the intensity of cutaneous symptoms (flush, itch, dandruff, skin malodor, dry fur, and skin lesions) in 71 *dogs* affected by atopic dermatitis ($^{***}p < 0.001$) [56]. On the other hand, a specific diet consisting of a mixture of milk enzymes, *Origanum vulgare*, chestnut, *Plantago psyllium*, MOS, FOS, electrolytes, and *Rosa canina*, significantly reduced the intensity of symptoms (dehydration, appetite loss, regurgitation, emesis, abdominal pain, flatulence, borborygma, diarrhea, weight loss, stool consistency, blood, and mucus presence in the stool) in 60 *dogs* with evident gastrointestinal issues ($^{***}p < 0.001$) [56]. Obviously, an inflammatory condition may also occur in other segments of the gastrointestinal apparatus, such as the mouth. In this case, the main clinical manifestation is halitosis, which is generally the sum of metabolic anomalies, poor oral hygiene, hyposalivation, dental appliances, as well as gingival, mucosal, and periodontal disease [60]. In this regard, pets can be affected by halitosis and a correct dietary approach becomes fundamental to manage and treat such complaints [50, 61]. We compared the efficacy of a specific nutraceutical diet and a standard diet in significantly reducing the concentration of three specific volatile sulfur compounds, hydrogen sulfide, methyl mercaptans, and dimethyl sulfide, in 16 *dogs* suffering from chronic halitosis [50]. More in detail, the diet consisted of a mixture of fish meal, rice carbohydrates, propolis, *Salvia officinalis*, lysozyme, bioflavonoids, *Thymus vulgaris*, *R. nigrum,* and an Omega 3/6 ratio of 1:4.

Then, by means of a portable gas chromatograph (OralChroma™), a syringe to collect the breath and specific software, a significant reduction in halitosis, were observed after 30 days from the beginning of the nutraceutical diet supplementation ($^{*}p < 0.05$). Further, a long-lasting effect was still observed even 20 days after the diet interruption.

As previously stated, *cats* and *dogs* can be affected by adverse food reactions, which can involve apparatuses including the gastroenteric and cutaneous but can be extended also to otological, ocular, urinary, and respiratory systems [49, 62]. We recently ascertained the effectiveness of a combined use of a nutraceutical diet and current pharmacological therapy in 15 adult *dogs* affected by chronic bilateral otitis externa [49]. The diet, which was composed of fish proteins, rice carbohydrates, *Melaleuca alternifolia, Tilia platyphyllos scapoli et cordata, Allium sativum L., Rosa canina L.* and Zinc and an Omega 3/6 ratio of 1:4, and the drug (Otomax®) significantly reduced the mean intensity of all clinical symptoms (occlusion of ear canal, erythema, discharge quantity, and odor) within 90 days ($^{***}p < 0.001$). This study can be considered a further example of the importance of the selection of substances endowed with anti-inflammatory and antioxidant activity in a pet food diet.

In some cases, substances endowed with anti-inflammatory as well as immune-modulatory activity can drastically influence the clinical outcome of lethal pathologies, that is, *Leishmania* [63].

A study conducted by Cortese et al. investigated the effect of an immune-modulating diet, based on fish- and vegetable-hydrolyzed proteins, minerals, *A. nodosum, C. melo, C. papaya, A. vera, Astaxanthin, C. longa, C. sinensis, P. granatum, P. nigrum, Poligonum* spp., *E. purpurea, G. frondosa, G. max* and an Omega 3/6 ratio of 1:1 along with an anti-*Leishmania* pharmacological therapy (meglumine antimoniate, and allopurinol) in 20 naturally infected *dogs* over a period of 12 months [63]. The diet results were particularly effective in restoring regulatory T cells and decreasing T helper cell percentage ($^{***}p < 0.001$).

In other cases, the selection of substances with a remarkable antioxidant activity also acquires a pivotal role in other clinical conditions, which are not strictly related to adverse food reactions, that is, cognitive impairment, as a consequence of aging or pathologies such as Alzheimer's and Parkinson's disease [52, 64, 65].

In this regard, we studied the effect of a nutraceutical diet based on fish proteins, rice carbohydrates, *G. frondosa, C. longa, C. papaya, P. granatum, A. vera, P. cuspidatum, Solanum lycopersicum, Vitis vinifera, R. officinalis,* and an Omega 3/6 ratio of 1:0.8 on cognitive decline of nine elderly *dogs* over a period of 6 months [52]. Specifically, derivatives of reactive oxygen metabolites, biological antioxidant potential levels, and brain-derived neurotrophic factor were evaluated in *dogs*' plasma samples at the beginning and at the end of the dietary regime. Results showed a significant decrease of dROMs ($^{*}p < 0.05$) and a significant increase in brain-derived neurotropic factor (BDNF) ($^{*}p < 0.05$) serum levels.

A recent study has also raised the possible key role of some selected ingredients (fish proteins, rice carbohydrates, *P. granatum, Valeriana officinalis, R. officinalis, Tilia* spp., tea extract, and L-tryptophan, with an Omega-3:-6 ratio of 1:0.8) in modulating behavioral disturbances in 12 *dogs* with chronic anxiety and stress caused by intense and restless activity over a period of

only 10 days [58]. By means of a sophisticated and extremely sensitive sensor, a mobile phone app, and a wireless router, it was possible to induce and monitor significant improvements in the time spent in activity and at rest ($^{**}p < 0.01$ and $^{*}p < 0.05$, respectively). Last but not least, all *dogs* also showed an overall significant improvement in clinical (dandruff, itchiness, flush, seborrhea, fur opacity, vomiting, diarrhea, flatulence, lachrymation, and anal sac repletion) and behavioral (marking, anxiety, diffidence, irregular biorhythm, reactivity, activation, irritability, alertness, environmental exploration, and attention requirement) symptoms.

5. The "market stand" of functional pet foods

The interest into the adequacy and safety of commercially available pet foods has been growing worldwide [66]. Functional foods such as prebiotics, for example, inulin, gluco-oligosaccharides, and galacto-oligosaccharides have shown to induce beneficial effects on biochemical parameters improving satiety and reducing postprandial glucose and insulin concentrations, thus reducing diabetes-related disorders [67–69]. Inulin and oligofructose, but also dietary fibers, can also modify the intestinal microflora in pets and humans by promoting commensal bacteria growth [70–72]. However, many *in vitro* studies highlighted other hidden properties of dietary fibers such as gastric emptying, gastric transit time and decrease in blood cholesterol concentrations, increase in satiety, glucose uptake rate, and fecal excretion as well as dilution in diet calorie density [73–76]. Another valuable fiber source is represented by corn fiber due to the lack of detrimental effects on palatability and nutrient digestibility, and the glycemic response lowering in adult *dogs* [71, 77]. Based on these novel and unexpected activities of dietary fibers, many commercially available pet diets moved to an accurate use of these along with novel sources of carbohydrates including cereal grains, which represents almost 90% of animal diet content, and whole grains [78]. These latter, whose main source are wheat, corn, oats, barley, and rye [79], are rich in dietary fibers, trace minerals, and vitamins B and E [80]. Furthermore, whole grains have bioactive compounds, for example, tocotrienols, lignans and polyphenols, lipotropes and methyl donors, such as choline, methionine, betaine, inositol and folate and antinutrients, that is, compounds that interfere with the absorption of nutrients such as phytic acid, tannins, and saponins endowed with antioxidant and anti-carcinogenic effect [79–82]. As to rice bran, the vitamin-rich outer layer that surrounds the endosperm of whole grain brown rice has bioactive molecules such as tocopherols, tocotrienols, polyphenols including ferulic acid and α-lipoic acid, phytoesterols, γ-oryzanol and carotenoids such as carotene, lycopene, lutein, and zeaxanthin, which was endowed with antioxidant, anti-inflammatory, and chemopreventive activity [83]. Rice bran is also an excellent source of essential amino acids (especially sulfur-containing amino acids) and micronutrients such as magnesium, manganese, and B-vitamins (especially B9 and B12) [83, 84]. It is worth noting that during pet food heat processing, known as the Maillard reaction, that is, a nonenzymatic browning and flavoring reaction, a reduction of essential amino acids, such as lysine, bioavailability occurs [85, 86]. Therefore, many pet diets might be at the risk of supplying less lysine than the animal may require. Hence, the understanding of nutritional benefits of functional foods currently available is of key importance for the owners to provide their pets with the

correct diet. Nevertheless, great attention has to be paid to pet food palatability along with adequacy and safety. For instance, Spears and coworkers examined the palatability and its effect on digestion of stabilized rice bran in a dry canine diet determining fecal characteristics, food intake, selected immune mediators, and blood lipid characteristics [87]. They observed that dry pet food containing 12% stabilized rice bran was well tolerated by *dogs* with no detrimental effect on nutrient digestibility, fecal characteristics, and changes in inflammatory/immune mediators. Moreover, the rice bran diet presented greater palatability compared to the defatted rice bran diet. Vitamin A (retinol), whose safe upper limit in complete diets for *dogs* ranges from 5.24 to 104.80 mmol, is an essential fat-soluble vitamin at the center of investigations in *dogs* in the context of immune stimulation, vision-supporting functions, reproduction, bone growth, and cellular differentiation [88–90]. In *dogs*, unlike humans where retinyl esters are only detected in plasma in cases of intoxication or following a vitamin A-rich meal [91], vitamin A is present in the plasma predominantly in the form of retinyl esters, in both adequate and vitamin A-deprived states [92]. Moreover, in *dogs* [93], which excrete vitamin A in the urine [91] along with retinyl esters [94], retinol concentrations are unaffected by dietary vitamin A intake (1.2 ± 0.03 vs. 1.0 ± 0.03 mg/l, respectively), whereas serum retinyl esters parallel the concentrations of vitamin A in the diet [91].

5.1. The role of microbiota

Pet's well-being and health also depend on gut microbiota, whose composition and activity is correlated to several diseases [95–97]. *Cats* and *dogs* harbor several bacterial species (with *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria,* and *Eubacterium* as the predominant phyla [98–102]), which differ from each other but also from the same species [103]. As shown in human studies, gut microbiota also plays a key role in immune and food intake regulation [104, 105]. However, only a few studies have examined the impact of the diet on canine gut microbial population [106–108]. For example, Kerr and coworkers evidenced the lack of negative alterations of the microbiome in healthy *dogs* of different species (*Golden Retriever, Hound Mix, Pitbull Mix, Mixed, St. Bernard, Australian Cattle, Dalmatian, Pointer, Standard Poodle, Terrier*) fed on a cooked navy bean powder [109]. Cooked beans decreased *Actinobacteria* and *Fusobacteria* and increased *Firmicutes*. Therefore, it is reasonable to think that future dietary products for *dogs* will modulate the gut microbial population in order to treat or prevent some food-related diseases (dysbiosis, leaky gut, intestinal bowel disease, irritable bowel syndrome, coeliac disease, sepsis, renal failure, autoimmune disease, peritonitis, and intestinal obstruction). Recently, Park and coworkers monitored healthy *dogs* for 6 months [110]. The first group was fed *ad libitum* on commercial food, while the second was fed on a restricted amount of the same commercial food. Animals fed *ad libitum* resulted in obesity with high levels of triglycerides and cholesterol. The microbiota presented differences between the two groups of animals, while *Actinobacteria* and *Bacteroidetes* were the predominant microflora in animals receiving a restricted amount of food, animals fed ad libitum presented *Firmicutes*, *Fusobacteria,* and *Actinobacteria*. Thus, a targeted diet may promote changes in gut microbiota affecting the activity of specific beneficial microbes resulting in benefits for the overall dog's health. In this sense, a targeted diet might also be based on the use of prebiotics, for example, chicory, fructooligosaccharides, pectin, and polydextrose. Zentek and coworkers demonstrated that

nine adult healthy *beagles* fed a diet supplemented with 3% chicory had more consistent stools with increased levels of *bifidobacteria* and decreased *Clostridium perfringens* and a lower fecal pH with respect to a protein-rich diet [111]. Further, *cats* fed diet supplemented with FOS (4% of diet) showed increased concentrations of *bifidobacteria* and reduced count of *Escherichia coli*, while pectins (4% of diet) increased *C. perfringens* and *lactobacilli* concentrations [112]. Conversely, *dogs* fed a diet with low level of dietary fiber (beet pulp) for 2 weeks decreased *Fusobacteria* and increased *Firmicutes* [107]. Interestingly, feline diets, particularly rich in animal proteins and low-carbohydrate plant-based additives, promoted fecal *Clostridium*, *Faecalibacterium*, *Ruminococcus*, *Blautia*, and *Eubacterium* growth affecting circulating hormones and metabolites of key importance in satiety and host metabolism [113, 114]. As to polydextrose, its consumption by *dogs* resulted in an increase of fecal acetate, propionate, and total SCFA concentrations, while fecal pH, indole, and *C. perfringens* population decreased [106]. Thus, incorporating prebiotics in pets' diet may beneficially modulate gut microbiota and intestinal health and possibly protect the animals from enteric infections.

6. Conclusions

Recent advances in pet food production have raised the potential of some functional ingredients to be useful in preventing and treating disease in pets. Although further work is required to better characterize the long-term effect of these substances on biological mechanisms, we reported some *in vivo* and *in vitro* examples of the synergic efficacy of selected ingredients, present in some commercially available nutraceutical diets, as a valid and reliable natural approach for the management of some pet diseases. Functional food development can be considered a promising new research area, which surely will be able to improve the health and quality of life of *dogs* and *cats* in the near future. However, great attention should be paid on pet food production and ingredient selection since, without a correct R&D process, diets might shift from the primary source of feeding to primary source of poisoning.

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