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Article

Nutrient Composition, Forage Parameters, and Antioxidant Capacity of Alfalfa (*Medicago sativa*, L.) in Response to Saline Irrigation Water

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Abstract: Although alfalfa is moderately tolerant of salinity, the effects of salinity on nutrient composition and forage parameters are poorly understood. In addition, there are no data on the effect of salinity on the antioxidant capacity of alfalfa. We evaluated four non-dormant, salinity-tolerant commercial cultivars, irrigated with saline water with electrical conductivities of 3.1, 7.2, 12.7, 18.4, 24.0, and 30.0 $dS \cdot m^{-1}$, designed to simulate drainage waters from the California Central Valley. Alfalfa shoots were evaluated for nutrient composition, forage parameters, and antioxidant capacity. Salinity significantly increased shoot N, P, Mg, and S, but decreased Ca and K. Alfalfa micronutrients were also affected by salinity, but to a lesser extent. Na and Cl increased significantly with increasing salinity. Salinity slightly improved forage parameters by significantly increasing crude protein, the net energy of lactation, and the relative feed value. All cultivars maintained their antioxidant capacity regardless of salinity level. The results indicate that alfalfa can tolerate moderate to high salinity while maintaining nutrient composition, antioxidant capacity, and slightly improved forage parameters, thus meeting the standards required for dairy cattle feed.

Keywords: alfalfa; salinity; forage quality; nutrient composition; antioxidant capacity; total phenolics

1. Introduction

Alfalfa (*Medicago sativa*, L.) is the most cultivated legume worldwide and the fourth most cultivated crop in the United States. Alfalfa is cultivated in most continents and in more than 80 countries occupying more than 35 million ha [1]. In the USA, it is among the top three field crops cultivated in 26 states, thus contributing more than US $10 billion a year to the farm economy, primarily as an animal feed [2]. Alfalfa is considered to be the most important forage crop for providing protein to dairy and beef cattle, sheep, horses, birds, and other livestock [1]. Feeding of alfalfa hay to lactating dairy cows has decreased sharply in the past 10 years, primarily as a result of economic issues associated with high water use, the costs of multiple harvests, and storage [3]. These authors also mentioned the increased use of corn and cereal silages in animal diets to replace alfalfa. However, dry matter intake is significantly higher for cows fed alfalfa and barley silages than for cows fed oat and triticale silages [4]. According to these authors, alfalfa silage contains higher concentrations of all minerals analyzed compared with cereal silages, except for Na. Moreover, the cows also absorbed K better from alfalfa silage (89%) than from cereal silages (74% to 83%). Alfalfa is highly important to livestock considering its fast canopy recovery after each harvest, its relative tolerance of salinity, its capacity to endure temperature extremes (e.g., hot days and cold nights), its nutritional value, and palatability to livestock.

In arid lands, irrigation is necessary for high forage mass production. However, this irrigation is often associated with salinization. Among the approximately 270 million hectares of irrigated land worldwide, about 40% is located in arid/semiarid zones [5] where soil salinization generally occurs. Some of the typical agronomic parameters used to evaluate the salinity tolerance of crops include yield, survival, plant height, and relative growth rate or reduction [6–8]. Few researchers have evaluated alfalfa forage mass production, nutrient composition, and forage parameters for livestock under high salinity stress [9–12]. Further, we found no published reports on the effects of salinity on the antioxidant capacity of alfalfa. It has been reported that salinity stress imposed on a model legume (*Lotus japonicus*) increased antioxidant enzyme levels in leaves [13], and that the expression of genes associated with antioxidant enzymes increased in response to excessive levels of reactive oxygen species (ROS) generated by salinity stress [14]. These authors postulated that these enzymes protect plant tissues from ROS damage triggered by salinity stress, but there are no reports on the biosynthesis of non-enzymatic antioxidants, such as flavonoids and phenolic compounds, by alfalfa in response to salinity. Alfalfa shoots are a rich source of antioxidant flavonoids, mainly apigenin, tricin, luteolin, and chrysoeriol glycosides [15], and of phenolic compounds reported to have anti-inflammatory [16], antioxidant, and neuroprotective activity in mice [17]. The ratio of alfalfa antioxidant flavones acylated with hydroxycinnamic acid to non-acylated (lower antioxidant capacity) flavones increases in summer when plants are exposed to a higher amount of UV-B radiation [15]. Antioxidant flavonoids in *Ligustrum vulgare* were reported to increase under both UV-B and NaCl salinity stress [18]. Thus, although

alfalfa is fed to livestock for its high protein content, digestibility, and palatability, there is a scarcity of information on the effects of salinity on alfalfa mineral composition and forage quality, while there is no information on its antioxidant capacity under salinity stress.

In this work, we evaluated four commercial alfalfa cultivars, tolerant to salinity, for their response to salinity when cultivated in outdoor sand tanks and irrigated at six salinity levels with water high in sodium, chloride, and sulfate. The goal of our work was to evaluate the effects of increasing salinity on the mineral nutritional composition, forage quality, and antioxidant capacity of alfalfa shoots.

2. Experimental Section

2.1. Plant Material and Growth Conditions

Four commercial non-dormant, salinity-tolerant, *Medicago sativa* L. cultivars "Salado", "SW8421S", "SW9215", and "SW9720" (S&W, Fresno, CA, USA, www.swseedco.com) were grown from seeds in 24 outdoor sand tanks from 23 June 2011 to 17 April 2012 at the Salinity Laboratory (USDA-ARS) in Riverside, California. Irrigation water at different levels of electrical conductivity (EC) was applied to four cultivars in a split-plot design. The irrigation water EC (measured in deciSiemens per meter) levels consisted of a control using Riverside tap water (EC = 0.6 $dS \cdot m^{-1}$) plus fertilizers (EC = 3.1 $dS \cdot m^{-1}$), and treatments of 7.2, 12.7, 18.4, 24.0 and 30.0 $dS \cdot m^{-1}$, with four tanks (replicates) per treatment. The tanks measured 82 cm wide by 202 cm long by 85 cm deep. Further details on sowing density per cultivar and irrigation frequency are described elsewhere [19]. Salinity treatments and the irrigation water control (EC of 3.1 $dS \cdot m^{-1}$) were designed to simulate the drainage water composition of the Central Valley, CA, with subsequent concentration of salts considering mineral precipitation (calcite and/or gypsum) using the UNSATCHEM model [20], which simulates typical soil water interactions. All reservoirs had modified Hoagland's solution, and added Na^+, SO_4^{2-}, and Cl^- (including control water) to reach the target EC; the detailed composition is described elsewhere [19]. The composition of Riverside tap water (EC = 0.6 $dS \cdot m^{-1}$) in $mmolc \cdot L^{-1}$ was: 3.4 Ca^{2+}, 0.8 Mg^{2+}, 1.6 Na^+, 0.1 K^+, 1.3 SO_4^{2-}, 0.8 Cl^-, and 0.49 NO_3^-. The water composition of all the treatment waters is shown in Table 1.

2.2. Plant Growth and Nutrient Composition

Growth and forage mass measurements were collected at seven harvest dates except for the plants that were irrigated with water with an EC = 24.0 $dS \cdot m^{-1}$, which were harvested three times (4th, 6th, and 7th harvests) during the 299 days of cultivation and are presented elsewhere [19]. For this work, we present data on ionic and nutrient composition at 84 days after seeding (DAS) (2nd harvest, on 15 September 2011) and at 299 DAS (7th harvest, on 17 April 2012). The second harvest was conducted when the control plants were at the early flowering stage, corresponding to morphological stage 5 [21]. The seventh harvest was conducted when the control plants were at a late vegetative stage (due to the absence of flowering). The shoot fresh and dry weights (dried at 60 °C for 48 h) were recorded at each harvest and all plants were cut back to 5–8 cm above the sand surface.

Table 1. Chemical composition of the water used in the six salinity treatments in this study. EC, electrical conductivity of irrigation water that defines each salinity level (in deciSiemen per meter); mmolc·L^{-1}, millimole of charge of each cation or anion listed.

Treatment	1	2	3	4	5
EC ($dS \cdot m^{-1}$)	3.1	7.2	12.7	18.4	24.0
Ion Concentration in mmolc·L^{-1}					
Ca^{2+}	6.4	19.2	25.0	29.4	28.4
Mg^{2+}	4.0	14.3	24.1	40.7	58.5
Na^{+}	15.5	54.2	101	169	229
K^{+}	6.4	6.4	6.2	6.4	6.6
SO_4^{2-}	15.3	53.3	85.0	132	182
Cl^{-}	8.0	31.8	62.9	104	133
PO_4^{3-}	0.3	0.3	0.3	0.4	0.5
NO_3^{-}	5.5	5.6	5.5	6.0	6.0

All salinity levels had the following added nutrients, (in mmolc·L^{-1}): 0.3 KH_2PO_4, 5.0 KNO_3, 3.1 $MgSO_4.7H_2O$, 3.0 $CaCl_2$, and 1.0 KCl. Table modified from [19]. Highest salinity level (30 $dS \cdot m^{-1}$) not shown as all plants died at this level.

The levels of the macronutrients N, P, K, Ca, Mg, and total S, and of the micronutrients Fe, Cu, Mn, Zn, and Mo were determined from nitric acid digestions of the dried and ground plant material using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, 3300DV, Perkin-Elmer Corp., Waltham, MA, USA). There was insufficient plant material to analyze samples from the EC = 24 $dS \cdot m^{-1}$ treatment at 84 DAS, and there are no data from the EC = 30 $dS \cdot m^{-1}$ treatment as all plants died at this salinity level.

2.3. Oxygen Radical Absorbance Capacity (ORAC) and Total Phenolics (TP) Analyses

Ground dried samples (0.5 g) of alfalfa tops were mixed with 5 g of sand. Each mixture was then extracted in a pressurized stainless steel cell (ASE 350, Thermo Scientific/Dionex, Sunnyvale, CA, USA) using hexane to extract the lipophilic fraction and acetone:water:acetic acid (70:29.5:0.5 by volume) for the hydrophilic fraction. The extraction time was 5 min, followed by a 100% flush, a 60-s purge with 2 cycles, at 80 °C and 1500 psi. The hexane extract was evaporated to dryness with nitrogen in an evaporator (N-EVAP, Organomation, Berlin, MA, USA) at 37 °C and then redissolved in 10 mL of pure acetone; a 50-µL aliquot was collected for dilution and lipophilic ORAC analysis. After extraction with aqueous acetone by the ASE 350, the samples were made up to a volume of 25 mL in the acetone-water-acetic acid solution. A 150-µL aliquot of the aqueous acetone extracts was diluted for hydrophilic ORAC analysis. The ORAC assay is based on the inhibition of the peroxyl-radical-induced oxidation initiated by thermal decomposition of azo-compounds such as [2,2′-azobis(2-amidino-propane) dihydrochloride (AAPH)] [22]. Samples were analyzed for their antioxidant capacity (ORAC) in triplicate. The same ASE 350 aqueous acetone extracts were used for quantification of TP according to the Folin-Ciocalteu method [23,24] using gallic acid (cat. No. 398225, Sigma-Aldrich, Saint Louis, MO, USA) as the standard. A 20-µL aliquot of the extracts or a gallic acid standard solution was pipetted into a cell of a 96-cell microplate, followed by the addition of 100 µL of 0.4 N Folin Ciocalteu phenol reagent (stock solution F9252, Sigma-Aldrich, Saint Louis, MO, USA) and the

addition of 80 μL of 0.94 M Na_2CO_3. The plate was covered with a plastic plate cover and allowed to develop color for 5 min at 50 °C. The absorbance was read at 765 nm using a microplate spectrophotometer (xMark™, BIO-RAD, Hercules, CA, USA).

2.4. Forage Quality

Shoots were dried at 60 °C for 48 h. Samples were ground to a size of 1.0 mm and analyzed for acid detergent fiber (ADF), neutral detergent fiber (NDF), and moisture by an independent laboratory (Analytical Feed & Food Laboratory, Visalia, CA, USA), according to AOAC International Methodology [25]. The parameters and analytical methods used were AOAC 973.18 for ADF, AOAC 2002.04 for NDF, and AOAC 930.15 for moisture. The parameters calculated according to ADF, NDF, and/or moisture include the net energy for lactation (NEL), calculated as NEL = 0.8611 – (0.00835 × ADF); relative feed value (RFV), calculated as RFV = (DMD × DMI)/1.29; dry matter intake (DMI), calculated as DMI = 120/NDF; and dry matter digestibility (DMD), calculated as DMD = 88.9 – (0.779 × ADF), according to National Forage Testing Association [26]. Crude protein (CP) was estimated as N% × 6.25 [27]. Nitrogen was determined by sample combustion in pure oxygen and measured by thermal conductivity detection (AOAC, 2000; ID 990.03) using a Vario Pyro Cube® (Elementar Americas, Inc., Mt. Laurel, NJ, USA).

2.5. Statistical Analysis

The nutrient composition data for each harvest were analyzed using a split-plot procedure, with the following statistical model:

$$Y_{ijk} = \mu + S_j + R_i + C_k + (SC)_{jk} + \varepsilon_{ijk}$$

where R, S and C represent the replicates (i = 1,…4), salinity level (j = 1,…5), and cultivars (k = 1,…4) respectively. All effects were considered as fixed. Thus, Y_{ijk} is the response to replicate i in S_j and C_k, μ is the overall mean; and ε_{ijk} represents the random error. The significance in the split-plot design was calculated by deriving the mean squares in the analysis of variance using the InfoStat program [28] with a completely randomized design (CRD). The significance of the main plot (salinity, S) was tested by S > R (salinity inside replicate) as an experimental error of the main plot, and the mean square error was used to test significance of the subplot (C) and the interaction S × C (salinity per cultivar). The mean differences were determined using the Fisher LSD test at $p \leq 0.05$. Chemical analyses for forage parameters were performed on two samples per cultivar, which were combined to represent each salinity level (n = 8) per harvest. These data (Figure 1) were subjected to a one-way (salinity) ANOVA with means compared by the Fisher LSD test. For total phenolics (TP) and antioxidant capacity (ORAC) analyses, samples were analyzed in triplicate, where total phenolics were quantified from a gallic acid standard curve. The effects of salt as a main plot, cultivar as a subplot, and the interaction between salt and cultivar (salt × cultivar) for ORAC and TP concentrations were analyzed at $p \leq 0.05$ using the GLM procedure with a standard split-plot test format in SAS (version 9.3; SAS Institute, Cary, NC, USA). The differences in ORAC and TP between the two harvests were analyzed at $p \leq 0.05$ using the *T*-test procedure in SAS (version 9.3; SAS Institute, Cary, NC, USA).

3. Results

3.1. Forage Quality

The impact of salinity on forage quality, expressed as the mean of the four cultivars at each salinity level per harvest, is presented in Figure 1. The parameters used to evaluate forage quality include acid detergent fiber (ADF), neutral detergent fiber (NDF), net energy for lactation (NEL), crude protein (CP), and relative feed value (RFV).

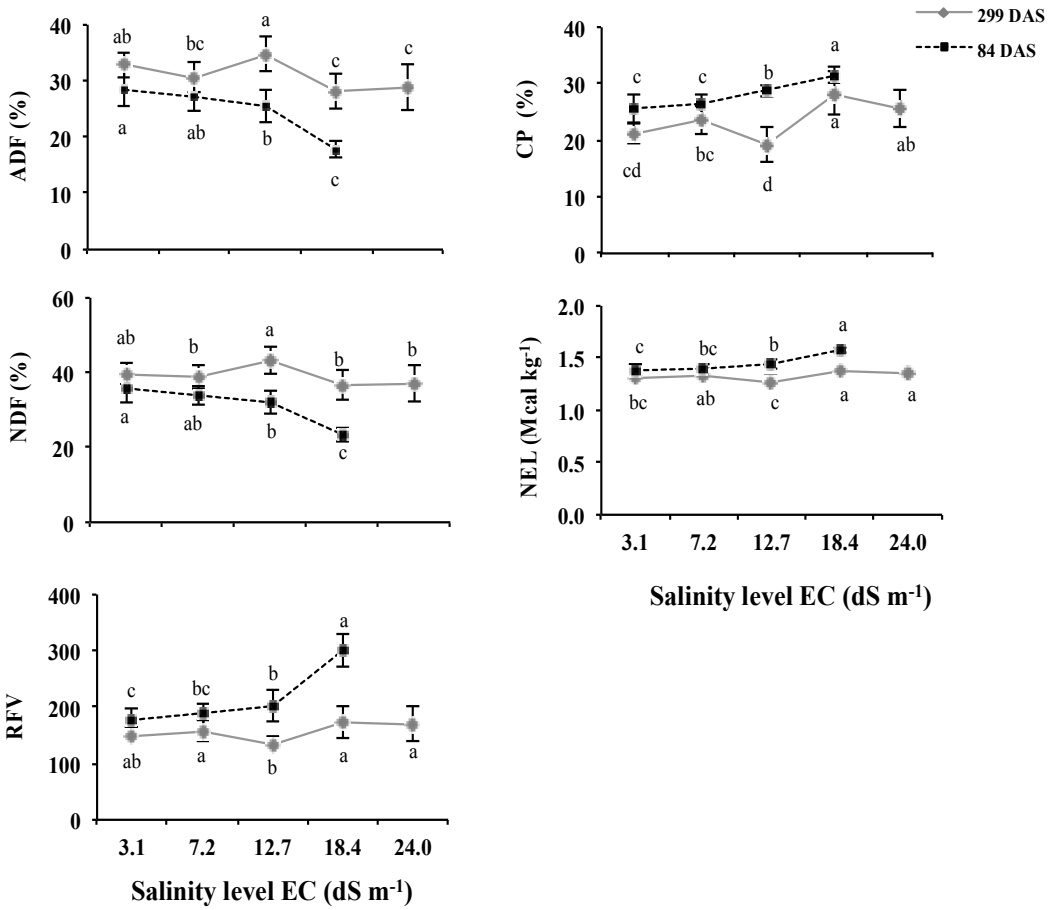

Figure 1. Impact of salinity increase on acid detergent fiber (ADF), neutral detergent fiber (NFD), net energy of lactation (NEL), crude protein (CP), and relative feed value (RFV) of salt-tolerant alfalfa. Data points represent the means (±SD) of the salinity-tolerant cultivars (n = 8). Means with the same letter are not significantly different according to a Fisher LSD test ($p \leq 0.05$). For the harvest at 84 DAS, the lack of data at 24 dS·m^{-1} was due to there being insufficient plant material for analysis because of growth limitations.

Salinity had a significant effect on the forage quality for both harvests ($p \leq 0.001$). At 84 DAS, there were no differences up to EC = 7.2 dS·m^{-1} for all parameters evaluated. Above that level, ADF and NDF decreased by approximately 8% and 9%, respectively, from 12.7 to 18.4 dS·m^{-1}. Consequently, the RFV (related to the ADF and NDF contents) increased sharply between those levels. CP increased by 5.2% from 7.2 to 18.4 dS·m^{-1} (Figure 1). In addition, the mean NEL increased as salinity increased. At 299 DAS, salinity also affected all forage parameters ($p \leq 0.05$). In contrast to 84

DAS, at 299 DAS significant differences between the control and salinity treatments generally were first observed at 12.7 $dS \cdot m^{-1}$ instead of at 7.2 $dS \cdot m^{-1}$ (Figure 1).

3.2. Nutrient Composition of Alfalfa

3.2.1. Macronutrients

The macronutrient (modified from [19]) data, including N and P, are expressed on a dry matter (DM) basis (Table 2). The main macronutrients found in alfalfa shoots ($g \cdot kg^{-1}$ DM) at both harvests were N, K, and Ca, while total S, Mg, and P were present at much lower levels (Table 2). Salinity had a significant effect on all macronutrients for both harvests, except for total S at 299 DAS. Nitrogen increased with salinity for both harvests, reaching levels that were significantly higher than those of the control at and above 12.7 $dS \cdot m^{-1}$ (84 DAS), and at and above 18.4 $dS \cdot m^{-1}$ (299 DAS). Shoot K decreased significantly ($P \leq 0.01$) for all cultivars and harvests as salinity increased. The calcium content remained constant up to 7.2 $dS \cdot m^{-1}$ (84 DAS) or up to 12 $dS \cdot m^{-1}$ (299 DAS), but decreased significantly for both harvests (more drastically at 299 DAS) as salinity increased. The Mg levels significantly increased for both harvests, with salinity, from the control to the highest level of salinity (84% and 48% increases for 84 DAS and 299 DAS, respectively). Sulfur concentrations increased with salinity, being significant ($p \leq 0.01$) at 84 DAS, but not at 299 DAS. Concentrations of P remained constant up to 12.7 $dS \cdot m^{-1}$, but increased significantly ($p \leq 0.01$) above that salinity level for both harvests (Table 2). There was a significant ($p \leq 0.01$) cultivar effect for all macronutrients (except for N) at 84 DAS, while at 299 DAS, there was a significant cultivar effect only for Ca and Mg (both at $p \leq 0.05$). Both Na and Cl increased significantly ($p \leq 0.01$) in shoots with increasing salinity, but these and detailed data by cultivar and salinity are presented in a companion paper [19].

Table 2. Average macronutrients (±SE) in alfalfa shoot dry matter (DM) according to salinity levels. EC, electrical conductivity of irrigation water in deciSiemens per meter. ND, not determined (insufficient biomass). Modified from [19].

	N	P	K	Ca	Mg	Total S
			DM ($g \cdot kg^{-1}$)			
			EC $dS \cdot m^{-1}$ Second Harvest (84 DAS)			
3.1	40.8 ^{c} ± 1.43	2.6 ^{b} ± 0.09	46.4 ^{a} ± 1.05	14.1 ^{a} ± 0.4	2.6 ^{c} ± 0.14	3.5 ^{d} ± 0.08
7.2	42.1 ^{c} ± 1.04	2.7 ^{b} ± 0.09	41.4 ^{b} ± 0.94	13.5 ^{a} ± 0.5	2.7 ^{c} ± 0.16	3.9 ^{c} ± 0.10
12.7	46.0 ^{b} ± 0.56	2.9 ^{b} ± 0.08	38.6 ^{c} ± 0.62	13.0 ^{c} ± 0.69	3.4 ^{b} ± 0.22	4.8 ^{b} ± 0.20
18.4	50.5 ^{a} ± 0.80	3.8 ^{a} ± 0.13	34.3 ^{d} ± 0.88	12.1 ^{b} ± 0.24	4.8 ^{a} ± 0.07	7.4 ^{a} ± 0.17
24	ND	ND	ND	ND	ND	ND
			Seventh Harvest (299 DAS)			
3.1	34.1 ^{d} ± 1.07	3.4 ^{b} ± 0.17	40.3 ^{a} ± 1.12	18.0 ^{a} ± 0.51	2.5 ^{c} ± 0.08	3.8 ^{a} ± 0.12
7.2	37.6 ^{bc} ±1.37	3.1 ^{b} ± 0.06	30.4 ^{bc} ± 0.74	18.3 ^{a} ± 0.61	2.8 ^{bc} ± 0.12	4.6 ^{a} ± 0.20
12.7	30.8 ^{d} ± 1.77	2.8 ^{b} ± 0.14	31.0 ^{b} ± 0.68	16.7 ^{a} ± 0.51	3.2 ^{ab} ± 0.12	4.8 ^{a} ± 0.15
18.4	45.3 ^{a} ± 2.11	4.1 ^{a} ± 0.12	27.3 ^{cd} ± 0.56	12.1 ^{b} ± 0.45	3.0 ^{bc} ± 0.10	4.8 ^{a} ± 0.15
24	40.8 ^{a} ±1.92	4.3 ^{a} ±0.16	26.7 ^{d} ± 0.61	11.0 ^{b} ± 0.83	3.6 ^{a} ± 0.20	5.3 ^{a} ± 0.39

Different small letters within each column, and between EC levels, represent significantly different means according to Fisher's LSD test ($p \leq 0.05$), where $n = 16$ (except for N, $n = 8$) for EC levels.

3.2.2. Micronutrients

Shoot micronutrients analyzed for the four alfalfa cultivars were iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), and molybdenum (Mo) (Table 3). At 84 DAS, there were no differences in mean Fe concentrations (ranging from 99.1 to 109.6 mg·kg^{-1} DM) or Cu (2.07–3.11 mg·kg^{-1} DM) as a function of increasing salinity (EC). Mean concentrations of Mn and Mo tended to increase with increasing salinity with significant ($p \leq 0.05$ and $p \leq 0.01$, respectively) differences between the control and the highest salinity level (18.4 dS·m^{-1}) at 84 DAS. There was a significant ($p \leq 0.01$) increase in Zn concentration at each level of salinity increase at 84 DAS. At 299 DAS, the Fe, Cu, Mn, and Zn levels remained mostly unchanged, but there was a small but significant ($p \leq 0.05$) decline (16%–28%) in the Fe levels between the 3.1 dS·m^{-1} control (116 mg·kg^{-1} DM) and the other saline treatments. Mn showed a transient increase of 42% (17.3 to 24.6 mg·kg^{-1} DM) as salinity increased from 3.1 to 7.2 dS·m^{-1}, and then declined to the salinity control levels. In general, the shoot Mo concentrations for all levels of salinity were significantly ($p \leq 0.05$) higher than those of the control (Table 3).

Table 3. Average micronutrient concentrations (±SE) in alfalfa shoot dry matter (DM), according to salinity levels. EC, electrical conductivity of irrigation water in deciSiemens per meter. ND, not determined (insufficient biomass).

	Fe	Cu	Mn	Zn	Mo
	DM (mg·kg^{-1})				
EC dS·m^{-1}	**Second Harvest (84 DAS)**				
3.1	104.0 ^{a} ± 6.29	2.1 ^{a} ± 0.27	25.5 ^{b} ± 3.38	40.9 ^{d} ± 1.32	2.0 ^{c} ± 0.09
7.2	99.1 ^{a} ± 4.90	2.3 ^{a} ± 0.10	31.7 ^{ab} ± 4.8	45.9 ^{c} ± 1.00	3.1 ^{b} ± 0.11
12.7	106.5 ^{a} ± 5.89	3.1 ^{a} ± 0.16	34.8 ^{a} ± 4.10	54.9 ^{b} ± 1.11	3.2 ^{b} ± 0.14
18.4	109.6 ^{a} ± 5.0	3.1 ^{a} ± 0.19	34.8 ^{a} ± 1.10	60.5 ^{a} ± 1.25	4.1 ^{a} ± 0.11
24	ND	ND	ND	ND	ND
	Seventh Harvest (299 DAS)				
3.1	116.1 ^{a} ± 6.35	5.8 ^{a} ±0.83	17.2 ^{b} ± 0.91	97.6 ^{a} ± 3.36	2.7^{c} ± 0.19
7.2	97.7 ^{b} ± 7.35	6.1 ^{a} ± 0.64	24.6 ^{a} ± 1.44	89.9 ^{a} ± 3.26	6.4 ^{a} ± 0.43
12.7	89.9 ^{b} ± 7.35	6.5 ^{a} ± 0.41	18.9 ^{b} ± 0.99	105.6 ^{a} ± 3.18	6.3 ^{a} ± 0.44
18.4	83.5 ^{b} ± 3.17	5.3 ^{a} ± 0.26	17.4 ^{b} ± 1.05	101.3 ^{a} ± 3.26	4.7 ^{c} ± 0.36
24	92.3 ^{b} ± 7.69	5.7 ^{a} ± 0.49	14.8 ^{b} ± 1.04	98.3 ^{a} ± 3.85	4.2 ^{c} ± 0.21

Different lower case letters within each column, and between EC levels, represent significantly different means according to Fisher's LSD test ($p \leq 0.05$), where $n = 16$.

3.3. Antioxidant Capacity of Alfalfa

Salinity had no effect ($p > 0.05$) on either the oxygen radical absorbance capacity (ORAC) or the total phenolic levels of the four alfalfa cultivars. The hydrophilic fractions of shoots had most (68%–99%) of the shoot total antioxidant capacity (Table 4). At early plant development (84 DAS), alfalfa shoots had hydrophilic ORAC ($ORAC_{Hydro}$) levels that ranged from 190–230 μmoles·TE·g^{-1} DM (Figure 2), while at 299 DAS, $ORAC_{Hydro}$ ranged from 229–274 μmoles·TE·g^{-1} DM, and the shoot total antioxidant capacity ranged from 244–287 μmoles·TE·g^{-1} DM (Figure 2, Table 4). Total phenolic (TP) concentrations ranged from 5.0–5.6 mg·GAE·g^{-1} DM for both harvests (Figure 2).

Table 4. Oxygen radical absorbance capacity of the lipophilic ($ORAC_{Lipo}$) and hydrophilic ($ORAC_{Hydro}$) fractions, and total antioxidant capacity ($ORAC_{Hydro}$ + $ORAC_{Lipo}$), in micromoles of trolox equivalents per gram of dry matter (μmoles·TE·g^{-1} DM) of alfalfa irrigated with water of different electrical conductivities (EC). Plants were sampled on 17 April 2012 (299 DAS). Data are means ± SE combined for the four cultivars with two replicated analyses per sample (n = 8).

EC	$ORAC_{Lipo}$	$ORAC_{Hydro}$	$ORAC_{Total}$
(dS·m^{-1})	(μmoles·TE·g^{-1} DM)		
3.1	15.0 ± 2.4	239.5 ± 12.8	254.5 ± 13.6
7.2	11.2 ± 1.5	252.1 ± 11.6	263.3 ± 11.0
12.7	13.4 ± 2.5	273.6 ± 14.3	286.9 ± 14.2
18.4	16.4 ± 1.7	268.4 ± 14.0	284.8 ± 15.5
24.0	15.3 ± 1.0	228.8 ± 18.3	244.1 ± 18.2

There was no effect of salinity (expressed as EC), cultivar, or the salt × cultivar interaction.

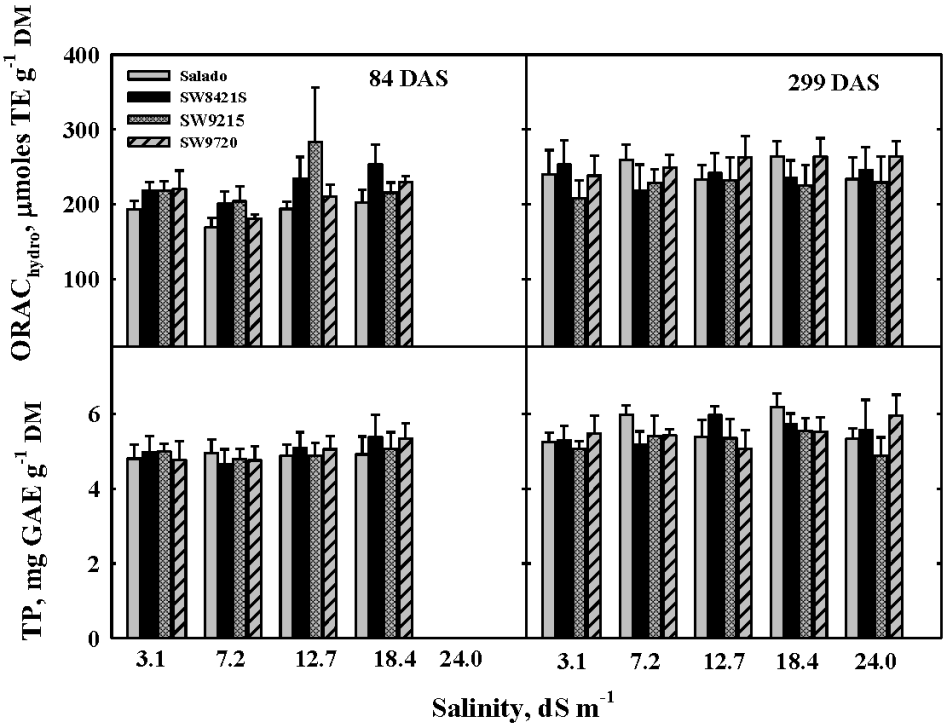

Figure 2. Total phenolics (TP) and hydrophilic shoot oxygen radical absorbance capacity (ORAC) of four salinity-tolerant alfalfa cultivars irrigated with saline water with different electrical conductivity levels. ORAC was measured in micromoles of trolox equivalents per gram of dry matter (μmoles·TE·g^{-1} DM). TP was measured as mg of gallic acid equivalents per gram of dry matter (mg·GAE·g^{-1} DM). Bars represent means (±SD), where n = 4. Plants were sampled at 84 and 299 days after sowing. For the harvest at 84 DAS, the lack of data at 24 dS·m^{-1} was due to growth limitations.

4. Discussion

4.1. Forage Quality

Forage quality was based on laboratory analyses of shoot biomass and evaluated in relation to recommended forage standards for livestock production output (e.g., milk, body weight gain) for animals consuming alfalfa of similar nutritional value and energy content [3,29]. Lower NDF translates into both increased DMI and milk yield within a forage family [3]. Regarding alfalfa protein, approximately 80% is degraded in the rumen of polygastric animals, but addition of tannins to alfalfa feed decreases rumen protein degradability and increases protein absorption [30].

Plant maturity is the main factor affecting forage quality [31], but the interaction between environmental and agronomic factors with maturity will influence the quality of alfalfa, even if harvested at the same stage of development [32]. Similarly, approaching harvest time, any stress that delays or accelerates alfalfa maturation affects the leaf-to-stem ratio and consequently, forage quality. The stems contain mostly structural components and are low in N, while the leaves contain mainly photosynthetic components and are richer in N than the stems. As a result, leaves have two to three times more CP than stems [33]. Increased leaf N leads to increased leaf area, thus increasing the leaf/stem ratio [34,35], but this could also be accounted for by the reduced stem height caused by salinity. The leaf-to-stem ratio increase leads to decreases in both ADF and NDF. Decreased ADF and NDF and increased shoot N lead to higher shoot CP levels in alfalfa irrigated with saline water. As reported in a previous study [19], plant height was significantly reduced by salinity only at 84 DAS, with the average difference in plant height between the control and EC = 18.4 $dS \cdot m^{-1}$ being 23 cm. Thus, we hypothesize that the decrease in height (shorter internodes) in salt-affected plants may have increased the leaf-to-stem ratio, shoot N, and CP by 61 $g \cdot kg^{-1}$ DM (6%). This decreased height of salt-affected plants also led to decreases in ADF and NDF of 107 and 122 $g \cdot kg^{-1}$ DM (10.7% and 12.2%) at 84 DAS and of 2.5% and 4% at 299 DAS, respectively, improving forage potential quality (Figure 1). This is in agreement with a previous report that salinity increased alfalfa leaf-to-stem ratio, slightly improving forage quality [36].

Al-Khatib and collaborators [7] reported that the leaf-to-stem ratio of alfalfa increased while forage mass decreased in response to increasing NaCl until 20 $dS \cdot m^{-1}$ (200 mM NaCl). At 299 DAS, there was also a significant increase in CP of 42.1 $g \cdot kg^{-1}$ DM (4.2%) between the control plants and those under 24 $dS \cdot m^{-1}$ (reflecting the increased accumulation of leaf N with increased salinity). This increase in CP was observed at both 84 and 299 DAS because N accumulation in shoots increased by 23% and 33%, respectively, in response to increased salinity (Table 2, Figure 1). Although plants had a fairly constant supply of N from NO_3^- in all irrigation treatments (Table 1), shoots significantly accumulated NO_3^--N, leading to higher CP. This could be due to morphological changes (e.g., increased leaf-to-stem ratio) under salinity stress or because the roots in the sand tanks were found to be associated with rhizobia. Despite the differences in developmental stages between the second and seventh harvest, there was a tendency for CP to increase with salinity levels up to 18.4 $dS \cdot m^{-1}$. Although plants irrigated with salinity levels higher than the control had different stages of maturity, plant height has been used to predict forage parameters under field conditions [33,37].

Differences in forage parameters changed more sharply at 84 DAS (late summer) with salinity than at 299 DAS (early spring). These changes were likely caused by differences in climatic conditions combined

with salinity [19]. Both climate and intervals between harvests (24 days before the second and 54 days before the seventh harvest) have a direct impact on maturity [33,38]. The RFV of alfalfa shoots in this experiment were similar to the values reported for alfalfa cultivars grown under field conditions with EC values ranging from 4–16 $dS \cdot m^{-1}$, although RFV did not change with salinity [39].

According to the classification of alfalfa hay [40], and judging from the parameters evaluated in this study, alfalfa herbage grown at the highest tolerated salinity fell within the "supreme" category. In comparison, forage grown at control salinity levels would be classified as "good" and "premium". Hence, our results indicated that forage quality improved with increasing salinity (despite some variation), independently of the changes between harvest seasons. Similar increases in CP and decreases in ADF in the salinity-tolerant cultivars Salado and SW9720 under salinity stress have been reported [9,11]. An increase in CP of alfalfa cultivars less tolerant to salinity was also reported when salinity increased from 2.1 to 7.8 $dS \cdot m^{-1}$ [41] or when salinity ranged from 0.3–4.5 $dS \cdot m^{-1}$ in one out of three years of cultivation [42]. Both drought and salinity restrict the growth of alfalfa, and mild drought also improves the forage quality of alfalfa [43]. These authors explained that the increase in quality with drought was due to a delay in plant maturation and an increase in the leaf-to-stem ratio; the latter is related to a reduction in stem length. However, the results of a 90-day pot experiment indicated that there were no differences in CP or N concentrations in alfalfa shoots when an EC of 15 $dS \cdot m^{-1}$ was applied using only NaCl [44].

The NEL values of alfalfa irrigated with increasing salinity, and ranging from 1.38–1.58 $Mcal \cdot kg^{-1}$ for the second harvest (84 DAS) and from 1.3 to 1.37 $Mcal \cdot kg^{-1}$ for the seventh harvest (299 DAS), were within the average (1.47 $Mcal \cdot kg^{-1}$) required for lactating cows [29], although some supplementation may be required to maintain the required energy levels.

4.2. Mineral Nutrient Composition

When irrigated with non-saline water, the predominant macronutrients in alfalfa are N, K, Ca, Mg, P, and S [45]. In our plants, which were fertilized to achieve the desired macro and micronutrients concentrations for ideal crop growth, and irrigated with saline water, the three main shoot macronutrients were also N, K, and Ca, followed by Cl and Na (data presented in [19]) and S, as these were added to the irrigation water to achieve high salinity, then followed by Mg and P at similar concentrations (Table 2). This suggests that alfalfa plants were provided adequate nutrients for growth, and our results express mostly the effects of salinity in a properly fertilized crop. The discussion on macro- and micronutrient requirements is based on the specifications for lactating dairy cattle provided by the Nutrient Requirements of Dairy Cattle [29]. The NRC requirement level for animals producing 35 kg $milk \cdot day^{-1}$ (Holstein or Jersey) was used, based on the average milk production for 2012 in California [46].

Macronutrients and sodium—Although adequate mineral nutrition alone will not prevent animal diseases, susceptibility to infectious diseases in response to malnourishment has been recognized for several centuries [47]. Thus, it is important to know if crop stress induced by salinity alters the nutrient composition of alfalfa.

The lowest Ca concentration in shoots in response to salinity (11 $g \cdot kg^{-1}$) was still above the daily dietary requirement (6.1 $g \cdot kg^{-1}$) for dairy cattle [29], while the highest Ca concentrations (18 $g \cdot kg^{-1}$)

were observed at ECs of 3.1 and 7.2 $dS \cdot m^{-1}$ at 299 DAS (Table 2). While dietary Ca concentrations above 10 $g \cdot kg^{-1}$ have been associated with reduced dry matter intake (Miller, 1983, in [29]), diets as high as 18 $g \cdot kg^{-1}$ have been fed to non-lactating dairy cows without problems (Beede *et al.*, 1991, in [29]). Feeding Ca in excess of daily dietary requirements is suggested to improve performance, mainly when cows are fed corn silage diets [29]. Potassium is the third most abundant element in mammals and is important for cellular osmotic balance. The cellular homeostasis of Na and K is maintained by Na^+/K^+ pumps located inside the cell membrane. These two cations play an important role in electrical activity of nerve and muscle cells, in the acid-base balance, and in water retention. Potassium is a cofactor for the activation of enzymes, including those involved in protein synthesis and carbohydrate metabolism [48]. Because of increasing levels of Cl^- in irrigation water, shoot absorption of potassium decreased significantly ($p \leq 0.01$) for both harvests (by 26%–33%). Sodium significantly increased (by 60%), both with salinity and harvest date (presented elsewhere [19]), which was expected due to its elevated concentration in the saline treatment water. The levels of K across harvests and salinity (2.6%–4.6%) were well above the required levels (1.04%) for average lactating cows [29]. However, diets supplemented with potassium carbonate increased K from 1.6% to 4.6% (w/w) and decreased milk yield and feed intake [49]. Thus, K levels in alfalfa shoots irrigated with saline water containing 6 to 6.5 $mmolc \cdot L^{-1}$ could be of concern, depending on forage intake.

A continuous supply of Mg from feed is desirable because a high K level in forage decreases Mg absorption from the rumen and can lead to tetany [50]. The frequency of tetany in cows, triggered by low Mg and/or Ca, and high K in forage, increases when the ratio of K: (Ca + Mg) exceeds 2.2 [51]. In our results, the ratio of K: (Ca + Mg) was higher than 2.2 at 84 DAS, but lower than 2.2 at 299 DAS, suggesting that Mg levels should be monitored in alfalfa irrigated with saline water. Thus, although our results indicate that salinity can lead to a small, but significant accumulation of Mg by alfalfa shoots, Mg supplementation is still a must due to its poor absorption (13% to 16% from ration) by cows [52].

Sulfur (S) is an important component of cysteine and methionine, of many enzymes, and of antioxidants such as glutathione and thioredoxin, but elevated concentrations of S in alfalfa shoots can be detrimental to animal feed intake and function. Although we discuss the concentrations of S in shoots of different ages, the saline water used here was sulfate-dominant to mimic the drainage waters of California's Central Valley. Thus, levels of S might not be of concern where waters are Cl^- dominant. However, the S levels in our experiment remained similar at 299 DAS across salinity treatments. The lack of significant S uptake at 299 DAS may be explained by cooler temperatures and lower evapotranspiration before that harvest. The S concentration in shoots ranged from 0.38%, at the lowest EC, to 0.54% at the highest EC observed at 299 DAS. Regardless of season, a decrease in S in a later harvest (as seen here) was reported previously for alfalfa irrigated with sulfate-dominant water at both 15 and 25 $dS \cdot m^{-1}$ [53]. The authors reported an S range in alfalfa of 0.5%–0.9% at 25 $dS \cdot m^{-1}$. In the S range recorded at 299 DAS for this study, and considering that the average consumption of alfalfa is 4.26 $kg \cdot cow^{-1}$ [3], the S consumption would be 16.2 to 23.0 $g \cdot day^{-1}$, well below the 32 g $S \cdot day^{-1}$ upper limit recommended for a mature grazing beef cow [54], but 1.9 to 2.7 times above the 8.52 g $S \cdot day^{-1}$ (0.2% S/day) required for dairy cows [29]. Although no S toxicity has been reported [29], it is important to balance the diet in order to maintain S intake at a safe level (below 0.4% of DM daily), as levels of S of 0.4% in bailed alfalfa can lead to molybdenosis and reduced uptake of Cu and Se in beef cattle if alfalfa is the only source of feed [45].

The P requirement in the daily diet of average-producing dairy cows is 0.35% [29], but P levels regarded as adequate in alfalfa shoots are 0.08% to 0.15% [45]. P deficiency will lead to osteomalacia (softening of the bones) and fragile bones. The average levels of P in our alfalfa shoots at 299 DAS (0.28% to 0.44% DW) are considered to be high for shoot levels, relative to alfalfa grown in soils of the Mediterranean and desert zones [45]. In addition, according to nutrient tables presented by these authors, our Mg levels (0.25%–0.37%) were adequate, while shoot K and S were high.

Salinity significantly increased Na and Cl levels for both harvest dates by 40%–60%, as presented in a companion paper [19], resulting in shoot Na levels two to five times higher than the level required (0.23%) for average-producing lactating dairy cows [29]. Our data showed that alfalfa accumulates more Na and Cl^- over time, even at the same irrigation salinity level. As previously reported [19], shoot Na ranged from 3.5–10 $g \cdot kg^{-1}$, and Cl from 7–14 $g \cdot kg^{-1}$, across salinity levels and harvest times. We found no reference reporting Na toxicity to livestock, but increasing Na in the diet from 5.5–8.8 $g \cdot kg^{-1}$ caused no reduction of feed intake, milk yield, or toxicity (Schneider *et al.* 1986, in [29]). NaCl, often added to feed mixes, can be tolerated up to 3% (lactating cows) or 4.5% (growing animals) of dietary dry matter. Thus, Na and Cl levels in alfalfa irrigated with saline water present no safety concern.

Micronutrients—Micronutrients and some vitamins are essential for animals to achieve optimal immune function, growth, and reproduction. Cattle can have sufficient amounts of these minerals for growth and reproduction, but not have enough for optimal immune function [47]. Examples are Cu and Zn, which are required for the activity of the antioxidant enzymes Cu-Zn superoxide dismutase (SOD) [55].

The average iron concentration was not affected by salinity and ranged from 83.5–116 $mg \cdot kg^{-1}$ across harvests, regardless of salinity treatment. Concentrations of 50 to 100 $mg \cdot kg^{-1}$ of Fe in a basal ration are within the requirements for the growth of grazing cattle [47,56] and concentrations of 15 $mg \cdot kg^{-1}$ in daily feed are recommended for average lactating cows [29]. Iron is essential for the formation of new red blood cells and only levels ≥4000 $mg \cdot kg^{-1}$ affect weight gain and cause diarrhea in young calves [47].

Copper (Cu) and zinc (Zn) are important micronutrients for immune function, and levels of 20 $mg \cdot kg^{-1}$ Cu and 40–60 $mg \cdot kg^{-1}$ Zn were suggested as optimal for feeding in the total diet of dairy cattle [57], while levels of 11 $mg \cdot kg^{-1}$ Cu and 48 $mg \cdot kg^{-1}$ Zn are recommended for average lactating dairy cows [29]. The Cu levels found in shoots for both harvests were below 7.0 $mg \cdot kg^{-1}$, indicating the need for supplementation. In addition, the ratio of Cu to Mo in shoots was always approximately 1:1, well below the ratio of 10:1 that is considered a threshold for potential Cu toxicity [58].

Salinity significantly increased the Zn concentration in young plants (84 DAS) but not in established alfalfa plants (299 DAS), with concentrations ranging from 90–106 $mg \cdot kg^{-1}$. Considering that a minimum Zn concentration of 48 $mg \cdot kg^{-1}$ is required for average lactating cows [29], our plants contained levels more than adequate to support a healthy immune function in livestock [57]. Manganese levels in alfalfa shoots were the third highest, after Fe and Zn. Manganese is important for its role in enzymatic systems but it is poorly absorbed (14%–18%) and if deficient, can reduce fertility and delay estrous [56]. This author mentions that Mn deficiency can lead to abortion and deformed calves at birth, but elevated Mn in the diet is generally not toxic. Levels of Mn in our alfalfa cultivars were at least 14 $mg \cdot kg^{-1}$, as recommended for average lactating cows (NRC 2001). However, considering the poor absorption of Mn, mineral supplementation would be recommended.

4.3. Antioxidant Capacity of Alfalfa

Antioxidant flavonoids in the diet are believed to have health-promoting benefits to both humans and animals. In addition to protein, alfalfa is a rich source of flavonoid antioxidants and phytoestrogens including luteolin, coumestrol, and apigenin [59]. Phenolic compounds (including flavonoids) protect plants against the damaging effects of excessive reactive oxygen species (ROS) triggered by abiotic stresses, including salinity [60,61]. Although oxygen radical absorbance capacity (ORAC) has been widely accepted by industry to gauge the total antioxidant capacity of fruits, vegetables, spices, and other items consumed by humans, ORAC has only recently been used to estimate the antioxidant capacity of plants destined for livestock consumption [62–64]. The total antioxidant capacity is the sum of the lipophilic ($ORAC_{Lipo}$) and hydrophilic ($ORAC_{Hydro}$) fractions extracted from plants by hexane (lipophilic) and 70:30 acetone:aqueous buffer (hydrophilic). Our ORAC data (Table 4) confirmed those of others [63,64] who reported that the hydrophilic fractions of plant extracts contain most (68%–99%) of the total antioxidant capacity of shoots. Alfalfa shoots grown with saline water had 94%–96% of the total antioxidant capacity in the hydrophilic fraction with only 4%–6% in the lipophilic fraction, indicating that alfalfa shoots are low in lipophilic antioxidants such as tocopherols, carotenes, and fatty acids. The oven-dried alfalfa plants in our study had $ORAC_{Hydro}$ values that ranged from 229–274 μmoles·TE·g^{-1} DM (Table 4, Figure 2). Although these values may seem small compared with those of other leguminous forages, such as *Lespedeza cuneata* ($ORAC_{Hydro}$ = 530 μmoles·TE·g^{-1} DM), previously reported [63] alfalfa flavonoids and isoflavonoids present in hydrophilic (aqueous) extracts reduced oxidative stress and exerted hepatoprotective activity in rats treated with the liver-damaging compound carbon tetrachloride [65]. These results indicate that when animals consume alfalfa on a regular basis, it can provide benefits other than nutritional value.

The values for both ORAC and total phenolics (TP) remained unaltered by increased salinity, without differences for either ORAC or TP among cultivars (Figure 2). Our results agree with a previous report where there were no differences in antioxidant compounds among different cultivars of alfalfa in the absence of salt stress [15]. These authors also reported that the major antioxidants in alfalfa shoots, determined by HPLC, were tricin and apigenin glycosides (each approximately 40% of the total HPLC peaks), and luteolin and chrysoeriol glycosides (10% or less of the total HPLC peaks). Our results suggest that the salinity levels tested did not highly stress these salt-tolerant alfalfa cultivars. Previously, mostly the aglycons (flavonoids stripped of sugar moieties by acidic or enzymatic hydrolysis) have been determined, but the determination of full glycosidic forms (flavonoid plus sugar moieties) has also been conducted [59]. Flavonoids from alfalfa have the typical structure of several other flavonoids reported as beneficial to human diets and found in fruits and vegetables. Although sun drying (used to produce alfalfa hay) drastically decreased the antioxidant capacity of the antioxidant herb *Artemisia annua*, oven drying at 45 °C only slightly reduced the antioxidant capacity compared with freeze drying [66]. Thus, we consider that our oven-dried alfalfa shoots had an antioxidant capacity close to that of freeze-dried (or fresh) shoots. We could not find any published work on the antioxidant capacity of alfalfa shoots determined by ORAC or TP, except that the total ORAC ($ORAC_{Hydro+Lipo}$) of alfalfa hay was 171 μmoles·TE·g^{-1}, and the $ORAC_{Lipo}$ was only 3% of the total ORAC [63]. The antioxidant capacity of all cultivars used here was not affected by salinity, thus expanding the value of alfalfa beyond its contents of CP and minerals.

Although the value of antioxidants in animal and human nutrition is still debated by some, several benefits (e.g., anti-cancer, anti-inflammatory, *etc.*) of antioxidant-rich diets have been proposed. Dairy cows supplemented daily with 500 g of oregano (2082 μmoles·TE·g^{-1} DM) increased their milk fat concentration, feed and milk NEL efficiencies, and fat-corrected milk yield by 3.5% [67]. Although oregano has an ORAC value 8–9 fold higher than our oven-dried alfalfa shoots (225 to 256 μmoles·TE·g^{-1}), the average consumption of alfalfa shoots by cows is 5.4 kg·day^{-1}, which is 10-fold higher than the 500 g·day^{-1} oregano supplement from the above-mentioned study. Thus, daily alfalfa consumption can provide as much antioxidant flavonoid intake as oregano, thus adding to the forage value of alfalfa.

5. Conclusions

The effect of salinity in irrigation water on the suitability of alfalfa as a forage was based on shoot levels of macro- and micronutrients, and the forage quality estimated from ADF, NDF, and CP. Additional forage value was based on the antioxidant capacity and total phenolics in response to salinity. The nutrient composition of alfalfa can vary with salinity. Although our saline irrigation waters provided 27%–87% more SO_4 than Cl and 60%–94% more Na than Cl, alfalfa shoots contained 20%–190% more Cl than total S and 20%–120% more Cl than Na. Although Na and Cl in shoots increased with salinity, reducing the K concentration by 26%–32% and Ca by 15%–32% in shoots, shoot K and Ca were considered high and adequate [1,45], respectively, at all salinity levels. Increased salinity also increased shoot N (23%–33%), P (21%–46%), Mg (20%–84%), and total S (100%–110%) for both harvests. In general, the levels of macro- and micronutrients were adequate or high for alfalfa forage [1,29,45] regardless of salinity. However, when irrigation water was sulfate-dominant, the S concentrations in alfalfa were close to the upper limits recommended for safe animal consumption and require monitoring for water EC higher than 12.7 dS·m^{-1}. Regarding forage potential quality, shoots from plants irrigated with salinity levels higher than the control remained unaltered, or slightly improved compared with the salinity control levels, with NDF and CP at levels recommended for various classes of milking cows, but below the NDF values required for bulls and dry cows [39]. The antioxidant capacity was 15–23 fold higher for hydrophilic than for lipophilic fractions, but remained mostly unaltered by salinity, indicating that total antioxidant compounds, including phenolics and flavonoids (postulated to neutralize reactive oxygen species triggered by salinity stress), may remain fairly constant in alfalfa cultivars that are tolerant to salinity. These constant antioxidant levels, regardless of salinity stress, may play an extra beneficial role in helping to maintain animal health, as accepted for antioxidants in humans. Except for numeric values (such as reduced K and increased S), salinity levels up to 24 dS·m^{-1} did not alter the potential nutritional value and antioxidant capacity of alfalfa for livestock. The nutrient composition and antioxidant capacity of alfalfa are expected to play a dual role in the maintenance of health, body index, and milk production in dairy cows. This is the first report we are aware of, which has determined the total antioxidant capacity of alfalfa in response to salinity. Further studies involving animal performance are required to confirm the potential feed value of salt-stressed alfalfa under field conditions.

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Author Contributions

Jorge Ferreira was responsible for the antioxidant method (ORAC), the data interpretation and discussion of forage nutritional value and antioxidant capacity, and the writing of the manuscript with Monica Cornacchione and Donald Suarez. Monica Cornacchione conducted the experiments, analyzed the data, and helped write the manuscript. Xuan Liu performed the tests for antioxidant activity (ORAC) and total phenolics (TP) and helped write the experimental section. Donald Suarez developed the experimental design, including the composition of the saline water, and assisted with the writing of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest. The mention of proprietary brands and names is solely for the convenience of the reader and does not imply endorsement by the authors or the USDA versus similar products. The USDA is an equal-opportunity employer.

Abbreviations

ADF, acid detergent fiber; NDF, neutral detergent fiber; NEL, net energy for lactation; CP, crude protein; RFV, relative feed value; ORAC, oxygen radical absorbance capacity; TP, total phenolics.

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