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Changes in Liver Glutathione *S*-Transferase Activities in Coturnix Quail Fed Municipal Sludge-Grown Cabbage with Reduced Levels of Glucosinolates (42315)

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Abstract. Cabbage, green beans, or seeds from sunflowers grown either on municipal sewage sludge-amended soil or soil alone were fed to male and female Coturnix quail, as 50% of a complete diet, for 5 weeks. Specific activities of liver glutathione *S*-transferase (GST) were similar in all quail fed the latter two plant diets and also similar to quail fed a nonplant, control diet. Sludge-grown cabbage-treated quail exhibited liver GST activities significantly higher ($P < 0.05$) than levels of liver GST in birds fed the other plants, with a further twofold activity increase in quail fed the soil-grown cabbage. This response seems to be correlated with the levels of glucosinolates present in the cabbage, i.e., 3040 and 9253 ppm (dry basis) in the sludge-grown and soil-grown cabbage, respectively.

Municipal sewage sludge disposal methods include ocean dumping, landfilling, and incineration. The need to find additional, inexpensive ways of disposal of the vast amounts of industrial sewage sludge production has led New York State, as well as other industrialized states, to consider its utility as a soil conditioner and fertilizer amendment on agricultural lands (1). Numerous biological changes and toxicologic effects have been observed in foods produced on sludge-amended soils (2), including alteration of the glucosinolate content of cabbage (3). Glucosinolates (thioglucosides) are natural sulfur-containing compounds present in many plants especially from the botanical family, cruciferae (4). They are important flavor components in cabbage, cauliflower, brussels sprouts, etc., but at high dose levels produce various toxicologic effects in laboratory and farm animals (5). Specific glucosinolates, or their metabolic products, have been shown to inhibit chemical carcinogenesis (6).

Glutathione *S*-transferase (GST) is a major detoxification enzyme system that catalyzes the binding of numerous electrophilic chemicals, including reactive forms of certain chemical carcinogens, to glutathione (7). The specific activity of liver cytosolic GST in Coturnix quail (*Coturnix coturnix japonica*) has been enhanced upon administration of various chemicals (8).

The purpose of this study was to observe possible changes in the specific activity of liver GST in Coturnix quail fed diets containing either cabbage, green beans, or sunflower seeds grown on a municipal sludge-soil mixture with known levels of glucosinolates.

Materials and Methods. Seven groups, 11–14 per group, of 1-day-old Coturnix quail (kindly supplied by the Department of Avian Sciences, Cornell University) were fed diets containing either cabbage, green beans, or sunflower seeds, grown on either a sludge-amended soil or soil only, and one group fed a basal, purified control diet. The vegetables were freeze-dried, ground, and added at 50%, by weight, to a semipurified diet (9). The diets were adjusted, with variable amounts of isolated soybean protein, to contain 33% total protein.

The municipal sludge, obtained from Syracuse, New York was added to experimental plots at the equivalent rate of 224 metric tons per hectare as has previously been described in detail (9).

The quail were sexed and wing-banded after 3 weeks on each treatment. After 5 weeks all quail were killed by cervical dislocation. Livers from the quail were homogenized in a phosphate buffer, pH 6.6, centrifuged at 2000*g* for 30 min, and the supernatant decanted and centrifuged at 100,000*g* for 60 min at 0–5°C. The specific activity of GST was calculated by monitoring the reaction product of recrystallized 1-chloro-2,4,-dinitrobenzene with 0.05 ml of cytosol (10) and recording the change in absorbance at 340 nm in a dual beam spec-

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trophotometer (Varian-Cary 219). A biuret method was used for cytosol protein (11).

The total glucosinolate analysis of all plant foods were performed (3) by measuring glucose (12) after hydrolysis with prepared myrosinase (thioglucoside, EC 3.2.3.1. (13)).

Results. As seen in Fig. 1, the cabbage-fed quail exhibited significantly higher ($P < 0.01$) levels of liver GST than the quail fed the other diets. The highest level of GST, in male quail fed the soil-grown cabbage, had a specific activity of 2195 ± 111 nmole/min/mg protein.

Glucosinolate analysis of the plant foods revealed that the soil-only grown cabbage contained 9253 ppm while the sludge-amended soils produced cabbage containing 3040 ppm. The only other plant food that contained glucosinolates was the soil-only grown green beans with 1530 ppm.

A significantly decreased ($P < 0.05$) body weight was observed in the quail fed the 50% cabbage diets (Table I) with the male quail exhibiting larger relative liver weights than the males fed the basal control diet (Table II). Also seen in Table II, the female quail, in general, exhibited significantly ($P < 0.05$) larger livers than the males. However, female quail fed the soil-grown green beans as the sunflower seeds, cultured on either sludge or soil, showed lowered relative liver weights as compared to basal-fed controls. The cabbage and green beans grown on sludge-amended soil produced quail with body weights significantly higher ($P < 0.05$) than quail fed these vegetables grown on soil alone. The highest body weights were observed in both male and female quail fed the 50% sunflower seed meal.

Discussion. There was approximately a three- to four-fold increase of the specific activity of liver GST from quail fed the soil-grown cabbage, as compared to quail fed the other three diets. Indeed, there was about a

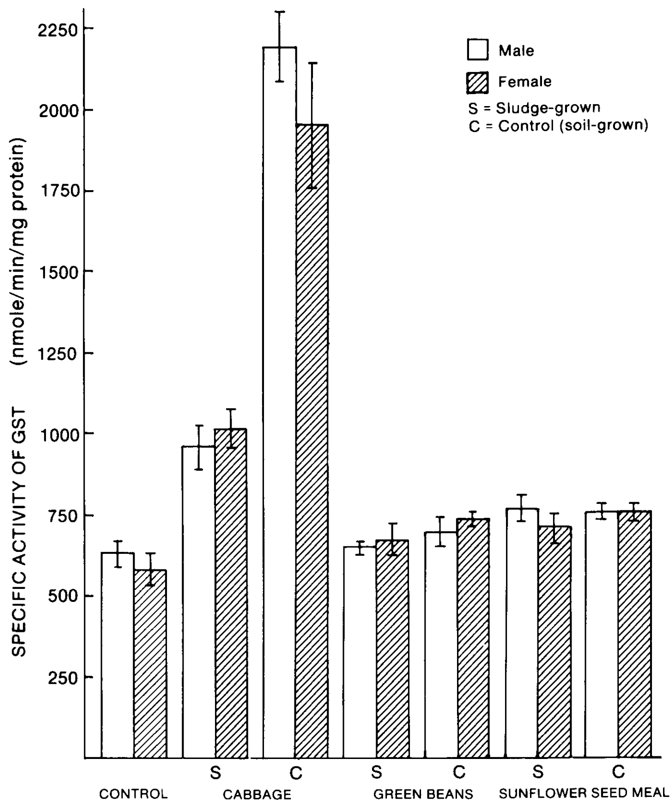

FIG. 1. Mean specific activities of liver GSTase in Coturnix quail fed plant foods grown on municipal sewage sludge. N shown in Table 1. Brackets indicate standard error of mean values.

TABLE I. MEAN BODY WEIGHTS, FOOD, AND GLUCOSINOLATE INTAKE OF COTURNIX QUAIL FED SLUDGE-GROWN PLANT FOODS FOR 5 WEEKS

Diet	Body wt (g) M[a]	Body wt (g) F	Food intake[c] (g)	Glucosinolate intake[c] (mg)
Basal–control	(3) 92 ± 2[b]	(4) 103 ± 6	286	0
Cabbage–sludge grown	(5) 88 ± 2	(4) 88 ± 3	310	471
Cabbage–soil grown	(4) 78 ± 3	(6) 70 ± 4	238	1100
Green beans–sludge grown	(5) 98 ± 1	(3) 102 ± 7	319	0
Green beans–soil grown	(4) 88 ± 3	(6) 87 ± 3	293	224
Sunflower seeds–sludge grown	(6) 96 ± 4	(6) 108 ± 3	322	0
Sunflower seeds–soil grown	(2) 100 ± 0	(8) 120 ± 4	332	0

[a] M = males; F = females. Number in parentheses represents the *N* per group.
[b] Values are means ± SD.
[c] Total intake/No. of quail.

twofold increase of GST in quail fed the soil-grown cabbage as compared to the quail fed the sludge-amended soil-grown cabbage. These differences appear to be correlated with glucosinolate intake (Table I). However, the relatively low intake of glucosinolates consumed in the soil-grown green bean treatment appeared to have no effect on liver GST levels. Sparnins *et al.* (14) have shown that feeding various vegetables, tea, or green coffee beans to mice enhanced liver and small intestinal mucosa GST levels. High glucosinolate fractions of brussels sprouts fed to rats elevated liver microsomal monooxygenase activity (15). Although GST is an important mechanism for carcinogen detoxification (7), and certain indoles, metabolized from indole glucosinolates, have been shown to enhance GST (14), as well as direct inhibition of polycyclic hydrocarbon-induced neoplasia (16), there is

TABLE II. RELATIVE LIVER WEIGHTS OF QUAIL FED SLUDGE-GROWN VEGETABLES

Diet	Relative liver wts (% body wt) M[a]	Relative liver wts (% body wt) F
Basal–control	1.88 ± 0.09	3.11 ± 0.16
Cabbage–sludge grown	2.42 ± 0.19*	2.80 ± 0.07
Cabbage–soil grown	2.65 ± 0.12*	2.95 ± 0.13
Green beans–sludge grown	2.26 ± 0.16	3.38 ± 0.13
Green beans–soil grown	2.08 ± 0.13	2.53 ± 0.13**
Sunflower seeds–sludge grown	1.71 ± 0.08	2.39 ± 0.19**
Sunflower seeds–soil grown	1.66 ± 0.04	2.53 ± 0.13**

[a] See first two footnotes in Table I.
* Significantly higher values ($P < 0.05$) than control males.
** Significantly lower values ($P < 0.05$) than control females.

evidence that nonglucosinolate fractions of cruciferous vegetables can also enhance liver GST (17).

Preliminary studies on the cabbage used in this investigation indicate that the total carotenoid content of the sludge-grown cabbage was lower than the carotenoid level present in the soil-grown cabbage. β-Carotene has been suggested to reduce human cancer rates although the experimental and epidemiological evidence appear equivocal (18).

The lowered body weights of quail fed the 50% cabbage diets, or the soil-grown green bean treatment, reflect the lowered food intake with the larger amounts of glucosinolate intake (Table I). Diets containing plant foods with high glucosinolate levels have produced experimental animals with depressed growth (19). The quail with the highest body weights in this study were fed sunflower seed meal. These results probably reflect the high nutritional content of this material (20).

Increased relative liver weights in male rats, correlated with increased levels of dietary cabbage, have been observed in our laboratory (21) which are similar to the results found in the male quail but not in the females (Table II). Liver enlargement without liver damage is known to be due to sustained induction of microsomal enzymes (22). The quail hens in this study, in general, exhibited larger relative liver weights than the males reflecting the pronounced effect of estrogen on avian liver (23). Estrogen may be a factor inhibiting any further liver enlargement by dietary cabbage. The lowered relative liver weights observed in the female quail fed sunflower seed meal or soil-grown green beans were unexpected.

This study presents evidence that Coturnix quail exhibit high levels of liver GST when consuming vegetables containing relatively high levels of glucosinolates. Additional studies with pure glucosinolates, as well as studies with other natural compounds known to be present, e.g., carotene, xanthophylls, etc. in cabbage, will have to be done to clarify these initial observations.

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