food being tested. The amount of exchange of extrinsic tracer iron with endogenous intestinal iron and intrinsic food iron will not necessarily be the same. Thus, a distinction must be made between the *amount of iron absorbed* by an individual of given iron status from a food and the *bioavailability* of the iron from the food, which can only be measured in an iron-deficient individual. This distinction has not always been fully and carefully made in the past. The data in Table III indicate clearly the need for such a distinction.

While some interesting differences in iron absorption were found among the various supplements in iron-replete animals (Table III), they are probably of little significance to the consumer. For a person with adequate iron stores the variations among brands would be unimportant; for an iron-deficient individual the differences among brands would probably not exist.

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Elemental Content of Tissues and Excreta of Lambs, Goats, and Kids Fed White Sweet Clover Growing on Fly Ash

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White sweet clover found voluntarily growing on a deep bed of soft coal fly ash was found to contain high concentrations of a number of elements including selenium, bromine, molybdenum, rubidium, strontium, and others. The clover was harvested and fed as 23.5% of a dry pelleted ration to lambs and pregnant goats for up to 173 days. High concentrations of selenium were found in 11 tissues, blood, goats' milk, and excreta of lambs, goats, and newborn kids. Molybdenum in liver, strontium in bone, and bromine and rubidium in animal tissues were also elevated over those in the corresponding tissues of animals fed an identical ration containing control clover grown on soil. No gross or histologic lesions were present in any of the animals.

Fly ash is trapped in electrostatic precipitators of soft coal-burning electric power-generating plants. It has been estimated that up to 36 million tons of the material will be produced in the United States by 1980 (Brackett, 1970). This estimate may be conservative owing to the recent renewed interest in coal as an energy source. Whereas a small percentage of the fly ash produced is used as a road base material or in concrete products, the bulk of it is disposed of in landfills.

Some studies have been made of the use of fly ash as an alkaline amendment to reclaim coal mine spoils (Adams et al., 1972). It has also been incorporated into soil to correct plant deficiencies of boron, phosphorus, zinc, potassium and molybdenum (Martens, 1971; Martens et al., 1970). Fly ashes may also contain toxic elements which are available to growing plants. Vegetables and millet cultured on potted soil containing 10% by weight of fly ash showed higher concentrations of a number of elements including boron, molybdenum, and selenium (Furr et al., 1976a). The extent of absorption of selenium was roughly proportional to the rate of application of fly ash. An analytical survey of 45 elements in fly ashes from 21 states was conducted (Furr et al., 1977). Cabbage grown on soil containing 7% by weight of 16 of these fly ashes absorbed arsenic, boron, molybdenum, selenium, and strontium to an extent that showed a high degree of correlation with the total content of the elements in the respective fly ashes (Furr et al., 1977).

Toxicologically, the use of fly ash in agriculture requires a knowledge of the magnitude of transfer of toxic elements in it to plants and finally to farm animals consuming these plants. Yellow sweet clover (*Melilotus officinalis*) found

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Table I. Composition of the Complete Pelleted Animal Rations

Constituent	% dry wt
White sweet clover	23.5
Alfalfa meal	32.5
Oats (crimped)	14.5
Corn (cracked)	14.5
Wheat bran	3.6
Soybean meal	3.6
Molasses	7.3
Salt (without minerals)	0.45
Vitamin supplement (A, D, and E)	0.05

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

Constituent	Percent (dry weight) in ration containing: Control clover	Fly ash grown clover
Ash	8.6	8.4
Crude fat	2.7	2.9
Crude protein	20.0	19.2
Total energy (cal/g)	4442	4435

growing on a deep bed of fly ash was found to contain 5.3 ppm of selenium. When the crop was fed to guinea pigs for 90 days, the animal tissues contained markedly higher levels of the element than those of control animals on a soil-grown clover ration (Furr et al., 1975). In the work reported, sheep and pregnant goats were fed for up to 173 days on a diet containing sweet clover grown on fly ash. The feeding trial was followed by multielement analysis of tissues, body fluids, and excreta as well as gross and histologic examination of organs.

EXPERIMENTAL SECTION

Gaudey Station, a soft coal-burning electric power-generating plant, is located near Johnson City, N.Y. Fly ash produced there is disposed of in nearly landfill areas. One such site in Endwell, N.Y., contained fly ash (pH 7.1) 15 to 22 m in depth and covered about 0.4 ha. In June, 1975, a thick stand of exclusively white sweet clover (*Melilotus alba*) approximately 1.2 m in height, was found voluntarily growing on the fly ash surface. Subsamples of the clover were taken and selenium concentrations up to 205 ppm (dry weight) were found. The entire field of clover (2 tons) was harvested, dried on slotted wagons using forced heated air (50 °C), milled to a fine powder, and thoroughly mixed. The mixed plant material was incorporated (23.5% dry weight) into the diet shown in Table I and pelleted (0.4-cm pellets). White sweet clover growing on Erie Channery silt loam soil (pH 6.2) near Ithaca, N.Y., was similarly harvested and processed to serve as the control. The rations were thoroughly mixed at the time of pelleting. Incorporation of 23.5% of this clover, which contained 66 ppm of selenium, yielded a pellet containing 16 ppm by analysis. The ash, fat, and protein contents of the diet are given in Table II. Subsamples of soil and fly ash to a depth of 8 in. were also collected from 20 stations throughout the control and fly ash plots, respectively, and combined and thoroughly mixed prior to analysis.

Four, 3-month-old Dorset wethers and four recently bred 1-year-old French Alpine goats were used in the feeding trial. Two lambs and two goats were fed the ration containing white sweet clover grown on fly ash and the remaining animals were fed the control diet. The animals were located in individual metabolic stalls throughout the feeding period. All animals were first adapted from a ration of hay and grain to a diet of hay and commercial pellets (containing no clover) over a period of 14 days. They were then fed the clover-containing pellets for 173 days. Salt (without iodine or trace minerals) and water were 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 each of the total mixed urine and mixed feces produced during the entire 9-day digestion trial was taken from each adult animal for analysis.

Table III. Data Pertaining to Animal Performance

Animal no.	Ration	Initial[a] animal weight, kg	Date feeding ended	Data on kids produced[b]	Average daily feed consumed,[c] g dry wt	Sheep average daily weight gain,[c] g	Goat average daily milk production,[c,d] g	Average ration digestibility in vivo,[c] %
				Sheep				
32	Control	16.7	5-30-76		1568 ± 235[e]	253 ± 47[e]		64 ± 2[e]
33	Control	20.7	5-30-76					
36	Fly ash grown clover	18.3	5-28-76		1333 ± 23[e]	205 ± 19[e]		65 ± 1[e]
37	Fly ash grown clover	20.7	5-28-76					
				Goats				
34	Control	30.6	5-21-76	One 3.3-kg female and one 3.2-kg female born on 3-28-76	1379 ± 80[e]		1708 ± 274[e]	65 ± 1[e]
35	Control	57.0	5-21-76	One 2.8-kg male and one 3.0 kg female born on 3-20-76[f]				
38	Fly ash grown clover	34.8	5-21-76	One 4.5-kg male born on 3-7-76	1612 ± 242[e]		2503 ± 815[e]	65 ± 1[e]
39	Fly ash grown clover	44.1	5-21-76	One 4.5-kg male and one 4.8-kg female born on 3-7-76				

[a] Weight of animal on date feeding began (Dec 8, 1975). [b] Gestation period for goats is 151 ± 3 days. [c] Mean ± standard deviation. [d] Milk produced from date of kidding to date feeding ended. [e] None of the paired means were significantly different ($P > 0.05$). [f] Kids were stillborn.

At the end of the feeding period the animals were killed and necropsied and tissue samples were taken for element analysis and histologic examination. The kids were killed and necropsied at birth. Liver, kidney, lung, thymus, lymph nodes, bone marrow, heart, muscle, tongue, adrenal, thyroid, pancreas, rumen, abomasum, small intestine, skin, and brain were taken for histopathological examination. 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 soil, fly ash, plant and animal tissues, body fluids, and excreta were analyzed for 42 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 39 elements using the procedure described previously (Furr et. al., 1976b). 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. Arsenic analysis was performed by dry ashing, distillation of arsine and determination using the silver diethyldithiocarbamate spectrophotometric procedure (Evans and Bandemer, 1954; Fisher Scientific Co., 1960). Boron was determined by the curcumin spectrophotometric procedure (Greweling, 1966). The measurement of pH was performed by the procedure of Peech et al. (1953). Ash and fat were determined in the rations by the procedures cited, respectively, in Official Methods of Analysis (1975). Protein was determined as Kjeldahl nitrogen × 6.25. Total energy in the rations was determined by oxygen bomb calorimetry. Comparison of means (Table III) was accomplished by Student's "t" test as described in Steel and Torrie (1960).

RESULTS AND DISCUSSION

Table III summarizes data on animal feed intake, weight gains, milk production, kidding, and ration digestibility. No significant differences ($P > 0.05$) were found between treatments as regards daily feed consumption, weight gains, milk production or ration digestibility. No significant gross or histologic lesions attributable to toxicity were observed in any of the adult or newborn animals.

The results of elemental analysis of soil, fly ash, and sweet clovers grown thereon are listed in Table IV. Every element was higher in the fly ash than the soil except Br, Hf, Hg, In, K, Mn, and Na. The elements Al, As, B, Br, Cl, Co, Cu, Fe, K, Na, Mo, Rb, Se, Sr, and V were notably higher in concentration in the sweet clover grown on fly ash as compared to the control crop. Interestingly, the rarer elements such as Ce, Eu, Hf, In, La, Lu, Sm, Ta, Th, W, and Yb were also higher in the fly ash grown crop. Several of these rarer elements (Ce, Hf, La, Sm, Th, W, and Yb) were found consistently at appreciable concentrations in fly ashes surveyed from 21 states (Furr et al., 1977). Many of these rarer elements are reported as present in coal (Bowen, 1966). A given power plant may buy coal from many different sources as determined by price and sulfur and ash content. A given fly ash landfill site thus usually represents coals from different geographic locations and may expectedly be quite variable in composition. As pointed out by Bowen (1966), a large number of elements are accumulated by coal but not by every coal seam. Many rare elements tend to concentrate at the boundaries of coal seams but the reasons for this are not clear (Bethell, 1962). Fly ash composition is also affected by conditions during coal combustion with some elements volatilizing through the stack while others deposit on the fly ash trapped above or remain in the bottom ash (Klein et al., 1975). Furthermore the efficiency of a particular electrostatic precipitator may affect the proportion of finer particles trapped which have a greater surface area and may have a higher concentration of deposited elements (Davison et al., 1974).

Table IV. Elemental Analysis of Soil, Fly Ash, and White Sweet Clovers Grown Thereon

Element	Element concentration, ppm dry weight in: Soil	Fly ash	Soil-grown clover	Fly ash grown clover
Al	55910	428550	139	237
As	13	356	0	1.4
Au	0.0	0.03	0.003	
B	5.2	7.5	45	51
Ba	427	913	39	28
Br	5.8	2.9	7.9	29
Ca	6349	12960	12000	13030
Cd	2.1	6.1	0.2	0.2
Ce	84	218	0.8	1.0
Cl	388	721	810	1380
Co	9.2	52	0.5	1.0
Cr	51	160	2.3	0.4
Cs	4.0	7.1	0.6	0.5
Cu	32	281	8.4	15
Eu	0.0	0.2	0.06	0.2
Fe	28210	108100	386	522
Hf	8.7	5.0	0.1	0.3
Hg	0.0	0.0	0.01	0.01
I	20	37	0.4	0.5
In	0.8	0.0	0.003	0.02
K	17810	14410	10600	16400
La	27	72	0.15	0.2
Lu	0.5	1.2	0.02	0.03
Mg	7304	12650	2200	1490
Mn	991	264	20	15
Mo	2.0	6.3	1.4	38
Na	5268	1297	31	44
Rb	120	135	16	50
Sb	1.0	10	0.2	0.2
Sc	6.4	22	0.05	0.05
Se	3.2	16	0.02	66
Sm	25	74	0.05	0.09
Sn	314	982	9	11
Sr	65	668	21	157
Ta	0.8	1.4	0.03	0.29
Th	16	42	0.01	0.8
Ti	3884	7044	40	32
U	2.9	7.9	0.03	0.01
V	100	195	0.3	1.4
W	1.3	6.4	0.3	2.5
Yb	2.7	6.2	0.2	0.3
Zn	101	169	52	45

Table V summarizes the average concentrations of selenium found in the tissues, fluids, and excreta of the replicated animals fed either of the two dietary treatments. Selenium concentrations are notably high in the animals fed the fly ash-grown clover as compared to the control clover ration. With goats fed a given ration, there were no obvious differences observed in the concentrations of selenium in the organs of the kids as a function of sex or whether born alive or stillborn.

The absorption, deposition, and excretion of various forms of selenium in animals has been reviewed by Underwood (1971) and the findings of others agree with much of the data on selenium presented in Table V. According to Underwood (1971), selenium locates in all body tissues including hair as determined by the nature of the tissue and the concentration of selenium in the diet. Kidney, liver, and pancreas are typically high in selenium with lower amounts occurring in muscle, bone, and blood. Cardiac muscle is usually higher than skeletal muscle. It has been shown that selenium retention in lambs is greater

Table V. Selenium in Animal Tissues, Fluids, and Excreta

Tissue, etc.	Selenium (ppm, dry wt)[a] in: Control sheep	FAC[b] sheep	Control goats	FAC goats	Control kids[c]	FAC kids[c]
Adrenal	1.3 ± 0.2	10.6 ± 0.1	1.4 ± 0.01	9.2 ± 1.1	1.2 ± 0.1	7.3 ± 0.4
Bone	0.04 ± 0.0	2.6 ± 0.0	0.02 ± 0.0	0.5 ± 0.2	0.1 ± 0.1	2.3 ± 0.1
Brain	0.6 ± 0.04	7.2 ± 0.6	0.7 ± 0.04	7.0 ± 0.2	0.8 ± 0.1	7.1 ± 0.7
Hair (or wool)	0.2 ± 0.01	4.5 ± 0.1	0.3 ± 0.06	6.3 ± 1.7	0.6 ± 0.2	10.3 ± 2.3
Heart	1.0 ± 0.1	16.9 ± 0.2	0.9 ± 0.2	13.1 ± 0.1	0.7 ± 0.1	8.1 ± 1.4
Kidney	5.7 ± 0.1	34.9 ± 2.8	5.5 ± 0.6	19.2 ± 3.3	2.4 ± 0.3	11.5 ± 0.4
Liver	1.4 ± 0.1	28.2 ± 0.5	1.2 ± 0.1	17.1 ± 0.7	1.6 ± 0.7	13.8 ± 0.7
Muscle	0.3 ± 0.0	11.4 ± 1.8	0.3 ± 0.04	9.9 ± 0.5	0.4 ± 0.1	11.2 ± 0.7
Pancreas	1.6 ± 0.1	11.2 ± 3.5	1.3 ± 0.04	12.9 ± 0.3	1.5 ± 0.2	8.4 ± 0.2
Spleen	1.3 ± 0.1	13.1 ± 0.9	1.2 ± 0.07	8.4 ± 1.7	1.0 ± 0.2	7.9 ± 0.1
Thyroid	0.6 ± 0.0	7.7 ± 0.7	0.5 ± 0.0	8.6 ± 0.3	0.7 ± 0.02	7.4 ± 0.3
Blood	1.3 ± 0.1	14.5 ± 0.6	1.2 ± 0.2	10.9 ± 0.6	0.9 ± 0.3	7.0 ± 0.6
Milk[d]			0.1 ± 0.1	6.0 ± 0.6		
Urine	0.3 ± 0.04	59.3 ± 4.6	0.3 ± 0.2	62.5 ± 5.7		
Feces	0.5 ± 0.3	21.8 ± 2.1	0.4 ± 0.01	18.9 ± 4.1		

[a] Mean ± standard deviation. [b] Diet containing fly ash grown white sweet clover. [c] See Table III for number and sexes of of kids. [d] Based on analysis of 16 to 21 daily milk samples from each goat.

Table VI. Other Elements Whose Concentrations Were Elevated (Above Controls) Both in Fly Ash Grown Clover and Tissues of the Respectively Fed Animals

Animal	Ration	Tissue	Parts per million (dry weight)[a] Br	Mo	Rb	Sr
Sheep	Control	Liver	7.3 ± 0.8	2.2 ± 0.6		
Sheep	Fly ash	Liver	12 ± 0.8	6.2 ± 0.3		
Sheep	Control	Bone				109 ± 23
Sheep	Fly ash	Bone				351 ± 21
Goat	Control	Liver	7.9 ± 0.7	3.8 ± 0.4	35 ± 0.0	
Goat	Fly ash	Liver	10 ± 1.3	5.7 ± 0.1	41 ± 0.0	
Goat	Control	Bone				14 ± 4.2
Goat	Fly ash	Bone				124 ± 69
Kid	Control	Bone	10 ± 0.0		3.3 ± 1.8	
Kid	Fly ash	Bone	81 ± 28		6.2 ± 1.4	

[a] Average ± standard deviation.

for selenomethionine (a major form of selenium along with lesser amounts of selenocystine found in plants grown on seleniferous soils) than for sodium selenite (Ehlig et al., 1967). Jacobson and Oksanen (1966) reported that when ewes are injected with selenomethionine or selenocystine, the selenium concentration in the lambs is higher than when selenite is injected and is nearly as high as in the mother. Selenium is known to be excreted in milk and probably occurs largely in the protein fraction (Underwood, 1971). Selenium is excreted largely in the urine and feces of ruminants. In urine, selenium has been shown to be present as trimethylselenonium ion (Palmer et al., 1969).

Whereas other animals show toxic symptoms at lower dietary levels of selenium, the ruminants in this study showed no toxic symptoms at concentrations of about 16 ppm of selenium in their rations. This agrees with the data of others showing sheep to be particularly tolerant of high levels of selenium in their diets (Underwood, 1971). High protein diets appear to offer protection against selenium toxicity (Underwood, 1971). Arsenic in their drinking water at 5 ppm has been shown to prevent the toxicity of selenium in rats (Moxon, 1938). The arsenic content of the fly ash grown clover was 1.4 ppm while no arsenic was detectable in the control clover (see Table IV). Selenium is an essential element for animals. Glutathione peroxidase isolated from bovine erythrocytes has been shown to contain four selenium atoms/mole (Hockstra, 1975). The mechanism of selenium toxicity is believed to involve its substitution for sulfur in sulfhydryl groups which are essential to oxidative cell processes (Underwood, 1971).

Table VI lists elements which were found higher in concentration both in the fly ash grown clover and in specific tissues of the respectively fed animals. Molybdenum is known to concentrate in liver and strontium in bone while rubidium and bromide tends to distribute in all tissues (Browning, 1969; Bowen, 1966). Bromine, rubidium, and strontium are toxicologically quite innocuous. Molybdenum in sufficiently high concentrations can induce a copper deficiency in animals (Browning, 1969).

This study indicates that crops growing on soft coal fly ash may accumulate a number of elements with selenium of particular concern. Although the clover crop was fed to ruminants and caused no apparent toxicity, it is doubtful that feeding such a high percentage of high selenium clover could be recommended for practice. Fly ashes and thus the crops grown thereon may vary widely in elemental composition. Many interactions among such elements in the animal body could cause antagonistic as well as synergistic reactions as regards toxicity. Toxic symptoms might have developed in this study had the time of feeding been lengthened. Other animals species might be more susceptible to the development of toxic reactions than were the ruminants studied here. Most importantly, the concentrations of selenium in the milk and meat of such animals might be far higher than what would be considered safe for human consumption. Indeed, a concentration of 5 ppm of selenium in common foods or 0.5 ppm in water or milk is considered potentially dangerous (Rosenfield and Beath, 1964). A more feasible recommendation is the possible use of fly ash in controlled amounts as a soil amendment to increase the selenium content of plants in areas where selenium deficiency is known to occur (Furr et al., 1976a).

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Amino Acid Analysis and Acrylamide Gel Electrophoresis Patterns of Bovine Hemopoietic Marrow

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Amino acid analysis and SDS polyacrylamide gels were used to characterize the proteins present in hemopoietic marrow. Amino acid composition of hemopoietic marrow remained relatively constant with changes in age of animal and proximate composition of the marrow. Major protein bands in hemopoietic marrow included albumin, a protein band at 20 000 daltons, and hemoglobin. Actin percentages in muscle and hemopoietic marrow mixtures increased in a linear manner as muscle content of the mixture increased. Since mechanically deboned meat (MDM) is a mixture of muscle and hemopoietic marrow, it is possible to determine the actin percentage in MDM and, from the actin percentage, estimate the amount of muscle or marrow present.

Marrow is the largest organ in the body, comprising 3.0 to 5.9% of the body weight (Reich, 1946; Winthrobe, 1974; Woodward and Holodny, 1960). At birth all bones contain red marrow (Custer, 1933) but in mature animals red marrow is found only in the proximal epiphysis of the long bones such as the femur and humerus and in flat bones such as the sternum, ribs, vertebrae, and pelvis. The metamorphosis of red to yellow marrow in long bones is a function of increasing age.

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Although the consumption of marrow by humans is well documented (Scrimshaw and Young, 1976; Souron, 1975; American National Cow Belles, Inc., 1973), the literature is devoid of data on marrow proteins. Characterization of marrow protein has become more important with the advent of mechanically deboned meat (MDM). The process of mechanical deboning removes much of the hemopoietic marrow from the interspaces of spongy bones at the same time the meat clinging to the outside of the bones is removed. Chang and Field (1977) believe that much of the variation in the protein quality of MDM is a result of variable amounts of marrow present.

This research was undertaken to characterize the protein

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