Flavonoids: Not Just for Cancer Anymore

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Increasingly, veterinary clients are using nutraceuticals as adjuvant treatment for a variety of ailments in their pets. Recent evidence suggests that more than 50% of clients who have companion animals with cancer are using a nutraceutical and/or supplement as part of the treatment plan, and 65% of these clients say their veterinarian approves.1 This trend does not extend to healthy pets, of which only 10% receive routine supplements.2 Unfortunately, few resources regarding the safety of nutraceutical use exist, and recent evidence has shown that doses of human-formulated supplements such as lipoic acid can be toxic to cats.3 Veterinarians need a better understanding of the metabolism and safe upper limits for commonly used nutraceuticals because many clients are giving these products to their pets, often in human formulations. This article focuses primarily on two flavonoids: epigallocatechin-3-O-gallate (EGCG), a flavanol, and genistein, an isoflavone.

Overview of Flavonoids

Of the nutraceuticals considered beneficial from in vitro and in vivo studies, flavonoids have become popular in human and veterinary medicine, with many commercial products touting their antioxidant potential. Flavonoids are a heterogeneous group of tricyclic compounds with a similar backbone structure (FIGURE 1); variations in double bonds, hydroxylations, methylations, or other carbon side chains lead to their distinct classifications. They are found in a wide range of commonly consumed fruits, vegetables, plants, legumes, and nuts. Overall, there are 12 different flavonoid subclasses; however, only six are abundantly expressed in commonly consumed foods. These flavonoids often occur naturally as glycosides, which renders them more water soluble and may potentiate rapid gastrointestinal absorption.

One major rationale for incorporating flavonoids into the diet is their ability to act as antioxidants. This ability is usually associated with the most proximal (right-side) phenolic ring. The fewer the number of hydroxyl groups (i.e., –OH) on this ring, the more potent the antioxidant activity. A hydroxyl group in the middle ring structure at carbon position 4 (FIGURE 1) also enhances antioxidant ability. In nature, however, this position is often occupied by a glucuronide, which severely hinders the antioxidant ability of the flavonoid.4 For many flavonoids, their antioxidant activity, while potentially important, may play a less clinically significant role than their other activities, including antiinflammatory, antiproliferative, and metabolic activities.

EGCG (FIGURE 2) and genistein (FIGURE 3) have known antioxidant capabilities. However, evidence also points toward the use of EGCG and associated flavanols found in green tea extracts (GTEs) for amelioration of the insulin resistance in dogs, whereas genistein may play a role in appetite suppression and maintenance of lean mass during weight loss.5–7 More importantly, sufficient bioavailability and toxicity data exist to safely suggest the use of these flavonoids as adjuvant treatments to clients considering nutraceutical therapy for their pets.

EGCG and Green Tea Extracts

Toxicity and Potential Dosing Regimens

EGCG and associated flavanols can be found in wine, tea, and cocoa. However, with the exception of unfermented green tea, extensive fermentation and processing of these products lead to theaflavin and other conjugates that are not absorbed well. EGCG is likely to be the most potent of the flavanols, although epicatechin and catechins are found at higher concentrations in green tea.

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Figure 1. Flavonoid backbone consisting of a tricyclic molecule. Note the numbering of the carbon positions. Positions 2 and 3 are important with regard to the structure of isoflavones, and carbon 4 is often modified by glucuronidation.

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Pharmacokinetic studies in dogs show the bioavailability of green tea polyphenols in this species to be comparable to that in humans and rodents, with ingestion of 12.5 mg/kg resulting in peak serum concentrations of around 1 µM and a serum half-life of 4 to 6 hours. Therefore, it is presumed that 3 to 4 times daily dosing would be required to maintain therapeutically significant serum concentrations of EGCG.

Despite the apparent safety of green tea polyphenols and EGCG, some concern about toxicity exists. One study provided a purified EGCG product used at ≥200 mg/kg. This study was ended early due to mortality in all groups, including the treatment group receiving the lowest dose. All dogs showed lack of weight gain, some degree of anorexia, and bone marrow erythroid hypoplasia and myeloid hyperplasia resulting in anemia and elevated white blood cell counts. Part of the toxic effect was attributed to giving the extract in an unfed state; when follow-up studies were performed with the 200 mg/kg dose, EGCG proved to be far less toxic but was still associated with significant adverse effects.

Another study using a similar purified EGCG product proved that a 50 mg/kg dose was safe in fasted dogs. Doses of 150 and 300 mg/kg proved to be toxic over time, with signs similar to those mentioned above (e.g., anorexia), overt renal tubule necrosis, and hepatocellular damage. However use of a complex GTE (not EGCG alone) in obese dogs at a dose of 80 mg/kg showed no adverse effects. While these data are limited in scope, they lead to the suggestion that clients who want to use GTE as an herbal supplement not administer more than 50 mg/kg of any GTE product.

**Cancer and Insulin Resistance**

Extensive rodent xenograft models and chemically induced carcinogenesis studies have shown diminished or eliminated tumor growth when EGCG or GTE is administered. Many epidemiologic studies in Chinese populations show significantly reduced relative risk of developing prostate or breast cancer as an inverse correlation with green tea consumption. The most promising human trial to date was a randomized placebo-controlled investigation examining encapsulated green tea catechins in men with high-grade preneoplastic prostate lesions. After 1 year, the incidence of prostate cancer was 3% in the men receiving green tea catechins and 30% in those receiving a placebo. By contrast, however, recent meta-analysis of epidemiologic data did not universally support green tea consumption across all neoplastic conditions, showing no benefit in many cancers other than prostatic neoplasia. Despite the intriguing human studies, no companion animal data exist on the use of green tea for neoplasia or preneoplastic conditions.

Metabolically, green tea polyphenols may be able to enhance insulin sensitivity. This finding is supported in a recent article examining the effects of GTE on obese, insulin-resistant beagles. Serum was collected before and after obesity induction, with one group of dogs receiving GTE (80 mg/kg). Markers of insulin, glucose, and lipid metabolism were examined, and adipose and muscle tissue samples were collected to examine markers of fat metabolism and adipokine status. Surprisingly, the dogs receiving the GTE supplement had improved insulin sensitivity, lower nonesterified fatty acids, and lower very-low-density lipoprotein particles, with all parameters being closer to the baseline values obtained when the dogs were in ideal body condition than to the values in the obese control group. Furthermore, adipose and muscle concentrations of adiponectin (a hormone released from fat to improve insulin sensitivity) were markedly increased, as were nuclear receptors (PPARα and PPARγ), which are involved in upregulation of lipoprotein lipase activity and GLUT 4 receptors. Therefore, green tea seems to have glucose sensitization properties that need to be examined in clinical settings to better understand how they might affect diabetic and obese veterinary patients.

**Genistein**

Isoflavones with phenolic rings attached at the carbon 3 position rather than the carbon 2 position (FIGURE 1) are found almost exclusively in legumes; their concentrations are highest in soybeans. The two major isoflavones in legumes are genistein and daidzein, which have estrogenic activity and have been studied for their bioavailability and toxicity in dogs and cats.
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Toxicity
Long-term toxicity studies of orally administered genistein were performed in beagles. Other than changes in reproductive tissue weights (increased uterine weight in females and increased testicular weight in males), no adverse effects were observed, even at the highest concentrations of 500 mg/kg/d. Reproductive performance was not evaluated, however. In cats, short-term postcastration toxicity studies showed no toxicity when genistein was given orally at 100 mg/kg. This dose resulted in maximal serum concentrations of 1 μM for genistein with a serum half-life of around 9 hours, making the logical dosing interval twice a day.

Cancer and Obesity
Genistein is of interest for its potential to inhibit or stimulate estrogen or androgen receptors, with evidence pointing to inhibition of estrogen receptors, particularly estrogen receptor β, which genistein binds to more favorably. The inhibitory effect of genistein has been observed in many breast and prostate cancer cell lines (androgen-sensitive cell lines), in which supraphysiologic doses of genistein caused diminished cell growth and even regression of tumors in rodent xenograft models. Most xenograft and carcinogenesis models are of breast and prostate cancer, and the data from these models show continual promise and positive results in either tumor growth rates or carcinogenesis. One exception is that certain models of xenograft breast cancer have shown enhanced metastasis, fueling the continual debate regarding genistein and breast cancer, particularly in ovariectomized mice. The ovariectomized mouse model may have some similarity to typical household pets, which are frequently neutered or spayed at an early age, making the role of genistein in carcinogenesis even more complicated for companion animals.

Some evidence points to improved lipid metabolism and decreased food intake in primates and rodents receiving genistein supplements. The effect of neutering on satiety has been debated as it relates to obesity in dogs and cats, with evidence suggesting increased ad lib consumption in these species after spaying and neutering. The evidence for estrogen use in cats is convincing, as estrogen treatment completely ablates the typical postneutering increase in food consumption in male and female cats. Genistein has also been examined as an appetite suppressant in a study in which male and female cats were gonadectomized and supplied genistein at 100 mg/kg for 10 days. Genistein did show some short-term effects at suppressing appetite compared with a control group. A follow-up study was then performed to examine how supplementation with estrogen and genistein affected lean mass, adiposity, and overall food consumption over a 35-day period. While estrogen suppressed food intake after gonadectomy, genistein’s actions appeared short lived, with nearly identical food consumption to control cats on days 8 through 35 after gonadectomy. Surprisingly, cats receiving 100 mg/kg of genistein daily maintained better lean body mass and had less overall fat mass than control cats. Therefore, even though the effects of genistein were transient, there may be benefits regarding lean mass that warrant further investigation.

Additionally, evidence has emerged that other flavonoids, such as those found in citrus fruits, may also have appetite-suppressing effects. These recent findings in companion animals are leading some therapeutic and commercial food manufacturers to add isoflavones and other flavonoids to their products to potentially aid in weight management and lean mass maintenance during weight loss. Currently, only one commercially available weight loss diet contains flavonoids, with a minimum flavonoid content of 850 mg/kg of food, which equates to doses of 10 to 20 mg/kg in a typical dog. These doses appear to be safe when incorporated into food.

Conclusion
The use of nutraceuticals in veterinary medicine is increasing, and the goal of veterinarians should be to provide safe and effective dosing regimens for these products. This is often difficult due to the paucity of available data and potential species differences; however, based on the present body of evidence, administered doses of GTE should not exceed 50 mg/kg for dogs because of the potential for toxicity at very high doses; a reasonable dosing frequency is 3 to 4 times/day. Dosing recommendations for isoflavones and other flavonoids with minimal or unreported toxicity are even trickier; therefore, starting at a minimum dose of 10 to 20 mg/kg is acceptable, with a much wider margin of safety based on current information. Although the use of these nutraceuticals is in its infancy, more products will become available, and as long as we have safe dosing guidelines, these nutraceuticals can be used in everyday practice to help ameliorate certain conditions without the danger of oversupplementation.

References
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* Purina OM Overweight Management Canine Formula.