Utilization of Selenium in Fly Ash and in White Sweet Clover Grown on Fly Ash by the Chick

Gerald F. Combs, Jr., Kingston T. Mandisodza,[1] Walter H. Gutenmann, and Donald J. Lisk*

The nutritional activity of selenium in soft coal fly ash and in white sweet clover grown on fly ash was studied by using a biological assay in which protection from exudative diathesis in the vitamin E deficient chick was measured. Fly ash selenium was found to have a biologic availability of ~7% of that of sodium selenite, whereas selenium in the high-selenium clover appeared to be well utilized. Both sources of selenium promoted the activity of selenium-dependent glutathione peroxidase in plasma and resulted in enhanced deposition of selenium in blood, liver, kidney, and M. pectoralis. The effects of fly ash selenium on plasma glutathione peroxidase and tissue selenium levels did not correlate with its apparent nutritional value determined on the basis of prevention of exudative diathesis, promotion of growth, and survival.

The nutritional essentiality of selenium for animals has been established through controlled experimentation and field experience with several animal species [see a review by Combs and Scott (1977)]. Among these, the chicken has been found to be a useful monogastric animal model. In the chicken, uncomplicated selenium deficiency results in pancreatic atrophy and fibrosis (Gries and Scott, 1972); selenium deficiency combined with deficiencies of vitamin E and/or sulfur-containing amino acids results in exudative diathesis (Noguchi et al., 1973), muscular dystrophy (Calvert et al., 1962), or reproductive failure (Cantor and Scott, 1975; Latshaw et al., 1977). Selenium deficiency can be prevented by feeding inorganic selenium compounds (e.g., sodium selenite) or organic selenium compounds (e.g., selenomethionine) as would be present in natural selenium-containing foodstuffs.

The efficacy of selenium in organic compounds and in foodstuffs for prevention of selenium-deficiency diseases of the chick has been found to be generally less than that of sodium selenite (Cantor et al., 1975a,b). Therefore, most organic selenium forms are assigned biologic availabilities for poultry less than that of selenite (Combs, 1977). Nevertheless, the organic forms of selenium have been found to be more effective dietary sources for increasing the selenium contents of tissues of chickens (Scott and Thompson, 1971; Cantor et al., 1975a; Latshaw, 1975; Osman and Latshaw, 1976; Latshaw et al., 1977). The lack of correlation of the deposition of organic forms of selenium in tissues and their utilization to prevent selenium-deficiency diseases appears to relate to the degree of incorporation of such selenium into proteins other than those of metabolic significance (i.e., the selenium-dependent glutathione peroxidases).

Fly ash, collected by electrostatic precipitation during the combustion of soft coal in power plants, has been found to contain significant levels of selenium (Furr et al., 1977). Fly ash selenium is in the form(s) which can be deposited in tissues of pigs (Mandisodza et al., 1979), sheep (Hogue et al., 1980), and dairy cows (Lein et al., 1980) fed diets containing fly ash; however, the nutritional value of fly ash selenium in prevention of specific deficiency diseases has not been evaluated. Plants can accumulate selenium from fly ash in the soil; the selenium in such plants occurs in forms which can be deposited in tissues of several species including guinea pigs (Furr et al., 1975), sheep and goats (Furr et al., 1978), and japanese quail (Stoewsand et al., 1978). Chicks fed corn grain produced on soil amended with fly ash can utilize the enhanced selenium in the corn to prevent exudative diathesis (Combs et al., 1980).

The present study was conducted to determine the nutritional value of selenium in soft coal fly ash and in white sweet clover grown on such fly ash. The efficacy of each source of selenium for protecting vitamin E deficient chicks from exudative diathesis, for sustaining plasma selenium-dependent glutathione peroxidase activity, and for increasing the selenium content of chick tissues was compared to that of sodium selenite.

EXPERIMENTAL SECTION

Fly ash was obtained as freshly produced material from Milliken Station, a coal-burning electric power generating plant in Lansing, NY. This facility burns bituminous coal strip-mined largely in Pennsylvania and West Virginia. The coal is burned in a Combustion Engineering cyclone-fired boiler and the fly ash is trapped in an electrostatic precipitator. The fly ash was mixed, subsampled, and analyzed for total selenium by the fluorometric method of Olson (1969). It contained 6.33 ppm of selenium on a dry weight basis.

Gaudey Station is a soft coal burning electric power generating plant near Johnson City, NY. Fly ash produced there is disposed of in nearby landfill areas. One such site in Endwell, NY, contained fly ash (pH 7.1) 15–22 m in depth covering ~0.4 ha. In July 1977, a thick stand of exclusively white sweet clover (*Melilotus alba*) ~1.2 m in height, growing voluntarily on the fly ash surface, was harvested. It was dried by using forced heated air (50 °C), milled to a fine powder, and thoroughly mixed. Fat was extracted from the clover before feeding by Soxhlet extraction with petroleum ether (bp 60–70 °C). The resulting dry material was found to contain 63 ppm of selenium by fluorometric analysis. White sweet clover growing on a low-selenium loam soil in Dryden, NY, was similarly harvested and prepared as a control; it was found to contain 0.06 ppm (dry weight) of selenium.

Selenium- and vitamin E depleted 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. Feed and water were provided ad libitum. Fly ash or white sweet clover grown on fly ash was fed to vitamin E deficient chicks in order to assess the relative biological availabilities of selenium from these sources. Dietary treatments were based on the selenium-deficient vitamin E free diet pre-

Departments of Poultry Science (G.F.C.), Animal Science (K.T.M.), and Food Science (W.H.G. and D.J.L.), New York State College of Agriculture and Life Sciences, Cornell University, Ithaca, New York 14853.

[1]Present address: Department of Agricultural Sciences, Tuskegee Institute, Tuskegee, AL 36088.

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Table I. Composition of Experimental Diets

ingredient	composition, %								
	Na_2SeO_3[a] series 1-5	fly ash series				clover series[b]			
		6	7	8	9	10 (14)	11 (15)	12 (16)	13 (17)
fixed ingredients[c]	70.006	70.006	70.006	70.006	70.006	70.006	70.006	70.006	70.006
fly ash		4.739	9.479	14.218	18.957				
clovers						0.476	0.962	1.429	1.905
stripped corn oil	2.500	11.000	11.000	11.000	11.000	2.500	2.500	2.500	2.500
glucose monohydrate	22.714					22.714	22.714	22.714	22.714
cellulose	4.780	14.255	9.524	4.775	0.037	4.304	3.828	3.351	2.875

[a] Na_2SeO_3 was added in aqueous solution to provide 0, 30, 60, 90, or 120 ppm of Se to diets 1, 2, 3, 4, and 5, respectively. [b] Diets 10-13 contained the fat-extracted high-Se clover (63 000 ppb of Se); diets 14-17 contained the fat-extracted low-Se clover (6 ppb of Se). [c] Contained the following (percent complete diet): torula yeast, 11.000; isolated soya protein, 14.050; glucose monohydrate, 34.716; L-tryptophan, 0.034; L-arginine hydrochloride, 0.213; glycine, 0.302; DL-methionine (98%), 0.750; $CaCO_3$, 1.020; $CaHPO_4 \cdot 2H_2O$, 1.900; KH_2PO_4, 0.306; NaCl, 0.180; trace element premix and vitamin E free vitamin premixes (Combs, 1978), 0.720 and 0.200, respectively; choline chloride (70% solution), 0.290.

viously reported (Combs, 1978), modified to include 0, 30, 60, 90, or 120 ppb of selenium as sodium selenite or 300, 600, 900, or 1200 ppb of selenium from either fly ash or clover. A series of dietary treatments in which low-selenium clover was incorporated in the diet at the same levels as the high-selenium clover was used as a control for possible effects of nonextractable factors other than selenium in clover which may alter chick performance. The low-Se clover control diets contained less than 10 ppb of Se. The compositions of experimental diets are presented in Table I. All diets were isocaloric and contained adequate amounts of all known nutrients except selenium and vitamin E. All diets were essentially free of vitamin E, making biologically available selenium the limiting factor for growth, protection against exudative diathesis, and survival of young chicks. Each dietary treatment was applied to triplicate lots of 10 chicks each for 2 weeks starting at 1 day of age; during this period, gain in body weight, feed consumption, incidence of exudative diathesis, and mortality were recorded.

At 2 weeks of age, blood was obtained from each of 10 chicks randomly selected from each treatment by anterior cardiac puncture using a heparinized syringe. Plasma was prepared from aliquots of whole blood from four chicks from each of the control (selenium-deficient basal diet), sodium selenite, fly ash, and high-selenium clover treatments by centrifugation at 1000*g* for 5 min. Plasma selenium-dependent glutathione peroxidase was determined by using the glutathione reductase coupled assay of Paglia and Valentine (1967) as modified by Lawrence and Burk (1976). Hydrogen peroxide was used as the substrate. Plasma protein was determined by the method of Lowry et al. (1951). Results were expressed as enzyme units [1 unit = 1 μmol of NADPH oxidized (mg of protein)$^{-1}$ min^{-1}].

After blood was collected, the same chicks were killed by cervical dislocation. The following tissues were quickly excised and packed in ice: liver; kidney; M. pectoralis. These tissues and aliquots of whole blood were lyophilized; total selenium was determined in each sample by the fluorometric method as described above. Results were expressed as parts per billion of selenium on a total dry matter basis.

All 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 76.6 Statistical Analytical System, SAS Institute, Inc., Raleigh, NC) on the Cornell computer.

RESULTS AND DISCUSSION

Selenium present in fly ash or in white sweet clover grown on fly ash was available to the vitamin E deficient chick for the prevention of the specific selenium vitamin E deficiency disease exudative diathesis (Table II). The protection from exudative diathesis was an exponential function of the total selenium added (as Na_2SeO_3 or high Se sweet clover) to the basal diet. Fly ash selenium provided substantial protection against exudative diathesis; however, selenium from this source appeared to be utilized at ~7% of the efficiency of selenium provided as sodium selenite (fly ash: $Y = 0.053X + 9.0$, $r = 0.743$; Na_2SeO_3: $Y = 0.817X + 13.0$, $r = 0.872$; Y = protection against exudative diathesis (100 - % exudative diathesis) and X = ppb of Se added). Selenium in the high-selenium clover was utilized by the chick to effect near-complete protection against exudative diathesis, which was achieved at the levels of substitution in the diet, all of which provided selenium in excess of the chicks' dietary requirement. That this effect was related to selenium in white sweet clover is indicated by the fact that low-selenium clover processed in the same manner did not confer any protection from exudative diathesis. For each source of selenium, protection from exudative diathesis was accompanied with significant improvements in chick growth and survival, although the efficiency of feed utilization (feed/gain) was not significantly ($P > 0.05$) affected by treatment.

Plasma selenium-dependent glutathione peroxidase activity increased with increasing levels of selenium from fly ash or sodium selenite in the diet. Fly ash was found to yield activities which averaged more than 3 times those produced by feeding sodium selenite although the latter source provided only 10% as much selenium as fly ash. Previous work in this laboratory (Noguchi et al., 1973) showed that the activity of this enzyme in plasma of young chicks was inversely correlated with their subsequent manifestation of exudative diathesis when dietary selenium was provided as sodium selenite or seleno-DL-methionine. The present results, however, show that selenium was utilized for the production of high glutathione peroxidase activities even though prevention of exudative diathesis was poorly effected. It, thus, appears that manifestation of selenium deficiency may not be directly related to circulating levels of glutathione peroxidase as originally suggested (Noguchi et al., 1973).

Selenium from each dietary source was readily deposited in chick tissues (Table III). The rates of deposition/retention of dietary selenium in tissue were greater for chicks fed fly ash or the high-selenium clover than for chicks fed sodium selenite (which provided only 10% as much dietary selenium). The enhanced apparent efficiency of tissue deposition of dietary selenium was greatest in liver and kidney, whereas muscle and blood showed weaker responses. In general, tissue selenium levels were about 60% and 200% greater when dietary selenium was provided as fly ash and clover, respectively, compared to sodium sel-

Table II. Response of the Vitamin E Deficient Chick to Selenium from Na_2SeO_3, White Sweet Clover, and Fly Ash

supplemental Se		2-week performance				
source	level, ppb	gain, g	feed/gain	mortality, %	exudative diathesis, %	plasma glutathione peroxidase, units
none	0	31.3 ± 5.7^{C} [a,b]	2.49 ± 0.37 [a,c]	60.0 ± 7.1^{ABC} [a,b]	100.0 ± 0.0^{A} [a,b]	0.32 ± 0.18^{F} [b,d]
Na_2SeO_3	30	51.2 ± 1.3^{ABC}	2.26 ± 0.04	10.0 ± 7.1^{DE}	55.0 ± 3.5^{ABC}	1.17 ± 0.18^{F}
	60	67.2 ± 5.2^{AB}	1.94 ± 0.00	15.0 ± 10.6^{DE}	25.0 ± 10.6^{DE}	4.24 ± 1.70^{EF}
	90	56.8 ± 3.4^{ABC}	2.03 ± 0.08	5.0 ± 3.5^{DE}	10.0 ± 3.3^{E}	4.72 ± 1.03^{EF}
	120	71.1 ± 2.4^{AB}	1.91 ± 0.01	10.0 ± 7.1^{DE}	0 ± 0^{E}	8.72 ± 0.48^{CDEF}
fly ash[g]	300	45.4 ± 12.4^{BC}	1.97 ± 0.01	45.0 ± 3.5^{ABCD}	70.0 ± 14.1^{AB}	6.92 ± 0.24^{DE}
	600	56.4 ± 0.2^{ABC}	2.29 ± 0.07	20.0 ± 0.0^{CDE}	40.0 ± 7.1^{BCD}	11.79 ± 1.50^{BCD}
	900	71.4 ± 3.8^{AB}	1.86 ± 0.08	42.2 ± 1.6^{ABCD}	45.0 ± 0^{BCD}	11.60 ± 1.44^{BCD}
	1200	64.5 ± 3.0^{AB}	2.11 ± 0.04	35.0 ± 3.5^{BCDE}	35.0 ± 3.5^{CD}	17.10 ± 2.95^{AB}
high-Se clover[e]	300	68.9 ± 0.8^{AB}	2.01 ± 0.03	0 ± 0^{E}	0 ± 0^{E}	16.27 ± 1.66^{ABC}
	600	76.3 ± 0.4^{AB}	1.87 ± 0.05	10.0 ± 7.1^{DE}	5.0 ± 3.5^{E}	13.93 ± 2.98^{ABCD}
	900	80.5 ± 2.2^{A}	1.98 ± 0.04	10.0 ± 7.1^{DE}	0 ± 0^{E}	17.35 ± 1.41^{AB}
	1200	80.4 ± 2.1^{A}	1.86 ± 0.01	5.0 ± 3.5^{DE}	0 ± 0^{E}	16.14 ± 1.02^{ABC}
low-Se clover[f]	3	31.1 ± 1.3^{C}	2.72 ± 0.11	55.0 ± 3.5^{ABC}	100.0 ± 0.0^{A}	
	6	47.7 ± 13.0^{BC}	2.23 ± 0.05	80.0 ± 7.1^{A}	100.0 ± 0.0^{A}	
	9	27.4 ± 2.9^{C}	2.45 ± 0.20	75.0 ± 3.5^{AB}	100.0 ± 0.0^{A}	
	11	27.0 ± 1.9^{C}	2.52 ± 0.04	60.0 ± 7.1^{ABC}	100.0 ± 0.0^{A}	

[a] Mean ± SE for triplicate lots of 10 chicks per treatment. [b] Means with like superscript within a column are not significantly different ($P > 0.05$). [c] Treatment effect was not significant ($P > 0.05$). [d] Mean ± SE for three composite samples from four chicks each per treatment. [e] Fat-extracted material contained 63 ppm total of Se. [f] Fat-extracted material contained 0.06 ppm total of Se. [g] Contained 6.33 ppm total of Se.

Table III. Influence of Dietary Sodium Selenite, High-Se Clover, and Fly Ash on Tissue Selenium Levels in Tissues of Two-Week-Old Chicks

supplemental Se		tissue Se levels, ppb			
source	level, ppb	blood	liver	kidney	M. pectoralis
none	0	12 ± 3^{h} [a]	128 ± 6^{f} [a]	165 ± 5^{f} [a]	60 ± 5^{hi} [a]
Na_2SeO_3	30	25 ± 3^{h}	173 ± 12^{f}	211 ± 16^{f}	46 ± 2^{i}
	60	61 ± 11^{g}	283 ± 41^{e}	346 ± 70^{e}	52 ± 5^{i}
	90	58 ± 10^{g}	287 ± 41^{e}	384 ± 35^{e}	61 ± 6^{hi}
	120	88 ± 5^{efg}	452 ± 27^{d}	457 ± 20^{cde}	64 ± 2^{hi}
fly ash[c]	300	71 ± 6^{fg}	312 ± 23^{e}	367 ± 22^{e}	65 ± 6^{hi}
	600	91 ± 7^{def}	491 ± 15^{cd}	432 ± 19^{de}	74 ± 3^{gh}
	900	122 ± 5^{cd}	547 ± 16^{c}	481 ± 17^{cde}	89 ± 6^{fg}
	1200	116 ± 14^{de}	519 ± 14^{de}	539 ± 14^{cd}	97 ± 2^{f}
high-Se clover[b]	300	148 ± 5^{c}	560 ± 11^{c}	582 ± 12^{c}	130 ± 3^{e}
	600	192 ± 6^{b}	677 ± 26^{b}	752 ± 23^{b}	188 ± 9^{d}
	900	220 ± 13^{b}	713 ± 16^{b}	811 ± 30^{b}	223 ± 12^{c}
	1200	257 ± 5^{a}	858 ± 15^{a}	840 ± 41^{ab}	281 ± 6^{b}

[a] Mean ± SE for 10 individual analyses of 10 chicks per treatment; means with like superscripts are not significantly different ($P > 0.05$). [b] Contained 63 ppm total of Se. [c] Contained 6.33 ppm total of Se.

enite, which was fed at levels providing 0.1 times as much selenium.

These results show that selenium in soft coal fly ash was nutritionally active in the chick. Although fly ash selenium was deposited and/or retained at least as well in tissues as sodium selenite (estimates based upon the regressions presented above indicated that the rates of deposition have enhanced about ninefold in liver and eightfold in kidney and were not decreased in muscle and blood), fly ash selenium was much less effective in preventing selenium deficiency than the latter form. On the basis of prevention of exudative diathesis, fly ash selenium had a biologic availability at ~7% of that of sodium selenite. The fact that fly ash feeding produced high levels of selenium-dependent glutathione peroxidase without producing comensurate prevention from exudative diathesis shows that the activity of this enzyme in chick plasma may not always be a valid indicator of nutritional selenium status. It is likely that the release of this enzyme into the plasma from other tissues in which it may be functional may be stimulated by a factor(s) contained in fly ash and perhaps other natural materials. This hypothesis is tenable on the basis of the questionable physiologic significance of the enzyme found in plasma which contains insufficient substrate (reduced glutathione) for its activity. It is also possible that other components of fly ash (i.e., heavy materials and polynuclear aromatics) may enhance the expression of exudative diathesis without affecting the Se-dependent glutathione peroxidase.

White sweet clover can apparently convert the poorly available selenium in fly ash to a form(s) which is effectively used by the chick both for prevention of selenium deficiency and for deposition/retention of selenium in tissues. Alternatively, it is possible that fly ash contains some soluble Se which can be utilized directly by the chick and which can be accumulated by plants, resulting in an apparent improvement in biological availability. The biologic availability of selenium in the high-selenium clover cannot be determined on the basis of these results; however, clover selenium was deposited in liver, kidney, and blood with apparent efficiencies two- to threefold those of sodium selenite. Cantor et al. (1975b) using a similar biological assay also observed that selenium in alfalfa had an apparent biologic availability of at least twice that of

sodium selenite when both sources were fed at equivalent selenium levels. Selenomethionine, presumed to be the predominant form of selenium in plant materials, has been shown by using a similar bioassay to be less biologically available than sodium selenite (Cantor et al., 1975b). The results of Cantor et al. (1975b) indicate that selenomethionine or a form of selenium with similar biological availability fed at high levels as in this study would effectively protect against selenium deficiency.

Although several studies have shown that selenium in fly ash or in plants grown on fly ash is deposited in the tissues of animals consuming such materials, the present study constitutes the first demonstration of the nutritional value of that selenium. Tissue selenium levels do not correlate with the nutritional value of selenium in fly ash. Therefore, tissue residue analyses may not be a valid indicator of the biologic availability of selenium for animals fed fly ash. Nevertheless, it can be inferred from these results that fly ash selenium can be of only limited nutritional significance for animals. Plants grown on fly ash appear to be able to convert its poorly available form(s) of selenium to a form(s) with superior biological availability, or they may be able to concentrate low levels of soluble selenium present in fly ash.

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Fate of Amide Nitrogen Added to Soils

William T. Frankenberger, Jr., and M. Ali Tabatabai*

The fate of nitrogen in amides (formamide, acetamide, and propionamide) was studied in five soils. The amounts of inorganic N ion species and NH_3 produced from each compound were compared with those produced from soils treated with $(NH_4)_2SO_4$ or urea and incubated under aerobic conditions at 30 °C for 14 days. Recovery of the inorganic N ion species and NH_3 produced was affected by the compound and soil used. More than 25% of the N added as formamide was evolved as NH_3 from one sandy soil. The nitrification rate of the amide N was the greatest with formamide, and those of acetamide and propionamide were similar to those of urea and $(NH_4)_2SO_4$. The total recovery of inorganic ion species of N and NH_3 produced in the five compounds and five soils studied ranged from 62 to 103%. The amounts of NH_3 voltatilized from the amide- and urea-treated soils were significantly but negatively correlated with cation-exchange capacity, organic C, and clay contents of the soils examined.

Numerous studies have been conducted on the manipulation of biochemical processes in soils for increasing the efficiency of N fertilizers, but there seems to be little progress in achieving that goal. A number of problems are encountered when N fertilizers are employed. Upon application to soils, these fertilizers may be subjected to (1) leaching and runoff losses, (2) denitrification losses through biological and chemical mechanisms, (3) dissolution rates too slow to keep pace with daily and normal crop requirements, and (4) NH_3 volatilization losses during or shortly after application.

One of the more popular solid N fertilizers available today is urea, an organic compound containing 46% N. The advantages of urea are manifold and include (1) a high analysis, (2) safety in handling, (3) application as either a solid or solution, and (4) relatively low cost (Gasser,

Department of Agronomy, Iowa State University, Ames, Iowa 50011.

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