Biologic Availability of Selenium in Corn Grain Produced on Soil Amended with Fly Ash

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The selenium content of corn grain produced in central New York was increased 670% by application of fly ash containing 6.5 ppm Se to soil at the rate of 500 tons/acre. Fat-extracted corn produced from untreated soil contained 0.038 ppm Se; corn produced from fly ash amended soil contained 0.296 ppm Se. The Se content of corn grain produced 1 year after soil amendment was comparable (0.306 ppm Se) to that of the first season crop, indicating the persistent effect of the soil treatment. Vitamin E deficient and Se-deficient chicks were fed vitamin-D free, Se-deficient semipurified diets containing incremental amounts of Na_2SeO_3 or fat-free ground corn produced from fly ash amended or control soil plots. Results showed that approximately 50% of the Se in fly-ash-soil corn was available to the chick for promoting growth and plasma Se-dependent glutathione peroxidase and for preventing the vitamin E–Se deficiency disease exudative diathesis.

Nutritional deficiencies of selenium in animals have been demonstrated in many parts of the world where the contents or availability of the mineral in soils, and therefore in crops grown on those soils, are extremely low. Selenium-related deficiency diseases of agricultural importance include exudative diathesis in chicks, reproductive failures and placental retention in ewes and cows; nutritional muscular dystrophies in lambs, calves, chicks, and poults; and others (see review by Combs and Scott, 1977). Selenium status, as indicated by blood selenium levels in humans in North America, is variable (usually 0.18–0.23 μg/mL) and generally reflects the soil and plant selenium status of the region inhabited (Allaway et al., 1968; Burk et al., 1967; Shamberger et al., 1973). Low blood selenium levels have been reported in humans in low-selenium areas with endemic selenium deficiency in farm animals: 0.12 μg/mL in Sweden (Brune et al., 1966), 0.05 μg/mL in New Zealand (Griffiths and Thompson, 1974). Similar levels (as low as 0.10 μg/mL) have been reported in the selenium-deficient central-midwestern United States (Allaway et al., 1968).

Recent advances in elucidating the metabolic roles of selenium and related nutrients have stemmed from the discovery by Rotruck et al. (1973) of the function of selenium as an essential component of glutathione peroxidase (glutathione:H_2O_2 oxidoreductase, EC 1.11.1.9), a critical enzyme in the antioxidant defense system of animals (Chow, 1979; Combs and Scott, 1977). Glutathione peroxidase purified from rat liver (Nakamura et al., 1974) and from ovine (Oh et al., 1974), bovine (Flohé et al., 1973), and human (Awasthi et al., 1975) erythrocytes contains approximately 4 g-atoms of selenium/mol of enzyme. The necessity of selenium-dependent glutathione peroxidase for metabolic protection from oxidative stress is now accepted as one explanation for the etiology of numerous selenium-responsive disorders of animals and has raised concerns that some human populations may also be at nutritional risk relative to this trace mineral.

High selenium content and plentiful supply have made fly ash attractive as a potential source of selenium for agricultural purposes in low-selenium regions such as the northeastern United States. Brackett (1970) has estimated that as much as 36 million tons of fly ash will be produced annually by 1980 by coal-burning electric power generating plants in the United States. Although a small amount is presently used in road construction and as a concrete additive, most fly ash is disposed in landfills or settling ponds at costs of $0.25–2.00 per ton (Plank and Martens, 1973; Capp and Spencer, 1970; Martens et al., 1970). Fly ash is generally high in selenium: Gutenmann et al. (1976) found that fly ashes from 21 states contained 1.2–16.5 ppm selenium.

Several reports have shown that at least a portion of the selenium in fly ash is available for uptake by plant species grown naturally or experimentally on fly ash or on soils amended with fly ash (Furr et al., 1975, 1976, 1977, 1978a,b; Gutenmann et al., 1976; Stoewsand et al., 1978). Furr et al. (1978b) found that sweet clover voluntarily growing in deep layers of fly ash at a landfill accumulated as much as 205 ppm Se (dry weight). Animals fed seleniferous feedstuffs produced from fly ash or fly ash amended soils have been shown to develop significant tissue selenium concentrations (Furr et al., 1975, 1978b; Stoewsand et al., 1978). Presumably this is chiefly due to increased plant selenomethionine content resulting from growth on fly ash containing media and to the subsequent incorporation of selenomethionine and/or its metabolites into tissue proteins of animals fed those plants. Signs of selenium toxicity have not been observed in sheep or goats fed fly ash produced high-selenium feedstuffs in the range approaching toxicity in other species (Furr et al., 1975, 1978b; Stoewsand et al., 1978). If the selenium in fly ash produced crops resides in compounds with limited biologic availability at high levels of feeding (in excess of 245 ppm), then relatively poor nutritional value would be expected for that selenium when those materials are fed at lower selenium levels (less than 0.1 ppm).

Agronomic considerations in using fly ash as a soil additive have been discussed (Capp and Spencer, 1970; Martens et al., 1970; Plank and Martens, 1973; Patterson et al., 1974; Plank et al., 1975). Of nutritional importance is the possibility of improving the selenium nutriture of animals and humans in selenium-deficient regions by the use of fly ash as a soil additive if such use will result in increasing the amount of biologically available selenium in feed crops. Implicit in a consideration of the nutritional implications of uses of fly ash is a full consideration of the toxicological aspects. The purpose of this investigation was to assess the amount of biologically available selenium in corn grain grown on fly ash amended soils.

EXPERIMENTAL SECTION

Experimental plots were established on moderately well drained Lima soils representative of the neutral glacial till

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Table I. Composition (Percent) of Experimental Diets

ingredients	treatments						
	1-4	5	6	7	8	9	10
fixed ingredients[a]	37.28	37.28	37.28	37.28	37.28	37.28	37.28
corn, 50% fly ash soil[b]		10.14	20.27	30.41			
corn, 0% fly ash soil[c]					10.14	20.27	30.41
glucose monohydrate	58.77	49.49	40.28	31.07	49.49	40.28	31.07
Solka-Floc[d]	3.95	3.09	2.17	1.24	3.09	2.14	1.24

[a] Casein, 5%; soya protein, 14.05%; torula yeast, 11.00%; stripped (vitamin E free) corn oil, 2.00%; L-arginine hydrochloride, 0.21%; glycine, 0.30%; DL-methionine, 0.07%; choline Cl, 70% solution, 0.29%; vitamin premix (Combs, 1978), 0.20%; trace mineral premix (Combs, 1978), 0.72%; $CaCO_3$, 1.02%; $CaHPO_4 \cdot 2H_2O$, 1.90%; KH_2PO_4, 0.31%; NaCl, 0.18%. [b] Fat-free dried corn contained 0.296 ppm total Se; levels included in diets 5, 6, and 7 provided 0.03, 0.06, and 0109 ppm Se, respectively. [c] Fat-free dried corn contained 0.038 ppm total Se; levels included in diets 8, 9, and 10 provided 0.004, 0.008, and 0.012 ppm, respectively. [d] Brown Co., Berlin, NH.

belt in central New York. Freshly produced (Milliken Station, NY State Electric and Gas Co., Lansing, NY) fly ash was applied at the rates of 0, 125, 250, 375, or 500 tons per acre for various treatments, as part of an agronomic study (Barrows and Swader, unpublished data, 1978); however, only the low (0 ton of fly ash per acre) and high (500 tons of fly ash per acre) treatments are considered in the present report. These levels represented approximately 0 and 50%, respectively, of total soil (8-in. depth). Fly ash was hand-spread over 15 × 30 ft plots in two applications. After each application the plots were plowed and disc-harrowed. Care was taken to prevent contamination between plots of different treatments.

A short-season variety corn (Pioneer 3977) was planted in six 30-in. spaced rows per plot. Corn ears were harvested at maturity from the center 20 ft of each of the two center rows. Ears were shelled, dried, and ground. One kilogram samples from each treatment were extracted 24 h with 60–70 °C petroleum ether, using a Soxhlet extractor to remove vitamin E. Solvent was allowed to evaporate from extracted corn meal at room temperature, after which duplicate 1-g aliquots of each sample were analyzed for total selenium content according to the fluorometric procedure of Olson (1969).

Vitamin E and selenium depleted day-old Single Comb White Leghorn male chicks, produced as described by Thompson and Scott (1969), were reared in thermostatically controlled battery brooders with raised wire floors, with feed and water provided ad libitum. Dietary treatments of chicks were based on the selenium-deficient, vitamin E free diet previously reported (Combs, 1978), modified to include 0, 0.03, 0.06, or 0.09 ppm selenium as sodium selenite or as the high-selenium, fly-ash-soil corn, or to include the control soil corn at levels equal to those used for the fly-ash-soil corn. The latter treatment series was included as a control for any other factors in corn which might have altered chick performance. The compositions of experimental diets are presented in Table I. All diets were isocaloric and contained adequate amounts of all known nutrients except vitamin E and selenium. Each dietary treatment was applied to triplicate lots of ten chicks each for 2 weeks, during which time body weight, feed consumption, incidence of exudative diathesis, and mortality were recorded.

At 2 weeks of age, blood was obtained by anterior cardiac puncture using a heparinized syringe from each of five chicks selected randomly from each of the sodium selenite and fly-ash-soil corn treatments. Plasma was prepared after centrifugation at 1000*g* for 5 min. Plasma protein was determined by the method of Lowry et al. (1951); plasma selenium-dependent glutathione peroxidase was determined by the method of Rotruck et al. (1973) as modified by Noguchi et al. (1973), using hydrogen peroxide as substrate. Performance and biochemical data were evaluated by analysis of variance; treatment means were ranked by a 5% new multiple range test using a catalogued statistical analysis system (SAS-76, Statistical Analytical System, SAS Institute, Inc., Raleigh, NC) on the Cornell computer.

The biologic availability of selenium in the fly-ash-soil corn was determined on the basis of its efficacy in preventing the vitamin E–selenium deficiency disease, exudative diathesis, relative to sodium selenite which was arbitrarily used as a standard and assigned a biologic availability of 100%. This procedure is similar to that of Cantor et al. (1975) and has been valuable as a means of evaluating the nutritional significance of selenium in practical feedstuffs.

RESULTS AND DISCUSSION

Application of fly ash containing 6.5 ppm Se at the rate of 500 tons per acre significantly reduced yield [74.5 bu/acre vs. 99.5 bu/acre (at 15% moisture) for control plots, $P < 0.05$], but increased the Se content [0.296 ppm vs. 0.038 ppm (fat-free basis), $P < 0.05$ of corn grain. Corn grain produced on the same control and fly ash treated plots 1 year later and Se concentrations comparable to those determined for the previous crop for respective soil treatments (0.306 ppm[6] vs. 0.042 ppm[2], $P < 0.05$). Therefore, use of fly ash amended soil resulted in approximately a sevenfold increase in the total Se content of corn grain produced for at least two seasons after application of fly ash.

The performance of vitamin E deficient chicks fed diets limiting with respect to selenium is shown in Table II. Significant ($P < 0.05$) dose–response relationships were observed for the reference standard sodium selenite and for the high-selenium, fly-ash-soil corn for promotion of growth, protection from exudative diathesis, and reduction in chick mortality. The low-Se corn fed at the levels of the fly-ash-soil corn did not significantly ($P > 0.05$) affect those parameters. That improvement in chick performance was a function of the Se provided by the fly-ash-soil corn and not due to other factors associated with corn grain is further demonstrated by the response of Se-dependent glutathione peroxidase in plasma of chicks fed diets containing either sodium selenite or fly-ash-soil corn (Table III).

Whereas plasma activities of this selenoenzyme demonstrated improvement in chick selenium status as a result of consumption of the fly-ash-soil corn, the response was not as great as that obtained with sodium selenite. The utilization of dietary selenium from fly-ash-soil corn for protection against exudative diathesis was also less than that of sodium selenite. The latter parameter, used previously by Cantor et al. (1975) to assess the biologic availability of Se in practical feedstuffs, was employed for that purpose as outlined in Table IV. Results show that

Table II. Evaluation of Selenium Availability in Corn Produced on Soils Amended with Fly Ash

treatment/supplemental selenium[a]			2-week performance			
	source	amount, ppm	gain, g	feed/gain	exudative diathesis, %	mortality, %
1	none	0	25.5 ± 8.2^{D}[b,c]	3.91 ± 1.23^{A}[b,c]	100.0 ± 0.0^{A}[b,c]	70.0 ± 0.0^{A}[b,c]
2	Na_2SeO_3	0.030	62.4 ± 7.4^{B}	2.00 ± 0.15^{C}	60.0 ± 5.8^{B}	16.7 ± 3.3^{C}
3	Na_2SeO_3	0.060	77.6 ± 1.4^{A}	1.87 ± 0.11^{C}	36.7 ± 8.8^{C}	10.0 ± 10.0^{C}
4	Na_2SeO_3	0.090	78.0 ± 1.4^{A}	1.95 ± 0.03^{C}	13.3 ± 6.7^{D}	6.7 ± 3.3^{C}
5	corn, 50% fly ash soil[d,e]	0.030	45.4 ± 0.2^{C}	2.50 ± 0.06^{BC}	93.3 ± 6.7^{A}	20.0 ± 5.8^{BC}
6	same as above	0.060	72.8 ± 2.7^{AB}	2.01 ± 0.03^{C}	60.0 ± 10.0^{B}	6.7 ± 3.3^{C}
7	same as above	0.090	79.2 ± 5.0^{A}	1.70 ± 0.21^{C}	33.3 ± 6.8^{C}	0.0 ± 0.0^{C}
8	corn, 0% fly ash soil[d,f]	0.004	34.3 ± 2.7^{CD}	3.22 ± 0.35^{AB}	100.0 ± 0.0^{A}	63.3 ± 8.8^{A}
9	same as above	0.008	29.3 ± 4.4^{D}	3.42 ± 0.33^{AB}	100.0 ± 0.0^{A}	36.7 ± 3.3^{B}
10	same as above	0.012	36.7 ± 4.6^{CD}	2.61 ± 0.30^{BC}	100.0 ± 0.0^{A}	56.7 ± 3.3^{A}
significance of treatment effect			$P < 0.0001$	$P < 0.0143$	$P < 0.0001$	$P \leq 0.0001$

[a] Basal diet was essentially free of vitamin E and contained less than 0.02 ppm total selenium. [b] Mean ± SE for ten chicks each per treatment. [c] Means with like superscripts are not significantly different ($P > 0.05$). [d] Corn samples were finely ground and fat extracted to remove all tocopherols. Diets were adjusted to be isocaloric. [e] Fat-free corn contained 0.296 ppm Se. [f] Fat-free corn contained 0.038 ppm Se.

Table III. Plasma Glutathione Peroxidase Activity in 2-Week-Old Chicks Fed Corn Produced on Fly Ash Amended Soil

	treatment/supplemental selenium			plasma GSHpx	
	source	amount, ppm	plasma protein, mg/mL	total activity, EU/mL	specific activity, EU/mg of protein
1	none	0	31.80 ± 5.33[b]	4.99 ± 2.46^{BC}[b,c]	0.127 ± 0.054^{C}[b,c]
2	Na_2SeO_3	0.03	33.73 ± 1.53	22.64 ± 3.07^{BC}	0.665 ± 0.082^{BC}
3	Na_2SeO_3	0.06	27.24 ± 3.15	34.12 ± 8.01^{B}	1.223 ± 0.181^{B}
4	Na_2SeO_3	0.09	33.08 ± 2.56	91.33 ± 18.43^{A}	2.823 ± 0.619^{A}
5	corn, 50% fly ash soil[d]	0.03	28.92 ± 1.64	2.76 ± 2.76^{C}	0.113 ± 0.113^{C}
6	same as above	0.06	36.90 ± 2.29	24.72 ± 5.74^{BC}	0.655 ± 0.115^{BC}
7	same as above	0.09	35.21 ± 1.81	29.69 ± 7.37^{BC}	0.819 ± 0.158^{BC}
significance of treatment effect			NS	$P < 0.0086$	$P < 0.0038$

[a] Basal diet was essentially free of vitamin E and contained less than 0.020 ppm total selenium. [b] Mean ± SE for five individuals per treatment. [c] Means with like superscripts are not significantly different ($P > 0.05$) according to Duncan's Multiple Range Test. [d] Fat-free dried corn contained 0.296 ppm total selenium.

Table IV. Calculation of Biological Availability of Selenium in Corn Grain

supplemental Se		exudative diathesis		avail Se (rel to Na_2SeO_3),[a]	Se avail, %
source	amnt, ppm	%	log		
none	0	100.0	2.000	0	
Na_2SeO_3[b]	0.03	60.0	1.778	0.03	100
	0.06	36.7	1.565	0.06	100
	0.09	13.3	1.124	0.09	100
corn, 50% fly ash soil	0.03	93.3	1.970	0.01	33
	0.06	60.0	1.778	0.03	50
	0.09	33.3	1.522	0.05	56
				av availability	46

[a] Determined by regression of Na_2SeO_3 standard curve: available Se = 0.099 log %ED + 0.203, $r = 0.93$. [b] Arbitrarily selected as a standard.

the Se in fly-ash-soil corn was better utilized relative to that of sodium selenite when included at higher levels in the diet. Relatively good utilization of low levels of dietary selenium also observed by Cantor et al. (1975) with corn grain and other feedstuffs is a general property of this biologic assay. When fly-ash-soil corn was incorporated at higher levels into the experimental diet, apparent biologic availability of 50–56% was observed. This availability was less than that of 86% reported by Cantor et al. (1975); however, their sample of corn was produced in a high-Se area (South Dakota) and contained more than three times the Se content (1.0 ppm) of the corn produced in this study. It is possible that improvements in Se content of corn grain due to increased Se content of the soil resulted primarily from increased levels of selenium compounds with high biologic availability.

These results indicate that the use of seleniferous fly ash as a soil additive can markedly increase the Se content of corn grain. Presumably the Se contents of other crops may be similarly increased as a result of this agricultural use of fly ash. In Se-deficient areas such as the northeastern and central-midwestern United States, increases in the Se contents of feedstuffs represent improvements in their nutritional value to the extent of the biological availability of that selenium. These results show that the Se content of corn grain produced in central New York was increased approximately sevenfold by using fly ash as a soil additive, and that approximately one-half of that selenium was available for utilization by the chick. Such an improvement in the available Se status of northeastern corn would have practical significance if it could be effected without depressing crop yield or resulting in heavy metal accomodation, etc., as it would obviate the need for supplemental sodium selenite in practical diets for poultry.

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Thermal Coagulation of Egg Albumin

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Turbidity was used to study thermocoagulation of egg albumin as a function of pH and protein concentration. Coagulation was dependent on pH and protein concentration. The net charge of egg albumin at the critical pH ($h_{T=0.5}$) upon coagulum formation increased linearly with protein concentration. The first step of coagulation reaction involved the formation of disulfide bonds and the exposure of hydrophobic groups. During further heating, egg albumin was polymerized by intermolecular sulfhydryl–disulfide exchange and the protein network structure was formed. The high net charge of proteins prevented the matrix from forming mainly by hydrophobic interaction. Succinylated egg albumin acted in a similar way with unmodified protein regarding relationship between protein concentration and the critical pH ($h_{T=0.5}$) upon coagulation.

The utilization of proteins as food is largely determined by their functional properties such as emulsifying activity, emulsion stability, foaming capacity, water-holding property, and gel formation.

Egg albumin is a key ingredient in many food products because of its ability to coagulate upon heating. The major proteins of egg white, ovalbumin and conalbumin, are heat coagulable and they are constituted of almost 70% of whole protein (Parkinson, 1966). Painter and Koening (1976) reported the Raman spectra of heat-coagulated ovalbumin. The spectral changes demonstrated the formation of extensive regions of antiparallel β-sheet between ovalbumin molecules. The heat denaturation of egg white and its component proteins was studied by differential scanning calorimetry (Donovan et al., 1975). At a heating rate of 10 °C/min, egg white at pH 7.0 showed two major endotherms, 65 and 84 °C, which were produced by the denaturation of conalbumin and ovalbumin, respectively. Seideman et al. (1963) reported that pH affected the coagulation temperature of egg white. Nakamura et al. (1978) confirmed that the heat-induced aggregation of ovalbumin depended on the degree of electrostatic repulsion on the denatured protein molecules. To prevent thermoaggregation and coagulation, an attempt was made to add anionic detergents (Hegg and Löfqvist, 1974, 1977) and sucrose (Seideman et al., 1963) to ovalbumin solution. In spite of these studies, the mechanism of coagulation is still not well understood.

In this paper, coagulum of egg albumin, an opaque and heat-irreversible gel, was investigated with respect to pH and protein concentration. The mechanism of three-dimensional network formation was also speculated.

MATERIALS AND METHODS

Materials. Egg albumin was purchased from Nakarai Chem. Ltd., Kyoto. This protein consisted of 80% ovalbumin and 20% conalbumin which were detected by NaDodSO$_4$–polyacrylamide gel electrophoresis method as described below. Other chemicals were reagent grade.

Succinylation of Egg Albumin. Succinylation was performed by the procedure of Groinger (1973). The succinylated protein was dialyzed against distilled water

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