Identification and concentration of soy phytoestrogens in commercial dog foods

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Objective—To identify and determine the concentrations of phytoestrogens in commercial dog foods.

Sample Population—24 commercial dog foods, including 12 moist or dry extruded commercial dog foods that contained soybeans or soybean fractions and 12 foods without any soybean–related ingredients listed on the label.

Procedure—Foods were analyzed for phytoestrogen content, including 4 isoflavones (genistein, glycitein, daidzein, and biochanin A), 1 coumestan (coumestrol), and 2 lignans (secoisolariciresinol and matairesinol) by use of acid-methanol hydrolysis and high-pressure liquid chromatography with UV-absorbance detection. Phytoestrogens were identified and quantified by reference to authentic standards.

Results—Isoflavones, coumestans, and lignans were undetectable in diets that did not list soybean–related ingredients on the label. Only 1 of the 12 diets that included soybean or soybean fractions had undetectable concentrations of phytoestrogens and that product contained soy fiber. The major phytoestrogens were the isoflavones daidzein (24 to 615 µg/g of dry matter) and genistein (4 to 238 µg/g of dry matter).

Conclusions and Clinical Relevance—Soybean and soybean fractions are commonly used ingredients in commercial dog foods. Dietary intake of phytoestrogens may have both beneficial and deleterious health effects. Our results indicated that certain commercial dog foods contain phytoestrogens in amounts that could have biological effects when ingested long-term. (*Am J Vet Res* 2004;65:592–596)

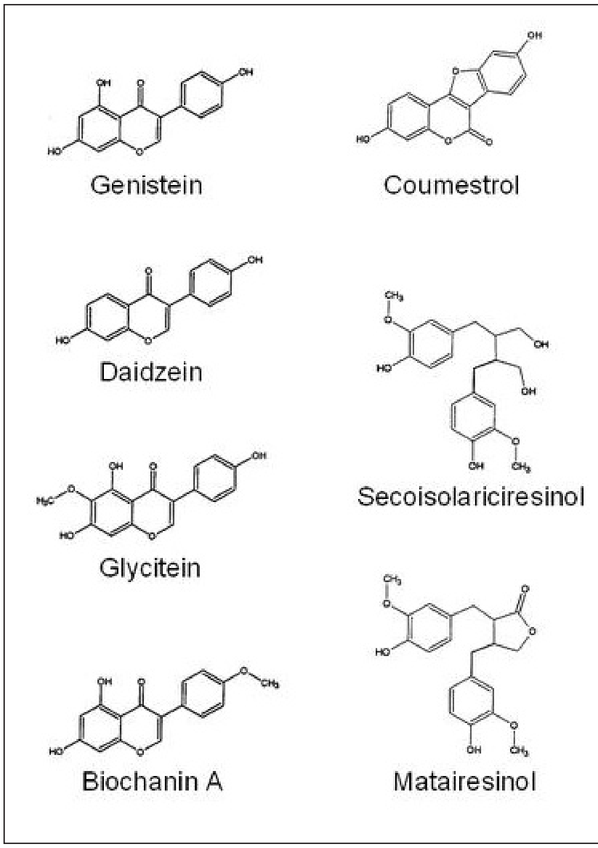

Figure 1—Diagrams of the structures of soy phytoestrogens.

Phytoestrogens are plant-derived, nonsteroidal compounds with estrogenic activity. They are classified as flavonoids (flavones, flavanones, and isoflavones), coumestans, and lignans. The principal dietary source for phytoestrogens is soybeans, which contain the isoflavones daidzein, genistein, glycitein, and biochanin A; the coumestan, coumestrol; and the lignans secoisolariciresinol and matairesinol (**Fig 1**).[1-3]

For centuries, soy has been part of the diet in many Asian countries, and phytoestrogen intake has been quite high among those populations. Phytoestrogens are reported to have both positive and negative effects on the health of humans and other animals. Effects on health depend on the frequency, duration, and amount of intake; effective intestinal absorption; and metabolism of these compounds. Soy has been a commonly used ingredient in the manufacture of commercial pet foods; however, there have been few investigations of the phytoestrogen content of companion animal diets, tissue concentrations, or the impact of phytoestrogen intake on animal health.[4,5] The objective of the study reported here was to identify and determine the concentrations of phytoestrogens in commercial dog foods.

Received July 31, 2003.
Accepted October 13, 2003.
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Supported by a departmental research grant, Department of Clinical Studies-Philadelphia, School of Veterinary Medicine, University of Pennsylvania, and by a R01-GM-61834 grant from the National Institutes of Health, Bethesda, Md (Court, Hao).
Preliminary data were presented as a poster at the American College of Veterinary Internal Medicine Forum, Charlotte, NC, June 2003.
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Materials and Methods

Commercial dog foods—During the fall of 2002, 24 commercially available dog foods[a-x] were obtained from either a local supermarket or the pharmacy of the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania. Products were selected to provide a cross section of commercially available dog foods, including therapeutic, premium, and supermarket varieties. There were 8 moist[a-h] and 16 dry extruded[i-x] dog foods. Information with regard to the soybean or soybean fraction content of each food was obtained from the label **(Table 1)**. Five products listed soybean meal as an ingredient alone or in combination with other soybean fractions (soybean mill run, soybean hulls, or soy flour). Three products contained soy flour (1 also contained soybean meal). Of the label ingredients for the 5 remaining dog foods, 2 listed soy fiber, 1 listed soybean oil, 1 listed textured soy protein, and 1 listed isolated soy protein. All 24 foods were labeled as nutritionally complete and balanced on the basis of **Association of American Feed Control Officials (AAFCO)** feeding trials or formulated to meet AAFCO nutrient profiles.

Soy phytoestrogen assay—Phytoestrogens in soybeans are present as glycosidic conjugates. Consequently, the free (unconjugated) flavonoids, coumestans, and lignans in dog food were identified and quantified by use of acid-methanol hydrolysis and extraction, followed by **high-pressure liquid chromatography (HPLC)** by use of modifications of described methods.[5-7] Briefly, samples of food (1 g) were weighed and added to 10-mL screw-topped glass tubes. After drying overnight in a vacuum oven at 40°C, samples were reweighed to determine dry weight. Methanol (9 mL) and concentrated hydrochloric acid (1 mL) were added to each tube, and the tubes were vortexed, capped, and placed in a boiling water bath for 5 hours (optimal time determined in initial experiments) with hourly mixing by inversion. After cooling to room temperature (22°C), 1-mL aliquots were transferred to polypropylene microcentrifuge tubes and centrifuged for 5 minutes at 14,000 $\times$ g. The supernatant was transferred to HPLC sample vials, and 5-µL aliquots were injected into the HPLC apparatus. This apparatus consisted of a quaternary pump[y] with autoinjector[y] serially connected to a column[z] and a diode array UV absorbance detector[y] set at a wavelength of 260 nm. The mobile phase was run at a flow rate of 2 mL/min, initially consisting of 80% solvent A (50mM potassium phosphate buffer [pH, 2.2] in water) and 20% solvent B (100% acetonitrile). At 5 minutes after injection, solvent B was linearly increased from 20% to 23% for over 5 minutes, held at 23% for a further 25 minutes, and then linearly increased to 70% for over 20 minutes. Analyte retention times were 9.5 minutes for secoisolariciresinol,[aa] 14.5 minutes for daidzein,[aa] 16.3 minutes for glycitein,[aa] 27.4 minutes for matairesinol,[aa] 31.1 minutes for genistein,[aa] 36.7 minutes for coumestrol,[aa] and 48.2 minutes for biochanin A.[aa]

For the purpose of peak identification and quantitation, a series of concentrations of pure standards (final concentrations, 1 to 40 µg/mL) were prepared in a 10% hydrochloric acid-methanol solution. Results of initial experiments indicated that recovery of analytes added to dog food matrix was > 90%. Sample peaks were identified by coelution with the purified compounds and by comparisons of UV absorption spectra from the diode array detector. Intra- and interassay variations were < 10% and 15%, respectively. Minimum quantifiable concentrations were 1 µg/g of food for genistein, glycitein, and daidzein, and 10 µg/g of food for coumestrol, biochanin A, secoisolariciresinol, and matairesinol.

Results

The moisture content of each dog food was determined to enable normalization of the phytoestrogen content for comparison among food types. Dry extruded foods contained (mean ± SD) 6 ± 0.8% moisture and moist foods contained 75 ± 5% moisture (Table 1).

The lignans secoisolariciresinol and matairesinol and the coumestan coumestrol were not found in detectable amounts in any of the diets. Isoflavones were undetectable in the diets that did not list soybean-related ingredients on the label (Table 1). Only 1 of the 12 diets that included soybean-related ingredients (soy fiber) had no detectable concentrations of any of the assayed isoflavones. The lowest concentrations of isoflavones were found in foods containing soy fiber or soybean oil. Genistein and daidzein were the major detected isoflavones. Glycitein, but not biochanin A, was also detected in 9 of the soy-containing diets.

Table 1—Concentrations of soy isoflavones in 24 commercial dog foods

Soy fraction	Food type	Dry matter (% wt/wt)	Daidzein (µg/g DM)	Glycitein (µg/g DM)	Genistein (µg/g DM)
No soy[a]	Moist	28	0	0	0
No soy[b]	Moist	29	0	0	0
No soy[c]	Moist	19	0	0	0
Soy fiber[d]	Moist	30	0	0	0
Soybean meal, and soy flour[e]	Moist	17	615	154	559
Textured soy protein[f]	Moist	23	133	20	168
Soy flour[g]	Moist	23	131	21	169
Soy flour[h]	Moist	24	213	32	276
No soy[i]	Extruded	94	0	0	0
No soy[j]	Extruded	95	0	0	0
No soy[k]	Extruded	94	0	0	0
No soy[l]	Extruded	94	0	0	0
No soy[m]	Extruded	93	0	0	0
No soy[n]	Extruded	95	0	0	0
No soy[o]	Extruded	94	0	0	0
No soy[p]	Extruded	94	0	0	0
No soy[q]	Extruded	94	0	0	0
Soy fiber[r]	Extruded	94	33	0	6
Soybean meal, soybean mill run[s]	Extruded	92	129	36	72
Soybean meal[t]	Extruded	95	64	25	51
Soybean meal, soybean hulls[u]	Extruded	93	279	56	238
Soybean oil[v]	Extruded	95	24	0	4
Isolated soy protein[w]	Extruded	94	102	14	191
Soybean meal[x]	Extruded	94	48	17	38

The lower limits of detection were 1 µg/g dry matter (DM) for daidzein, genistein, and glycitein.
[a-x]*See* text footnotes.

Discussion

Analysis of 24 commercial dog foods found that products formulated with soybeans or soybean fractions contained phytoestrogens in amounts comparable to those that have biological effects in other species.[8,9] The isoflavones genistein and daidzein were the major phytoestrogens detected, and this is consistent with the fact that they are the predominant classes of isoflavones found in soybeans.[9] Another soy isoflavone, glycitein, was also found as a minor constituent in the analyzed foods, whereas other phytoestrogens were not detected. Biochanin A, coumestrol, secoisolariciresinol, and matairesinol have been identified in soy products, although reported concentrations are < 1 µg/g of food, which is substantially below the detection limit of our assay (10 µg/g of food).[9,10] Phytoestrogens were not found in any of the foods that lacked soybean-related ingredients. Only 1 of 12 foods that listed soybean-related ingredients on their labels did not contain detectable amounts of any of the assayed isoflavones, and that food contained soy fiber. Although the actual amount of soy fiber contained in the food is not obtainable from nonproprietary sources of information, it is known that the crude fiber content of the product is low (0.7% dry matter); therefore the content of soybean-related ingredients is probably minimal. The product with the lowest amounts of detectable isoflavones also contained soy fiber in presumably small amounts as well (crude fiber, 1.2% dry matter).

Although, on the basis of the findings of this investigation, it appears that dogs that eat soy-containing commercial dog foods are exposed to phytoestrogens, little is known about the bioavailability of these compounds in dogs. The isoflavones found in soybeans exist principally in the form of glycosidic conjugates.[11] Studies[4,12,13] in a number of mammalian species, including dogs, have found that hydrolyzed isoflavone glycosides, known as aglycones, and their metabolites can be detected in the serum and urine of animals that consumed soy-containing diets. Investigations in humans suggest that the isoflavone glucosides are not absorbed from the intestinal tract intact[14]; aglycones, however, are readily absorbable.[11,14] There is evidence that hydrolysis of soy isoflavone glucosides can occur during food processing and preparation and with exposure to gastric acid.[11,15] However, the principal mechanism for hydrolysis of soy isoflavone conjugates involves intestinal microflora.[14] Because dietary components other than soybeans and food processing can influence gastrointestinal microflora, these factors need to be considered along with the actual soy isoflavone content of a food when attempting to assess the impact of phytoestrogen ingestion.

As mentioned, lignans are found in soybeans in relatively low concentrations; much higher concentrations of these compounds are found in flaxseed and lentils.[10,16] They are also found in more modest concentrations in a variety of fruits and vegetables.[16] Lignans, like isoflavones, are altered by gastrointestinal microflora before being absorbed systemically.[17] Although the biological effects of lignans have received little attention, in comparison to isoflavones, they may be an important source of phytoestrogens in diets that contain relatively little or no soy.

Although phytoestrogens are nonsteroidal compounds, their chemical structure includes a phenolic ring that confers estrogen-receptor binding capacity and the ability to act as partial estrogen agonists and antagonists.[18,19] The potency of phytoestrogens is low, compared with physiologic estrogens.[20,21] However, this lower potency may be offset by the observation that serum concentrations of phytoestrogens in humans consuming soyfoods are several orders of magnitude higher than those of physiologic estrogens.[15] Most of the investigations of the biological effects of phytoestrogens have focused on their role as estrogen receptor modulators; however, they also have antioxidant properties, and genistein inhibits protein tyrosine kinases, DNA topoisomerases I and II, and cytokine-stimulated nitric oxide formation in vitro.[22-24] Therefore, the biological effects of these compounds that have been observed in vitro and in vivo may be attributable to mechanisms other than their estrogenic properties.

There has been much speculation that dietary phytoestrogens would exert biological effects in animals susceptible to estrogen-dependent diseases, such as some forms of cancers or age-related conditions. Epidemiologic observations that show the rate of breast cancer is significantly lower in Asia, where soyfoods are commonly consumed, in comparison with the United States, have led to the hypothesis that soy intake decreases risk of breast cancer.[25,26] Similar hypotheses have been proposed for prostatic and thyroid cancer.[27,28] Much in vitro investigation of the role of phytoestrogens, particularly genistein, on cell growth and metastatic activity in both hormone-dependent and -independent cell lines has been peformed.[27] In general, there is evidence that genistein inhibits the growth of a wide range of cancer cells with an IC_{50} (concentration at which growth is inhibited by 50%) from 5 to 40 µm.[22,27,29] However, it is unclear whether cellular concentrations of genistein in vivo would reach these in vitro concentrations. Further prospective interventional studies will be required to better understand the role of dietary estrogens in cancer prevention.

There is also the possibility of deleterious effects from the long-term consumption of a diet rich in phytoestrogens. It has been reported that phytoestrogens inhibit some of the enzymes involved in sex hormone steroidogenesis.[30,31] The infertility seen in sheep grazing on subterranean clover, which is associated with increased blood equol concentration (a daidzein metabolite) and hyperestrogenism, is the best documented example of this.[12] Precocious or delayed puberty has been suspected in humans exposed to phytoestrogens.[32] It has also been suggested that serum genistein concentrations found in soy-fed infants may be capable of causing thymic and immune abnormalities.[33] Furthermore, phytoestrogens may influence hair quality in humans and mice, causing hair miniaturization.[34] Genistein decreased hair growth by 60% to 80% when hair follicles isolated from scalp biopsy specimens were treated in culture.[35]

An investigation similar to that reported here has been published by 1 of the authors; it analyzed the isoflavone content of commercial cat foods.[5] The same isoflavones (daidzein, genistein, and glycitein) identified in dog foods were also found in soy-containing cat foods. The total isoflavone intake of a cat fed 1 of the soy isoflavone-containing diets would be from 0.4 to 6 mg/kg of body weight. The same analysis was done for a 15-kg dog by use of the isoflavone content of the foods investigated in this study. Assuming that a typical 15-kg dog would consume from 750 to 1,000 kcal/d and, by the use of caloric content information provided by the manufacturers of the products, the dog's total daily isoflavone intake would range from 0.4 to 39 mg/kg. Because the exact formulation of pet foods is proprietary information, it is not possible to say whether the diets analyzed in these 2 studies reflect the phytoestrogen content of pet foods in general. However, on the basis of this limited sample, dog foods may have considerably higher phytoestrogen content than cat foods. Furthermore, as was stated in the investigation of the isoflavone content of cat foods, measurable effects on serum concentrations of steroid and thyroid hormones have been detected in women consuming up to 2 mg of isoflavones/kg/d.[8] Another review examining investigations of the physiological effects of isoflavones suggested that the threshold intake necessary to achieve biological effects in humans would be on the order of 30 to 50 mg/d, which is equal to 0.4 to 0.7 mg/kg/d for a 70-kg human.[9] Consequently, a dog consuming any of the soy-containing diets analyzed in this investigation would meet the latter criterion (0.4 to 0.7 mg/kg/d isoflavone intake) and the former criterion (2 mg/kg/d for isoflavone intake) as well, for all diets except those containing either soybean oil or soy fiber alone.

Whether or not dietary intake of phytoestrogens of the magnitude described in this investigation would have any biological effect in dogs is not known at this time. Clinical studies will be necessary to evaluate the bioavailability of dietary phytoestrogens and the impact of short- and long-term ingestion of a soy-based diet on serum and urine concentrations of phytoestrogens in dogs. Areas that deserve further investigation include the effects of ingestion of phytoestrogens on the immune response in puppies and adult dogs; effects on steroidogenesis, in particular, sex hormones; and possible effects on skin and coat quality.

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[a] IVD Canine Potato and Duck (canned), IVD, Pittsburgh, Pa.
[b] Eukanuba Veterinary Diet Response FP/Canine (canned), Iams Co, Dayton, Ohio.
[c] Pedigree Little Champions Meaty Strips in Gravy with Beef (pouch), Kal Kan Foods, Vernon, Calif.
[d] Prescription Diet Canine i/d (canned), Hill's Pet Nutrition Inc, Topeka, Kan.
[e] Skippy Premium Strips in Gravy with Chicken (canned), Heinz Pet Products, Pittsburgh, Pa.
[f] Cadillac Chunky Stew with Beef (canned), Menu Foods Inc, Pennsauken, NJ.
[g] Friskies Mighty Dog Carved Chicken Dinner in Gravy (canned), Friskies Petcare Co, Glendale, Calif.
[h] Friskies Mighty Dog Ground Beef Dinner (canned), Friskies Petcare Co, Glendale, Calif.
[i] Prescription Diet Canine d/d, Rice & Egg (extruded), Hill's Pet Nutrition Inc, Topeka, Kan.
[j] IVD Canine Potato and Duck (extruded), IVD, Pittsburgh, Pa.
[k] Waltham Veterinary Diet Canine Low Fat (extruded), Waltham, Vernon, Calif.
[l] Waltham Veterinary Diet Canine Selected Protein (extruded), Waltham, Vernon, Calif.
[m] Waltham Veterinary Diet Canine Calorie Control (extruded), Waltham, Vernon, Calif.
[n] Eukanuba Veterinary Diet Response FP/Canine (extruded), Iams Co, Dayton, Ohio.
[o] Eukanuba Adult Maintenance Formula (extruded), Iams Co, Dayton, Ohio.
[p] IAMS Chunks (extruded), Iams Co, Dayton, Ohio.
[q] Eukanuba Veterinary Diet Response KO/Canine (extruded), Iams Co, Dayton, Ohio.
[r] Prescription Diet Canine i/d (extruded), Hill's Pet Nutrition Inc, Topeka, Kan.
[s] Prescription Diet Canine r/d (extruded), Hill's Pet Nutrition Inc, Topeka, Kan.
[t] Science Diet Canine Adult (extruded), Hill's Pet Nutrition Inc, Topeka, Kan.
[u] Purina Veterinary Diet OM Overweight Management Canine Formula (extruded), Nestlé Purina, St Louis, Mo.
[v] Purina Veterinary Diet EN GastrENnteric Canine Formula (extruded), Nestlé Purina, St Louis, Mo.
[w] Purina Veterinary Diet HA HypoAllergenic Canine Formula (extruded), Nestlé Purina, St Louis, Mo.
[x] Purina Dog Chow Nutritional Excellence Formula (extruded), Nestlé Purina, St Louis, Mo.
[y] Model 1100, Agilent, Palo Alto, Calif.
[z] Synergy Hydro-RP (10 µM C_{18}; 4.6 mm × 25 cm), Phenomenex, Torrance, Calif.
[aa] Sigma-Aldrich, St Louis, Mo.

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