Ecotoxicology and Environmental Safety **46**, 322–328 (2000)
Environmental Research, Section B
doi:10.1006/eesa.1999.1909, available online at http://www.idealibrary.com on IDEAL®

Absorption and Distribution of Selenium in Animals Consuming Canola Grown for Selenium Phytoremediation

G. S. Bañuelos* and H. F. Mayland†

*USDA, ARS, Water Management Research Laboratory, 2021 S. Peach Avenue, Fresno, California 93727; and
†USDA-ARS, 3793N 3600E, Kimberly, Idaho 83341

Received September 27, 1999

Canola (*Brassica napus*) grown as a selected plant species for field phytoremediation of selenium (Se) may be harvested and utilized as Se-enriched forage for marginally Se-deficient lambs and cows. Two field studies were conducted under controlled conditions to evaluate the accumulation of Se into different animal tissues, including blood, excreta, and milk. In Study 1, treatments consisted of feeding lambs freshly cut Se-enriched canola (containing ≈ 4 mg Se kg^{-1} DM) or control canola (containing <0.1 mg Se kg^{-1} DM), respectively, for 64 days. In Study 2, treatments consisted of feeding cows dried Se-enriched canola (containing ≈ 3.5 mg Se kg^{-1} DM) as part of their daily ration for 20 days. In Study 1 at postmortem, Se concentrations were significantly greater in all tested tissues and in excreta from lambs fed Se-enriched canola. In Study 2, Se values were slightly higher in blood and excreta, but not significantly higher in milk from cows sampled throughout the study. Significant differences in total live animal weight were not observed between treatments in either study. Based on these results, canola plants (not including seeds) used for field phytoremediation of Se may be harvested and safely fed to lambs and cows to help meet normal Se intake requirements.

Key Words: **canola; selenium; phytoremediation; lambs; cows.**

INTRODUCTION

Selenium bioconcentration and apparent toxicity in the wetland food chain at Kesterson National Wildlife Refuge (Ohlendorf *et al.*, 1990) prompted researchers to evaluate phytoremediation as a technology to lower Se concentrations in the soil (Wu *et al.*, 1995; Bañuelos *et al.*, 1998; Parker and Page, 1994; Bell *et al.*, 1992). Bañuelos and Meek (1990) demonstrated that plant species that require high concentrations of sulfur (S), such as *Brassica*, will indiscriminately accumulate high concentrations of Se when grown in Se-rich soils. Bañuelos *et al.* (1997) proposed that plants that accumulate Se could be cultivated, harvested, and removed as Se-enriched plant material, resulting in lower Se concentrations in the Se-rich soils.

Selenium, while not required by plants, is an essential trace element for normal nutrition and health of animals (Mayland, 1994). It is a component of the enzyme glutathione peroxidase, which is an antioxidant capable of reducing peroxides and thereby inhibiting the propagation of cell-damaging free radical species produced during metabolism or from an oxidant stress (Flohe *et al.*, 1973). Generally, animal diets containing 0.1 to 0.3 mg Se kg^{-1} will provide adequate Se for animals (Mayland, 1994). Selenium deficiencies are generally a far greater problem than Se toxicities in animals in the United States (Mayland, 1994). Animal producers wishing to ensure an adequate supply of Se to their livestock have a variety of techniques at their disposal, which include giving Se by injecting or by its incorporation as a supplement in the diet. Livestock producers in New Zealand, for example, often use high Se-concentrated boluses (up to 10% elemental Se) that may last for a few years in the reticulo-rumen of cattle and sheep. More recently, they have added small amounts of Se to phosphate fertilizers that are broadcast over the extensive pasturelands resulting in temporary increases in whole blood and plasma Se concentrations in sheep feeding upon the pasture (Whelan *et al.*, 1994). Alternatively, Bañuelos *et al.* (1997) have suggested that plant material harvested from the phytoremediation of Se, e.g., *Brassica* species, may be carefully mixed with other animal feedstuffs (amount depends on tissue Se concentration) and fed to animals in Se-deficient areas. Using Se-rich vegetative plant material as supplemental animal feed may be considered as a potential disposal option for plants used for phytoremediation of Se, pending approval by regulating agencies (Bañuelos *et al.* 1997).

Combs and Combs (1986) list bioavailability estimates from nearly 300 inorganic and organic Se compounds. Assuming sodium selenite to be 100% bioavailable, Se in animal by-product feeds have low availability (9–25%), while that in various plant products has a bioavailability of about 80%. Incorporation of Se-enriched *Brassica* feedstuffs

0147-6513/00

into mixed diets would provide growers with a disposal option for plants used in phytoremediation of soils or waters rich in Se. Canola (*Brassica napus*) has long been used as a forage crop, as grazing or silage (Bell, 1995). Some forage canola cultivars may contain up to 22% of digestible protein (DP), which is comparable to alfalfa. The addition of canola meal to hay or silage has been reported to the improve forage nutritive value for lambs and improve daily weight gains (Agbossamey *et al.*, 1998). There is, to the authors' knowledge, no information regarding absorption or excretion of Se after providing animals with Se-enriched plant material that was previously used for phytoremediation of naturally occurring Se. Moreover, Se absorption by the animal may vary for many reasons including type of animal, tissue or animal product tested, quantity and Se concentration of mixture provided, and duration of feeding.

Using canola that was field-grown for the phytoremediation of Se, the objectives of the two field trials were to: (i) determine the feasibility of feeding lambs only freshly cut canola; (ii) determine the feasibility of feeding cows dried canola as part of their daily ration; and (iii) evaluate the accumulation of Se into different tissues of both animals, including blood, milk, and excreta.

MATERIALS AND METHODS

Study 1

Purebred Southdown lambs were fed daily freshly cut Se-enriched canola (*B. napus*) or a control canola (low Se content) during January to April 1998. This type of lamb, a low-maintenance breed, was selected, because they are commonly raised for meat in central California. Se-enriched canola was grown on a 5 ha field site [Oxalis silty clay loam (fine montmorrillonitic, thermic Pachic haploxeralls)], on the west side of the central valley in California, where Se-laden drainage effluent containing 75–100 μg Se L^{-1} was used as a source of irrigation water. "Control canola" (control) was grown at an adjacent 2 ha site, where water lower in Se (< 3 μg Se L^{-1}) was used as a source of irrigation water.

Both sites were planted in November of 1997. Plants were hand-harvested from each site during the growth cycle of the canola, when the leaf: stem ratio was approximately 2:1. Leaves were removed from the plants and fed to the lambs on a daily basis. A schedule of rates, concentrations of Se, and "free" amino acid content in both types of canola are presented in Tables 1 and 2.

Ten lambs (five ewes and five wethers) were randomly assigned to two treatment groups of five lambs each. Lambs were housed in individual pens (3 × 4 m) in an insulated well-ventilated barn with concrete floors. Lambs were fed daily at approximately 06:00 h. Fresh drinking water was available at all times (Se content was measured to be less than 5 μg L^{-1}). Ambient temperature averaged 11°C and relative humidity averaged 85% during the study. During some weeks in February, canola field sites were impassable due to extraordinarily wet conditions caused by El Niño weather patterns in central California. Consequently,

TABLE 1
Mean Daily Fresh and Dry Weight and Se Concentrations in Seleniferous and Nonseleniferous Canola Fed to Lambs

Julian day of supplying noted amount of canola	Mean daily amounts fed: Fresh (kg FM)	Mean daily amounts fed: Dry (kg DM)	Se concentrations in: Se-enriched (mg kg^{-1} DM)	Se concentrations in: Control
15	1.68[a]	0.200	2.00	0.045
18	1.68	0.200	2.25	0.048
21	2.58	0.280	2.09	0.056
26	2.56	0.280	1.94	0.048
30	2.50	0.280	2.53	0.052
33	2.64	0.290	2.21	0.043
40	2.62	0.290	2.38	0.041
44	—[b]	—[b]	—[b]	—[b]
48	—[b]	—[b]	—[b]	—[b]
52	—[b]	—[b]	—[b]	—[b]
58	4.95	0.620	2.65	0.042
62	4.92	0.620	2.94	0.038
64	5.44	0.700	3.19	0.041
74	5.58	0.700	3.63	0.052

[a]Values are the means from three subsamples of canola fed on the noted day.

[b]Due to El Niño weather patterns, it was not possible to harvest canola; consequently ≈ 1 kg DM alfalfa was temporarily fed to lambs. Selenium concentration in alfalfa was < 100 μg kg^{-1} DM.

TABLE 2
Free Amino Acid Composition in Seleniferous and Nonseleniferous Canola Fed to Lambs

Free amino acid detected in canola	Free amino acid concentration in: Se-enriched (ng g^{-1} DM)	Free amino acid concentration in: Control (ng g^{-1} DM)
Cysteine	29 ± 1.4[a]	36 ± 2.2
Methionine	63 ± 13	82 ± 10
S-Methylcysteine	61 ± 15	73 ± 11
Cystine	29 ± 3.3	40 ± 2.2
Selenocysteine	28 ± 3.3[d,*]	ND[b]
Selenomethionine	110 ± 10*	Trace[c]
Se-Methylselenocysteine	81 ± 11*	Trace
Selenocystine	37 ± 4.4*	ND

[a]Values represent the means followed by standard deviation of canola samples fed to lambs throughout the study.

[b]ND, not detected at 1 ng g^{-1} DM.

[c]Concentrations were too low to accurately report (< 3 ng g^{-1} DM).

[d]*t* test used for determination of significance between treatments;

*Significance between treatments at $P < 0.05$ level.

canola plants could not be collected at either site for feeding. During this time, canola was replaced in the lambs' diets from both treatments with a daily ration of about 1 kg DM alfalfa (*Medicago sativa*) (see Table 1). At each canola collection, six plants were collected, dried at 45°C for 7 days, and the leaves were ground, acid digested, and analyzed for Se, as described later. Two core samples were taken from each alfalfa bale, processed similarly as canola, and analyzed for Se content.

Each lamb's pen was cleaned and feces removed daily. A weekly feces and urine sample was collected from all lambs for qualitative detection of Se (Tables 3 and 4). It was not the intent to quantify the total amounts of feces and urine, which would be necessary to created a Se mass balance. Any contaminated material in the collected feces subsample, such as wood, uneaten feed, or any foreign contaminant visually observed, was removed by hand. Feces that appeared to have been trampled or sprayed with urine was not collected. The fecal sample was frozen, ground to pass a 1-mm screen using a Wiley mill, and stored for Se analysis at a later date. Urine samples were also collected weekly by placing a 4-L container under each secured lamb and then startling the lamb. Approximately a 50-ml urine sample was then collected, frozen, stored, and analyzed at a later date for Se.

Animals were killed after 64 days on experiment of feeding canola including days when canola was not available. Select animal tissues were sampled and freeze-dried (Table 5). Whole blood samples were collected via jugular venipuncture into heparinized tubes prior to the feeding trial and at the end of this study (Table 6).

TABLE 3
Mean Se Concentrations in Urine Samples Collected from Lambs Fed Seleniferous and Nonseleniferous Canola

	Se concentrations in urine of lambs fed	
Julian day of collection	Se-enriched ($\mu g\ L^{-1}$)	Control
22	15 ± 2^{a}	ND[b]
30	29 ± 5	ND
37	45 ± 8	ND
43	96 ± 16	ND
49	9 ± 2	ND
58	11 ± 2	4
65	86 ± 14	1
72	152 ± 25	ND
79	301 ± 50	ND

[a]Means from five replications are presented with 95% confidence limits: *t*-test was used for determination of significance between treatments; there was significance between treatments for all collection days at least at the $P < 0.05$ level.
[b]ND, not detected: less than 1 μg Se L^{-1}.

TABLE 4
Mean Se Concentrations in Fecal Samples Collected from Lambs Fed Seleniferous and Nonseleniferous Canola

	Se concentrations in feces of lambs fed	
Julian day of collection	Se-enriched ($\mu g\ kg^{-1}$ DM)	Control
22	10 ± 2^{a}	11 ± 2
26	32 ± 5	21 ± 3
33	$331 \pm 58^{c,*}$	51 ± 9
37	657 ± 110*	71 ± 10
40	594 ± 104*	71 ± 11
42	704 ± 120*	59 ± 10
47	353 ± 61*	27 ± 4
54[b]	31 ± 5	17 ± 3
58[b]	21 ± 4	9 ± 2
61	513 ± 99*	40 ± 7
68	486 ± 85*	34 ± 6
75	395 ± 69*	28 ± 5
79	358 ± 63*	29 ± 5

[a]Means from five replications are presented with confidence limits at the 95% level.
[b]Alfalfa was fed to lambs during this time (see Materials and Methods).
[c]*t* test used for determination of significance between treatments:
*Significance between treatments at $P < 0.05$ level.

Study 2

Late lactating dairy cows were fed dried and coarsely ground Se-enriched canola as part of their daily ration during April and May 1999. The amounts of Se-enriched canola added to the other feed rations of alfalfa and grain, and Se concentrations, are presented in Table 7. Plant material was obtained from the same field site receiving Se-laden drainage effluent, already described in Study 1.

TABLE 5
Mean Se Concentrations in Different Freeze-Dried Tissues of Lambs Fed Seleniferous and Nonseleniferous Canola

	Se concentrations in tissues of lambs fed	
Tissue	Se-enriched ($\mu g\ kg^{-1}$ DM)	Control
Heart	682(14)[a,b,***]	360(11)
Liver	809(42)***	438(25)
Kidney	2100(99)***	1507(59)
Spleen	525(66)***	216(10)
Longissimuss muscle	213(11)***	68(4)
Toenail	227(27)***	98(20)

[a]Values are the means from five replications followed by the standard error in parentheses.
[b]*t* test used for determination of significance between treatments.
***Significance between treatments at $P < 0.001$ level.

TABLE 6
Selenium Concentrations in Blood Samples from Lambs Fed Seleniferous and Nonseleniferous Canola

Replication	Se concentrations in blood of lambs fed[a,t] Se-enriched (mg L^{-1})	Control
I	0.229[b]	0.047
II	0.273	0.099
III	0.287	0.102
IV	0.299	0.079
V	0.301	0.089
Mean	0.278(0.01)[c,d,***]	0.083(0.01)

[a]Mean Se concentration in blood at onset of study was 0.050 mg Se L^{-1}.
[b]Values are blood-EDTA for Se.
[c]t test used for determination of significance between treatments. All values were significantly different at the ***$P < 0.001$ level.
[d]Mean value from all replications followed by standard error in parentheses.

Both leaves and stem were dried, coarsely ground, and stored in large sealable plastic bins for feeding of the cows.

Eight cows were randomly assigned to two treatment groups of four cows each. Cows were housed openly in a large open pen (20 × 20 m) with adequate covering. Although both groups were housed together, feeding cows their respective treatments were performed separately. Those cows receiving Se-enriched canola were first fed canola, followed by alfalfa, and lastly by grain. Amounts of canola added to feed were gradually increased to allow for the cows to adapt to a new taste. Ambient temperature averaged 20°C and relative humidity averaged 40% during the study.

Blood, milk, and excreta samples were collected periodically from cows for qualitative detection of Se at a later date (See Table 8). Cows were milked twice daily with an automatic milker. Fifty-milliter milk subsamples were collected, frozen, stored, and analyzed later for Se, as well as for other selected elements, i.e., sulfur, because of its potential contribution to causing a bitter taste in milk by S-related compounds (Table 9).

TABLE 7
Amount of Feed and Selenium Concentrations in Alfalfa, Canola, and Grain Fed to Cows

Julian day of supplying feed[a]	Amount of feed provided as[b] Alfalfa	Canola (kg DM)	Grain	Concentration of Se in Alfalfa	Canola (μg kg^{-1} DM)	Grain
119–126	22.7[c]	0	6.8	100	NA[d]	230
127–130	20.4	2.3	6.8	125	3750	265
131–141	17.7	5.0	6.8	137	3750	278
142–153	22.7	0	6.8	116	NA	246

[a]Cows were obtained on Julian Day 118.
[b]Amount provided daily to each cow for the Se treatment during the designated Julian days. Control cows did not receive Se-enriched canola, but rather 22.7 kg of alfalfa and 6.8 kg of grain on a daily basis.
[c]Values were collected from subsampling of each respective feed.
[d]Not applicable.

TABLE 8
Selenium Concentrations in Milk, Blood, and Excreta from Cows Fed Seleniferous Canola and Alfalfa (Control)

Julian day of tissue sampling	Treatment	Se concentrations in Milk (μg L^{-1})	Blood (μg L^{-1})	Urine (μg L^{-1})	Feces (mg kg^{-1} DM)
124	Control	31(1)[a]	63(2)	23(1)	35(1)
	Se-enriched	32(1)	65(2)	21(1)	38(1)
130	Control	42(1)	65(2)	24(1)	46(2)
	Se-enriched	43(2)	62(2)	25(1)	48(2)
141	Control	54(2)	67(2)	33(1)	48(1)
	Se-enriched	57(2)	88(3)	48(1)	67(2)
145	Control	65(3)	65(2)	31(1)	52(1)
	Se-enriched	71(2)	90(3)[b,*]	59(2)*	73(2)*

[a]Values represent mean from four replications followed by standard error in parentheses.
[b]t test used for determination of significance between treatments.
*Significance between treatments at $P < 0.05$ level.

Analysis for Both Studies

Freeze-dried samples and blood were wet-digested with nitric/perchloric acid, and EDTA-Se levels were analyzed by inductively coupled plasma atomic emission using hydride generation, while samples of forages and excreta were wet-acid digested as described by Bañuelos and Pflaum (1990) and Bañuelos and Akohoue (1994) and analyzed for total Se by atomic absorption spectrophotometry with an automatic

TABLE 9
Mean Elemental Concentrations in Milk Samples Collected from Cows Fed Seleniferous Canola and Alfalfa (Control)

Treatment	Elemental concentrations of Ca	Mg	K	Na (mg L^{-1})	P	S
Control	1440(31)[a]	121(4)	1820(31)	600(23)	835(22)	329(18)
Se-enriched	1250(29)	124(4)	1410(32)	884(25)	920(24)	355(17)

[a]Values represent the mean and standard error in parentheses from all milk samples collected throughout the study for all cows in each respective treatment.

vapor accessory (Thermo Jarrell Ash, Model Smith-Hieftji-1000). The precision and accuracy of the Se analysis were monitored using the NIST Wheat Flour Standard (SRM 1567; Se content of 1.1 ± 0.2 mg kg^{-1}, 94% recovery) as an external quality control for Se analyses. Identification of "free" nonprotein selenoamino acids in plant tissue was initially extracted from both Se-enriched and control canola (Table 2), using both the esterification reagents HFB-IBA (heptafluorobutyric anhydride-isobutanobutyric anhydride) derivatization kit (Alltech, Dearfield, IL) and the ethyl chloroformate esterification method, and then later identified by gas chromatography (Janak *et al.*, 1994; Husek, 1993). The method for final quantification of "free" selenoamino acids was described by Wu *et al.*, (1997).

Data were analyzed as a completely randomized design. Treatment differences were tested by Student *t* test using SAS version 6.03 (SAS, 1998).

RESULTS

Study 1

Total plant Se concentrations increased slightly over time in harvested Se-enriched canola fed to lambs (Table 1). Due to unforeseen weather conditions which created saturated field soils, it was necessary to replace canola with alfalfa in the diets for 14 days in February. Selenium concentrations in the alfalfa were less than 0.100 mg kg^{-1} DM. Among the four "free" nonprotein selenoamino acids (Se-methylselenocysteine, selenocysteine, selenomethionine, and selenocystine) identified, only selenomethionine and Se-methylselenocysteine were found in high concentrations in Se-enriched canola and in control canola (Table 2). Overall, concentrations of "free" selenoamino acids were much lower in control canola compared to Se-enriched canola. In contrast, "free" sulfur-based aminoacids were slightly higher in control canola than in Se-enriched canola (Table 2).

Lambs remained free of clinical signs of disease and consumed the canola material from both treatments throughout the study. Diarrhea was observed only once on one lamb in the Se-treatment. Significant differences in total live weight were not observed between treatments at the end of the study. The mean final live weights and standard error (SE) were 45 (2.5) kg for lambs feeding upon Se-enriched canola and 49 (3.1) kg for those feeding upon control canola.

Selenium concentrations were greatest in all samples collected from lambs fed-enriched canola. These samples included the excreta (Tables 3 and 4), selected animal tissues, especially kidneys (Table 5), and blood (Table 6).

Study 2

The cows readily consumed the canola ration. Concentrations of Se were consistent for canola used as feed throughout the study, because all canola was harvested, dried, chopped, and then stored for daily feeding (Table 7). Concentrations of Se in grain and alfalfa rations were also consistent throughout study (Table 7). Significant differences in total live weight were not observed between treatments at the end of the feed trial; the mean weight was approximately 500 (80) kg for cows in both treatments (data not provided).

Due to the primary objective of this study (effect of Se-enriched canola on milk quality), Se was only measured in milk, blood, and excreta. Selenium concentrations were slightly greater in blood, urine, and fecal samples from cows fed Se-enriched canola as part of the feed ration (Table 8). Selenium and sulfur, or related elemental concentrations, were, however, not significantly higher in milk samples from cows fed Se-enriched canola (Table 9).

DISCUSSION

Due to excessively wet growing conditions, irrigating canola with Se-laden effluent was infrequent and intensive leaching and surface runoff of soluble Se likely occurred. Inaccessibility to the wet field site reduced the number of plant harvests that could occur. Consequently, there were lower extractable Se concentrations in the soil which resulted in lower Se concentrations in canola than have been measured in past investigations (Bañuelos, 1997, unpublised). However, higher Se concentrations in harvested plant material would have prompted blending with low Se plant material in order to bring Se concentrations to a safe level (Mayland, 1994). In this regard, using canola may indirectly control Se absorption by the animal, because of its naturally high concentrations of S in plant tissue. Sulfur, which is chemically and physically similar to Se, may reduce absorption of Se as its comparatively lower concentrations.

In these studies, Se concentrations in the Se-enriched canola were always less than 5 mg Se kg^{-1} DM. Cattle and sheep may consume seleniferous plant tissues up to 5 mg kg^{-1} without suffering from Se toxicity (Mayland *et al.*, 1989). Echevarria *et al.* (1988) have reportedly fed sheep up to 9 mg Se kg^{-1} diet without observing any clinical signs of toxicity. Because blood Se levels were less than 0.5 mg Se L^{-1} in the present study (blood levels greater than this level may indicate a potential Se toxicity), the amount of Se provided to the lambs or cows via canola was not harmful. Blood Se concentrations have been as high as 1.4 mg Se L^{-1} in other animals fed forage having a Se concentration between 30 and 64 mg Se kg^{-1} DM (Mitra *et al.*, 1996). It was not the intent of these studies to include dose-response trials to investigate the upper limit of Se concentrations in canola, which can be fed to lambs and cows without observing Se-induced disturbances.

Selenium absorption by the cows fed a partial ration of Se-enriched canola was evaluated primarily in milk. Because S concentrations should not exceed 0.4% in feed

provided to dairy cows (concentrations of S greater than this may cause polyoencephalomalacia, which interfers with absorption of copper (Eduardo, 1999, personal communication), sulfur-rich canola was provided only as a part of their daily ration. Neither Se nor S levels were significantly greater in the milk measured from the cows fed Se-enriched canola compared to alfalfa-fed cows. Similarly, other studies have reported the proportion of dietary Se transferred to cow's milk decreases with increasing Se intake (Miller *et al.*, 1988). Thus unless Se is fed at levels toxic to the cow, its transfer to milk is too low to pose a potential hazard to human health or to be detected by humans. In this regards, a preliminary tasting test of the milk was performed with five persons (data not provided). They could not distinguish a difference in taste between milk produced from cows fed Se-enriched canola and those fed alfalfa.

Selenium absorption by animals fed Se-enriched canola plant material is not only affected by the animal species, but also duration of feeding, composition of diet, and ruminal microbial population, i.e., *Prevotella ruminicola* (Koenig *et al.*, 1997). Microorganisms within the rumen can enhance the availability of Se by incorporating Se into selenoamino acids of bacterial protein. Selenium accumulation by the animals may vary depending on their exposure to different selenoamino acids. Peter *et al.* (1982) reported that the absorption and retention of selenoamino acid, selenomethionine, was greater than that of inorganic Se as selenite. In the current study, selenomethionine was the predominate "free" selenoamino acid identified in the Se-enriched canola fed to the animals, while other forms of "free" selenoamino acids were detected at lower concentrations. Research is currently in progress to accurately identify "free" and "bound" selenoamino acids in plant tissue (Uden *et al.*, 1998; Irgolic and Abegaz, 1997, unpublished) and identify those chemical forms of Se which are more readily absorbed by animals.

Animal Se supplementation practices have been questioned because of the possibility of an increase in the environmental burden of Se derived from animal manure (USFDA, 1993; Oldfield *et al.*, 1994). In this study, animals fed Se-enriched canola as a means of plant disposal and/or as a means of supplementing the Se status of animals excreted some Se in collected urine and feces. It is noteworthy to mention that Se concentrations did increase in the urine of the lambs and slightly in cows. The maximum level of Se in the urine may not have been reached in either animal. This means that after a certain period of feeding animals Se-enriched plant material, their body tissues may become saturated and excess Se has to be excreted. Organ tissues analyzed in the lamb study, especially the liver and the other glandular tissue, absorbed Se similarly as reported by Miller *et al.* (1988). Kidneys used for processing urine did contain the highest tissue Se concentration among all organs tested. Selenium not absorbed by the selected organs may have been excreted because some rumen microorganisms transform Se into unbioavailable forms of Se (Mayland, 1994; NAS, 1980). Selenium was still being excreted to a small extent by the lambs after the temporary halt of providing them Se-enriched canola. Insoluble Se compounds generally pass through the digestive tract intact and are excreted. Various forms of Se excreted (Lopes *et al.*, 1969; Olson *et al.*, 1976; NRC, 1983) are not readily available for plant uptake (Butler and Peterson, 1961; Ajwa *et al.*, 1998). Generally, Se solubility or Se bioavailability in ruminal feces is less than in original diet.

Overall, emphasis should be on meeting animal's nutritional needs with the addition of Se-enriched phytoremediation plants to the diet. However, supplying Se-rich plants to meat animals is not recommended until more research has been performed, especially the monitoring of Se uptake by plants. Moreover, the effects of prolonged feeding of Se-enriched canola should be conducted in future studies.

CONCLUSIONS

The study demonstrates a practical option for plants used in a Se phytoremediation strategy. Canola plants grown for phytoremediation of Se may be harvested and used as a source of supplemental Se in forage fed to lambs and cows. Organic Se provided as Se-enriched canola at the tested levels will lead to increases in organ and tissue concentrations of Se, as well as small increases in excreted Se. Because the experiment did not include different sources of Se, nor did it include canola with varied concentrations of Se, the rate of Se accumulation from the Se-enriched canola cannot be quantified or compared to other sources of Se. Strict monitoring of Se concentrations in plants harvested from phytoremediation is, in any case, highly recommended before this method of plant disposal is considered. Plants disposed of as animal forage could be economically important in Se-depressed/deficient regions. Future efforts must examine the economics of transporting and processing Se-enriched canola from phytoremediation sites to Se-deficient areas sustaining animal production.

REFERENCES

Agbossamey, Y. R., Petit, H. V., Seoane, J. R., and St.-Laurent, G. J. (1998). Performance of lambs fed either hay or silage supplemented with canola or fish meals. *Can. J. Anim. Sci.* **78**, 135–141.

Ajwa, H. A., Bañuelos, G. S., and Mayland, H. F. (1998). Selenium uptake by plants grown in soils treated with inorganic and organic materials. *J. Environ. Qual.* **27**, 1218–1227.

Bañuelos, G., and Akohoue, S. (1994). Comparison of wet digestion and microwave digestion on selenium and boron analysis in plant tissues. *Comm. Soil Sci. Plant Anal.* **25**, 1655–1670.

Bañuelos, G. S., and Meek, D. W. (1990). Accumulation of selenium in plants grown on selenium-treated soil. *J. Environ. Qual.* **19**, 772–777.

Bañuelos, G. S., and Pflaum, T. (1990). Determining selenium in plant tissue with optimal digestion conditions. *Comm. Plant Soil Anal.* **21**, 1717–1726.

Bañuelos, G. S., Ajwa, H. A., Wu, L., and Zambruski, S. (1998). Selenium accumulation by *Brassica napus* grown in Se-laden soil from different depths of Kesterson Reservoir. *J. Contam. Soil* **7**, 481–496.

Bañuelos, G. S., Ajwa, H. A., Mackey, B., Wu, L., Cook, C., Akohoue, S., and Zambruski, S. (1997). Evaluation of different plant species used for phytoremediation of high soil selenium. *J. Environ. Qual.* **26**, 639–646.

Bell, J. M. (1995). Meal and by-product utilization in animal nutrition. In *Brassica Oil Seeds—Reduction and Utilization* (D. Kimber and D. McGregor, Eds.), pp. 301–338. CAB International, Oxon, UK.

Bell, P. F., Parker, D. R., and Page, A. L. (1992). Contrasting selenate-sulfate interactions in selenium-accumulating and nonaccumulating plant species. *Soil Sci. Soc. Am. J.* **56**, 1818–1824.

Butler, G. W., and Peterson, P. J. (1961). Aspects of fecal excretion of selenium by sheep. *N. Zeal. J. Agric. Res.* **4**, 484–491.

Combs, G. F., Jr., and Combs, S. B. (1986). *The Role of Selenium in Nutrition.* Academic Press, Orlando, FL.

Echevarria, M. G., Henry, P. R., Ammerman, C. B., and Rao, P. V. (1988). Effects of time and dietary Se concentrations as sodium selenite on tissue Se uptake by sheep. *J. Anim. Sci.* **66**, 2299–2305.

Flohe, L. W., Gunzler, A., and Schock, H. H. (1973). *FEBS Lett.*, 32–132.

Hendreck, K. A., and Godwin, K. O. (1970). Distribution in the sheep of selenium derived from 75 Se-labelled ruminal pellets. *Aust. J. Agric. Res.* **21**, 71–75.

Husek, P. (1993). Improved procedure for the derivatization and gas chromatographic determination of hydroxycarboxylic acids treated with chloroformates. *J. Chromatogr.* **630**, 429–437.

Janak, J., Billiet, A. H., Frank, J., Ch, K., Luyben, A. M., and Husek, P. (1994). Separation of selenium analogs of sulphur-containing amino acids by high-performance liquid chromatography and high resolution gas chromatography. *J. Chromatogr.* **677**, 192–196.

Koenig, K. M., Rode, L. M., Cohen, R. D. H., and Buckley, W. T. (1997). Effects of diet and chemical form of selenium on selenium metabolism in sheep. *Can. J. Anim. Sci.* **75**, 817–827.

Lopez, P. L., Prestion, R. L., and Pfander, W. H. (1969). Whole-body retention, tissue distribution and excretion of selenium-75 after oral and intravenous administration in lambs fed varying selenium intakes. *J. Nutr.* **97**, 123–128.

Mayland, H. F. (1994). Selenium in plant and animal nutrition. pp. 29–46. In *Selenium in the Environment* (W. T. Frankenberger and S. Benson, Eds.). Marcel Dekker, New York.

Mayland, H. F., James, L. J., Panter, K. E., and Sonderegger, J. L. (1989). Selenium in seleniferous environments. pp. 15–50. In *Selenium in Agriculture and the Environment* (L. W. G. Jacobs, Ed.). SSSA Spec. Publ. 23, ASA and SSSA, Madison, WI.

Miller, J. K., Ramsey, N., and Neadson, F. C. (1988). The trace elements. In *The Ruminant Animal: Digestive Physiology and Nutrition* (D. C. Church, Ed). Prentice Hall, Englewood Cliffs, NJ.

Mitra, M., Ghosh, A., Basak, D. N., Sarkar, S., Basak, D. K., and Bawmik, M. K. (1996). Spontaneous selenosis in cattle: Clinicopathological studies. *Indian J. Vet. Pathol.* **20**, 40–42.

National Academy of Sciences (1980). Selenium. In *Mineral Tolerance of Domestic Animals.* Subcommittee on Mineral Toxicity in Animals. N. Acad. Press, Washington, DC.

National Research Council (NRC) (1983). *Selenium in Nutrition.* N. Acad. Sci., Washington, DC.

Ohlendorf, H. M., Hothem, R. L., Bunck, C. M., and Marois, K. C. (1990). Bioaccumulation of selenium in birds at Kesterson Reservoir California. *Arch. Environ. Contam. Toxicol.* **19**, 495–507.

Oldfield, J. E., Burau, R., Moller, G., Ohlendorf, H. M., and Ullrey, D. (1994). *Risks and Benefits of Selenium in Agriculture.* Council for Agricultural Science and Technology, No. 3.

Olson, O. E., Cary, E. E., and Allaway, W. H. (1976). Absorption of trimethylselenoniurn by plants. *Agron. J.* **68**, 805–809.

Parker, D. R., and Page, A. L. (1994). Vegetation management strategies for remediation of selenium contaminated soils. In *Selenium in the Environment.* (W. T. Frankenberger and S. Benson, Eds.), pp. 327–342. Dekker, New York.

Peter, D. W., Whanger, P. D., Lindsay, J. P., and Buscall, D. J. (1982). Excretion of selenium, zinc, and copper by sheep receiving continuous intraruminal infusions of selenite or selenomethionine. *Proc. Nutr. Soc. Aust.* **7**, 178–186.

SAS (1988). *SAS/STAT User's Guide*, Release 6.03. SAS Institute, Inc., Cray, NC.

UC California Extension (1989). *Testing Alfalfa for Its Feeding Value.* Leaflet 21457 WREP 109. Western Regional Extension Publication.

Uden, P. C., Bird, S. M., Kotrebai, M., Nolibas, P., Tyson, J. F., Block, E. and Denoyer E. (1998). Analytical selenoamino acid studies by chromatography with interfaced atomic mass spectrometry and atomic emission spectral detection. *J. Anal. Chem.* **362**, 447–454.

Ullrey, D. E., Dado, R. G., DeBar, S. R., and Ku, P. K. (1992). Unpublished research. Department of Animal Science, Michigan State University, East Lansing, MI.

U.S. Food and Drug Administration (USFDA) (1993). *Fed. Regist.* **58**, 47962–47973.

Whelan, B. R., Peter, D. W., and Barrow, N. J. (1994). Selenium fertilizers for pastures grazed by sheep. I. Se concentrations in whole blood and plasma. *Aust. J. Agric. Res.* **45**, 863–875.

Wu, L., Guo, X., and Bañuelos, G. S. (1997). Accumulation of seleno-amino acids in legume and grass plant species grown in selenium laden soil. *J. Environ. Toxicol. Chem.* **16**, 491–497.