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Received for review June 23, 1987. Accepted October 29, 1987. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Metabolism of Selenium from Soybean and Egg Products in Rats[1,2]

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The utilization of selenium (Se) from soy flour and eggs was evaluated by comparing whole-body absorption, retention, and tissue accumulation of ^{75}Se in rats from radiolabeled test meals. Selenium-depleted male Sprague–Dawley rats were fed selenium-adequate repletion diets containing either egg, soy, combined egg/soy, or sodium selenite supplemented torula yeast as the protein sources. The first meal of the repletion period was radiolabeled. Intrinsically labeled egg protein was obtained by gavaging hens with [^{75}Se]selenomethionine or sodium [^{75}Se]selenite. Soy was intrinsically labeled via nutrient culture with sodium [^{75}Se]selenite ($Na_2{}^{75}SeO_3$) or sodium [^{75}Se]selenate ($Na_2{}^{75}SeO_4$). The combined egg/soy diet contained egg from hens gavaged with $Na_2{}^{75}SeO_3$ and soy grown with $Na_2{}^{75}SeO_3$. Torula yeast was extrinsically labeled by adding $Na_2{}^{75}SeO_3$. A significantly greater amount of ^{75}Se was absorbed and retained from egg than from soy protein. The ^{75}Se from mixed egg/soy protein diets was absorbed and retained at a level intermediate to that of the egg or soy protein diets alone, suggesting the formation of a common pool of selenium within the intestinal tract.

Many foods of animal origin (e.g., meat, fish, and egg) contain high levels of selenium. Plant foods, on the other hand, normally contain less selenium than foods of animal origin. However, the selenium content of food does not necessarily reflect the utilization of the mineral from the food.

Many studies have compared the utilization of selenium from different foods. Soybean meal has been shown to be more effective than fish in preventing exudative diathesis in chicks (Cantor et al., 1975a), but less effective in restoring glutathione peroxidase activity (Gabrielsen and Opstvedt, 1980). Selenium from wheat was more effective than selenium from tuna in preventing pancreatic fibrosis in chicks (Cantor et al., 1975b) and in restoring tissue glutathione peroxidase activities in rats (Douglass et al., 1981; Alexander et al., 1983). No clear distinction can be made at this time as to the utilization of selenium from foods of animal versus plant origin.

The Food and Nutrition Board has established a 50–200 μg/day safe and adequate range of selenium intake for healthy individuals (Food and Nutrition Board, 1980). This range of selenium intake is not difficult to obtain in a varied American diet but may present problems for individuals on restricted nutritional regimens. Examples of such individuals are infants and enterally or parenterally fed patients. Selenium deficiency symptoms have been shown in persons receiving total parenteral nutrition. Symptoms of selenium deficiency reported are cardiomyopathy and muscle weakness (Quercia et al., 1984; Kien and Ganther, 1983). Selenium status of these individuals has been reported as very low with erythrocyte levels of glutathione peroxidase only 6–7% of normal (Baker et al., 1983).

Selenium in food sources is concentrated in protein. A protein source being used commercially in the formulation of infant and enteral nutrition is soy protein. Soy is used because of its high protein quality and content and because it is generally less allergenic than other vegetable proteins.

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[1] Journal Paper No. 11,050, Purdue Agricultural Experiment Station, West Lafayette, IN 47907.

[2] Supported in part by a grant from the American Egg Board.

0021-8561/88/1436-0256$01.50/0

Table I. Experimental Groups

group code	description	radioact in test meal, dpm
SI	intrinsically labeled soy flour; plants exposed to [^{75}Se]selenite	211, 843
SA	intrinsically labeled soy flour; plants exposed to [^{75}Se]selenate	265, 166
EI	intrinsically labeled egg diet; chickens exposed to [^{75}Se]selenite	277, 636
EM	intrinsically labeled egg diet; chickens exposed to [^{75}Se]selenomethionine	116, 736
TY	torula yeast diet extrinsically labeled with [^{75}Se]selenite	298, 156
MS	egg/soy mixed diet with soy flour; plants exposed to [^{75}Se]selenite	255, 410
ME	egg/soy mixed diet with egg; chickens exposed to [^{75}Se]selenite	151, 370

However, little is known about the utilization of selenium from soy protein. In a previous study from our laboratory, the absorption and retention of selenium from extrinsically labeled soy isolate were measured in rats (Mason and Weaver, 1986). Selenium absorption from the soy source was high. In the current study, we compared the utilization of selenium from an animal protein and a plant protein. The absorption and retention of ^{75}Se from intrinsically labeled soy flour was compared to that from intrinsically labeled egg.

MATERIALS AND METHODS

Preparation of Intrinsically Labeled Soybean Flour and Eggs. Soybeans (*Glycine max* L. Merr cv. Century) were grown under conditions described previously (Mason and Weaver, 1986). At flowering, one set of plants was labeled with sodium [^{75}Se]selenite and another set with sodium [^{75}Se]selenate added weekly to the nutrient solution at a concentration of 1.0 μCi/L. Soybeans were harvested, dehulled, ground into a fine powder, and defatted. Defatted soy flour was autoclaved to inactivate trypsin inhibitors and used as the protein source in radioactive test diets. Nonradioactive defatted soy flour was prepared from locally field-grown Century soybeans by the procedure of Levine et al. (1982).

To obtain intrinsically labeled chicken eggs, two White Leghorn chickens were dosed daily for 14 days. One chicken was given 1.0 μCi of sodium [^{75}Se]selenite, and the other received 1.0 μCi of [^{75}Se]selenomethionine by intubation directly into the crop. Radioactive eggs were collected. Nonradioactive eggs of White Leghorn chickens were also obtained from the Purdue Poultry farm. All chickens received the same commercial poultry feed during the experimental period. Both radioactive and nonradioactive eggs were cooked by scrambling, with no added fat, freeze-dried, and ground into a fine meal. Egg was chosen because it is food of animal origin, its protein is of high quality, and the average selenium content of whole eggs is high with an average of 0.317 ppm, most of which is concentrated in the yolk (Hadjimarkos and Bonhorst, 1961).

Preparation of Experimental Diets. A depletion diet was prepared to contain 30% torula yeast protein estimated by Kjeldahl nitrogen content according to Section 7.015 AOAC (Horowitz, 1984) and <0.001 ppm selenium, measured fluorometrically by the technique of Hoffman et al. (1968). Radioactive test meals contained the same components with the substitution of torula yeast with intrinsically labeled soy, intrinsically labeled egg, extrinsically labeled torula yeast, or a soy/egg mixture where either the soy or the egg was intrinsically labeled in two separate diets. The soy and egg each provided half of the selenium in the total diet. The experimental groups are described in Table I. Repletion diets consisting of 30% protein were prepared from soy, egg, soy/egg mixture, or sodium selenite supplemented torula yeast. The composition of all diets is shown in Table II.

Experimental Protocol. Male weanling Sprague–Dawley rats (Harlan Industries, Indianapolis, IN) were randomly assigned to seven experimental groups of six animals each. Rats were housed in individual stainless steel cages, allowed free access to deionized water, and fed the torula yeast based depletion diet for 28 days. On day 28 or day 0 of the experimental period, all animals were fasted for 18 h and then given a 5-g radioactive test meal. Whole-body radioactivity was determined for each animal immediately after the radioactive dose was ingested, in a large-well whole-body γ spectrometer as described by Meyer et al. (1983). Whole-body radioactivity was also measured on days 1, 3, 5, 10, 15, and 21 of the experimental period to determine the retention of the nuclide. Radioactivity was measured using a window of 60–670 keV. Instrument efficiency was measured at 30%. After the test diets, rats were allowed nonradioactive experimental repletion diet of the same composition as the test meal ad libitum.

At the end of 21 days, animals were anesthetized with sodium pentobarbital. The thorax was opened by midline sternotomy, and 0.25 mL of anticoagulant (35 mM citric acid, 75 mM sodium citrate, 0.14 M glucose) was injected directly into the heart to prevent blood coagulation. After 1 min a 5.0-mL sample of blood was drawn by cardiac

Table II. Composition of Experimental Diets

	% of diet				
	depletion diet	repletion diets			
diet component		torula yeast	soy	egg[a]	soy/egg[a]
soy flour[b]			70.87		35.44
egg[c]				13.34	6.7
torula yeast[d,e]	56.7	56.7		39.1	19.47
sucrose	26.3	26.3	14.8	33.59	24.2
fiber (Alphacel)[e,f]	5.0	5.0	5.0	5.0	5.0
corn oil[g]	7.0	7.0	4.42	3.97	4.19
AIN mineral mix[e] (Se free)	3.5	3.5	3.5	3.5	3.5
AIN vitamin mix[e]	1.0	1.0	1.0	1.0	1.0
DL-(+)-methionine mix[e]	0.09	0.09	0.09	0.09	0.09
choline bitartrate mix[e]	0.2	0.2	0.2	0.2	0.2
sodium selenite,[h] mg/6 kg		1.0998			
selenium, ppm	0.001	0.0824	0.1160	0.0638	0.1010

[a] Due to the high selenium content of egg, it was diluted with torula yeast to increase protein content but not selenium content. [b] Lowfat soy flour contained 42.3% protein and 5.05% fat. [c] Scrambled freeze-dried whole egg contained 70.3% protein and 30.19% fat. [d] Torula yeast contained 52.9% protein and 5.0% fat. [e] ICN Nutritional Chemicals, Cleveland, OH. [f] Nonnutritive bulk. [g] Mazola, purchased locally. [h] Sigma Chemical Co., St. Louis, MO.

Table III. Absorption and Retention of ^{75}Se[a]

group code[b]	calcd % absorption, day 0	% retention, day 21
SI	76.9 ± 1.9[b]	69.0 ± 2.5[a]
SA	71.5 ± 0.9[a]	68.4 ± 1.4[a]
EI	91.9 ± 2.0[d]	77.2 ± 1.7[bc]
EM	94.9 ± 1.1[d]	81.1 ± 1.7[c]
TY	84.9 ± 1.6[bc]	72.2 ± 1.8[ab]
MS	81.3 ± 2.4[bc]	75.3 ± 1.7[abc]
ME	86.6 ± 1.4[c]	73.9 ± 1.6[ab]

[a] Mean ± SE. Within columns, means followed by the same letter are not significantly different, $p < 0.05$; Student–Newman–Keuls test. [b] Key to group code in Table I.

puncture. The animals were then perfused with 0.9% saline through the left ventricle with emission of blood through the right atrium. After perfusion, the heart, liver, and kidneys were removed, washed with deionized water, blotted dry, and weighed. One milliliter of blood and a sample of each tissue were assayed for radioactivity in a Beckman 8000 multisample γ counter using a photopeak of 60–670 keV with a calculated efficiency of 27%.

Data Analysis and Statistical Tests. All radioactivity values were corrected for background, nuclide decay, and daily γ spectrometer fluctuations. Values of samples measured in the Beckman 8000 were normalized to the whole-body counter values by a conversion factor determined by measuring a ^{75}Se standard in both devices.

Percent retention of the administered dose was calculated for each of the days the animals were measured for radioactivity. On day 0, not all the radioactive selenium given to a rat is actually absorbed; thus, the percentage of administered dose absorbed was calculated by linear regression analysis to extrapolate to day 0. Absorption was then expressed as a percent by eq 1. Percent retention of absorbed dose was calculated for comparison of nuclide utilization over the experimental period (eq 2).

$$\% \text{ absorp} = \frac{\text{calcd absorbed dose (dpm)}}{\text{radioact, day 0 (dpm)}} \times 100 \quad (1)$$

$$\% \text{ retention absorbed dose} = \frac{\text{radioact (dpm)}}{\text{calcd absorbed dose (dpm)}} \times 100 \quad (2)$$

Tissue and whole-blood radioactivity were calculated as radioactivity/unit weight or volume measure and expressed as a percent of the final day whole-body radioactivity.

Homogeneity of variance and normality of each data set were tested by the Bartlett–Box homogeneity of variance test and by the Wilkes normality test, respectively. Comparisons among groups were accomplished by one-way analysis of variance using Student–Newman–Keuls multiple-range test at the level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Table III shows the calculated percent absorption of ^{75}Se from egg, soy, and torula yeast meals and the percent retention of the absorbed radioactivity after 21 days. Overall absorption of the ^{75}Se from all protein sources was high. Absorption of ^{75}Se from egg diets was significantly higher than from the other diets. There was no significant difference in the absorption of ^{75}Se from egg intrinsically labeled with [^{75}Se]selenite or [^{75}Se]selenomethionine.

^{75}Se from the soy diets was absorbed least efficiently, and there was a significant difference in the absorption between the two soy groups. ^{75}Se from soy intrinsically labeled with selenite was absorbed more efficiently than soy intrinsically labeled with selenate. Thus, availability of selenium from a plant source may be dependent on the form of the mineral present during the growth of the plant and the form deposited in the plant.

Table IV. ^{75}Se in Tissues[a] (Percent of Whole-Body ^{75}Se/g of Tissue)

group[b] code	tissue			
	whole blood	liver	kidney	heart
SI	1.28 ± 0.03[ab]	1.06 ± 0.04[a]	3.24 ± 0.16[a]	0.73 ± 0.03[a]
SA	1.42 ± 0.15[b]	1.35 ± 0.10[b]	3.67 ± 0.46[a]	0.86 ± 0.10[a]
EI	1.19 ± 0.09[ab]	0.79 ± 0.07[c]	3.20 ± 0.19[a]	0.73 ± 0.04[a]
EM	1.20 ± 0.10[ab]	0.75 ± 0.08[c]	3.20 ± 0.24[a]	0.69 ± 0.07[a]
TY	1.19 ± 0.06[ab]	1.03 ± 0.09[a]	3.26 ± 0.22[a]	0.75 ± 0.07[a]
MS	0.98 ± 0.07[a]	0.79 ± 0.04[c]	3.02 ± 0.17[a]	0.66 ± 0.02[a]
ME	1.06 ± 0.03[ab]	0.71 ± 0.02[c]	2.74 ± 0.13[a]	0.64 ± 0.05[a]

[a] Mean ± SE. Within columns, means followed by the same letter are not significantly different, $p < 0.05$; Student–Newman–Keuls test. [b] Key to group code in Table I.

^{75}Se from the soy/egg mixed diets and the selenite-supplemented torula yeast diet was absorbed at an intermediate level compared to the egg and soy diets. Absorption of ^{75}Se from the soy/egg diets was identical whether the labeled component was soy or egg. Similar results have been reported for the absorption of zinc and iron from mixed-protein diets. Meyer et al. (1983) and Stuart et al. (1986) have shown intermediate absorption of ^{65}Zn from soy/egg and soy/chicken mixed diets compared to diets of the plant or animal protein source alone. Intermediate levels of absorption of iron were observed when black beans or corn were combined with veal (Layrisse et al., 1968) and when eggs and wheat were combined (Bjorn-Rasmussen et al., 1973). These results suggest a common pool of these minerals combined in a single meal was formed in the gastrointestinal tract of the animal. Absorption of the minerals was determined by the composite effects of factors in the meal.

Retention of the absorbed dose after 21 days is also shown in Table III. The trend of selenium retention was similar to absorption data, but there were fewer significant differences.

Of the tissues removed and assayed for radioactivity, only the liver and blood showed statistically significant differences among groups (Table IV). After 21 days the percent of whole-body count retained/g of liver tissue was greater from the soy groups than the egg or mixed-diet groups. The greatest selenium retention in the liver occurred in the soy group intrinsically labeled with selenate. This was in contrast to the whole-body retention of ^{75}Se. These data suggested that the liver selectively retains selenium of the form present in selenate intrinsically labeled soy. The retention of radioactivity in the blood of animals fed intrinsically selenate labeled soy flour was significantly greater than that of animals fed a mixed soy/egg diet where the soy was intrinsically selenite labeled. This difference is consistent with reports that selenate is better absorbed than selenite. The actual form of selenium in these products is however not known.

The results of this study show distinct differences in absorption and retention of selenium from animal sources of protein when compared to a plant protein. A possible explanation of this could be that the soy protein contains enough phytic acid to interfere with selenium absorption. Sodium phytate has been shown to increase fecal selenium losses in humans (Morris et al., 1985). The interference of mineral absorption by high levels of phytic acid is well documented for other minerals such as zinc and calcium. Controlled experiments are necessary to determine the role of phytic acid in selenium absorption from soy.

Registry No. Se, 7782-49-2; Na_2SeO_4, 13410-01-0; Na_2SeO_3, 10102-18-8.

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Received for review May 12, 1987. Accepted October 6, 1987.

Phytate Hydrolysis in Soybean and Cottonseed Meals by *Aspergillus ficuum* Phytase

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Microbial phytase was produced by solid-state cultivation of *Aspergillus ficuum*, the enzyme (crude culture filtrate) was applied to soybean and cottonseed meals, and factors affecting the hydrolysis of phytate were studied. Soybean and cottonseed meals contain about 60% and 50% of total phytate as water-soluble forms, respectively. The water-insoluble portion of phytate was further hydrolyzed and removed by application of microbial phytase. Treatment at higher temperature (50 °C), pH 4–5.5, and heating the substrate (1 h at 121 °C) prior to enzyme treatment facilitated the hydrolysis of phytate by the microbial phytase. The heat treatment also reduced the level of total extractable phytate from these substrates. Hydrolysis and removal of phytate in soybean meal was more effective than that for cottonseed meal. About 85% of phytate in soybean meal was hydrolyzed by the microbial phytase whereas only 67% of the phytate in cottonseed meal was destroyed by the same enzyme treatment.

Phytic acid is the principal storage form of phosphate in plants, particularly in cereal grains and legumes. The interaction of phytic acid with protein, vitamins, and several minerals is considered to be one of the primary factors limiting the nutritive values of cereal grains and legume seeds. For instance, soybean and cotton seed meals are the major protein supplement in poultry feeds and also are a source of phosphorus. However, two-thirds of soybean meal phosphorus is bound as phytate and unavailable for poultry (Whitaker and Brunnert, 1977). Hydrolyzing plant phytate prior to animal consumption would increase the availability of inositol and inorganic phosphorus in animal diet. Thus, attempts have been made to hydrolyze dietary phytate by microbial phytase to improve the feed quality (Chang et al., 1977; Liener, 1977; Nelson et al., 1968; Whitaker and Brunnert, 1977). Although beneficial effects of the enzyme treatment were evident, the high cost of enzyme production and lack of a practical method for enzyme application were cited as limiting factors in using the enzyme in animal diets.

The enzyme phytase, which hydrolyzes phytic acid, is widely distributed among plant and animal tissues. It is also produced by a variety of microorganisms, and its characteristics have been studied (Cosgrove et al., 1970; Greaves et al., 1967; Han et al., 1987; Shieh et al., 1969; Yamada et al., 1968). Although the enzyme activity has been previously studied with pure phytic acid or its derivatives as a substrate, the factors affecting hydrolysis of phytate in native seeds are not well elucidated. In the native state, phytate exists in close association with other plant components that make it difficult to be hydrolyzed by microbial enzyme. Thus, the present investigation was conducted to establish the factors affecting hydrolysis and removal of phytate in soybean and cottonseed meals by *Aspergillus ficuum* phytase.

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MATERIALS AND METHODS

Materials. Wheat bran, soybean meal, and cottonseed meal were obtained from a local feed store and stored at 18 °C and 50% relative humidity. The moisture content of the meals was about 11%, and the total phosphorus content was 0.24 and 0.34% for soybean meal and cottonseed meal, respectively. Commercial phytase (6-phytase; phytate 6-phosphatase; myoinositol hexakisphosphate 6-phosphohydrolase; EC 3.1.3.26) prepared from wheat by Sigma Chemical Co. (St. Louis, MO) was used as a standard for phytase activity.

Phytase Production and Enzyme Assay. *A. ficuum* (NRRL 3135) was grown and maintained on potato dextrose agar at 30 °C. The conidia formed on the agar