

Producing Nitrate-free Endive Heads: Effect of Nitrogen Form on Growth, Yield, and Ion Composition of Endive

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ABSTRACT. In a growth chamber, endive (*Cichorium endivia* L. var. *crispum* Hegi) plants were grown using a solution culture method to evaluate the influence of four ammonium : nitrate ($\text{NH}_4\text{-N} : \text{NO}_3\text{-N}$) percentage ratios (100:0, 70:30, 30:70, and 0:100) on growth (leaf area, dry mass, crop growth rate, relative growth rate, and net assimilation rate), yield characteristics (head and root fresh mass and root length), quality (dry matter, nitrogen, and nitrate), and inorganic ion content. No symptoms of NH_4^+ toxicity were detected in endive plants 8 weeks after beginning nutrient treatments. Moreover, by feeding N in mixed form, the growth indices increased compared to indices from feeding with any of the two N forms alone. Ammonium-fed plants produced nitrate-free heads with a fresh mass (171 g) similar to nitrate-fed plants. Compared to the other treatments, the heads of NH_4^+ -fed plants were darker green and more succulent. Mixed N improved yield but caused a remarkable accumulation of nitrate in heads. Following an increase in $\text{NO}_3\text{-N}$ from 30% to 70% in the nutrient solution, head fresh mass rose from 196 to 231 g and NO_3^- concentration more than doubled (from 2.4 to 6.1 $\text{g}\cdot\text{kg}^{-1}$ fresh mass). With 100% of $\text{NO}_3\text{-N}$, NO_3^- concentration was 5.5 $\text{g}\cdot\text{kg}^{-1}$ fresh mass. With higher $\text{NO}_3\text{-N}$ percentages in the nutrient solution, the difference in the concentration of inorganic cations and anions increased, but K^+ concentration was also high in ammonium-fed plants (on average 77 $\text{g}\cdot\text{kg}^{-1}$ dry mass). Head total N accumulation was increased by the presence of NH_4^+ in the nutrient solution and decreased with 100% $\text{NO}_3\text{-N}$. From the commercial viewpoint, the produce obtained from 100% $\text{NH}_4\text{-N}$ was good, with the value-added factor of the absence of nitrate. This may be an extremely remarkable factor because of the commercial limits on the allowable nitrate content in leafy vegetables already enforced by many European countries and those the European Union is going to adopt in a directive.

The consumption of vegetables is recommended for their low energy value and high content of dietary fiber, vitamins, and minerals. Despite the contribution of vegetables to human health, consumers are worried about some cultivation techniques that might reduce the quality of produce, e.g., the presence of pesticide residues or nitrate content.

The presence of nitrates in vegetables, as in water and generally in other food products, is a serious threat to human health, not so much due to their toxicity, which is low, but for the compounds they induce in the organism. Nitrites, produced by nitrate reduction, induce methaemoglobinemia or can form nitrosamines and nitrosamides by reacting with amines and amides, whose carcinogenic action is well known (Walker, 1990).

In 1973, in evaluating nitrate and nitrite, the Food Agricultural Organization (FAO)/World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) set an acceptable daily intake (ADI) for NO_3^- of 0 to 3.65 $\text{mg}\cdot\text{kg}^{-1}$ body weight and an ADI for NO_2^- of 0 to 0.13 $\text{mg}\cdot\text{kg}^{-1}$ body weight (WHO, 1973). Subsequently, the Commission of European Communities Scientific Committee for Food (CECSCF) also set an ADI for NO_3^- of 0 to 3.65 $\text{mg}\cdot\text{kg}^{-1}$ body weight but established a lower temporary ADI for NO_2^- of 0 to 0.07 $\text{mg}\cdot\text{kg}^{-1}$ body weight (CECSCF, 1992).

Compared with these ADIs, the ingestion of only 100 g of raw vegetables (with a NO_3^- concentration of 2500 $\text{mg}\cdot\text{kg}^{-1}$ fresh mass) accounts for an intake of 250 mg NO_3^- . If a 60-kg person consumes this item alone, the ADI for NO_3^- would be exceeded by 14%. By calculating the partial conversion of NO_3^- to NO_2^- (5% according to Choi, 1985), the JECFA and CECSCF ADI for NO_2^- would be exceeded by 60% and 200%, respectively. However, some components of vegetables (e.g., ascorbic acid, phenols, etc.) have been reported to inhibit the bad effects of nitrites (Walker, 1990). In the meantime, some European governments have already introduced official limits for nitrates in certain vegetables, paralleling horticultural adaptations leading to a decrease in nitrate content. Also, the European Union is drafting a directive on the maximum nitrate content in vegetables.

In leafy vegetable crops, nitrate concentration can be partially reduced by eliminating $\text{NO}_3\text{-N}$ in the nutrient solution a few days before crop harvesting (Martignon et al., 1994; Santamaria et al., 1996; Van der Boon et al., 1990) or with a partial $\text{NO}_3\text{-N}$ substitution with $\text{NH}_4\text{-N}$ (Gunes et al., 1994; Santamaria et al., 1996; Steingröver et al., 1993; Van der Boon et al., 1990).

The form of N used by a plant may affect its morphology (Ganmore-Neumann and Kafkafi, 1980a; Gashaw and Mugwira, 1981; Gigon and Rorison, 1972) and chemical composition: NH_4^+ causes lower concentrations of K^+ , Ca^{2+} , and Mg^{2+} and higher concentrations of P, S, and organic N (Ganmore-Neumann and Kafkafi, 1980b; Gashaw and Mugwira, 1981; Haynes and Goh, 1978; Troelstra et al., 1985). The concentration of carboxylates in the plant is enhanced with nitrate nutrition (Haynes and Goh, 1978).

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Table 1. Composition of nutrient solutions for different N-form ratios.

NH ₄ ⁺ :NO ₃ ⁻ (% ratio)	Compound (mM) ²								
	(NH ₄) ₂ SO ₄	Ca(NO ₃) ₂	NaNO ₃	KNO ₃	CaCl ₂	MgSO ₄	K ₂ HPO ₄	K ₂ SO ₄	H ₃ PO ₄
100:0	4	0	0	0	2	0.5	0.5	1	0.5
70:30	2.8	0.4	0	1.6	1.6	0.5	0.5	0.2	0.5
30:70	1.2	2	0	1.6	0	0.5	0.5	0.2	0.5
0:100	0	2	2	2	0	0.5	0.5	0	0.5

²The other micronutrients were the same for all treatments according to half-strength Hoagland type solutions (Hoagland and Arnon, 1950).

Comparison of NH₄⁺ and NO₃⁻ as sources of N for uptake and growth has received considerable attention. Even in purely agromomic terms, this comparison is not surprising given the importance of maximizing the efficiency of uptake and use of fertilizer N by crop plants and minimizing losses of N from soil-plant-animal systems.

In our previous work in solution culture (Santamaria et al., 1995) with endive plants supplied with a 1 NH₄⁺:1 NO₃⁻ molar ratio, N yield efficiency increased and leaf nitrate content decreased compared to nitrate-fed plants. Therefore the aims of the present paper were to evaluate 1) endive capability to use NH₄⁺ even in the absence of nitrate in the nutrient solution and 2) the chances it offers to produce nitrate-free endive heads.

Materials and Methods

'Pancalieri castello' endive plants (a cold-tolerant, selection of Pancalieri type with big heads and crispy midribs) were started from seeds in seedling trays containing a mixture of 7 peat : 1.5 vermiculite : 1.5 perlite (by volume). At the fifth true leaf stage (45 d from seeding), the medium was washed from roots and plants were transferred into rectangular prismatic plastic pots (60 cm long, 17 cm deep, 13 cm wide; filled with about 8 L of nutrient solution). Each pot represented an experimental unit with seven plants. The pots were suitably covered to avoid evaporation of the nutrient solution and placed for another 56 d in a growth chamber [relative humidity ±75%, temperature 20/16 ±0.5 °C (day/night), photoperiod 12 h, photosynthetic photon flux 300 μmol·m⁻²·s⁻¹].

Treatments consisted of placing plants in one of four nutrient solutions having the same N concentration (8 mM) but a different NH₄⁺:N: NO₃⁻:N (NH₄⁺:NO₃⁻) ratio (100:0, 70:30, 30:70, and 0:100). Treatments were arranged in a randomized complete-block design with three replications within the growth chamber. Compounds used to create the different N-ratio treatments are reported in Table 1. Distilled water was used for preparing all solutions.

Solution pH was checked daily and, if necessary, adjusted to 5.5 with NaOH (2 N) or H₂SO₄ (1 N). Distilled water was added daily as needed to each pot to replace transpiration losses. Air was continuously bubbled through pots to supply oxygen. All solutions were renewed ex novo weekly.

Three plants per pot were sampled 21 d after beginning treatment (DABT) and the others were sampled after 7, 21, 28, and 35 d more to measure leaf area (LI-3100 leaf area meter; LI-COR, Lincoln, Neb.), leaf fresh mass, root fresh mass, and root length.

All plant material was dried in a thermoventilated oven at 65 °C until reaching a constant mass. Material of the last sample was then finely ground and used for the quantitative chemical analyses of total N (only head) and major inorganic anions (Cl⁻, NO₃⁻, H₂PO₄⁻, and SO₄²⁻) and cations (Na⁺, K⁺, Mg²⁺, and Ca²⁺).

Total N was determined by Kjeldahl method. Anions, previously extracted from dry matter samples of 0.5 g with 50 mL of sodium carbonate (1.8 mM) and sodium bicarbonate (1.7 mM)

solution, were determined by ion chromatography (model QIC; Dionex Corp., Sunnyvale, Calif.), using a conductivity detector, guard-column IonPac AG4A, and analytical column IonPac AS4A. Cations, extracted from samples of 2 g of dry matter ashed in muffle furnace at 450 °C and digested with 1 N HCl in boiling water for 30 min, were determined through ion chromatography using a conductivity detector, the guard-column IonPac CG12, and the analytical column IonPac CS12 (American Society for Testing Materials, 1992).

Growth analysis indices were calculated by the functional method (Hunt, 1978). To describe leaf area and dry mass patterns over time, different degrees of polynomial and exponential equations were tested, and those having the highest determination coefficient and the best residual distribution (Draper and Smith, 1981) were chosen.

The function adopted is leaf area (or dry mass) = exp(a + bt + ct²), where a, b, and c are constants and t = DABT.

Using dry mass and leaf area functional values, crop growth rate (CGR), relative growth rate (RGR), and net assimilation rate (NAR) were calculated. Formulas for these calculations are CGR = dM/dt; RGR = (1/M)·dM/dt; and NAR = (1/LA)·dM/dt.

Yield and quality data were subjected to SAS's (Cary, N.C.) general linear model procedure. Orthogonal polynomials were used to study changes with increasing NO₃⁻:N percentage in nutrient solution by partitioning the sums of the squares into components that were associated with linear, quadratic, and cubic terms (Steel and Torrie, 1980).

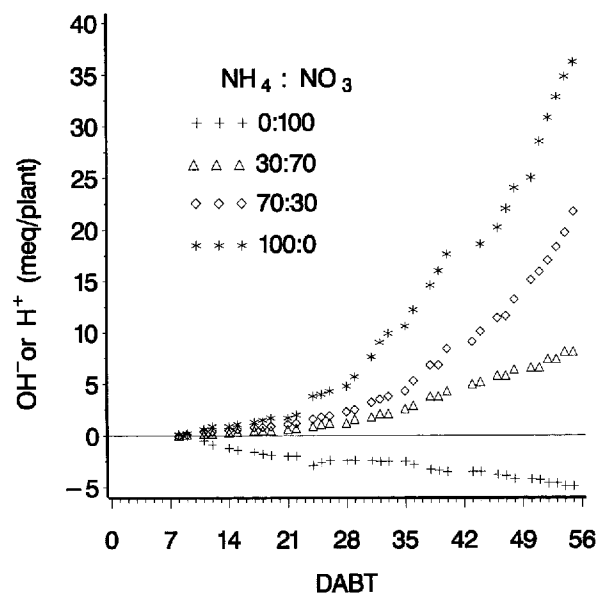


Fig. 1. Cumulative quantities of H⁺ (positive values) or OH⁻ (negative values) released from endive roots as a function of NH₄⁺:NO₃⁻ ratios in the endive growing solution (DABT = days after beginning treatment).

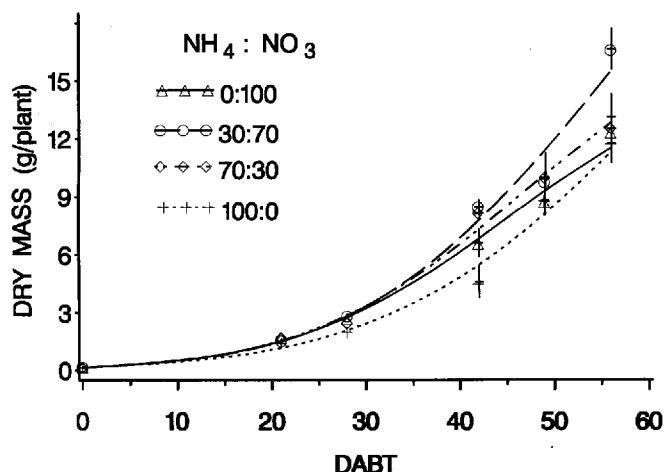


Fig. 2. Observed total dry mass and curves predicted by the exponential model with time of endive plants grown in nutrient solution with different $\text{NH}_4 : \text{NO}_3$ ratios (DABT = days after beginning treatments; vertical lines indicate \pm SE, no error line indicates a SE smaller than the symbol size; each point is a mean of three replicates; for all functions $R^2 > 0.99$ with $P < 0.01$).

Results

NUTRIENT SOLUTION PH. The amounts of H^+ or OH^- added to the nutrient solutions to correct pH during the trial (Fig. 1) show that there was a total net release of H^+ ions of 39, 22, and 7 meq/plant with 100:0, 70:30, and 30:70 $\text{NH}_4 : \text{NO}_3$ ratios, respectively, and of 5 meq/plant of OH^- with a 0 $\text{NH}_4 : 100 \text{ NO}_3$ ratio. Despite daily corrections, the pH of the ammonium solution often dropped below 4 during 24 h.

GROWTH ANALYSIS. Plant dry mass 56 DABT, increased 111-fold (16.6 vs. 0.15 g/plant) with 30% $\text{NH}_4\text{-N}$ and 70% $\text{NO}_3\text{-N}$, whereas it was \approx 81-fold with the three other ratios (12.3 vs. 0.15 g/plant) (Fig. 2).

Leaf area reached on average 3387 cm^2 /plant with mixed N and 2941 cm^2 /plant with 100% $\text{NO}_3\text{-N}$ or $\text{NH}_4\text{-N}$ (Fig. 3).

CGR was higher with the 30:70 ratio. After 2 weeks, with mixed forms or 100% $\text{NO}_3\text{-N}$, absolute growth rate showed convex trends and peaked before the end of the trial; whereas, with 100% $\text{NH}_4\text{-N}$, CGR steadily increased and became higher than the values for 70:30 and 0:100 ratios (Fig. 4).

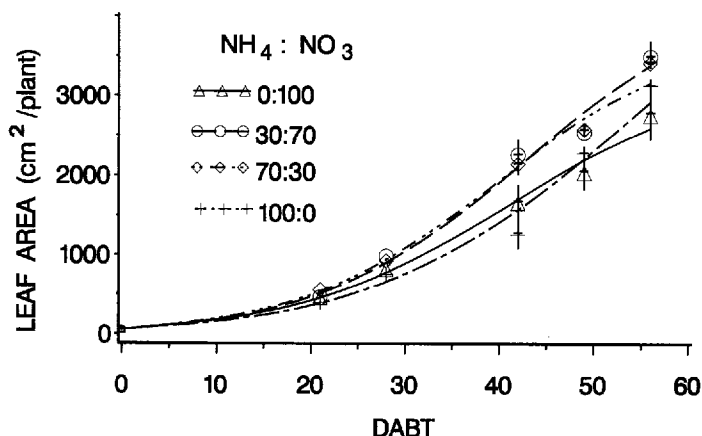


Fig. 3. Observed leaf area and curves predicted by the exponential model with time of endive plants grown in nutrient solution with different $\text{NH}_4 : \text{NO}_3$ ratios (DABT = days after beginning treatment; vertical lines indicate \pm SE, no error line indicates a SE smaller than the symbol size; each point is a mean of three replicates; for all functions $R^2 > 0.99$ with $P < 0.01$).

RGR decreased with time, less with 100% $\text{NH}_4\text{-N}$ than with the three other treatments. Indeed, ammonium-fed plants gave the lowest value in the first 28 d of the trial and the highest in the last 10 d (Fig. 5).

NAR decreased in time and marked a clear-cut difference between 100% $\text{NH}_4\text{-N}$ and the three other treatments. Compared to the initial values, at the end of the trial, NAR decreased 3.3, 3.2, 2.0, and 1.8-fold with the 0:100, 70:30, 30:70, and 100:0 ratios, respectively (Fig. 6).

MORPHOLOGIC FEATURES, YIELD, AND QUALITY. With the increase of $\text{NO}_3\text{-N}$ percentage in the nutrient solution, root length and fresh mass increased linearly and quadratically, respectively (in this latter case, the peak was attained with a 30:70 ratio), whereas root dry mass percentage decreased linearly (Table 2).

Head fresh mass and the relevant NO_3^- content increased as $\text{NO}_3\text{-N}$ was added up to 30 $\text{NH}_4 : 70 \text{ NO}_3$ ratio, but with 100% $\text{NH}_4\text{-N}$ heads were nitrate free. Head dry mass percentage increased with higher percentages of nitrate in the solutions. The heads of ammonium-fed plants were dark green.

ION CONCENTRATION. Head and root NO_3^- concentration increased as $\text{NO}_3\text{-N}$ was added in a higher ratio to the nutrient solution (Table 3). The highest values were recorded with a 30 $\text{NH}_4 : 70 \text{ NO}_3$ ratio in heads and with 100% $\text{NO}_3\text{-N}$ in roots.

The Cl^- concentration increased with increasing $\text{NH}_4\text{-N}$ percentage in the nutrient solution more in heads than in roots.

The average concentration of H_2PO_4^- in heads was 19 $\text{g}\cdot\text{kg}^{-1}$ dry mass and was not affected by N form; in the roots, it was on average higher than the H_2PO_4^- in the heads, and the maximum concentration was attained with a 70:30 ratio.

The SO_4^{2-} concentration decreased more in roots than in heads as $\text{NO}_3\text{-N}$ fraction in the nutrient solution was increased.

The highest concentration of Na^+ in the heads was recorded with 100% $\text{NO}_3\text{-N}$. Root Na^+ concentration was not affected by N form and was almost 2-fold the head concentration.

The K^+ concentration was on average higher in the heads than in roots. In the former, the highest concentrations were attained with prevailing $\text{NH}_4\text{-N}$, whereas in the latter it decreased linearly with increasing ammonium fraction in the nutrient solution.

Also, Ca^{2+} concentration was on average higher in heads than in roots and rose when $\text{NO}_3\text{-N}$ was present in the nutrient solution.

Root Mg^{2+} concentration increased linearly with increasing nitrate fraction, but in the heads it was not affected by N-form ratio.

The total concentration of inorganic cations (C) in the heads and

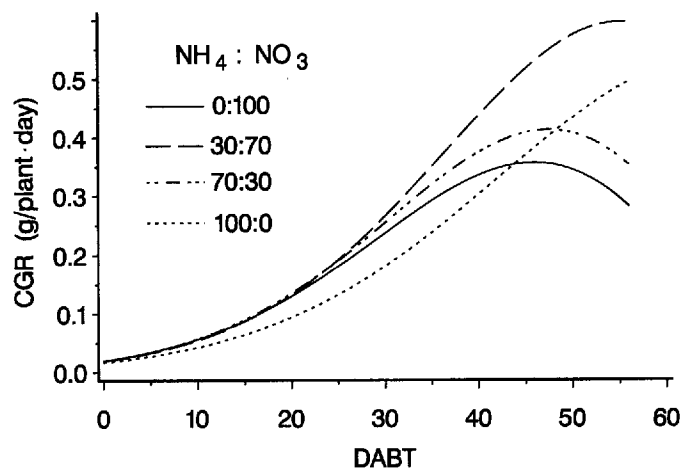


Fig. 4. Derived crop growth rate (CGR) of endive plants grown in nutrient solution with different $\text{NH}_4 : \text{NO}_3$ ratios, based on fitted exponential relation of dry matter with time (DABT = days after beginning treatment).

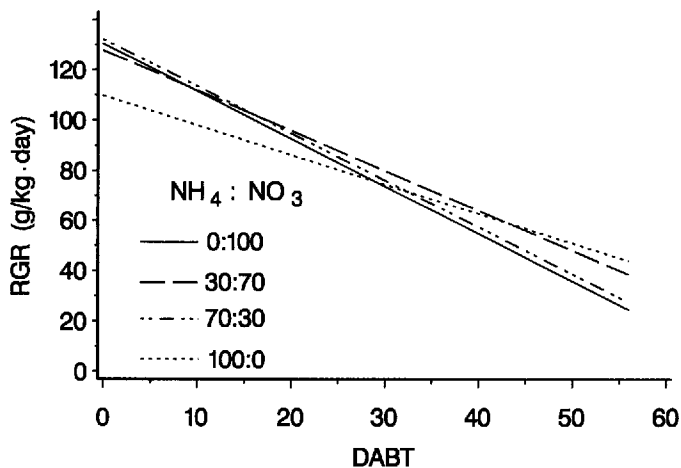


Fig. 5. Derived relative growth rate (RGR) of endive plants grown in nutrient solution with different $\text{NH}_4^+ : \text{NO}_3^-$ ratios, based on fitted exponential relation of dry matter with time (DABT = days after beginning treatments).

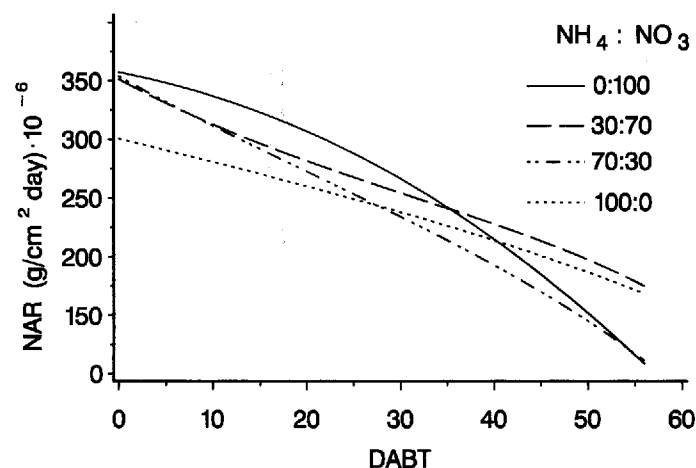


Fig. 6. Derived net assimilation rate (NAR) of endive plants grown in nutrient solution with different $\text{NH}_4^+ : \text{NO}_3^-$ ratios, based on fitted exponential relations between dry matter and leaf area with time (DABT = days after beginning treatments).

of inorganic anions (A) in the heads and roots were not affected by the treatments. Root inorganic cations increased linearly only with increasing nitrate percentage in the nutrient solution. The difference in concentration of the two ion forms (C–A) always increased with increasing nitrate in the nutrient solution, following a quadratic trend in the heads and a linear trend in roots.

Nitrogen form affected total N content in the endive heads. When changing from 100% to 70% and 30% of $\text{NH}_4^+\text{-N}$, total N increased from 47 to 50 and to 55 $\text{g}\cdot\text{kg}^{-1}$ dry mass, respectively, and then dropped again with 100% $\text{NO}_3^+\text{-N}$ (49 $\text{g}\cdot\text{kg}^{-1}$ dry mass).

Discussion

The N-form ratios of the nutrient solutions distinctly affected plant release of H^+ or $\text{OH}^-/\text{HCO}_3^-$ ions. The release of H^+ , observed in ammonium-fed plants or with mixed N forms, is typical of ammonium nutrition and is associated with a higher uptake of cations, especially NH_4^+ , than of anions. The release of OH^- , instead, occurs in plants fed with NO_3^- to counterbalance the higher uptake of anions (Van Beusichem, 1981). The low pH tolerance of endive was very impressive. Many plants cannot survive when the pH of the medium is lower than 4 (Islam et al., 1980), but endive grew normally in the ammonium solution even with pH temporarily dropping below 4 during the day. The specific mechanism of such an outstanding tolerance to low pH in this species is obscure and needs further investigation. Furthermore, it is likely that, by keeping pH constantly at ≈ 5.5 , endive yield could

increase, as it has been demonstrated with lettuce (Moritsugu et al., 1983) and other species (Findenegg, 1987).

Growth analysis allowed us to observe how, by feeding N in mixed form, the growth indices increased compared to feeding with any of the two N forms alone. In the first part of the growing cycle, NH_4^+ -fed plants increased at a lower rate than with other treatments. In the second half, however, NH_4^+ -fed endive showed higher growth and net uptake rates. This result is explained by the greater sensitivity of the plant to NH_4^+ in the first vegetative stages, due to the inadequate content of sugar providing energy and carbon skeletons for NH_4^+ assimilation (Barker and Mills, 1980; Chaillou et al., 1991). The benefits of mixed N nutrition compared to NO_3^- become more relevant on account of the lower energy cost of NH_4^+ uptake and of the different distribution of assimilated N between leaves and roots (Raven, 1985; Salsac et al., 1987).

The main finding of this work is that ammonium-fed endive produced nitrate-free heads with a mass similar to nitrate-fed plants. Mixed N improved yield quantity response but caused a remarkable accumulation of NO_3^- in heads. The amount of NO_3^- in the endive grown with 70% or 100% $\text{NO}_3^+\text{-N}$ was much above the maximum allowable NO_3^- content in some European countries, e.g., 3.5 $\text{g}\cdot\text{kg}^{-1}$ fresh mass in the Netherlands for endive in the winter (November–April), and diverge from most other studies, which generally report relatively low levels of nitrate in endive compared to other nitrate-accumulating leafy vegetables, such as lettuce, spinach, and celery (Roorda van Eysinga and Spaans, 1986; Vegter and Tanis, 1990). However, in the experiments of

Table 2. Fresh and dry mass, root length, and head nitrate content of endive as influenced by N-form ratio.

$\text{NH}_4^+:\text{NO}_3^-$ (% ratio)	Head			Roots		
	Fresh mass (g)	Dry mass ² ($\text{g}\cdot\text{kg}^{-1}$)	NO_3^- ($\text{mg}\cdot\text{kg}^{-1}$)	Fresh mass (g)	Dry mass ($\text{g}\cdot\text{kg}^{-1}$)	Length (cm)
100:0	171	58	0	35.0	51	28
70:30	196	57	2,417	41.8	48	37
30:70	231	62	6,070	53.9	43	50
0:100	171	63	5,513	40.7	46	55
Significance						
Linear	NS	*	NS	NS	*	**
Quadratic	*	NS	*	*	NS	NS

²Dry mass is based on fresh mass.

NS,*,** Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 3. Effect of N-form ratio on concentration of main inorganic ions (fresh mass basis).

NH ₄ : NO ₃ (% ratio)	Anions					Cations					
	NO ₃	Cl ⁻	H ₂ PO ₄ ⁻	SO ₄ ²⁻	Total (A)	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	Total (C)	C-A
	(g·kg ⁻¹)				(meq·kg ⁻¹)	(g·kg ⁻¹)				(meq·kg ⁻¹)	
	<i>Head</i>										
100:0	0	57	19	20	2245	3.9	78	9	2.8	2877	632
70:30	42	46	21	16	2528	3.6	85	12	3.5	3193	665
30:70	98	3	18	14	2146	4.2	71	15	1.7	2899	753
0:100	87	2	18	16	1999	7.2	73	15	1.9	3085	1085
Significance											
Linear	***	***	NS	**	NS	***	NS	***	NS	NS	**
Quadratic	**	***	NS	*	NS	**	NS	*	NS	NS	*
						<i>Roots</i>					
100:0	0	15	26	23	1188	8.0	32	3	1.3	1452	264
70:30	35	8	32	12	1362	7.9	41	5	1.3	1730	368
30:70	58	1	26	5	1334	6.8	48	6	2.2	2032	699
0:100	77	1	20	5	1563	8.1	50	11	2.4	2403	840
Significance											
Linear	***	***	**	***	NS	NS	***	***	**	***	*
Quadratic	*	***	**	*	NS	NS	NS	*	NS	NS	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Schonbeck et al. (1991) and Reinink et al. (1994), the level of nitrate in endive was similar to the levels found in lettuce cultivars.

The possibility of reducing nitrate content in endive through ammonium nutrition is of major importance. Reinink et al. (1994), reporting on genetic variations in the nitrate content between cultivars of endive, found that there is only a slight possibility of reducing the nitrate content of endive by cultivar choice. From the commercial viewpoint, nitrate-free produce would be a value-added factor.

Head dry matter percentage decreased with increasing ammonium in the nutrient solution, suggesting that the effects of the two N forms on growth could partially originate from cell osmotic regulation and expansion.

With higher NO₃-N levels in the nutrient solutions, the difference between concentration of inorganic cations and anions, which assess organic anion content, increased. In most plants, carboxylic acid concentration is higher with nitrate nutrition to balance cation charges accompanying NO₃⁻ assimilation (Haynes and Goh, 1978; Kirkby and Mengel, 1967). The NH₄-fed plants, instead, normally have lower organic acid concentrations as a consequence of organic N compounds formation (Haynes and Goh, 1978). The mechanism that allows endive to tolerate NH₄⁺ could be connected with the greater capability of endive to synthesize organic acids in the absence of NO₃⁻ than other nontolerant plants (Salsac et al., 1987).

The NH₄⁺ uptake increased Cl⁻ absorption mainly in leaves to make up for the lack of NO₃⁻. The Cl⁻ concentration increase was also due to the higher Cl⁻ concentration in the nutrient solution. In leaves, when changing from nitrate to ammonium solution, NO₃⁻ dropped from 71% to 0% and Cl⁻ rose from 4% to 72% of the total inorganic anions. Its impact on H₂PO₄⁻ and SO₄²⁻ was unvaried, whereas, in roots, SO₄²⁻ also contributed to make up for the lack of NO₃⁻, much as Cl⁻.

The accumulation of Cl⁻ to replace organic anions and NO₃⁻ allowed the restoration of the anion level needed to keep the osmotic potential at optimal values for growth (Blom-Zandstra and Lampe, 1983; Gill and Reisenauer, 1993; Soltani et al., 1989).

The K⁺ concentration accounted for 60% (with 100% NO₃-N) to 70% (with 100% NH₄-N) of total cations in the head and for 58%

on average in roots. The Ca²⁺ concentration reached the maximum value of 24% in heads and in roots and increased with higher concentrations of NO₃⁻ in the nutrient solution. The Mg²⁺ and Na⁺ concentration variations were of lesser importance.

The K⁺ concentration had special significance, as it was high also in ammonium-fed plants. In endive, thus, K⁺ is not inhibited by NH₄⁺ uptake, rather it favors the assimilation-detoxification mechanism of NH₄⁺. Several authors have underlined the importance of suitable K⁺ feeding when using mixed N to increase yield and protein content. A scarcity of K⁺ would increase the chances of toxic effects in the plant at high concentrations of NH₄⁺. For instance, Ajayi et al. (1970) indicated that lesions on tomato plant stems fed with NH₄-N are inhibited by the addition of K⁺. In two more experiments, maize and wheat yields were not affected by K⁺ when N was in the form of NO₃⁻ but increased with higher K⁺ when fed with NH₄⁺ (Dibb and Welch, 1976; Shaviv and Hagin, 1988). An adequate supply of K⁺ should speed up the activity of the enzymes involved in NH₄⁺ assimilation and, thus, would reduce NH₄⁺ accumulation in plants and its toxic effects (Hagin et al., 1990). Gigon and Rorison (1972) suggested that the ability of *Deschampsia flexuosa* to maintain a high rate of K⁺ uptake with ammonium nutrition might explain its NH₄⁺ tolerance, whereas NH₄⁺-intolerant species could not maintain an adequate K⁺ uptake.

Head total N accumulation was increased by the presence of NH₄-N in the nutrient solution and decreased with 100% NO₃-N. Consequently, reduced N accounted for 81% of total N with NH₄ : NO₃ ratios of 70:30 and 30:70 and for 60% of total N with 100% NO₃-N.

Conclusions

Data from this research indicate that endive can use NH₄⁺ if the pH of nutrient solution is controlled. The yield of ammonium-fed plants was similar to that of nitrate-fed plants but 25% lower than plants supplied with 30 NH₄⁺ : 70 NO₃⁻. The heads of NH₄⁺-fed plants were also darker green and were more succulent. From the commercial viewpoint the produce was good and had the value-added factor of the absence of nitrate. The latter is an extremely remarkable factor because the European Union is drafting a

directive on the allowable NO_3^- content in leafy vegetables that could be similar to the already used official limits in Germany, The Netherlands, Switzerland, Belgium, and Austria.

The use of NH_4^+ as a means of depressing nitrate concentrations in endive heads is worthy of further consideration in soilless growing systems because NH_4^+ reduces the need to add acids to lower the pH of the nutrient solution and ammonium-based fertilizers cost less than nitrate-based ones.

Literature Cited

- American Society for Testing and Materials. 1992. Standard test method for anions in water by chemically suppressed ion chromatography, p. 336–341. In: Annual Book of ASTM Standards, 11.01, Water (1), Designation: D 4327–91.
- Ajayi, O., D.N. Maynard, and A.V. Barker. 1970. The effect of potassium on ammonium nutrition of tomato (*Lycopersicon esculentum* Mill.). *Agron. J.* 62:818–821.
- Barker, A.V. and H.A. Mills. 1980. Ammonium and nitrate nutrition of horticultural crops. *Hort. Rev.* 2:395–423.
- Blom-Zandstra, M. and J.E.M. Lampe. 1983. The effect of chloride and sulphate salts on the nitrate content in lettuce plants (*Lactuca sativa* L.). *J. Plant Nutr.* 6:611–628.
- Chaillou, S., J.K. Vessey, J.F. Morot-Gaudry, C.D. Raper, Jr., L.T. Henry, and J.P. Boutin. 1991. Expression of characteristics of ammonium nutrition as affected by pH of the root medium. *J. Expt. Bot.* 42:189–196.
- Choi, B.C.K. 1985. N-nitroso compounds and human cancer: A molecular epidemiologic approach. *Amer. J. Epidem.* 121:737–743.
- Commission of the European Communities Scientific Committee for Food. 1992. Report of the scientific committee for food on nitrate and nitrite. 26th series. 19 Oct., EUR 13913.
- Dibb, D.W. and L.F. Welch. 1976. Corn growth as affected by ammonium vs. nitrate absorbed from soil. *Agron. J.* 68:89–94.
- Draper, N.R. and H. Smith. 1981. Applied regression analysis. 2nd ed. Wiley, New York.
- Findenegg, G.R. 1987. A comparative study of ammonium toxicity at different constant pH of the nutrient solution. *Plant Soil* 103:239–243.
- Ganmore-Neumann, R. and U. Kafkafi. 1980a. Root temperature and percentage $\text{NO}_3^-/\text{NH}_4^+$ effect on tomato plant development. I. Morphology and growth. *Agron. J.* 72:758–761.
- Ganmore-Neumann, R. and U. Kafkafi. 1980b. Root temperature and percentage $\text{NO}_3^-/\text{NH}_4^+$ effect on tomato plant development. II. Nutrients composition of tomato plants. *Agron. J.* 72:762–766.
- Gashaw, L. and L.M. Mugwira. 1981. Ammonium-N and nitrate-N effects on the growth and mineral compositions of triticale, wheat and rye. *Agron. J.* 73:47–51.
- Gigon, A. and I.H. Rorison. 1972. The response of some ecologically distinct plant species to nitrate and ammonium nitrogen. *J. Ecol.* 60:93–102.
- Gill, M.A. and H.M. Reisenauer. 1993. Nature and characterization of ammonium effects on wheat and tomato. *Agron. J.* 85:874–879.
- Gunes, A., W.N.K. Post, E.A. Kirkby, and M. Aktas. 1994. Influence of partial replacement of nitrate by amino acid nitrogen or urea in the nutrient medium on nitrate accumulation in NFT grown winter lettuce. *J. Plant Nutr.* 17:1929–1938.
- Hagin, J., S.R. Olsen, and A. Shaviv. 1990. Review of interaction of ammonium-nitrate and potassium nutrition of crops. *J. Plant Nutr.* 13:1211–1226.
- Haynes, R.J. and K.M. Goh. 1978. Ammonium and nitrate nutrition of plants. *Biol. Rev.* 53:465–510.
- Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. *Calif. Agr. Expt. Sta. Circ.* 347.
- Hunt, R. 1978. Plant growth analysis. Edward Arnold, London, England.
- Islam, A.K.M.S., D.G. Edwards, and C.J. Asher. 1980. pH optima for crop growth: results of a flowing culture experiment with six species. *Plant Soil* 54:339–357.
- Kirkby, E.A. and K. Mengel. 1967. Ionic balance in different tissues of tomato plant in relation to nitrate, urea or ammonium nutrition. *Plant Physiol.* 42:6–14.
- Martignon, G., D. Casarotti, A. Venezia, M. Schiavi, and F. Malorgio. 1994. Nitrate accumulation in celery as affected by growing system and N content in the nutrient solution. *Acta Hort.* 361:583–589.
- Moritsugu, M., T. Suzuki, and T. Kawasaki. 1983. Effect of nitrogen source on growth and mineral uptake in plants under constant pH and conventional culture conditions. *Ber. Ohara Inst. Landw. Biol. Okayama Univ.* 18:125–144.
- Raven, J.A. 1985. Regulation of pH and generation of osmolarity in vascular plants: A cost-benefit analysis in relation to efficiency of use of energy, nitrogen and water. *New Phytol.* 101:25–77.
- Reinink, K., M. van Nes, and R. Groenwold. 1994. Genetic variation for nitrate content between cultivars of endive (*Cichorium endivia* L.). *Euphytica* 75:41–48.
- Roorda van Eysinga, J.P.N.L. and L. Spaans. 1986. Factorum van invloed op het nitraat- en bromidegehalte in andijvie en spinazie onder glas. *Inst. Soil Fertility, Haren, The Netherlands, Rpt.* 15–85.
- Salsac, L., S. Chaillou, J.F. Morot-Gaudry, C. Lesaint, and E. Jolivet. 1987. Nitrate and ammonium nutrition in plants. *Plant Physiol. Biochem.* 25:805–812.
- Santamaria, P., A. Elia, and M. Gonnella. 1995. Azoto, imbianchimento, produzione e accumulo di nitrati in indivia allevata in idrocoltura. *Italus Hortus* 2(3):32–38.
- Santamaria, P., A. Elia, and M. Gonnella. 1996. $\text{NH}_4^+/\text{NO}_3^-$ ratio changes, withdrawal of N before the harvest and reduction of nitrate leaf content in endive (*Cichorium endivia* L. var. *crispum* Hegi). *Proc. IX Intl. Congr. on Soilless Culture, St Helier, Jersey*, 12–19 Apr. 1996. (In press.)
- Schonbeck, M.W., R. Rivera, J. O'brein, S. Ebinger, and R.E. Degregorio. 1991. Variety selection and cultural methods for lowering nitrate levels in winter greenhouse lettuce and endive. *J. Sust. Agr.* 2 (1):49–75.
- Shaviv, A. and J. Hagin. 1988. Interaction of ammonium and nitrate nutrition with potassium in wheat. *Fert. Res.* 17:137–146.
- Soltani, A., M. Hajji, and C. Grignon. 1989. Nécessité d'un anion exogène mobile en cas de nutrition ammoniacale. *Agronomie* 9:777–784.
- Steel, R.G. and J.H. Torrie. 1980. Principles and Procedures of Statistics. 2nd ed. Macmillan, New York.
- Steingröver, E., J.W. Steenhuizen, and J. Van Der Boon. 1993. Effect of low light intensities at night on nitrate accumulation in lettuce grown on a recirculating nutrient solution. *Neth. J. Agr. Sci.* 41:13–21.
- Troelstra S.R., C. Van Dijk, and T. Blacquiere. 1985. Effects of N-source on proton excretion, ionic balance and growth of *Alnus glutinosa* (L.) Gaertner: comparison of N_2 fixation with single and mixed sources of NO_3^- and NH_4^+ . *Plant Soil* 84:361–385.
- Van Beusichem, M.L. 1981. Nutrient absorption by pea plants during dinitrogen fixation. I. Comparison with nitrate nutrition. *Neth. J. Agr. Sci.* 30:317–330.
- Van Der Boon, J., J.W. Steenhuizen, and E. Steingröver. 1990. Growth and nitrate concentration of lettuce as affected by total nitrogen and chloride concentration, $\text{NH}_4^+/\text{NO}_3^-$ ratio and temperature of the recirculating nutrient solution. *J. Hort. Sci.* 65:309–321.
- Vegter, J. and C. Tanis. 1990. Bladgewassen. Nitraatgehalte vertoont grillig verloop in winter. *Groenten en Fruit*. 46(19):40–41.
- Walker, R. 1990. Nitrates, nitrites and N-nitroso compounds: a review of the occurrence in food and diet and the toxicological implications. *Food Add. Cont.* 7:717–768.
- World Health Organization. 1973. Toxicological evaluation of certain food additives with a review of general principles and of specifications. 17th Rpt. Joint FAO/WHO Expert Committee on Food Additives. FAO nutrition report series 53, Geneva.