NaH_2PO_4 and LiH_2PO_4. Commerical production of K_2HPO_4, Na_2HPO_4, etc. may also be possible if higher temperatures and pressures are employed; higher pressures are probably required to minimize thermal decomposition of the phosphates.

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Elemental Content of Tissues of Sheep Fed Rations Containing Coal Fly Ash

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Sheep were fed up to 7.5% by weight of fly ash in complete pelleted rations for 124 days. Among 34 elements determined in rations and animal tissues, selenium increased slightly in specific tissues and blood as a function of the percentage of fly ash in the ration. Selenium concentration in blood increased slowly as the time of feeding progressed. There was no significant effect from the inclusion of fly ash in the ration on quantity of feed consumed, animal weight gain, or in vivo ration digestibility.

Whereas high concentrations of dietary selenium are known to be toxic to farm animals (Rosenfield and Beath, 1964), traces of the element are essential. Glutathione peroxidase isolated from bovine erythrocytes has recently been shown to contain four selenium atoms per molecule of the enzyme (Hoekstra, 1975). This enzyme is believed to destroy H_2O_2 and other organic hydroperoxides which may produce oxidative damage in living cells. Decreases in glutathione peroxidase correlate with lesions caused by selenium deficiency.

Between 0.03 and 0.1 ppm of selenium in the diet appears to satisfy the essential requirements of the element for animals (Allaway et al., 1967). Since soils in many areas of the world are deficient in selenium available for plants, supplementation of the diets of farm animals with selenium has become common (Perry et al., 1976; Frost, 1973; Kuchel and Buckley, 1969; Handrick and Godwin, 1970). Recent studies here have shown that fly ashes produced in soft coal-burning electric power generating plants contain appreciable quantities of selenium which is readily available to plants grown in it (Gutenmann et al., 1976; Furr et al., 1975, 1976a) or to aquatic organisms when fly ash contaminates their water (Gutenmann et al., 1976). It

Table I. Composition of the Complete Pelleted Sheep Rations

constituent	% dry wt	% dry wt	% dry wt	% dry wt	% dry wt
fly ash	0[a]	1	2.5	5	7.5
alfalfa meal	45	44	41.5	38	34.5
oats (crushed)	10	10	10	10	10
corn (ground)	13	13	13	13	13
wheat (middlings)	5	5	5	5	5
soybean meal	11.5	11.5	12.5	13.5	14.5
molasses	15	15	15	15	15
salt (without minerals)	0.45	0.45	0.45	0.45	0.45
vitamin supplement (A, D, and E)	0.05	0.05	0.05	0.05	0.05

[a] Control ration.

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Table II. Content of Ash, Fat, and Protein in Pelleted Rations Fed

ration	% dry wt ash	fat	protein	energy[a]
control	8.2	2.4	18.4	4407
1% fly ash	8.6	2.4	18.0	4351
2.5% fly ash	9.8	2.5	18.2	4311
5% fly ash	12.0	2.3	17.7	4172
7.5% fly ash	14.3	1.5	17.4	4064

[a] Calories/gram (dry weight).

was therefore reasoned that perhaps fly ash could be added to rations of farm animals to serve as a supplementary source of selenium in rations deficient in the element. In the work reported, sheep were fed rations containing increasing percentages of soft coal fly ash for 124 days after which elemental analysis was conducted on tissues, body fluids, and excreta.

EXPERIMENTAL SECTION

Fly ash was obtained as freshly produced material from Milliken Station, a coal-burning electric power-generating plant in Lansing, N.Y., about 20 miles north of Ithaca on the eastern shore of Cayuga Lake. The coal burned there is bituminous obtained largely from strip mines 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 thoroughly mixed and incorporated at levels of 1, 2.5, 5, and 7.5% by weight into complete rations for lambs. A ton of each ration (including a control diet containing no fly ash) was mixed and pelleted (0.4 cm pellets). The composition of the rations is given in Table I.

Twenty 3-month-old Dorset wethers were used in the feeding trial. Individual groups of four sheep were fed one of the rations, i.e., four replicated animals per dietary treatment, for 124 days. The animals were located in individual metabolic stalls throughout the feeding period. All animals were first adapted from their normal rations of hay and grain to the pelleted diets over a period of 14 days. Salt (without iodine or trace minerals) and water was provided ad libitum. The digestibility of the rations was measured in vivo over a 9-day period during the last 2 weeks of the experiment. A subsample of the total mixed feces produced during the entire 9-day digestion trial was taken from each animal for analysis. At the end of the feeding period the animals were killed and necropsied and tissue samples were taken for element analysis and pathologic examination. The tissues included adrenal, heart, intestine, kidney, liver, lung, lymph nodes, pancreas, skeletal muscle, spleen, thymus, thyroid, and tongue. The tissues were fixed in 10% neutral formalin and embedded in paraffin. They were sectioned at 6 μm and stained with hematoxylin and eosin.

The control ration, fly ash used in the rations, and animal tissues were analyzed for 34 nutrient and toxic elements. Tissues were freeze dried, milled, and mixed prior to subsampling for analysis. Nondestructive neutron activation analysis was used for the determination of 31 elements using the procedure described previously (Furr et al., 1976b). Selenium was determined by a modification of the fluorometric method of Olson (1969). Arsenic analysis was performed by dry ashing (Evans and Bandemer, 1954) and determination of distilled arsine spectrophotometrically (Fisher Scientific Co., 1960). Boron was determined by the curcumin spectrophotometric procedure (Greweling, 1966). Ash and fat were determined by the procedures cited, respectively, in "Official Methods of Analysis" (AOAC, 1975). Protein was determined as Kjeldahl nitrogen × 6.25. Total energy in the rations was determined by oxygen bomb calorimetry (see Table II). Comparison of means (Tables III and V) was accomplished by analysis of variance and calculation of least significant difference as described in Steel and Torrie (1960).

Table III. Data Pertaining to Animal Performance

animal no.'s	ration	av. daily feed consumed[a,b] (dry wt, g)	av. daily wt gain,[a,b] g	av. ration digestibility (in vivo),[a] %
25-28	control	1395 ± 238	176 ± 16	64 ± 2
33-36	1% fly ash	1450 ± 220	208 ± 30	67 ± 2
37-40	2.5% fly ash	1394 ± 161	171 ± 32	64 ± 2
41-44	5% fly ash	1474 ± 188	189 ± 28	61 ± 3
45-48	7.5% fly ash	1417 ± 82	185 ± 25	62 ± 0.0

[a] Mean ± standard deviation for the replicated animals. [b] Average for the entire 124-day feeding period.

Table IV. Elemental Analysis of Fly Ash, Control Ration, and Ration Containing 7.5% Fly Ash

element	element concentration (ppm dry weight) in: fly ash	control ration	7.5% fly ash ration
Al	120500	115	8001
As	195	0.2	13
B	21	10	13
Ba	672	13	49
Br	1	20	19
Ca	15650	5220	5961
Ce	183	0.5	9.7
Co	33	0.4	2.2
Cr	134	0.6	10
Cs	10	0.1	0.9
Dy	23	0.4	1.6
Eu	2.1	0.1	0.4
Fe	64820	186	3489
I	42	2.0	4.0
K	24730	14390	17110
La	61	0.1	3.8
Mg	11650	3078	3290
Mn	175	45	59
Mo	13	1.7	1.3
Na	1809	2493	2250
Rb	166	16	22
Sb	4.8	0.1	0.4
Sc	18	0.01	1.0
Se	6.8	0.1	0.6
Sm	58	0.1	3.4
Sn	3205	10	141
Sr	3845	98	195
Ta	1.4	0.03	0.09
Th	39	0.2	2.2
Ti	4930	11	414
U	5.5	0.1	0.4
V	243	0.6	8.1
Yb	4.4	0.03	0.2
Zn	191	48	60

RESULTS AND DISCUSSION

The magnitudes of feed consumed, animal weight gains, and in vivo ration digestibility are given in Table III. Analysis of variance showed no significant differences ($p > 0.05$) from inclusion of fly ash in the ration on quantity of feed consumed, animal weight gain, or in vivo ration digestibility. No gross or microscopic lesions attributable to toxicity were observed in any of the sheep. It has been reported that ingested soil can cause excessive wear of

Table V. Selenium in Animal Tissues, Body Fluids, Excreta, and Pelleted Rations

sample	Se (ppm dry weight) in sheep fed rations containing: no fly ash (control)	1% fly ash	2.5% fly ash	5% fly ash	7.5% fly ash
blood					
30[a]	1.5 ± 0.2[b]	1.6 ± 0.2	1.9 ± 0.1	1.8 ± 0.03	1.8 ± 0.1
60	1.7 ± 0.2	1.8 ± 0.2	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
90	1.9 ± 0.1	2.0 ± 0.1	2.2 ± 0.2	2.0 ± 0.2	2.1 ± 0.1
120[c]	1.9 ± 0.1	2.0 ± 0.1	2.2 ± 0.2	2.0 ± 0.2	2.1 ± 0.1
average	1.75[a a]	1.85[ac]	2.08[b]	1.93[bc]	1.98[b]
brain	0.7 ± 0.03[a]	0.7 ± 0.1[a]	0.7 ± 0.04[a]	0.7 ± 0.04[a]	0.7 ± 0.1[a]
heart	1.2 ± 0.1[a]	1.2 ± 0.01[a]	1.4 ± 0.04[b]	1.4 ± 0.03[b]	1.4 ± 0.1[b]
kidney	5.9 ± 0.5[a]	5.7 ± 0.3[a]	5.6 ± 0.7[a]	5.7 ± 0.3[a]	5.9 ± 0.4[a]
liver	1.5 ± 0.3[a]	1.6 ± 0.3[ab]	1.9 ± 0.2[bc]	2.1 ± 0.4[c]	2.0 ± 0.3[bc]
muscle	0.4 ± 0.2[a]	0.5 ± 0.3[a]	0.5 ± 0.1[a]	0.5 ± 0.1[a]	0.4 ± 0.03[a]
spleen	1.5 ± 0.1[a]	1.8 ± 0.2[b]	1.9 ± 0.1[bc]	1.9 ± 0.2[bc]	2.0 ± 0.1[c]
thyroid	0.5 ± 0.03[a]	0.7 ± 0.02[ab]	0.9 ± 0.2[bc]	0.8 ± 0.1[bc]	0.9 ± 0.2[c]
adrenal	1.4 ± 0.1[a]	1.6 ± 0.1[b]	1.6 ± 0.04[b]	1.6 ± 0.1[b]	1.6 ± 0.1[b]
lung	1.2 ± 0.1[a]	1.3 ± 0.1[a]	1.5 ± 0.1[a]	1.5 ± 0.1[a]	1.5 ± 0.1[a]
pancreas	1.8 ± 0.1[a]	2.0 ± 0.1[a]	2.0 ± 0.2[a]	1.8 ± 0.1[a]	2.0 ± 0.2[a]
wool	0.2 ± 0.01[a]	0.2 ± 0.1[a]	0.2 ± 0.02[a]	0.3 ± 0.03[a]	0.3 ± 0.03[a]
tibia	0.05 ± 0.01[a]	0.04 ± 0.00[a]	0.05 ± 0.02[a]	0.04 ± 0.00[a]	0.08 ± 0.05[a]
feces	0.4 ± 0.1[a]	0.5 ± 0.03[b]	0.7 ± 0.1[c]	1.0 ± 0.04[d]	1.2 ± 0.1[e]
pelleted ration	0.1	0.2	0.3	0.5	0.6

[a] Days after feeding began. [b] Average ± standard deviation for the four replicated animals. [c] Day of slaughter. [d] Means with dissimilar letter exponents were significantly different ($p < 0.05$).

sheep's teeth (Healy and Ludwig, 1965), but no such effect was noted in teeth of the animals in this study which were fed fly ash.

Table IV lists the results of elemental analysis of the fly ash, the control ration, and the ration containing 7.5% fly ash. Liver and tibia samples from the sheep fed these latter two rations were also analyzed for the elements in Table IV but none of the elements except selenium showed an increase (above the controls) in tissues from the sheep fed the fly ash ration.

Table V lists the average concentrations of selenium in a variety of tissues, feces, and the pelleted rations. Analysis of variance and calculation of least significant differences at the 5% level among treatment means yielded the statistical comparisons shown. The relationship between selenium concentration in blood (BSe) and time (T) of sampling in days was linear and was described by the relationship:

$$BSe = 1.642 + 0.108T$$

There was no interaction between the effect of fly ash in rations and the time of blood sampling on the concentration of selenium in blood.

The concentration of selenium in the fly ash added to the rations in this study was 6.8 ppm (Table IV). Selenium in fly ash is believed to exist in the elemental state (Andren et al., 1975). Selenium would presumably have to undergo preliminary oxidation to more soluble forms prior to assimilation and incorporation into animal tissue protein. The kinetics of this preliminary oxidation in a ruminant perhaps coupled with diffusion limited availability of the selenium in the hollow fly ash microspheres (Fisher et al., 1976) may account for the only slight increase in animal tissue selenium observed in this study. Feeding fly ash containing rations to sheep for periods longer than we did may result in further increases in tissue selenium. Whereas selenium in the control ration (Table IV) in this study would be considered sufficient to meet animal nutritional requirements for the element (Allaway et al., 1967), sheep receiving rations basically deficient in selenium, but then fortified with fly ash, might incorporate the fly ash released selenium more efficiently in their body tissues. Fly ashes which contain higher concentrations of selenium than the approximately average content (Furr et al., 1977) of that used here might also promote greater incorporation of selenium in tissues. Elevating the percentage of fly ash in the animal diet much above 7.5 might be impractical in terms of preparing balanced nutrient rations and animal acceptance.

In conclusion, although no adverse effects were observed by including fly ash in sheep rations, the practice would appear to be of limited value as a means of substantially increasing tissue levels of selenium in deficient animals. Use of fly ash in sheep rations might, however, serve to furnish a continuing maintenance level of selenium for animals which are not deficient in the element.

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Effect of L-Glutamic Acid and Siapton Leaf Organic Fertilizer on Oxidized Nicotinamide Adenine Dinucleotide Dependent Glutamate Dehydrogenase of Different Maize Genotypes

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Leaves of 14-day-old plants of inbred maize lines and their F_1 hybrid were vacuum infiltrated with solutions of L-glutamic acid (1.25×10^{-2} M, pH 6.67), Siapton leaf organic fertilizer (7 g L^{-1}, pH 6.27), and potassium sodium phosphate buffer (1.25×10^{-2} M, pH 6.58) as control. The molecular heterogeneity of nicotinamide adenine dinulcleotide–glutamate dehydrogenase (NAD^+–GDH) was determined by polyacrylamide gel electrophoresis. Differences were established between the investigated genotypes concerning changes in the isoenzyme spectra of NAD^+–GDH, caused by exogenous L-glutamic acid. It was established that the enzyme activity increases under the action of this acid in all three genotypes, an increase which is not due to increased H^+ ion concentration. The effect of L-glutamic acid on the deaminating activity of GDH in leaves of 14-day-old maize plants proved to be nonspecific. The Siapton leaf organic fertilizer, whose biologically active part is a mixture of free amino acids obtained after protein hydrolysis, has a similar effect.

The possibilities of regulating the action of glutamate dehydrogenase under the influence of different metabolites and physical agents have been investigated in animal tissues, microorganisms, bacteria (Frieden, 1963; Stadtman, 1966), and also more recently in higher plants (Kretovich et al., 1970, 1971, 1972; Barash et al. 1973; Bayley et al., 1972; Hartmann, 1973; King and Yung-Fan Wu, 1971; Pahlich, 1971, 1972; Pahlich and Hoffman, 1975; Sahulka et al., 1975; Sahulka and Gaudinova, 1976). The effects of adenylate metabolites, as well as some inorganic ions, growth regulators, herbicides, and amino acids, have been studied.

The effect of NH_4^+ ions and L-glutamic acid on changes in the isoenzyme spectra of nicotinamide adenine dinucleotide–glutamate dehydrogenase (NAD^+–GDH), extracted from roots of 6- and 14-day-old plants of maize inbreds and their F_1 hybrid, were studied in a previous work (Mladenova, 1977). Here we submit data on the effects of exogenously supplied L-glutamic acid and Siapton leaf organic fertilizer (a polypeptic amino acidic mixture, obtained after partial protein hydrolysis) on isoenzyme spectra of NAD^+–GDH extracted from leaves of 14-day-old plants of different maize genotypes. We assume that investigation of the effect of this fertilizer on the metabolism of different maize genotypes is expedient as our previous work (Mladenova and Dankov, 1976) showed that the effect of Siapton on the yield depended not only on the type of the culture but also on the genotype within the scope of a given culture.

MATERIAL AND METHODS

Fourteen-day-old plants hydroponically grown on Hoagland-Arnon I nutritive solution of the W-32 and W-187 inbred lines and of the F_1 hybrid W-32 × W-187 were investigated. They were cultivated in a growth chamber at 23 °C, 65–70% humidity, and 10 000-lx light intensity with 14-h "day" and 10-h "night". A solution of glutamic acid (1.25×10^{-2} M), potassium–sodium phosphate buffer with the same molarity (as control) and a Siapton solution (7 g L^{-1}) were infiltrated in 1 g of leaf mass under vacuum (10^{-2} Torr). The vacuum infiltration procedure has been described elsewhere (Mladenova, 1975). Solution pH was determined with a CP2 pH meter with glass and calomel electrodes. The enzyme was extracted (4 °C) by homogenizing 1 g of fresh material with 3 mL of 10^{-2} phosphate buffer (pH 7.4) containing 0.5 M sucrose and 6 mM ascorbate. The homogenization was performed as follows: the sample was put in a glass mortar, and then 1 mL from the above buffer and 0.5 g of ion-exchange resin Dowex 1 × 8 (200–400 mesh), equilibrated to pH 7.4 with the same buffer, were added. Next, the material was ground (3 min) and the remaining buffer (2 mL) was added, followed by final and full homogenization (2 min). After storing the samples for 1 h at 4 °C, followed by centrifugation (Janetzki K-24 centrifuge) with cooling (50 min, 18 000g), the clear supernatant was used to determine the protein content (Lowry, 1951) and for electrophoresis of 400 μg of protein. The isoenzymes were separated by means of disc electrophoresis in 7.5% polyacrylamide gel, pH 8.9 (Davis, 1964). The isoenzyme

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