*Determining the Major Forms of Selenium in Jose Tall Wheatgrass Herbage**

Final Report

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**this project was an extension of an earlier study (Agreement # 4600003430) entitled "Animal Evaluation of Salt Tolerant Forages Irrigated with Saline Drainage Water: Forage quality, persistence under grazing, digestibility and intake by ruminants.*

I. ABSTRACT

A study conducted at Red Rock Ranch in 2007 and 2008 concluded that beef cattle could be grazed for 6 to 7 months on pastures of 'Jose' tall wheatgrass and creeping wildrye (*Leymus triticoides*) containing up to 4 ppm total Se in the dry matter without developing symptoms of Se toxicity or other adverse effects on animal health. A dairy cattle feeding study was then conducted in 2012 to examine the potential of using Se-enriched tall wheatgrass hay as a substitute for mineral supplements (sodium selenite) fed to dairy cattle in Se-deficient areas. Results from this study suggested that although the mineral source had higher bio-availability, there were benefits of utilizing Se-enriched TWG hay as a bioavailable Se source for dairy cattle, including increased milk production as compared to no supplementation.

Both of these studies revealed the need to determine the chemical forms of Se in the tall wheatgrass hay so as to better assess its bio-availability for cattle. For the Se speciation study reported here, samples of tall wheatgrass grown in the greenhouse at different levels of selenium and salinity in the soil, and a tall wheatgrass hay sample from the Panoche Water District, were submitted to three qualified laboratories for Se speciation. Due to the complex nature of Se biochemistry in plants, not all of the Se could the speciated, but Applied Speciation Lab (Bothell, WA) provided the best results. A step-wise, acid-peroxide digestion (HNO_3 /HCl/H_2O_2, heating to 100°C after each addition) was used to extract the samples and reverse phase, inductively coupled mass spectrometry (***RP-ICP-CRC-MS***) was used for Se detection. This process extracted 45 – 60%, and identified 31 – 47%, of the total-Se measured in the forage samples.

The major form of Se in the extractable portion was selenomethionine (Se-Met) which accounted for 21 to 31% of the total Se in the TWG samples. Se-methionine is considered to be a bio-available form for animals/cattle, based on reports in the literature (Whanger et al., 2006, Knowles et al., 1999). After Se-Met, the next most abundant species in TWG herbage was selenate, an inorganic form which accounted for 2 – 16% of the extractable Se. Both of these results agree with Whanger (2002) who reported that Se-Met is the major selenocompound in cereal grains (same family as tall wheatgrass- Poaceae) and grassland legumes and that selenate is the predominant inorganic form of Se in both plants and animals. In vegetables such as garlic, onion, broccoli florets and leeks, however, methylselenocysteine (another Se-amino acid) has been reported to be the major species.

Bio-availability is a key characteristic to assess the potential for Se toxicity when cattle are grazing Se-enriched forage and to assess the potential of using Se-enriched tall wheatgrass hay as a substitute for sodium selenite supplements added to the diets of dairy cattle in Se-deficient areas. The benefit of this substitution would be to reduce the importation of 'new' Se into the San Joaquin Valley in the form of feed supplements.

II. ABBREVIATIONS

CWR	creeping wildrye (*Leymus triticoides*)
DM	dry matter
NRC	National Research Council
ppm	part per million (= mg/L, water and mg/kg dry weight, for plant tissue and soil)
ppb	part per billion (= ug/L, water and ug/kg dry weight, for plant tissue and soil)
RP-ICP-CRC-MS	reverse phase, inductively coupled plasma, mass spectrometry using a collision reaction cell.
SJV	San Joaquin Valley
TMR	total mixed ration (for a confined animal)
TWG	tall wheatgrass (*Thinopyrum ponticum* var. 'Jose')
Se-Met	selenomethionine
MeSe-Cys	methylselenocysteine
MeSe(IV)	methylseleninic acid
SeMetO	selenomethionine oxide
Tot-Se	total selenium

III. PROJECT DESCRIPTION

The research described in this report is an extension of a larger study (Agreement 4600003430) funded by the Proposition 204 Agricultural Drainage Program which was entitled "*Animal Evaluation of Salt Tolerant Forages Irrigated with Saline Drainage Water: forage quality, persistence under grazing, digestibility and intake by Cattle*". The objectives of this study conducted at Red Rock Ranch in 2007 and 2008 were to investigate selenium (Se) accumulation in beef cattle (blood, liver, muscle tissue) grazing forages ('Jose tall wheatgrass and creeping wildrye) irrigated with saline drainage water high in Se and sulfur. Potential risks to ruminant health were examined in the context of the exposure period and forage dry matter (DM) consumption. Results of this study were submitted in ten reports with the last submitted in August 2010. They are also summarized in the Introduction (section IV) of this report and they were published as a peer-reviewed article in Science of the Total Environment (Juchem et al. 2012). *Grazing as an Alternative for Utilization of Saline-sodic Soils in the San Joaquin Valley: Selenium accretion and performance of beef heifers*. Sci. Total Environ. 419(1): 44-53 which is provided in Appendix 1.

Upon completion of the grazing study, we identified the need to identify the chemical forms of Se in 'Jose' tall wheatgrass so as to better assess its bio-availability in a nutritional sense, and the potential for Se toxicity in grazing cattle. Se Speciation was also a key issue for a companion study funded by the California State University Agricultural Research Initiative (CSU-ARI) which investigated the potential of using Se-enriched tall wheatgrass (TWG) hay as a substitute for the mineral supplement (sodium selenite) which is fed to dairy cattle in the eastern San Joaquin Valley (SJV) where soils and irrigation water can be deficient

in selenium. To substitute for these mineral Se supplements, the Se in TWG hay would need to have similar bio-availability to sodium selenite.

Thus, the task order objective for this study (agreement 4600008986) which ran from Nov. 2010 to Jan. 2014 was to:

- Determine the major forms of selenium (Se) present in 'Jose' tall wheatgrass herbage (forage samples conducted during the period that the beef cattle grazed the pastures at Red Rock Ranch).

IV. INTRODUCTION

Grazing Study (Se toxicity)

The aim of the "Red Rock Ranch Grazing Study" was to investigate selenium (Se) accumulation in beef cattle grazing 'Jose' tall wheatgrass (*Thinopyrum ponticum, var. 'Jose' = TWG*) and creeping wildrye (*Leymus triticoides = CWR*) pastures irrigated with saline drainage water enriched in selenium and sulfur. Prior research (Suyama et al., 2007a) had shown very high levels of Se in these forages relative to animal requirements. Indeed from 2002 to 2004 in pastures abundantly irrigated with Se-enriched drainage water (300-600 ppb), 'Jose' Tall Wheatgrass had an average of 6.12 ppm total Se in the herbage and creeping wild rye had 10.7 ppm which are far above the dietary requirement of 0.3 ppm Se (NRC, 2001).

This study was run for two grazing seasons (2007 and 2008; 190 and 165 days) with a new herd of heifers (6-8 months old at the start of grazing) each year. Unfortunately, these grazing seasons corresponded to drought years when saline drainage water was scarce and the pastures, particularly in 2008 were deficit-irrigated, which reduced Se concentrations in the forage to 4 ppm (TWG) and 2.5 ppm (CWR) and thus, the Se exposure for the cattle grazing them. Nevertheless, the cattle demonstrated large increases in Se in blood (2 to 3 fold), liver (8 to 12 fold) and muscle (9 to 17 fold) during the grazing seasons. In spite of what were clinically toxic levels of Se in the animal tissues based on published guidelines, the heifers had moderate weight gains (1.30 lb/day (0.59 kg/day) for TWG-grazed and 0.60 lb/day (0.27 kg/day) for CWR-grazed heifers) in 2007. In 2008 with a new group of heifers, weight gains were lower overall (0.60 lb/day (0.27 kg/day) for TWG-grazed and 0.79 lb/day (0.36 kg/day) for CWR-grazed) which was likely due to low forage availability and low crude protein in the forage, rather than Se toxicity. Nitrogen fertilizer had not been applied to these pastures because the saline drainage water used in earlier years contained high levels of nitrate. However, with the small volume of drainage water applied to the pastures in 2008, the forages had insufficient levels of crude protein to support higher body weight gains. In 2008, the beef heifers were also border-line copper deficient, but after careful analysis of the literature and the potential impacts of moderate Cu deficiency on cattle weight gains, it was concluded that the low body weight gains in 2008 were primarily due to insufficient nutrient content in the forages rather than the low Cu levels in the forages.

The overall conclusion of the grazing study was that TWG and CWR forage containing up to 4 ppm total Se in the dry matter did not cause beef heifers to develop signs of Se toxicity (deformed hooves, loss of hair, diarrhea or sudden death) and that exposing non-pregnant, non-breeding cattle to these high Se forages for a grazing season of 6 to 7 months would not likely result in death, or even negative impacts on animal health or productivity, due to Se intoxication. At this very high level of Se exposure, potential micro-mineral imbalances in forages grown in soils irrigated with saline drainage water were judged to be a more relevant concern for animal health and performance (i.e. body weight gain). Therefore, depending on the outcome of forage quality analyses, macro- and micro-mineral supplementation should be considered for cattle grazing these Se-enriched forages so as to optimize their performance and to minimize the risk of undesirable metabolic conditions, particularly hypomagnesia and copper deficiency. While subsurface agricultural drainage water can provide high levels of nitrogen (N) to growing forages, application of supplemental N sources should be considered if needed to sustain adequate forage production and crude protein, as well as body weight gains for the cattle.

This production system could also provide an economic opportunity for ranchers to produce "value-added beef" with higher levels of Se (an antioxidant with potential anti-cancer activity, Whanger et al. (2006), which is considered to be low in the diets of Americans), utilizing saline soils and/or saline drainage waters not suitable for the production of higher value, salt-sensitive crops. One should point out, however, that not all saline drainage waters are enriched in selenium—some may be high in molybdenum or other trace elements—thus utilization of saline waters for this type of niche product requires adequate testing of soil, water, forage, and even animal tissues.

Dairy cattle feeding study (bio-availability of Se in tall wheatgrass hay)

Unlike the western San Joaquin Valley (SJV), soils in the eastern SJV tend to be deficient in Se as are the forages grown on them. A common practice for dairy producers in the eastern SJV is to add a supplemental Se source to the diet, which is often sodium selenite. Thus, the question arose as to whether Se-enriched tall wheatgrass hay could substitute for these dietary supplements and thereby reduce importation of 'new' Se into the SJV. This also raised the issue of the bio-availability of the Se in TWG hay as compared to that in sodium selenite, and ultimately there arose the need to speciate the selenium in order to assess its bio-availability. Our dairy cattle feeding study is briefly described here as the speciation work funded by DWR is relevant to this study.

This study was conducted from February to May 2012 on a commercial dairy farm located near Hanford, CA (U.S.A). There were three dietary treatments: (1) **Control** = baseline diet Se, targeted at 0.3 - 0.4 ppm Se from the normal feedstuffs in the diet, (2) **TWG** = supplemental Se in the form of Se-enriched (4.65 ppm) tall wheatgrass hay added to the diet at 4.6% of dry matter (DM) and (3) Sodium selenite (SS) = supplemental Se in the form of a premix (10 g SS/kg ground corn) added to the diet at 4.3 g/kg DM. Both Se supplements were designed to increase the dietary Se level by 0.3 ppm of DM over the control. However due to variability in Se content between the initial sample and the 45 tons of TWG hay used in the study, the final diets had slightly more Se in the SS diet (0.65 mg Se/kg DM) than in the TWG diets (0.53 mg/kg), but both were substantially increased over the control diet (0.35 mg/kg). Three similar pens,

each containing 310 primiparity, pregnant, mid-lactation dairy cows were used. The experiment was a 3 x 3 Latin square design consisting of the three dietary treatments and three pens. A dietary treatment was randomly assigned to each pen prior to the first period and the treatments were then rotated after 28 and 56 d such that each pen of cows sequentially received all dietary treatments. Se accumulation in blood, urine and feces was measured to assess its bioavailability amongst the three diets. Se output in milk was also measured in order to calculate a mass balance for Se for each of the three diets.

Feeding Se-enriched TWG hay increased Se in the dairy cattle whole blood by 6.4% over the control, whereas sodium selenite increased it by only 4.8%, which suggested higher bioavailability for Se in TWG hay vs. sodium selenite. However, a complete Se response in whole blood may require a time span equal to the average life span of red blood cells, which in cattle is 90-120 d, longer than the 28 day exposure periods for each of our diets. Furthermore, based on the fecal and urine data, a different conclusion was reached. Several methods for determining the amount of dietary Se that was digested (and not excreted) were examined and it was concluded that calculation of the "marginal outputs" (i.e. Se from the TWG hay or the SS supplement, rather than Se in the whole diet, that was excreted in milk, urine and feces) was the best measure because its inverse (that not excreted) specifically measures the bioavailability of the supplemented Se. Using marginal Se not excreted in feces as a measure of Se bioavailability, the Se in SS had a higher Se bioavailability (72.5%) than did the Se in TWG (55.1%).

Interestingly, marginal Se output data also showed that when the dietary Se supplement was from TWG hay, the milk output was 3.0% of the supplemented Se, whereas for the inorganic Se source (SS), the milk Se output was only 0.6%. This higher milk output with the TWG vs. SS diet suggests that the Se in TWG hay may have been more effectively incorporated into milk than was the Se in SS. Similar to Se-enriched meat, milk enriched in Se could have some health benefits due to its antioxidative properties which can protect against cancer and its involvement in the immune system (Combs, 2001; Whanger, 2006). In fact, supplementation of cow's milk with Se to create a dietary Se supplement for humans already occurs in some countries.

Overall, results from this study suggested that the inorganic Se source (sodium selenite) was more bioavailable than the organic Se source (TWG hay); although both would be considered to have high bioavailability, in general. However, our results also indicated potential benefits of utilizing Se-enriched TWG hay as a bioavailable Se source in dairy cattle, including increased milk production as compared to no supplementation. TWG hay can by produced using saline irrigation water and if that water is high in Se, a value-added, Se-enriched forage product could be produced. Use of Se-enriched TWG hay in place of SS represents a translocation option for Se from the westside to the eastside of the SJV, thereby reducing importation of 'new' Se into the Valley in the form of dietary supplements for dairy cattle. This is important because virtually all of this 'new' Se is excreted in animal urine and feces and can only leave the SJV in surface waters or by leaching.

A manuscript describing this work has been drafted and will be submitted to the academic journal "Science of the Total Environment" (Elsevier Publishing) in October, 2014.

Se biochemistry in plants and animals

Se biochemistry is extremely complex with two main forms of inorganic Se (selenate and selenite), but several more complex organic forms (Lauchli, 1993; Sors et al. 2005; Terry et al., 2000). Figure 1 shows that in non-accumulator plants, which includes most crop plants and the forages investigated in our studies, inorganic Se can be incorporated into Se-amino acids such as selenocysteine (Se-Cys) and selenomethionine (Se-Met) and in turn, these forms can be incorporated in selenoproteins in plants.

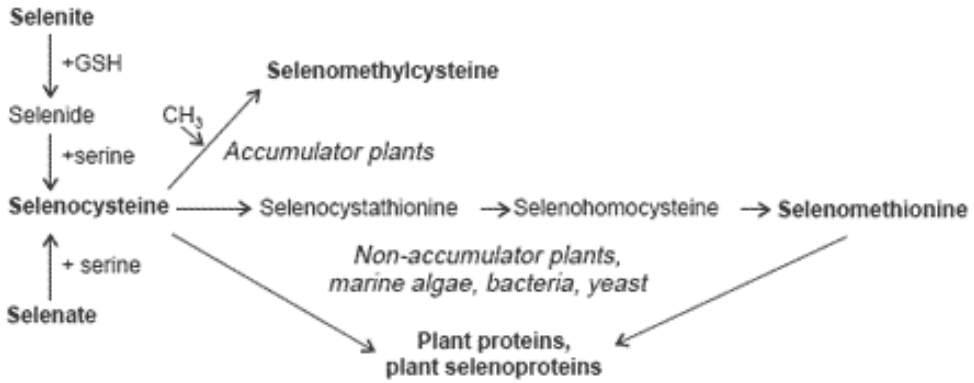

Fig. 1. Selenium assimilation in accumulator plants *vs.* assimilation in non-accumulator species, marine algae, many bacteria and yeast (from Marschner, 1995 and Schrauzer, 2000).

When present in high concentrations, incorporation of Se-amino acids can lead to dysfunctional amino acids in plants and other organisms, but at low concentrations, beneficial selenoproteins occur, such as glutathione peroxidase which protects cells against reactive oxygen species. This enzyme is present in animals and algae, but its occurrence in plants has not been clearly demonstrated (Lauchli, 1993).

Whanger, who has published extensively on the forms and functions of Se compounds in plants and animals, states that the selenocompounds present in plants have a profound effect on the health of animals and humans, and that the total Se content in plants cannot be used as an indicator of its efficacy, but that speciation (measurement of individual selenocompounds) is critical to interpretation of Se effects in the environment. And, because animals and humans consume plant materials either directly or indirectly, this makes the knowledge of the specific selenocompounds in plants even more critical (Whanger, 2006; Rayman, 2008).

Studies have shown the total net apparent absorption (*i.e.,* bioavailability) of Se from the small intestine was about 85% of ingested Se in pigs but only 35% in sheep (Wright and Bell, 1966) and 51% in lactating dairy cows (Ivancic and Weiss, 2001). The less efficient intestinal Se absorption in ruminants *vs.* monogastrics is largely due to the reduction of selenite to insoluble forms such as selenide and elemental Se by rumen microorganisms (Butler and Peterson, 1961; Van Ryssen, 1989). Total net absorption (digestibility) calculated in our dairy cattle feeding study was similar for the diet supplemented with TWG hay and the control diet (49.4% vs. 46.7%, respectively), but it was higher (58.0 %) for the diet supplemented with sodium selenite.

The most prevalent organic forms of Se consumed by ruminants are SeMet and SeCys, both of which occur in most normal animal feedstuffs at some level (Shrift, 1969; Peterson and Butler, 1962), but inorganic dietary supplements generally provide selenium as selenite and selenate. Once Se from selenate or selenite is incorporated into an amino acid (*i.e.,* methionine, cysteine) it is then considered to be in the organic form which is thought to be more readily absorbed by animals from the digestive tract (*i.e.,* has a higher bioavailability). Interestingly, the results of our dairy cattle feeding study may not be supportive of this conclusion. The Se in Se-Met appears to undergo fewer alterations during rumen fermentation than does selenite (Whanger et al., 1968). Knowles et al. (1999) compared effects of organic and inorganic forms of Se added to the diet of dairy cows and found that organic Se increased whole blood, milk and liver Se concentrations 2 to 3 times more than inorganic Se. Another study found that a Se yeast supplement resulted in a 130 % increase in milk Se, while the selenite and selenate groups only had ~20% higher Se as compared to the control diet (Ortman and Pehrson, 1999). This is not surprising as, in selenized yeast, Se mainly occurs as SeMet (reviewed by Rayman, 2004; Polatajko et al., 2005), the form that is naturally synthesized by plants. Given that numerous authors have emphasized that the chemical form of Se can have a large impact on its bioavailability, this project was initiated to speciate the Se found in 'Jose' tall wheatgrass, given that this forage has emerged as a top candidate for saline drainage water reuse systems in the western SJV.

V. METHODS

Dried and ground shoots from 'Jose' tall wheatgrass (TWG) grown in a greenhouse and irrigated with saline drainage water containing different combinations of selenium (low = LSe and high = HSe) and salinity (low = LS and high = HS) was submitted to qualified laboratories for Se speciation. The plants were part of another study examining Se accumulation in TWG herbage as impacted by irrigation water chemistry and cutting height. Although the amount of Se accumulated by the forage can be influenced by the ratio of Se to salinity, due to interactions between Se and sulfate (Wu and Huang, 1991; Cartes, 2006), it was not expected that this ratio would affect the speciation. Hence, the forage samples submitted for speciation were chosen so as to span a range of total-Se concentrations from 4 to 20 ppm. The last sample (labeled as TWG hay) was collected from bales harvested at the San Joaquin River Improvement Project (SJRIP) operated by Panoche Water District. This was also the source of hay used in our dairy cattle feeding study. Likewise, we did not expect that the speciation would be different in the TWG grown at this location versus in the samples from the greenhouse-grown plants.

Three laboratories speciated the Se in our TWG samples. They were 'Applied Speciation' and 'Frontier Global Sciences', both located in Bothell, WA (U.S.A.) and a subset of the samples was also analyzed by Dr. Bill Maher of Canberra University in Australia (http://iae.canberra.edu.au/html/staffmember.php?staffmember=Bill%20Maher). Due to the high cost of the analysis by the commercial labs (~$700/ sample), only nine forage samples were submitted which included a duplicate for two of the samples. The samples were selected to have a range of total-Se concentrations from 4 to 20 ppm, but because we did not expect that the speciation would differ amongst

them, it was concluded that nine samples would be sufficient to identify the major forms of extractable Se in 'Jose' tall wheatgrass herbage.

The methodology employed by 'Applied Speciation' was:

***Sample Reception*:** Nine TWG samples were submitted for total Se and Se speciation analyses. The lab reported that the samples were received in acceptable condition on March 6, 2012 in a sealed container at ambient temperature. They were received in a laminar flow clean hood void of trace metals contamination and ultra-violet radiation, assigned discrete sample identifiers and acid-digested. All samples were then stored in a cryofreezer with a temperature of less than -80°C, known to be free from trace metal contamination, until Se speciation could be completed.

***Sample Preparation*:** All sample preparation was conducted in laminar flow clean hoods known to be free from trace metal contamination. All applied water for dilutions and sample preservatives was monitored for contamination to account for biases associated with sample results.

***Total Selenium Analysis by ICP-DRC*-MS**: Approximately 0.1 g of each sample was transferred to a polypropylene centrifuge tube followed by the addition of **2mL HNO3.** All sample digests were placed in a **hot block digestion** apparatus, the temperature of which was slowly increased to 100°C. After 1 hour at 100°C, all samples were removed from the hot block, cooled, and 6.7mL of **HCl** was added. All sample digests were placed back in the hot block digestion apparatus at 100°C for 30 minutes. The sample digests were then boiled down to a thin film. Samples were allowed to cool, 5mL of H_2O_2 was added, and the sample digests were heated at 100°C for 1 hour. Samples were removed from the hot block digestion apparatus and allowed to cool. All sample digests were analyzed by inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS).

Selenium Speciation by RP-ICP-CRC-MS: Approximately 0.1 g of each sample was transferred to a polypropylene centrifuge tube followed by the addition of 10 mL of ultra pure deionized water and enzymes. The solution was mixed with a sonic wand for 5 minutes. The resulting solutions were filtered and analyzed by RP-ICP-CRC-MS (reverse phase, inductively coupled plasma mass spectrometry using a collision reaction cell).

Sample Analysis: All sample analysis was precluded by a minimum of a five-point calibration curve spanning the concentration range of interest. Calibration curves were performed at the beginning of each analytical day. All calibration curves, associated with each species of interest, were standardized by linear regression resulting in the response factor.

All sample results were instrument blank-corrected to account for any operational biases associated with the analytical platform. Prior to sample analysis, all calibration curves were verified using second source standards identified as initial calibration verification standards (ICV). Ongoing instrument performance was identified by analysis of continuing calibration verification standards (CCV) and continuing calibration blanks (CCB) at a minimal interval of every ten analytical runs.

Total Selenium Quantification by ICP-DRC-MS: All samples for total selenium quantification were analyzed by inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) on April 19, 2012. Aliquots of each sample were introduced into a radio frequency (RF) plasma where energy-transfer processes cause desolvation, atomization and ionization. Ions were extracted from the plasma through a differentially-pumped vacuum interface and then they traveled through a pressurized chamber (DRC) containing a reactive gas which preferentially reacted with interfering ions of the same target mass to charge ratios (m/z). A solid-state detector detects ions transmitted through the mass analyzer, on the basis of their mass-to-charge ratio (m/z), and the resulting current is processed by a data handling system.

Selenium Speciation Analysis by RP-ICP-CRC-MS*:* Each sample for selenium speciation was analyzed by reverse phase chromatography inductively coupled plasma collision reaction cell mass spectrometry (IC-ICP-CRC-MS) on May 8, 2012. An aliquot of each sample was injected onto a reverse phase column and mobilized by a neutral pH gradient. The eluting selenium species were then introduced into a radio frequency (RF) plasma where energy-transfer processes cause desolvation, atomization, and ionization. The ions are extracted from the plasma through a differentially-pumped vacuum interface and travel through a pressurized chamber (CRC) containing a reaction gas which preferentially reacts with interfering ions of the same target mass to charge ratios (m/z). A solid-state detector detects ions transmitted through the mass analyzer and the resulting current is processed by a data handling system. Retention times for each eluting species were compared to known standards for species identification.

The methodology employed by Bill Maher of Canberra University:

This laboratory used a protease to extract the samples (0.1 g protease/ 0.2 g of sample), incubating them for 24 hours and then the Se species were separated using a PRP X100 anion exchange column. Detection was by ICP-MS.

VI. RESULTS

Speciation results from 'Applied Speciation Lab' and from Bill Maher of Canberra University are in Tables 1 and 2, respectively. We are not presenting the results or describing above the analytical methods employed by 'Frontier Global Sciences' because they had lower recoveries than did 'Applied Speciation' and they had poorer detection of Se-methionine which based on the literature should be a major Se species in TWG. However, this lab was very helpful in discussing the low recoveries and the potential reasons for them.

Applied Speciation Lab

This lab recovered (extracted) 45 – 60% and identified 31 – 47% of the total-Se measured in the forage samples. The difference between these two percentages is due to the presence of unidentified Se in the extract. **The major form of Se in the extractable portion was selenomethionine (Se-Met) which accounted for 21 to 31% of the total Se in the TWG samples**. This agrees with Whangher (2002) who

reported that Se-Met is the major selenocompound in cereal grains, grassland legumes and soybeans. The forages used in our studies (tall wheatgrass and creeping wildrye) are members of the same family as cereal grains (Poaceae).

Table 1. Se speciation results for tall wheatgrass samples submitted to Applied Speciation and Consulting, LLC (Bothell, Washington).

		Applied Speciation											
Sample #	code	Tot- Se mg/kg	Selenate (VI) mg/kg *	Selenite (IV) mg/kg	Se-Met mg/kg *	Methyl seleno cysteine mg/kg	Methyl seleninic acid (mg/kg)	Seleno methionine Oxide mg/kg	Unknown Se Species mg/kg	Extracted % of total	Identified % of total	Selenate % of total	Se-Met % of total
1	LSe/ LS/ 60-2	7.80	0.819	0.055	2.45	0.067	0.232	0.008	0.926	58.4%	46.6%	10.5%	31.4%
2	LSe/ HS/ 60-4	7.98	0.144	0.110	2.17	0.085	0.341	0.015	0.941	47.7%	35.9%	1.8%	27.2%
3	-duplicate of #2	8.51	0.152	0.099	2.18	0.077	0.371	0.011	0.883	44.3%	34.0%	1.8%	25.6%
4	HSe/ LS/ 60-3	15.90	2.49	0.155	3.34	0.421	0.793	ND	1.69	55.9%	45.3%	15.7%	21.0%
5	HSe/LS/ 60-2	15.50	1.71	0.154	4.06	0.193	1.04	0.023	2.05	59.5%	46.3%	11.0%	26.2%
6	HSe/ HS /60-3	19.10	0.283	0.177	4.38	0.427	1.18	ND	3.35	51.3%	33.8%	1.5%	22.9%
7	HSe/ HS/ 60-2	22.20	0.289	0.202	4.87	0.314	1.19	ND	3.2	45.3%	30.9%	1.3%	21.9%
8	TWG- dairy study	4.15	0.196	0.104	1.30	0.047	0.126	0.008	0.2	47.7%	42.9%	4.7%	31.3%
9	-duplicate of #8	4.12	0.191	0.099	1.20	0.048	0.12	ND	0.199	45.1%	40.2%	4.6%	29.1%

After Se-Met, the next most abundant species in most of the extracts was selenate, an inorganic form of Se, which accounted for 2 – 16% of extractable Se. Selenate represents Se that has not yet been assimilated (converted into organic form). This also agrees with Whanger (2002), who reported that selenate is the predominant inorganic form of Se in both plants and animals and that selenite is not found in in any biological materials at high levels. This result also agreed with reports in the literature (Gissel-Nielsen and Hamdy, 1977) that, in alkaline soils, inorganic Se occurs mainly as selenate which is rarely fixed in soil and more available for uptake by plants. In contrast, a low soil pH favors the selenite form, which is strongly fixed to soil clay particles and iron hydroxides. The soil in which the TWG was grown in the greenhouse was alkaline (pH 7.8), as was true for the TWG pastures in Panoche Water District where samples 8 and 9 were obtained.

For samples #2, 3, 6 and 7, methylseleninic acid, rather than selenate, was the second most abundant species. Methaneseleninic acid, has been shown to have potent anticancer activity (Zhao, 2004), having superior inhibitory activity *in vivo* against prostate cancer, compared to selenomethionine or selenite (ion) (Li, 2008), and it enhances the efficacy of paclitaxel for treatment of breast cancer in women (Qi, 2012). However, methylseleninic acid can be a breakdown product of Se-methylselenocysteine, and for our samples, its levels were highest in the samples with higher levels of methylselenocysteine. Hence, it is possible that this species formed during the sample digestion.

Bill Maher Canberra University

This researcher is very experienced with analyzing Se in sediment samples and he offered to extract and analyze Se from our TWG samples. Dr. Maher's recovery (% extraction) was slightly higher (60 – 72%) than that of 'Applied Speciation', but the % of total Se identified was very low (12- 26%). With his

extraction and detection procedures, Se-Met was the only form identified, and it accounted for 8 - 22% of total Se. Given that he identified a much lower % of the total Se, we feel that the results obtained by 'Applied Speciation', in which Se-Met accounted for 21 to 31% of the total Se in TWG, are more reliable. The procedure used by Dr. Maher to extract the samples was probably not as suitable for plant samples which have complex structural carbohydrates in their cell walls, as was the acid/peroxide digestion process used by Applied Speciation.

Table 2: Se speciation results for tall wheatgrass samples submitted to Dr. Bill Maher, Department of Environmental Chemistry, Canberra University, Australia.

Sample #	code	Tot- Se mg/kg	Se-Met mg/kg	Unknown Se Species mg/kg	Extracted % of total	Identified % of total	Se-Met % of total
1	LSe/ LS/ 60-2	8.16	1.77	0.36	60.0%	26.1%	21.7%
2	LSe/ HS/ 60-4	8.41	0.96	0.23	64.8%	14.2%	11.4%
3	-duplicate of #2	.	.	.	.	.	.
4	HSe/ LS/ 60-3	17.41	1.66	0.91	64.0%	14.8%	9.5%
5	HSe/LS/ 60-2	17.48	2.35	0.42	63.2%	15.8%	13.4%
6	HSe/ HS /60-3	20.23	1.68	0.75	65.2%	12.0%	8.3%
7	HSe/ HS/ 60-2	21.38	2.31	0.75	71.8%	14.3%	10.8%

VII. EVALUATION OF RESULTS

'Applied Speciation' recovered 45 to 60% of the total Se in the TWG samples which means that 40 to 55% of the Se is unaccounted. One explanation is that some of this 'missing Se' is in selenoproteins that were not degraded in the acid digestion and are retained on the columns and not eluted. 'Frontier Global Sciences' also tried a basic (alkaline) digestion, but this it did not substantially increase the recoveries.

The fraction labeled as 'unknown Se species' in both Table 1 and 2 may include selenoamino acids other than Se-methionine and methylselenocysteine. The separation protocols used by 'Applied Speciation' and 'Frontier Global Sciences' were meant for determination of these two species as well as selenate, selenite, selenocyanate and methylseleninic acid. Separation schemes could be devised for other species, but it would be very difficult to narrow them down without a generalized idea of what they are and because of limitations in obtaining reference materials.

Whanger (2002) reported that conjugation of Se to different amino acids affects both its bioavailability and metabolism. The most prevalent organic forms of Se consumed by ruminants are SeMet and SeCys, both of which occur in most normal animal feedstuffs at some level (Shrift, 1969; Peterson and Butler, 1962), but inorganic dietary supplements generally provide Se as selenite and selenate. Once Se from selenate or selenite is incorporated into an amino acid (*i.e.,* methionine or cysteine) it becomes an organic form, which is thought to be more readily absorbed by animals from the digestive tract (*i.e.,* has a higher bioavailability). Although results of our dairy cattle feeding study did not support a higher bioavailability

for the organic Se provided in TWG hay (as compared to the mineral supplement), we did find potential benefits of utilizing Se-enriched TWG hay as a bioavailable Se source in dairy cattle, including increased Se concentrations in whole blood and increased milk production as compared to no supplementation.

The National Research Council (NRC) has published guidelines on maximum tolerable concentration of Se for animal diets (2 ppm, beef cattle (1996), and 5-40 ppm having the potential for chronic toxicity for dairy cattle (2001)). Although Se is essential to animal metabolism, there are currently no guidelines regarding the optimum Se concentration in cattle diets in the United States. Only a legal limit on additional supplementation to the diet (over and above that in the feed ingredients) of 0.3 mg Se/kg DM (= ppm) has been issued by the FDA. This would apply to mineral supplements such as sodium selenite, but not to Se-enriched tall wheatgrass hay if incorporated into the total mixed ration (TMR) of dairy cattle. Care would need to be taken in order to supplement at beneficial and not toxic concentrations. However, the Se supplementation levels recommended by the NRC (2001) of 0.3 ppm may not be sufficient for optimal animal metabolism because in our dairy cattle feeding study, increased milk and milk fat production occurred in the cows fed both of our Se-supplemented diets (sodium selenite, 0.65 ppm and TWG, 0.53 ppm in the TMR). As milk production responses can only be expected in Se-deficient animals, these responses suggest that the higher diet Se levels are needed to optimize animal metabolism. This could legally be achieved by incorporating Se-enriched TWG hay into a TMR at a concentration greater than 0.3 ppm, but it could not be done with mineral supplements

VIII. SUMMARY AND CONCLUSIONS

Our TWG Se speciation indicates that in 'Jose' tall wheatgrass hay, the predominant form of extractable Se is selenomethionine (Se-Met) and the second most abundant form is selenate, an inorganic form. In some samples methylseleninic acid, rather than selenate, was the second most abundant species. Se-Met is a form of Se consumed by ruminants in normal feedstuffs and selenate, along with selenite, is often fed as a dietary supplement. Thus both of these Se species would be considered 'bioavailable' and neither should pose a hazard to ruminant animals if fed at appropriate concentrations. In the case of grazing animals, our study at Red Rock Ranch demonstrated that TWG and CWR forage containing up to 4 ppm total Se in the dry matter did not cause beef heifers to develop signs of Se toxicity and exposing non-pregnant, non-breeding cattle to these Se-enriched forages for a grazing season of 6 to 7 months would not result in death due to Se intoxication, or even negative impacts on animal metabolism. Weight gains were acceptable for the cattle as long as the forage availability and nutrient content was adequate in the TWG and micromineral levels were adequate in the cattle. Methylseleninic acid should also have high bioavailability given that it has been shown to increase the efficacy of a drug used to protect against breast cancer in women. With regard to the Se not recovered by our extraction procedures, it is likely that much of it is present in selenoproteins in the forage tissues. In the digestive tract of ruminants, these selenoproteins would be degraded into simpler forms such as Se-Met and selenate, making it unlikely that the Se in TWG that was not extracted and speciated would have negative impacts on ruminant animal health.

The reduction of biologically-active Se from the environment by immobilization into sediments in wetlands, or its incorporation into plant tissues used as ruminant feed is generally considered to reduce the potential for ecotoxicological effects of Se in the environment (Wu, 2004), particularly for aquatic organisms and waterfowl that have a greater risk of Se toxicity (Ohlendorf, 1986).

Forage production using Se-laden drainage waters for irrigation represents a viable option to translocate Se from areas in the SJV where it is present in concentrations that pose an environmental hazard and move it into animal feeds that have agronomic value or in the case of Se-enriched beef or milk, that may be beneficial to human health. In the case of substituting Se-enriched tall wheatgrass hay for sodium selenite supplements, this would reduce importation of 'new' Se into the eastern SJV in the form of dietary supplements for dairy cattle.

IX. FUTURE WORK

To identify a portion of what remains as the unknown species in our Se speciation work with 'Jose' tall wheatgrass, Dr. Joy Goto (Department of Chemistry at Fresno State) has agreed to work on improving the extraction procedure. This research does not have funding at present, but we would like to continue, perhaps as a thesis project for a master's degree student. Her work plan can be described as:

> Assuming the selenium in the forage sample is bound as a selenoprotein, I plan to use a combination of one-dimensional isoelectric focusing (1-D IEF), two-dimensional sodium dodecyl sulfate-poly acrylamide gel electrophoresis (2D SDS-PAGE), followed by laser ablation inductively coupled plasma-mass spectrometry (LA ICP-MS), and then finally with an high performance liquid chromatography with tandem mass spectrometry (HPLC with quadrupole MS-MS) methods. **The main idea is there are many proteins in the forage samples, and if we can first separate the sample to localize just the selenium-containing regions then we can narrow our focus to identify the selenium-bound proteins.**
>
> These various separation, isolation and mass instruments are used to first define the range of important selenium specific proteins in the forage samples. Forage samples will first be extracted with sonication in a neutral pH buffer and the solubilized material will be discarded. Then the final pellet will be extracted with detergent to release the insoluble or Se-bound proteins which will then be separated by isoelectric point (IEF range of pH range 3-10) followed by a second dimension separation using an SDS-PAGE gel to separate proteins based on molecular weight. This gel will then be stained with Coomassie blue and laser ablated to sample various spots for their selenium metal content (LA ICP-MS), to produce a 'map' of the selenium containing proteins. A second 2D forage sample gel (also Coomassie stained to identify the spots) will then be used to pick selenium-containing spots from the identical gel for analysis by HPLC MS-MS. This final mass spectrometry step will allow us to identify the proteins co-localizing, or perhaps even binding, to selenium.

The protocol and procedures are based on the research of Bianga, et al., (*Analytical Chemistry* 2013, 85:2037-2043) which used the same sequence of instruments to characterize selenium incorporated into wheat proteins. The key to this sequence of separations and identifications is using LA ICP-MS (capable of detecting metals in the attomole range (10^{-18} moles)), to narrow the region of interest to only the selenium-containing proteins. Bianga et al., report on analyzing wheat with only a 43 microgram/g (ppb) dry weight of Se, with 53% in the water-insoluble portion. Our preliminary metal content data also shows that ~50% of the total materials contain selenium in the insoluble portion of the extracted sample. If we utilize these same techniques, we will hopefully be able to identify the selenium-containing proteins in the TWG forage samples.

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XII. APPENDIX 1 – *publication of results of our grazing study*

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Grazing as an alternative for utilization of saline-sodic soils in the San Joaquin Valley: Selenium accretion and performance of beef heifers

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ABSTRACT

Two experiments were conducted to evaluate Se accumulation and health of non-pregnant, non-breeding beef cattle grazing on forages with a high Se content due to irrigation with saline drainage water. Heifers grazed experimental pastures of "Jose" tall wheatgrass (TWG; *Thinopyrum ponticum* var. "Jose") and creeping wildrye (CWR; *Leymus triticoides* var. "Rio") for190 days in Experiment 1 (2007) and for 165 days in Experiment 2 (2008). In experiment 1, mean Se concentrations were similar in TWG and CWR herbage (4.0 versus 3.7 ± 0.26 mg/kg dry weight; $p = 0.34$) as was crude protein (113 versus 114 ± 7.9 g/kg dry weight; $p = 0.94$). Concentrations of Se in blood increased by 300% during the grazing period, and were similar for heifers grazing the TWG or CWR pastures (0.94 versus 0.87 ± 0.03 mg/kg; $p = 0.89$). Heifers grazing on TWG gained more body weight than did heifers grazing on CWR (0.59 versus 0.27 ± 0.07 kg/days; $p < 0.01$). In experiment 2, concentration of Se (4.0 versus 2.8 mg/kg ± 0.19 mg/kg dry weight; $p < 0.01$) and crude protein (79 versus 90 ± 5.6 g/kg dry weight; $p < 0.01$) differed, for TWG and CWR, respectively. Within 20 days, Se concentrations in blood had increased by 300% and by nearly 200% in heifers grazing on TWG or CWR. All data cited are least square means ± standard error of the mean. Data from our two grazing seasons are consistent in demonstrating the safety of grazing beef cattle for a period of up to 6 months on TWG and CWR forages having high levels of Se due to irrigation with saline drainage water. This suggests that forage production using saline drainage water is a viable alternative for saline soils with limited potential for producing high value, salt-sensitive, crops.

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1. Introduction

The San Joaquin Valley of California is one of the most productive agricultural areas in the United States relying on intensive irrigation and fertilization in order to reach high crop yields. Soils from the western San Joaquin Valley typically have high levels of soluble salts, boron, and Se and their saline-sodic condition creates a chronic problem for adequate drainage to reduce salt accumulation in the root zone. Subsurface drainage systems were utilized for water table control prior to the 1980s, but the occurrence of embryonic deformities in fish and migratory waterfowl traced to Se in agricultural drainage water stored in the Kesterson reservoir, near Los Banos, CA (Ohlendorf et al., 1990; Ohlendorf et al., 1986) greatly curtailed the use of drainage systems for salinity and water table management in the region (Letey et al., 2002). Recognizing the importance of good soil drainage to minimize soil salinization and maintain agricultural productivity numerous strategies, including re-use for irrigation, have been tested to allow farmers to use subsurface drainage systems in an environmentally responsible manner (DWR, 2003).

Former work by this group identified salt-tolerant forages suitable for drainage water reuse systems in the western San Joaquin Valley, such as "Jose" tall wheatgrass (TWG; *Thinopyrum ponticum* var. "Jose") and creeping wildrye (CWR; *Leymus triticoides* var. "Rio") (Suyama et al., 2007a, 2007b; Grattan et al., 2004a). Although TWG can have high metabolizable energy under well-watered conditions (Suyama et al., 2007b), both of these forages are characterized by a high fiber content (560 to 650 g/kg dry weight) which reduces forage quality and lowers their market value. Utilization of TWG and CWR for grazing, however,

Abbreviations: ADF, acid detergent fiber; ADICP, acid detergent insoluble crude protein; aNDF and aNDF (om), neutral detergent fiber including residual ash and without residual ash, respectively; CP, crude protein; CWR, creeping wildrye; EC, electrical conductivity; ECe, electrical conductivity of a saturated soil paste extract; ICP-AES, inductively coupled plasma atomic emission spectrometry; Lignin(sa), lignin determination utilizing sulfuric acid; ME, metabolizable energy; NRC, National Research Council; SAR, sodium adsorption ratio; TWG, tall wheatgrass.

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Table 1
Chemical composition of the saline drainage water and tailwater used to irrigate the tall wheatgrass (TWG) and creeping wildrye (CWR) pastures in 2007 and 2008. Data are means ± standard error (S.E.) with n = the number of water analyses conducted.

Forage	Irrigation source	n	EC[a] (dS/m)	SAR[a]	B (mg/l)	Se (mg/l)	Mo (mg/l)	NO_3-N (mg/l)	Cl^- (mmol/l)	SO_4^{2-} (mmol/l)	Na^+ (mmol/l)	Ca^{2+} (mmol/l)	Mg^{2+} (mmol/l)
TWG	Drainage	2	6.9	11.9	9.3	0.098	0.033	131	22.4	25.9	48.6	12.9	3.7
	S.E.		(0.4)	(0.1)	(1.7)	(0.03)	(SS)	(SS)	(0.5)	(5.9)	(2.2)	(0.6)	(0.7)
	Tailwater	5	1.8	8.2	2.3	0.011	0.028	0.6	6.78	5.5	13.5	2.2	0.6
	S.E.		(0.3)	(0.9)	(0.4)	(0.005)	(0.012)	(0.3)	(0.9)	(1.0)	(2.2)	(0.4)	(0.1)
CWR	Drainage	4	7.7	13.5	12.0	0.144	0.030	131	25.9	30.2	55.9	12.9	4.2
	S.E.		(0.5)	(0.9)	(1.6)	(0.032)	(SS)	(SS)	(2.1)	(3.6)	(4.5)	(0.5)	(0.4)
	Tailwater	6	2.0	7.9	2.3	0.012	0.028	1.2	7.22	6.0	13.9	2.7	0.7
	S.E.		(0.2)	(0.7)	(0.2)	(0.003)	(0.006)	(0.7)	(0.5)	(0.6)	(1.1)	(0.4)	(0.1)

[a] Water pH for the drainage and tailwater was 7.5 to 7.7; SS, single sample; EC, electrical conductivity.

would reduce the costs of hay cutting and processing while harvesting the value of these salt-affected lands as beef.

Forages grown in saline soils from the western San Joaquin Valley are frequently high in Se, Mo and S and simultaneously, can have very low concentrations of Cu and Mg (Grattan et al., 2004b; Suyama et al., 2007b) relative to the requirements of growing beef cattle (NRC, 2000). Because of the mineral imbalances in these forages, serious issues related to toxicity, nutrient imbalance and cattle health have been raised, particularly Se toxicosis, hypomagnesemia, and Cu deficiency (Grattan et al., 2004b). Questions were also posed by local beef farmers regarding the utilization and suitability of these forages as the major source of feed for cattle.

The primary objective of this 2-year study was to determine the impacts of grazing salt tolerant forages containing high concentrations of Se (i.e., > 2 mg/kg dry weight) on beef cattle performance and accretion of Se into blood and liver tissues. The secondary objectives were to determine body weight gain and Se incorporation into muscle tissue, with the latter only evaluated during the second grazing season (2008).

2. Materials and methods

2.1. Experimental site, soils, and forage swards

Two experiments, Experiment 1 in 2007 and Experiment 2 in 2008, were conducted at Red Rock Ranch, a commercial farm located in the Westlands Water District about 10 km southwest of the town of Five Points in Fresno County (CA, USA). The grazing areas utilized were part of an Integrated On-farm Drainage Management demonstration project that started in 1995 with the objective of sequentially re-using saline, subsurface drainage water to reduce its volume prior to final disposal in solar evaporation systems (Jacobsen and Basinal, 2004; Suyama et al., 2007b).

Two adjacent ~9 ha pastures of "Jose" tall wheatgrass (TWG; *Thinopyrum ponticum* var. "Jose") and creeping wildrye (CWR; *Leymus triticoides* var. "Rio") were utilized for grazing in both the 2007 and 2008 seasons. These were the same areas utilized in a previous study (referred as "TWG1" and "CWR1") in which the forages were cut for hay and evaluated for biomass yield, quality, and mineral composition under irrigation with saline drainage water (Suyama et al., 2007b). The pasture planted to CWR was in its fifth year and the TWG pasture in its eighth year of irrigation with saline drainage water when this grazing study began. At this time, however, irrigation water supplies were drastically reduced in this area due to drought and environmental restrictions, thus the irrigation water applied during this study was a combination of saline drainage water and low salinity tail water (surface run-off collected and recycled). The chemical characteristics of both of these irrigation waters and the soils in the TWG and CWR pastures in 2007 and 2008 are shown in Tables 1 and 2.

In the current study, each pasture was divided into four paddocks which were further divided into north and south sub-paddocks that were rotationally grazed at 14 day intervals for most of the season. However at the end of the grazing season, shorter (7 days) or longer (21 days) grazing cycles were used to maximize the utilization of available forage biomass. Throughout the grazing seasons, the TWG and CWR paddocks were irrigated approximately 28 days before heifers were moved into that paddock for grazing.

2.2. Cattle and grazing management

Two groups of 20 Angus heifers were utilized to rotationally graze the experimental pastures in Experiment 1 (2007) and Experiment 2 (2008). In each experiment, the heifers were divided into four groups of 5 animals based on initial body weight with the objective of having groups that were as homogenous as possible in average body weight, yet large enough to support group grazing dynamics. Two groups of 5 heifers were then randomly selected to graze either the TWG or CWR pasture in each experiment. Within a pasture, one group of 5 heifers grazed the northern set of sub-paddocks and the other group simultaneously grazed the southern set. Grazing pressure in both experiments was ~1.1 heifers/ha for each pasture. The heifers were de-wormed ~3 weeks before the grazing season began in both 2007 and 2008. During the spring and summer months the heifers were treated three times with 10 to 15 ml of a pour-on insecticide formulation containing 1% lambdacyhalothrin (Saber™ Pour-On, Schering-Plough Animal Health Corporation, Union, NJ, USA)[1] to control horn flies. Heifers that developed clinical signs of pink eye were treated with an oxytetracycline solution (Liquamycin® LA-200, Pfizer Animal Health, Exton, PA, USA) injected intramuscularly at 20 mg/kg of body weight which was repeated after 72 h.

2.2.1. Experiment 1 (2007)

The 20 Angus heifers, approximately 6 months of age, were purchased from local farmers in the San Joaquin Valley. The two groups of 5 heifers assigned to the CWR pasture initially weighed 205 ± 49.9 and 185 ± 28.4 kg, whereas TWG heifers weighed 187 ± 27.7 and 188 ± 31.8 kg (mean ± standard deviation). All heifers received a copper bolus (Copasure boluses 12.5 g, Walco International, Inc., Westlake, TX, USA) 3 weeks before grazing was initiated to avoid Cu deficiency in their diet. Four heifers died during 2007 due to heat stress and exhaustion when they inadvertently escaped from the grazing site. Four additional heifers were then purchased in order to maintain the same grazing pressure and group dynamics, but their body mass, blood and liver measurement data were not included in the analysis since their exposure to high selenium forage was shorter in duration. The grazing season began on May 27th 2007 for both the CWR and TWG pastures.

[1] Mention of company names or products is for the benefit of the reader and does not imply endorsement by the University of California or California State University, Fresno.

Table 2
Soil salinity (ECe), sodium adsorption ratio (SAR) and ion composition for the 0–45 cm soil depth in tall wheatgrass (TWG) and creeping wildrye (CWR) pastures grazed by beef heifers in 2007 and 2008. Data[a] are means ± standard error of the mean (SEM).

Forage	Year	ECe[b] (dS/m)	pH	SAR[b]	B (mg/l)	Total Se (mg/l)	Total Mo[c] (mg/l)	Cl^- (mmol/l)	SO_4^{2-} (mmol/l)	Na^+ (mmol/l)	Ca^{2+} (mmol/l)	Mg^{2+} (mmol/l)
TWG	2007	22.2	8.3	50.9	31.6	2.98	1.31	84.5	106.4	240.0	12.4	9.4
	SEM	(1.2)	(0.02)	(2.4)	(1.7)	(0.12)	(0.04)	(7.3)	(4.9)	(14.3)	(0.1)	(0.6)
TWG	2008	14.5	8.3	34.4	24.8	3.99	–	40.0	75.6	148.3	12.0	6.5
	SEM	(1.0)	(0.03)	(2.6)	(2.0)	(0.30)	–	(6.6)	(4.3)	(12.1)	(0.4)	(0.3)
CWR	2007	15.5	8.3	36.0	25.4	1.86	0.94	52.8	72.8	161.2	13.6	6.4
	SEM	(0.5)	(0.03)	(1.5)	(1.0)	(0.05)	(0.02)	(3.3)	(2.6)	(7.7)	(0.2)	(0.2)
CWR	2008	13.1	8.3	30.7	21.8	2.24	–	30.8	69.7	131.5	12.0	6.1
	SEM	(0.8)	(0.05)	(2.0)	(1.0)	(0.04)	–	(3.6)	(4.7)	(10.9)	(0.4)	(0.5)

[a] n = 16.
[b] ECe, electrical conductivity of the saturated soil paste extract; SAR, sodium adsorption ratio.
[c] "–" not measured.

2.2.2. Experiment 2 (2008)

Twenty-three Angus heifers that were approximately 8 months of age were purchased from local farmers in Northern California (USA). Twenty heifers were then selected based on uniformity of their body weight to be utilized in the study. The two groups of 5 heifers assigned to the CWR pasture initially weighed 273 ± 27.1 and 265 ± 36.1 kg, whereas the TWG heifer groups initially weighed 280 ± 24.5 and 277 ± 21.8 kg (mean ± standard deviation). The heifers were moved to the grazing areas on May 9th and 17th for the CWR and TWG pastures, respectively.

2.3. Sample collection and analysis

2.3.1. Water and soil

Several times during each season, irrigation water samples were collected for chemical analysis (Table 1). The samples were kept cold (4 °C) and processed for analysis within 24 h. Soil samples (0–45 cm depth) were taken twice each year (August and November in 2007 and April and November in 2008). For each pasture, a composite sample consisting of four cores was taken from each of the eight sub-paddocks.

For the irrigation water samples, salinity was measured using an electrical conductivity (EC) meter (YSI Model 3100 Conductivity system, Yellow Springs, OH, USA) and pH using a standard pH meter (UB-5 Ultra basic benchtop, Denver Instrument, Bohemia, NY, U.S.A). The samples were then filtered through a 0.22 μm pore size, nylon filter prior to chemical analysis and the portion used for analysis of Na^+, Ca^{2+}, Mg^{2+}, Se and Mo was acid-fixed using 1 ml of 70% nitric acid. Chloride, SO_4^{2-} and NO_3^- were measured on the non-acidified portion using a Dionex ICS-2000 ion chromatograph (Dionex, Sunnyvale, CA, USA) according to EPA method 300.0. Sodium, Ca^{2+}, Mg^{2+} and B were measured using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES; Perkin Elmer Optima 4300 DV, Waltham, Massachusetts, USA) according to EPA method 200.7. Se and Mo were quantified using an ICP-Mass Spectrometer (Perkin Elmer ELAN DRC-e) according to EPA method 200.8 (EPA, 1994). The water analysis was conducted at the California Department of Water Resource's Bryte Laboratory utilizing standard operating procedures developed by the U.S. Environmental Protection Agency (EPA) which also comply with the California Department of Public Health's Environmental Laboratory Accreditation Program.

Soil samples were air-dried and ground to pass a 1 mm screen. Saturated soil pastes were prepared with deionized water and allowed to stand overnight prior to vacuum filtration. The filtrate (i.e. paste extracts) represented the water-soluble fractions of ion constituents in the soil solution. Soil salinity (ECe) and pH were measured on the paste extracts. The paste extracts were then filtered using 0.22 μm syringe filters. The portion used for analysis of Na^+, Ca^{2+}, and Mg^{2+} was acid fixed and analyzed as described for the water samples. Chloride, SO_4^{2-} and B were also measured on the saturated paste extracts using the method described for water samples. For each submittal of samples, two blanks (non-filtered and filtered) consisting of the deionized water used for cleaning the filtration apparatus were included, along with a standard reference for each analyte.

A sub-sample of soil was oven-dried at 55 °C for 48 h to measure total Se and total Mo. For total Se, a wet digestion of organic forms using nitric, perchloric and sulfuric acid was done after which the inorganic forms were quantified using ICP-AES (Tracy and Moeller, 1990). For total Mo, microwave digestion with nitric acid and hydrogen peroxide was used with quantitative determination by ICP-AES. The analysis of total Se and Mo was conducted at the University of California Davis Analytical Laboratory (2010). A reagent blank was analyzed with every set of samples and a duplicate was analyzed at every tenth sample. At least one standard reference material was analyzed with each set of samples.

2.3.2. Forage sampling and determination of available forage biomass

In Experiment 1, a "frame" method was utilized (Vendramini et al., 2008), in which a 0.5 m^2 square was thrown in 4 locations of each sub-paddock to create 4 forage samples per sub-paddock. In each sample area, forage was cut and weighed fresh, and then dried at 55 °C in a forced air oven for dry weight determination. Available forage biomass prior to grazing was calculated based on the total area of each sub-paddock and the average dry weight of forage per m^2 as estimated by this "frame" method. The procedure was repeated before the heifers were moved to a new sub-paddock, which resulted in a total of 7 to 8 replicates of forage biomass in each sub-paddock to estimate CWR and TWG pasture chemical composition during the 2007 grazing season. In addition, the procedure was repeated at the end of each grazing cycle in each sub-paddock in order to estimate residual biomass and intake by animals in each pasture due to grazing.

Due to the extremely heterogeneous nature of these pastures in 2007 and concerns with the efficacy of the "frame" method to estimate forage biomass, the method was changed in 2008 (i.e., Experiment 2). In this procedure, each sub-paddock was systematically walked through before grazing and visually scored every 10 m, on a two-dimensional 10 m grid, by two scorers which yielded 70 to 90 individual scores per sub-paddock. The scores, which ranged from 0 to 3 at 0.25 unit increments, represented the relative abundance of forage (i.e., 0 = no forage, 1 = lowest forage, 2 = medium forage, and 3 = highest forage abundance) at each scoring site. Once the scores were determined, the 0.5 m^2 frame was used twice in each score area (1, 2 and 3) to generate six forage samples per sub-paddock for each measurement period. These samples were weighed fresh and then dried. A regression equation plotting forage dry weight per m^2 by score was constructed in which the intercept of the regression line was forced through zero, as a zero score represented sites with no biomass. Utilizing these regressions, the total dry weight of forage per sub-paddock prior to grazing was calculated. A total of 12 and 16 estimates of forage biomass before grazing were generated during 2008 (i.e., Experiment 2) for the CWR and TWG pastures, respectively.

2.3.3. Forage quality and mineral analysis

After drying, the forage samples taken for available biomass determination were ground to pass a 1 mm screen on a model 4 Wiley Mill. Moisture content in the forage was determined gravimetrically by

measuring the weight difference before and after heating samples to 105 °C in a forced air oven for 2 h (Reuter et al., 1986). Neutral detergent fiber analysis was performed with addition of sodium sulphite and a heat stable amylase (Van Soest et al., 1991) and expressed including residual ash (aNDF) and without residual ash (aNDFom). Acid detergent fiber (ADF) was determined according to methods of the Association of Official Analytical Chemists (AOAC, 2000; #973.18) and is expressed including residual ash. Lignin (sa) was determined by the sulfuric acid procedure (Robertson and Van Soest, 1981). Ash was determined as the gravimetric residue after heating to 550 °C for 8 h. Total fat in the forage was determined by extraction with ether (AOAC, 2000; #930.39). The fatty acid profile was then determined by direct methylation utilizing 10% methanolic HCl (Palmquist and Jenkins, 2003). Methyl esters of fatty acids were separated in a Hewlett Packard 5890 gas chromatograph equipped with a 100 m capillary column (0.32 mm, 0.20 mm film thickness; Supelco 2560, Supelco Inc., Bellefonte, PA, USA), utilizing hydrogen as the carrier gas. Gas chromatographic conditions and standards were as described in DePeters et al. (2001).

The P, S, Ca, Mg, Na, B, Zn, Mn, Fe, Cu, Co and Mo concentrations in dried forage samples were determined utilizing nitric acid/hydrogen peroxide microwave digestion and atomic absorption spectrometry (AAS) or ICP-AES (Sah and Miller, 1992; Meyer and Keliher, 1992). Total K and Cl were extracted using 20 g/l acetic acid with K determined by atomic emission spectrometry (AES; Johnson and Ulrich, 1959) and Cl by a chloridometer (Johnson and Ulrich, 1959). Total Se was extracted by nitric/perchloric acid digestion/dissolution and determined by vapor generation using ICP-AES (Tracy and Moeller, 1990). The total N and acid detergent insoluble crude protein (ADICP) levels were determined with a nitrogen gas analyzer utilizing induction furnace and thermal conductivity (LECO FP-528; AOAC (2000) #990.03). For the determination of NO_3-N, forage samples were extracted in acetic acid followed by reduction to nitrite in a copper/cadmium column (Carlson et al., 1990). Analyses were conducted at the University of California Davis Analytical Laboratory (2010) with quality control procedures as described for total Se and total Mo in soil.

The energy status of the forage material was also assessed. *In vitro* gas production was assayed in graduated glass syringes where 200 mg of ground forage tissue were incubated with 30 ml of buffered rumen fluid (Menke and Steingass, 1988). The amount of gas produced during the first 24 h was recorded and corrected for blank incubation (i.e., buffered rumen fluid without forage sample). Metabolizable energy values were estimated using 24 h *in vitro* gas values combined with crude protein and fat content (Menke and Steingass, 1988). However, as these plants contain substantial quantities of ether soluble material which is not fatty acids, the equation of Menke and Steingass (1988) noted above, was modified by replacing the assayed ether extract level with the assayed fatty acid level (g/kg dry weight) plus 10 g/kg, as suggested by NRC (2001).

2.3.4. Animal body weight, sampling and analysis of blood, liver and muscle

Animal weight gains were determined at various times by measuring their biomass. Heifers were weighed at 40 days prior and 139, 155, 159 and 190 days after grazing began in 2007 and at 25 days prior and 20, 45, 70, 91, 134 and 165 days after grazing began in 2008.

Blood samples were also collected at various times. One whole blood sample from the jugular vein was collected at each sampling time and dispensed into two evacuated tubes (Vacutainer®, Becton Dickinson, Franklin Lakes, NJ, USA) for collection of whole blood (BD Hemogard™ Lavender, 10.8 mg of K_2EDTA) and serum (BD Hemogard™ Royal Blue). Serum was separated from whole blood through centrifugation at 1500 × *g* for 10 min at 5 °C, after which it was removed with a plastic pipette and transferred into a plastic microtube. Whole blood and serum were stored at 5 °C until submission for analysis to the California Animal Health and Food Safety Laboratory (Davis, CA, USA) no later than 72 h after collection. In Experiment 1, blood was drawn at 40 days prior and 55, 95, 135 and 190 days after grazing began. In Experiment 2, blood was drawn at 25 days prior and 20, 45, 70, 91, 134 and 165 days after grazing began and serum was separated for the samples taken at 25 days prior and 91 and 165 days after grazing. All heifers were individually inspected at each blood sampling in both years for occurrence of deformed hoofs, alopecia, and diarrhea.

Samples of liver were collected utilizing a biopsy needle (Sontec Instruments, Inc., Englewood, CO, USA). Hair on the right flank between the 12th and 10th rib was trimmed with a 0.2 mm blade (Golden A5 veterinary clipper, Oster, McMinnville, TN, USA) and cleaned with a paper towel. The area was then evaluated with ultrasound unit equipped with a 3.5 MHz convectional mechanical probe (WED-200A, Well.D Electronics Co., Ltd, Shenzhen, China) to identify the limits of the liver, avoid major blood vessels and determine liver thickness. This information was then used to choose the best incision site which in most cases was the 11th intercostal space. The skin was thoroughly disinfected with a scrub solution of 7.5% povidone-iodine (Purdue Products L.P., Stanford, CT, USA) for approximately 3 min, rinsed with water and then with 700 ml/l alcohol in water. A local anesthesia of skin and muscle layer was performed with 2% lidocaine HCl (First Priority, INC., Elgin, IL, USA), and the area was disinfected again with a solution of 10% povidine-iodine for 3 min, followed by 700 ml/l alcohol in water. An incision of approximately 4 cm was made through the skin and the biopsy needle was utilized to puncture the muscular layers and peritoneum to enter the abdominal cavity cranio-ventrally towards the left elbow until it reached the liver. The incision was subsequently closed with an interrupted cruciate suture pattern. On average, 1 to 3 punctures were necessary to obtain 100 to 300 mg of liver tissue. Liver tissue was individually stored in plastic microtubes and kept on ice until submittal within 72 h of collection for chemical analysis at the California Animal Health and Food Safety Laboratory (University of California, Davis, CA, USA). All heifers received one intramuscular injection of penicillin (22,000 IU / kg of BW) 20 min before each biopsy. Liver samples were collected at 40 days prior and 190 days after the onset of grazing in 2007 and at 25 days prior and 91 and 165 days after grazing in 2008. In order to compare our measurements expressed on a wet weight basis to literature values reported on dry weight basis, a liver moisture content of 500 mg/g was utilized for correction. Seventeen samples of liver from heifers at 190 days of grazing in Experiment 1 were submitted for histopathological examination at the California Animal Health and Food Safety Laboratory (University of California, Davis, CA, USA).

Muscle biopsies from the semitendinosus muscle were performed in the right rear leg following the same procedure described for liver, except that muscle samples were collected utilizing a scalpel. Two parallel incisions about 6 cm long, 1 cm apart, and 2 cm deep, were made in the semi-membranous muscle. The muscle tissue was clamped with a curved hemostatic forceps, the dorsal and ventral extremities of the muscle fibers were cut, the sample was removed, and the incision was sutured with a simple continuous suture pattern. Samples of muscle tissue were processed and stored as described for liver tissue. Muscle samples were collected at 25 days prior and 91 and 165 days after the onset of grazing in 2008 only.

Liver and muscle tissues were digested with nitric acid at 180 °C and subsequently analyzed for Pb, Mn, Ca, Cu, Fe, Zn, Mo, Ar and Hg by ICP-AES (FISONS, Accuris Model, Thermo Optek Corporation, Franklin, MA, USA) according to Martin et al. (1987). After precipitation of proteins in serum samples, protein free supernatants were analyzed simultaneously for Zn, Cu, Fe, Mn, Na, P, Ca and K as described by Melton et al. (1990) utilizing ICP-AES. For Se determination, the liver and muscle tissues were digested in a solution of nitric, sulfuric and perchloric acids at 350 °C, followed by reduction with 5 M HCl at 95 °C and quantified by hydride vapor generation using ICP-AES (FISONS, Accuris Model, Thermo Optek Corporation, Franklin, MA, USA) (Tracy and Moeller, 1990). Whole blood Se concentrations were measured by ICP-AES, as described previously (Tracy and Moeller, 1990).

A certified standard reference sample, dogfish liver (National Research Council of Canada, DOLT-4) and lobster hepatopancreas

(National Research Council of Canada, TORT-2), and 2 mg/kg overspikes of tissue were utilized to determine ICP accuracies for quantification of Se, Pb, Mn, Ca, Cu, Fe, Zn, Mo, Ar and Hg. Readings that were within 2 standard deviations of the certified reference values and recoveries within 80–120% were required for a valid run. Accuracy of ICP determinations of trace minerals in serum was determined with standard reference sera obtained from the Veterinary Laboratory Association Quality Assurance Program (Genzyme Diagnostics, Blaine, MD, USA). Readings within two standard deviations of the reference values were necessary for a valid batch of serum determinations.

2.4. Statistical analysis

Data sampled over time, such as blood or serum, but not body weight, were analyzed as repeated measures utilizing the PROC MIXED procedure of SAS (2009) with the experimental unit (heifers) nested within sub-paddocks. The statistical model included effects of heifer, time, forage and the forage by time interaction. Sub-paddock was the experimental unit for forage chemical composition data, and the statistical model included effects of forage, sub-paddock, time and the forage by time interaction. For forage composition data, the variable "time" was used to identify forage samples collected at the beginning and end of the grazing season in each year. Forage samples collected until May 21st, 2007 or June 27th, 2008 were categorized as "early" in Experiments 1 and 2, respectively; whereas forage samples collected after those dates were categorized as "late" grazing season. Differences with $p \leq 0.05$ were considered significant whereas $0.05 < p \leq 0.10$ were considered to be a tendency toward a difference. All data reported are least square of the means ± standard error of the mean, unless stated otherwise.

3. Results

Although we tried to estimate the voluntary dry matter intake by the heifers, the extreme heterogeneity of forage biomass in the field made this assessment impossible. Therefore neither the biomass frame-method used in 2007 nor the biomass ranking-method in 2008 were adequate to estimate biomass intake. Nonetheless, the heifers grazed the experimental pastures from May to November (190 days) in 2007 and from May to October (165 days) in 2008. In neither experiment were clinical signs of Se toxicity such as loss of tail hair or abnormal growth of the hoof observed. Se accretion due to intake of soil was considered to be negligible in the present study. Although it represents a true aspect of the interface between the soil, plant and animal, it is not one that can be interpreted independently.

3.1. Forage composition

In Experiment 1, chemical composition of CWR and TWG was similar (Table 3A) for ether extract, fatty acids, lignin (sa) and crude protein contents. No interaction between forage and stage of grazing season (early or late) occurred for any of the analytes, indicating that forage chemical composition was relatively constant throughout the grazing period. However, fiber content and metabolizable energy differed between forage types, independent of the stage of the grazing season. CWR had higher ($p<0.01$) neutral detergent fiber (675 versus 600 ± 5.8 g/kg dry weight), acid detergent fiber (371 versus 340 ± 4.0 g/kg) and lower metabolizable energy (6.6 versus 7.6 ± 0.09 MJ/kg). The mineral content differed between forages (Table 3B), where P (1.21 versus 1.45 ± 0.05 g/kg), Mg (1.1 versus 1.5 ± 0.05 g/kg), S (2.4 versus 5.0 ± 0.41 g/kg), and Na (2.5 versus 15.1 ± 2.54 g/kg dry weight) were lower ($p<0.01$) for CWR as compared to TWG. In contrast, the content of Zn (30.6 versus 19.1 ± 0.41 g/kg) and Cu (6.8 versus 5.5 ± 0.22 g/kg dry weight) were higher ($p<0.01$) for CWR than for TWG, Se, however, was similar ($p=0.34$) for CWR and TWG (3.7 versus 4.0 ± 0.26 g/kg dry weight, respectively).

In Experiment 2, forage chemical composition was highly affected by stage of the grazing season (early or late; Table 3C), particularly for crude protein, metabolizable energy and Se. The metabolizable energy was lower ($p<0.01$) for CWR as compared to TWG (5.8 versus 6.8 ± 0.13 MJ/kg dry weight, respectively) during the early grazing season, but it was similar ($p>0.10$) to TWG at the end of grazing season in 2008 (6.2 versus 6.5 ± 0.13 MJ/kg dry weight, respectively; Table 3C). The level of crude protein was low for both CWR and TWG during 2008 (less than 100 g/kg dry weight at beginning of grazing) and it decreased ($p<0.01$) in TWG pastures to as low as 63.5 g/kg dry weight towards the end of grazing, likely due to reduced irrigation with the saline drainage water which normally carries high levels of NO_3-N. The content of neutral detergent fiber (683 versus 642 dry weight for CWR and TWG, respectively, Table 3A) was not affected by stage of grazing and was consistently higher for CWR as compared to TWG forage. Concentrations of K, B, Zn and Cu varied between stages of the grazing season (Table 3C) with B being lower and K, Zn, and Cu being higher in CWR forage as compared to TWG. The Se level was consistently higher ($p<0.05$) in TWG forages (4.0 mg/kg dry weight) throughout the grazing season in 2008, but Se concentration in CWR

Table 3A
Organic chemical composition (dry weight basis) of the forages creeping wildrye (CWR) and tall wheatgrass (TWG) during the grazing seasons of 2007 and 2008. Data are least square means ± standard error of the mean (SEM).

	Experiment 1 (2007)[a]					Experiment 2 (2008)[a]				
	CWR	TWG	SEM	Forage[a]	F*time[a]	CWR	TWG	SEM	Forage[a]	F*time[a]
n	14	16	–	–	–	60	66	–	–	–
Ether extract, g/kg	43.5	42.4	0.02	0.73	0.08	–	–	–	–	–
Fatty acids, g/kg	15.0	14.4	0.87	0.63	–	–	–	–	–	–
aNDF, g/kg[b]	675	600	5.82	< 0.01	0.17	683	642	6.9	< 0.01	0.13
aNDFom, g/kg[b]	661	586	5.9	< 0.01	0.17	672	630	7.4	< 0.01	0.10
ADF, g/kg[c]	371	340	4.0	< 0.01	0.13	–	–	–	–	–
Lignin(sa), g/kg[d]	43.0	41.5	2.00	0.59	0.96	–	–	–	–	–
24h gas, ml/g	135	169	3.0	< 0.01	0.48	116	141	3.5	< 0.01	< 0.01
ME, MJ/kg[e]	6.65	7.61	0.088	< 0.01	0.45	6.05	6.66	0.118	< 0.01	< 0.01
CP, g/kg[f]	114.3	113.5	7.92	0.94	0.90	90.2	78.8	5.62	0.17	< 0.01
ADICP, g/kg CP[f]	92.0	69.3	2.98	< 0.01	0.62	–	–	–	–	–

[a] *p*Values for the effects of forage and the interaction of forage by time; "–," not applicable.
[b] aNDF and aNDF (om), neutral detergent fiber including residual ash and without residual ash, respectively. BW, body weight; CP, crude protein.
[c] ADF, acid detergent fiber.
[d] Lignin(sa), lignin determination utilizing sulfuric acid.
[e] ME, metabolizable energy.
[f] CP, crude protein; ADICP, acid detergent insoluble crude protein.

Table 3B
Concentration of minerals in the forages (dry weight basis) creeping wildrye (CWR) and tall wheatgrass (TWG) during the grazing seasons of 2007 and 2008. Data are least square means ± standard error of the mean (SEM).

	Experiment 1 (2007)					Experiment 2 (2008)				
	CWR	TWG	SEM	Forage[a]	F*time[a]	CWR	TWG	SEM	Forage[a]	F*time[a]
Samples (n)	14	16	–	–	–	60	66	–	–	–
Ash, g/kg	68.6	102.3	5.84	< 0.01	0.20	74.6	84.5	3.8	0.09	0.07
Ca, g/kg	3.12	3.12	0.01	0.98	0.16	2.29	2.15	0.16	0.53	0.45
P, g/kg	1.21	1.45	0.05	< 0.01	0.88	1.20	1.09	0.08	0.31	0.33
K, g/kg	17.4	13.0	1.14	0.02	0.20	21.4	11.0	0.71	< 0.01	< 0.01
Mg, g/kg	1.10	1.52	0.05	< 0.01	0.29	0.92	0.97	0.05	0.47	0.75
S, g/kg	2.40	5.02	0.41	< 0.01	0.35	2.39	2.88	0.16	0.06	0.30
Na, g/kg	2.48	15.1	2.54	< 0.01	0.13	–	–	–	–	–
Cl, g/kg	9.64	19.1	2.34	0.02	0.12	–	–	–	–	–
B, mg/kg	185	480	67	< 0.01	0.38	337	558	42	< 0.01	< 0.01
NO_3 N, mg/kg[b]	80.7	181.6	39.2	0.08	0.25	247	143	17	< 0.01	–
Zn, mg/kg	30.6	19.1	0.41	< 0.01	0.92	22.9	13.8	1.06	< 0.01	< 0.01
Mn, mg/kg	34.2	38.6	2.64	0.26	0.23	36.8	36.6	2.15	0.95	0.68
Fe, mg/kg	3078	262	13	0.03	0.26	279	279	18	0.97	0.02
Cu, mg/kg	6.76	5.51	0.22	< 0.01	0.19	6.65	4.12	0.35	< 0.01	< 0.01
Se, mg/kg	3.68	4.05	0.26	0.34	0.38	2.82	3.97	0.19	< 0.01	< 0.02
Mo, mg/kg	1.15	3.56	0.36	< 0.01	0.27	–	–	–	–	–

[a] *p*Values for the effects of forage and the interaction of forage by time; "–," not applicable or not determined.
[b] NO_3-N, nitrate nitrogen.

did increase ($p<0.05$) from the beginning to the end of grazing season (2.4 versus 3.2 ± 0.21 mg/kg dry weight).

3.2. Blood, tissue composition and animal performance

Upon entering the pastures in 2007, Se concentrations were similar ($p>0.60$) in blood (0.15 versus 0.16 ± 0.039 mg/g wet weight; Fig. 1) and liver (0.40 versus 0.37 ± 0.044 mg/g) for CWR and TWG grazed heifers. Likewise in 2008, initial Se concentrations in blood (0.095 mg/g; Fig. 1), liver (0.23 mg/g; Fig. 2), and muscle (0.06 mg/g; Fig. 3) tissues were similar ($p>0.70$) for CWR and TWG grazed heifers.

Accumulation of Se in blood occurred quickly in both years. In 2008, after only 20 days of grazing, Se concentrations in whole blood increased more than three fold in heifers grazing TWG and almost twofold in heifers grazing CWR, as compared to blood Se 25 days prior to grazing (Fig. 1). About 50 days after grazing, the Se concentration in whole blood was higher ($p<0.01$) for heifers grazing TWG forages in both the 2007 (0.67 versus 0.48 ± 0.040 mg/g) and 2008 (0.73 versus 0.37 ± 0.024 mg/g) years. In 2007, after 190 days of grazing, the concentration of Se in whole blood was similar ($p>0.10$) between heifers that grazed TWG and CWR forages (0.94 versus 0.87 ± 0.042 mg/g); whereas in 2008, after 165 days of grazing, the Se concentration was considerably higher for TWG than CWR-grazed heifers (1.2 versus 0.8 ± 0.024 mg/g).

Accumulation of Se in liver and muscle showed a pattern that was similar to that which occurred for whole blood. In these tissues, there was an increase in Se concentration with increased exposure time of animals to high Se forages. Concentrations of Se in wet liver tissue were one- to threefold higher than concentrations in blood at the end of grazing. Heifers that grazed CWR had almost twice as much Se in liver

Table 3C
Metabolizable energy (ME) and selected chemical compounds (dry weight basis) in forages during Experiment 2 (2008) according to time of grazing. Data are least square means ± standard error of the mean (SEM).

	Early grazing			Late grazing			
	CWR	TWG	SEM	CWR	TWG	SEM	F*time[c]
ME, MJ/kg DM	5.8[b]	6.8[a]	0.13	6.2	6.5	0.13	< 0.01
CP, g/kg	93.2	94.1	6.0	87.3[A]	63.5[B]	6.0	< 0.01
K, g/kg	2.3[a]	1.4[b]	0.08	2.0[a]	0.8[b]	0.08	< 0.01
B, mg/kg	242	363	48	433[b]	754[a]	47	< 0.01
Zn, mg/kg	21.3[a]	15.8[b]	1.18	24.6[a]	11.7[b]	1.16	< 0.01
Fe, mg/kg	239	186	24	320	371	22	< 0.03
Cu, mg/kg	6.7	5.0	0.37	6.6	3.2	0.37	< 0.01
Se, mg/kg	2.4[b]	4.0[a]	0.22	3.2[B]	4.0[A]	0.21	< 0.02

[A, B] $p<0.05$; [a,b] $p<0.01$.
[c] F*time, *p* values for the interaction of forage by time.

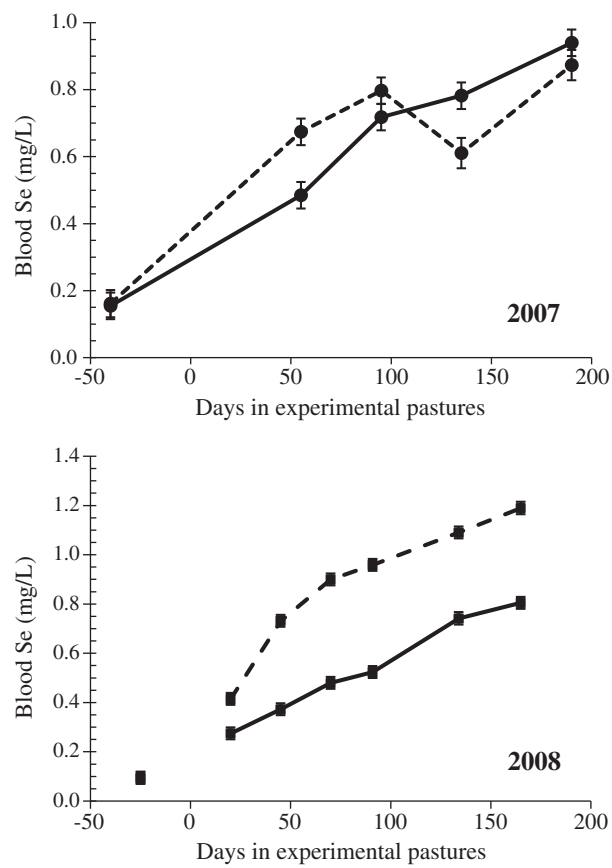

Fig. 1. Changes over time in concentrations of Se (mg/l) in whole blood of heifers that grazed creeping wildrye (CWR; solid line) or tall wheatgrass (TWG; dashed line) in 2007 or 2008. Heifers that grazed TWG had higher concentrations of Se in whole blood at 55 days, but lower at 135 days after grazing, than did CWR heifers in 2007 (forage by day interaction; $p<0.01$). During the grazing season of 2008, heifers grazing TWG had consistently higher ($p<0.01$) Se in whole blood throughout the grazing period than CWR heifers. Pooled SEM are 0.03 and 0.01 in 2007 and 2008, respectively. Data are least square means ± standard error of the mean (SEM).

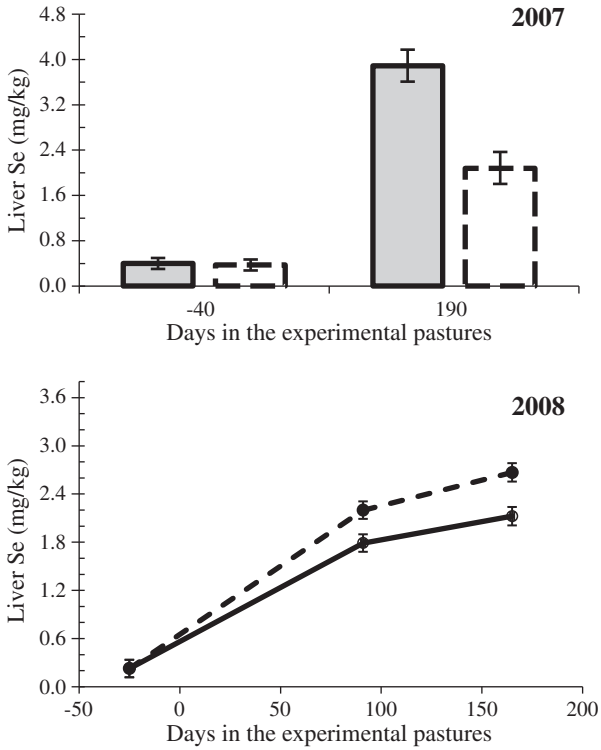

Fig. 2. Concentrations of Se (mg/kg wet weight) in liver tissue from heifers that grazed creeping wildrye (CWR; solid lines) or tall wheatgrass (TWG; dashed lines) in 2007 (bars) or 2008 (lines). Concentrations of Se in liver were higher for heifers grazing CWR in 2007 ($p<0.01$; forage effect), but not in 2008. Heifers that grazed TWG in 2008 accumulated more Se in liver during the grazing period than CWR heifers ($p<0.01$; forage by day interaction), even though liver Se concentrations were similar at 25 days before grazing. Pooled SEM are 0.11 and 0.064 in 2007 and 2008, respectively. Data are least square means ± standard error of the mean (SEM).

tissue in 2007 after 190 days of grazing than did those of TWG-grazed heifers (Fig. 2). The opposite occurred in 2008, when heifers grazing TWG had higher concentrations of Se in the liver at 91 and 165 days of grazing as compared to heifers grazing CWR. Despite the large increase in Se concentrations in blood and liver during 2007 and 2008, none of the 40 heifers demonstrated clinical signs of Se toxicity, such as deformed hooves, alopecia (loss of hair), diarrhea or sudden death.

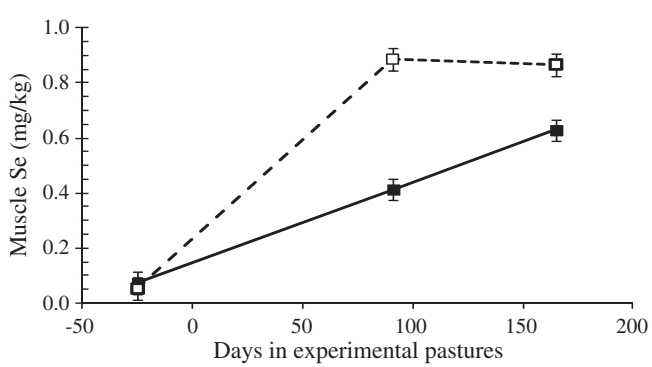

Fig. 3. Concentration of Se (mg/kg wet weight) in muscle tissue from heifers that grazed creeping wildrye (CWR; solid) or tall wheatgrass (TWG; dashed) in Experiment 2 (2008). Incorporation of Se into muscle occurred faster in heifers grazing TWG, which resulted in higher concentrations of Se in muscle at 91 and 165 days of grazing (forage by day interaction; $p<0.01$; Pooled SEM = 0.02). Muscle biopsies were not performed in 2007. Data are least square means ± standard error of the mean (SEM).

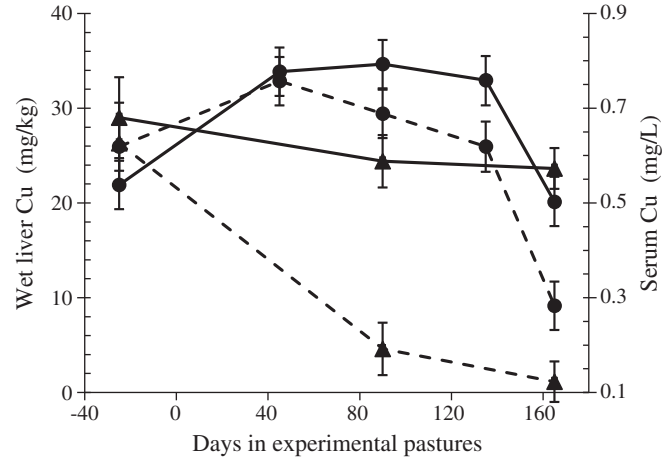

Fig. 4. Concentrations of Cu in liver tissue (mg/kg wet weight; ▲) and blood serum (mg/l; ●), respectively, from heifers that grazed creeping wildrye (CWR; solid) or tall wheatgrass (TWG; dashed) in Experiment 2 (2008). Heifers that grazed TWG had lower Cu concentrations in serum at 165 days of grazing ($p<0.01$) and in liver tissue at 90 and 165 days of grazing ($p<0.01$). Pooled SEM are 2.6 and 0.04 in liver and serum, respectively. Data are least square means ± standard error of the mean (SEM).

Histopathological examination of 17 liver samples collected in 2007 at 190 days of grazing revealed no relevant histological alterations in 12 samples, whereas 4 liver samples showed minor and focal infiltration of mononuclear inflammatory cells.

In 2008, grazing TWG resulted in higher ($p<0.01$) incorporation of Se into heifer muscle tissue than did grazing CWR (Fig. 3), an outcome similar to that observed for blood and liver. Muscle tissue had a larger relative change in Se concentration as compared to blood, with Se concentrations increased by 17 and 9 fold after heifers had grazed TWG or CWR pastures for 165 days, respectively. Other trace minerals in serum differed between TWG and CWR heifers, particularly Cu (Fig. 4), Mg and Na (Table 4), and in some cases, these differences were time dependent. Heifers that grazed TWG had lower levels o Cu (10.7 versus 25.4 ± 0.04 mg/kg; $p<0.01$) in liver, higher levels o Zn (57.9 versus 35.0 ± 2.5 mg/kg; $p<0.01$), whereas the concentration of Mn (2.6 versus 2.4 ± 0.10 mg/kg; $p=0.2$) and Fe (97.5 versus 96.3 ± 6.1 mg/kg; $p=0.9$) were similar for heifers that grazed CWR or TWG pastures, respectively.

Mean body weight before grazing was similar in the TWG and CWR heifer groups in 2007 (181.4 versus 195.0 ± 12.62 kg) and 2008 (264.7 versus 270.6 ± 9.02 kg), respectively ($p>0.10$). In 2007, heifers grazing TWG gained more weight than did heifers grazing CWR (0.59 versus 0.27 ± 0.040 kg/days; $p<0.01$). However during 2008, body weight gains were low for both groups and did not differ ($p=0.62$) between heifers grazing TWG or CWR (0.27 versus 0.36 kg/days; Fig. 5).

4. Discussion

4.1. Forage composition

Chemical composition of forages is dependent on their genetic background, growth stage, fertilization and the soil and irrigation water chemistry in their environment. TWG and CWR are tolerant to salinity and therefore have an advantage of performing well in saline soils as compared to more salt-sensitive forages such as alfalfa (Robinson et al., 2004; Suyama et al., 2007a; Semple et al., 2008) and typically, their nutritional value is not greatly affected by salinity (Suyama et al., 2007a). Indeed, the effects of salinity on yield, quality and chemical composition observed in earlier studies, suggest these forages are good candidates for production systems utilizing saline water or soils.

The mineral content of the TWG and CWR forage was reflective of the soil conditions in which they were grown, in particular saline soils

Table 4
Concentrations of Ca, P, Cu, Fe, Mg, K, Na and Zn in serum from heifers that grazed creeping wildrye (CWR) or tall wheatgrass (TWG) in 2007 and 2008. Data are least square means ± standard error of the mean (SEM).

	Experiment 1 (2007)					Experiment 2 (2008)				
	CWR	TWG	SEM	Forage[a]	F*time[a]	CWR	TWG	SEM	Forage[a]	F*time[a]
Heifers (n)	10	6	–	–	–	10	10	–	–	–
Ca, mg/l	99.1	99.4	0.89	0.79	0.07	92.0	92.6	1.17	0.69	0.10
P, mg/l	63.7	63.6	2.50	0.98	0.84	60.6	50.5	1.41	< 0.01	< 0.01
Cu, mg/l	0.66	0.69	0.036	0.61	< 0.01	0.67	0.59	0.037	0.14	< 0.01
Mg, mg/l	18.5	19.5	0.47	0.14	0.08	16.2	18.9	0.58	< 0.01	0.16
Fe, mg/l	1.36	1.40	0.057	0.61	0.18	1.04	0.88	0.060	0.08	0.02
Zn, mg/l	0.94	0.99	0.036	0.42	0.19	0.85	0.94	0.044	0.15	0.98
K, mg/l	203	211	3.9	0.23	0.18	222	226	6.2	0.64	0.88
Na, g/l	3.42	3.38	0.01	0.02	0.40	3.31	3.41	0.01	< 0.01	< 0.01

[a] *p*Values for the effects of forage and the interaction of forage by time; "–," not applicable.

irrigated primarily with the saline drainage water (Table 1). Total Se in soil in the TWG pasture (2.98 to 3.99 mg/kg) was 1.6 to 1.8 times higher than in the CWR pasture (1.86 to 2.24 mg/kg) (Table 2) and likewise, Se content was higher ($p<0.01$) in TWG forage as compared to CWR, particularly in 2008 (3.97 versus 2.82 ± 0.19 mg/kg dry weight, respectively) (Table 3B). The higher sulfur levels in TWG forage were also reflective of higher sulfate concentrations in the soil of the TWG pasture. The TWG pasture had been irrigated with saline drainage water for a longer period of time (7 years at the start of grazing) thus salinity (ECe), sodium adsorption ratio, Na^+, Cl^-, B and total Se were higher in the soil of the TWG pasture and the TWG forage as compared to CWR soil and forage. Therefore the forage composition reflected the soil chemical environment at the experimental site.

4.2. Se toxicity and content of Se in whole blood and animal tissues

Selenium is an essential mineral necessary to support animal health and growth, but it is required in very small quantities, National Research Council (NRC, 2000). Intoxication with Se can be acute or chronic and the most common signs associated with chronic intoxication in cattle are loss of hair from the end of the tail (i.e., rat tail), lameness, weight loss and deformed hooves; while the most common finding in acute cases is sudden death (Rebhun, 1995; Radostits et al., 2007). Heifers that grazed TWG and CWR showed none of these signs of Se intoxication during experiment 1 or 2, and no deaths related to Se intoxication occurred despite the heifers exclusively consuming forages containing 2.8 to 4.0 mg Se/kg dry weight, the latter being twice the maximum tolerable concentration of Se suggested for beef cattle by the NRC (2000).

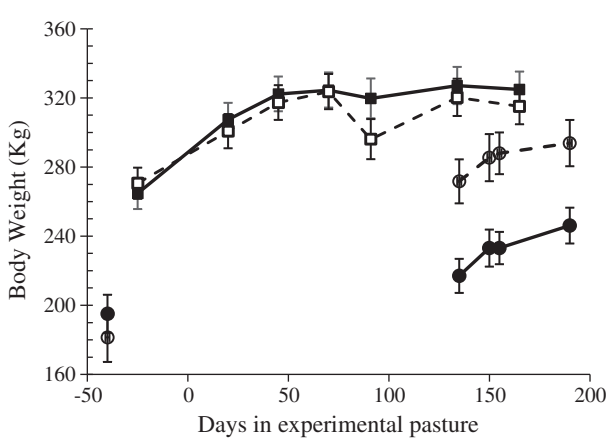

Fig. 5. Body weight (BW; kg) in heifers that grazed creeping wildrye (CWR; solid line) or tall wheatgrass (TWG; dashed line) in 2007 (circles) or 2008 (squares). Heifers that grazed TWG gained more BW than CWR heifers in 2007 (forage by day interaction $p=0.02$; Pooled SEM = 10.0). However, BW gains were similar during 2008 grazing season (forage effect $p=0.62$; Pooled SEM = 9.4). Data are least square means ± standard error of the mean (SEM).

The toxic oral threshold of Se—as well as that in whole blood and liver—varies according to the reference and the findings from toxicological studies conflict (O'Toole and Raisbeck, 1995). Rebhun (1995) suggested 1.5 mg Se/l of whole blood as the threshold to diagnose Se intoxication in dairy cattle, whereas Radostits et al. (2007) stated that: "clinical illness is evident at blood levels of 3 mg/l." However, when referring to dietary thresholds for Se intoxication, both references reported wide ranges such as 5 to 40 mg/kg dry weight, or more than 25 mg/kg in forage. The NRC (2000) proposed a more conservative recommendation, i.e. a minimum nutritional requirement of 0.10 mg of Se/kg dry weight and 2 mg of Se/kg dry weight as the maximum tolerable concentration (MTC) in diets for beef cattle, whereas the minimal requirement for dairy cattle is 0.30 mg/kg dry weight (NRC, 2001). In a factorial study that investigated several levels of Se in the diet (0, 5, 10, and 25 mg Se/kg dry weight) and sources (selenomethionine or sodium selenite) fed to beef steers for a period of 120 days, only 2 of 3 steers fed at the highest level with selenomethionine developed gross lesions on the hooves (O'Toole and Raisbeck, 1995) and those steers also had hair loss from the tail. However, these authors observed histological alterations of hoof epithelia only in steers fed a 10 mg Se/kg dry weight diet, which suggests that some of the subclinical signs of Se intoxication develop at levels of Se intake that are much higher than those fed in our study.

The highest concentration of Se in whole blood occurred for TWG heifers at 165 days during Experiment 2 in which case whole blood Se was 1.19 mg/l and wet liver Se was 2.67 mg/ kg which placed these heifers below the toxicity thresholds suggested by Radostits et al. (2007) and Rebhun (1995). Because of the fast and large load of Se through pasture ingestion, the between animal variation in Se accumulation in all tissues was very small. Whole blood Se maxima were even lower during Experiment 1 (CWR heifers, 0.94 mg Se/l), but Se concentration in liver was higher (CWR heifers, 3.89 mg Se/kg wet liver). In contrast, Lawler et al. (2004) reported that beef steers fed diets containing ~2.8 mg Se/kg dry weight, mainly from supplementation of the diet with high Se feeds (i.e., hay or wheat grain), or sodium selenate, for 126 days had much higher levels of Se in liver (9.9 to 10.8 mg Se/kg dry weight), which is approximately twice that observed in our study. These authors also reported no clinical signs of Se toxicity and furthermore, dry weight intake and body weight gain were not affected by the high Se intake and high Se in liver tissue in their study. Because clinical signs of Se intoxication were not observed in our study, and similar or higher blood concentrations of Se reported in the literature did not impact steer growth and performance, it is reasonable to conclude that during Experiments 1 and 2, our heifers were not clinically affected by the high levels of Se ingested by grazing TWG and CWR forage irrigated with saline drainage water.

Feeding high levels of Se to cattle (Lawler et al., 2004) and lambs (Taylor, 2005) increased incorporation of Se in all tissues, although the

rate of incorporation varied between organs, and it was dependent on the time of exposure and molecular form of Se (Lawler et al., 2004). In the study by Taylor, wether lambs fed a diet containing 2.9 mg Se/kg dry weight as organic Se for 56 days had higher concentrations of Se in kidney, liver, spleen, muscle, duodenum, heart, lungs and wool than lambs fed the control diet (i.e., 0.2 mg Se/kg dry weight). Taylor (2005) reported that Se concentration in the muscle increased 6 fold, as compared to 3 fold in the liver, due in part to the higher initial concentration in the liver (~9 mg Se/kg liver) as compared to muscle (~3.5 mg Se/kg liver). In our Experiment 1 and 2, Se incorporation had similar features; primarily a rapid increase in Se levels in the blood, particularly noticeable in Experiment 2, followed by substantial increases in liver and muscle Se content.

Heifers that grazed TWG in 2007 had similar ($p = 0.30$) Se concentrations in blood at 190 days as those which grazed CWR (0.87 versus 0.94 ± 0.042 mg/kg dry weight); however in 2008, the TWG heifers had higher ($p < 0.05$) levels of Se in blood (Fig. 1), liver (Fig. 2) and muscle (Fig. 3) throughout the 165 days grazing period. In 2007, the heifers grazing TWG had higher blood Se during the early grazing season, yet at the end of grazing (i.e., 190 days) Se concentrations in their liver tissue were much lower than those of the CWR heifers (2.1 versus 3.9 ± 0.181 mg Se/kg liver wet tissue). Forage was in short supply in the TWG pasture at the end of the 2007 grazing season. Reduced intake of forage by the TWG heifers may have prompted mobilization of Se from the liver during the last months of grazing thus depleting liver Se with less impact on blood Se, as the blood pool receives inputs from both the liver and digestive tract.

4.3. Mineral deficiency and animal weight gain

Forages grown in soils irrigated with saline DW can be highly unbalanced in micro-minerals which can impact cattle mineral requirements. Grattan et al. (2004b) described the potential detrimental effects of feeding such forages to cattle without appropriate mineral supplementation,, such as the risk for hypomagnesaemia due to high levels of K, and Cu deficiency resulting from high levels of Mo in the forage. Even though Mg concentrations in TWG and CWR forages were marginal (NRC, 2000) during Experiment 1 and 2 (i.e., 0.92 to 1.52 mg/kg), the K concentrations were not high (NRC, 2000) being below 21.5 mg/kg dry weight which probably explains the absence of any occurrences of grass tetany in either Experiment 1 or 2. Concentrations of Ca, P, Zn, K and Na in blood serum were within expected physiological ranges for healthy cattle (Radostits et al., 2007) in both of our experiments. Different than observed for Se, the between animal variation in micro-mineral concentration in serum was much larger, which most likely is related to the low concentration of most micro-minerals in these forages.

The nutritional requirements of Cu can be variable, e.g. 4 to 15 mg/kg diet dry weight of forage, because of interactions with other minerals such as Mo, Mg and S (NRC, 2000). Mo levels lower than 2 mg/kg dry weight and sulfur lower than 2500 mg/kg dry weight should not interfere with Cu absorption (NRC, 2000). Cu concentrations in our forages were consistently marginal during both experiments, and were particularly low at the end of the 2008 grazing season for TWG, which exposed this group of heifers to Cu deficiency, but not those grazing CWR. In Experiment 1, supplementation of Cu through a slow releasing bolus appeared to alleviate Cu depletion during the grazing period given that CWR and TWG heifers finished the grazing season with similar ($p = 0.61$) Cu levels in serum (0.66 versus 0.69 ± 0.036 mg/l).

According to Wikse et al. (1992), levels of Cu in serum between 0.2 and 0.4 mg/l, or liver concentrations from 0.5 to 10 mg Cu/kg wet weight can reduce body weight gains. Indeed, Cu concentrations in the livers of our TWG heifers were low at 165 days of grazing in 2008, averaging 1.14 mg/kg, and 6 of 10 TWG heifers had Cu levels below the minimum detectable level of 1 mg/kg.

Ward and Spears (1997) found that feeding a silage based diet containing 5.2 mg/kg dry weight of Cu and 1.16 mg/kg Mo for 196 days was sufficient to maintain adequate levels of Cu in the serum (0.9 mg/l Cu). The addition of 5 mg sodium molybdate/kg of diet dry weight reduced Cu concentrations in serum to 0.2 mg/l and liver to 7.5 mg/kg dry weight after 196 days of feeding; however, body weight gains were not reduced. Hansen et al. (2009) reported that when growing calves were supplemented with Mg (i.e., 500 mg/kg dry weight) and Mo (2 mg/kg dry weight) to induce a Cu deficiency, body weight gains were similar to a Cu-sufficient group (i.e., 120 mg Cu/kg liver dry weight) up to 239 days of feeding. Reduced body weight gains occurring just after this period suggested to these authors that chronic Cu deficiency could in fact, impact body weight gain.

In experiments 1 and 2 of our study, Se concentrations increased in all tissues, including blood, more than any other mineral measured in both CWR and TWG heifers. The literature, however, has no indications that these levels of Se in blood and liver depress voluntary intake or body weight gain (Lawler et al., 2004; Taylor, 2005).

According to the NRC recommendations for beef cattle (2000), a diet containing 71 to 98 mg crude protein/kg dry weight and 500 to 600 g/kg total digestible nutrients (equivalent to 7.4 to 8.8 MJ metabolizable energy/kg dry weight) would support daily body weight gains of 300 to 600 g/days, respectively. As the crude protein level was considerably above animal requirements (but not necessarily the estimated energy content of forages) during Experiment 1 and body weight gains for TWG heifers of 0.59 kg/days were in close agreement with estimates based on NRC (2000) equations, it is likely that the heifers had an intake slightly higher than predicted by NRC (2000). Heifers grazing CWR had lower body weight gains (0.27 kg/days) than TWG heifers in 2007, probably due to the lower metabolizable energy content of CWR (i.e., 6.65 MJ /kg dry weight) because the crude protein content was definitely not the growth-limiting nutrient in Experiment 1. The chemical composition of the forages in Experiment 2 was quite different between CWR and TWG, particularly because of the strong effect of time on crude protein content. However, the lower nutritive character (metabolizable energy) of CWR persisted, albeit at a smaller magnitude. During Experiment 2, body weight increased in both TWG and CWR heifers up to 91 days of grazing when it stabilized which coincided with the onset of suboptimal crude protein content of the forages (Table 3C), particularly TWG, along with low levels of Cu in serum and liver.

It is important to recognize the unavoidable confounding of low Cu levels and low crude protein content of forages; however, based on Cu levels in our animal tissues, a Cu deficiency could only have compromised body weight gains later in the grazing period. Due to sufficient crude protein levels in CWR, but a low metabolizable energy, it is likely that the low body weight gains in Experiment 2 were, in large part, due to an insufficient nutrient content of the forages rather than a micro-mineral deficiency and/or imbalance.

It would be a very simplistic interpretation to state that Cu supplementation, as done in Experiment 1, would be sufficient to accommodate the micro-mineral imbalances observed in these forages that were irrigated with saline drainage water high in selenium. The micro-mineral composition of these forages is a consequence of the mineral profile of the soil and the drainage water, which can be highly variable. Micro-mineral supplementation based on a recent chemical analysis of the forages to be grazed is therefore strongly recommended if animal health and performance are to be optimized.

4.4. Implications for human health

Possible benefits of utilizing areas that are naturally high in Se to produce Se-enriched beef have been suggested (Hintze et al., 2001; Lawler et al., 2004). Meat products, particularly beef and poultry, comprise one of the main sources of Se in the diets of U.S. adults (Cotton et al., 2004). The typical content of Se in fresh beef ranges from 200 to 250 μg/kg on a wet basis (Hintze et al., 2001). The USA recommended

dietary allowance (RDA) for Se of 70 and 50 μg of daily Se intake for adult men and women, respectively, suggests that an intake of 100 g/day of beef would provide 36% to 50% of the RDA of Se. Consumption of the same amount of semitendinosus muscle from Se-enriched beef from our CWR and TWG grazed heifers (i.e., 0.63 versus 0.87 mg Se/kg wet muscle) heifers would provide about 90% to 125% of the RDA of an adult man, which makes Se-enriched beef a promising source of Se in human diets.

5. Conclusions

Our TWG and CWR forage containing up to 4 mg Se/kg dry weight did not cause beef heifers to develop signs of Se toxicity. It is therefore unlikely that exposing non-pregnant, non-breeding cattle to these high Se forages for a grazing season of 6 to 7 months would result in death due to Se intoxication. Potential micro-mineral imbalances in forages grown in soils irrigated with these saline drainage waters are likely to be a more relevant concern for animal health and performance than is their high Se content. Therefore, cattle grazing these Se-enriched forages should receive macro- and micro-mineral supplementation during the grazing period to optimize their performance and minimize the risk of undesirable metabolic conditions, particularly hypomagnesaemia and Cu deficiency. Although drainage water can provide high levels of N to growing forages, application of supplemental sources of N should be considered if needed to maintain desirable levels of crude protein in the forage so as to sustain adequate plant biomass yield, as well as body weight gains of the cattle. In addition, such a production system creates an opportunity for farmers to produce "value-added" beef that contains higher levels of Se, while creating an economic opportunity to utilize saline soils and/or saline drainage waters that are not suitable for the growth of higher value, salt-sensitive crops.

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