Nitrogen Form and Ratio Impact Swiss Chard (*Beta vulgaris* subsp. cicla) Shoot Tissue Carotenoid and Chlorophyll Concentrations

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Introduction

Swiss chard (*Beta vulgaris* subsp. cicla) is a leafy green rich in dietary antioxidants and commonly consumed fresh, frozen, or canned. The leaves and stalkscontain high quantities of chlorophyll (CHL) and carotenoid (CAR) pigments, and other nutrients such as vitamins A, C, and K.[1] Enhancement of these nutritive qualities by varying cultural practices, such as mineral nutrient levels, has become common practice in vegetable production systems. Nitrogen (N) metabolism is regulated by supply, demand for growth, and amino acid content in plant. Two forms of available N are mineralized from organic matter or inorganic fertilizers are ammonium (NH_4^+) and nitrate (NO_3^-), which influence growth, photosynthesis, yield, and quality. Nitrogen form and ratio will influence CAR and CHL concentrations in kale (*Brassica oleracea* var. acephala).[2] However, research is lacking on influences of N on CAR and CHL in the leaf tissue of Swiss chard. Therefore, the purpose of this study was to determine the effects of N form and ratio on CAR and CHL concentrations in Swiss chard leaf tissue.

Table 1. Mean carotenoid and chlorophyll pigment concentrations expressed in fresh mass (mg/100 g) and biomass accumulation (mg·g^{-1}) in leaf tissue of 'Rhubarb Red' or 'Oriole Orange' Swiss chard (*Beta vulgaris* subsp. cicla) grown under varying %NH_4-N:%NO_3-N ratios in nutrient solution culture.

	Carotenoids [a]					
Cultivar	**LUT**	**BC**	**NEO**	**VIO**	**ANTH**	**ZEA**
Rhubarb Red	9.23 a	6.41 a	3.63 a	3.01 a	2.60 a	0.43 a
Oriole Orange	8.21 b	5.87 b	3.46 a	2.62 b	2.76 a	0.47 a
P-Value [b]	**	*	ns	**	ns	ns
	Xanthophylls [a]		**Chlorophylls [a]**		**Biomass [a]**	
	ZAV	**ZA/ZAV**	**Chl *a***	**Chl *b***	**FM**	**DM**
Rhubarb Red	6.03 a	0.50 b	111.32 a	32.73 a	13.31 a	0.82 b
Oriole Orange	5.85 a	0.56 a	99.33 b	31.61 a	14.79 a	1.32 a
P-Value [b]	ns	**	**	ns	ns	***

[a] Abbreviations: LUT = lutein; BC = β-carotene; NEO = neoxanthin; VIO = violaxanthin; ANTH = antheraxanthin; ZEA = zeaxanthin; ZAV = ZEA + ANTH + VIO; ZA/ZAV ratio = ZEA + ANTH/ZEA + ANTH + VIO; Chl *a* = Cholrophyll *a*; Chl *b* = Cholrophyll *b*; FM = Fresh Mass; DM = Dry Mass. The standard error of the mean was LUT ± 0.61, BC ± 0.61, NEO ± 0.31, VIO ± 0.21, ANTH ± 0.27, ZEA ± 0.08, ZAV ± 0.27, ZA/ZAV ± 0.27, Chl *a* ± 0.27, Chl *b* ± 0.27, Fresh Mass ± 0.27, Dry Mass ± 0.27. [b] ns, *, **, and *** indicate nonsignificant or significant at $P \leq 0.05, 0.01, 0.001$, respectively.

Table 2. Mean carotenoid pigment concentrations expressed on a fresh mass (mg/100 g) basis in leaf tissue of Swiss chard (*Beta vulgaris* subsp. cicla) grown under varying %NH_4-:%NO_3-N in nutrient solution culture.

%NH_4-N:	Carotenoids[a]					
%NO_3-N	**LUT**	**BC**	**NEO**	**VIO**	**ANTH**	**ZEA**
100:0	6.22 d	4.13 d	2.29 d	1.55 c	2.06 c	0.52 a
75:25	8.75 b	6.42 b	3.61 b	3.27 b	2.72 ab	0.34 b
50:50	10.32 a	7.52 a	4.42 a	3.81 a	3.21 a	0.46 ab
25:75	10.72 a	7.41 a	4.49 a	3.48 ab	3.22 a	0.48 ab
0:100	7.57 c	5.19 c	2.91 c	1.98 c	2.18 bc	0.44 ab
P-Value[b]	***	***	***	***	***	ns
Contrast						
Quadratic	***	***	***	***	***	ns

[a] Abbreviations: LUT = lutein; BC = β-carotene; NEO = neoxanthin; VIO = violaxanthin; ANTH = antheraxanthin; ZEA = zeaxanthin. The standard error of the mean was LUT ± 0.61, BC ± 0.61, NEO ± 0.31, VIO ± 0.21, ANTH ± 0.27, ZEA ± 0.08.
[b] ns, *, **, and *** indicate nonsignificant or significant at $P \leq 0.05, 0.01, 0.001$, respectively.

Table 3. Mean carotenoid and chlorophyll pigment concentrations expressed in fresh mass (mg/100 g) and biomass accumulation (mg·g^{-1}) in leaf tissue of Swiss chard (*Beta vulgaris* subsp. cicla) grown under varying %NH_4-:%NO_3-N in nutrient solution culture at Mississippi State University (MS State) or the University of Tennessee (Univ. TN).

	Carotenoids [a]					
Location	**LUT**	**BC**	**NEO**	**VIO**	**ANTH**	**ZEA**
MS State	7.27 b	5.86 b	3.13 b	3.13 a	3.59 a	0.69 a
Univ. TN	10.17 a	6.41 a	3.96 a	2.50 b	1.77 b	0.20 b
P-Value [b]	***	*	***	***	***	***
	Xanthophylls [a]		**Chlorophyll [a]**		**Biomass [a]**	
	ZAV	**ZA/ZAV**	**Chl *a***	**Chl *b***	**FM**	**DM**
MS State	7.41 a	0.56 a	94.30 b	29.27 b	14.72 a	1.37 a
Univ. TN	4.47 b	0.48 b	116.35 a	35.17 a	14.38 a	0.77 b
P-Value [b]	***	***	***	***	ns	***

[a] Abbreviations: LUT = lutein; BC = β-carotene; NEO = neoxanthin; VIO = violaxanthin; ANTH = antheraxanthin; ZEA = zeaxanthin; ZAV = ZEA + ANTH + VIO; ZA/ZAV ratio = ZEA + ANTH/ZEA + ANTH + VIO; Chl *a* = Cholrophyll *a*; Chl *b* = Cholrophyll *b*; FM = Fresh Mass; DM = Dry Mass. The standard error of the mean was Lutein ± 0.61, β-carotene ± 0.61, Neoxanthin ± 0.31, Violaxanthin ± 0.21, Antheraxanthin ± 0.27, Zeaxanthin ± 0.08, ZAV ± 0.27, ZA/ZAV ± 0.27, Chlorophyll *a* ± 0.27, Chlorophyll *b* ± 0.27, Fresh Mass ± 0.27, Dry Mass ± 0.27.
[b] ns, *, **, and *** indicate nonsignificant or significant at $P \leq 0.05, 0.01, 0.001$, respectively.

Materials and Methods

- Seeds of 'Rhubarb Chard' and 'Oriole Orange' Swiss chard were sown into 2.5 x 2.5-cm growing cubes, under greenhouse conditions and grown at 25/20 °C (day/night). At 21 d plantlets were transferred to 11-L containers filled with 10-L of nutrient solution. Nutrient solution consisted of elemental concentrations of (mg·L^{-1}): NO_3-N (98.0), NH_4-N (7.0), P (15.3), K (117.3), Ca (80.2), Mg (24.6), S (32.0), Fe (0.5), B (0.25), Mn (0.25), Zn (0.025), Cu (0.01), and Mo (0.005).
- Treatments consisted of N at 105 mg·L^{-1} supplied as 1) 100% NH_4-N: 0% NO_3-N, 2) 75% NH_4-N: 25% NO_3-N, 3) 50% NH_4-N: 50% NO_3-N, 4) 25% NH_4-N: 75% NO_3-N, 5) 0% NH_4-N: 100% NO_3-N. NH_4-N was supplied as ($(NH_4)_2SO_4$) and NO_3-N was supplied as ($Ca(NO_3)_2$) and potassium nitrate (KNO_3). Plants were harvested at 54 d and fresh mass (FM), dry mass (DM), and plant height data were recorded.
- Swiss chard shoot tissue CHL and CAR concentrations were analyzed by High Pressure Liquid Chromatography (HPLC).

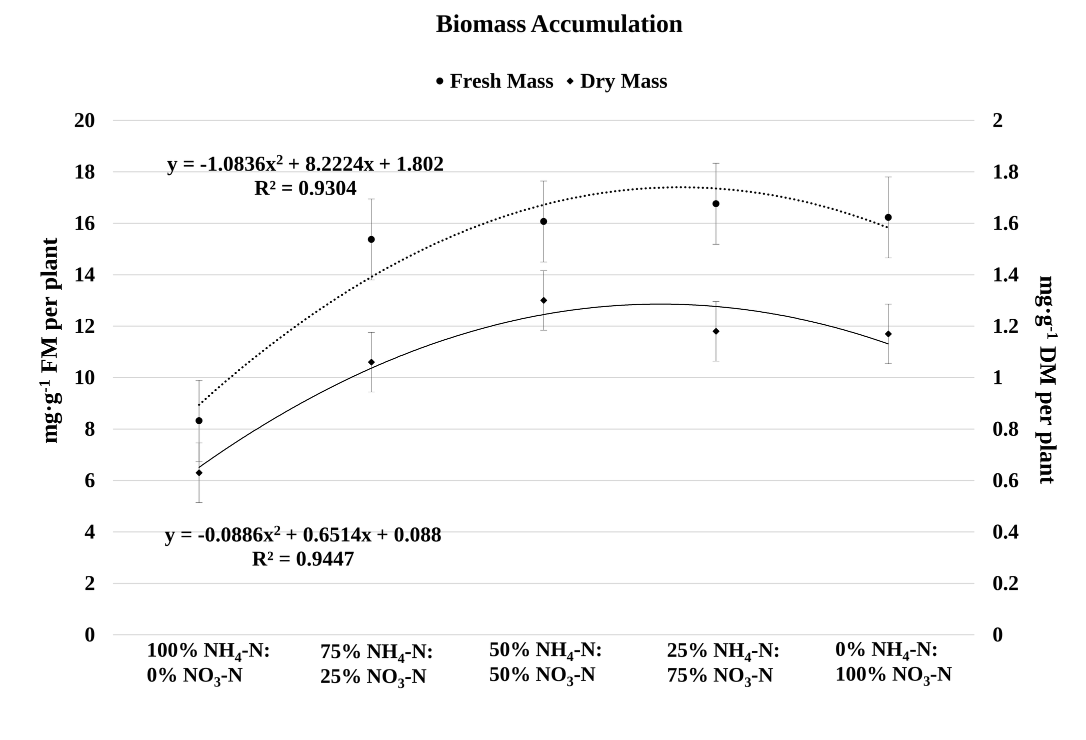

Figure 1. Effect of NH_4-N and NO_3-N on fresh mass (FM) and dry mass (DW) accumulation in two genotypes (Rhubarb Red and Oriole Orange) of Swiss chard (*Beta vulgaris* subsp. cicla) leaf tissue.

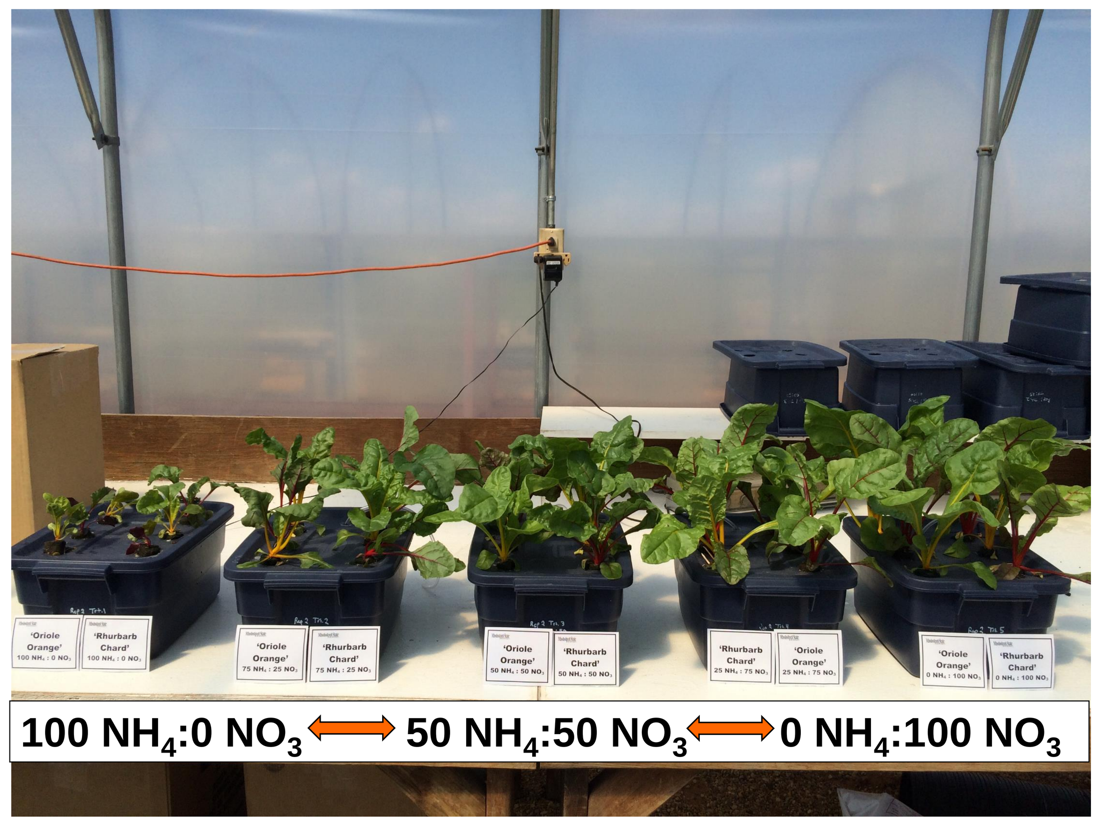

Figure 2. 'Rhubarb Red' and 'Oriole Orange' Swiss chard grown under varying %NH_4-N:%NO_3-N ratios in nutrient solution culture.

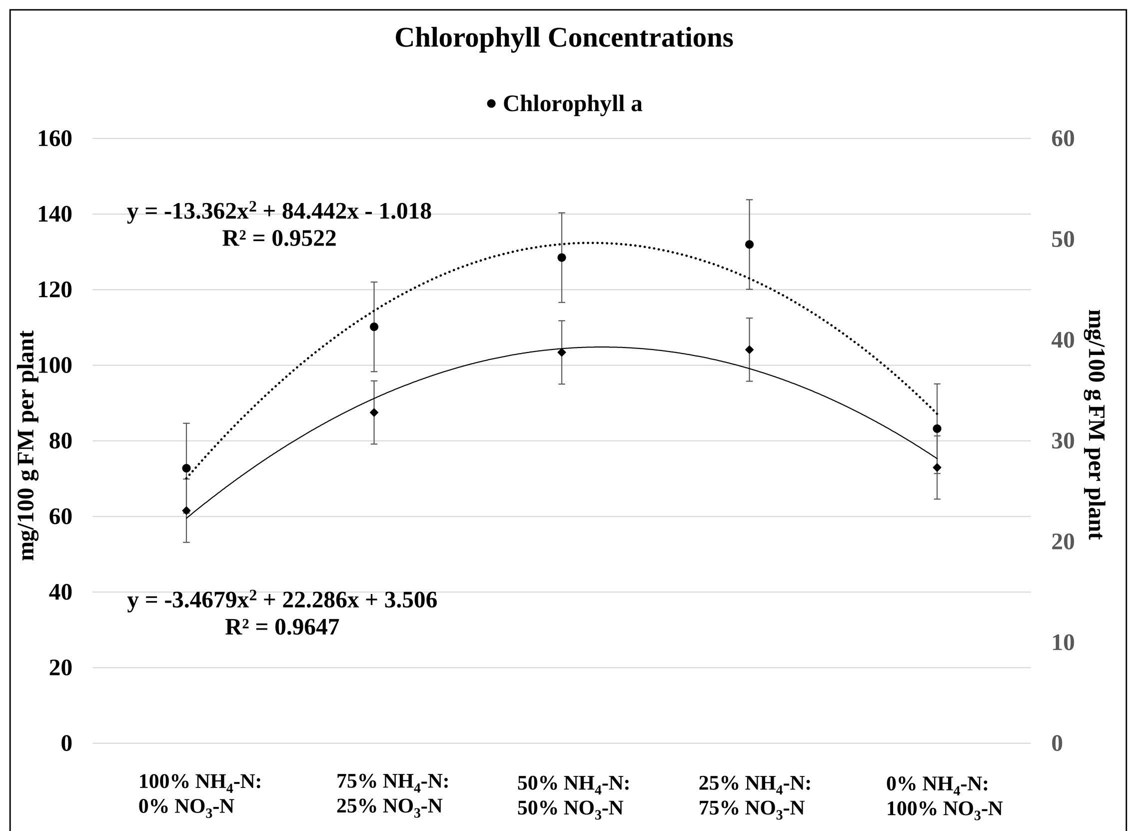

Figure 3. The effect of NH_4-N and NO_3-N on the chlorophyll concentration in two genotypes (Rhubarb Red and Oriole Orange) of Swiss chard (*Beta vulgaris* subsp. cicla) leaf tissue.

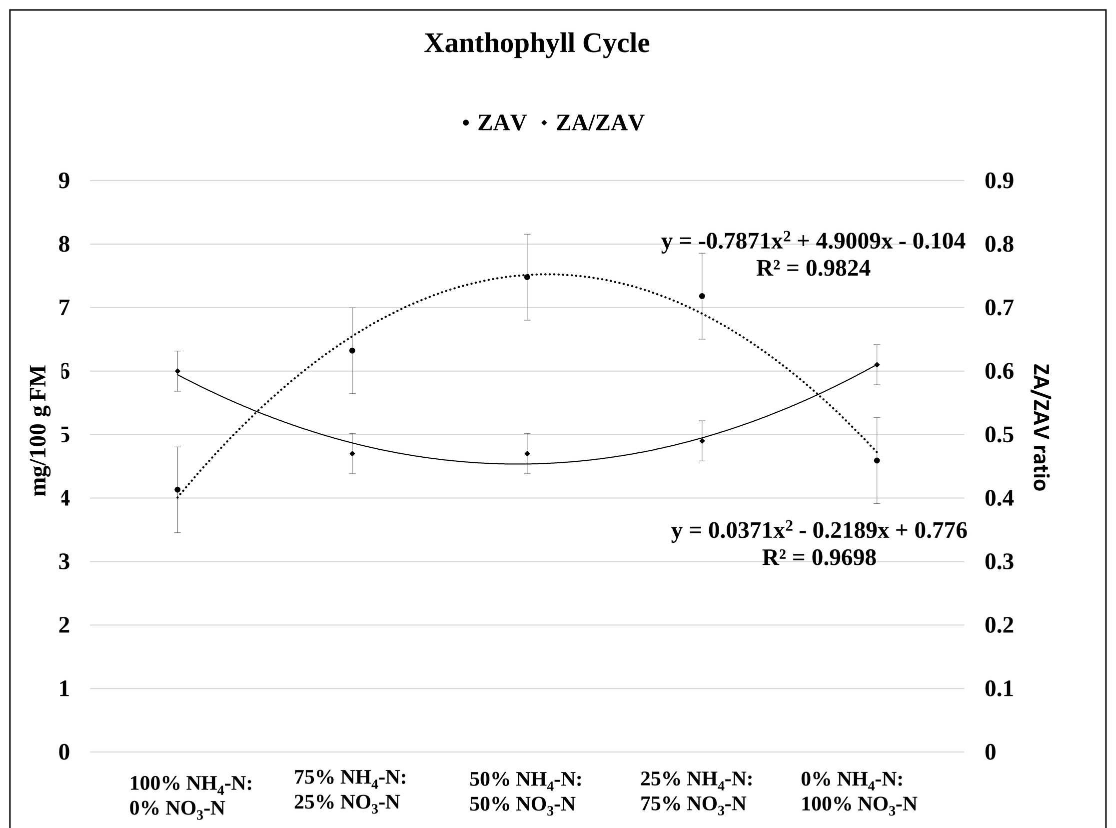

Figure 4. The effect of NH_4-N and NO_3-N on the xanthophyll cycle pigment ratios in two genotypes (Rhubarb Red and Oriole Orange) of Swiss chard (*Beta vulgaris* subsp. cicla) leaf tissue. Abbreviations: ZAV = Zeaxanthin + Antheroxanthin + Violaxanthin; ZA/ZAV ratio = Zeaxanthin + Antheroxanthin/Zeaxanthin + Antheroxanthin + Violaxanthin.

Results

- 'Rhubarb Red' had the highest concentrations of LUT, BC, and VIO in leaf tissues compared to the 'Oriole Orange' (**Table 1**).
- Swiss chard CAR concentrations, except for ZEA, responded significantly to N form (**Table 2**).
- There were significant differences in LUT, BC, NEO, VIO, ANTH, and ZEA between the MSU and UT locations (**Table 3**).
- Swiss chard FM responded significantly to N form, and the interaction of N form and location (**Figure 1**).
- Swiss chard CHL concentrations had a significant positive quadratic response to N form in the leaf tissue (**Figure 3**).
- There were significant differences in the total pool of xanthophyll cycle pigments of ZEA + ANTH + VIO when treated with different N forms in Swiss chard (**Figure 4**).

Conclusions

Results indicated that decreasing NH_4-NO_3 ratio had a positive quadratic effect of biomass accumulation, CHL, and CAR concentrations. There were also differences among the two genotypes of Swiss chard. The highest concentrations of CAR and CHL occurred at 25% NH_4-N:75% NO_3-N and lowest at 100% NH_4-N:0% NO_3-N. Since N fertility is important to overall plant health, it is crucial to understand the influence of managing N in a production system aimed to increase yields and nutritionally important pigmentation in Swiss chard. Thus, the recommendation for increased biomass accumulation and health promoting CAR and CHL concentrations in the leaf tissue is to decrease NH_4-NO_3 ratios in a fertility program to 25% NH_4-N:75% NO_3-N.

1) Reif, C., E. Arrigoni, H. Scharer, L. Nystrom, and R.F. Hurrell. 2013. Carotenoid database of commonly eaten Swiss vegetables and their estimated contribution to carotenoid intake. J. Food Comp. Analysis 29:64-72. 2) Kopsell, D. A., D. E. Kopsell, M. G. Lefsrud, and J. Curran-Celentano. 2007. Carotenoid pigments in kale are influenced by nitrogen concentrations and form. J. Sci. Food Agric. 87: 900-907.

NOTE: This This material is based upon work that is supported by the NIFA, USDA Hatch project under accession number MIS 149160.