

Areas with High Concentrations of Selenium in the Soil and Forage Produce Beef with Enhanced Concentrations of Selenium

Korry J. Hintze,[†] Gregory P. Lardy,[†] Martin J. Marchello,[†] and John W. Finley*[‡]

Grand Forks Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Grand Forks, North Dakota 58202-9034, and Department of Animal and Range Sciences, North Dakota State University, Fargo, North Dakota 58105

Beef provides a significant portion of human dietary selenium (Se), and it is possible that modest portions of beef produced in areas with high-Se soil and forage could provide the entire Recommended Dietary Allowance (RDA) for Se. The present study has addressed the environmental conditions that resulted in the production of high-Se beef. One hundred and thirty-eight cull cows were obtained from 21 ranches in five distinct geographic regions that, on the basis of soil parent material, reports of Se deficiency, and previous soil and forage Se surveys, were likely to have high or low Se concentrations in the soil. Grass and soil samples were taken from ranch sites, and hair, whole blood, skeletal muscle, diaphragm muscle, and liver samples were obtained from the animals. Hair and whole blood samples were taken 1 day prior to shipping. Selenium concentrations of all samples were determined by hydride generation atomic absorption spectroscopy. Geographic origin affected Se content of all samples ($p < 0.05$). Selenium concentrations in soil ($r = 0.53$; $p < 0.01$) and grass ($r = 0.63$; $p < 0.01$) were correlated to Se content of skeletal muscle. Selenium concentrations in whole blood, diaphragm, hair, and liver also were significantly correlated to Se content of skeletal muscle ($p < 0.01$). Cows that received Se in mineral supplements did not have significantly higher concentrations of Se in sampled tissues ($p > 0.05$). Results of this study suggest that the greatest source of variation in Se content of bovine skeletal muscle was the geographic region from which the beef originated and not production or management practices. Results also demonstrated that a 100 g serving of high-Se beef could provide 100% of the RDA for Se.

Keywords: Selenium; beef; soil

INTRODUCTION

Selenium (Se) was demonstrated to be nutritionally essential in 1957 (1). Selenium deficiency may result in fatal disease conditions in humans and animals (2). Selenium functions in the active site of selenoproteins and also is involved in immune and neuropsychological function. Recent evidence indicates that consumption of Se in excess of the Recommended Dietary Allowance (RDA) may provide substantial cancer-protective benefits for humans. In a long-term, double-blind study (3), 200 μg of supplemental Se supplied daily as high-Se yeast resulted in reductions in lung, colorectal, and prostate cancer. An RDA of 55 $\mu\text{g}/\text{day}$ for men and women has been determined on the basis of the maximization of glutathione peroxidase (GSH-Px) enzyme activity (4).

Publication of the cancer-protective benefits of Se has resulted in many people seeking to increase their Se intakes, but there are relatively few choices available for accomplishing this. Tablets of Se as selenite or high-Se yeast are available, but guidelines from the American Dietetic Association encourage people to consume nutrients through food whenever possible. However, the

Se concentration of a particular food may be variable and dependent on the geographic origin of the raw agricultural product (5).

On average, beef is the single largest source of Se in the North American diet and provides almost 20% of total dietary Se (6, 7). Similar to other foods, the Se concentration of beef is quite variable (5, 8). Because Se from beef comprises such a large portion of total dietary Se and because the Se content of beef is variable, consumption of beef may greatly influence total dietary Se intake. For example, 97% of ranchers from seleniumiferous areas in western South Dakota and eastern Wyoming reported consuming beef raised on their ranches; the Se intake of these ranchers was reported to be 54% greater than the American average (9).

Hoffman et al. (8) reported large differences in the concentrations of Se in beef from research stations across Canada, and the differences were related to the geographic origin of the animal. However, a direct link between causative factors such as Se concentration in soil and ingested forages was not established, and it was assumed that animals with elevated carcass Se concentrations were the result of diets high in Se.

The Western Plains of the Dakotas are underlain by a geographic formation that contains high concentrations of Se (10). This geological formation results in high concentrations of Se in the soil of parts of North and South Dakota and, consequently, the beef raised in this region may contain high concentrations of Se. The demonstrated health benefits of Se, the importance of

* Address correspondence to this author at the USDA, ARS, Grand Forks Human Nutrition Research Center, P.O. Box 9034, Grand Forks, ND 58202-9034 [telephone (701) 795-8416; fax (701) 795-8220; e-mail jfinley@gfhnrc.usda.ars.gov].

[†] U.S. Department of Agriculture.

[‡] North Dakota State University.

Table 1. Se Content of Tissue and Organ Samples Collected from Cattle Carcasses Originating from Five Distinct Geographical Locales of North Dakota^a

sample	region				
	northwest	south central	southwest	central	southeast
muscle	0.67 ± 0.04 ^a (23)	0.47 ± 0.03 ^b (20)	0.40 ± 0.02 ^{bc} (69)	0.38 ± 0.05 ^{bcd} (8)	0.27 ± 0.03 ^d (14)
liver	0.68 ± 0.06 ^a (23)	0.76 ± 0.06 ^a (19)	0.49 ± 0.04 ^b (63)	0.61 ± 0.08 ^{ab} (8)	0.47 ± 0.06 ^b (14)
blood	0.49 ± 0.02 ^a (21)	0.36 ± 0.02 ^b (21)	0.35 ± 0.01 ^b (71)	0.29 ± 0.02 ^c (7)	0.27 ± 0.02 ^c (15)
diaphragm	0.54 ± 0.03 ^a (23)	0.42 ± 0.03 ^b (21)	0.38 ± 0.02 ^c (69)	0.35 ± 0.04 ^c (8)	0.26 ± 0.03 ^d (14)
hair	1.78 ± 0.07 ^a (21)	1.51 ± 0.07 ^b (20)	1.17 ± 0.04 ^c (71)	1.01 ± 0.11 ^{cd} (8)	0.72 ± 0.08 ^d (15)

^a Values are means (mg/kg) ± SE; means with different superscripts in the same row are significantly different ($p < 0.05$). Se concentrations are expressed on a wet weight basis. Number in parentheses is number of samples.

beef in Se nutriture, and the potential enrichment of Se in the beef of animals raised on the high-Se soils of the western Dakotas suggest that consuming beef raised in these areas may be an ideal way of increasing dietary Se intakes. Consequently, the overall objectives of this study were to determine (a) whether there is a direct connection between Se concentrations in soil and forage and Se concentrations in beef, (b) whether areas predicted to have high soil and plant Se concentrations also result in the production of high-Se beef, and (c) which accessible tissues in live animals and carcasses could be used to predict the Se content of skeletal muscle.

MATERIALS AND METHODS

Experimental Design. Twenty-one ranches in five distinct geographic regions throughout North Dakota participated in this study. Regions overlying geologic formations known to produce forages either high or low in Se were chosen as target areas. The ranches were located in western Bowman and Slope Counties (southwestern North Dakota; SW), Sioux County (south central North Dakota; SC), Williams County (northwestern North Dakota; NW), Morton and southern Oliver Counties (central North Dakota; C), and the sandhill region of Richland and Ransom Counties (southeastern North Dakota; SE). The SW and SC regions were chosen because the parent soil material is Cretaceous aged shale, primarily Pierre Shale (11), that is associated with high-Se soils (10). The NW sampling area was selected because soil and forage Se surveys in the late 1940s showed it was a high-Se area (12). The C and SE regions were chosen because of scattered reports of Se deficiency, and the lack of seleniferous geologic material suggested they may be low-Se regions (11). Producers were contacted in each region and recruited on a voluntary basis.

Grass and Soil Sampling. Grass and soil samples were taken from the final pasture of the grazing rotation. Pastures were divided into two to six similarly sized quadrats, and five grass and five soil samples (each soil sample was a composite of 10 subsamples) were taken per quadrant. A 1-m clipping square was randomly placed to collect and separate grass, standing dead grass, and broadleaf plants. A soil probe was used to collect soil samples from 0 to 25 cm in depth. Feed labels from mineral supplements were examined to determine if Se sources other than forages were present in the diet.

Tissue Sampling. Carcass and organ samples were collected from 138 cull cattle that were shipped from the participating ranches to a commercial abattoir. Hair (from the tail) and blood (jugular venipuncture into EDTA) samples were collected 1 day prior to slaughter. Carcass and organ samples (50–150 g of liver, skeletal muscle, and diaphragm muscle) were collected at the time of slaughter. All samples were frozen at -30°C until analysis.

Selenium Analyses. Liver, skeletal muscle, and diaphragm samples were lyophilized and ground into a powder prior to analysis. Hair samples were cleaned with acetone and distilled-deionized water using the method of van Ryssen et al. (13). Forage and soil samples were oven-dried at 60°C for 72 h and ground through a 2 mm screen. Soil samples were also homogenized in a soil roller mill prior to analysis. Tissue, hair, and grass samples (0.3–0.5 g) were digested in concentrated

nitric acid (J. T. Baker, Inc., Phillipsburg, NJ) according to a previously described procedure (5). Selenium was determined by hydride generation atomic absorption spectroscopy (HGAAS).

Soil was analyzed for soluble Se according to a modification of the procedure described by Black et al. (14). A suspension of ~5 g of ground, homogenized soil was refluxed in 30 mL of deionized distilled water for 1 h, centrifuged, and filtered. Concentrated hydrochloric acid was added to the filtrate, and samples were heated to 60°C .

Quality control was maintained by analysis of triplicate standards of bovine liver (NIST 1577b) or apple leaves (NIST 1515 for forage analysis; U.S. Department of Commerce National Institute of Standards and Technology, Gaithersburg, MD) with each batch run of samples. Runs were acceptable if the NIST analyzed values fell within the stated range. The run-to-run coefficient of variation averaged 1.3%. The within-run coefficient of variation averaged 1.1%. The Se content of blank samples averaged 0.14 ng/mL. The detection limit for Se analyzed by using this method was 0.001 ng/mL. All samples were analyzed in duplicate.

Statistical Analysis. Data were analyzed as a nested design with ranch within region. The experimental unit was individual animal for tissue samples and ranch for forage and soil samples. Data were analyzed by using the GLM procedure of SAS (15), and Tukey's Studentized range test was used to separate means. Pearson correlation coefficients were computed to determine intersample relationships. *t* tests were used to compare means of animals exposed to Se supplements and animals without Se supplements and to compare Se concentration means between skeletal and diaphragm muscle (16). Regression equations were determined with the REG procedure of SAS using the best R^2 option (15). Selenium content of hair was influenced by hair color; consequently, hair analyses were blocked on hair color.

RESULTS

Selenium Concentration in Animal Samples. Muscle, blood, diaphragm, and hair samples collected from animals from the NW region contained the greatest concentrations of Se (Table 1), whereas animal tissue and organ samples from the SE region always contained the least Se. The rank order of Se concentrations in muscle, blood, diaphragm and hair was NW > SC > SW > C > SE, and for liver it was SC > NW > C > SW > SE. The Se concentration of forage (Table 2) also was highest in the NW and lowest in the SE regions; the rank order was NW > SC > SW > C > SE. The NW region had the highest concentration of soluble Se in the soil, but the rank order was not similar to that of Se in animal tissues or forage.

Associations between Carcass Se Concentration and Se Concentrations of Other Organs and Tissues. Se concentrations in skeletal muscle (round) from the carcass were most strongly associated with Se concentrations of whole blood ($r = 0.66$, $p = 0.0001$, Figure 1a) diaphragm ($r = 0.65$, $p = 0.0001$, Figure 1b), and grass ($r = 0.63$, $p = 0.0017$, Figure 1c). Associations between muscle Se concentrations and Se concentrations in the liver ($r = 0.36$, $p = 0.0001$ Figure 1d), hair

Table 2. Se Content of Dried Forage and Soil Collected from the Pastures of Cull Cattle^a

sample	region					p value
	northwest	south central	southwest	central	southeast	
grass	0.85 ± 0.08 ^a (4)	0.48 ± 0.07 ^{ab} (4)	0.40 ± 0.05 ^{ab} (9)	0.20 ± 0.13 ^{ab} (2)	0.17 ± 0.11 ^b (3)	0.02
soil	0.84 ± 0.12 ^a (5)	0.07 ± 0.04 ^b (4)	0.14 ± 0.03 ^b (9)		0.39 ± 0.06 ^{ab} (3)	0.04

^a Pastures were on ranches scattered throughout five distinct geographical locales of North Dakota. Values are means (mg/kg) ± SE; means with different superscripts in the same row are significantly different ($p < 0.05$). Se concentration of grass samples is expressed on a dried weight basis. Number in parentheses is number of sampled ranches.

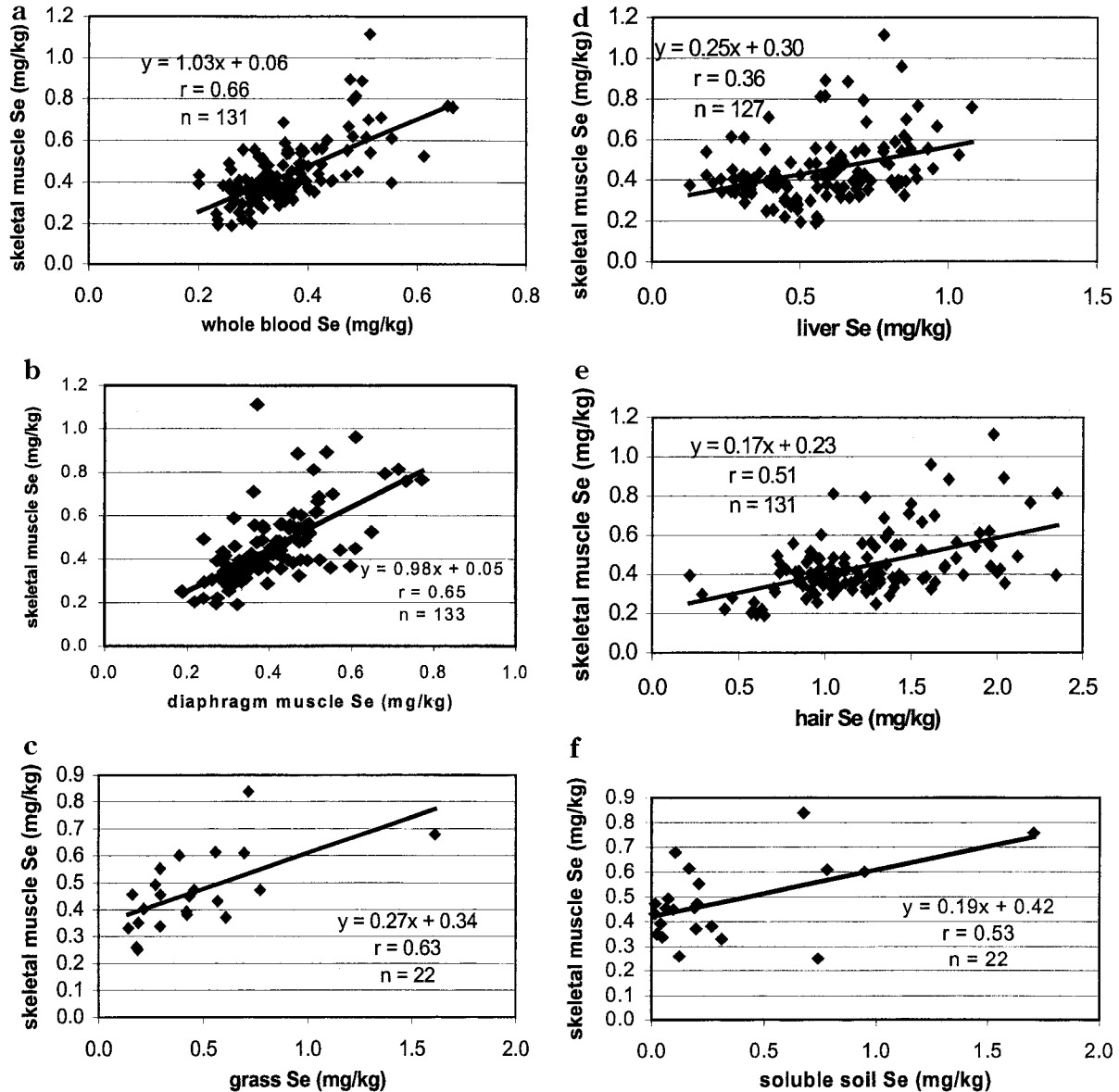


Figure 1. Association of Se concentrations in the skeletal muscle with Se concentrations in other organs, tissues, grass, and soil. Cull cattle from different geographic areas of North Dakota were slaughtered in a commercial abattoir, and organ and tissue samples were collected. Grass and soil samples were collected from the pasture that the animals were taken from. The association between Se concentrations in the carcass and Se concentrations in (a) whole blood, (b) diaphragm, (c) grass, (d) liver when all animals were used, (e) hair, and (f) soluble soil Se is shown

($r = 0.51$, $p = 0.0001$, Figure 1e), and soil ($r = 0.53$, $p < 0.01$, Figure 1f) were significant but not as strong.

Supplemental Se did not increase the concentration of Se in any tissue or organ ($p > 0.05$, Table 3), but the association between Se in liver and skeletal muscle of cattle not exposed to Se supplements was much stronger ($r = 0.50$) than for supplemented animals ($r = 0.23$). The age of the animal was not significantly associated with Se concentration of skeletal muscle ($p > 0.05$). Dark hair had a significantly higher Se concentration (1.47 μg of Se/g) than light hair (1.04 μg of Se/g; $p <$

0.0001, Table 4), and there was a stronger association between Se concentration in light hair and muscle ($r = 0.65$) than between Se concentration in dark hair and muscle ($r = 0.46$).

When all variables were included in a linear regression model, the best predictor of Se in skeletal muscle was $[-0.022 + 0.60(\text{whole blood Se}) + 0.567(\text{diaphragm Se}) + 0.094(\text{soluble soil Se})]$ ($R^2 = 0.80$). When only noninvasively collected variables were considered, the best predictor was $[-0.0086 + 0.98(\text{whole blood Se}) + 0.057(\text{hair Se}) + 0.094(\text{soluble soil Se})]$ ($R^2 = 0.75$).

Table 3. Effect of Se Supplementation on Se Content of Lyophilized, Ground Skeletal Muscle; Liver; Diaphragm Muscle; and Whole Blood Taken from Cull Cows from Five Distinct Geographic Regions in North Dakota^a

tissue	Se in supplement	<i>N</i>	no Se in supplement	<i>N</i>	<i>p</i> value
skeletal muscle	0.46 ± 0.02	58	0.41 ± 0.02	58	0.07
liver	0.56 ± 0.03	58	0.56 ± 0.02	52	1.00
whole blood	0.36 ± 0.01	58	0.35 ± 0.01	59	0.40
diaphragm muscle	0.41 ± 0.01	60	0.38 ± 0.01	57	0.09

^a Values are means (mg/kg) ± SE.

Table 4. Effect of Hair Color on Se Content of Hair Washed with Acetone and Distilled-Deionized Water, Taken from the Switch of the Tail of Cull Cows from Five Distinct Geographic Regions of North Dakota^a

hair color	<i>N</i>	Se content
black	39	1.47 ± 0.06 ^a
brown	44	1.20 ± 0.05 ^b
blonde	59	1.04 ± 0.05 ^c

^a Values are means (mg/kg) ± SE; means with different superscripts are significantly different. Means are blocked by region. *p* value < 0.001.

DISCUSSION

Studies of the health benefits of Se include reports that Se supplementation of an extremely deficient human population in the People's Republic of China prevents the occurrence of potentially fatal Keshan disease (17). Also, intakes of Se in excess of the RDA may improve neuropsychological function (18), and optimal Se status may be necessary for optimal immune function (19). These reports, and the anticancer benefits of Se reported by Clark et al. (3), have created much interest in increasing Se intakes. The present study demonstrates that beef from high-Se areas could be used to greatly increase dietary Se, and identifying such high-Se beef also may be a means of improving the often negative image of beef as a food.

Per capita consumption of red meat has declined substantially in the past few decades. Although the reasons for the decline are complex, certainly a primary reason is the popular misconception of red meat as a food with only negative health effects, that is, as a primary contributor to heart disease and major cancers. Because of the negative publicity, the health benefits of red meat are often ignored. Perhaps one of the most positive nutritional aspects of beef is the amount of trace elements, including Se, that it contributes to the diet. Meat enriched in Se may provide an opportunity for positive marketing and perhaps an opportunity to develop a specialty nutrient-enhanced product.

The present study clearly demonstrates that the concentration of Se in edible beef is consistently higher when animals are raised in areas where the underlying geologic features are known to be high in Se. The area of the state that did not have high-Se geological features (SE region) consistently produced animals with the lowest tissue Se concentrations, and areas with geologic features known to be high in Se consistently produced cattle with the highest tissue Se concentrations. The geographic origin of the animals was a more important determinant of the Se concentration of beef than the presence or absence of supplemental Se. Geographical area resulted in a range of Se concentrations from 0.27 to 0.67 μg of Se/g, whereas providing supplemental Se

increased mean Se concentrations only from 0.41 μg/g (no supplemental Se) to 0.46 μg of Se/g (*p* = 0.07). Others have reported that the concentration of Se in beef varies with geographical region (8); however, that study was a survey of beef carcasses raised in many areas. The uniqueness of the present report is that we have selected areas a priori on the basis of evidence of high or low soil Se, and we have demonstrated that the Se concentration of beef is elevated in areas predicted to have high soil Se.

The potential significance of increasing the Se concentration of beef can be appreciated when its contribution to total dietary Se intake is calculated. Assuming a national average of ~0.2 μg of Se/g of beef, and assuming an intake of 100 g of this beef/day, then an individual's Se intake from beef would be 20 μg/day, or less than half the adult female and male RDA. Conversely, the beef of individual animals from NW North Dakota exceeded 1.0 μg of Se/g of beef, and if a person consumed 100 g of such beef, his/her Se intake would be in excess of 100 μg/day, or in excess of the RDA for either men or women.

Linear regression models were used to determine the best predictors of Se concentrations of skeletal muscle. Among samples that could be obtained in the field, Se concentration of whole blood, hair, and soil Se solubles had the greatest correlation to Se concentration of skeletal muscle. Although the association between Se concentration of grass and Se concentration of skeletal muscle was stronger (*r* = 0.63) than the association between skeletal muscle Se and soluble soil Se (*r* = 0.51), the model included Se in soil, but not Se in grass, as a predictor. [Soluble Se in soil was measured as opposed to total Se as it more accurately reflects Se available to plants (20).] On the basis of this model, one could quickly identify potential areas that would produce high-Se beef carcasses by sampling soil from the area and blood and hair from the animals. Another regression model was used to show that the concentration of Se in an individual carcass could be well predicted by measuring Se in whole blood and the diaphragm; both are samples that could be easily obtained from a slaughterhouse and without damage or loss of value to the carcass.

Although the liver is a major pool of Se in the body, the Se concentration of liver was not a good predictor of Se in skeletal muscle. It is unclear why liver Se concentrations did not follow the same pattern as muscle. Liver and skeletal muscle represent the two largest Se pools (21) but differ in Se metabolism. In rat liver, 82% of Se is associated with GSH-Px compared to 5% of Se in muscle (21).

There are few reports of the association between whole blood Se and skeletal muscle Se of cattle grazing on pasture. Whole blood is considered to be indicative of long-term Se status (22, 23) because of the relatively long half-life of erythrocytes (21, 23). Hair also can be obtained easily by noninvasive means, but the association between muscle Se and hair Se was not as strong (*r* = 0.51) as that of muscle Se and whole blood Se (*r* = 0.66). Using the Se concentration of hair to predict Se concentration of muscle may be complicated by hair color. In this study darker hair was higher in Se than lighter hair. This has also been noted in steers grazing native range (13) and in swine (24). The concentration of Se in both hair and muscle is affected by

the chemical form of Se in the diet. van Ryssen et al. (23) reported that the Se concentration of wool from sheep fed high-Se wheat was almost 3-fold higher than that of animals given the same amount of Se as selenite.

Although the dietary form of Se consumed by cattle in this study was not determined, many assume that the speciation of Se in forages is similar to that in wheat grain, that is, SeMet (25). However, this assumption may not be correct. Peterson and Butler (26) demonstrated that 60–80% of Se in pasture grass was associated with the protein fraction (probably SeMet) and 20–30% was in the form of selenoamino acids. Wu et al. (27) demonstrated that grasses contain several different Se species including Se-methylselenocysteine, SeMet, and selenocysteine. Brown and Shrift (28) hypothesized that inorganic Se is reduced to selenite and incorporated into organic compounds in the leaves. Thus, it is probable that the ratio of organic to inorganic forms of Se in grass is dependent on the maturity and dry matter of the plant.

Although the present study has demonstrated that the total Se concentration of beef may be increased by raising the animal in a high-Se area, it has not addressed the potential health benefits to humans of Se from beef. The health benefits of Se apparently depend in part on the chemical form of the Se consumed. Selenium salts are most effective for producing selenoproteins (29), SeMet is most effective for increasing the body stores of Se, and forms of Se most easily metabolized to methyl selenol may be the most efficacious against cancer (30). Selenium is present in proteinaceous tissue primarily as SeMet and selenocysteine, and the ratio of these forms depends on the food consumed by the animal (29, 31). Thus, determination of the health benefits to humans of Se from beef will depend on further experimentation and require complete and accurate determination of the chemical forms of Se in beef.

LITERATURE CITED

- Schwarz, K.; Foltz, C. M. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc.* **1957**, *79*, 3292–3293.
- Levander, O. Selenium. In *Trace Elements in Human and Animal Nutrition*; Mertz, W., Ed; Academic Press: Orlando, FL, 1986; pp 209–280.
- Clark, L. C.; Combs, G. F., Jr.; Turnbull, B. W.; Slate, E. H.; Chalker, D. K.; Chow, J.; Davis, L. S.; Glover, R. A.; Graham, G. F.; Gross, E. G.; Krongard, A.; Leshner, J. L., Jr.; Park, H. K.; Sanders, B. B., Jr.; Smith, C. L.; Taylor, J. E. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. *J. Am. Med. Assoc.* **1996**, *276*, 1957–1962.
- National Research Council. *Dietary Reference Intakes*; National Academy Press: Washington, DC, 2000.
- Finley, J. W.; Mathys, L.; Shuler, T.; Korynta, E. Selenium contents of food purchased in North Dakota. *Nutr. Res.* **1996**, *16*, 723–728.
- Shi, B.; Spallholz, J. E. Selenium from beef is highly bioavailable as assessed by liver glutathione peroxidase activity (EC 1.11.1.9) and tissue selenium. *Br. J. Nutr.* **1994**, *72*, 873–881.
- Holden, J. M.; Gebhardt, S.; Davis, C. S.; Lurie, D. G. A nationwide study of the selenium contents and variability in white bread. *J. Food Compos. Anal.* **1991**, *4*, 183–195.
- Hoffman, I.; Jenkins, K. J.; Meranger, J. C.; Pigden, W. J. Muscle and kidney selenium levels in calves and lambs raised in various parts of Canada: relationship to selenium concentrations in plants and possible human intakes. *Can. J. Anim. Sci.* **1972**, *53*, 61–66.
- Longnecker, M. P.; Taylor, P. R.; Levander, O. A.; Howe, M.; Veillon, C.; McAdam, P. A.; Patterson, K. Y.; Holden, J. M.; Stampfer, M. J.; Morris, J. S. Selenium in diet, blood, and toenails in relation to human health in a seleniferous area. *Am. J. Clin. Nutr.* **1991**, *53*, 1288–1294.
- Rosenfeld, I.; Beath, O. A. *Selenium Geobotany, Biochemistry, Toxicity, and Nutrition*; Academic Press: New York, 1964.
- Clayton, L.; Moran, S. R.; Bluemle, J. P.; Carson, C. G. *Geologic Map of North Dakota*; Interior National Geologic Survey: Reston, VA, 1980.
- Byers, H. G.; Miller, J. T.; Williams, K. T.; Lakin, H. W. *Selenium Occurrence in Certain Soils in the United States with a Discussion of Related Topics*; Seventh Report; U.S. Department of Agriculture Technical Bulletin 758; U.S. GPO: Washington, DC, 1948.
- van Ryssen, J. B.; Whanger, P. D.; Turner, H. A.; Tinsley, I. J. Mineral and vitamin interactions of steers in a Mediterranean climate. *Livestock Prod. Sci.* **1994**, *38*, 107–115.
- Black, C. A.; Evans, D. D.; White, J. L.; Ensminger, L. E.; Clark, F. E. Methods of soil analysis. Part 2. *Agronomy* **1965**, *9*, 1122.
- SAS User's Guide*, version 6, 4th ed.; SAS Institute: Cary, NC, 1990; Vol. 2.
- Steel, R.; Torrie, J. *Principles and Procedures of Statistics*; McGraw-Hill: New York, 1980.
- Chen, X.; Yang, G.; Wen, Z.; Chen, J.; Ge, K. Relation of Selenium Deficiency to the Occurrence of Keshan Disease. In *Selenium in Biology and Medicine*; Spallholz, J., Martin, J., Ganther, H., Eds.; Van Nostrand Reinhold: New York, 1981; pp 171–175.
- Finley, J.; Penland, J. Adequacy or deprivation of dietary selenium in healthy men: Clinical and psychological findings. *J. Trace Elem. Exp. Med.* **1998**, *11*, 11–27.
- Beck, M.; Kolbeck, P.; Rohr, L.; Shi, Q.; Morris, V.; Levander, O. Benign human enterovirus becomes virulent in selenium-deficient mice. *J. Med. Virol.* **1994**, *43*, 166–170.
- Olson, O. E.; Moxon, A. L. The availability to crop plants of different forms of selenium in the soil. *Soil Sci.* **1939**, *47*, 305–311.
- Behne D.; Wolters, W. Distribution of selenium and glutathione peroxidase in the rat. *J. Nutr.* **1983**, *113*, 456–461.
- Ullrey, D. E. Biochemical and physiological indicators of selenium status in animals. *J. Anim. Sci.* **1987**, *65*, 1712–1726.
- van Ryssen, J. B.; Deagen, J. T.; Beilstein, M. A.; Whanger, P. D. Comparative metabolism of organic and inorganic selenium by sheep. *J. Agric. Food Chem.* **1989**, *37*, 1358–1363.
- Wahlstrom, R. C.; Goehring, T. B.; Johnson, D. D.; Libal, G. W.; Olson, O. E.; Palmer, I. S.; Thaler, R. C. The relationship of hair color to selenium content of hair and selenosis in swine. *Nutr. Rep. Int.* **1984**, *29*, 143–146.
- Olson, O. E.; Novacek, E.; Whitehead, E.; Palmer, I. Investigations on selenium in wheat. *Phytochemistry* **1970**, *9*, 1181–1188.
- Peterson, P. J.; Butler, G. W. The uptake and assimilation of selenite by higher plants. *Aust. J. Biol. Sci.* **1962**, *15*, 126–146.
- Wu, L.; Guo, X.; Banuelos, G. S. Accumulation of selenoamino acids in legume and grass plant species grown in selenium-laden soils. *Environ. Toxicol. Chem.* **1997**, *16*, 491–497.

- (28) Brown, T. A.; Shrift, A. Selenium: Toxicity and tolerance in higher plants. *Biol. Rev.* **1982**, *57*, 59–84.
- (29) Beilstein, M.; Whanger, P. Glutathione peroxidase activity and chemical forms of selenium in tissues of rats given selenite or selenomethionine. *J. Inorg. Biochem.* **1988**, *33*, 31–46.
- (30) Ip, C.; Ganther, H. Activity of methylated forms of selenium in cancer prevention. *Cancer Res.* **1990**, *50*, 1206–1211.
- (31) Beilstein, M. A.; Whanger, P. D. Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. *J. Nutr.* **1986**, *116*, 1701–1710.

Received for review June 6, 2000. Revised manuscript received November 3, 2000. Accepted November 10, 2000. The U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

JF000699S