Identification and concentration of soy isoflavones in commercial cat foods

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Objective—To determine the absolute and relative soy isoflavone content in commercial cat foods.

Sample Population—14 dry, 6 semimoist, and 22 moist commercial cat foods.

Procedure—Soy isoflavone content of each food was determined by use of acid-methanol hydrolysis and high-pressure liquid chromatography with ultraviolet absorbance detection. Isoflavones were identified and quantified by reference to authentic standards.

Results—Genistein and daidzein were the major soy isoflavones identified in 24 of 42 foods, with concentrations ranging from 1 to 163 µg/g of food. Foods labeled as containing soybean solids (16/42) had isoflavone concentrations > 11 µg/g. More dry (13/14) and semimoist (6/6) foods contained isoflavones than moist foods (5/22). Isoflavone content and food cost were negatively correlated for dry and semimoist foods but not for moist foods. Total amount of isoflavone consumed by cats fed these soy-containing foods as a sole maintenance diet was estimated to be between 0.6 and 4.5 mg/kg of body weight/d, which is comparable to concentrations in humans that result in a measurable although modest effect on serum concentrations of steroid and thyroid hormones.

Conclusions and Clinical Relevance—Genistein and daidzein are common constituents of commercial cat foods. Predictors of isoflavone content included ingredient labeling, food type, and food cost. Soy isoflavones in some commercial cat foods were detected in amounts predicted to have a biological effect. (*Am J Vet Res* 2002;63:181–185)

Hyperthyroidism (toxic nodular goiter) is considered the most common endocrinopathy in cats in the United States.[1] The etiopathogenesis of this disease is currently unknown. Since it was first diagnosed and reported in the late 1970s,[2] the prevalence of hyperthyroidism in cats appears to have increased dramatically. Recent estimates indicate that as many as 1 in 300 cats are affected.[1,3] Although this increase in disease prevalence may simply be the result of increased awareness of owners, diagnostic acuity of veterinarians, and the longer lifespan and increased popularity of cats, it may also indicate the emergence of 1 or more etiologic factors in the disease process.[1]

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A possible cause of hyperthyroidism in cats is the inadvertent inclusion of a goitrogenic compound in the diet.[1] Because many potential goitrogens are phenolic in structure, this could also explain the relatively high incidence of hyperthyroidism in cats, compared with other species of domestic animals, because cats have a profoundly reduced ability to metabolize and excrete phenolic compounds by glucuronidation.[4] Soybean is a potential dietary goitrogen that is used as a source of high quality vegetable protein in commercially available pet foods. The goitrogenic effect of soybeans has been attributed to an inhibitory effect of the soy isoflavones genistein and daidzein on thyroid peroxidase, an enzyme essential to thyroid hormone synthesis.[5,6] Furthermore, glucuronidation appears to be an important pathway in the elimination of soy isoflavones in humans and laboratory animals.[7-9] In particular, UDP-glucuronosyltransferase 1A9 is the most active soy isoflavone glucuronosyltransferase in human liver[7] but is deficient in feline liver.[4]

Soy isoflavones in commercial cat foods could therefore play an etiologic role in thyroid disease in cats. However, it is not presently known whether cat foods contain soy isoflavones, which types of cat foods contain soy isoflavones, what specific soy isoflavones are in cat foods, or whether the isoflavone concentration is sufficient to result in a biological effect. Consequently, the objectives of the study reported here were to develop assay methods for identification of commercial cat foods that contain soy isoflavones and to determine the absolute and relative concentrations of isoflavones in these foods.

Materials and Methods

Commercial cat foods—Between January and March 2000, 42 commercially available cat foods[a-pp] representing at least 12 different manufacturers were obtained from local supermarkets, specialty pet food stores, or the Tufts University School of Veterinary Medicine. Foods were classified as dry (foods D1 to D14), moist (foods M1 to M22), or semimoist (foods S1 to S4). All but 3 foods were described on the label as complete and balanced on the basis of results of **Association of American Feed Control Officials (AAFCO)** feeding trials or formulation to meet AAFCO profiles. The remaining 3 foods (S4, S5, and S6) were designated cat treats and were not intended as the sole source of nutrition. Four foods (S2, M1, M4, M12) were store brands, so the manufacturer could not be identified. Except for the store-brand foods for which we used the label price, manufacturers' list prices were obtained in April 2000.[10] Information with regard to the soybean content of each food was obtained from the label. Soy flour or meal was listed as ingredients for foods D1 to D6, S1 to S3, and M1 and M2. Foods S4 to S6 contained soybean protein isolate, whereas food M6 contained soybean oil. Textured vegetable protein (assumed to be a soybean product) was also listed as an ingredient of food M3.

Soy isoflavone content—Soybean contains isoflavones as glucoside conjugates. Consequently, the soy isoflavone concentration of cat food was determined by use of acid hydrolysis and extraction followed by **high-pressure liquid chromatography (HPLC)** and quantitation of the derived aglycones by use of modifications of described methods.[7,11-13] Briefly, 10-ml screw-topped glass tubes were prepared containing 12.5 µg of naringenin[qq] (the internal standard). Samples of food (1 g) were weighed and added to these 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 (approx 25 C), 1-ml aliquots were transferred to microcentrifuge tubes and centrifuged for 5 minutes at 14,000 X g. The supernatant was transferred to sample vials, and 5- to 10-µl aliquots were injected into the HPLC apparatus. This apparatus consisted of a dual-head pump[rr] with autoinjector[ss] serially connected to a column[tt] (3.9 mm X 15 cm) and a UV absorbance detector[uu] set at a wavelength of 260 nm. The mobile phase consisted of 85% 50 m*M* potassium phosphate buffer in water (pH 4.5) and 15% acetonitrile with a flow rate of 1.2 ml/min.

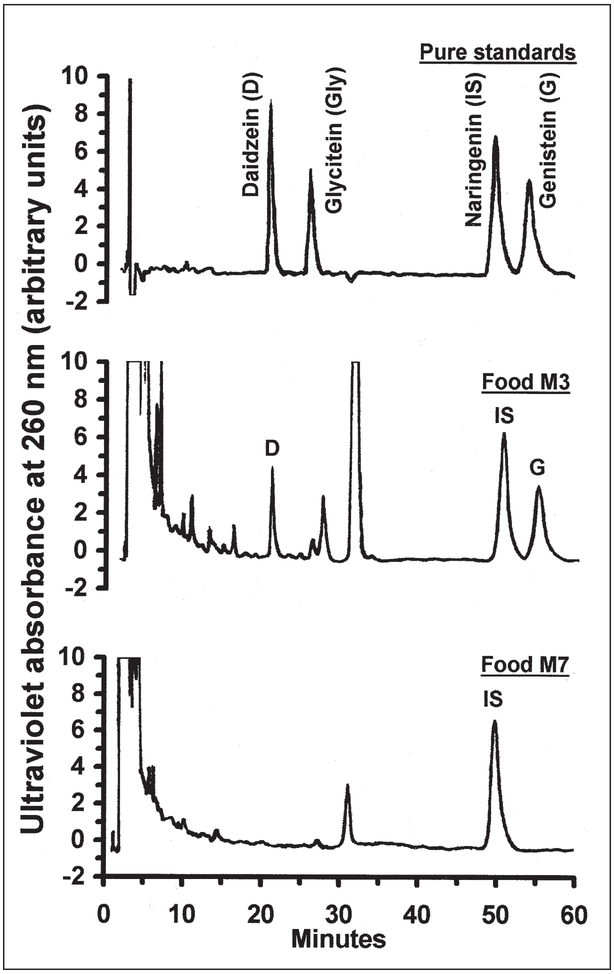

Figure 1—Chromatograms of pure standards (top panel) and acid-methanol extracts of 2 commercially available moist cat foods (M3, middle panel; M7, bottom panel) derived by use of high-pressure liquid chromatography. D = Daidzein. Gly = Glycitein. IS = Internal standard (naringenin). G = Genistein.

For the purpose of peak identification and quantitation, a series of concentrations of pure daidzein,[qq] genistein,[qq] and glycitein[qq] (final concentration, 1 to 10 µg/ml) were prepared with naringenin in a 10% hydrochloric acid-methanol solution. Results of initial experiments indicated that recovery of both daidzein and genistein added to an isoflavone-free cat food matrix was > 90%. Sample peaks were identified by co-elution with the purified compounds and by detection of similar ultraviolet absorption characteristics as the standard compounds at low and high wavelength settings (200 to 300 nm). Intra- and interassay variations were < 10 and 15%, respectively. The minimum quantifiable concentration was 1 µg/g of food (dry weight) for both genistein and daidzein.

Statistical analyses—Isoflavone assays were performed in duplicate, and results were averaged. Concentrations were normalized to food dry weight. The relationship between food cost and total isoflavone content (sum of genistein and daizein content) was assessed by use of Pearson product-moment correlation analysis for each food type. The frequencies with which dry, semimoist, and moist food types contained soy isoflavones (defined as a concentration > 1 µg

Table 1—Concentration of soy isoflavones (genistein and daidzein) in 42 commercially available cat foods

Food	DW (%; wt/wt)	Cost ($/kg of DW)	Soy*	Genistein (µg/g of DW)	Daidzein (µg/g of DW)
D1[a]	96.0	1.30	Y	154	147
D2[b]	94.4	2.59	Y	94	90
D3[c]	95.7	3.13	Y	84	72
D4[d]	93.6	2.70	Y	73	73
D5[e]	96.3	3.14	Y	36	28
D6[f]	96.3	3.14	Y	32	27
D7[g]	96.0	3.15	Y	21	18
D8[h]	92.4	5.11	N	5	3
D9[i]	95.9	4.43	N	5	4
D10[j]	93.8	3.32	N	4	5
D11[k]	95.6	4.94	N	3	2
D12[l]	96.9	3.13	N	3	3
D13[m]	94.1	3.31	N	1	3
D14[n]	94.9	4.92	N	0	0
S1[o]	64.6	6.33	Y	147	140
S2[p]	85.9	3.39	Y	126	123
S3[q]	63.8	6.41	Y	125	121
S4[r]	70.9	21.41	Y	53	39
S5[s]	70.8	21.43	Y	14	8
S6[t]	72.6	20.90	Y	13	9
M1[u]	24.9	5.73	Y	163	104
M2[v]	28.0	18.71	Y	111	86
M3[w]	23.8	6.74	Y	109	78
M4[x]	26.2	5.44	Y	102	135
M5[y]	25.1	18.04	N	12	11
M6[z]	24.3	8.71	N	0	0
M7[aa]	32.8	4.88	N	0	0
M8[bb]	29.2	7.63	N	0	0
M9[cc]	27.5	7.53	N	0	0
M10[dd]	28.1	18.18	N	0	0
M11[ee]	24.6	19.52	N	0	0
M12[ff]	28.2	5.05	N	0	0
M13[gg]	27.2	7.60	N	0	0
M14[hh]	25.7	22.40	N	0	0
M15[ii]	26.3	8.02	N	0	0
M16[jj]	28.5	5.05	N	0	0
M17[kk]	23.3	26.96	N	0	0
M18[ll]	27.5	24.91	N	0	0
M19[mm]	22.6	23.22	N	0	0
M20[nn]	25.1	20.86	N	0	0
M21[oo]	21.5	20.68	N	0	0
M22[pp]	22.3	19.98	N	0	0

*Indicates whether ingredient list contained soy solids. Most listed as soy flour or meal. Soy protein isolate listed for foods S4 through S6, textured vegetable protein for food M3, and soybean oil for food M6.
DW = Dry weight. D = Dry. Y = Yes. N = No. S = Semimoist. M = Moist.

of daidzein or genistein/g of food [dry weight]) was evaluated by use of χ^2 analysis. Significance was set at $P \leq 0.05$.

Results

Moisture content of each cat food was determined to enable normalization of soy isoflavone concentration for comparison among food types. As expected, foods that were classified as dry contained the lowest amount of moisture (mean ± SD, 5 ± 1% wt/wt), moist foods contained the highest amount (74 ± 3%), and semimoist foods contained an intermediate amount of moisture (29 ± 8%).

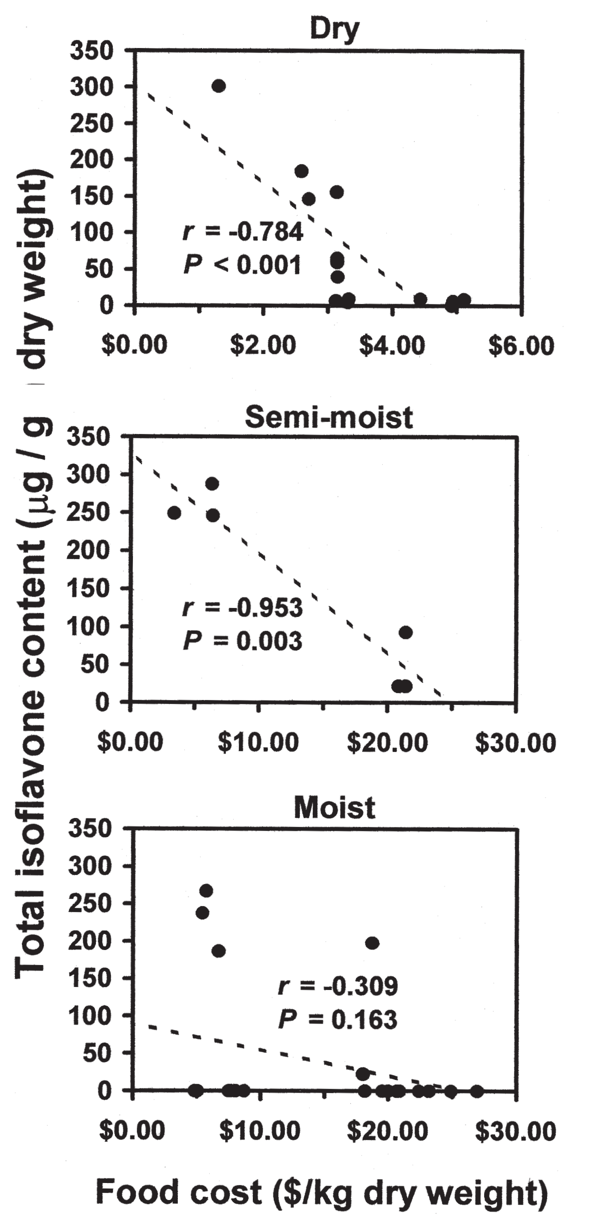

Figure 2—Scatterplots of total isoflavone content versus food cost normalized to dry weight for 14 commercially available dry cat foods (top panel), 6 commercially available semimoist cat foods (middle panel), and 22 commercially available moist cat foods (bottom panel). The dashed line represents the linear regression line of best fit for each comparison. Correlation coefficients (*r*) were determined by use of Pearson product-moment correlation analysis.

Daidzein and genistein were detected (> 1 µg of isoflavone/g of food [dry weight]) in 24 of the 42 foods assayed by use of HPLC (**Fig 1**). Concentrations were as high as 163 µg/g for genistein and 147 µg/g for daidzein (**Table 1**). Although concentrations of the 2 isoflavones were similar, most (20/24) isoflavone-containing foods contained slightly more genistein than daidzein. In many of the isoflavone-containing foods, a small peak relative to the size of the genistein and daidzein peaks was observed with a retention time similar to the glycitein standard. However, this peak could not be adequately separated from interfering unknown peaks to allow identification or quantitation. Dry (13/14) and semimoist (6/6) foods more frequently contained isoflavones than did moist foods (5/22; $P < 0.001$; $\chi^2 = 22.432$).

Labels on 17 of 42 foods listed soybean as an ingredient, and all but 1 of these foods contained > 12 µg of genistein/g and > 11 µg of daidzein/g (Table 1). No soy isoflavones were detected in food M6 even though soybean oil was listed as an ingredient. Labels on 25 of 42 foods did not list soybean as an ingredient; isoflavones were either not detected in these foods (n = 18), or concentrations of each isoflavone were between 1 and 12 µg/g (7). Food cost was significantly and negatively correlated with isoflavone content of dry ($r = -0.784$; $P < 0.001$) and semimoist ($r = -0.953$; $P = 0.003$) cat foods but not with moist cat foods ($r = -0.309$; $P = 0.163$; **Fig 2**).

Discussion

Results of this study indicate that the soy isoflavones genistein and daidzein are common constituents of commercially available cat foods. Only 1 of 20 dry and semimoist foods examined did not contain detectable concentrations of either isoflavone. Moist cat foods also contained soy isoflavones, but the incidence (5/22 foods) was lower than for dry or semimoist. Glycitein, which is also a soy isoflavone, was only detected in small amounts and could not be adequately separated by use of HPLC to allow accurate quantitation. In most soybean products, however, daidzein and genistein are the principal isoflavones.[14] Glycitein typically constitutes only 5 to 10% of total soy isoflavones but can exceed 40% in certain products such as soy germ.[15] Other soy isoflavones may also be present; however, chromatographic standards are only commercially available for the 3 isoflavones that we assayed in the present study.

In all but 1 case, foods that had labels listing soybean products as an ingredient (n = 17) had isoflavone concentrations > 11 µg/g. We could not detect isoflavones in 1 food that was labeled as containing soybean oil, presumably because of the low hexane solubility of the native isoflavone conjugates; soybean oil, which is derived from soybean by use of hexane extraction,[14] contains little or no soy isoflavones. Assuming that soybean meal typically contains approximately 1 mg of genistein/g of dry weight,[12] foods with labels listing soybean as an ingredient, which we found had > 0.011 mg of genistein/g, may contain > 1.1% (ie, 0.011/1) soybean meal by weight. Similarly, we predicted that foods with the highest isoflavone content (eg, D1, S1, and M1) contain as much as 15% soybean meal by weight.

A potential influence on the amount of soybean used in cat foods may be cost, because protein derived from vegetable and grain sources is typically less expensive than protein derived from animal sources. In support of this hypothesis, we found that foods containing soybean were less expensive; the cost of dry and semimoist foods was strongly and negatively correlated with isoflavone content (Fig 2). Furthermore, there appeared to be a critical food cost threshold of approximately $3.20/kg for the dry foods; foods costing less than this had high isoflavone concentrations and, presumably, high concentrations of added soybean. Such a cost threshold may be dictated by market influences, especially the relative cost of raw ingredients used as a protein source. Although the relationship between isoflavone content and food cost was not significant for moist foods, these foods appeared to segregate into low-cost (< $10/kg;11) and high-cost (> $15/kg; n = 11) groups, with 3 of the 4 soybean-containing moist foods in the low-cost group. It is also possible that soybean was added to the moist foods for reasons other than cost minimization, perhaps to enhance the aesthetic appeal to the consumer.

To our knowledge, this is the first study to examine the soy isoflavone content of commercial diets intended for domestic cats. However, authors of a previous study[16] identified and quantified soy isoflavones in a commercial diet intended for consumption by exotic cats. In agreement with our results, genistein and daidzein were identified in the exotic cat diet by use of a highly specific gas-liquid chromatography-mass spectrometry assay. Furthermore, quantities of each of these isoflavones in the exotic cat diet ranged from 18 to 35 µg/g. Isoflavone concentrations in 15 of the 42 foods that we examined in this study were equal to or exceeded these concentrations, with 1 food (D1) containing concentrations as much as 8-fold higher (163 µg of genistein/g and 147 µg of daidzein/g).

Assuming that an average 5-kg cat consumes 50 to 100 g of food/d,[17] the total amount of isoflavone consumed by a cat fed 1 of these isoflavone-containing diets could range from 0.4 to 0.8 mg/kg of body weight (food D7) to 3 to 6 mg/kg of body weight (food D1). Modest but measurable effects on serum concentrations of steroid and thyroid hormones have been documented in women who consumed up to 2 mg of isoflavone/kg of body weight/d.[18,19] If the dose-effect relationship for soy isoflavones in cats and humans is comparable, we would predict a similar hormonal effect in cats consuming a diet with a high isoflavone content. Furthermore, because elimination of isoflavones by glucuronidation may be less efficient in cats, compared with humans, cats may be exposed to relatively higher isoflavone concentrations following consumption of the same amount as humans.

The role of dietary soy in hyperthyroidism in cats is speculative at present. The etiopathologic mechanism that we propose is as follows: inhibition of thyroid peroxidase by soy isoflavones leads to reduced thyroid hormone synthesis and a compensatory increase in **thyroid stimulating hormone (TSH)** release via the hypothalamic-pituitary axis. Although thyroid hormone synthesis returns to normal, continued hyperstimulation of the thyroid gland by TSH may lead to a hypermetabolic state with increased intracellular concentrations of hydrogen peroxide, an essential cofactor for thyroglobulin iodination. Hydrogen peroxide is also a potential DNA mutagen.[20] Excessive amounts of hydrogen peroxide may cause mutation and dysfunction of the genes responsible for coupling TSH to thyroid hormone synthesis and controlling cellular proliferation with resultant formation of autonomous hyperplastic thyroid nodules. High serum thyroid hormone concentrations and associated clinical signs would then follow either withdrawal of the isoflavones or nodule development beyond the suppressive capacity of the isoflavones.

Interestingly, results of 2 retrospective studies[21,22] indicate that the risk of developing hyperthyroidism in cats is related to the composition of the diet. In the study by Kass et al,[22] the risk of hyperthyroidism decreased as the proportion of dry food relative to moist food in the diet increased. Although these results may argue against an etiologic role for soy-containing dry foods in hyperthyroidism, many of the cats in this study consumed both dry and moist foods, and cats that had consumed dry food prior to moist food were not excluded. Furthermore, because soy isoflavones have a direct inhibitory influence on thyroid hormone synthesis similar to the antithyroid drugs methimazole and carbimazole, an increase in serum thyroid hormone concentrations and associated clinical signs may only become evident following a change in diet to 1 low in isoflavones (eg, a moist diet). In the more recent study by Martin et al,[21] cats that consumed fish-flavored canned foods had a higher risk of hyperthyroidism than cats that consumed foods without fish flavoring. These authors speculated that the high iodine content of ocean fish may have contributed to the disease, although a mechanism was not proposed. Iodine has been shown to antagonize the thyroid inhibitory effects of soy.[23] Consequently, we conjecture that consumption of cat foods with relatively high iodine content, such as fish-flavored canned foods, may have unmasked functional adenomatous thyroid tissue previously suppressed by soy isoflavones. Finally, it should be acknowledged that soy isoflavones have other biological activities that may have contributory adverse or beneficial effects in hyperthyroidism or other diseases in cats. These include inhibition of tyrosine kinase,[24] angiogenesis,[25] and topoisomerase II,[26] interaction with the estrogen receptor,[18,19] and induction of apoptosis.[27]

[a]Alley Cat, Ralston Purina Co, St Louis, Mo.
[b]Meow Mix, Ralston Purina Co, St Louis, Mo.
[c]Whiskas Original Recipe, Kal Kan Foods, Vernon, Calif.
[d]Purina Cat Chow, Ralston Purina Co, St Louis, Mo.
[e]Friskies Ocean Fish Flavor, Friskies Petcare Co, Glendale, Calif.
[f]Friskies Poultry Platter, Friskies Petcare Co, Glendale, Calif.
[g]Friskies Gourmet Flavor, Friskies Petcare Co, Glendale, Calif.
[h]Science Diet Feline Maintenance Light, Hill's Pet Nutrition Inc, Topeka, Kan.
[i]Iams Original Adult, Iams Co, Dayton, Ohio.
[j]Purina One Salmon & Tuna, Ralston Purina Co, St Louis, Mo.

[k]Science Diet Feline Maintenance, Hill's Pet Nutrition Inc, Topeka, Kan.
[l]Friskies Special Diet, Friskies Petcare Co, Glendale, Calif.
[m]Purina One Special Formula Adult Cats, Ralston Purina Co, St Louis, Mo.
[n]Max Cat Adult, Nutro Products, City of Industry, Calif.
[o]Tender Vittles Gourmet, Ralston Purina Co, St Louis, Mo.
[p]Moist Dinner for Cats Tender Tuna Flavor, Stop & Shop Supermarket, Boston, Mass.
[q]Tender Vittles Beef, Ralston Purina Co, St Louis, Mo.
[r]Whisker Lickin's Turkey Treats, Ralston Purina Co, St Louis, Mo.
[s]Whisker Lickin's Salmon Treats, Ralston Purina Co, St Louis, Mo.
[t]Whisker Lickin's Chicken Treats, Ralston Purina Co, St Louis, Mo.
[u]Sliced Chicken in Gravy Dinner, Stop & Shop Supermarket, Boston, Mass.
[v]Fancy Feast Chicken Feast in Savory Juices, Friskies Petcare Co, Glendale, Calif.
[w]9 Lives Tuna Select & Cheese Bits in Sauce, Heinz Pet Products, Newport, Ky.
[x]Whitefish & Tuna Dinner, Stop & Shop Supermarket, Boston, Mass.
[y]Nature's Recipe Beef Maintenance Feline Formula, Heinz Pet Products, Newport, Ky.
[z]Figaro Cat Food Tuna, Bumblebee Seafoods, San Diego, Calif.
[aa]9 Lives Tuna in Sauce, Heinz Pet Products, Newport, Ky.
[bb]Beef & Liver Dinner, Friskies Petcare Co, Glendale, Calif.
[cc]Alpo with Beef, Friskies Petcare Co, Glendale, Calif.
[dd]Natural Beef Cat Dinner, Old Mother Hubbard, Lowell, Mass.
[ee]Science Diet Feline Maintenance Beef, Hill's Pet Nutrition Inc, Topeka, Kan.
[ff]Beef & Liver Dinner, Stop & Shop Supermarket, Boston, Mass.
[gg]Whiskas Ground with Bits O' Beef, Kal Kan Foods, Vernon, Calif.
[hh]Beef Formula, Iams Co, Dayton, Ohio.
[ii]Optimum Simmered Stew with Beef, Kal Kan Foods, Vernon, Calif.
[jj]9 Lives Beef Dinner, Heinz Pet Products, Newport, Ky.
[kk]Beef Stew Formula, Triumph Pet Industries, Warwick, NY.
[ll]Gourmet Classics Beef & Egg Skillet, Nutro Products, City of Industry, Calif.
[mm]Fancy Feast Fish & Shrimp Feast, Friskies Petcare Co, Glendale, Calif.
[nn]Fancy Feast Beef & Chicken Feast, Friskies Petcare Co, Glendale, Calif.
[oo]Sheba Salmon, Kal Kan Foods, Vernon, Calif.
[pp]Sheba Tender Beef, Kal Kan Foods, Vernon, Calif.
[qq]Sigma Chemical Co, St Louis, Mo.
[rr]Model 515, Waters, Milford, Mass.
[ss]Model 717, Waters, Milford, Mass.
[tt]5-µm C_{18} Novapack, Waters, Milford, Mass.
[uu]Model 486, Waters, Milford, Mass.

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