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Nutrigenomic activity of plant derived compounds in health and disease: Results of a dietary intervention study in dog

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ABSTRACT

The study was conducted to investigate the effects of dietary administrations of four nutraceuticals in dogs. Seventy four dogs were enrolled in the trials, 24 healthy dogs were fed with a control diet (CT) and the experimental groups received for 60 days the same diet supplemented with nutraceuticals, namely *Echinacea angustifolia* (EA, 0.10 mg/kg live weight as echinacoside; 14 dogs), *Vaccinium myrtillus* (VM, 0.20 mg/kg live weight as anthocyanidin, 13 dogs), *Curcuma longa* (CL, 6.60 mg/kg live weight as curcumin, 18 dogs with arthrosis), and *Sylibum marianum* (SM, 1.5 mg/kg live weight as sylibin, 8 dogs with hepatopathy). Dogs were weighted at the beginning of study and blood samples were collected at the beginning (T0) and at the end (T60) of the study. VM significantly down regulated *TNF*, *CXCL8*, *NFKB1* and *PTGS2* and decreased plasma ceruloplasmin (CuCp). The activity of EA was evidenced by the significant decrease of *TNF* and *NFKB1* expression and CuCp levels and by the increase of plasma Zn. Administration of CL caused a significant decrease of CuCp and increase of Zn and a down regulation of *TNF*, *CXCL8*, *NFKB1* and *PTGS2*, corroborating the anti-inflammatory action of curcuminoids. After 60 days of treatment with SM, plasma ALT/GPT activity was reduced and paraoxonase was increased, supporting the antioxidant activity of silymarin, also confirmed by the significant up regulation of *SOD2*. Results indicated that nutraceutical administrations in dogs can be an interesting approach to modulate immune response in order to improve health condition of animals.

1. Introduction

The inclusion of nutraceuticals in the diet of human, livestock and companion animals has gained popularity (Colitti and Grasso, 2014; Swanson et al., 2003) in the last decades and in the market there is a large variety of supplements or foods containing a broad type of plant extracts, pure compounds and, more in general, functional compounds. Among the other, the activity of nutraceuticals can be directed towards immune system, arthrosis, Alzheimer, obesity, ageing, oxidative stress, depression, intestinal bowel disease and intestinal bowel syndrome or metabolic syndrome (Wichtl, 2004). Often, the same nutraceutical has several positive effects, as the case of curcumin (Ara et al., 2016; Colitti et al., 2012) or resveratrol (Tomé-Carneiro et al., 2013; Woode et al., 2015). According to the EU Regulation 1831/2003 (Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003) on additives for use in animal nutrition, only the herbal extract registered in the annexes can be used for animals, and the inclusion in this list has to follow a standard protocol. In the last published list (Annex I list of additives Regulation (EC) No 1831/2003, Released 18.04.2016), natural products are registered mainly in the technological category of sensory additives, subcategory "flavouring compounds: substances the inclusion of which in feedstuffs increases feed smell or palatability". Nevertheless, several petfoods have claim for specific activities of natural products, but scientific evidences of their benefits are not always conclusive. To assess the activity of plant nutraceutical, researches have published studies using laboratory animals or *in vitro* cell cultures (Farinacci et al., 2008; Farinacci et al., 2009; Pomari et al., 2013; Pomari et al. 2014; Pomari et al., 2015; Stefanon et al., 2015), and scientific evidences are also available for companion animals (Colitti et al., 2012; Comblain et al., 2015).

Plants contain an almost infinitive list of compounds with pharmacological and nutraceutical properties and in the present study we concentrated on 4 purified extracts, *Vaccinium myrtillus*, *Curcuma longa*, *Echinacea angustifolia* and *Sylibum marianum*, with documented activity for specific biological functions.

Vaccinium myrtillus (VM) is a shrub belonging to *Ericaceae* family growing spontaneously in Europe, Northern Asia, Iceland, Canada and United States. Its dry and powdered fruits and leaves have been used in European traditional medicine for the treatment of many disorders. It is generally named "Bilberry" or "European Blueberry" to distinguish from "American Blueberry". Anthocyanins chemical group, responsible for the typical bilberry' color, represent the main active compounds contained in this fruit (Kähkönen et al., 2003), which have a well-recognized antioxidant property. Among berries, bilberry or *Vaccinium*

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myrtillus specie is the richest in anthocyanins, furthermore showing the most effective antioxidant activity (Nakajima et al., 2004).

Curcuma longa (CL), also known as turmeric, is a perennial plant of the Zingiberaceae family and the rhizome contains compounds called curcuminoids, which include curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin. Therapeutic uses suggested for curcumin refer to a huge range of biological activities, like cancer chemopreventive, antitumor and neuroprotective, primarily based on its recognized antioxidant and anti-inflammatory properties (Venugopal and Sudheer, 2007). Among the activities of CL, a strong anti-inflammatory activity has been reported in dogs (Colitti et al., 2012; Comblain et al., 2015).

Echinacea angustifolia (EA) is a perenneal herb belonging to Asteraceae family, that includes 9 species, but only *E. pallida*, *E. angustifolia* and *E. purpurea* have shown pharmacological properties. American Indians were the first to use Echinacea species for many different ailments, including cough, sore throat and tonsillitis (Caruso and Gwaltney, 2005).

An extensive description of Echinacea genus was made in a study for EFSA (European Food Safety Authority) evaluating its use as additive in animal production. In particular, the report concerned Echinacea' chemical active constituents, pharmacology, toxicology and efficacy either in human than in veterinary studies (Franz et al., 2005). Most relevant Echinacea active constituents, principally found in the roots, are echinacoside, cichoric and chlorogenic acids, caffeic acid derivatives and different polysaccharides like fructans, xyloglucans and heteroxylanes. Among the 3 species, *E. angustifolia* shows the highest concentration in phytochemicals, in particular echinacoside content, absent in the others (Senchina et al., 2006; Zhai et al. 2007). Known immune modulatory effects of EA are the activation of macrophages (Barrett, 2003) and polymorphonuclear granulocytes (Farinacci et al., 2009), resulting in an increased phagocytic activity. This immune stimulation activity of EA can be used not only to cope with infectious diseases but also when stressful events temporary lowering immune defenses (Sgorlon et al., 2012).

Silybum marianum (SL), also known as milk thistle, is a biennial plant of the Asteraceae family, which can be typically found in middle-south European regions, where it grows spontaneously. The main active component of SM is silymarin, a phytocomplex comprising different flavonoids among which the most important are silybin, isosilybin, silychristin and silydianin, concentrated in fruits and seeds (Pradhan and Girish, 2006). Silymarin has several beneficial actions useful in the treatment of hepatobiliary disease, including antioxidant, anti-inflammatory, and antifibrotic properties (Trappoliere et al., 2009; Verma and Thuluvath, 2007), also in dogs (Vandeweerd et al., 2013).

The present study reports the results of a dietary intervention study which involved the phytocomplexes of VM, CL, EA and SM. Pure extracts were added to a semi moist complete diet and fed for 60 days to dogs to assess the effects on plasma biochemical parameters and target gene expression in the peripheral leukocytes.

2. Materials and methods

2.1. Dog recruitments and allocation to groups

All dogs were adult and were recruited in one shelter and in three kennels. The shelter (S0) was a private Dog Shelter under regular veterinary monitoring for clinical diseases and therapeutic protocols and the dogs were for at least from 1 year hosted in the shelter. Kennel 1 (K1) was a private kennel, with resident utility and defense working dogs. Kennel 2 (K2) was a training center, where owners regularly carried dogs for education and to reinforce physical conditions. Kennel 3 (K3) was a facility for hunting dogs breeding, with some animals regularly involved in hunting sessions. Dogs were under the control of the respective veterinarian and all data were obtained from medical records, including a standard complete physical examination performed by the veterinarians, which were asked to follow the dogs during the study. All dogs had to fulfill the following criteria: aged >2 years, medium to large size, resident in the shelter or attending regularly the kennels for at least 1 year.

Within each site, the dogs with no evidence of clinical diseases for at least 1 year and without any therapeutic protocol for at least 6 months were considered healthy and randomly allotted to the control group (CTRL) or to *Echinacea angustifolia* (Polinacea® - EA) group. Exclusion criteria were the presence of infectious and parasitic diseases, systemic, neurological or traumatic diseases or general symptoms of intolerance/allergy to nutraceutical diets administered in this dietary intervention study. Dogs actively involved in training were allocated to *Vaccinium myrtillus* (VM) group, those with history of arthrosis were allocated to *Curcuma longa* group (CL) and those suffering from liver diseases to *Sylibum marianum* group (SM). At the end, according to dogs clinical conditions and history and owners availability, a total of 74 dogs were enrolled (Table 1), 23 hosted in the shelter (S0), 14 resident in K1, 25 resident in K3 and 12 regularly attending K2. Crossbreeds were the most representative individual in S0 (18 dogs), whilst in K1 and K2 the majority of dogs were German Shepherd (11 dogs in K1 and 5 in K2) and in K3 English Setter (21 dogs). Age, breed, sex and live weight at the beginning of the study of all the dogs are reported in the Supplementary Table 1.

2.2. Dietary Intervention Study

For the study, 5 diets were formulated to satisfy the nutrients requirements according to NRC (2006) indications and produced in a form of semi moist food. For the formulation, energy, protein, linoleic acid, eicosopentaenoic acid, mineral and vitamins requirements were considered. All the 5 diets contained the same ingredients with the same proportions (Table 2), the only variation being the inclusion of minerals and vitamins of the different diets according with the nutraceuticals supplementation and the specific clinical conditions. The commercial name of nutraceuticals, main active compounds, purity and daily dose (mg/kg live weight) added to the experimental diets are reported in Table 3. The nutraceuticals were purchased from Indena (Indena Spa, Milan, Italy).

From to the beginning of the study, within each site dogs were fed with different commercial diets. Starting from 15 days before the beginning of the study, they were fed with the control diet (CTRL), consisting of a semi moist canned food without inclusion of nutraceuticals. After this adaptation period, each group received a different diet, as indicated in Table 1, for 60 days. The amount of diet offered was calculated on the basis of the live weight.

At the beginning (T0) and end (T60) of the study, blood samples were collected in the morning just before meal from the cephalic vein in 5 ml vacuum tubes with Li-heparin (Venoject, Terumo Europe N.V., Leuven, Belgium) and in PAXgene Blood RNA System tube (Preanalytix, Hombrechtikon, Switzerland), for biochemical analysis and RNA extraction, respectively.

For biochemical analysis, blood was immediately refrigerated to 6–8 °C, transported to the laboratory within 2 h and centrifuged at

Table 1
Number of dog allotted for the treatments in each of the four sites of study.

Group	S0	K1	K2	K3	Total
CTRL	7	4	6	4	21
VM	–	4	4	5	13
CL	6	3	–	9	18
EA	5	–	2	7	14
SM	5	3	–	–	8
Total	23	14	12	25	74

CTRL = control; VM = *Vaccinium myrtillus*; CL = *Curcuma longa*; EA = *Echinacea angustifolia*; SM = *Sylibum marianum*.
S0 = shelter; K1 = Kennel 1; K2 = Kennel 2; K3 = Kennel 3.