The decline in milk production in cattle with advancing lactation can be influenced by many factors. However, it is clear that one important factor is the level of acetyl-CoA synthetase and acetyl-CoA hydrolase, which in turn affects the level of acetyl-CoA. The data suggest that the activity of the enzymes is under the control of a complex system of hormones which are also intimately involved in the initiation and maintenance of lactation. Prolactin, growth hormone, thyroid hormone, and glucocorticoids are generally accepted as being of major significance in this respect.

We propose that as lactation advances and acetyl-CoA synthetase activity declines, there is a fall of acetyl-CoA levels in the cell in both the mitochondrial and extramitochondrial pools. As a consequence more glucose is oxidized via glycolysis and the citric acid cycle and so less is available for lactose synthesis. When lactose synthesis is inhibited, milk secretion declines (Rook and Hopwood, 1970). In this way acetyl-CoA synthetase plays a major role in the control of milk production.

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Mineral Composition of Liver and Kidney of Rats Fed Corn, Sorghum, and Soybean Grain Grown with Sewage Sludges and NPK Fertilizers

Josephine Miller* and Fred C. Boswell

Weanling rats were fed grains of corn, sorghum, and soybeans that were grown on soils treated with sewage sludge from an industrial area known to be high in heavy metals. The diets contained 80% grain and adequate quantities of essential nutrients. Grains and tissues were analyzed for P, K, Mg, Mn, Fe, Zn, Cu, Cr, Cd, Ni, and Pb. Of these, differences in tissue composition associated with type of soil treatment were found only for Mn, Cu, and Fe and even for these elements all values were within normal ranges. Measurable quantities of Cd, Ni, and Pb were not present in either grain or tissue samples. Cr was found in trace quantities in the grains but not in the animal tissues.

The disposal of sewage sludge is of great concern to municipalities because of limited areas available for disposal and current governmental regulations relative to incineration and/or discharge into surface waters. An alternative disposal operation, which is currently being considered, is recycling via land application. Possible problems that may arise from use of sewage sludge as a soil additive are that substances, such as heavy metals, present in the sludge may enter the food chain and become a hazard to animals consuming the plants grown on sludge treated soils (Somers, 1974). Numerous reports in the literature attest to the uptake of heavy metals by plants grown on soils treated with sludge (e.g., Kirkham, 1975) but few of these explore the eventual effects on animals consuming such plants. After a 3-year study of effects of

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Table I. Composition of Grains

	%		g/kg		mg/kg					
	N[a]	Tannin	P	K	Mg	Mn	Fe	Zn	Cu	Cr
					Corn Grain					
LSS	1.27	T[b]	1.9	1.9	760	2.5	8	18.8	T	T
HSS	1.29	T	2.2	2.2	830	2.2	19	23.4	T	T
NPK	1.54	T	2.6	2.0	880	2.8	10	19.5	T	T
					Sorghum Grain					
LSS	1.28	3.71	2.5	2.5	1150	15.0	41	22.1	2.0	T
HSS	1.32	3.81	2.4	2.4	1090	13.2	34	20.4	0.8	T
NPK	1.90	3.12	2.8	2.1	1250	9.3	33	14.2	0.2	T
					Soybean Grain					
LSS	6.45	T	5.3	17.3	2010	36.0	140	63.2	12.8	T
HSS	6.35	T	5.6	17.0	2060	33.0	73	68.6	12.8	T
NPK	6.31	T	5.7	17.0	2060	26.0	105	49.0	15.0	T

[a] Kjeldahl nitrogen. [b] Trace.

spray irrigation of chlorinated sewage effluent in wildlife areas, Wood et al. (1975) concluded that, if concentrations of Cd, Cr, Cu, Ni, Pb, and Zn were increasing in the bodies of small herbivorous mammals feeding there, the changes were quite small. In order to further evaluate potential hazards under controlled feeding conditions, rats were fed diets containing corn, sorghum, or soybean grain from plants grown on soils treated with sewage sludge, from an industrial area, known to be high in heavy metals. The diets contained about 80% grain and were supplemented with adequate amounts of essential nutrients. Feeding trials were accomplished during the first few weeks after weaning the animals when rate of growth is most rapid and the animals are under the greatest nutritional stress. This protocol offered the possibility of detecting acute or sub-acute effects of the treatments before the animals could adapt to the dietary conditions.

MATERIALS AND METHODS

Corn (*Zea mays* L.) hybrid Pioneer 3009, grain sorghum [*Sorghum bicolor* (L.) Moench] hybrid McNair 546, and soybeans [*Glycine max* (L.) merr.] variety Hutton were grown to maturity on Piedmont soils (Davidson cl and Cecil scl). The corn treatments were NPK (168, 25, and 93 kg/ha, respectively), 2.8 mtons/ha sewage sludge (low sewage sludge, LSS) and 5.6 mtons/ha (high sewage sludge, HSS) per year which had been applied each of 3 years (a total for 1972–1974 of 8.4 and 16.8 mtons/ha for the LSS and HSS, respectively). The sorghum received NPK (125, 25, and 93 kg/ha, respectively), 5.6 mtons/ha sewage sludge (LSS), and 11.2 mtons/ha sewage sludge (HSS) for the 1974 growing season only. The soybeans received treatments only for the 1974 growing season and they were the same as for the sorghum except no N was applied. The sewage sludge source was from an industrialized metropolitan area (Boswell, 1975), and typically contained, in ppm: P, 8200; K, 1200; Mg, 1200; Mn, 890; Zn, 11 800; Cu, 640.

Soybeans were autoclaved at 15 lb pressure (121 °C) for 30 min and oven dried overnight at 60 °C. The corn, sorghum, and autoclaved soybeans were ground in a hammer mill to a particle size no greater than 1.5 mm. Composition of the grains is shown in Table I and ingredients of the diets are listed in Table II. Casein supplemented with methionine provided the animals' protein requirement. A complete vitamin mixture was added to all diets and corn oil was added to diets containing corn and sorghum to provide essential fatty acids and to increase the caloric density of the diets. Inorganic elements were added to the diets on the basis of preliminary analyses of the crop grains in such quantity that the total diet would provide the rat's requirement for these.

Table II. Formulation of Diets

	g/kg			
	Corn	Sorghum	Soybeans	Control
Crop	775	776	833	
Casein	143	143	143	143
1-Methionine	2	2	2	2
Vitamin mix[a]	10	10	10	10
Corn oil	50	50		50
Cellulose				15
Dextrin				400
Sucrose				333
$CaHPO_4$	13.75	11.00		11.34
$CaCO_3$	4.38	6.25	10.00	7.28
Na_2HPO_4				6.54
KCl	1.00			7.33
NaCl	1.50	1.50	1.50	
$MgSO_4$				2.31
	mg/kg			
$MnSO_4 \cdot H_2O$	150.00	150.00	112.50	154.60
$FeSO_4 \cdot 7H_2O$	150.00	60.00		87.50
$ZnCO_3$				25.50
$CuSO_4$	15.75	11.75		20.20
$KClO_3$	0.30	0.30	0.30	0.70

[a] Provided per kg of diet: 20 000 IU of vitamin A, 2000 IU of vitamin D, 110 mg of α-tocopherol, 990 mg of ascorbic acid, 110 mg of inositol, 1650 mg of choline chloride, 49.5 mg of menadione, 110 mg of *p*-aminobenzoic acid, 99 mg of niacin, 22 mg of riboflavin, 22 mg of pyridoxine, 22 mg of thiamine, 66 mg of calcium panthothenate, 2 mg of folic acid, 440 μg of biotin, 30 μg of vitamin B_{12}.

In addition to the test diets, a typical diet containing a mixture of salt to provide all required inorganic elements was given to one group of animals for comparison.

Weanling Sprague-Dawley male rats obtained from Charles River Breeding Laboratories (Wilmington, Mass.) were placed on the diets at 4 weeks of age. The animals were housed individually, 10 rats per treatment, and provided with food and deionized water ad libitum for 3 weeks.

After the feeding trial the animals were weighed and anesthetized with an intraperitoneal injection of pentobarbital sodium and exsanguinated by heart puncture. The liver and kidneys were excised, rinsed with deionized water, blotted dry, weighed, and frozen until analyzed. Animal tissues, aliquants of the diets, and appropriate standard were wet ashed with sulfuric, nitric, and perchloric acids. Inorganic elements were determined by atomic absorption spectroscopy, utilizing a Deuterium Background Corrector. Percent nitrogen by Kjeldahl (AOAC, 1955) and tannin (Burns, 1973) were determined on grains utilized in the diets.

Table III. Mineral Content (per g Fresh Weight) of Livers and Kidneys of Rats Fed Crops Fertilized with Sewage Sludge or Inorganic Fertilizer

	P, mg	K, mg	Mg, μg	Mn, μg	Fe, μg	Zn, μg	Cu, μg
				Corn Grain			
				Liver			
LSS[a]	3.34	3.81	233	2.44	82.0	27.1	3.46
HSS	3.59	3.78	229	2.22	63.3	28.8	3.81
NPK	3.33	3.93	239	2.40	71.7	27.8	3.75
P[b]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
				Kidney			
LSS	2.89	0.59	241	0.69	47.6	25.9	4.70
HSS	2.88	0.61	230	0.79	45.3	23.2	4.30
NPK	3.01	0.59	228	0.70	48.9	23.0	4.78
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
				Sorghum Grain			
				Liver			
LSS	3.42	3.60	226	2.57[b]	52.1	27.2	4.31
HSS	3.51	3.61	235	2.52[b]	53.7	26.5	4.00
NPK	3.42	3.59	227	3.15[a]	56.0	27.7	3.61
	n.s.	n.s.	n.s.	0.01	n.s.	n.s.	n.s.
				Kidney			
LSS	2.93	0.59	227	1.02[a]	42.5[b]	19.6	4.52
HSS	3.02	0.60	239	0.87[a]	45.8[ab]	21.1	4.79
NPK	3.06	0.60	241	0.65[b]	51.9[a]	17.6	4.75
	n.s.	n.s.	n.s.	0.01	0.01	n.s.	n.s.
				Soybean Grain			
				Liver			
LSS	3.42	3.66	235	3.27	81.8	30.1	4.23[b]
HSS	3.41	3.62	239	3.17	77.2	31.8	4.02[b]
NPK	3.79	3.70	247	3.04	82.7	31.1	4.79[a]
P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.01
				Kidney			
LSS	2.96	0.61	228	0.93	43.7	26.3	5.92
HSS	3.01	0.63	230	0.94	41.7	27.5	5.82
NPK	2.91	0.61	233	0.76	46.6	26.3	6.43
P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
				Standard Diet[c]			
				Liver			
Mean	3.17	3.91	231	2.13	66.6	27.1	4.30
SD[d]	0.40	0.17	17	0.30	7.0	2.1	0.57
				Kidney			
Mean	3.01	0.64	234	1.17	41.7	26.4	5.19
SD[d]	0.09	0.03	7	0.24	4.2	2.2	0.75

[a] LSS, low sewage sludge; HSS, high sewage sludge; NPK, inorganic fertilizer. [b] Probability that differences due to fertilizer treatment are significant (n.s., not significant). Where applicable, values in a column not having a common superscript are different according to Duncan's multiple range test. [c] Standard diet providing minerals at levels recommended for normal rat growth. [d] Standard deviation of mean for ten animals.

The data obtained were subjected to analyses of variance (Snedecor, 1946) to compare results of the three treatments (LSS, HSS, and NPK fertilizer) for each crop. Where appropriate, Duncan's multiple range test (Duncan, 1955) was applied to the means.

RESULTS AND DISCUSSION

Nitrogen content of the corn and sorghum grain was increased by the larger application of nitrogen in the NPK treatments than in the sewage sludge treatments (Table I). Otherwise, the fertilizer treatments had little effect on content of the major minerals, N, P, K, and Mg, in the crops. There were differences in minor element content of the crops associated with fertilizer treatments but these were not necessarily in proportion to the amount of mineral applied to the crops. Boswell (1975) has postulated that chelation of the minor elements in the sludge may affect their uptake by plants.

Albino rats fed the diets containing large proportions of these crops adapted well to the feeding regimes and showed no overt signs of digestive disturbances, nutritional inadequacies, or toxicities. Of the 11 minerals measured in liver and kidney tissues of animals fed the three crops, there were only four cases of statistically significant differences in the tissue mineral content associated with fertilizer treatment of the crop (Table III). The elements involved were Mn, Fe, and Cu. In tissues of rats fed diets containing corn grain, there were no differences in mineral composition related to the imposed crop treatments.

Copper content of liver tissue was higher in animals fed soybean grain from the plots treated with NPK fertilizer than in rats fed this legume grain from plots to which sewage sludge had been applied. This difference in copper content of the animal tissue was paralleled by a similar difference in copper content of the soybean grain. However, the mean values for liver copper content of all three groups of animals fed the soybean diets were within one standard deviation of the mean value of copper concentration in liver tissue of rats fed a standard diet.

Animals fed the LSS-sorghum diet gained more weight with a higher rate of feed conversion than did those given the diet containing sorghum grain from plots treated with NPK fertilizer. These differences in growth performance of the animals were highly significant statistically despite the higher nitrogen content of the grain produced with optimum NPK fertilization. The increased concentrations of manganese in liver and iron in kidney of animals fed the NPK-sorghum diets may be related to reduced size of these organs in the slower growing animals since the total content of the minerals in the tissues was not greater than for animals fed the sorghum treated with sewage sludge. However, the manganese content of the kidneys of rats fed the LSS-sorghum diet was higher than that of animals given the diets with sorghum grown on plots treated with NPK fertilizer whether expressed as concentration or total content of the mineral in the tissue. The manganese content of the LSS grain was higher than that of the NPK grain (Table I). Though the values for kidney manganese are statistically different, they are all within normal values reported in the literature (Underwood, 1971).

In addition to the elements listed in Table III, the grain and tissue samples are analyzed for Cd, Ni, Pb, and Cr. None of these were present in the animal tissues in detectable levels and only Cr was found in trace amounts in the grains. Thus, the application of sewage sludge to plots on which the crops were grown appeared to have no adverse effects on rats fed diets containing a high proportion of either corn, sorghum, or soybean grain.

SUMMARY

With the possible exception of Mn, Fe, and Cu, sewage sludge treatments applied to the crops had no effect on mineral composition of liver and kidney of rats fed diets containing about 80% of either corn, sorghum, or soybeans. Differences in mineral composition of the tissues, including Mn, Fe, and Cu, were within normal physiological ranges and no overt symptoms of toxicities or deficiencies were observed.

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Isolation of Lipoxygenase from Split Pea Seeds, Snap Beans, and Peas

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Lipoxygenase was isolated from dried split pea seeds, frozen raw peas, and snap beans by ammonium sulfate fractionation, gel filtration, and ion-exchange chromatography. Split pea seed lipoxygenase was purified 19-fold; 22% of the original activity was recovered. Raw vegetable lipoxygenases were partially purified by a modified procedure using Ca^{2+} in the extraction, which appeared to stabilize the enzyme. Snap bean lipoxygenase was purified threefold, recovering 8% of the original activity. Pea lipoxygenase was purified ninefold, recovering 3% of the activity. The enzymes were characterized by determination of pH optima, behavior on polyacrylamide gels, and limited kinetic studies.

An active lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12) system has been identified in many vegetables including legumes (Dillard et al., 1960) corn (Fritz and Beevers, 1955), potatoes (Galliard, 1970), flax seed (Zimmerman, 1966), alfalfa (Siddiqi and Tappel, 1956a), and peas (Lee and Wagenknecht, 1958). The bitterness of soybeans has been attributed to the action of lipoxygenase (Rackis et al., 1972). In frozen raw or unblanched vegetables, progressive deterioration during storage was associated with changes in the lipid fractions which were thought to be catalyzed by lipoxygenase (Wagenknecht and Lee, 1958).

Many of the purification procedures used for isolation of soybean lipoxygenase are based on the work of Theorell et al. (1947). Modifications of that method have been used for isolating green pea seed lipoxygenase by a number of workers (Mapson and Moustafa, 1955; Siddiqi and Tappel, 1956b; Wagenknecht and Lee, 1956; Dillard et al., 1961; Eriksson, 1967). More extensive purification of pea seed lipoxygenase was reported by Eriksson and Svensson (1970) and other investigators have used similar procedures (Haydar and Hadziyev, 1973; Anstis and Friend, 1974).

Differentiation of lipoxygenase isoenzymes is partially based on metal activation of the enzyme. According to Koch (1968) and Koch et al. (1971), calcium is required for activation of lipoxygenase in crude extracts from navy beans and soybeans. The presence of calcium activated lipoxygenase isoenzymes in soybeans has been reported by a number of workers (Holman et al., 1969; Christopher et al., 1972; Restrepo et al., 1973; Zimmerman and Snyder, 1974), but the way in which calcium ions function has not been clarified.

The present investigation was undertaken to develop a method for purifying lipoxygenase from seeds and raw vegetables for use in further studies of the effects of Ca^{2+} on lipoxygenase activity and of the effects of lipoxygenase systems on color, odor, and flavor changes in frozen raw or unblanched vegetables. The work described here deals with the partial purification and some properties of lipoxygenase isolated from dried split peas, frozen raw snap beans, and raw peas.

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