Wheat Grown on Fly Ash: High Selenium Uptake and Response When Fed to Japanese Quail

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Winter wheat was grown to maturity on a deep bed of fly ash from a coal-burning electric power plant. The harvested grain was fed as 60% of a complete diet to Japanese quail for 112 days. The wheat grain contained 5.7 ppm (dry weight) of selenium as compared to 0.02 ppm in control wheat grown on soil. The tissues and eggs from the quail fed the fly ash-grown wheat contained greatly elevated levels of residual selenium compared to control birds. Eggshell thickness was significantly greater in the birds fed the fly ash-grown wheat. No significant differences were observed in liver microsomal enzyme activities between quail on the fly ash or control wheat rations.

About 30 million tons of fly ash results annually from coal burning in electric power generating stations in the United States. The bulk of this waste material is disposed in landfills. In an earlier study, yellow sweet clover (*Melilotus officinalis* (L.) Pall.) was found growing voluntarily on a fly ash landfill site in Lansing, N.Y. Analysis of this clover showed selenium, as well as 19 other elements, to be higher than clover grown on soil. The concentration of selenium in clover grown on fly ash was 5.3 ppm. These clovers were formulated at 45% into a diet and fed to guinea pigs for 90 days. Organs of the guinea pigs fed the fly ash grown clover contained elevated selenium levels (Furr et al., 1975). In a later study, white sweet clover (*Melilotus alba* Desr.), found growing on a fly ash landfill near Binghamton, N.Y., contained selenium at concentrations exceeding 200 ppm (Gutenmann et al., 1976).

In the present investigation, winter wheat was planted and grown to maturity on a fly ash landfill. It was then harvested and incorporated into a complete diet and fed to Japanese quail (*Coturnix coturnix japonica*), followed by analysis of plant and avian tissues for residual total selenium. Observations were also made on eggshell thickness and hepatic microsomal enzyme activity of quail fed this wheat.

EXPERIMENTAL SECTION

The fly ash landfill site is located in Lansing, N.Y., about 32 km north of Ithaca. The fly ash was derived from coal burned at Milliken Station, an electric power generating plant about 3 km west of the landfill on the eastern shore of Cayuga lake. In September, 1975, a plot 15.2 m wide and 45.7 m long was plowed. This bed of fly ash (pH 5.0) was approximately 4.6 m deep. The concentration of 36 elements including selenium in this fly ash was reported earlier (Furr et al., 1975). Lime (291 kg) and 36 kg of 10-20-20 fertilizer was then spread on and tilled in with a rotary cultivator. After liming, the pH of the fly ash was 7.3. The plot was then seeded with "York Star" winter wheat (*Triticum aestivum* L.). Wheat grown on a Honeoye silt loam soil, pH 7.1 and similarly fertilized, served as the control crop. In July, 1976, the grain was harvested, dried, threshed, milled to a powder, and thoroughly mixed.

Thirty, unsexed day-old Japanese quail were placed either on a diet containing 60% of the fly ash grown wheat or soil-grown winter wheat as described in Table I. At the end of 6 weeks, egg production commenced. Eggs were collected during the first, third, fifth, and eighth week of production (15 eggs/treatment), weighed, and measured for shell thickness (Stoewsand et al., 1977). At the end of 112 days, the birds were killed by cervical dislocation. Hepatic *N*-demethylase activity was measured in male quail by following microsomal production of formaldehyde from aminopyrine (Nash, 1952), and *O*-demethylase activity was determined by measuring the production of *p*-nitrophenol from *p*-nitrosoanisole (Kato and Gillette, 1965).

Samples of fly ash, wheat, and quail tissues and eggs were analyzed for selenium. The avian tissues were freeze-dried, milled to a powder, mixed, and subsampled

Table I. Composition of Basal Diet

Ingredient	Percent dry weight
Wheat[a]	60.00
Isolated soy protein	26.00
Corn oil	5.00
Minerals[b]	6.67
DL-Methionine	0.70
Glycine	0.23
Choline chloride	0.40
Vitamin mix[c]	1.00
Vitamin B_{12}	30 mcg/kg
Antioxidant[d]	0.0125

[a] Fly ash grown wheat contained 5.7 ppm Se. Soil-grown wheat contained 0.02 ppm Se. [b] In percent: $CaHPO_4 \cdot 2H_2O$, 3.00; $CaCO_3$, 1.50; KH_2PO_4, 1.00; KCl, 0.10; NaCl, 0.60; $NaHCO_3$, 0.08; $MgSO_4$, 0.30; $FeSO_4 \cdot 7H_2O$, 0.0333; $MnSO_4$, 0.0333; KI, 0.0003; ZnO, 0.0125; $CuSO_4 \cdot 5H_2O$, 0.0017; $CoCl_2 \cdot 6H_2O$, 0.0002; $NaMoO_4 \cdot 2H_2O$, 0.0008. (Ca = 1.30%; P = 0.77%). [c] In g/kg of mix; thiamin hydrochloride, 7.00; riboflavin, 2.00; nicotinic acid, 9.00; pyridoxine hydrochloride, 2.00; calcium pantothenate, 6.00; inositol, 25.00; PABA, 10.00; ascorbic acid, 1.00; menadione sodium bisulfate, 0.10; folic acid, 1.00; biotin, 0.05. In IU/kg of diet: vitamin A, 5000; vitamin D_3, 1600; α-tocopheryl acetate, 50. Glucose added to 1 kg. [d] Santoquin, Monsanto Chemical Co., St. Louis, Mo.

Table II. Selenium in Soil, Fly Ash, Wheat, and Wheat Rations

Sample	Selenium, ppm, dry weight
Soil	2.1
Fly ash	21.3
Soil-grown control wheat	0.02
Fly ash grown wheat	5.7
Control wheat ration	0.06
Fly ash grown wheat ration	3.7

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Table III. Selenium in Tissues of Japanese Quail Fed Winter Wheat Grown on Fly Ash

Wheat grown on	Quail sex	No. of reps.	Selenium[a] (ppm dry weight) in Brain	Heart	Kidney	Liver	Muscle
Fly ash	M	9	3.4 ± 1.6	4.4 ± 0.7	9.5 ± 1.1	12.7 ± 2.4	4.1 ± 0.6
Soil (control)	M	8	0.8 ± 0.1	0.7 ± 0.2	3.6 ± 0.4	1.6 ± 0.0	0.3 ± 0.2
Fly ash	F	7	3.4 ± 0.2	4.4 ± 0.4	9.4 ± 0.8	9.8 ± 3.0	3.7 ± 0.4
Soil (control)	F	9	0.8 ± 0.1	0.4 ± 0.1	1.4 ± 0.3	0.7 ± 0.1	0.2 ± 0.02

[a] Average ± standard deviation.

Table IV. Eggshell Thickness of Japanese Quail Hens Fed Fly Ash Grown Wheat

Week of laying	Treatment[a]	Eggshell thickness, mm	Egg weight, g
1	Fly ash	0.193 ± 0.003[b]	8.1 ± 0.2
	Control	0.178 ± 0.004	7.9 ± 0.3
3	Fly ash	0.182 ± 0.003	9.5 ± 0.3
	Control	0.173 ± 0.002	9.3 ± 0.1
5	Fly ash	0.183 ± 0.002	9.8 ± 0.2
	Control	0.168 ± 0.003	9.2 ± 0.1
8	Fly ash	0.183 ± 0.002	10.0 ± 0.2
	Control	0.175 ± 0.002	9.2 ± 0.3

[a] Wheat grown on fly ash or soil (control). [b] Mean ± SEM 15 eggs/treatment. Eggshells are significantly thicker ($P < 0.05$) from hens fed fly ash grown wheat during each laying period.

prior to analysis. Selenium was determined by a modification of the method of Olson (1969) employing wet digestion of the sample and measurement of the fluorescence of piazselenol resulting from reaction of selenium with 2,3-diaminonaphthalene.

RESULTS AND DISCUSSION

The concentration of selenium in the soil and fly ash, in the respectively grown wheats and in the respective quail diets, are given in Table II. The concentrations of selenium found in the avian tissues are listed in Table III. Selenium is markedly higher in all tissues of male and female quail fed the wheat grown on fly ash as compared to control wheat. The livers of male quail fed seleniferous wheat contained the highest level of selenium. Brain, heart, and kidneys of both male and female quail fed the fly ash grown wheat contained similarly high levels of selenium, with female quail containing slightly lower residues of selenium in liver and breast muscle. It was interesting to note that the tissues from female quail fed the soil-grown wheat, with the exception of the brain, contained lower selenium levels than the respective tissues of the control males. These slightly decreased selenium tissue residues in the quail hens could be attributable to selenium present in the eggs (Figure 1).

Figure 1 presents the concentration of selenium in the whites and yolks of the quail eggs produced by the birds as a function of time while consuming either of the diets. Selenium is consistently higher in the egg whites of the quail fed the wheat grown on fly ash but not the control wheat. Moxon and Poley (1938) showed that increasing the selenium content of the diet of hens caused a greater increase in the selenium content of egg whites than of yolks. Interestingly, Latshaw and Osman (1975) reported that, after feeding selenomethionine to hens, selenium was higher in egg white than yolk. Conversely, feeding selenite or selenocystine resulted in a higher selenium content in egg yolks than whites. Selenomethionine and, to a much lesser extent, selenocystine are believed to comprise important forms of selenium in plants (Olson et al., 1970). From a nutritional standpoint it should be noted that average concentrations of selenium in ppm fresh weight for a number of foods are reported by Morris and Levander (1970) as follows: vegetables, 0.01; fruits, 0.006; cereals, 0.38; egg whites, 0.05; egg yolks, 0.18; cheeses, 0.08; whole milk, 0.01; meats (excluding kidney), 0.22; and seafoods, 0.53. It should be noted, however, that concentrations of selenium in the range of 5 ppm in common foods are considered potentially hazardous (Rosenfeld and Beath, 1964).

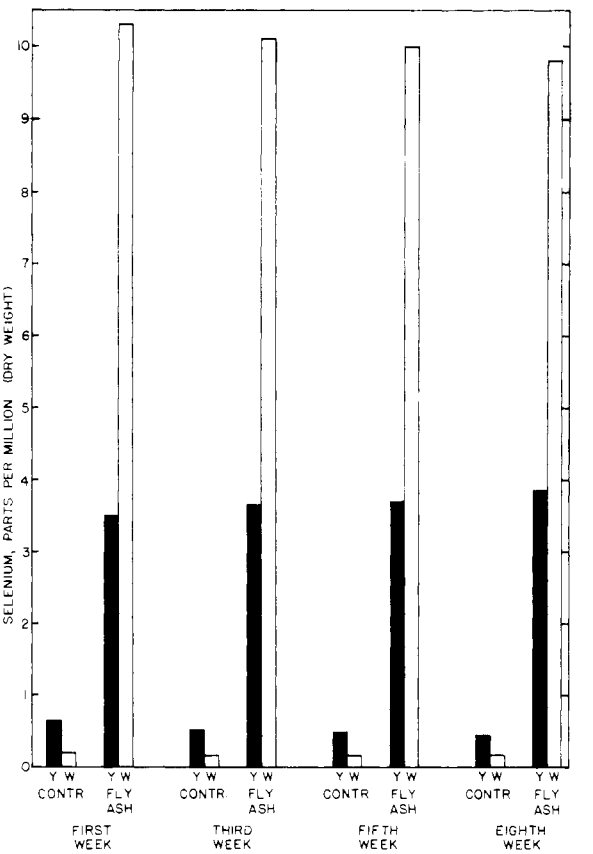

Figure 1.

Table V. Mean Body Weight, Liver Weight, and Hepatic Microsomal Enzyme Activities of Male Quail Fed Fly Ash Grown, Seleniferous Wheat or Soil-Grown Control Wheat

Treatment	Body weight, g	Liver weight, g	*p*-Nitroanisole[b] *O*-demethylase, nM (mg of protein)$^{-1}$ h^{-1}	Aminopyrine[b] *N*-demethylase, nM (mg of protein)$^{-1}$ h^{-1}
Fly ash	114 ± 3[a]	1.9 ± 0.1	19.9 ± 1.3	10.0 ± 1.6
Control	118 ± 4	1.8 ± 0.1	18.4 ± 0.9	9.4 ± 0.6

[a] Mean ± SEM. [b] Six quail/treatment.

Table IV shows eggshell thickness and egg weights of the quail hens fed the seleniferous, fly ash grown wheat. In agreement with our previous observations (Stoewsand et al., 1977), selenium-containing wheat or sodium selenite fed to Japanese quail hens significantly increased eggshell thickness, as compared to eggs produced by hens consuming low selenium diets. It should be noted that in both of these studies the dietary level of selenium was high, i.e., between 1.8 or 3.4 ppm, in great excess of an avian species nutritional requirement (Scott et al., 1969). Dietary calcium was relatively low for optimal egg production and quality (Table I).

Japanese quail body and liver weights, as well as hepatic microsomal enzyme activity, were unaltered in the high selenium, fly ash grown, wheat-fed group (Table V). It has been reported that dietary selenium may alter hepatic microsomal enzyme activities in the rat (Burk et al., 1974). These tissue–enzyme systems, so important in the metabolism of foreign compounds, may interact with a myriad of dietary elements (Becking, 1976).

These data show that fly ash, a waste material from coal-burning electric power plants, may contain selenium readily available to crops. Uptake of this element by wheat can occur, as it does in wheat grown in high selenium soils (Olson et al., 1970). Diets containing large amounts of this wheat produce some biological changes in Japanese quail, such as enhanced tissue storage of selenium, with dietary selenium levels as high as 3.4 ppm. Indeed, if further studies confirm that selenium additions to deficient diets increases eggshell thickness, this could add support to the argument for adding this element to layer diets. Fly ash high in selenium and low in toxic elements may be a desirable amendment to low selenium soils and a practical source of this element in grains and other plant foods.

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LITERATURE CITED

Becking, G. C., *Med. Clin. North Am.* **60**, 813 (1976).
Burk, R. F., Mackinnon, A. M., Simon, F. R., *Biochem. Biophys. Res. Commum* **56**, 431 (1974).
Furr, A. K., Stoewsand, G. S., Bache, C. A., Gutenmann, W. H., Lisk, D. J., *Arch.* Environ. Health **30**, 244 (1975).
Gutenmann, W. H., Bache, C. A., Youngs, W. D., Lisk, D. J., *Science* **191**, 966 (1976).
Kato, R., Gillette, J. R., *J. Pharmacol. Exp. Ther.* **150**, 279 (1965).
Latshaw, J. D., Osman, M., *Poult. Sci.* **54**, 1244 (1975).
Morris, V. C., Levander, O. A., *J. Nutr.* **100**, 1383 (1970).
Moxon, A. L., Poley, W. E., *Poult. Sci.* **17**, 77 (1938).
Nash, T., *Biochem. J.* **55**, 416 (1953).
Olson, O. E., *J. Assoc. Off. Anal. Chem.* **52**, 627 (1969).
Olson, O. E., Novacek, E. J., Whitehead, E. I., Palmer, I. S., *Phytochemistry* **9**, 1181 (1970).
Rosenfeld, I., Beath, O. A., "Selenium-Geobotany, Biochemistry, Toxicity, and Nutrition", Academic Press, New York, N. Y., 1964.
Scott, M. L., Nesheim, M. C., Young, R. J., "Nutrition of the Chicken", M. L. Scott & Associates, Ithaca, N.Y., 1969, p 318.
Stoewsand, G. S., Anderson J. L., Gutenmann, W. H., Lisk, D. J., *Nutr. Rep. Int.* **15**, 81 (1977).

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Removal of the Growth Inhibitor(s) from Acid and Pressure Hydrolyzed Sawdust

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Sawdust hydrolyzed (HSD) with 2.3% H_2SO_4 at 42.2 kg/cm^2 pressure for 40 s caused reduced growth when included in the diets of rats. Furfural produced by the acid hydrolysis of the sawdust did not appear to cause the growth depression in rats. Extraction of the HSD with 95 and 80% ethanol removed substance(s) which inhibited cellulose digestion by rumen microorganisms. Ninety-five percent ethanol extraction of HSD was also effective in removing the compound(s) inhibiting growth in rats.

Sawdust offers great potential as an energy source for ruminants, but wood polysaccharides usually are not sufficiently degraded by rumen microorganisms to provide energy unless some manner of delignification is used. Several methods of improving wood cellulose degradation are: irradiation with high-energy electrons (Lawton et al., 1951), reduction in physical size to micron-sized particles (Stranks, 1959; Dehority and Johnson, 1961), and heating in dilute alkali (Wilson and Pigden, 1964; Mellenberger et al., 1971). These processes increased the utilization by bacteria or isolated enzymes.

Sawdust has also been subjected to modification by acid and pressure hydrolysis. Inclusion of this modified sawdust in cattle, sheep, or rat diets reduced performance in all cases (Butterbaugh and Johnson, 1974; Erlinger and Klopfenstein, 1975; Hudson, 1971). The present experiments were designed to determine the factor(s) in the hydrolyzed sawdust (HSD) responsible for the reduced performance.

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MATERIALS AND METHODS

Sawdust (80% hardwood and 20% pine) was hydrolyzed with 2.3% H_2SO_4 at 42.2 kg/cm^2 pressure for 40 s. The HSD was neutralized to pH 7 with NaOH.

In Vitro Experiments and HSD Extractions. Two in vitro dry matter disappearance (IVDMD) experiments were utilized to determine whether extraction procedures would remove materials inhibiting cellulose digestion by rumen microorganisms. Initially, petroleum ether was used for extraction, but negligible amounts of dry matter were extracted and it was not investigated further. A sequential ethanol (ETOH) extraction (Figure 1) of the HSD was performed starting with absolute ETOH, then 95% ETOH, and finally 80% ETOH. Upon refrigeration of the extracts, precipitates formed in each extract. The precipitates were resolubilized in 0.2 N NaOH. All extracts

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