Selenium Incorporation in "*Jose*" Tall Wheatgrass and Bio-availability for Dairy Cattle

Final Report*

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Executive Summary

Re-use of saline drainage water (DW) for irrigation is a useful strategy to address problems of irrigation water shortage and salinity management in the western San Joaquin Valley (SJV) of California. However, utilizing this selenium (Se)-enriched DW with minimal impact to wildlife and the environment has been a major challenge. 'Jose' tall wheatgrass (*Thinopyrum ponticum;* TWG) is a highly salt tolerant forage that has performed well under irrigation with saline DW, having adequate dry matter (DM) production and quality, but in some cases Se concentrations in the herbage were >10 mg Se/kg DM (= ppm (part per million)). Se accumulation in the forage raised concerns for its safety for animal feeding, but it also presented the opportunity to use the Se-enriched TWG hay as an organic feed supplement in Se-deficient areas where dairy cattle are commonly given dietary Se in the form of sodium (Na) selenite. Utilization of Se-enriched TWG hay in place of Na selenite supplements would reduce the importation of 'new' Se into the SJV and reduce the risks associated with this trace element.

A greenhouse study conducted at Fresno State University from June 2010 to June 2011 assessed selenium accumulation in TWG as affected by different cutting heights (20, 40, 60 cm) using irrigation water combinations consisting of two salinities (electrical conductivity (EC_w) of 3 and 12 dS m^{-1}) and two selenium concentrations (350 and 1000 µg/L (= ppb (part per billion)) arranged in a split-plot design. Se accumulation was compared at both low and high salinity because it has been reported in the literature that with sulfate-dominated salinity, as found in the western SJV, high levels of sulfate can suppress Se uptake by plants (Terry et al., 2000).

The plants were cut six times and the herbage was analyzed for Se and other major and minor ions. Results of the greenhouse study showed that high Se concentrations (>10 mg/kg) in TWG herbage could be obtained when irrigating with high Se waters that were either low or high in salinity except, perhaps, for the CH20 plants which accumulated more Se when irrigated with high Se water that was low in salinity, rather than high. Dry matter yield was highest for the TWG plants cut at 60 cm and accordingly, the accumulation of Se on a mass basis was also highest in these plants, as compared to those cut at 40 or 20 cm. Mineral composition was at or near optimum levels for most of the minerals, but high concentrations of S and Cu in the herbage would require attention when feeding this hay to ruminant animals. However, if used as a dietary Se supplement and mixed into a ration at < 5%, high concentrations of S and Cu in the hay should not be problematic.

A dairy cattle feeding study was also conducted at a commercial dairy which utilized Se-enriched (4.65 mg/kg) TWG hay as a dietary Se source for lactating dairy cows and compared Se accumulation patterns in blood, urine and feces to determine its bioavailability. Three pens of ~310 cows each were fed a similar total mixed ration (TMR) in a 3 x 3 Latin Square design over 4-week periods, except that the supplemental Se source differed (*i.e.,* none; TWG or Na selenite). The chemical composition of the diets was the same, except for Se which was higher ($P<0.01$) in the TWG and Na selenite diets (0.53 and 0.65 ppm) *versus* 0.35 ppm in the control diet. Feeding Se-enriched TWG hay increased

blood Se by 6.4% over the control whereas Na selenite increased blood Se by only 4.8%, suggesting slightly higher bioavailability for Se from TWG hay *versus* Na selenite. In contrast, the amount of dietary Se which was apparently digested increased from 47 to 58% with Na selenite, but with TWG supplementation there was no increase over the control which suggested higher bioavailability for Na selenite as compared to TWG hay. As Se outputs in the urine did not differ among treatments (P=0.07), the apparent metabolizability of Se was higher for the Na selenite diet as compared to Control and TWG diets. Overall, the similar apparent metabolizability for Se from the TWG and the base diet suggests that Se-enriched TWG hay can be used as a value-added Se feed supplement for cattle producers in the eastern SJV who are currently challenged by environmental regulations to reduce the use of trace minerals imported to the SJV in their cattle rations.

Results from both experiments indicate that agricultural DW of 350 µg Se/L (ppb) (equivalent to the LSe treatments in our greenhouse experiment) can, after 10 months of frequent irrigation, produce TWG hay with 5 to 7 ppm total Se or higher in the dry matter. If mixed into a dairy ration at approx. 5%, this would provide adequate Se and negate the need for Na selenite supplements and the importation of 'new' Se into the San Joaquin Valley. Irrigation waters with higher Se content (≥750 µg/L Se, equivalent to the HSe treatments in our greenhouse experiment) produced TWG hay with 10 to 17 ppm Se. Thus, unless salinity is much greater than 12 dS/m EC, concentrated drainage waters with higher Se levels could be used to irrigate TWG and produce a value-added hay suitable for use as a Se supplement in the diets of dairy cattle.

I. Greenhouse Study

The objective of this study was to investigate the effect of irrigation water quality (selenium/salinity combination) and cutting height (CH) on selenium incorporation into tall wheatgrass herbage.

Methodology

Experimental set-up and design

The study was conducted in a greenhouse at California State University, Fresno. Soils were collected from Red Rock Ranch in Five Points in western Fresno County, CA and passed through a screen. The soil thus screened was mixed with sand in a 60:40 (soil:sand) ratio to ensure better drainage in the pots while maintaining the cracking clay characteristics common to soils in the western SJV. Four irrigation water combinations consisting of two levels of salinity (3 and 12 dS/m EC_w) representing low (LS) and high (HS) salinity and two levels of Se (target 350-400 µg/L and 1000 µg/L) for the low (LSe) and high (HSe) selenium levels were utilized as the main plot factor. The Se treatment levels could not be set precisely because they were determined by the amount of source water needed to reach the target salinity levels. Because Se measurement requires sophisticated laboratory analysis, it could not be measured at the time of mixing, as could salinity levels. Cutting heights of 20 cm, 40 cm, and 60 cm were used as sub-plot factor which resulted in a split-plot design (Fig. 1).

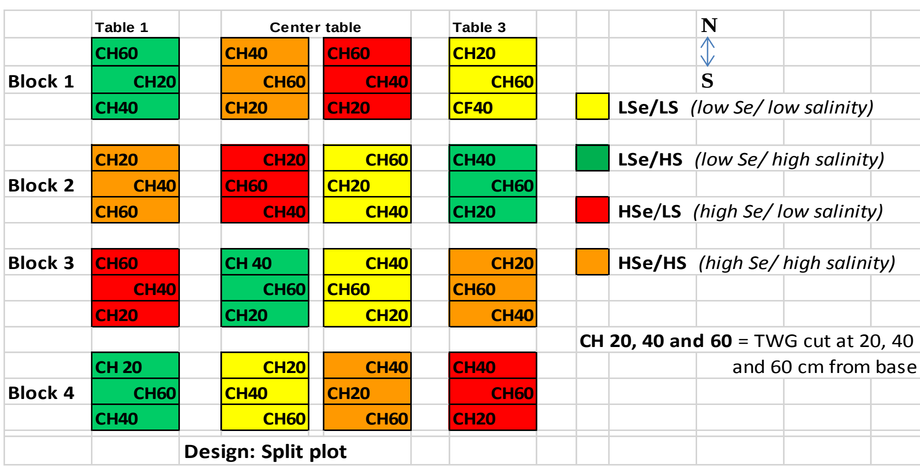

Fig. 1: split-plot design for arrangement of pots in the greenhouse.

Plant establishment, salinization, and irrigation

The pots used in the study (25.5 cm diameter x 30 cm deep = 15.3 L volume) were seeded directly and thinned to 12 plants per pot once the seedlings were 8-10 cm tall. The tall wheatgrass variety used was “Westside Wheatgrass” from S&W Seed, Five Points, CA. For the first several weeks all the plants were irrigated with non-saline tap water supplemented with basic nutrients (3 mM KNO_3, 0.5 mM KH_2PO_4 and 20 µM Fe-DTPA).

Concentrated DW from Panoche Water District, CA was collected from a drainage sump, analyzed and then used as the saline water source. To reach the target salinities, the saline source water was introduced weekly in step-wise increments (¼, ½, full strength). The Se input from the DW was calculated for each irrigation water treatment and then supplementary Se in the form of sodium selenate was added as needed to reach the target Se levels. After calculating the NO_3-N from the saline DW additions, KNO_3 and $Ca(NO_3)_2$ fertilizers were added in a 3:2 ratio to reach 56 ppm NO_3-N (= 1/4 strength Hoagland's solution) in all irrigation tanks and KH_2PO_4 and Fe-DTPA were added at the concentrations listed above. Because the HS treatments had more NO_3-N coming from DW additions and less from the fertilizers, KCl and $CaCl_2$ were added in small amounts to the HS tanks so as to balance K and Ca levels amongst all treatments. Once the desired salinity, selenium and nutrient levels were reached, irrigation water samples were taken for complete chemical analysis. The irrigation tank waters were changed every 6 to 8 weeks, at which time fresh nutrients were added.

Large plastic irrigation tanks (378.5 L = 100 gal.) were used in a re-circulating system in which all the drainage water from the pots returned to the source tank by gravity flow (Photos 1 & 2). Tap water was used to replenish the water lost to evapotranspiration when the water level in the tank fell below 90%. Irrigation water salinity and nitrate concentrations were measured weekly and the targets levels were maintained. If NO_3-N levels dropped below 25 mg/L, a spike of KNO_3 was added to restore the concentration to near 50 mg/L. Beginning with the second tank mix, it was observed that Se levels in the irrigation water were depleting substantially during the period between tank mixes, likely due to adsorption to the soil. Thus beginning with tank mix 2, Se spikes (50 ug/L for LSe and 150 ug/L for HSe treatments) in the form of Na selenate were added to the irrigation tanks every two weeks.

Photo 1: Tall Wheatgrass plants in greenhouse experiment.
Photo 2: 100 gal. tanks from irrigation water was pumped and drainage returned by gravity flow

The pots were irrigated 3-4 times a week initially and then daily or twice daily during the peak of summer to maintain a sufficient leaching fraction to keep soil salinities in the pots close to the irrigation water salinities. Drip emitters (Netafim PC drippers) with capacity of 0.5 and 1 gallon per hour (GPH) were used in combination to water the plants. Due to the slow infiltration characteristics of the soils and the differences in ET amongst the frequently cut plants in the CH20 treatment, as compared to the CH40 and CH 60 cm plants which accumulated more biomass in between cuttings, different combinations of drip emitters had to be used to avoid water-logging the CH20 plants. To calculate the leaching fraction (LF), drainage was collected periodically from selected pots and the LF was calculated as the ratio of the drainage volume to the volume of irrigation water. A LF of approximately 20-30 % was targeted so as to keep the soil salinity in the high salinity treatment at or below 15 dS/m.

Water and soil sampling

Irrigation tank water samples were collected one day after mixing and at the end of each tank mix. These water samples were analyzed for EC, pH, B, Se, Cl-, SO_4^{2-}, Ca^{2+}, Mg^{2+}, Na^+, and NO_3-N. At the end of the experiment soil samples representing the entire depth of the pot were taken from each pot and saturated soil pastes were prepared. Salinity (EC_e), pH, B, Cl^-, SO_4^{2-}, Ca^{2+}, Mg^{2+}, and Na^+ were measured on the saturated paste extracts and total Se and NO_3^- were measured on dry soil samples using established procedures.

Forage Sampling

The forage was cut when the plants grew to 20 cm, 40 cm, and 60 cm height. A harvest was considered to be completed when the 60 cm plants reached their full height. Samples thus obtained were rinsed three times in deionized water to remove surface salts and dust. Samples were then air-dried in forced air oven at 60ºC for 48 hours and weighed. The dried tissue was then ground to pass a 1 mm sieve using a Wiley mill. The samples were analyzed individually for total Se, but for the analysis of other mineral nutrients (Cu, Zn, B, S, Ca, Mg, total N and crude protein) samples from multiple cuts within a harvest period, as occurred for the 20 cm plants, were composited.
Forage Se is presented on a concentration basis (mg/kg) as well as a mass basis (mg), the latter of which was determined by multiplying the Se concentration by the dry matter yield for each pot.

Data and statistical analysis

Total Se concentrations (mg/kg) and mineral nutrient concentrations in plant tissue were statistically analyzed using a general linear model with irrigation treatment (Se/Salinity level), cutting height, and the interaction (irrigation treatment x cutting height) as fixed factors and block as a random factor using SPSS 17 (SPSS, Inc., Chicago, Illinois). The data sets were tested to see if they meet the assumptions of the analysis of variance (ANOVA), but no transformation was required. When the 2-way ANOVA indicated significant differences at the 0.05 significance level amongst irrigation water treatments and cutting heights, Tukey's HSD test was used for mean separation.

Results and Discussion

Irrigation water composition

The average salinity (EC_w), pH and ionic composition of the water used to irrigate the pots are shown in Table 1. Low Se treatments averaged 313 and 369 µg/L (= ppb) of Se for the LS and HS treatments, respectively, and high Se treatments averaged 761 and 787 µg/L Se for the LS and HS treatments. The low salinity (LS) treatments had salinities of 3.3 to 3.4 dS/m EC_w, boron concentrations of 4.2 mg/L and SAR of 10.3. In contrast, the high salinity (HS) treatments had an $EC_{w.}$ of 11.1 to 11.5 dS/m, boron concentrations of 17-18 mg/L and SAR of 27. Nitrate (NO_3-N) levels were well-balanced amongst the treatments being 36-40 mg/L for the LS and 41-47 mg/L for the HS irrigation waters.

Table 1: Irrigation water composition (averages for six tank mixes and for samples taken at the beginning and end of each mix).

Irrigation treatment	Se (ug/L)	EC_w (dS/m)	pH	B (mg/L)	NO_3-N (mg/L)	Cl^-	SO_4^{2-}	Na^+	Ca^{2+}	Mg^{2+}	SAR
						..............................(meq/l)......................................					
LSe/LS	313	3.3	7.9	4.2	39.9	12.4	15.6	23.0	5.1	5.9	10.2
LSe/HS	369	11.5	8.0	17.8	47.5	59.2	70.7	103.2	14.1	13.5	27.8
HSe/LS	761	3.4	8.0	4.2	36.1	13.0	16.1	23.5	5.4	5.9	10.4
HSe/HS	787	11.1	8.2	18.3	41.3	56.5	67.7	98.2	13.2	12.8	27.3

Soil chemical composition

Total Se in soil was lowest in the LSe/LS treatment (0.88 mg/kg) and LSe/HS treatments (1.14 mg/kg) and higher in the HSe treatments (1.49 and 2.55 mg/kg for HSe/LS and HSe/HS, respectively) (Table 2). Soluble Se (measured on the saturated soil paste extract) was very low in all treatments (~100 µg/L) which suggests that much of the Se applied remained associated with the soil and that total Se concentrations, rather than soluble, are a better indicator of the Se potentially available for plant uptake.

Table 2: Soil chemical composition (samples taken at end of experiment)

Irrigation Treatment	Total Se (mg/kg)	Soluble Se (ug/L)	EC_e (dS/m)	pH	B mg/L	Cl^-	SO_4^{2-}	Na^+	Ca^{2+}	Mg^{2+}	SAR
						-------------------------------- meq/L---------------------------------------					
LSe/LS	0.88 ± 0.05	100 ± 10	4.7 ± 0.4	7.7 ± 0.03	6.2 ± 0.2	16.0 ± 0.7	28.4 ± 4.6	32.4 ± 1.9	7.0 ± 1.3	4.4 ± 0.7	13.8 ± 0.4
LSe/HS	1.14 ± 0.06	100 ± 5	13.3 ± 1.8	7.6 ± 0.1	19.8 ± 1.9	56.6 ± 10.3	86.7 ± 12.9	93.1 ± 17.9	17.1 ± 2.1	14.5 ± 2.1	23.6 ± 3.9
HSe/LS	1.49 ± 0.12	110 ± 3	4.5 ± 0.2	7.9 ± 0.1	6.4 ± 0.7	17.5 ± 2.8	23.4 ± 3.9	32.4 ± 3.6	6.7 ± 0.5	4.4 ± 0.4	13.7 ± 1.0
HSe/HS	2.55 ± 0.28	100 ± 10	12.8 ± 0.1	7.7 ± 0.1	18.1 ± 1.3	52.8 ± 2.8	77.1 ± 6.5	111.9 ± 17.9	15.3 ± 1.2	12.8 ± 0.1	29.9 ± 4.1

At the end of the experiment, soil salinities were 4.5 to 4.7 dS/m EC_e for the low salinity (LS) treatments and 12.8 to 13.3 dS/m ECe for the HS treatments. Measured leaching fractions were 42-44% for LS treatments and ~50% for the HS treatments, higher than our target of 20 to 30%. The high LF was the reason for the relatively modest increases in soil salinity (EC_e) as compared to the irrigation water salinity (EC_w).

Boron concentrations were high (17-18 mg/L) in the irrigation water for the HS treatments and accordingly, soluble B was high in the soil at the end of the experiment (Table 2) being

18-20 mg/L. In both the irrigation water and the soil, SO_4^{2-} was more predominant than was Cl^-. High levels of sulfate in soil and irrigation water are of particular importance because sulfate has been shown to inhibit Se uptake by plants (Grieve et al., 2001; Bañuelos et al., 2003) and high levels of sulfur in forage tissue can be detrimental to ruminant health (Grattan et al., 2004).

Selenium accumulation in forage (concentration basis)

Irrigation water composition (selenium/salinity level) had a significant effect on Se accumulation in TWG forage ($P \leq 0.001$). For plants cut at 40 and 60 cm, total Se in the herbage was greatest under HSe irrigation, reaching 11.2 to 17.1 mg/kg (= ppm) for harvests 5 and 6 (Fig. 2 c,d) and for most harvests, it was not significantly different for low vs. high salinity conditions. Under LSe irrigation, plants cut at 40 and 60 cm, accumulated 5.2 to 9.5 mg/kg of Se in the herbage (Fig. 2 a,b).

For the 20 cm plants, however, the effect of salinity on Se accumulation was more significant as plants in both the LSe and HSe treatments generally accumulated more Se under low salinity (LS) vs. high salinity (HS) conditions. This was particularly true for the HSe treatments (Fig. 2 c,d) where Se concentrations in the forage were significantly higher for HSe/LS plants as compared to HSe/HS plants at most harvests and for the final harvest (H6), total Se in the forage was 16.9 mg/kg for the HSe/LS plants as compared to 10.1 mg/kg for HSe/HS plants. It could not be determined whether the reduced Se accumulation under high salinity observed in the 20 cm plants was due to sulfate inhibition of Se uptake, or an effect of salinity on evapotranspiration (ET) which could have reduced Se uptake. Overall, it was concluded that except under very frequent cutting, high sulfate in the irrigation water did not significantly depress Se accumulation in the forage.

Cutting height (CH) showed mixed effects on Se accumulation in TWG herbage as it was significant only for the H3, H4 and H5 cuts. When cutting height did have a significant impact on total Se in herbage the highest accumulation of Se was obtained with the CH40 and 60 plants, as compared to CH20 plants, and this difference was greatest for the H5 cut (Fig. 2). Differences in Se accumulation in response to cutting height were greatest for the HSe/HS treatment (Fig. 2 c,d) and for this treatment, Se accumulation was lowest for plants cut at 20 cm suggesting that the osmotic effect of salinity may have impacted water and Se uptake to a greater extent in these frequently cut plants with younger tissue.

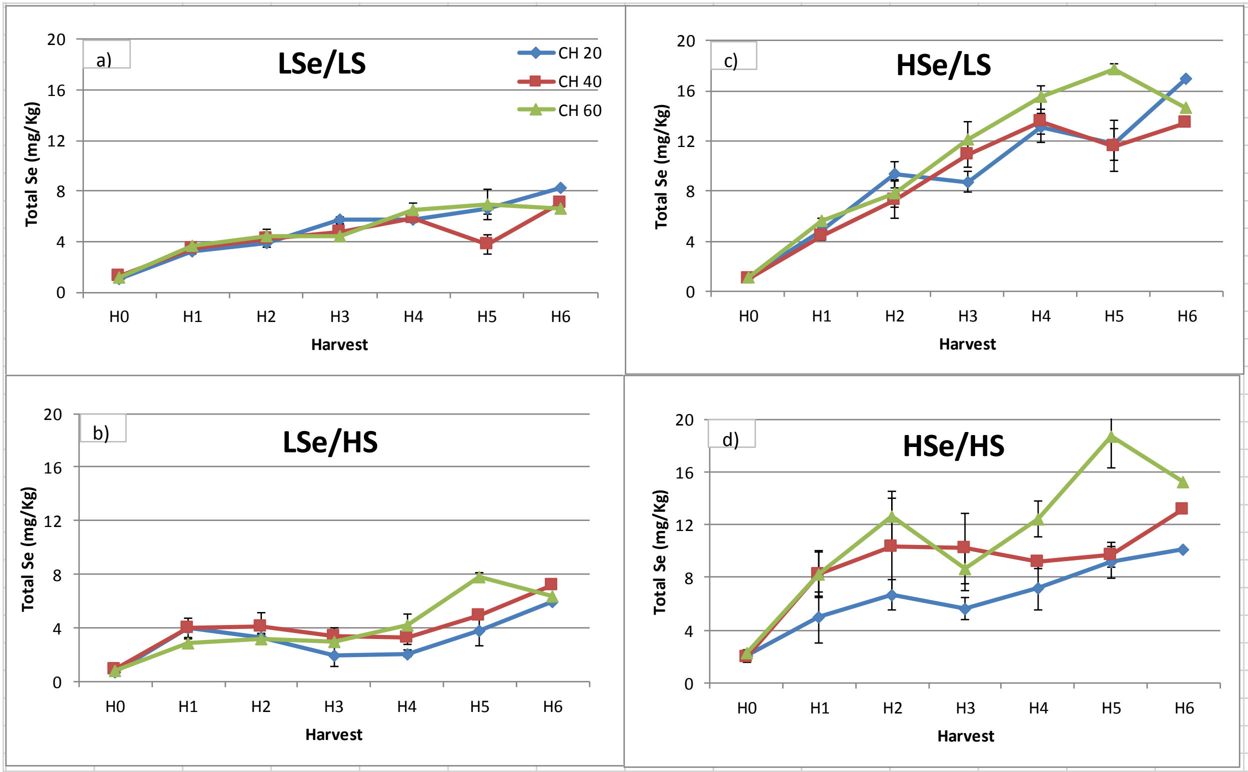

Fig.2. Total Se accumulation (mg/kg) in "Jose" tall wheat grass irrigated with a) low selenium/low salinity (LSe/LS), b) low selenium/high salinity (LSe/HS), c) high selenium/low salinity (HSe/LS) and d) high selenium/high salinity (HSe/HS) water for plants cut at 20, 40 or 60 cm heights in a greenhouse experiment. Six cuts of the forage were made over the one year period (June 2010 to 2011).

For the plants cut at 40 and 60 cm, Se concentrations in the herbage generally increased over time likely due to the increased exposure to the selenium-enriched irrigation water. Interestingly, there was little difference in final concentrations of Se in the herbage of plants cut at 40 vs. 60 cm which could mean that as the tissue started to age, the uptake and accumulation of Se slowed down. It is important to note that with 20 cm plants, frequent cuttings resulted in low biomass production per pot and the death of a number of plants initially. With frequent cutting these plants became fewer and finer. With the overall objective of harvesting tall wheatgrass as an organic Se supplement for dairy cows, the greater biomass production obtained from the plants cut at 40 and 60 cm would be desirable.

Forage Biomass accumulation

Cutting height (CH) had an overwhelming effect on dry matter yield (P<0.0001) in the pooled analysis with all harvests combined. In general, the effect of irrigation water on dry matter yield was not significant (Fig. 3). The relatively small difference in DM yield between LS and HS treatments reflects the high level of salt tolerance observed for tall wheatgrass both in earlier work by our research group (Suyama *et al*. 2007a,b) and by other authors (Bennett *et al.* 2009, Rogers *et al*. 2005).

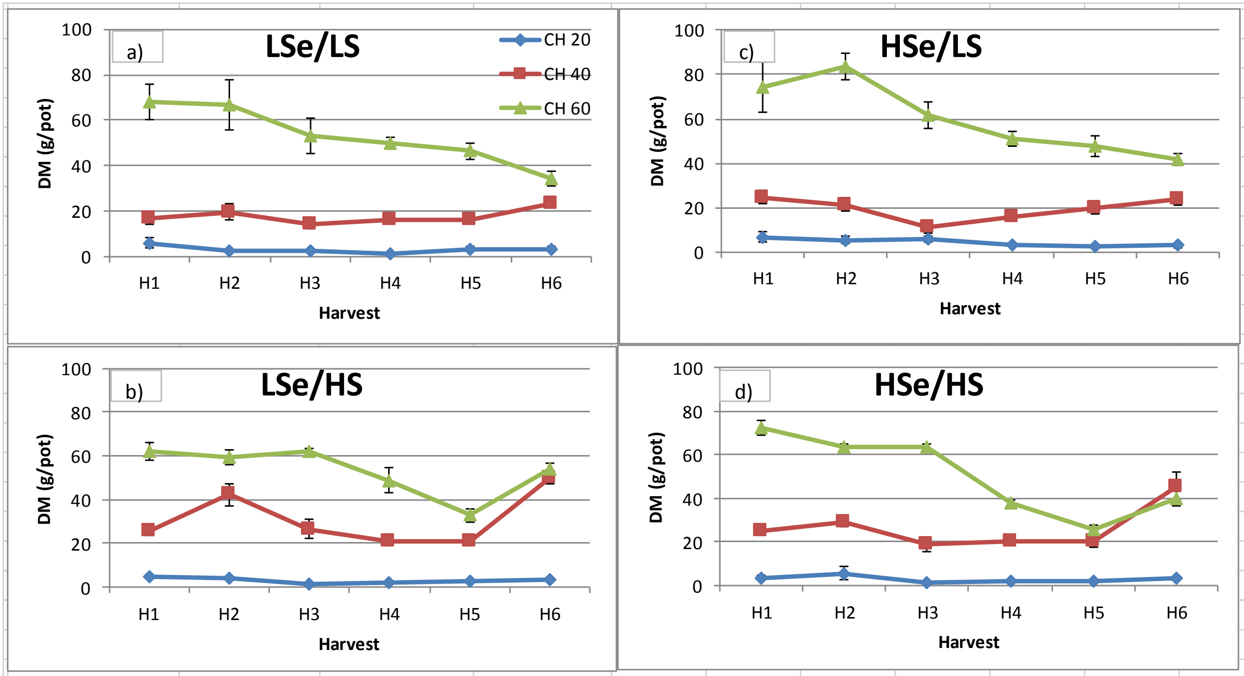

Fig. 3. Dry matter yield (g/pot) for 'Jose' tall wheatgrass irrigated with a) low selenium/ low salinity (LSe/LS), b) low selenium/high salinity (LSe/HS), c) high selenium/low salinity (HSe/LS) and d) high selenium/high salinity (HSe/HS) waters for plants cut at 20, 40 or 60 cm heights in a greenhouse experiment. Six cuts of the forage were made over the one year period (June 2010 to 2011).

Selenium accumulation in forage (mass basis)

The mass of Se accumulated in TWG herbage was directly related to the dry matter yield. As the dry matter yield was greatest for the CH60 plants, so was the mass of Se accumulated in the herbage (Table 3). The accumulation pattern for total Se on a mass basis was CH60 > CH40 > CH20, in contrast to data on a concentration basis where differences amongst the cutting heights were not as great. For total Se on a mass basis, the higher accumulation by CH60 plants in both HSe treatments is likely due to the longer exposure of the TWG herbage to high Se irrigation for the CH60 plants, as compared to CH40 and CH20 plants.

Table 3. Average mass Se (µg) accumulated in TWG herbage irrigated with saline drainage water for harvest three (H3) to harvest 6 (H6) in a greenhouse experiment.

Variable	H3			H4			H5			H6		
Irrigation Water	CH20	CH40	CH60	CH20	CH40	CH60	CH20	CH40	CH60	CH20	CH40	CH60
LSe/LS	14a	65a	230a	8a	95a	320a	20a	150a	310a	28a	160a	230a
LSe/HS	2a	90a	180a	3a	67a	210a	30b	100a	250a	20a	350ab	340a
HSe/LS	50b	120a	740b	39b	200b	790b	32b	220a	810b	50a	320a	610b
HSe/HS	7a	170a	550b	14a	180b	470a	15a	220a	460a	30a	590b	590b
Two factor ANOVA Irrigation Water (IW)												
Mean squares		0.13			0.15			0.12			0.16	
F value		10.39			16.5			11.3			10.2	
P_r>F		0.003			0.001			0.002			0.003	
Current height (CH)												
Mean squares		0.73			0.80			0.80			0.75	
F value		120.4			174.5			97.1			128.9	
P_r>F		<0.001			<0.001			<0.001			<0.001	
IWxCH												
Mean squares		0.08			0.06			0.07			0.05	
F value		13.15			14.05			8.3			9.41	
P_r>F		<0.001			<0.001			<0.001			<0.001	

Means within the same column and with the same letter are not significantly different at $P < 0.05$.

Conclusions

Se concentrations of >10 mg/kg in TWG herbage were easily achievable when TWG was grown under frequent irrigation with high Se water (~750 µg/L). The FDA-approved Se supplementation for dairy and beef cattle is to not exceed 0.3 mg of supplemental Se/kg of DM (NRC, 2001) which would require that TWG hay with 10 mg/kg Se be mixed into a cattle ration at less than 5%. Dry matter yield was highest for the TWG plants cut at 60 cm and accordingly, the accumulation of Se on a mass basis was also highest in CH60 plants, as compared to CH40 and CH20 plants. Allowing the forage to grow for a longer period of time to accumulate more Se would likely reduce its forage quality, but if this Se-enriched hay were to be used as a dietary supplement and mixed into a ration at 5% or less, then organic forage quality would not be an important criterion.

The irrigation water salinity used in this experiment was as high as 12 dS/m EC which did not significantly impact the growth and development of the forage, supporting the high salt tolerance reported for TWG in our earlier work (Suyama *et al.*, 2007b) and that of other researchers. High selenium irrigation water (~750 µg/L) resulted in significantly higher Se concentrations ($P < 0.05$) in the TWG herbage and this was not greatly influenced by the salinity level of the irrigation water, except perhaps, for the CH20 plants. Although it has been reported in the literature that high levels of sulfate (e.g. as found in the sulfate-dominated salinity of the western SJV) can inhibit the uptake of Se, this was not consistently observed in this experiment. Consequently, with either low salinity (~3 dS/m) or high irrigation waters (10-12 dS/m EC), TWG can accumulate high levels of Se in the herbage.

The mineral composition of the TWG hay produced under irrigation with this saline DW was at or near optimum levels for most of the minerals. However, results showed high concentrations of S and Cu in the forage tissue which would require that care be taken when feeding this Se-enriched hay to ruminant animals as a large proportion of their diet.. However, as an organic Se supplement added at 5% or less to a mixed ration, imbalances of the other minerals should not present a problem. And because of the complex interactive nature of Cu with different minerals, it is hard to predict the exact outcome should this forage be fed at higher percentages in the ration. High Cu and Mo present in the herbage could counteract the high S because of the antagonistic properties between them.

In summary, results of this one year greenhouse experiment indicate that “Jose” tall wheatgrass (TWG) has good potential to be used as an organic Se supplement for livestock production in areas where soils are naturally deficient in Se and animals are routinely provided dietary Se supplements. Furthermore, Se-enriched tall wheatgrass hay can be produced using a saline drainage water that is not suitable for the irrigation of most conventional crops and for agricultural areas that use subsurface drainage for salt and water management, it solves a disposal problem as this drainage water cannot be discharged into local waterways. Thus, the liability of these Se-enriched drainage waters can be turned into a value-added product which recycles Se within the San Joaquin Valley, obviating the need to import more Se in the form of dietary supplements for beef and dairy cattle. Furthermore, production of Se-enriched hay for dietary supplements could be allocated to low quality soils not suitable for the production of higher value, salt-sensitive crops.

II. Dairy cattle feeding study

The objectives of this study were to:

a) Evaluate the feasibility of using Se-enriched TWG hay as a dietary supplement for dairy cattle.
b) Examine patterns of Se concentration accumulation in blood, fecal matter, and urine.
c) Determine the bio-availability of Se in TWG hay as compared to sodium selenite, the industry standard.

Methodology

Forty-five tons of Se-enriched (4.7 ppm) TWG hay was purchased from Panoche Water District in Firebaugh, CA. The hay came from pastures irrigated with saline DW in the San Joaquin River Improvement Project. TWG hay with a higher concentration of Se was preferred, but could not be obtained in the quantity required. The animal feeding study was conducted at Cloverdale Dairy, in King's County, CA (Photo 3) from Jan. 26 to June 21, 2012.

Photo 3 (left): corrals at commercial dairy where study was conducted.

Experimental Design and Duration

Three corrals, each containing 310 non-pregnant, mid-lactating dairy cows were used. Each corral received one of three diets which were the standard total mixed ration (TMR) without Se supplementation (Control), with TWG hay as the Se source (TWG) or with sodium selenite, as the Se source (SS). There was also an initial and final period during which the animals were maintained on a baseline diet.

For the TWG treatment, chopped TWG hay was added to the pre-mix for the TMR at 4.6% of diet dry matter (DM) and an equivalent portion of wheat straw was removed from the pre-mix to keep proportions of all other ingredients the same amongst the diets. For the SS diet, a 50 lb. bag of SodSel was added to the TMR for both the AM and PM loads so as to deliver the same amount of Se as in the TWG diet. The mixing wagon had weighing sensors to record the weight of each ingredient added to the ration and a vertical mixing bar to ensure a uniform mixture (Photo 2).

Corrals were rotated amongst the three diets every 4 weeks which resulted in a 3 x 3 Latin Square design as shown in Table 4. The animals were returned to the baseline diet on May 18^{th} and blood, urine and fecal sampling continued one month longer so as to track the depletion of Se (data not shown) from these biological materials.

Table 4. Experimental design (3 x 3 Latin Square) for dairy cattle feeding study.

	Period 1	**Period 2**	**Period 3**
Corral 25	1	3	2
Corral 26	2	1	3
Corral 27	3	2	1
Treatment Codes			
1	Control (WS)	P1	Feb 23-Mar 22
2	Tall wheatgrass (TWG)	P2	Mar 23-Apr 19
3	Sodium Selenite (SS)	P3	Apr 20-May 17

Sampling and Measurements

1) Feed Monitoring and Dry Matter Intake

- TMR Tracker, a software program, recorded the weight of all ingredients placed into the mixing wagon (Photo 4) and the amount fed to each pen.
- TMR and feed ingredients were sampled twice during the last week of each period (i.e., day 21 and 27) (Photo 5). Samples were frozen immediately at -20°C, and analyzed for dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), fat, starch and minerals, including Se
- DM intake was calculated as total amount of feed offered to each pen divided by the number of days in a period and by the number of cows in the pen. It was determined for the final 7 days of each experimental period.

Photo 4 (left): adding ingredients into the mixer wagon for the TMR (total mixed ration).
Photo 5 (right): TMR samples were collected in a bucket and then subsampled for chemical analysis.

2) Blood Sampling

- Blood was sampled from a pre-determined group of 28 cows with the objective of creating final sets of 18 cows/pen which were also sampled for feces, urine and milk.
- Blood samples were taken weekly from the tail vein for total Se into vacu-tainer tubes with EDTA and stored at 5°C. Samples were submitted to the California Animal Health and Food Safety (CAHFS) laboratory (Davis, CA, USA) for total Se analysis.

Photo 3: blood sampling from tail vein (left) and samples prior to analysis (right).

3) Fecal Sampling

- Taken on the last day of each experimental period as a rectal grab and placed in pre-marked container and analyzed for total Se.

4) Urine Sampling

- Taken on the second to last day of each period. Samples were collected in a specimen container, cooled with ice, subsampled, and analyzed for total Se

5) Milk Sampling

- Milk samples were taken from individual cows every fourth Thursday through the DHIA (Dairy Herd Improvement Association) milk testing program. Data were obtained for fat, lactose, true protein and somatic cell counts (SCC). Samples were obtained from DHIA for the set of 18 cows (same for which blood, feces and urine were measured) and total Se was determined on these milk samples.

Statistical Analysis

Results were statistically analyzed as a 3 x 3 Latin Square crossover design using the statistical package SAS 9.3 with corrals as the experimental unit for DM intake, and cows within pens used as the experimental unit for animal-based parameters. Sources of variation included treatment, period and pen.

Results

The chemical composition of the diets was the same, except for Se which was higher (P<0.01) in the TWG and SS diets (0.53 and 0.65 mg/kg DM) *versus* 0.35 mg/kg DM in the control diet (Table 5). This represents a 51 and 86% increase in Se for the TMG and SS diets respectively. Total Se turned out to be higher in the SS diet due to over estimation of weekly dry matter intake in that treatment.

Table 5. Chemical composition of the total mixed ration (TMR) of dairy cattle fed a control (C) level of Se, supplemental Se in the form of tall wheatgrass hay (TWG) or sodium selenite (SS) dietary supplement.

	Treatment				*P*		
	C	TWG	SS	SEM	C vs TWG	C vs SS	TWG vs SS
DM	52.29	52.92	52.97	2.920	0.79	0.78	0.98
CP, (%DM)	16.58	17.14	16.8	0.357	0.08	0.46	0.27
Lig, (%DM)	4.58	4.52	4.68	0.274	0.77	0.66	0.47
aNDom, (%DM)	34.13	33.52	33.57	0.676	0.28	0.32	0.93
aND, (%DM)	35.05	34.58	34.5	0.702	0.43	0.35	0.89
Ash, (%DM)	8.32	8.1	8.21	0.246	0.28	0.60	0.57
Fat, (%DM)	4.27	4.41	4.3	0.304	0.58	0.88	0.68
Se, (mg/kg DM)	0.35	0.53	0.65	0.031	<.0001	<.0001	0.0002

The TWG diet increased blood Se by 6.4%, whereas the SS diet increased it by only 4.8% (Fig. 4), suggesting slightly higher bioavailability for Se from TWG hay *versus* Na selenite. This was a noteworthy finding given that dietary Se turned out to be higher in the SS diet.

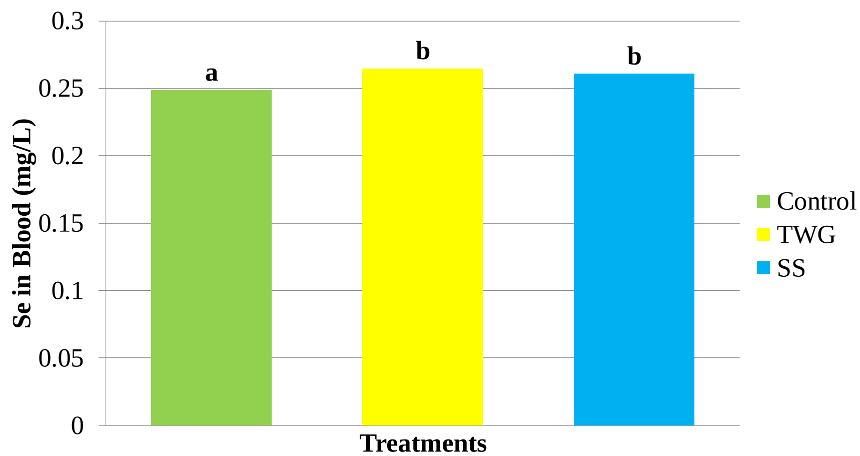

Fig. 4. Total Se in the blood of dairy cattle fed of dairy cattle fed a control level of Se, supplemental Se in the form of tall wheatgrass hay (TWG) or sodium selenite (SS) dietary supplement.

Se outputs in the urine did not differ among treatments (Fig. 5). Se loss in fecal matter was less for the SS diet (42%) as compared to the TWG diet (50.6%) suggesting lower bioavailability for Se from TWG hay *versus* Na selenite. Total output of Se (milk, urine, fecal matter) was 75% of the dietary intake for both the control and TWG diet, but lower (68.6%) for the SS diet. These data suggest lower bioavailability for Se from TWG hay, in contrast to the blood data which indicated slightly higher bio-availability for the Se from TWG hay *versus* Na selenite.

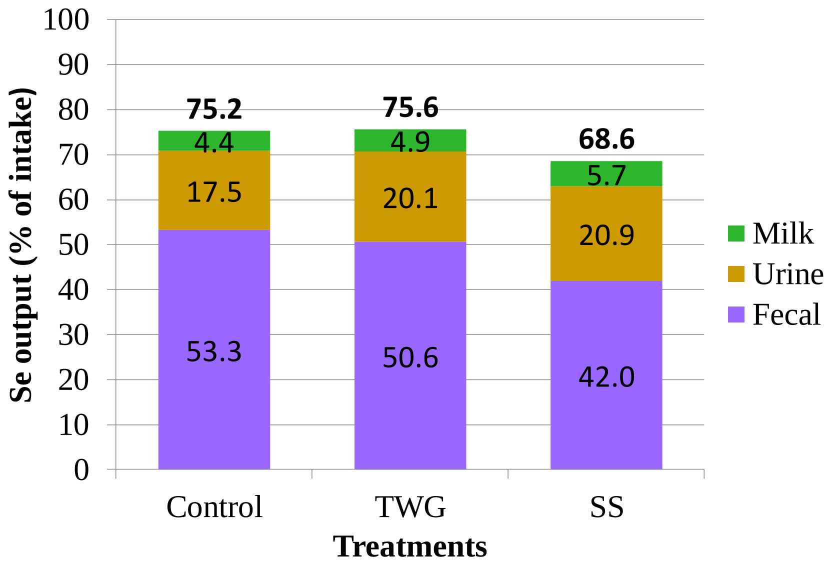

Fig. 5. Se output in milk, urine and fecal matter as a percentage of total Se intake in dairy cattle fed a control level of Se, supplemental Se in the form of tall wheatgrass hay (TWG) or sodium selenite (SS) dietary supplement.

Conclusions

- Feeding Se-enriched TWG increased blood Se by 6.4% over the control; whereas Se provided in the form of sodium selenite (SS) increased blood Se by only 4.8%, suggesting higher bioavailability for Se from TWG hay vs. SS.
- In contrast, the amount of dietary Se excreted in fecal matter was less (68.6%) with SS supplementation, as compared to 75.6% excretion for Se from TWG hay. In contrast to the blood data, these data suggest lower bioavailability for TWG as compared to SS.
- Bio-availability of Se is also influenced by the form of Se, e.g. inorganic vs. organic and the specific organic form (Se-methionine, seleno-methylcysteine, Se-protein, etc.). Future work will attempt to speciate the Se in TWG hay.
- Se outputs in the urine did not differ among treatments, thus the metabolizability of Se from SS appears to be higher than that from TWG.
- Further analysis of total Se output in the milk is required to fully assess the potential for Se-enriched TWG hay to be grown as a value-added forage product in the western SJV.
- Potential exists to develop an economic market for TWG as a 'natural' and SJV-originating selenium source

Impact Statements

- Results indicate that "Jose" tall wheatgrass (TWG) has good potential to be used as an organic Se supplement for livestock production in areas where soils are naturally deficient in Se and animals are routinely provided dietary Se supplements.
- Se-enriched TWG hay can be produced using a saline drainage water (or shallow groundwater) not suitable for the irrigation of most conventional crops.
- For agricultural areas that use subsurface ('tile') drainage for salt and water management, TWG cultivation using saline drainage water solves a disposal problem as this drainage cannot be discharged into local waterways.
- The liability of high Se drainage or groundwaters can be turned into a value-added product, (Se-enriched hay), which recycles Se within the San Joaquin Valley, obviating the need to import more Se in the form of dietary supplements for beef and dairy cattle.
- Production of Se-enriched hay for dietary supplements could be allocated to low quality soils not suitable for the production of higher value, salt- sensitive crops. Likewise, these soils could be used to produce TWG hay for general ruminant feeding, as long as levels of Se, S and Cu were monitored so as to not exceed recommended levels in the final ration.

Literature Cited

1. Bañuelos, G.S., Sharmarsakar, S., Cone, D. and G. Stuhr. 2003. Vegetative approach for improving the quality of water produced from soils in the Westside of central California. Plant Soil 249, 229–236.

1. Diaz, F.J. and S.R. Grattan. 2009. Performance of tall wheatgrass (*Thinopyrum ponticum*, cv. 'Jose') irrigated with saline-high boron drainage water: Implications on ruminant mineral nutrition. Ag Ecosyst Envi. 131: 128–136

2. Grattan, S.R., Grieve, C.M., Poss, J.A., Robinson, P.H., Suarez, D.L. and S.E. Benes. 2004. Evaluation of salt-tolerant forages for sequential reuse systems: III. Implications for ruminant mineral nutrition. Agric. Water Manage. 70, 137–150.

3. Grieve, C.M., Poss, J.A., Suarez, D.L. and D.A. Dierig. 2001. Lesquerella growth and selenium uptake affected by saline irrigation water composition. Ind. Crops Prod. 13, 57–65.

4. Minson, D.J., 1990. Forage in Ruminant Nutrition. Academic Press, New York, NY, USA.

5. National Research Council (NRC), 2001. Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001. The National Academies Press.

6. Robinson, P.H., Grattan, S. R., Getachew, G., Grieve, C.M., Poss, J.A., Suarez, D.L., Benes S.E. 2004. Biomass accumulation and potential nutritive value of some forages irrigated with saline-sodic drainage water. *Animal Feed Sci Technol* 111, 175-189.

7. Suyama, H., Benes, S.E., Robinson, P.H., Getachew, G., Grattan, S.R., and C.M. Grieve. 2007a. Productivity and quality of forages under long-term irrigation with saline-sodic drainage water

on the Westside San Joaquin Valley. Animal Feed Sci Technol 135, 329–345.

8. Suyama H., Benes, S. E., Robinson, P. H., Grattan, S. R., Grieve, C. M. and G. Getachew. 2007b. Forage yield and quality under irrigation with saline-sodic drainage water: Greenhouse evaluation. Agricultural Water Management 88, 159-172.

9. Terry, N., Zayed, A.M., de Souza, M.P., Tarun, A.S., 2000. Selenium in higher plants. Annual Review of Plant Physiology and Plant Molecular Biology 51, 401-432.

Abbreviations used in this report

DM = dry matter
DW = drainage water
SJV = San Joaquin Valley
TMR = total mixed ration
TWG = tall wheatgrass (*Thinopyrum ponticum*)

Treatment codes used in this report

Greenhouse experiment

LSe/LS = low selenium / low salinity
LSe/HS = low selenium / high salinity
HSe/LS = high selenium / low salinity
HSe/HS = high selenium / high salinity

CH20 = forage cut at 20 cm (8 inches)
CH40 = forage cut at 40 cm (16 inches
CH60 = forage cut at 60 cm (24 inches)

Dairy cattle feeding study

C = control level of Se supplementation
SS = diet using sodium selenite for Se supplementation
TWG = diet using tall wheatgrass hay for selenium supplementation

Scientific measures and units used in this report

EC_e = electrical conductivity of the saturated soil paste extract
EC_w = electrical conductivity of water (irrigation or source water)
ppm = mg/L (for waters and for soil analyses made on a saturated soil paste extract)
ppm = mg/kg (for plant tissue or for soil analyses made on dried soil)
ppb = µg/L (for waters and for soil analyses made on a saturated soil paste extract)