Effect of dietary soy on serum thyroid hormone concentrations in healthy adult cats

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Objective—To compare effects of short-term administration of a soy diet with those of a soy-free diet on serum thyroid hormone concentrations in healthy adult cats.

Animals—18 healthy adult cats.

Procedure—Cats were randomly assigned to receive either a soy or soy-free diet for 3 months each in a crossover design. Assays included CBC, serum biochemical profile, thyroid hormone analysis, and measurement of urinary isoflavone concentrations.

Results—Genistein, a major soy isoflavone, was identified in the urine of 10 of 18 cats prior to dietary intervention. Compared with the soy-free diet, cats that received the soy diet had significantly higher total thyroxine (T_4) and free T_4 (fT_4) concentrations, but unchanged total triiodothyronine (T_3) concentrations. The T_3/fT_4 ratio was also significantly lower in cats that received the soy diet. Although the magnitudes of the increases were small (8% for T_4 and 14% for fT_4), these changes resulted in an increased proportion of cats (from 1/18 to 4/18) that had fT_4 values greater than the upper limit of the laboratory reference range. There was no significant effect of diet on any other measured parameter.

Conclusions and Clinical Relevance—Short-term administration of dietary soy has a measurable although modest effect on thyroid hormone homeostasis in cats. Increase in T_4 concentration relative to T_3 concentration may result from inhibition of 5′-iodothyronine deiodinase or enhanced T_3 clearance. Soy is a common dietary component that increases serum T_4 concentration in cats. (*Am J Vet Res* 2004; 65:586–591)

Hyperthyroidism is the most common endocrinopathy in cats in the United States.[1] The etiology of this disease is unknown. Prior to the late 1970s, feline hyperthyroidism was considered a rare disease, but has dramatically increased in incidence during the 1980s and 1990s suggesting involvement of an emerging casual factor.[2] Factors that have been proposed include an epizootic infectious agent, a chemical agent introduced into the environment in the 1970s, and a nutritional component.[1-4] Three case-control studies have investigated possible causative factors for feline hyperthyroidism.[2-4] All studies failed to support a role for infectious agents. In 1 study,[3] environmental toxins such as fertilizers, herbicides, and flea treatments were implicated, but not corroborated in later studies involving larger numbers of animals. That study and another[2] did find that cats with diets that contained a high proportion of commercial foods (> 80%, particularly canned foods) were at increased risk of hyperthyroidism. The second study[2] also identified use of cat litter as a significant risk factor. A more recent study[4] suggested that consumption of certain types of commercial cat foods, particularly those that are fish flavored or liver and giblets flavored, were associated with increased risk of the disease. The most consistent finding appears to be an association of hyperthyroidism with diet.

It has been hypothesized that there could be a common component of the feline diet, perhaps a goitrogen, which is responsible for the development of feline hyperthyroidism.[1] The only potential goitrogen that has been studied to date in cats is iodine.[5,6] One study[5] determined iodine concentrations in 28 varieties of commercially available cat foods and found highly variable concentrations (more than 100-fold difference). Another study[6] revealed that serum **free thyroxine (fT_4)** concentrations in cats are acutely responsive to changes in iodine intake.

Soybean (soy) is another potential dietary goitrogen[7-11] that is commonly used as a source of high quality vegetable protein in commercial pet foods. A recent study[12] revealed that 17 of 42 commercial cat foods surveyed listed soy as an ingredient, whereas an additional 7 foods (total, 24/42) contained substantial amounts of soy isoflavones, measured as an indicator of soy content. The soy isoflavones, including daidzein, genistein, and glycitein, are polyphenolic compounds found in soy with weak estrogenic properties among other endocrine effects.[10,13,14]

Although soy was initially identified as a goitrogen more than 70 years ago,[10,15] the exact mechanism for this effect has not been elucidated. Several possibilities have been proposed including inhibition of T_4 synthesis in the thyroid gland,[8,9] inhibition of **triiodothyronine (T_3)** synthesis from T_4,[16] and induction of phase II enzymes, such as UDP-glucuronosyltransferase, which are responsible for T_3 and T_4 clearance.[17-20] The resultant decrease in available T_3 would then stimulate

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increased **thyroid-stimulating hormone (TSH)** release from the pituitary, which in turn leads to thyroid hypertrophy (goiter) and restoration of thyroid hormone concentrations to reference range.

To our knowledge, the effect of soy on thyroid function in cats has not been evaluated. Consequently, the purpose of this study was to compare effects of short-term administration of a soy diet with those of a soy-free diet on serum thyroid hormone concentrations in healthy cats. On the basis of the known inhibitory effects of soy on thyroid peroxidase,[8,9] we hypothesized that there would be an initial decrease in circulating T_4 and T_3 concentrations and that homeostatic mechanisms would return T_4 and T_3 concentrations to reference ranges through increased stimulation of the thyroid gland by TSH.

Materials and Methods

Study subjects—Twenty adult cats were recruited for this study from the Tufts University staff, students, and faculty. To be eligible for the study, the cats had to live indoors only, be from 1 to 10 years of age, and be deemed healthy. The health of the cats was ascertained before the start of the study by use of physical examination, CBC, serum biochemical profile, total T_4 concentration, and urinalysis. Cats with palpable thyroid nodules or heart murmurs detected on physical examination were excluded. Cats with any laboratory evidence of underlying metabolic disease were excluded. The study was approved by Tufts University Institutional Animal Care and Use Committee. Owners of the cats provided written consent for the cats to be used.

Experimental diets—Both diets (soy and soy-free; **Tables 1 and 2**) were custom manufactured[a] and were formulated to be complete and balanced for all feline life stages in accordance with **American Association of Feed Control Officials (AAFCO)** profiles. The iodine contents of the diets (determined by direct analysis) were similar and were approximately 3 times the minimum AAFCO recommendation. The taurine contents of both diets were also determined to be similar on the basis of the content of ingredients.

Experimental design—A crossover design was used with cats randomly assigned to receive either the soy or the soy-free diet first. Cats were fed the assigned diet for 3 months and then crossed over to the other diet for a further 3 months. The 2 diets were formulated and coded such that the type of diet would remain unknown to the investigators and study participants until the completion of the study. Owners were instructed to feed the diet exclusively, with no other cat foods, treats, or table food for the duration of the study.

Table 1—Ingredients (percentage by weight values) used to formulate soy and soy-free study diets fed to 18 cats for 3 months each in a crossover design

Ingredient	Soy-free diet	Soy diet
Corn	19.8	17.5
Rice	11.0	8.0
Wheat	7.5	6.5
L-lysine	0.3	0
Taurine	0.1	0
KCl	0.9	0.5
Corn gluten meal	24.25	11.72
Vitamin mix	0.075	0.075
Soybean meal	0	10.25
Soy flakes	0	7.5
Fish meal	0	5.0
Poultry by-product	16.3	5.0
Calcium carbonate	0.83	0.75
Phosphoric acid	0.75	0.75
Mineral mix	0.105	0.105
Choline	0.25	0.25
Chicken (whole)	18.0	18.0
Tallow	7.5	9.0
Palatability coating	2.0	2.0

Table 2—Results of analysis of formulated soy and soy-free study diets fed to 18 cats for 3 months each in a crossover design

Analyte	Soy-free diet	Soy diet
Moisture (%)	9.52	9.15
Protein (%)	34.2	34.3
Fat (%)	14.2	14.2
Ash (%)	5.84	6.76
Iodine (µg/g)	0.94	1.2
Metabolizable energy (kcal/g)	4.13	4.00

A physical examination and collection of blood was performed on each cat at entry into the study and at 6-week intervals after initiation of each diet. Urine was collected by cystocentesis at entry into the study, before switching diets (3 months), and at the end of the study (6 months) for measurement of concentration of free and conjugated soy isoflavones. Complete blood count and serum biochemical profiles were determined at each visit to evaluate the health of the cats. Serum was stored at –80°C and all samples were analyzed for T_3, T_4, and fT_4 concentrations. Cat owners maintained a logbook and recorded the amount of food consumed and any problems potentially related to the study.

Analytical methods—All assays were conducted in duplicate, and mean results were determined. Unless otherwise indicated, results for multiple measurements are given as mean ± SD (for normally distributed data) or median and range. Concentrations of total (free plus conjugated) soy isoflavones were measured in the diets and in urine by use of high-performance liquid chromatography with UV absorbance detection[b] following either acid-methanol hydrolysis (food) or enzymatic deconjugation (urine) on the basis of described methods.[12,21,22] Identity of analyte peaks was confirmed by comparison of retention times and diode-array absorption scans of sample peaks with that of authentic standards of daidzein,[c] genistein,[c] and glycitein.[c] The limit of detection for the assay was 1 µg of isoflavones/g of food and 0.05 µg of isoflavones/mL of urine.

Commercial assay kits were used to measure total T_4[23,d] and fT_4 after equilibrium dialysis.[24,e] Total T_3 was measured by use of an in-house charcoal separation method.[f]

Statistical analyses—Statistical analyses were conducted with commercial statistical software.[g] Comparisons of CBC, serum biochemical, and thyroid hormone data between diets were performed with repeated-measures ANOVA. The fT_4 concentrations were also evaluated by use of χ^2 analysis. For all comparisons, values of $P < 0.05$ were considered significant.

Results

Eighteen of the 20 cats that entered the study completed the study. One cat that had previously been an outdoor cat developed behavioral problems when kept indoors and was removed from the study on day 7. The second cat was removed from the study after developing lower urinary tract disease 84 days into the study. Data analysis was performed on the 18 cats that completed the study. Median age of the cats was 5 years (range, 2 to 9 years). There were 11 spayed females and 7 castrated males. Mean ± SD

body weight was 5.1 ± 1.2 kg. Both diets were readily consumed by all cats. There was no effect of diet on body weight ($P > 0.05$).

The isoflavone content of the soy diet per gram of food (dry weight) was 198 µg of genistein, 182 µg of daidzein, and 29 µg of glycitein. The soy-free diet contained 19 µg of genistein/g, 16 µg of daidzein/g, and 3 µg of glycitein/g. Analysis of individual components used to formulate the soy-free diet revealed genistein and daidzein in the corn gluten meal (50 and 32 µg/g, respectively) and the poultry meal (9 and 12 µg/g, respectively), but not in any other constituent. On entry into the study, 10 of 18 cats had measurable urinary concentrations of genistein (median, 0.11 µg/mL; range, 0.05 to 1.26 µg/mL), whereas 2 of 18 cats had measurable urinary concentrations of daidzein (0.29 and 0.51 µg/mL). Glycitein was not detected in the urine of any of the cats at entry or while receiving any of the experimental diets. Of the 10 cats with measurable urinary genistein concentrations, 4 cats received the soy diet first and 6 cats received the soy-free diet first. All cats that received the soy diet had measurable urinary concentrations of genistein (median, 1.27 µg/mL; range, 0.35 to 4.7 µg/mL), whereas 17 of 18 cats had urinary concentrations of daidzein (median, 0.30 µg/mL; range, 0.08 to 1.53 µg/mL). All cats that received the soy-free diet also had measurable but much lower urinary concentrations of genistein (median, 0.15 µg/mL; range, 0.07 to 0.43 µg/mL), whereas only 2 of these cats had measurable daidzein concentrations (0.05 and 0.10 µg/mL).

There was no significant effect of the order of diet administration on any of the measured parameters. Complete blood count and serum biochemical parameters were essentially unaffected by diet except for serum creatinine concentration and alkaline phosphatase activity. Compared with cats on the soy-free diet, creatinine concentrations were significantly ($P = 0.004$) higher and alkaline phosphatase activities were significantly ($P < 0.001$) lower in cats fed the soy diet at the end of the 3-month treatment period. However, these values were still within the laboratory reference ranges.

Plasma thyroid hormone concentrations were determined at 6 and 12 weeks after initiation of dietary treatments (**Table 3**). After 6 weeks of treatment, only total T_4 concentrations were significantly ($P = 0.01$) higher in cats that consumed the soy diet, compared with cats that consumed the soy-free diet. However, after a further 6 weeks of treatment, fT_4 ($P = 0.004$; **Fig 1**) as well as total T_4 ($P = 0.02$) concentrations were significantly higher in cats that received the soy diet.

Table 3—Serum thyroid hormone values (mean ± SD) measured in 18 cats after being fed a soy diet and a soy-free diet for 6 and 12 weeks

Variable	Reference Range	6 weeks		12 weeks	
		Soy-free diet	Soy diet	Soy-free diet	Soy diet
fT_4 (pmol/L)	10–50	41 ± 10	44 ± 8	37 ± 9	42 ± 9*
T_4 (nmol/L)	10–49	40 ± 9	43 ± 6*	39 ± 8	42 ± 9*
T_3 (nmol/L)	0.6–1.9	0.81 ± 0.20	0.79 ± 0.16	0.80 ± 0.12	0.76 ± 0.16
T_3/fT_4 (ratio)	ND	0.021 ± 0.008	0.018 ± 0.004	0.023 ± 0.007	0.019 ± 0.005*

*Significant ($P < 0.05$) difference from soy-free diet. fT_4 = Free T_4. T_4 = Thyroxine. T_3 = Triiodothyronine. ND = Not determined.

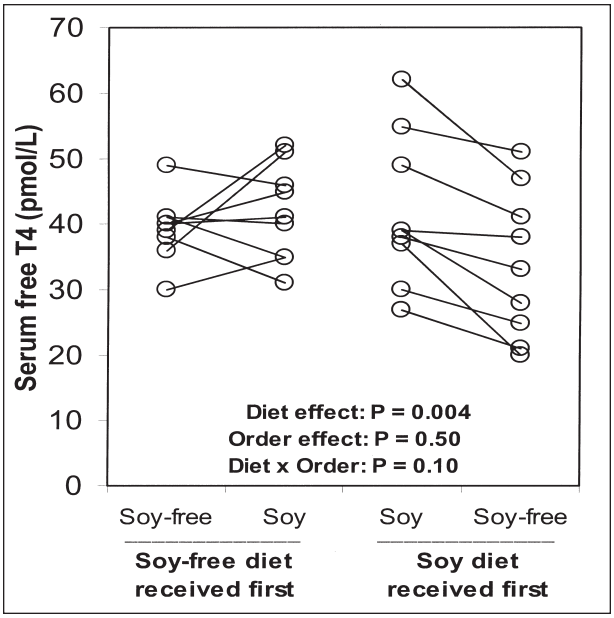

Figure 1—Mean serum free thyroxine (T_4) concentrations in 18 cats fed soy-free and soy diets for 3 months each in a crossover design.

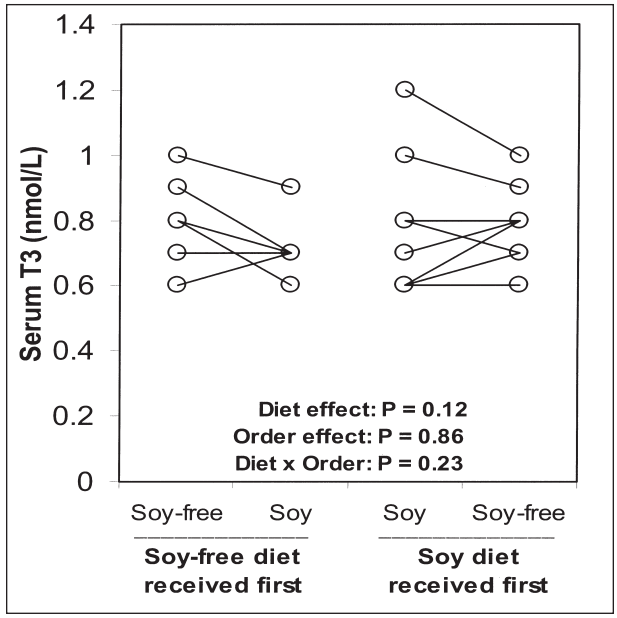

Figure 2—Mean serum triiodothyronine (T_3) concentrations in 18 cats fed soy-free and soy diets for 3 months each in a crossover design.

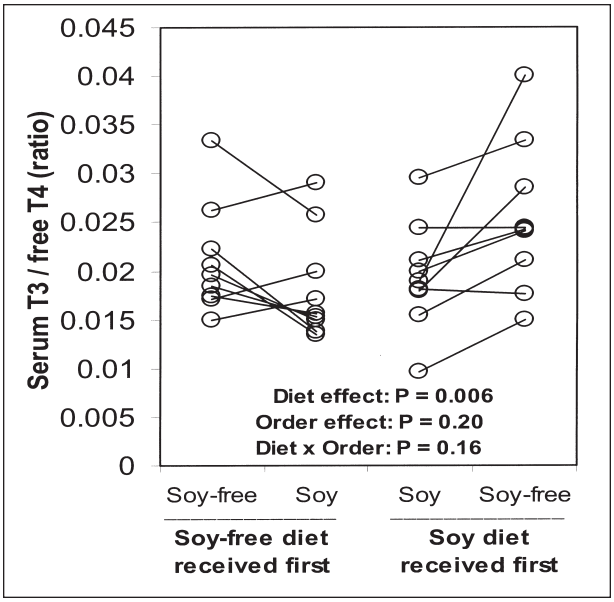

Figure 3—Mean serum T_3/free T_4 concentration ratios determined in 16 cats fed soy-free and soy diets for 3 months each in a crossover design.

Increases were small (mean increase, 8% and 14% for total T_4 and fT_4 concentrations, respectively). In cats that received the soy diet, 4 of 18 fT_4 measurements at the end of the treatment period were greater than the upper limit of the laboratory reference range (reference range, 10 to 50 pmol/L), whereas only 1 of 18 fT_4 measurements were greater than this range in cats that received the soy-free diet ($P < 0.001$). Total T_3 concentrations were unaffected by dietary treatments regardless of treatment duration (**Fig 2**). The ratio of total T_3 to fT_4 was significantly lower in cats fed the soy diet, compared with the soy-free diet, after 12 weeks of treatment (mean difference, 19%; **Fig 3**), but not at the 6-week time point ($P = 0.22$).

Discussion

Results of this study indicate that a soy diet has a measurable although modest effect on serum thyroid hormone concentrations. However, the effect was not as we had originally hypothesized on the basis of inhibition of thyroid peroxidase (decrease in T_4 and T_3 concentrations) in that we observed increases of fT_4 and total T_4 concentrations without apparent change in total T_3 concentration. A review of the literature indicates that these findings are, in fact, in agreement with the reported effects of soy on thyroid hormone concentrations in most other species. Compared with a casein diet, miniature pigs fed soy protein had 26% to 34% higher T_4 concentrations without a change in T_3 concentrations.[26] Similarly, hamsters fed soy protein had higher T_4 concentrations as well as lower T_3 concentrations, compared with those fed a casein diet.[27,28] Increased serum T_4 concentrations and unchanged T_3 concentrations were also reported for rats that received a soy protein diet, compared with a casein diet.[18] Conversely, in humans, soy is reported to either have no effect[29] or result in reduced T_3 concentrations but unchanged T_4 concentrations.[30] However, exposure to soy protein in the human studies was much less than that in the experimental animal studies.

Increase in serum T_4 concentrations relative to T_3 concentrations could result from inhibition of the enzyme responsible for conversion of T_4 to T_3 (5'-iodothyronine deiodinase). Although the soy isoflavones have not yet been investigated as inhibitors of deiodinase, related flavonoid compounds found in plants such as biochanin A, rutin, and quercetin are potent inhibitors, with concentrations causing 50% decrease in enzyme activity in the low micromolar range.[16,31] There is also substantial evidence that amiodarone, a drug used to treat cardiac dysrhythmias, causes thyrotoxicosis as an important adverse effect with prolonged usage through inhibition of T_4 deiodination.[32,33] Deiodinase inhibition initially would be expected to decrease T_3 concentrations. However, because T_3 is the primary determinant of TSH release by the pituitary gland, homeostatic mechanisms should rapidly restore T_3 concentrations nearly to reference range, but with a higher output of T_4 from the thyroid gland, as required to overcome enzyme inhibition. With amiodarone administration in humans, a substantial increase in serum T_4 concentration occurs as soon as 1 week after initiation of treatment.[34] In our study, we observed a significant increase in total T_4 concentrations within 6 weeks of treatment and in both total T_4 and fT_4 concentrations after 12 weeks of treatment.

A similar effect on serum thyroid hormone concentrations (higher T_4 with unchanged T_3 concentrations) could also be hypothesized to occur if soy resulted in enhanced clearance of T_3 (but not of T_4) through induction of the enzymes responsible for metabolism of T_3. Inducers of T_3 glucuronidation in rats (such as pregnenolone-16-alpha-carbonitrile) have been identified that result in thyroid hyperplasia.[20] Interestingly, pregnenolone-16-alpha-carbonitrile administered to mice increases serum T_4 concentrations without affecting T_3 concentrations, similar to the effect on thyroid hormones in our study.[35] Finally, the finding of increases in both fT_4 and total T_4 concentrations with the soy diet indicated that increased total T_4 concentrations were not the result of increased thyroid hormone-protein binding.

Dietary iodine is known to modify the effects of soy on the thyroid gland.[7,17,36] Iodine deficiency enhances the goitrogenic effects of soy, whereas iodine supplementation is protective. Iodine concentrations in the diets in our study were approximately 3 times the minimum AAFCO recommended concentration; therefore, any effect of diet on the thyroid gland was unlikely to have resulted from iodine deficiency. Indeed, it is possible that this concentration of iodine may have minimized the observed differences in thyroid hormone concentrations between the diets. There was a small difference in iodine content between the soy (1.2 μg/g) and soy-free (0.94 μg/g) diets, which could be argued to have affected the results. However, based on the findings of a previous study,[6] the higher iodine content of the soy diet should have resulted in lower fT_4 concentrations rather than the observed higher fT_4 concentrations, compared with the lower iodine content of the soy-free diet.

Measurement of serum TSH concentrations would have helped to substantiate the potential goitrogenicity of the diet by revealing increased TSH concentrations with soy consumption. Although the commercial T_3 and T_4 assays that we used are well established for use in cats, a feline TSH assay is not yet available. In preliminary studies[37] by 1 of our coauthors, an expected increase in serum TSH values in a sample of treated hyperthyroid cats was detected with the commercial canine TSH assay. However, this assay has not been fully validated for use in cats and also appears to be somewhat insensitive, probably because of differences in the molecular structure of canine and feline TSH. Consequently, our findings will need to be confirmed with a more sensitive and specific feline TSH assay, should it become available.

The primary purpose for measuring the soy isoflavone content of the experimental diets was to verify that we had concentrations that were comparable to concentrations in commercial diets. Indeed, the isoflavone content of the soy diet (genistein, 198 μg/g; daidzein, 182 μg/g) was similar to, although slightly higher than, the highest values we have reported for commercial cat foods (genistein, 163 μg/g; daidzein, 147 μg/g).[12] Unexpectedly, the soy-free diet contained low but measurable concentrations of soy isoflavones (approx 10% of the soy diet), despite being formulated with ingredients that were not identified as containing soy products. Subsequent analysis identified 2 components (chicken meal and corn gluten meal) that contained soy isoflavones. Conceivably, the chicken meal may have included gastrointestinal contents with residual soy-containing chicken feed. However, there is no apparent explanation for the soy isoflavones in the corn gluten meal. These findings are consistent with our previous study,[12] which identified 7 of 42 commercial cat foods that contained low concentrations of isoflavones but did not list soy as an ingredient on the food packaging.

The urinary soy isoflavone content of the cats reported here was measured to ensure compliance with treatment. Consistent with results of the dietary isoflavone analysis, we also found urinary isoflavones in all cats that received the soy-free diet; these were approximately 10% that of cats that received the soy diet. We also determined that many of the cats (10/18) had measurable urinary isoflavones at entry into the study and prior to receiving any of the experimental diets. In fact, 2 of the cats at entry had urinary isoflavone concentrations that were similar to the median values obtained for cats that received the soy diet. These findings indicated that the experimental diets were similar to available commercial diets in terms of isoflavone bioavailability and that exposure of cats to dietary soy was common, at least among the population of cats that provided the study subjects.

One limitation of the present study was that the diets differed in components other than soy that could have affected the results. For example, fish meal was added to the soy diet, but not to the soy-free diet, to ensure similar iodine content. Despite this, there could be effects of fish meal (other than the iodine) on thyroid gland function. A more stringent approach, from a scientific standpoint, may have been to use diets based on a single major protein source such as casein and supplemented with soy and essential micronutrients. However, it could be argued that these types of experimental diets are not representative of diets that pet cats consume.

The magnitudes of increases in total T_4 and fT_4 concentrations in cats that consumed the soy diet were admittedly small, although these changes resulted in an increased number of cats (from 1/18 to 4/18) that had values greater than the upper limit of the laboratory reference range. The clinical implication of this effect, particularly with regard to feline hyperthyroidism, is presently unclear and will require further study before a definitive conclusion can be reached. However, it should be noted that the duration of exposure to the soy diet was short (only 3 months), especially considering that feline hyperthyroidism typically is not apparent until cats are > 7 years of age. It is therefore possible that chronic low-level hyperstimulation of the thyroid gland during a long period could lead to formation of thyroid adenoma and feline hyperthyroidism. Such a mechanism would need to be evaluated by dietary intervention during a longer time frame (probably years) and with use of a more sensitive feline-specific TSH assay.

[a]Nestlé Purina Co, St Louis, Mo.
[b]Model 1100, Agilent, Palo Alto, Calif.
[c]Sigma-Aldrich, St Louis, Mo.
[d]Gamma Coat M total T_4 radioimmunoassay kit, Diasorin Inc, Stillwater, Minn.
[e]Free T_4 equilibrium dialysis kit, Nichols Institute Diagnostics, San Clemente, Calif.
[f]Diagnostic Center for Population and Animal Health (AHDL), Michigan State University, Lansing, Mich.
[g]Systat 10.0, SPSS, Chicago, Ill.

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