

# **Selenium Accumulation in a Rapid-Cycling *Brassica oleracea* Population Responds to Increasing Sodium Selenate Concentrations**

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## **ABSTRACT**

Because of their short life cycle, rapid-cycling base populations (RCBP) of *Brassica* can act as model systems for investigating selenium (Se) metabolism in high sulfur (S) accumulating plants. To establish treatment responses for a *B. oleracea* RCBP, plants were grown in nutrient solutions containing 0, 3, 6, and 9 mg sodium selenate ( $\text{Na}_2\text{SeO}_4$ )  $\text{L}^{-1}$ . Depletion of Se from nutrient solutions increased linearly with increasing  $\text{Na}_2\text{SeO}_4$  concentrations. Selenium accumulation ranged from 551 to 1,916  $\mu\text{g Se g}^{-1}$  dry weight for leaf tissue, 267 to 1,165  $\mu\text{g Se g}^{-1}$  dry weight for stem tissue, and 338 to 1,636  $\mu\text{g Se g}^{-1}$  dry weight for root tissue. Selenium additions also resulted in linear increases in S accumulation in leaves and stems. Selenium supplementation has been shown to improve the health of individuals with low Se status. Because *Brassica* species are important vegetable and forage crops, their enrichment with Se may be a good delivery system for mammalian diets.

## INTRODUCTION

Although Se is one of the most widely distributed elements on earth, considerable variability exists for its soil concentration from location to location (Mayland et al., 1989). The accumulation of Se by plants is dependent on soil pH and redox potential, and a species' affinity to absorb and metabolize Se. The form and concentration of Se present, along with competing ions, also affect Se uptake. A close chemical and physical similarity exists between Se and S. Selenium and S are present in the environment in 2-, 0, 4+, and 6+ oxidation states (Mikkelsen et al., 1989). Most plants are able to substitute Se for S during plant uptake and its subsequent metabolism (Brown and Shrift, 1982). Selenium and S compete for the same binding sites in plants during active absorption (Bryant and Laishley, 1988). Sulfate ( $\text{SO}_4^{2-}$ ) and selenate ( $\text{SeO}_4^{2-}$ ) ions may be in direct competition for a high-affinity S permease (Shrift and Ulrich, 1969).

Selenium is absorbed by plants as  $\text{SeO}_4^{2-}$ , selenite ( $\text{SeO}_3^{2-}$ ), or as organic Se complexes (Mikkelsen et al., 1989). The incorporation of  $\text{SeO}_4^{2-}$  into organic compounds in plants is believed to occur in the leaves and begins with its reduction to  $\text{SeO}_3^{2-}$  (Brown and Shrift, 1982). In a similar manner, S is taken up by plants as  $\text{SO}_4^{2-}$  and is reduced to sulfide ( $\text{S}^{2-}$ ) in the leaves before being assimilated into cysteine (Landcaster and Boland, 1990). Selenite is incorporated into various selenoether amino acids, such as Se-methylselenocysteine, selenocystathione, and Se-methylselenomethionine (Anderson and Scarf, 1983). In most plant species, Se-amino acids replace corresponding S-amino acids and are incorporated into proteins. The toxic effect of Se to plants results mainly from interferences of Se with S metabolism (Mikkelsen et al., 1989). Slight chlorosis and decreases in protein synthesis and dry matter production are most often associated with Se toxicity (Mengel and Kirkby, 1987). Some plant species methylate Se-amino acids and prevent their incorporation into proteins, thereby avoiding Se-induced phytotoxicity (Brown and Shrift, 1982).

Plants have been classified by their ability to accumulate Se (Rosenfeld and Beath, 1964). Most plants are 'non-concentrator' species and will accumulate Se in amounts  $< 25 \text{ mg kg}^{-1}$  dry weight. 'Secondary absorbers' normally grow in areas with low to medium soil Se and can accumulate Se from 25 to  $100 \text{ mg kg}^{-1}$  dry weights. The 'primary indicators' are plant species, which accumulate from 100 to  $10,000 \text{ mg Se kg}^{-1}$  dry weight and are the suspects in acute selenosis of range animals. 'Primary indicators', such as some *Astragalus* and *Neptunia* species, may require Se for optimal growth (Ernst, 1982). 'Primary indicators' are often high S-accumulating plants, such as the *brassic*as and other species of *Cruciferae* (Mayland et al., 1989).

Selenium is an essential trace element in mammalian nutrition but has not been classified as an essential plant micronutrient (Mayland et al., 1989). Beneficial and toxic effects to mammals have brought much attention to Se as a nutrient element. Health benefits associated with Se intake include carcinoma suppression, immune

system enhancement, and reduced cardiovascular disease (Levander, 1982). Selenium status in a population correlates highly with the Se content of locally produced crops (Combs, 1989). In areas of the world with low Se in the soil, Se fertilization is practiced to avoid Se deficiencies in animals and humans (Gissel-Nielsen et al., 1984). Consumption of traditional dietary vegetables would be a good way to deliver organic Se to a population, and insure that its beneficial effects may be realized over the life span of an individual (Ip and Lisk, 1994). Several *Brassica* species are important vegetable crops that could be enriched with Se to supplement the human diet.

Model systems are often used to investigate plant metabolic and genetic processes. Arabidopsis is probably the most widely used. Rapid-cycling base populations (RCBPs) of different *Brassica* species have also been developed for use in teaching and as model systems for investigative studies (Williams and Hill, 1986). The reproductive cycle of these plants ranges from 30 to 60 days. Although the plants within each RCBP were originally selected for rapid life cycles, large amounts of heterogeneity remain in each population for other traits. Because vegetable *Brassic*as could be used to deliver Se to a mammalian diet, RCBPs could be used to study Se and S metabolism and its genetic control in these species.

The objectives of the study, therefore, were: i) to establish selenate-Se treatments for the RCBP of *B. oleracea*; ii) to establish accumulation patterns of selenate-Se in the leaf, stem, and root tissues; and iii) to examine effects on growth and dry matter production under increasing selenate-Se.

## MATERIALS AND METHODS

On 10 December 1997, seed of a genetically heterogeneous RCBP of *B. oleracea* (Crucifer Genetics Cooperative, Department of Plant Pathology, University of Wisconsin, Madison, WI) were sown into growing cubes (Grodan A/S Dk-2640, Hedehusene, Denmark). The cells were filled with fine vermiculite and watered twice daily. Seeds were germinated in a growth chamber (Model E15; Conviron, Asheville, NC) at 23°C and 24-h photoperiod. Two L of a 200 mg L<sup>-1</sup> solution of Peter's 20 nitrogen (N)-20 phosphorus (P)-20 potassium (K) (Grace-Sierra Company, Milpitas, CA) were applied five days after sowing. The first true leaves were observed on 17 December 1997 and plants were transferred to 38-L containers (Rubbermaid, Inc., Wooster, OH). Fifteen plants were placed in 2.2-cm holes at 10.6 x 9.5-cm spacing in each container lid. The containers were filled with 30 L of half-strength modified Hoagland's nutrient solution (Hoagland and Arnon, 1950) and the solutions were aerated. The magnesium sulfate (MgSO<sub>4</sub>·7H<sub>2</sub>O) concentration was 246.48 mg L<sup>-1</sup> (96 mg SO<sub>4</sub><sup>2-</sup> L<sup>-1</sup>). The containers were placed into growth chambers set at 21°C with a 24-h photoperiod. Water was added as needed to maintain the initial solution volume.

On 19 December 1997, four Na<sub>2</sub>SeO<sub>4</sub> (ICN Biochemicals, Cleveland, OH) treatments were initiated. The Na<sub>2</sub>SeO<sub>4</sub> concentrations were 0.0, 3.0, 6.0, and 9.0

mg L<sup>-1</sup> which provides SeO<sub>4</sub><sup>2-</sup>:SO<sub>4</sub><sup>2-</sup> ratios of 1:42, 1:21, and 1:14, respectively. The experimental design was a randomized complete block with four replications and 15 plants per replication. On 7 January 1998, ten plants were harvested just prior to flowers opening from each replication and treatment combination. Each 10-plant combination was subdivided into leaf, stem, and root tissues, and their fresh weights were recorded. The plant tissues were placed in paper bags and dried in a forced-air oven at 70°C for no less than 36 h to obtain dry weights. The dried plant material was ground through a 0.5-mm screen (Cyclotec Sample Mill; Model 1093; Tector, Höganäs, Sweden).

To measure Se and S depletion from nutrient solutions by the plants, 20-mL solution samples were taken from each container at Se treatment inception and at plant harvest. At the end of the experiment, all the plants were removed and the solutions were returned to a 30-L volume before final solution samples were taken. Each solution sample was filtered (Whatman #1 filter paper; Maidstone, England) to remove particulates. Selenium in solution samples was measured using graphite furnace atomic absorption spectrophotometry (GFAA; Model 4100ZL; Perkin-Elmer Corporation, Norwalk, CT). The detection limit for Se by GFAA was 4.0 µg L<sup>-1</sup>. Sulfate in solution samples was measured by a turbidimetric method (Gains and Mitchell, 1979). Selenium and S depletion were calculated by subtracting final solution values from initial solution values. Depletion of Se and SO<sub>4</sub><sup>2-</sup> from solutions is reported on a per plant basis.

A wet acid digest was used for Se analysis. Ground tissues were placed into 125-mL flask with 10 mL conc. nitric acid (70% HNO<sub>3</sub>) and placed on a hot plate (Model 2200; Thermolyne, Dubuque, IA) for 4 h at 165°C. The flasks were allowed to cool to room temperature and brought to a final volume of 50 mL with deionized water. The solutions were filtered (Whatman #1 filter paper) before total Se was measured by GFAA. Total S in the plant tissue samples was determined using a LECO Sulfur Determinator (Model SC-232; LECO Corporation, St. Joseph, MI). One g of ground tissue was combined with vanadium pentoxide accelerator (LECO Corporation, St. Joseph, MI) and combusted at 1371°C with oxygen (O<sub>2</sub>). Total S was measured as sulfur dioxide (SO<sub>2</sub>) with an infrared cell detector.

Data were analyzed by the GLM procedure of SAS (Cary, NC). A correlation matrix and Spearman rank correlation were calculated for all variables tested within each Se treatment. Orthogonal polynomials were used to study changes associated with increasing Na<sub>2</sub>SeO<sub>4</sub> concentrations by partitioning the sums of squares into linear components (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

Significant decreases in stem fresh weight and stem dry weight were found under increasing selenate-Se according to GLM. While not statistically different, decreases in total fresh weight relative to the control (0 mg Na<sub>2</sub>SeO<sub>4</sub> L<sup>-1</sup>) treatment for 3.0, 6.0, and 9.0 mg Na<sub>2</sub>SeO<sub>4</sub> L<sup>-1</sup> were 4.7%, 34.7%, and 41.7%, respectively,

TABLE 1. Fresh and dry weight of plant tissues of a rapid-cycling *B. oleracea* grown with 0, 3, 6, and 9 m L<sup>-1</sup> Na<sub>2</sub>SeO<sub>4</sub> in the nutrient solutions.

Na <sub>2</sub> SeO <sub>4</sub>	Leaf	Stem	Root	Total
<i>Fresh weight (g)</i>				
0	249.5 ± 71.1	158.7 ± 32.0	63.6 ± 10.1	471.8 ± 103.8
3	248.7 ± 58.6	129.6 ± 24.0	71.1 ± 24.4	449.4 ± 103.8
6	188.7 ± 32.0	84.5 ± 10.4	34.7 ± 7.3	307.9 ± 49.4
9	182.8 ± 31.4	65.4 ± 12.3	26.8 ± 6.0	275.0 ± 46.9
Contrasts				
Linear	P=0.54	P=0.03	P=0.23	P=0.19
<i>Dry weight (g)</i>				
0	25.8 ± 7.5	12.5 ± 3.1	3.6 ± 0.8	42.0 ± 10.5
3	24.2 ± 5.6	10.3 ± 1.9	3.8 ± 1.2	38.3 ± 8.6
6	19.3 ± 2.7	7.2 ± 0.8	2.0 ± 0.4	28.4 ± 3.8
9	18.6 ± 2.6	5.4 ± 0.9	1.6 ± 0.3	25.6 ± 3.6
Contrasts				
Linear	P=0.63	P=0.05	P=0.25	P=0.26

whereas dry weight decreases were 9.0%, 32.4%, and 39.0%, respectively (Table 1). The heterogeneous nature of the RCBP could have led to the high variability for fresh and dry weights associated with each treatment level. Reductions in dry matter yields with Se additions have been shown for alfalfa (*Medicago sativa* L.) and subterranean clover (*Trifolium subterranean* L.) (Broyer et al., 1966), for several *B. juncea* land races (Bañuelos et al., 1997b), and for Raya (*B. juncea* Cos) (Singh, 1979). Incorporation of Se-amino acids in normal plant metabolic pathways is most likely the cause of lowered dry weight when plants are grown in the presence of high Se (Brown and Shrift, 1982). Even though *Brassica* species accumulate high amounts of Se, limited plant growth and lowered protein synthesis are common symptoms for plants grown in seleniferous environments (Bañuelos et al., 1997a).

Selenium depletion from the nutrient solutions during the experiment differed in response to increasing Na<sub>2</sub>SeO<sub>4</sub> treatments according to GLM procedures (F=343.6; P=0.001). Total depletion of Se increased linearly with increasing Na<sub>2</sub>SeO<sub>4</sub> treatments (P=0.01; Table 2). Selenium present in the solution without Na<sub>2</sub>SeO<sub>4</sub> addition was below GFAA detection. Increased Se depletion from nutrient solutions with increasing Se concentrations was reported for onion (Kopsell and Randle, 1997) and for broccoli (Bañuelos and Meek, 1990). Sulfate depletion from nutrient solutions did not statistically differ with Na<sub>2</sub>SeO<sub>4</sub> concentrations.

Selenium concentration in the leaf, stem, and root tissues differed in response to the Na<sub>2</sub>SeO<sub>4</sub> L treatments according to GLM procedures (P=0.001). Selenium

TABLE 2. Selenium and sulfate depletion from nutrient solution culture for a rapid-cycling *B. oleracea* grown with 0, 3, 6, and 9 mg L<sup>-1</sup> Na<sub>2</sub>SeO<sub>4</sub> in the nutrient solutions.

Na <sub>2</sub> SeO <sub>4</sub>	Se depletion	SO <sub>4</sub> <sup>2-</sup> depletion
	<i>mg L<sup>-1</sup> plant<sup>-1</sup></i>	
0	nd	3.79 ± 0.12
3	0.075 ± 0.003	3.97 ± 0.03
6	0.148 ± 0.002	3.85 ± 0.24
9	0.196 ± 0.030	3.53 ± 0.44
<b>Contrasts</b>		
Linear	P=0.01	P=0.16

nd=nondetectable.

accumulation increased linearly in leaf, stem, and root tissues as Na<sub>2</sub>SeO<sub>4</sub> concentration increased in the solutions (Table 3). Leaves generally had higher total Se than stem or root tissues (Table 3). Mean Se accumulation ranged from 551 to 1,915 µg Se g<sup>-1</sup> dry weight for leaf tissue, 267 to 1,165 µg Se g<sup>-1</sup> dry weight for stem tissue, and 338 to 1,636 µg Se g<sup>-1</sup> dry weight for root tissue. With 4.0 mg L<sup>-1</sup> Na<sub>2</sub>SeO<sub>4</sub> in solution culture, the range for Se accumulation in the shoots of several *Brassica* land races was 501 to 1,017 µg Se g<sup>-1</sup> dry weight (Bañuelos et al., 1997b). Several forages and vegetable crops also concentrated Se in greatest amount in the leaves (Gupta, 1991).

The highest accumulation of S was in the leaf tissues (Table 3). Leaf S (P=0.001) and stem S (P=0.001) differed in response to Na<sub>2</sub>SeO<sub>4</sub> treatments, whereas root S was not significant in the GLM model. A significant linear increase in leaf S with increasing Na<sub>2</sub>SeO<sub>4</sub> treatment concentrations was found (Table 3). Sulfate is trans-located in an acropetal direction, and reduction takes place in the chloroplasts (Mengel and Kirkby, 1987). It is believed that SeO<sub>4</sub><sup>2-</sup> follows similar translocation and incorporation patterns as SO<sub>4</sub><sup>2-</sup>. An antagonistic relationship between SO<sub>4</sub><sup>2-</sup> and SeO<sub>4</sub><sup>2-</sup> was reported during absorption and translocation (Leggett and Epstein, 1956; Ferrari and Renosto, 1972). These experiments, however, used high concentrations of Se to S (a 1 SeO<sub>4</sub><sup>2-</sup>:1 SO<sub>4</sub><sup>2-</sup> ratio). Kopsell and Randle (1997) reported if the ratio of SeO<sub>4</sub><sup>2-</sup>:SO<sub>4</sub><sup>2-</sup> is decreased to 1:125 or 1:500, Se actually enhanced SO<sub>4</sub><sup>2-</sup> uptake and accumulation in onions. In the current study, leaf S concentration increased, as the ratio of SeO<sub>4</sub><sup>2-</sup>:SO<sub>4</sub><sup>2-</sup> decreased from 1:42 to 1:12 over increasing Na<sub>2</sub>SeO<sub>4</sub> concentrations. The ratio of SO<sub>4</sub><sup>2-</sup> to SeO<sub>4</sub><sup>2-</sup> therefore appears to be important in the relationship between S and Se uptake and metabolism.

Uptake of SeO<sub>4</sub><sup>2-</sup> by the *B. oleracea* plants in this experiment, as measured by Se depletion from solutions, was very efficient. Plants in the 3.0, 6.0, and 9.0 mg L<sup>-1</sup>

TABLE 3. Selenium and sulfur accumulation in the plant tissues of a rapid-cycling *B. oleracea* grown with 0, 3, 6, and 9 m L<sup>-1</sup> Na<sub>2</sub>SeO<sub>4</sub> in the nutrient solutions.

Na <sub>2</sub> SeO <sub>4</sub>	Leaf	Stem	Root
<i>Selenium (μg g<sup>-1</sup> dry weight)</i>			
0	nd	nd	nd
3	552 ± 74.5	267 ± 111	338 ± 36
6	1275 ± 116	721 ± 80	857 ± 30
9	1916 ± 144	1165 ± 98	1636 ± 361
<b>Contrasts</b>			
Linear	P=0.001	P=0.001	P=0.001
<i>Sulfur (mg g<sup>-1</sup> dry weight)</i>			
0	13.4 ± 2.8	8.0 ± 1.6	9.3 ± 3.0
3	21.6 ± 3.1	6.0 ± 0.9	8.7 ± 2.7
6	24.1 ± 2.5	8.5 ± 0.9	10.4 ± 4.4
9	26.5 ± 1.8	11.2 ± 1.5	7.3 ± 1.4
<b>Contrasts</b>			
Linear	P=0.06	P=0.12	P=0.79

nd=nondetectable.

Na<sub>2</sub>SeO<sub>4</sub> treatment concentrations removed an average of 90%, 88%, and 78% of the total Se available over the course of the experiment, respectively. The fate of Se depleted from the nutrient solutions can be tracked using the Se concentrations in the tissues and total dry weight of the plant tissues. For the 3.0 mg Na<sub>2</sub>SeO<sub>4</sub> L<sup>-1</sup> treatment concentration, 76% of the Se depleted from the nutrient solutions was deposited in the plant tissues. The remaining 24% was apparently lost by volatilization (Terry and Zayed, 1994). Similar accumulation/volatilization values were found for the 6.0 and 9.0 mg Na<sub>2</sub>SeO<sub>4</sub> L<sup>-1</sup> treatments. A distinctive metallic odor characteristic of Se was detected in the growth chambers during the experiment. The odor was most likely the gaseous volatile dimethylselenide (Terry and Zayed, 1994). In a previous experiment *Brassica* species volatilized Se at rates ranging from 280 to 340 μg Se m<sup>-2</sup> leaf area per day (Terry and Zayed, 1994). In another study, *B. napus* (canola) removed as much as 47% of the total soil Se present from a seleniferous soil (Bañuelos et al, 1997a). The researchers accounted for 57% of the removed Se the plant tissues. The remaining 43% was thought to be lost by through volatilization. In the current study, the relative proportion of accumulated Se to volatilized Se did not change in response to increasing Na<sub>2</sub>SeO<sub>4</sub> treatment concentrations.

TABLE 4. Correlation coefficients for Se and S accumulation in leaf, stem, and root tissues, Se and  $\text{SO}_4^{2-}$  depletion from nutrient solutions, and total fresh weight and total dry weight for a rapid-cycling *B. oleracea* grown with 0, 3, 6, and 9 mg  $\text{L}^{-1}$   $\text{Na}_2\text{SeO}_4$  in the nutrient solution.

Variable	Leaf Se	Stem Se	Root Se	Leaf S	Stem S	Root S	Se uptake <sup>2</sup>	$\text{SO}_4^{2-}$ uptake <sup>2</sup>	Total FW	Total DW
Leaf Se		0.98***	0.97***	0.87***	0.67**	-0.14 <sup>ns</sup>	0.97***	-0.36 <sup>ns</sup>	-0.77***	-0.74***
Stem Se			0.97***	0.83***	0.71**	-0.14 <sup>ns</sup>	0.95***	-0.41 <sup>ns</sup>	-0.73***	-0.70**
Root Se				0.81***	0.74***	-0.22 <sup>ns</sup>	0.91***	-0.44 <sup>ns</sup>	-0.75***	-0.72**
Leaf S					0.53*	-0.14 <sup>ns</sup>	0.85***	-0.20 <sup>ns</sup>	-0.78***	-0.80***
Stem S						-0.25 <sup>ns</sup>	0.52*	-0.66**	-0.72**	-0.68***
Root S							-0.13 <sup>ns</sup>	0.26 <sup>ns</sup>	0.46 <sup>ns</sup>	0.46 <sup>ns</sup>
Se uptake <sup>2</sup>								-0.20 <sup>ns</sup>	-0.71**	-0.69**
$\text{SO}_4^{2-}$ uptake <sup>2</sup>									0.40 <sup>ns</sup>	0.37 <sup>ns</sup>
Total FW										0.99***
Total DW										

<sup>2</sup>Uptake measured as depletion from nutrient solutions.

<sup>ns</sup>, \*, \*\*, \*\*\*Nonsignificant and significant at  $P=0.05$ ,  $0.01$ , and  $0.001$ , respectively.

As expected, Se uptake (depletion from solution) was positively correlated with leaf, stem, and root Se (Table 4). Selenium accumulations in leaf, stem, and roots of the *B. oleracea* plants were highly correlated with leaf S and stem S. Selenium uptake was negatively correlated with fresh weight ( $r=-0.71^{**}$ ) and dry weight ( $r=-0.69^{**}$ ). Total fresh weights were negatively correlated with leaf, stem, and root Se, and leaf and stem S. Total dry weights were also negatively correlated with leaf, stem, and root Se, and leaf and stem S.

The vegetable *Alliums*, e.g., onions and garlic, have been identified as good delivery systems for organic Se in human diets (Ip and Lisk, 1994; Ip et al., 1992). Organic Se delivered through enriched vegetables not only retains the positive health benefits, but also reduces the risk of excessive Se accumulation in muscle and organ tissues. Toxic or excessive accumulation of Se in mammalian tissues can be associated with inorganic  $\text{SeO}_3^{2-}$  and yeast derived selenomethionine, respectively (Ip and Lisk, 1994). Selenite or  $\text{SeO}_4^{2-}$  caused mammalian toxicity by inhibiting protein synthesis through changes in an enzyme's catalytic activity (Ganther and Lawrence, 1997). Organic Se products in the selenized *Alliums* also displayed greater anticarcinogenic activity in mammals than traditional inorganic Se supplements. As with the vegetable *Alliums*, the vegetable *Brassicacae* have worldwide importance as nutritional and economic crops and could be as useful in

delivering Se to the mammalian diet. The chemical form and dose of Se consumed are the most important factors determining its biological activity (Ip et al., 1991). Unlike traditional Se supplements, enriched *Brassica* and *Allium* vegetables could deliver Se in a multitude of forms (Block et al., 1996), thereby reducing the risks associated with any one single form.

## CONCLUSIONS

Members within the *Brassicaceae* have been classified as 'primary Se accumulators'. Similar to other members of the *Brassicaceae*, the RCBP of *B. oleracea* accumulated high amounts of Se in their tissues and accumulation increased with increasing  $\text{Na}_2\text{SeO}_4$  concentrations. Significant increases in S accumulation with increasing  $\text{Na}_2\text{SeO}_4$  concentrations were found for the leaf tissues. The unique reproductive characteristics of the RCBPs may allow them to be used as a model for investigations into Se accumulation and metabolism, and its genetic control. The metabolism of Se by the *Brassicaceae* holds promise for the delivery of beneficial Se forms in mammalian diets.

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