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# Yield, Fructans Accumulation, and Nutritional Quality of Young Chicory Plants as Related to Genotype and Nitrogen Fertilization

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**Abstract:** Growth and quality attributes were quantified in *Cichorium intybus* L. and *Cichorium endivia* L. in response to the nitrate supply. Chicory was grown in Italy, in a cold greenhouse from the 11 of February 2020, in a pot with commercial soil and sand with and without 12 mM of $Ca(NO_3)_2$. Seventy-six days after sowing, the growth variables, contents of leaf and root carbohydrates (glucose, fructose sucrose starch and fructans), carbon, nitrate sulphate and phosphate were measured. Fertilization significantly increased the yield and specific leaf dry weight (SLDW) of the *C. endivia*. The shoot/root ratio was increased in *C. intybus* with high N; this also increased the carbohydrate content in leaves and roots of *C. endivia* compared to the value measured in *C. intybus*. The interaction between fertilization and genotype significantly affected fructans and nitrate accumulation in taproots. Fertilization decreased the sulphate and phosphate contents in the leaves and roots of both chicory species. The yield and quality of young chicory plants can be modulated by species selection and nitrate fertilization. The genotype and nitrogen supply interact in modulating the yield and the inulin and nitrate accumulation in the taproot, thereby affecting its nutritional value and representing a powerful tool to cultivate this new produce for healthy human nutrition.

**Keywords:** plant yield; carbohydrate; inulin-type polymers dietary; fibers; nitrogen; protein; nitrate; sulphate; phosphate; organic acids; early-stage chicory

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## 1. Introduction

*Cichorium intybus* L. and *Cichorium endivia* L. are two closely related species of the Cichorium genus, from the Asteraceae family, which share the common name of chicory. They represent two traditional European horticultural crops, spread worldwide, with great differences in cultural practices and utilization [1]. Plant leaves and stems in the early bolting phase are consumed as a fresh salad or cooked depending on alimentary traditions [2], while roots can be used in food preparations and in the food industry, dried and roasted for use as coffee substitutes and additives. Despite the differences detectable in the cultivation and use of two botanical varieties of chicory, the leaves and roots of both species are characterized by high nutritional value. In chicory leaves, high levels of health-promoting phytochemical compounds can be detected, such as vitamins, polyphenols, proteins, and minerals [3]. In addition, carbohydrates, organic acids, and different secondary metabolites define the unique taste of chicory plants [4]. Chicory taproots contain a high quantity of inulin, so they are used as a model system to study or modify inulin metabolism in plants, and as industrial raw material for the extraction of inulin and inulin hydrolysis by-products, used in food and non-food applications for their prebiotic and health properties [5–7].

Inulin is a dietary fiber, constituted by fructan-type polymers with low energy characteristics (1.5 kcal/g) and with fructose subunits linked by a β-2,1 bond, ending with

α-linked glucose. The degree of subunit polymerization (DP) varies from 2 to 60, with its partial enzymatic hydrolysis products (DP 2–10) as oligofructose or fructooligosaccharides (FOS) [8]. Fructans can accumulate in the vacuole, apoplast, phloem, and xylem tissues, representing energy storage for plants, possessing antioxidant properties, and with a cryoprotectant role in cold stress protection [9]. In chicory leaves, inulin is less abundant, or it occurs at much lower levels and DP than in roots. For instance, leaves can contain inulin at less than 11%, compared to roots up to 70.5% on a dry weight basis [3,10], while DP was recorded as 3–5 and 10–15 on average, in leaves and roots, respectively [3,11]. Moreover, chicory fructans are completely fermented by bacteria in the large intestine, and inulin increases the relative abundance of the *Bifidobacterium*, *Faecalibacterium*, and *Lactobacillus* population, protects the digestive system, stimulates the immune system, and prevents inflammatory illnesses, infection, and cardiovascular and carcinogenic diseases [12]. Inulin and fructans were tolerated at doses lower than 20 g/day; however, any more than this intake of these prebiotic compounds would induce gastrointestinal disorders [12,13].

Different factors such as the density of plant cultivation, environmental parameters, fertilization practices, and harvest date impact the yield of leaves and root biomass in chicory, affecting the content of different health-promoting phytochemicals [14,15]. The level of nitrogen supply can affect the chicory crop yield, biomass partitioning between shoot and root organs, and the amounts of different metabolites accumulated in vegetable parts. For this reason, the nitrogen requirement is generally carefully defined in chicory cultivation. Nitrogen deficiency is known to reduce the total biomass of plants and decreases the shoot/root ratio, enhancing the C allocation and the carbohydrate accumulation in the roots through the increase in metabolic sink strength. However, when the nitrogen supply exceeds the plant's requirements for growth and development, an accumulation of nitrate in plants tissues can occur, and despite the effects of nitrate compounds on the human organism currently being under discussion, nitrate intake in the human diet is still associated with some serious health threats [16]. Chicory can accumulate large amounts of nitrate in leaves compared to roots. Since both shoot and root organs are valuable in chicory for different reasons, the chemical nature and dose of nitrogen fertilization are essential with regard to the balance between shoot/root development, phytochemical accumulation, food security for human health, and the minimization of environmental impacts. Efforts to optimize nitrogen fertilization are intended to improve crop production, ensuring adequate shoot development, which provides high photosynthetic C assimilation, sufficient root growth, nutrient uptake enhancement, and reserve storage. Furthermore, the vegetable's quality can be improved through fertilization management, increasing the levels of nutraceutical compounds and reducing the accumulation of anti-nutritional components in different edible plant tissues.

In nitrate fertilizers, calcium nitrate $Ca(NO_3)_2$ is largely used as a nitrogen source for vegetable growing, due to its chemical properties (i.e., pH neutral or slightly alkaline), low toxicity, high solubility, and low electrical conductivity [17]. Moreover, the use of calcium nitrate can also act as a source of $Ca^{2+}$, an essential mineral involved in important physiological processes such as the preservation of the structural and functional integrity of the plant membrane and cell wall, regulation of ion transport, and enzymatic activities [18].

Chicory can be grown under limited or full environmental control, in a short-cycle growth to produce young plants with limited-size tap roots, containing about 50% of the dry weight as fructans [19]. Due to the rising interest in controlled environment agriculture to grow health-promoting produce in greenhouses, in vertical farming systems, and even in facilities designed as life support during space missions [19], it is important to understand how agronomical tools can influence early-stage chicory production and quality. Young chicory taproots may contain large quantities of fructans, a confirmed prebiotic class of molecules, and can then represent a new food with particularly relevant health attributes and commercial potential. However, no research has been conducted so far on the role of the genotype and nitrogen availability on the quality and safety of such new produce. Considering this objective, our works aimed to verify the effects of

nitrogen fertilization (i) on the yield of chicory's leaf and root, both representing a valuable resource of the high marketable vegetable crop; (ii) on the shoot/root ratio, which can be considered a physiological indicator of carbon allocation in chicory plants; and (iii) on the nutritional quality of chicory plants, particularly regarding fructans accumulated in chicory root, potentially usable as functional foods and dietary supplements due to their prebiotic content.

## 2. Materials and Methods

### *2.1. Plant Material and Treatments*

The experiment was carried out in a cold greenhouse at CNR-IRET, in Porano (TR) (Lat. 42°41′3″, Long. 12°5′57″, 444 m asl), between February 2020 (sowing date) and April 2020 (harvesting date). Plants of *Cichorium intybus* L. cv. Pan Zucchero and *Cichorium endivia* L. cv. Romanesca were grown in 1.5 L plastic pots, in commercial topsoil and sand (3:1). Three weeks after sowing, thinning was carried out to a final plant population of 50 plants per $m^2$; harvest occurred 76 days after sowing (DAS), and chicory plants were supplied with water (control plants—*C. intybus*-C and *C. endivia*-C) or nitrate solution containing 12 mM of $Ca(NO_3)_2$ (fertilized plants -*C. intybus*-N and *C. endivia*-N) every week for an equivalent rate of 80 kg $ha^{-1}$. Five plants from each treatment were harvested; the roots were washed with deionized water and carefully dried with paper. Shoots and roots were separated and used for the determination of the shoot and root fresh weight (FW) and dry weight (DW), dry matter (DM) percentage, shoot/root (S/R) ratio, and specific leaf dry weight (SLDW) obtained by a subsample of 3 leaf disks making up a 1.8 $cm^2$ area. Plant material was dried by a freeze-dryer to constant weight. Dried samples were reduced to a fine powder in a mill before the analysis. The nitrogen (N) content of leaves and roots was analyzed using an elemental analyzer (Model NA 1500, Carlo Erba, Milan, Italy) and expressed as a percentage (%) of the dry matter. The total protein content was obtained from the total N concentration corrected by subtracting the N due to nitrate ion and applying a conversion factor of 6.25 [20].

### *2.2. Non-Structural Carbohydrates (NCS) Analysis*

Non-structural carbohydrates (NSC) in chicory leaves were measured using 10 mg lyophilized powder extracted in 80% ethanol at 80 °C for 45 min under continuous shaking. The extract was centrifuged at 16,000× *g* for 5 min, soluble sugars (glucose, fructose, and sucrose) were recovered in the supernatant, and starch was contained in the pellet. Soluble sugar and starch determination, by spectrophotometric coupled enzymatic assay, was performed as in Proietti et al. [21]. The pellet, containing starch, was washed four times with 50 mM NaAcetate buffer (pH 4.5) and then suspended and autoclaved at 120 °C for 45 min in 1 mL of the same buffer. After autoclaving, the sample was incubated at 50 °C for 1 h with amyloglucosidase (70 U) and α-amylase (4U) to hydrolyze the starch to glucose. The glucose produced by starch hydrolysis was then measured as described before by a spectrophotometric coupled enzymatic assay.

### *2.3. Fructans Analysis*

Fructans, sucrose, glucose, and fructose from chicory taproots were extracted according to Verspreet et al. [22] with some variations: 20 mg of powdered root tissue was extracted on 0.5 mL 100% ethanol for 20 min at 80 °C until evaporation, then the residue obtained was dissolved in 2 mL of deionized water and the solution was treated at 80 °C for 1 h, in a thermomixer with shaking. After cooling to room temperature, the extract was centrifuged at 6000× *g* for 10 min and then filtrated through a nylon 0.45 µm PPII syringe filter (Whatman Inc., Maidstone, UK). The fructan concentrations and fructan average DP were determined after mild acid hydrolysis of the extracts, carried out with 60 mM HCl (final concentration) at 70 °C for 2 h. The non-hydrolyzed and hydrolyzed samples were diluted with water and analyzed through high-performance anion exchange chromatography, with pulsed amperometric detection (HPAEC-PAD), using a Dionex™ ICS-5000.

An analytical CarboPac PA-100 column (4 × 250 mm) with a relative guard column was used (all equipment was ThermoFisher Scientific Dionex™, Waltham, MA, USA). All runs were carried out at 30 °C, at a flow rate of 1 mL $min^{-1}$, using a mobile phase gradient with two aqueous solutions: (A) NaOH 1 mol $L^{-1}$; and (B) NaAcetate 1 mol $L^{-1}$ under the following conditions: 0–8 min, 100 mM NaOH; 8–18 min, 100 mM NaOH with 100 mM NaAcetate; 18–25 min, 500 mM NaOH with 500 mM NaAcetate, maintained until 35 min; 35–45 min 100 mM NaOH, maintained until 60 min. Sucrose, glucose, and fructose were quantified against a carbohydrate standard curve prepared using HPLC-grade reagents (Sigma, Steinheim, Germany). Fucose was used as an internal standard.

### *2.4. Inorganic Anions and Organic Acids Analysis*

The ethanolic extracts of NSC determination were used to quantify inorganic anions and organic acids in chicory leaves and roots. After centrifugation, the supernatants were filtrated through a 0.2 µm nylon PPII syringe filter before injection on an ion chromatography system Dionex™ ICS-5000 equipped with a conductivity detector, an analytical IonPac AS11-HC column (4 × 250 mm) with a related guard column and an IonPac Anion Trap Column (ATC). The system was coupled with an ERSTM 500 Electrolytically Regenerated Suppressor. All chromatography equipment was a ThermoFisher Scientific Dionex™ (Waltham, MA, USA) product. The IC analysis was performed as described in Proietti (2021) [21]. The eluents and inorganic anion and organic acid standard solutions were prepared using HPLC-grade reagents (Merck KGaA, Darmstadt, Germany). ICS-5000 chromatography system control, data acquisition, and processing were performed with the software Chromeleon Data System 7.1 (DionexTM ICS-5000; ThermoFisher Scientific, Waltham, MA, USA).

### *2.5. Statistical Analysis*

Statistical analysis was performed by two-way ANOVA using the STATISTICA software package (StatSoft 8 for Windows, 1998), with the species (*C. intybus*, and *C. endivia*) and treatment with or without nitrogen (TR: C, control; N, $Ca(NO_3)_2$ 12 mM) as factors. Differences between averages were tested by Fisher's post hoc test, with a significance level of $p = 0.05$, and designated with different letters.

## 3. Results

### *3.1. The Growth and Productivity of Chicory Plants*

The growth and productivity of chicory plants were affected by the interaction between the nitrogen supply and genotypic traits of Pan Zucchero and Romanesca (Figure 1A,B). The highest yield, in Kg of fresh and dry biomass per $m^2$, occurred in Romanesca-N (2.60 ± 0.11 and 0.27 ± 0.01 Kg $m^{-2}$, respectively), while Pan Zucchero-C showed the lowest value of fresh biomass production (0.81 ± 0.11 Kg FW per $m^2$). Pan Zucchero-N and Romanesca-C showed values of fresh biomass statistically similar with 1.23 ± 0.17 and 1.13 ± 0.13 Kg $m^{-2}$, respectively (Figure 1A). The values of dry biomass production of Pan Zucchero-C and -N and Romanesca-C did not show statistical differences (0.08 ± 0.01, 0.12 ± 0.02, 0.10 ± 0.01 Kg FW $m^{-2}$, respectively) (Figure 1B).

Leaf and tap root growth parameters significantly differed between species of chicory for both Pan Zucchero and Romanesca and in interaction with nitrogen fertilization (Table 1).

The highest amount of the leaf's fresh and dry weight occurred in Romanesca -N, while control plants of both cultivars showed the lowest value for these parameters (Table 1). The higher dry matter percentage (DM%) value was obtained in leaves of Romanesca -N and of Pan Zucchero -C. The specific leaf dry weight (SLDW) was 36.3% higher in Romanesca -N, compared to control leaves, while the SLDW of Pan Zucchero was unaffected by nitrogen supply (Table 1). The fresh and dry weight of tap roots was significantly affected by the nitrogen supply in Romanesca plants (Romanesca -N), increasing by 3.5 and 3.8 times, respectively, concerning the value measured in Romanesca -C. These parameters measured on Pan Zucchero were not affected by nitrogen supply. The DM% of chicory tap root was

33.6% higher in Romanesca -N than in Romanesca-C, while it was 21.3% higher in Pan Zucchero -C than in Pan Zucchero -N plants (Table 1).

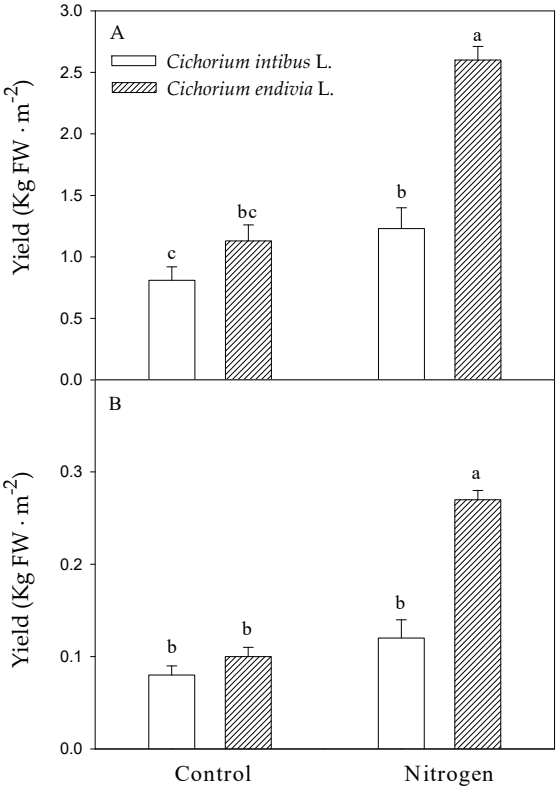


**Figure 1.** Effects of nitrogen supply on yield of chicory plants (*Cichorium intybus* L.) cv Pan Zucchero and (*Cichorium endivia* L.) cv Romanesca. Data were expressed in Kg/m$^2$ of plant fresh weight (**A**) and plant dry weight (**B**). C, control; Nitrogen, $Ca(NO_3)_2$ 12 mM. Different letters indicate significant differences between the means (n = 5) for each parameter, according to two-way ANOVA and Fischer's LSD test at $p = 0.05$.

**Table 1.** Effects of nitrogen supply on yield of chicory plants (*Cichorium intybus* L.) cv Pan Zucchero and (*Cichorium endivia* L.) cv Romanesca.

| | **Leaf FW (g/Plant)** | **Leaf DW (g/Plant)** | **Leaf DM%** | **SLDW (mg cm$^{-2}$)** | **Tap Root FW (g/Plant)** | **Tap Root DW (g/Plant)** | **Tap Root DM%** | **Shoot/Root** |
|---|---|---|---|---|---|---|---|---|
| Species | | | | | | | | |
| *C. intybus* | 13.19 ± 1.91 | 1.33 ± 0.20 | 10.04 ± 0.21 | 2.31 ± 0.08 | 1.02 ± 0.13 | 0.19 ± 0.03 | 18.02 ± 0.98 | 8.36 ± 1.53 |
| *C. endivia* | 22.22 ± 4.22 | 2.25 ± 0.46 | 9.71 ± 0.37 | 2.35 ± 0.22 | 1.61 ± 0.30 | 0.32 ± 0.07 | 18.36 ± 1.06 | 7.29 ± 0.54 |
| TR | | | | | | | | |
| C | 9.21± 0.53 | 0.87 ± 0.05 | 9.52 ± 0.33 | 2.09 ± 0.10 | 0.93 ± 0.08 | 0.17 ± 0.02 | 17.73 ± 1.00 | 5.68 ± 0.57 |
| N | 26.19 ± 3.12 | 2.70 ± 0.34 | 10.23 ± 0.22 | 2.57 ± 0.17 | 1.70 ± 0.30 | 0.34 ± 0.07 | 18.65 ± 1.01 | 9.96 ± 1.17 |
| Species × TR | | | | | | | | |
| *C. intybus*-C | 8.63 ± 0.57 c | 0.87 ± 0.04 c | 10.10 ± 0.31 a | 2.35 ± 0.06 b | 1.04 ± 0.13 b | 0.21 ± 0.03 b | 19.75 ± 1.11 a | 4.48 ± 0.55 c |
| *C. intybus*-N | 17.74 ± 2.40 b | 1.79 ± 0.28 b | 9.98 ± 0.33 ab | 2.27 ± 0.15 bc | 1.01 ± 0.25 b | 0.17 ± 0.05 b | 16.29 ± 1.25 b | 12.23 ± 1.66 a |
| *C. endivia*-C | 9.79 ± 0.88 c | 0.88 ± 0.10 c | 8.94 ± 0.48 b | 1.83 ± 0.08 c | 0.83 ± 0.08 b | 0.13 ± 0.02 b | 15.72 ± 1.13 b | 6.88 ± 0.65 bc |
| *C. endivia*-N | 34.65 ± 1.47 a | 3.62 ± 0.11 a | 10.48 ± 0.29 a | 2.87 ± 0.26 a | 2.39 ± 0.31 a | 0.50 ± 0.07 a | 21.01 ± 0.50 a | 7.70 ± 0.90 b |

C, control; N, $Ca(NO_3)_2$ 12 mM. Different letters indicate significant differences between the means (n = 5) for each parameter, according to two-way ANOVA and Fischer's LSD test at $p = 0.05$.

### *3.2. Non-Structural Carbohydrate and Fructans Content*

In chicory leaves, the nitrogen supply increased the content of soluble carbohydrates and total NSC by 1.4 and 1.3 times, respectively, compared to the control plants. The starch content was 1.5 times higher in control plants than in fertilized plants. Romanesca leaves showed a higher value of all the parameters mentioned above than the content measured in Pan Zucchero (Table 2).

**Table 2.** Effects of nitrogen supply on the carbohydrate content on leaves of chicory (*Cichorium intybus* L.) cv Pan Zucchero and (*Cichorium endivia* L.) cv Romanesca. Data were reported as a percentage (%) of dry matter (DM).

| | **Chicory Leaves** | | |
|---|---|---|---|
| | **Total Soluble** | **Total NSC** | **Starch** |
| Species | | | |
| *C. intybus* | 9.13 ± 1.05 b | 10.10 ± 1.08 b | 0.97 ± 0.13 b |
| *C. endivia* | 11.51 ± 0.75 a | 13.89 ± 0.64 a | 2.39 ± 0.19 a |
| TR | | | |
| C | 8.51 ± 0.52 b | 10.54 ± 0.73b | 2.03 ± 0.29 a |
| N | 12.13 ± 0.99 a | 13.46 ± 1.17a | 1.33 ± 0.23 b |
| Species × TR | | | |
| *C. intybus*-C | 7.71 ± 0.95 | 8.96 ± 1.03 | 1.25 ± 0.12 |
| *C. intybus*-N | 10.55 ± 1.75 | 11.24 ± 1.88 | 0.69 ± 0.15 |
| *C. endivia*-C | 9.31 ± 0.09 | 12.11 ± 0.30 | 2.80 ± 0.23 |
| *C. endivia*-N | 13.70 ± 0.31 | 15.68 ± 0.41 | 1.97 ± 0.14 |

C, control; N, $Ca(NO_3)_2$ 12 mM, Different letters indicate significant differences between the means (n = 5) for each parameter, according to two-way ANOVA and Fischer's LSD test at $p = 0.05$.

Sucrose and glucose were the most abundant soluble sugars in chicory leaves, with average values of 4.0% and 3.7%, respectively, on a dry matter basis, followed by fructose at 2.58% (Table S1). No detectable fructans were found in the leaves of young chicory plants. On average, the soluble carbohydrate levels (glucose, fructose, and sucrose) were found to be 8% lower in chicory roots than in leaves (regardless of the cultivar or nitrogen supply). In chicory roots, fertilization increased the content of soluble carbohydrates by 20.5% compared to the control plants, while the roots of Romanesca plants showed 23.1% higher levels of soluble sugars than Pan Zucchero roots (Table 3).

**Table 3.** Effects of nitrogen supply on the carbohydrate content on roots (*Cichorium intybus* L.) cv Pan Zucchero and (*Cichorium endivia* L.) cv Romanesca. Data were reported as a percentage (%) of dry matter (DM). The yield of fructans in g per $m^2$ was also reported.

| | **Chicory Roots** | | | |
|---|---|---|---|---|
| | **Total Soluble** | **Total NSC** | **Fructans** | **Fructans (g $m^{-2}$)** |
| Species | | | | |
| *C. intybus* | 4.57 ± 0.48 b | 43.52 ± 2.39 b | 38.94 ± 2.30 | 4.02 ± 0.44 |
| *C. endivia* | 5.94± 0.25 a | 50.16 ± 2.94 a | 44.20 ± 2.92 | 7.77 ± 2.04 |
| TR | | | | |
| C | 4.66± 0.47 b | 43.16 ± 1.69 b | 38.49 ± 1.80 | 3.26 ± 0.40 |
| N | 5.86± 0.31 a | 50.52 ± 3.30 a | 44.65 ± 3.16 | 8.53 ± 1.86 |
| Species × TR | | | | |
| *C. intybus*-C | 3.52 ± 0.50 | 43.58 ± 2.57 | 40.05 ± 2.37 b | 4.03 ± 0.41 b |
| *C. intybus*-N | 5.62 ± 0.50 | 43.46 ± 4.36 | 37.83 ± 4.18 b | 4.01 ± 0.84 b |
| *C. endivia*-C | 5.79 ± 0.32 | 42.74 ± 2.49 | 36.92 ± 2.77 b | 2.49 ± 0.48 c |
| *C. endivia*-N | 6.09 ± 0.40 | 57.58 ± 2.26 | 51.47 ± 2.08 a | 13.06 ± 2.17 a |

C, control; N, $Ca(NO_3)_2$ 12 mM. Different letters indicate significant differences between the means (n = 5) for each parameter, according to two-way ANOVA and Fischer's LSD test at $p = 0.05$.

Sucrose was found in chicory roots on average at 4.32% on a dry matter basis, followed by glucose and fructose at 0.55% and 0.39%, respectively (Table S1). Fructans were the most abundant carbohydrates in chicory roots, reaching an average value of 38.9% in Pan Zucchero and 44.2% in Romanesca plants; however, their content was negligible in leaves. The percentage of oligosaccharides on a dry weight basis was 19.6% higher in Romanesca -N roots compared

with the Romanesca -C leaves and 26.5% and 22.19% higher than in Pan Zucchero -N and Pan Zucchero -C, respectively (Table 3). The total NSC content in roots was defined by the fructans level: it was 13.2% higher in Romanesca than in Pan Zucchero, and 14.5% higher in fertilized plants than in control plants. The yield of fructans in g per $m^2$ was 81% higher in Romanesca -N roots compared with the control. In Pan Zucchero -C and -N roots, a similar content of fructans was found: about a third compared to the content measured in Romanesca -N plants (Table 3).

### *3.3. Nitrogen, Protein, Anions, and Organic Acids Content*

Both in the leaves and tap roots of chicory plants, the nitrogen supply increased the N percentage compared to the control plants (Table 4).

**Table 4.** Effects of nitrogen supply on C%, N%, and protein% on dry matter basis of chicory plants (*Cichorium intybus* L.) cv Pan Zucchero and (*Cichorium endivia* L.) cv Romanesca.

| | **Chicory Leaves** | | **Chicory Roots** | |
|---|---|---|---|---|
| | **N (% DM)** | **Protein (% DM)** | **N (% DM)** | **Protein (% DM)** |
| Species | | | | |
| *C. intybus* | 3.04 ± 0.35 | 11.15 ± 0.91 | 1.18 ± 0.20 | 5.64 ± 0.85 |
| *C. endivia* | 2.68 ± 0.32 | 9.95 ± 0.91 | 1.02 ± 0.16 | 5.62 ± 0.74 |
| TR | | | | |
| C | 2.01 ± 0.12 b | 9.66 ± 0.87 | 0.78 ± 0.12 b | 4.68 ± 0.75 |
| N | 3.71 ± 0.13 a | 11.44 ± 0.88 | 1.42 ± 0.16 a | 6.57 ± 0.67 |
| Species × TR | | | | |
| *C. intybus*-C | 2.16 ± 0.13 | 10.54 ± 1.10 | 0.76 ± 0.23 | 4.52 ± 0.31 |
| *C. intybus*-N | 3.92 ± 0.21 | 11.77 ± 1.60 | 1.60 ± 0.16 | 6.75 ± 0.70 |
| *C. endivia*-C | 1.87 ± 0.20 | 8.70 ± 1.40 | 0.73 ± 0.12 | 4.41 ± 0.70 |
| *C. endivia*-N | 3.49 ± 0.09 | 11.03 ± 1.00 | 1.51 ± 0.05 | 7.63 ± 0.30 |

C control; N, $Ca(NO_3)_2$ 12 mM. Different letters indicate significant differences between the means (n = 5) for each parameter, according to two-way ANOVA and Fischer's LSD test at $p = 0.05$.

The percentage of proteins was not significantly affected by the factors considered, nor in the leaves or the tap roots of chicory plants. Moreover, on average, the percentage of N and proteins was 59% and 44% lower in the tap root than in the chicory leaves, respectively (Table 4).

The nitrate content was about three times higher in the leaves than in the roots (Table 5).

Moreover, there was no significant difference in the nitrate content in the leaves of the two chicory species, whereas, in the roots, Pan Zucchero had a nitrate content 15% higher than that of Romanesca. Fertilization with $Ca(NO_3)_2$ increased the nitrate content in both leaves and roots of the two chicory species considered by 93% and 65%, respectively (Table 5). The sulphate and phosphate content measured in the leaves and roots of chicory plants was reported in Table 5. In both leaves and roots, nitrogen fertilization decreased the levels of sulphate and phosphate, which were 71% and 59% lower in fertilized than in control leaves, while they were, respectively, 71% and 66% lower in fertilized than in control roots. Malic acid content was double in both leaves and roots of Pan Zucchero compared to Romanesca tissues, while nitrogen fertilization increased the level of malate only in roots by 40%. The citric acid content was similar in the leaves of both Pan Zucchero and Romanesca, and the nitrogen fertilization did not affect the citrate level in either leaves or roots within each species (Table 5).

**Table 5.** Effects of nitrogen supply on the nitrate, sulphate, and phosphate inorganic anions and the malic and citric organic acids on leaf and root (*Cichorium intybus* L,) cv Pan Zucchero and (*Cichorium endivia* L,) cv Romanesca. Data were reported in ppm (mg $kg^{-1}$ FW) for the nitrate content and in mg $g^{-1}$ FW for the other inorganic anions and organic acids.

| | Chicory Leaves | | | | | Chicory Roots | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Nitrate (ppm)** | **Sulphate** | **Phosphate** | **Malic Acid** | **Citric Acid** | **Nitrate (ppm)** | **Sulphate** | **Phosphate** | **Malic Acid** | **Citric Acid** |
| Species | | | | | | | | | | |
| *C. intybus* | 5332 ± 1121 | 0.28 ± 0.08 | 0.31 ± 0.06 | 1.86 ± 0.25 a | 2.73 ± 0.39 | 1805 ± 525 a | 0.13 ± 0.12 | 0.23 ± 0.12 | 1.40 ± 0.12 a | 3.10 ± 0.12 a |
| *C. endivia* | 4542 ± 996 | 0.21 ± 0.06 | 0.27 ± 0.05 | 0.85 ± 0.12 b | 2.23 ± 0.16 | 1471 ± 441 b | 0.18 ± 0.12 | 0.18 ± 0.12 | 0.66 ± 0.12 b | 0.56 ± 0.12 b |
| TR | | | | | | | | | | |
| C | 2564 ± 641 b | 0.38 ± 0.11 a | 0.42 ± 0.04 a | 1.39 ± 0.31 | 2.59 ± 0.40 | 215.3 ± 21.7 b | 0.24 ± 0.12 a | 0.31 ± 0.12 a | 0.86 ± 0.12 b | 1.80 ± 0.12 |
| N | 7310 ± 788 a | 0.11 ± 0.03 b | 0.17 ± 0.03b | 1.32 ± 0.19 | 2.37 ± 0.17 | 3062 ± 162.7 a | 0.07 ± 0.12 b | 0.10 ± 0.12 b | 1.20 ± 0.12 a | 1.86 ± 0.12 |
| Species × TR | | | | | | | | | | |
| *C. intybus*-C | 3343 ± 1240 | 0.41 ± 0.12 | 0.42 ± 0.08 | 2.02 ± 0.46 | 3.13 ± 0.73 | 266 ± 23.0 | 0.21 ± 0.02 | 0.34 ± 0.03 | 1.11 ± 0.10 | 2.97 ± 0.18 |
| *C. intybus*-N | 7321 ± 1461 | 0.15 ± 0.05 | 0.21 ± 0.06 | 1.70 ± 0.23 | 2.34 ± 0.27 | 3346 ± 2367 | 0.05 ± 0.01 | 0.12 ± 0.03 | 1.69 ± 0.13 | 3.23 ± 0.43 |
| *C. endivia*-C | 1785 ± 590 | 0.35 ± 0.09 | 0.42 ± 0.04 | 0.76 ± 0.17 | 2.06 ± 0.22 | 165 ± 17.9 | 0.26 ± 0.04 | 0.28 ± 0.05 | 0.61 ± 0.22 | 0.63 ± 0.10 |
| *C. endivia*-N | 7299 ± 812 | 0.07 ± 0.01 | 0.13 ± 0.01 | 0.94 ± 0.19 | 2.40 ± 0.23 | 2778 ± 151 | 0.09 ± 0.02 | 0.09 ± 0.01 | 0.72 ± 0.04 | 0.49 ± 0.08 |

C, control; N, $Ca(NO_3)_2$ 12 mM, Different letters indicate significant differences between the means (n = 5) for each parameter according to two-way ANOVA and Fischer's LSD test at $p = 0.05$.

## 4. Discussion

### *4.1. The Effect of Fertilization on the Early Growth Phase and Quality of Chicory*

A plant's nitrogen requirement is a genetic trait that differs greatly between species and organs and can also vary with abiotic and biotic factors in the growth environment [23,24]. The effect of calcium nitrate fertilization was evaluated on the early growth phase and quality of two botanical varieties of chicory, Pan Zucchero (*Cichorium intybus* L.) and Romanesca (*Cichorium endivia* L.), which grows worldwide, and characterized by the high marketable value of both leaves and roots, useful for food consumption and industrial food production. Our data showed a greater response of *C. endivia* cv Romanesca to calcium nitrate fertilization than *C. intybus* cv Pan Zucchero, in accordance with evidence underlining the high genetic variability of different plant species and cultivars on the use efficiency and metabolism of nitrogen [24,25]. Cassan et al. [23] distinguished different genotypes of chicory, from 140 to 184 days after sowing, by dividing them into nitrogen-sensitive, tolerant, or demanding cultivars, presenting negative, negligible, and positive responses, respectively, in relation to the high levels of nitrogen. In *C. endivia* cv Romanesca, a higher nitrate supply promoted the plant yield, in kg plants per $m^{-2}$ (Figure 1); sustained a higher production of both leaves and roots, as grams of fresh and dry weight (Table 1); and increased the value of SLDW, compared to fertilized Pan Zucchero plants, and to controls of both chicory species. The higher nitrate supply caused a different balance between the shoot and root biomass on both botanical varieties considered. The leaf and root growth of *C. endivia* cv Romanesca was equally promoted by the nitrogen supply, so the value of the shoot/root ratio increased slightly compared to the value of control plants. Conversely, the adjustments in shoot-to-root partitioning of dry matter in *C. intybus* cv Pan Zucchero were in favor of leaf growth, with the shoot/root ratio tripling compared to the control plants. According to many authors, the fresh and dry matter yields of chicory leaf and root change in response to nitrate fertilization across cultivars: Ameziane et al. [26] showed that in witloof chicory (*Cichorium intybus* L.) grown with low nitrate fertilization, the yield of tuberized roots was not greatly affected, while the higher nitrate fertilization increased the N concentration of tuberized roots, delayed maturation, and decreased the quality of chicory roots. Moreover, moderate rates of N fertilization positively affected the yield and quality of chicory roots, while excessive N fertilization modified the shape and solidity of tissues, increasing the looseness of chicory roots, which lead to half-open tips [27]. The increasing nitrogen doses differentially affected the growth and marketable yield of two different endivia cultivars (*Cichorium endivia* L. cv Cigal and Excel) used as leafy vegetables [28]. Biesiada and Kołota [29] showed that the nitrogen dose, when applied

early to the plant growing period, significantly affected the yielding of red leaf chicory (radicchio, *Cichorium intybus* L. var. foliosum). Conversely, Ćustić et al. [30] did not observe significant differences in the yield of radicchio leaf chicory under different levels of nitrogen fertilization. Chicory fertilization with high nitrate solution could be responsible for the incidence of leafy tip burns and root breaks [27,31]. However, in the presented trial, both botanical varieties considered did not show any visible damage to the leaves or roots, such as foliar tip burn or radical disorders. The fertilization of chicory plants with $Ca(NO_3)_2$ may have also acted as a source of $Ca^{2+}$, an essential nutrient that supports the stability of the surface of the cell membrane, playing an important role in the elongation and the division of plant cells, cell membrane permeability, metabolism of nitrogen and translocation of carbohydrates [32]. Several authors reported a reduction in tip-burn incidence with foliar application of $Ca^{2+}$ [33,34], as in lettuce, in which the tip-burn incidence and severity could be reduced by frequent foliar application of $Ca^{2+}$ [32]. Calcium is absorbed as divalent $Ca^{2+}$ ions by young root tips in which the cell walls of the epidermis are not yet suberized. The $Ca^{2+}$ uptake by roots is associated with water uptake and stimulated by high levels of $NO_3^-$ ions. The nitrogen fertilization applied during the cultivation of chicory plants seems to have ensured sufficient root growth, which was essential for the $Ca^{2+}$ uptake, avoiding related Ca-deficiency disorders [35].

### *4.2. Carbon Partitioning, NSC, and Fructans Content in the Leaves and Roots of Chicory*

Our data highlight the key role of fertilization in the dry matter partitioning between aerial and underground parts of plants, which acts as a physiological indicator of carbon allocation in chicory plants, differentially expressed in relation to the varieties considered. The mechanism by which the nitrogen supply influences carbon allocation in chicory plants can be seen through the carbohydrate content in the leaves and roots of *C. endivia* L. cv Romanesca and *C. intybus* L. cv Pan Zucchero. In chicory leaves, nitrogen fertilization increased the soluble NSC (glucose + fructose + sucrose) in both chicory species whilst decreasing the starch content (Table 3). Moreover, in *C. endivia* cv Romanesca leaves, calcium nitrate promoted a higher concentration of both soluble NSC and starch (Table 3). A higher nitrogen availability is favorable for higher leaf expansion and shoot growth, promoting the photosynthetic rate in mature leaves and the accumulation of carbohydrates, which can be translocated in the sink organs of chicory roots. Our data showed a positive effect of nitrogen fertilization in NSC accumulation in chicory roots, with a more evident impact on plants from Romanesca compared to the Pan Zucchero plants. The higher nitrate supply and the resulting N availability were shown to be beneficial for the radial growth of chicory taproots with a direct effect on phloem unloading and sucrose-metabolizing enzyme, particularly the sucrose:sucrose 1-fructosyltransferase (1-SST) [5]. This enzyme is recognized to play a crucial role in sink strength in fructans-forming sink organs in chicory roots and represents a limiting enzyme in fructan synthesis [36]. Nitrogen availability increased the root activity with polymerization and sequestering of fructans in the vacuole of fertilized *C. endivia* L. cv Romanesca. The highest fructans concentration was in its roots compared to control plants and to the fructans amount accumulated in *C. intybus* L. cv Pan Zucchero, both in fertilized and control plants. Our data showed that the selection of chicory species, in relation to nitrogen fertilization responses, is one of the most important premises to improve the yield and content of healthy metabolites, such as fructans, in chicory roots, even at the early growth stage investigated in this experiment.

The prebiotic action of fructans in both human and animal models is widely recognized, with beneficial effects on the growth and functionality of gut microbiota, that ferment the inulin-type fructans in the colon with the synthesis of different metabolites, as the short-chain fatty acids (SCFAs), which play an important health role both locally, inside the gut, and in systemic actions. Fructans are also a form of dietary fiber and, considering that several prebiotics are also dietary fiber, these compounds are gaining greater attention for their high nutritional properties [37]. Chicory is a prevalent source of purified fructans on the market; furthermore, its leaf and root tissues contain other important metabolites, such

as components of the cell wall, and various phytochemicals, contributing to the nutritional value of these vegetable organs. For this reason, chicory can be considered a relevant functional food that is health-promoting phytochemicals and can improve the human diet by evidence that encourages the daily consumption of prebiotics in addition to fiber, polyphenols, and antioxidants, to improve human and animal health [19,38]. According to EFSA, the recommended daily intake of "chicory-type inulin" for humans is 12 g/day [EFSA Regulation (EC) No 1924/2006. EFSA J. 2015 [39]). However, it was evaluated that the consumption of the full chicory plant is the most natural way to take prebiotics compared to nutritional supplements based on purified inulin [37], favoring the right assumption of prebiotics together with other vegetable fibers and phytochemicals, thus avoiding detrimental effects on gut functioning. Our results showed that species selection together with careful management of fertilization can allow the control of inulin production in a serving of early-stage chicory fresh root that can be included in the diet.

### *4.3. Nitrogen, Protein, Anions, and Organic Acids Content*

It is known that in plants, the nitrogen content is directly related to the leaf protein content, and hence, nitrogen fertilization may affect the protein content thereby increasing the nutritional value of vegetable food. In the experimental chicory species, nitrate fertilization increased the N% in root and leaves for both *C. endivia* cv Romanesca and *C. intybus* cv Pan Zucchero. However, this was not accompanied by a corresponding increase in the protein quantity (Table 4). Ćustić et al. [40] studied the effects of nitrogen fertilization on the content of essential amino acids in head chicory (*Cichorium intybus* L. var. foliosum), claiming that most plants were not able to metabolize high levels of nitrates to incorporate them into amino compounds. Therefore, surplus nitrate accumulates in the form of amides within the plants, becoming a limiting factor for nitrogen metabolism, resulting in a decrease in protein quality and an increase in nitrate levels. Our data seemed to reflect this plant-nutrient interaction, showing an accumulation of nitrate in the chicory plants (Table 4). Chicory can accumulate a high content of nitrate in the leaves and roots, and although the effects of nitrate compounds on human health are currently under discussion [41], nitrate intake in the human diet is still associated with serious pathologies, and, for this reason, the international authorities (European Commission's Scientific Committee on Food–SCF 1997; JECFA, 2008) [42] have set the Acceptable Daily Intake (ADI) for nitrate at 3.7 mg/kg body weight per day [43].

Nitrogen fertilization has been identified as the major driver of nitrates in vegetable crops, with a strong correlation between the increase in nitrogen fertilizers and higher nitrate content in the edible parts of crops, despite the yield improvement [44]. Moreover, nitrates can be differentially accumulated in relation to the genotypic characteristics and plant organs. This was concordant with the present research, which found that the nitrate accumulation varied significantly, in both chicory leaves and roots, between fertilized and control plants (Table 5). Furthermore, the nitrate accumulation was different between species, with the roots of *C. endivia* cv Romanesca showing a significantly lower accumulation of nitrates compared to the roots of *C. intybus* cv Pan Zucchero. In leaves, the nitrate content was about double that of the roots of the fertilized plants and 92% higher than in control chicory roots (Table 5). The differences in nitrate accumulation between the botanical varieties of chicory and between the leaves and roots corroborated the findings reported by many authors: Burns et al. [45] showed, on 48 different lettuce accessions, a variation in the average nitrate concentrations between genotypes, with the higher concentration in butterhead and lower concentration in crisphead cultivars, regardless of growing seasons. Luo et al. [46] compared two pakchoi cultivars (*Brassica rapa* L.) showing that the low nitrate-accumulating cultivar Shanghaiquing had a significantly higher nitrate reductase activity and gene expression of the putative nitrate reductase gene compared to high nitrate-accumulating cultivar Liangbaiye-1. Razgallah et al. [47] compared four lettuce cultivars and found that high nitrate accumulators had higher nitrate transporter transcripts than the low accumulators, suggesting a stronger ability to take up and transport nitrates in

the higher accumulators. Moreover, the nitrate concentration in aerial plant organs was higher than in the roots of rape, cabbage, and spinach [48]. In general, hypogeal storage organs, such as roots, rhizomes, and tubers, are recognized to accumulate relatively smaller nitrate concentrations than epigeal plant organs such as petioles, leaves, and stems [49]. The genetic variation in the nitrate accumulation capacity suggests that breeding for a low nitrate content in vegetables could be useful for reducing nitrate levels in food crops. In the present experiment, nitrate and fructan accumulation in taproots showed a significant interaction with the genotype and rate of fertilization. Since fructans and nitrate have opposite nutritional values, care must be taken while promoting fructans accumulation with an increased nitrate fertilization rate to avoid an excessive accumulation of nitrate. A daily intake of 12 g of fructans was suggested for a healthy human [39], while an acceptable daily intake of 3.7 mg of nitrate $kg^{-1}$ of body weight (corresponding to 259 mg for a 70 kg weight person) was recognized as safe by EFSA (Efsa 2017) [50]. Using data obtained in the present work, we can calculate that while eating the amount of tap root necessary for an intake of 12 g of fructans of unfertilized Romanesca chicory, a consumer would ingest 34 mg of nitrate equivalent to only 14% of the accepted daily intake of a 70 kg person (Table S2). The same person would ingest 757.8 mg of nitrates equivalent to 293% of the accepted daily intake if obtaining the same 12 g of fructans from eating the taproot of the fertilized Pan Zucchero chicory (Table S2). This highlights the relevant role of the genetic background and fertilization regime on the health value and safety of young chicory produce.

Phosphate and sulphate ions are involved in major chemical and metabolic reactions in plants and can influence or be influenced by the availability or utilization of other nutrients such as nitrogen, applied as fertilizer. Pi uptake by plants from the external environment can be increased in *Zea mays* roots by pre-treatment of soil with ammonium and nitrate [51]. In the leaves of wild rocket grown in a greenhouse under two cover materials and three nitrogen regimes, the availability of N in the growth medium showed a synergic effect on the absorption of other macroelements with a lower uptake of S but no effect on the P uptake [52]. The characteristics of sulphate transport into *Pisum sativum* L. seedlings changed if grown with a source of sulfur were as follows: the sulphate influx into the seedlings, when seeds were grown in the absence of a sulfur source, was inhibited by the application of external nitrates, while seedlings grown in the presence of a sulfur source were insensitive to nitrate application [53]. Nitrogen fertilization negatively affected the sulphate and phosphate content in chicory leaves and roots of botanical varieties of chicory, regardless of the genotype, highlighting the effect of nitrogen supply in the uptake of other macronutrients.

Plants of all species contain a combination of organic acids, although the carboxylate composition varies among species, and their content in plant tissues can be dependent on nitrogen fertilization. A higher organic acid content was observed in nitrate-fed plants due to the need for a charge balance of the cations that accompany the nitrate ion uptake once the nitrate is assimilated [54]. This distinction between plant species was observed with *C. intybus* cv Pan Zucchero leaves and roots showing a significantly higher content of malic acid compared to *C. endivia* cv Romanesca. In Pan Zucchero chicory roots, only the citric acid content was higher than in Romanesca roots. In chicory leaves, the nitrogen source did not affect the malic and citric acid contents; however, in roots, the malate concentration was affected by nitrogen fertilization.

The results of our research indicated that the application of nitrogen fertilizer was a significant factor in determining the leaf and root yield of early-stage chicory plants, with high metabolite contents with nutritional value in roots and leaves, both suitable for consumption. Genetic variability in inulin-type fructans and nitrate accumulation should also be considered in the choice of species and cultivars to enhance the potential nutritional benefits of vegetables for human nutritional consumption. Cultivation in controlled environmental conditions allows for better control of the production cycle, and for year-round growth of chicory plants, suitable for the production of ready-to-eat fresh vegetables, maintaining high qualitative standards, particularly related to the prebiotic

potential of produce. The production of chicory plants in a greenhouse and early season and in early stage growth showed the versatility of this species within innovative agricultural systems such as smart greenhouses, vertical farming systems, and innovative facilities designed to support life in space.

## 5. Conclusions

The taproot of the young chicory plant can be eaten boiled or steamed and be a relevant source of the recognized prebiotic inulin for the human diet; hence, it represents a potential new product of high nutritional value for the market. Our data indicate that, with appropriate genetic material and nitrate fertilization, young, fructans-rich chicory plants can be obtained under greenhouse conditions, opening new production opportunities for CEA agriculture. We also showed that nitrogen fertilization interacts with the chicory genetic background, modulating the yield and the concentration of fructans and nitrate in young chicory plants. Hence, while showing that young chicory can be a new and healthy food with a strong potential contribution to the prebiotic intake for humans, we also demonstrated that excessive nitrate accumulation in tap roots should be avoided with appropriate nitrate fertilization. More research in this area can increase the feasibility and efficiency of prebiotic-rich production and availability in the market of vegetables.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13071752/s1, Table S1: Effects of nitrogen supply on the glucose, fructose, and sucrose contents of leaves and roots of chicory (*Cichorium intybus* L.) cv Pan Zucchero and (*Cichorium endivia* L.) cv Romanesca; Table S2: Amount of taproot per serving providing 12 g of inulin, amount of nitrate ingested with the same amount of taproot served, and percentage of the acceptable daily intake of nitrate ingested with the 12 g inulin serve.

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**Data Availability Statement:** The data presented in this study are available on request from the corresponding author (SM and SP).

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