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# Agronomic Performance and Phytochemical Profile of Lettuce Grown in Anaerobic Dairy Digestate

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**Abstract:** Anaerobic liquid dairy digestate is a by-product of dairy waste anaerobic digestion from dairy operations and is associated with environmental risks if not handled properly, particularly nutrient leaching losses, water contaminations, and greenhouse gas emissions. We tested the applications of anerobic digestate (AD) as a biofertilizer and water source in greenhouse vegetable production to integrate food production and industry waste management for sustainable environments. We used a deep water culture system to assess the effects of AD effluent alone, inorganic nutrient solution (NS), and a combination of AD and NS on the growth, yield, and phytonutrient profile and heavy metal contamination assessment of hydroponically produced lettuce. Lettuce produced in AD had a lower leaf area, total chlorophyll content, and fresh biomass; however, it displayed significantly higher chicoric acid (200%), chlorogenic acid (67%), luteolin (800%), quercetin-3-O-β-d-glucuronide (378%), quercetin-3-glucoside (200%), quercetin-3-O-(6″-O-malonyl)-β-D-glucoside (1077%), folate (248%), pantothenic acid (200%), total phenolics (111%), total antioxidants (44%), and soluble sugars (253%) compared to control (inorganic feed). The AD-produced lettuce also showed significantly lower heavy metal bioaccumulation risks associated with the human consumption. Based on various results, we may conclude that AD utilization in hydroponics can offer a sustainable solution to harvest a better lettuce yield, higher phytonutrients, and environmental benefits.

**Keywords:** anaerobic digestate; bioactive compounds; food production; industrial waste handling; inorganic feed; nitrate; organic production; phytonutrient profile



**Citation:** Faran, M.; Nadeem, M.; Manful, C.F.; Galagedara, L.; Thomas, R.H.; Cheema, M. Agronomic Performance and Phytochemical Profile of Lettuce Grown in Anaerobic Dairy Digestate. *Agronomy* **2023**, *13*, 182. https://doi.org/10.3390/agronomy13010182

Academic Editor: Vincenzo Candido

Received: 29 November 2022
Revised: 29 December 2022
Accepted: 1 January 2023
Published: 6 January 2023



## 1. Introduction

There has been tremendous growth in the dairy industry in recent decades to meet the growing needs of human population [1]. Such tremendous increases in the dairy industry also caused the production of significant volumes of dairy manure everyday [1,2]. For instance, on average, an animal unit of 450 kg produces 0.05 $m^3$ of fresh dairy manure with almost 6.5 kg of total solids per day [3]. Such large volumes of dairy industry waste create challenges that are difficult for farmers to handle in an environmentally sustainable way. One of the most common dairy manure handling methods is applying manure as a nutrient source, either liquid or semi liquid forms, to farmlands. However, dairy manure applications to farmlands have several associated environmental risks such as greenhouse gases emissions, nutrient leaching losses, and the contamination of water resources.

Anaerobic digestors are adds-on to the dairy farms in recent years and are used to produce biogas, electricity, bedding material, and a nutrient laden liquid dairy stream termed as anaerobic digestate (AD). Anerobic digestate is a fully or partially digested byproduct of anaerobic digestion based on the retention time and partially degraded organic matter, microbial biomass, and various inorganic compounds, making it a potentially valuable biofertilizer [1,4]. The AD contains significant amounts of nutrients essential for plant growth and development; therefore, it is usually applied to farmlands to produce

agronomic and horticultural crops. However, the use of AD in hydroponic settings under controlled environmental conditions is very limited and needs detailed investigation. It may enhance plant biomass production through improved nutrient use efficiencies [5], minimize losses, and thus minimize environmental risks. Additionally, the growing of vegetables and other high-value crops in semi-enclosed systems provides an opportunity to reuse and circulate drained nutrient solution, tightly control inputs, as well as reduce N contaminants and greenhouse gas (GHG) emissions compared to the recycling of manure in open-land application systems.

Lettuce (*Lactuca sativa* L.) is the most popular leafy vegetable currently cultivated across the globe due to short growing season, low production cost, and rich in nutrients with low calories [6]. It is a good source of fibers and minerals (KP, Ca, Zn, Fe, Mg, and Cu) which are essential for better human health. For instance, K intake helps to lower blood pressure [7], P is indispensable for the building of cell membranes (in phospholipids), intercellular energy metabolism, adenosine triphosphate (ATP), and sugar generation [8]. Lettuce also contains an ample amount of vitamins that are essential for metabolism [9]; however, minerals and vitamins may vary in different varieties grown in different nutrient formulations.

In current study, we hypothesized that the addition of AD to a hydroponic system as a nutrient feed will not only sustain plant growth, fresh biomass production, and phytonutrient profile, but also display no heavy metal contamination risks in greenhouse vegetable production systems. To test this hypothesis, we cultivated two lettuce cultivars in 100% AD, 100% inorganic fertilizer feed (100%), and their combination (50% + 50%). Lettuce was selected as a model leafy vegetable due to its short growing season as well as its popularity among green leafy vegetables for worldwide production and consumption. It is mainly consumed as a salad, and considered as a significant source of bioactive compounds, such as minerals, vitamins, polyphenols, and antioxidants [10]. These biologically active substances are known to enhance human immunity by scavenging the free radicals, modify metabolic activation, detoxify various carcinogens, as well as help to reduce chronic disease risks [11–14]. We also hypothesized that AD would express higher phytonutrients of lettuce and would provide a sustainable solution for dairy industry waste management, vegetable production, and climate change mitigation.

## 2. Materials and Methods

### *2.1. Assessment of Heavy Metal Risks and Nutrient Compositions of AD*

Prior to the start of the experiments, heavy metals and mineral nutrients composed of AD were evaluated. AD was collected from an anaerobic digestor located at New World Dairy Inc., St. David's (51.8812° N, 5.2660° W), Newfoundland, Canada. The digestate was filtered with a 2 mm mesh to remove inert matter and other impurities, and was then analyzed to determine heavy metals and other basic properties. AD analyses showed lower concentrations of heavy metals except copper (Cu = 619 ± 18.28 mg $kg^{-1}$) and zinc (Zn = 438 ± 33.68 mg $kg^{-1}$) which were higher than the allowable limits set by Canadian Council of Ministers of the Environment (CCME) [15–17] (Supplementary Table S1). Additionally, analyses showed significant concentrations of other essential nutrients required for plant growth and development (Supplementary Table S2). However, AD displayed significantly higher concentrations of N in the form of ammonium ($NH_4^+$ = 2376.67 ± 133.86 mg $L^{-1}$); therefore, AD was diluted 10 times to lower $NH_4^+$ N, according to lettuce requirements (Supplementary Table S2). This dilution also helped to reduce Cu and Zn concentrations within the allowable limits (Supplementary Table S2).

### *2.2. Experimental Design and Treatments*

To assess the potential of AD as a nutrient feed, greenhouse experiments were conducted in a deep-water culture (DWC) hydroponic system with two lettuce cultivars and three nutrient feed solutions (NFSs). Three NFSs included were: (i) 100% AD; (ii) 100% inorganic nutrient solution NS) as a control; and (iii) 50% AD + 50% NS based on 50%

total N from each AD and NS source (Supplementary Table S2). The experiment was laid out in a completely randomized design in factorial settings with three replications. The AD was supplied to a DWC system after sieving and diluting, and the NS was prepared from water-soluble fertilizers, following the method described by Hoagland with few modifications [18], and 50% AD + 50% NS feed solution was prepared by mixing 50% AD with 50% NS. Throughout the execution of experiments, the pH of nutrient solutions was maintained between 5.8 and 6.2 by adding phosphoric acid whenever needed.

### *2.3. Lettuce Nursery, Transplantation, and Plant Sampling*

Newham and Romaine lettuce cultivar seed was purchased from High Mowing Organic Seeds, Wolcott, VT, USA, and was raised for nursery in Jiffy-7 pre-soaked peat pellets (Canadian Garden Supply, Castlegar, BC, Canada), in a walk-in growth chamber (BioChambers Inc., Winnipeg, MB, Canada) at Grenfell Campus, Memorial University of Newfoundland, Canada. The standard growth conditions for lettuce production were maintained in the walk-in growth chamber including 14 h/10 h day/night duration, 21 °C/19 °C day/night temperature, and 75–80% relative humidity [19]. One-week-old lettuce nursery seedlings were then transplanted in a Styrofoam sheet in DWC floating hydroponic system (14 L containers: 28 cm × 37 cm × 20 cm) in a greenhouse (Figure S1a,b). In each crop cycle, the DWC floating hydroponic system comprised of nine containers and a set of three containers was considered as one replication for each NFS treatment. Air pumps (Uniclife aquarium air pumps, 4 Watt, 4 LPM) were installed inside the containers to provide continuous airflow/oxygen to enhance roots growth. The NFSs were replaced with fresh NFSs weekly; EC and pH were monitored daily and were adjusted as needed. The lettuce crop was harvested 45 days after seeding and separated into roots and shoots. The fresh weight of lettuce in each experimental unit was recorded. The leaf area (LA) was measured with a portable leaf area meter (LI-3000C–LI-COR Biosciences, Lincoln, NB, USA). Briefly, leaves from two randomly selected plants were removed and placed in the encoding cord. Then, the encoding cord was pulled with the same rate as the leaves were being pulled through scanning head and the leaf was removed from the scanning head to prevent the additional area from being accumulated and means of two plants leaves were taken. Then, samples were oven-dried at 65 °C for 72 h or until a constant weight was achieved. Thereafter, the root–shoot ratio was calculated on a dry weight basis.

### *2.4. Phytonutrient Profile and Heavy Metal Uptake*

Phytomineral concentrations in lettuce plants were determined by following the methods of [20]. Briefly, the dried and grounded lettuce leaf samples were converted to ash at 530 °C and digested in hydrochloric acid, and the resulting digestates were analyzed for mineral content using inductively coupled plasma atomic emission spectrometry (ICP-OES 725, Agilent Technologies, Santa Clara, CA, USA). The concentrations were calculated based on standard curves generated from authenticated standards and results were reported on a dry-weight (DW) basis. The heavy metal concentrations in lettuce leaves were determined using inductively coupled plasma mass spectroscopy (ICP-MS, ThermoFisher Scientific, Waltham MA, USA). Briefly, 250 g dried-ground leaf samples were digested using concentrated nitric acid and hydrochloric acid and resulted digestates were analyzed using ICP-MS.

For ammonium ($NH_4^+$) and nitrate ($NO_3^-$) concentration assessment in lettuce leaves, a method proposed by Bottoms et al. [21] was adopted. The $NH_4^+$ and $NO_3^-$ in fresh lettuce leaves were extracted using KCl and were analyzed using QuickChem automated ion analyzer (Lachat Instruments Inc., Milwaukee, WI, USA). The $NH_4^+$ and $NO_3^-$ concentrations were calculated using the Fishman and Friedman equation [22] and the results were expressed on a fresh-weight (FW) basis.

For vitamin analysis, fresh leaves were homogenized with diatomaceous earth and extracted on a Dionex 350 ASE (Thermo Fisher Scientific, Waltham, MA, USA) using methanol–water (60:40 % *v/v*) based on the following program (temperature = 0 °C;

cycles = 2; pressure = 1500 psi; static time = 5 min; flush ratio = 50% rinse). The vitamin B concentration (riboflavin, pantothenic acid, and folate) was measured following the method described by Akhavan and Barzegar [23] with little modification, whereas vitamin C analysis was conducted based on ascorbic acid content, as reported by Boonpangrak et al. [24]. The extracted analytes were resolved on a Polar Acclaim II C18 column (150 × 4.6 mm I.D., particle size: 5 µm, pore diameter: 120 Å; Thermo Fisher Scientific, Mississauga, ON, Canada) coupled to a Dionex Ultimate 3000 ultra-high-performance liquid chromatography (UHPLC) system and a LTQ Orbitrap high-resolution accurate mass spectrometer (Thermo Fisher Scientific, Mississauga, ON, Canada). The HPLC were set up to determine vitamins as follows: column temperature: 30 °C; solvent A: water containing 0.1% *v/v* formic acid, solvent B: acetonitrile containing 0.1% *v/v* formic acid; solvent gradients: 0–90% (*v/v*) B for 0–10 min, 90% (*v/v*) B for 10–12.5 min, and 90%–0% (*v/v*) B for 12.5–13 min; flow rate: 0.3 mL $min^{-1}$. Moreover, 5 µL of the sample or standards was injected in the instrument. The mass spectrometer operated in selected ion monitoring (SIM) mode for enhanced ion sensitivity, with m/z 175 used for vitamin C (ascorbic acid), whereas m/z 442, 377, and 220 were used for folate, riboflavin, and pantothenic acid, respectively. Vitamin C (ascorbic acid) was ionized by the ESI source in the negative ion mode, whereas vitamin B was determined in the positive ESI mode. Mass spectrometer tuning parameters were as follows: sheath gas: 40, ion spray voltage: 3.0 kV, capillary temperature: 300 °C, capillary voltage: 100 V, tube lens: −250 V, and mass range: 50–500 m/z. The vitamin concentrations in lettuce leaf samples were based on standard curves developed from 1 mg/mL stock solution of ascorbic acid, riboflavin, pantothenic acid, and folate standards, and the results were expressed in µg $g^{-1}$ and mg $g^{-1}$ fresh weight (FW).

Polyphenols such as chlorogenic acid, chicoric acid, luteolin, quercetin-3-glucoside, quercetin-3-O- β-D-glucuronide, and quercetin-3-O-(6″-O-malonyl)-β-D-glucoside were analyzed in lettuce leaves following the method developed by Gavrilova et al. [25]. Briefly, leaf extracts were prepared using accelerated solvent extractor as previously described for vitamins. The extracts were freeze-dried (LABCONCO, FreeZone 2.5 Plus; Vacuum (0.051 torr, −90 °C), lyophilized, and then extracted with methanol–formic acid (9:1% *v/v*) containing hippuric acid as the internal standard. Following centrifugation (10,000 rpm, 10 min, 0 °C), the supernatants were pooled and analyzed by ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS/MS), as previously described for vitamin analysis with minor modifications. The solvent gradients used for separating polyphenols were: 0–2 min, 10–20% (*v/v*) B; 2–15 min, 20% (*v/v*) B; 15–20 min, 80–100% (*v/v*) B; 20–25 min, 100–0% (*v/v*) B; and 25–35 min, 0% (*v/v*) B. The Orbitrap mass spectrometer was operated in negative ESI conditions in the SIM mode and the following ions were monitored: m/z 353, 285, 473, 447, 463, and 549. Polyphenol concentrations in the leaf samples were determined based on standard curves developed from 1 mg $mL^{-1}$ stock solutions of chlorogenic acid, chicoric acid, luteolin, quercetin-3-glucoside, quercetin-3-O-β-D-glucuronide, and quercetin-3-O-(6″-O-malonyl)-β-D-glucoside, and the results were expressed in µg $g^{-1}$ dry weight (DW).

The total phenolic content and total antioxidant activity in lettuce leaves were determined. For this, lettuce leaf sample extraction was carried out according to the studies by Manful et al. [26] and Vidal et al. [27]. Briefly, a one-gram lettuce leaf sample was weighed in the centrifuge tube and mixed with 5 mL of 50 mM sodium phosphate buffer (pH 7.5), and the mixture was homogenized with a handheld homogenizer (VWR, Porcelain, Emonton AB, Canada). The mixture was then incubated in darkness for 30 min and centrifuged (5500 rpm at 10 °C for 10 min). The supernatant was pooled carefully without disturbing the pellet and designated as the hydrophilic extracts. The residue was re-suspended in 5 mL of 0.7% acidified ethanol and vortexed to homogenize. The resultant mixture was incubated for 30 min in darkness and then centrifuged (5500 rpm at 10 °C for 10 min), and the supernatant collected and designated as the lipophilic extract. The lipophilic and hydrophilic extracts were stored in darkness at −20 °C. The total phenolic content (TPC) in the lettuce leaf samples was determined using the Folin–Ciocalteu method, as described

by Cano et al. [28], and results were expressed as mg quercetin equivalent (QE)/g fresh weight (FW). Total antioxidant activity (TAA) analysis was based on the ferric-reducing ability of plasma (FRAP) method described by Benzie and Strain [29], and the results were expressed in μM Trolox equivalents (TE)/g fresh weight (FW), as described by our research group [27].

The total soluble-reducing sugar (soluble sugars) content in the lettuce leaf was determined following the method proposed by Shao and Lin [30] with little modifications. Briefly, soluble sugars were assessed using the Somogyi–Nelson assay with two freshly prepared working solutions and a color reagent. Solution 1 was composed of sodium potassium tartrate tetrahydrate, sodium carbonate, sodium bicarbonate, and sodium sulphate. Solution 2 was prepared using copper sulphate pentahydrate and sodium sulphate. The chromophore (color reagent) was prepared by mixing ammonium molybdate, sodium arsenate dibasic pentahydrate, and concentrated $H_2SO_4$. Thereafter, 45 μL samples and a 45 μL working solution were mixed in microplates, covered with lid, and placed in an oven (Shell Labs, Cornelius, OR, USA) at 90 °C for 20 min. After heating, microplates were packed in the ziplock bags and placed in cool running water for 5 min. The color reagent (45 μL) was added to each micro-well and placed on an even surface for 15 min to develop color. The bubbles were removed from samples and absorbance was recorded at 600 nm using a microplate reader (Biotek, Fisher Scientific, Mississauga, ON, Canada), and the results were reported as a fresh weight (FW) basis.

### *2.5. Statistical Analysis*

The dataset was subjected to two-way analysis of variance (ANOVA) to determine the effects of NFS treatments on growth, fresh biomass, phytonutrients, heavy metal concentrations, vitamins, and total antioxidant activities using Statistix-10 (Analytical Software, Tallahassee, FL, USA). The Shapiro-Wilk test was conducted to test the data normality. Data trends were similar during both crop cycles; therefore, the dataset was pooled, and analyzed without considering two growth cycles. Where the treatment effects were significant, treatment means were compared with Fisher's least significant difference (LSD) method at an alpha value of 0.05. The main effects of NFSs and cultivars were presented where interactive effects were non-significant. To visualize the overall association between NFS treatments, lettuce cultivars, growth, fresh biomass and phytonutrient parameters, principal component analysis (PCA) was performed using XLStat software (Premium 2017, Version 19.5; Addinsoft, Paris, France). Pearson's correlation coefficients were used to determine the strength of the relationships among phytonutrient parameters in lettuce cultivars. Figures were prepared using SigmaPlot 13.0 software program (Systat Software Inc., San Jose, CA, USA).

## 3. Results

### *3.1. Effect of AD on Plant Growth and Fresh Biomass*

The NFSs, cultivars, and their interaction had significant ($p < 0.05$) effects on the leaf area (LA), the total chlorophyll content, fresh biomass, and the root–shoot ratio of lettuce cultivars (Table 1). Interactive effects (NFS × cultivars) showed a significantly higher leaf area (3087.00 ± 37.48 $cm^2$ $plant^{-1}$), total chlorophyll content (46.42 ± 0.38 mg $g^{-1}$), and fresh biomass (410.20 ± 20.39 g $plant^{-1}$) of Romaine lettuce grown in NS compared to the lowest leaf area (1111.40 ± 16.03 $cm^2$ $plant^{-1}$), total chlorophyll content (33.15 ± 0.38 mg $g^{-1}$), and fresh biomass (89.07 ± 1.99 g $plant^{-1}$) observed in AD-grown Newham lettuce (Table 1). A higher root–shoot ratio (0.29 ± 0.02) was observed in Newham grown in NS, whereas the lowest (0.10 ± 0.01) was recorded in Romaine grown in AD (Table 1).

**Table 1.** Leaf area, chlorophyll content, fresh biomass, and root–shoot ratio of hydroponically grown lettuce cultivars in relation to nutrient feed solutions. Data are expressed as mean ± standard error of six replications.

| Source of Variation | Leaf Area ($cm^2$ $plant^{-1}$) | Total Chlorophyll Content (mg $g^{-1}$ DW) | Fresh Biomass (g $plant^{-1}$) | Root–Shoot Ratio |
|---|---|---|---|---|
| Nutrient feed solutions (NFSs) | | | | |
| AD | 1301.10 ± 57.86 $^{c}$ | 33.45 ± 0.26 $^{c}$ | 101.58 ± 4.86 $^{c}$ | 0.12 ± 0.01 $^{b}$ |
| AD+NS | 1818.40 ± 75.12 $^{b}$ | 38.13 ± 0.34 $^{b}$ | 255.34 ± 19.46 $^{b}$ | 0.12 ± 0.00 $^{b}$ |
| NS | 2704.60 ± 118.33 $^{a}$ | 44.82 ± 2.00 $^{a}$ | 356.39 ± 22.93 $^{a}$ | 0.22 ± 0.02 $^{a}$ |
| Cultivars | | | | |
| Newham | 1678.10 ± 123.63 $^{b}$ | 38.06 ± 1.03 $^{b}$ | 186.50 ± 23.54 $^{b}$ | 0.19 ± 0.02 $^{a}$ |
| Romaine | 2204.70 ± 161.23 $^{a}$ | 39.84 ± 1.28 $^{a}$ | 269.04 ± 30.32 $^{a}$ | 0.12 ± 0.01 $^{b}$ |
| NFSs × Cultivars | | | | |
| AD × Newham | 1111.40 ± 16.03 $^{e}$ | 33.15 ± 0.44 $^{d}$ | 89.07 ± 1.99 $^{d}$ | 0.14 ± 0.02 $^{bc}$ |
| (AD+NS) × Newham | 1600.50 ± 74.59 $^{d}$ | 37.83 ± 0.54 $^{c}$ | 167.86 ± 15.69 $^{c}$ | 0.13 ± 0.00 $^{bcd}$ |
| NS × Newham | 2322.20 ± 40.58 $^{b}$ | 43.21 ± 0.54 $^{b}$ | 302.58 ± 27.20 $^{b}$ | 0.29 ± 0.02 $^{a}$ |
| DD × Romaine | 1490.80 ± 8.88 $^{d}$ | 33.75 ± 0.27 $^{d}$ | 114.09 ± 6.13 $^{d}$ | 0.10 ± 0.01 $^{d}$ |
| (AD+NS) × Romaine | 2036.30 ± 16.82 $^{c}$ | 38.44 ± 0.42 $^{c}$ | 282.82 ± 9.88 $^{b}$ | 0.11 ± 0.00 $^{cd}$ |
| NS × Romaine | 3087.00 ± 37.48 $^{a}$ | 46.42 ± 0.38 $^{a}$ | 410.20 ± 20.39 $^{a}$ | 0.15 ± 0.00 $^{b}$ |
| Significance | | | | |
| NFSs | *** | *** | *** | *** |
| Cultivars | *** | *** | *** | *** |
| NFSs × Cultivars | *** | *** | * | *** |

AD, AD+NS, NS, and DW represent anaerobic digestate, anaerobic digestate + inorganic nutrient solution, inorganic nutrient solution, and dry weight, respectively. *, *** represent significant differences at alpha values of 0.05 and 0.001, respectively. Different letters within each column indicate significant differences among two cultivars, three nutrient feed solutions, or their interaction according to Fisher's least significant difference test.

### *3.2. Phytonutrient Profiles of Lettuce Leaves*

NFSs × cultivars had non-significant effects on all macronutrients in lettuce except S which had significant ($p < 0.05$) effects (Tables 2 and 3). Among micronutrients, the interactive effects of 'NFS × cultivars' were significant except Fe (Table 3). The lettuce plants cultivated in NS had significantly higher P (6.41 ± 0.27 mg $g^{-1}$), K (t), Ca (12.91 ± 0.51 mg $g^{-1}$), S (2.86 ± 0.14 mg $g^{-1}$), and Mg (2.61 ± 0.13 mg $g^{-1}$) values, followed by AD+NS, and were lowest in AD (Table 2). In general, concentrations of macronutrients P, K, Ca, Mg, and S varied in order of NS > AD+NS > AD (Table 2). Romaine showed significantly higher P (5.13 ± 0.38 mg $g^{-1}$), K (49.86 ± 6.20 mg $g^{-1}$), S (2.42 ± 0.18 mg $g^{-1}$), and Mg (2.22 ± 0.15 mg $g^{-1}$) values than Newham cultivar under the conditions evaluated in this study (Table 2).

Romaine cultivated in NS exhibited significantly higher B (57.92 ± 2.15 µg $g^{-1}$), Zn (93.67 ± 2.06 µg $g^{-1}$), and Mn (285.50 ± 0.01 µg $g^{-1}$) concentrations compared to 'AD × Newham' which showed lower concentrations of B, Zn, and Mn (Table 3). However, the interactive effects of 'NFS × cultivars' were non-significant for Fe, whereas NFSs and cultivars were significant as main factors. Among NFS treatments, NS exhibited higher Fe (300.42 ± 4.33 µg $g^{-1}$) as main factors followed by AD+NS, and lower values were observed in AD (Table 3).

The NFSs and cultivars had significant ($p < 0.05$) effects on $NH_4^+$ and $NO_3^-$ concentrations in lettuce leaves, whereas the interaction between NFSs × cultivars was non-significant (Table 2). Meanwhile, comparing NFS treatments, AD showed the lowest $NO_3^-$ concentration (398.69 ± 47.68 mg N $kg^{-1}$) whereas NS exhibited higher $NO_3^-$ concentration (596.79 ± 46.96 mg N $kg^{-1}$) (Table 2). As expected, the $NH_4^+$ concentration was higher (591.90 ± 25.05 mg N $kg^{-1}$) in lettuce grown in AD compared to lettuce cultivated in NS or AD+NS (Table 3). In Romaine lettuce $NH_4^+$ (433.20 ± 37.49 mg N $kg^{-1}$) and $NO_3^-$ (564.56 ± 53.25 mg N $kg^{-1}$) uptake was higher than Newham cultivar (Table 2).

**Table 2.** Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), ammonium ($NH_4^+$), and nitrate ($NO_3^-$) profiles of hydroponically grown lettuce cultivars in relation to nutrient feed solutions. Data are expressed as mean ± standard error of six replications.

| Source of Variation | P (mg $g^{-1}$ DW) | K (mg $g^{-1}$ DW) | Ca (mg $g^{-1}$ DW) | Mg (mg $g^{-1}$ DW) | $NH_4^+$ (mg N $kg^{-1}$ FW) | $NO_3^-$ (mg N $kg^{-1}$ FW) |
|---|---|---|---|---|---|---|
| Nutrient feed solutions (NFSs) | | | | | | |
| AD | 2.85 ± 0.33 $^{c}$ | 25.10 ± 0.97 $^{c}$ | 5.87 ± 0.15 $^{c}$ | 1.46 ± 0.05 $^{c}$ | 591.90 ± 25.10 $^{a}$ | 398.69 ± 47.68 $^{b}$ |
| AD+NS | 3.73 ± 0.23 $^{b}$ | 34.78 ± 1.44 $^{b}$ | 7.50 ± 0.40 $^{b}$ | 1.97 ± 0.09 $^{b}$ | 390.65 ± 14.59 $^{b}$ | 460.24 ± 50.10 $^{b}$ |
| NS | 6.41 ± 0.27 $^{a}$ | 80.57 ± 2.03 $^{a}$ | 12.91 ± 0.51 $^{a}$ | 2.61 ± 0.13 $^{a}$ | 248.10 ± 7.50 $^{c}$ | 596.79 ± 46.96 $^{a}$ |
| Cultivars | | | | | | |
| Newham | 3.53 ± 0.39 $^{b}$ | 43.78 ± 5.70 $^{b}$ | 7.95 ± 0.68 $^{b}$ | 1.81 ± 0.11 $^{b}$ | 387.23 ± 35.50 $^{b}$ | 405.92 ± 15.64 $^{b}$ |
| Romaine | 5.13 ± 0.38 $^{a}$ | 49.86 ± 6.20 $^{a}$ | 9.57 ± 0.85 $^{a}$ | 2.22 ± 0.15 $^{a}$ | 433.20 ± 37.49 $^{a}$ | 564.56 ± 53.25 $^{a}$ |
| NFSs × Cultivars | | | | | | |
| AD × Newham | 3.78 ± 0.20 | 23.53 ± 0.52 | 5.62 ± 0.05 | 1.34 ± 0.05 | 559.38 ± 46.76 | 338.38 ± 14.32 |
| (AD+NS) × Newham | 3.05 ± 0.18 | 31.38 ± 0.95 | 6.48 ± 0.47 | 1.76 ± 0.04 | 362.88 ± 12.27 | 406.62 ± 11.47 |
| NS × Newham | 5.62 ± 0.11 | 76.42 ± 2.01 | 11.74 ± 0.39 | 2.33 ± 0.12 | 239.43 ± 9.97 | 472.75 ± 18.78 |
| AD × Romaine | 3.78 ± 0.20 | 26.67 ± 1.69 | 6.12 ± 0.28 | 1.58 ± 0.05 | 624.42 ± 12.70 | 459.00 ± 91.33 |
| (AD+NS) × Romaine | 4.40 ± 0.15 | 38.18 ± 1.91 | 8.52 ± 0.28 | 2.18 ± 0.14 | 418.43 ± 21.85 | 513.85 ± 98.81 |
| NS × Romaine | 7.20 ± 0.24 | 84.72 ± 2.70 | 14.08 ± 0.68 | 2.88 ± 0.18 | 256.76 ± 10.87 | 720.83 ± 56.52 |
| Significance | | | | | | |
| NFSs | *** | *** | *** | *** | *** | ** |
| Cultivars | *** | *** | *** | *** | * | *** |
| NFSs × Cultivars | NS | NS | NS | NS | NS | NS |

AD, AD+NS, NS, and DW represent anaerobic digestate, anaerobic digestate + inorganic nutrient solution, inorganic nutrient solution, and dry weight, respectively. $^{NS}$, *, **, *** represent non-significant and significant differences at alpha values of 0.05, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences among two cultivars, three nutrient feed solutions, or their interaction according to Fisher's least significant difference test.

**Table 3.** Sulfur (S), boron (B), zinc (Zn), manganese (Mn), and iron (Fe) profiles of hydroponically grown lettuce cultivars in relation to nutrient feed solutions. Data are expressed as mean ± standard error of six replications.

| Source of Variation | S (mg $g^{-1}$ DW) | B (μg $g^{-1}$ DW) | Zn (μg $g^{-1}$ DW) | Mn (μg $g^{-1}$ DW) | Fe (μg $g^{-1}$ DW) |
|---|---|---|---|---|---|
| Nutrient feed solutions (NFSs) | | | | | |
| DD | 1.53 ± 0.09 $^{c}$ | 33.71 ± 0.70 $^{c}$ | 30.45 ± 4.75 $^{b}$ | 52.00 ± 2.41 $^{c}$ | 154.42 ± 7.44 $^{c}$ |
| AD+NS | 2.24 ± 0.08 $^{b}$ | 43.33 ± 0.62 $^{b}$ | 28.78 ± 3.29 $^{b}$ | 77.33 ± 8.33 $^{b}$ | 255.00 ± 4.11 $^{b}$ |
| NS | 2.86 ± 0.14 $^{a}$ | 54.96 ± 1.41 $^{a}$ | 56.02 ± 6.28 $^{a}$ | 231.79 ± 17.53 $^{a}$ | 300.42 ± 4.33 $^{a}$ |
| Cultivars | | | | | |
| Newham | 1.99 ± 0.11 $^{b}$ | 42.92 ± 1.84 $^{b}$ | 49.03 ± 4.75 $^{a}$ | 91.08 ± 15.28 $^{b}$ | 227.00 ± 16.60 $^{b}$ |
| Romaine | 2.42 ± 0.18 $^{a}$ | 45.08 ± 2.57 $^{a}$ | 27.81 ± 3.73 $^{b}$ | 149.67 ± 23.95 $^{a}$ | 246.22 ± 13.81 $^{a}$ |
| NFSs × Cultivars | | | | | |
| DD × Newham | 1.48 ± 0.13 $^{d}$ | 34.08 ± 1.00 $^{d}$ | 43.88 ± 4.90 $^{b}$ | 45.33 ± 1.45 $^{d}$ | 138.83 ± 10.40 |
| (AD+NS) × Newham | 2.04 ± 0.06 $^{c}$ | 42.67 ± 0.95 $^{c}$ | 30.05 ± 2.47 $^{cd}$ | 49.83 ± 0.91 $^{d}$ | 246.50 ± 6.00 |
| NS × Newham | 2.46 ± 0.13 $^{b}$ | 52.00 ± 0.82 $^{b}$ | 73.15 ± 2.53 $^{a}$ | 178.08 ± 10.23 $^{b}$ | 296.17 ± 7.84 |
| AD × Romaine | 1.57 ± 0.14 $^{c}$ | 33.33 ± 1.05 $^{d}$ | 17.02 ± 1.78 $^{d}$ | 58.67 ± 2.36 $^{d}$ | 170.50 ± 5.66 |
| (AD+NS) × Romaine | 2.43 ± 0.10 $^{c}$ | 44.00 ± 0.79 $^{c}$ | 27.52 ± 6.39 $^{cd}$ | 104.83 ± 1.28 $^{c}$ | 263.50 ± 3.10 |
| NS × Romaine | 3.27 ± 0.05 $^{a}$ | 57.92 ± 2.15 $^{a}$ | 38.88 ± 7.06 $^{bc}$ | 285.50 ± 9.70 $^{a}$ | 304.67 ± 3.74 |
| Significance | | | | | |
| NFSs | *** | *** | *** | *** | *** |
| Cultivars | *** | * | *** | *** | *** |
| NFSs × Cultivars | * | * | *** | *** | NS |

AD, AD+NS, NS, and DW represent anaerobic digestate, anaerobic digestate + inorganic nutrient solution, inorganic nutrient solution, and dry weight, respectively. $^{NS}$, *, *** represent non-significant and significant differences at alpha values of 0.05, and 0.001, respectively. Different letters within each column indicate significant differences among two cultivars, three nutrient feed solutions, or their interaction according to Fisher's least significant difference test.

The NFSs had significant ($p < 0.05$) effects on chlorogenic acid, chicoric acid, and quercetin-3-O-β-D-glucuronide content (Table 4), whereas cultivars had significant ($p < 0.05$) effects on chicoric acid and non-significant effects on chlorogenic acid and quercetin-3-O-β-D-glucuronide (Table 4). NFSs × cultivars had significant effects on luteolin, quercetin-3-glucoside, and quercetin- 3-O-(6″-O-malonyl)-β-D-glucoside content, and non-significant effects on chlorogenic acid, chicoric acid, and quercetin-3-O-β-D-glucuronide content (Table 4). Higher luteolin (0.90 ± 0.03 μg $g^{-1}$), quercetin-3-glucoside (3.10 ± 0.20 μg $g^{-1}$), and quercetin- 3-O-(6″-O-malonyl)-β-D-glucoside (211.87 ± 57.21 μg $g^{-1}$) biosynthesis activity was observed in Romaine cultivar grown in AD, followed by Newham grown in AD, whereas NS-treated Newham cultivar produced the lowest polyphenols (Table 4). Similarly, significant positive correlations between $NH_4^+$-N and TPC (r = 0.92 **), TAA (r = 0.89 ***), chicoric acid (r = 0.49 *), chlorogenic acid (r = 0.90 **), luteolin (r = 0.89 **), quercetin-3-O-β-D-glucuronide (r = 0.69 **), quercetin-3-glucoside (r = 0.88 **), and quercetin-3-O-(6″-O-malonyl)-β-D–glucoside (r = 0.57 **) were observed (Figures S4a,b and S5a–f).

**Table 4.** Polyphenol profiles of hydroponically grown lettuce cultivars cultivated in different nutrient feed solutions. All data are expressed as mean ± standard error of six replications.

| Source of Variation | Chlorogenic Acid (μg $g^{-1}$ DW) | Chicoric Acid (μg $g^{-1}$ DW) | Luteolin (μg $g^{-1}$ DW) | Quercetin-3-O-β-D-glucuronide (μg $g^{-1}$ DW) | Quercetin-3-glucoside (μg $g^{-1}$ DW) | Quercetin-3-O-(6″-O-malonyl)-β-D-glucoside (μg $g^{-1}$ DW) |
|---|---|---|---|---|---|---|
| Nutrient feed solutions (NFSs) | | | | | | |
| AD | 5.50 ± 0.21 $^{a}$ | 52.71 ± 10.68 $^{a}$ | 0.78 ± 0.04 $^{a}$ | 86.13 ± 10.02 $^{a}$ | 2.74 ± 0.15 $^{a}$ | 114.27 ± 40.15 $^{a}$ |
| AD+NS | 3.34 ± 0.14 $^{b}$ | 29.88 ± 5.28 $^{b}$ | 0.27 ± 0.01 $^{b}$ | 63.26 ± 16.66 $^{a}$ | 1.60 ± 0.07 $^{b}$ | 42.30 ± 17.76 $^{b}$ |
| NS | 2.25 ± 0.13 $^{c}$ | 28.71 ± 6.02 $^{b}$ | 0.13 ± 0.01 $^{c}$ | 17.82 ± 7.72 $^{b}$ | 0.68 ± 0.14 $^{c}$ | 11.57 ± 2.78 $^{b}$ |
| Cultivars | | | | | | |
| Newham | 3.76 ± 0.34 | 49.34 ± 7.59 $^{a}$ | 0.36 ± 0.06 $^{b}$ | 42.08 ± 8.75 | 1.45 ± 0.21 $^{b}$ | 9.35 ± 1.67 $^{b}$ |
| Romaine | 3.63 ± 0.36 | 0.03 ± 0.00 $^{b}$ | 0.43 ± 0.08 $^{a}$ | 69.39 ± 13.60 | 1.90 ± 0.58 $^{a}$ | 102.74 ± 28.170 $^{a}$ |
| NFSs × Cultivars | | | | | | |
| AD × Newham | 5.49 ± 0.42 | 61.40 ± 21.68 | 0.66 ± 0.01 $^{b}$ | 81.26 ± 13.23 | 2.38 ± 0.10 $^{b}$ | 16.67 ± 3.09 $^{b}$ |
| (NS+DD) × Newham | 3.35 ± 0.18 | 38.48 ± 7.97 | 0.29 ± 0.02 $^{c}$ | 40.45 ± 3.88 | 1.66 ± 0.12 $^{c}$ | 7.17 ± 1.19 $^{b}$ |
| NS × Newham | 2.45 ± 0.10 | 48.13 ± 1.05 | 0.12 ± 0.01 $^{d}$ | 4.53 ± 1.53 | 0.31 ± 0.02 $^{e}$ | 4.22 ± 0.68 $^{b}$ |
| DD × Romaine | 5.52 ± 0.11 | 44.02 ± 1.17 | 0.90 ± 0.03 $^{a}$ | 91.53 ± 16.03 | 3.10 ± 0.20 $^{a}$ | 211.87 ± 57.21 $^{a}$ |
| (AD+NS) × Romaine | 3.33 ± 0.22 | 21.29 ± 5.44 | 0.25 ± 0.02 $^{c}$ | 86.06 ± 31.59 | 1.55 ± 0.09 $^{c}$ | 77.42 ± 29.89 $^{b}$ |
| NS × Romaine | 2.05 ± 0.23 | 9.29 ± 2.77 | 0.14 ± 0.10 $^{d}$ | 31.10 ± 13.76 | 1.05 ± 0.16 $^{d}$ | 18.92 ± 3.46 $^{b}$ |
| Significance | | | | | | |
| NFSs | *** | * | *** | *** | *** | *** |
| Cultivars | NS | *** | *** | NS | *** | *** |
| NFSs × Cultivars | NS | NS | *** | NS | *** | *** |

AD, AD+NS, NS, and DW represent anaerobic digestate, anaerobic digestate + inorganic nutrient solution, inorganic nutrient solution, and dry weight, respectively. $^{NS}$, *, *** represent non-significant and significant differences at alpha values of 0.05, and 0.001, respectively. Different letters within each column indicate significant differences among two cultivars, three nutrient feed solutions, or their interaction according to Fisher's least significant difference test.

Among the NFSs, AD exhibited significantly higher chlorogenic acid (5.50 ± 0.21 μg $g^{-1}$), chicoric acid (52.71 ± 10.68 μg $g^{-1}$), and quercetin-3-O-β-D-glucuronide (86.13 ± 10.02 μg $g^{-1}$) concentrations, whereas NS expressed the lowest values for these polyphenols. Newham showed higher chicoric acid (49.34 ± 7.59 μg $g^{-1}$) concentrations than Romaine, as shown in Table 4.

The NFSs, lettuce cultivars, and their interaction (NFSs × cultivars) had significant effects on vitamin C, riboflavin, and folate. However, cultivars and the interactive effects of 'NFS × cultivars' had no significant effects on pantothenic acid. As a main factor, NFSs significantly influenced pantothenic acid accumulation in lettuce leaves (Table 5). Results from the significant interactions showed higher vitamin C (9.84 ± 0.06 μg $g^{-1}$) and riboflavin (5.69 ± 0.25 μg $g^{-1}$) values in the Romaine cultivar when grown in NS,

whereas the lowest concentrations were noticed in AD-treated Romaine cultivar (Table 5). Folate, however, expressed inverse trends where AD produced higher folate concentrations (757.50 ± 71.53 μg $g^{-1}$) in the Romaine cultivar, whereas the lowest was observed in NS-treated Newham lettuce (177.83 ± 15.23 μg $g^{-1}$) (Table 5). Considering the individual NFS effects, AD-fed plants produced higher pantothenic acid concentrations (32.71 ± 2.73 μg $g^{-1}$), whereas the NS solution resulted in the lowest concentration (14.96 ± 1.84 μg $g^{-1}$), although statistically at par with AD+NS treatment, as depicted in Table 5.

**Table 5.** Vitamin C, riboflavin, pantothenic acid, folate, total phenolic content, total antioxidant activity, and soluble sugar profile of hydroponically grown lettuce cultivars cultivated in nutrient feed solutions. Data are expressed as mean ± standard errors of six replications.

| Source of Variation | Vitamin C (μg $g^{-1}$ FW) | Riboflavin (μg $g^{-1}$ FW) | Folate (μg $g^{-1}$ FW) | Pantothenic Acid (μg $g^{-1}$ FW) | Total Phenolic Content (mg $g^{-1}$ FW) | Total Antioxidant Activity (mg $g^{-1}$ FW) | Soluble Sugars (mg $g^{-1}$ FW) |
|---|---|---|---|---|---|---|---|
| Nutrient feed solutions (NFSs) | | | | | | | |
| AD | 0.68 ± 0.08 $^{c}$ | 2.15 ± 0.06 $^{c}$ | 510.92 ± 81.84 $^{a}$ | 32.71 ± 2.73 $^{a}$ | 3.11 ± 0.07 $^{a}$ | 33.44 ± 0.07 $^{a}$ | 17.01 ± 2.32 $^{a}$ |
| AD+NS | 2.14 ± 0.18 $^{b}$ | 3.16 ± 0.13 $^{b}$ | 207.17 ± 7.98 $^{b}$ | 17.78 ± 1.45 $^{b}$ | 2.24 ± 0.05 $^{b}$ | 27.31 ± 0.05 $^{b}$ | 10.51 ± 0.95 $^{b}$ |
| NS | 8.09 ± 0.55 $^{a}$ | 4.91 ± 0.27 $^{a}$ | 197.67 ± 22.61 $^{b}$ | 14.96 ±1.84 $^{b}$ | 1.49 ± 0.03 $^{c}$ | 23.31 ± 0.03 $^{c}$ | 6.44 ± 0.34 $^{c}$ |
| Cultivars | | | | | | | |
| Newham | 3.17 ± 0.58 $^{b}$ | 2.98 ± 0.21 $^{b}$ | 211.83 ± 11.28 $^{b}$ | 20.90 ± 1.59 | 2.13 ± 0.15 $^{b}$ | 26.93 ± 0.15 $^{b}$ | 7.66 ± 0.38 $^{b}$ |
| Romaine | 4.12 ± 1.00 $^{a}$ | 3.83 ± 0.35 $^{a}$ | 398.67 ± 66.88 $^{a}$ | 22.73 ± 3.17 | 2.42 ± 0.17 $^{a}$ | 29.04 ± 0.17 $^{a}$ | 14.98 ± 1.81 $^{a}$ |
| NFSs × Cultivars | | | | | | | |
| AD × Newham | 0.86 ± 0.06 $^{d}$ | 2.04 ± 0.08 $^{e}$ | 264.33 ± 5.87 $^{b}$ | 28.96 ± 1.76 | 2.91 ± 0.03 $^{b}$ | 32.38 ± 0.03 | 9.63 ± 0.13 $^{c}$ |
| (AD+NS) × Newham | 2.29 ± 0.31 $^{c}$ | 2.79 ± 0.09 $^{d}$ | 193.33 ± 13.39 $^{b}$ | 16.84 ± 1.64 | 2.09 ± 0.02 $^{d}$ | 26.28 ± 0.02 | 7.39 ± 0.04 $^{d}$ |
| NS × Newham | 6.35 ± 0.34 $^{b}$ | 4.12 ± 0.04 $^{b}$ | 177.83 ± 15.23 $^{b}$ | 16.88 ± 0.73 | 1.41 ± 0.01 $^{f}$ | 22.14 ± 0.01 | 5.97 ± 0.26 $^{d}$ |
| DD × Romaine | 0.51 ± 0.10 $^{d}$ | 2.26 ± 0.08 $^{e}$ | 757.50 ± 71.53 $^{a}$ | 36.46 ± 4.91 | 3.31 ± 0.05 $^{a}$ | 34.51 ± 0.05 | 24.40 ± 1.37 $^{a}$ |
| NS × Romaine | 9.84 ± 0.06 $^{a}$ | 5.69 ± 0.25 $^{a}$ | 217.50 ± 43.12 $^{b}$ | 13.03 ± 3.54 | 1.57 ± 0.05 $^{e}$ | 24.28 ± 0.05 | 6.91 ± 0.58 $^{d}$ |
| (AD+NS) × Romaine | 2.00 ± 0.20 $^{c}$ | 3.52 ± 0.10 $^{c}$ | 221.00 ± 4.91 $^{b}$ | 18.71 ± 2.48 | 2.39 ± 0.02 $^{c}$ | 28.34 ± 0.02 | 13.63 ± 0.31 $^{b}$ |
| NS × Romaine | 9.84 ± 0.06 $^{a}$ | 5.69 ± 0.25 $^{a}$ | 217.50 ± 43.12 $^{b}$ | 13.03 ± 3.54 | 1.57 ± 0.05 $^{e}$ | 24.28 ± 0.05 | 6.91 ± 0.58 $^{d}$ |
| Significance | | | | | | | |
| NFS | *** | *** | *** | *** | *** | *** | *** |
| Cultivars | *** | *** | *** | NS | *** | *** | *** |
| NFSs × Cultivars | *** | *** | *** | NS | *** | NS | *** |

AD, AD+NS, NS, and DW represent anaerobic digestate, anaerobic digestate + inorganic nutrient solution, inorganic nutrient solution, and dry weight, respectively. $^{NS}$, *** represent non-significant and significant differences at an alpha value of 0.001, respectively. Different letters within each column indicate significant differences among two cultivars, three nutrient feed solutions, or their interaction according to Fisher's least significant difference test.

The NFS × cultivar interaction had significant ($p < 0.05$) effects on the TPC and soluble sugars and non-significant effects on TAA. The NFSs and cultivars as main factors had significant effects on TAA (Table 5). Interactive effects displayed significantly higher TPC (3.31 ± 0.05 mg $g^{-1}$) in Romaine lettuce cultivated with AD; however, NS-treated Newham lettuce produced the lowest TPC (1.41 ± 0.01 mg $g^{-1}$) (Table 5). Among NFS treatments, lettuce plants cultivated in AD showed significantly higher TAA (33.44 ± 0.07 mg $g^{-1}$) compared to the lowest TAA observed in NS (23.31 ± 0.03 mg $g^{-1}$) (Table 5). Furthermore, Romaine showed higher TAA (29.04 ± 0.17 mg $g^{-1}$) compared to Newham (26.93 ± 0.15 mg $g^{-1}$). Overall, Romaine lettuce produced 14% higher TAA than Newham (Table 5). The AD and Romaine interaction displayed significantly higher soluble sugar (24.40 ± 1.37 mg $g^{-1}$) values, followed by Romaine × AD+NS, whereas the lowest soluble sugar was observed in Newham grown in NS (5.97 ± 0.26 mg $g^{-1}$). The Romaine lettuce cultivated in AD produced soluble sugar that was four times higher than Newham cultivated in NS (Table 5).

### *3.3. Heavy Metal Concentrations in Lettuce Leaves*

The NFSs and cultivars had significant ($p < 0.05$) effects on heavy metal concentrations in lettuce leaves except arsenic (As) and nickel (Ni) (Table 6). Higher concentrations of Cd, Co, and Cu were observed in NS, whereas the lowest value was recorded in AD,

though this was statistically similar in the NS+AD combination. The bioaccumulation of heavy metals such as Cd, Co, and Mo was significantly higher in Newham than Romaine cultivar. Interactive effects (NFSs × cultivars) were only significant for Cd, as shown in Table 6. Overall, heavy metal concentrations in lettuce (As: 0.10–0.12 mg $kg^{-1}$; Cd: 0.00–0.03 mg $kg^{-1}$; Co: 0.03–0.0.07 mg $kg^{-1}$; Cu: 3.08–6.29 mg $kg^{-1}$; Pb: 12–0.17 mg $kg^{-1}$; Mo: 0.27–1.35 mg $kg^{-1}$; and Ni: 0.24–0.46 mg $kg^{-1}$) were significantly lower than the allowable limits, as suggested by CCME, for the safe use for human consumption (Table 6).

**Table 6.** Arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), molybdenum (Mo), and nickel (Ni) profiles of hydroponically grown lettuce cultivars in relation to nutrient feed solutions. Data are expressed as mean ± standard error of six replications.

| Source of Variation | As (mg $kg^{-1}$ DW) | Cd (mg $kg^{-1}$ DW) | Co (mg $kg^{-1}$ DW) | Cu (mg $kg^{-1}$ DW) | Pb (mg $kg^{-1}$ DW) | Mo (mg $kg^{-1}$ DW) | Ni (mg $kg^{-1}$ DW) |
|---|---|---|---|---|---|---|---|
| Nutrient feed solutions (NFSs) | | | | | | | |
| AD | 0.11 ± 0.01 | 0.02 ± 0.00 $^{ab}$ | 0.04 ± 0.01 $^{b}$ | 3.65 ± 0.17 $^{c}$ | 0.14 ± 0.03 | 0.43 ± 0.07 $^{a}$ | 0.36 ± 0.07 |
| AD+NS | 0.10 ± 0.00 | 0.02 ± 0.00 $^{b}$ | 0.05 ± 0.01 $^{b}$ | 4.51 ± 0.15 $^{b}$ | 0.14 ± 0.03 | 0.36 ± 0.04 $^{b}$ | 0.28 ± 0.03 |
| NS | 0.10 ± 0.00 | 0.03 ± 0.00 $^{a}$ | 0.07 ± 0.01 $^{a}$ | 5.75 ± 0.18 $^{a}$ | 0.14 ± 0.01 | 1.16 ± 0.10 $^{b}$ | 0.35 ± 0.02 |
| Cultivars | | | | | | | |
| Newham | 0.11 ± 0.01 | 0.03 ± 0.00 $^{a}$ | 0.07 ± 0.01 $^{a}$ | 4.12 ± 0.21 $^{b}$ | 0.14 ± 0.01 | 0.76 ± 0.11 $^{a}$ | 0.38 ± 0.04 |
| Romaine | 0.10 ± 0.00 | 0.02 ± 0.00 $^{b}$ | 0.04 ± 0.00 $^{b}$ | 5.16 ± 0.22 $^{a}$ | 0.15 ± 0.02 | 0.54 ± 0.10 $^{b}$ | 0.29 ± 0.03 |
| NFSs × Cultivars | | | | | | | |
| AD × Newham | 0.12 ± 0.02 | 0.02 ± 0.00 $^{b}$ | 0.06 ± 0.00 | 3.08 ± 0.04 | 0.13 ± 0.02 | 0.48 ± 0.04 | 0.46 ± 0.12 |
| (AD+NS) × Newham | 0.10 ± 0.00 | 0.02 ± 0.00 $^{b}$ | 0.06 ± 0.01 | 4.07 ± 0.03 | 0.12 ± 0.02 | 0.44 ± 0.06 | 0.33 ± 0.03 |
| NS × Newham | 0.10 ± 0.00 | 0.05 ± 0.00 $^{a}$ | 0.09 ± 0.01 | 5.22 ± 0.06 | 0.17 ± 0.02 | 1.35 ± 0.08 | 0.35 ± 0.02 |
| AD × Romaine | 0.10 ± 0.00 | 0.02 ± 0.00 $^{b}$ | 0.03 ± 0.00 | 4.22 ± 0.07 | 0.15 ± 0.06 | 0.38 ± 0.14 | 0.27 ± 0.05 |
| (AD+NS) × Romaine | 0.10 ± 0.00 | 0.01 ± 0.00 $^{b}$ | 0.03 ± 0.01 | 6.29 ± 0.16 | 0.17 ± 0.05 | 0.27 ± 0.03 | 0.24 ± 0.03 |
| NS × Romaine | 0.10 ± 0.00 | 0.01 ± 0.00 $^{b}$ | 0.05 ± 0.01 | 4.96 ± 0.13 | 0.12 ± 0.02 | 0.97 ± 0.16 | 0.36 ± 0.04 |
| Allowable limits in lettuce/plants | 0.33 mg $kg^{-1}$ | 0.20 mg $kg^{-1}$ | | 40 mg $kg^{-1}$ | 0.3 mg $kg^{-1}$ | | 1.5 mg $kg^{-1}$ |
| Significance | | | | | | | |
| NFSs | NS | *** | *** | *** | NS | *** | NS |
| Cultivars | NS | *** | *** | *** | NS | ** | NS |
| NFS × Cultivars | NS | *** | NS | NS | NS | NS | NS |

AD, AD+NS, NS, and DW represent anaerobic digestate, anaerobic digestate + inorganic nutrient solution, inorganic nutrient solution, and dry weight, respectively. $^{NS}$, **, *** represent non-significant and significant differences at alpha values of 0.01, and 0.001, respectively. Different letters within each column indicate significant differences among two cultivars, three nutrient feed solutions, or their interaction according to Fisher's least significant difference test.

### *3.4. Does Dairy Digestate Improve Lettuce Quality?*

To test our hypothesis, we performed PCA to explore the effects of NFSs on the growth, fresh biomass, phytonutrients, and bioactive compounds in hydroponically grown lettuce cultivars in greenhouse settings (Figure 1a,b). PCA accounted for 79.92% of the overall variability in the initial dataset, whereas PCA1 and PCA2 showed 67.70% and 12.23% variability, respectively (Figure 1a,b). An observation plot depicted the clear segregation of NFSs and cultivars based on the investigated variables including growth, yield, phytonutrients, minerals, vitamins, soluble sugars, TPC, and TAA (Figure 1a).

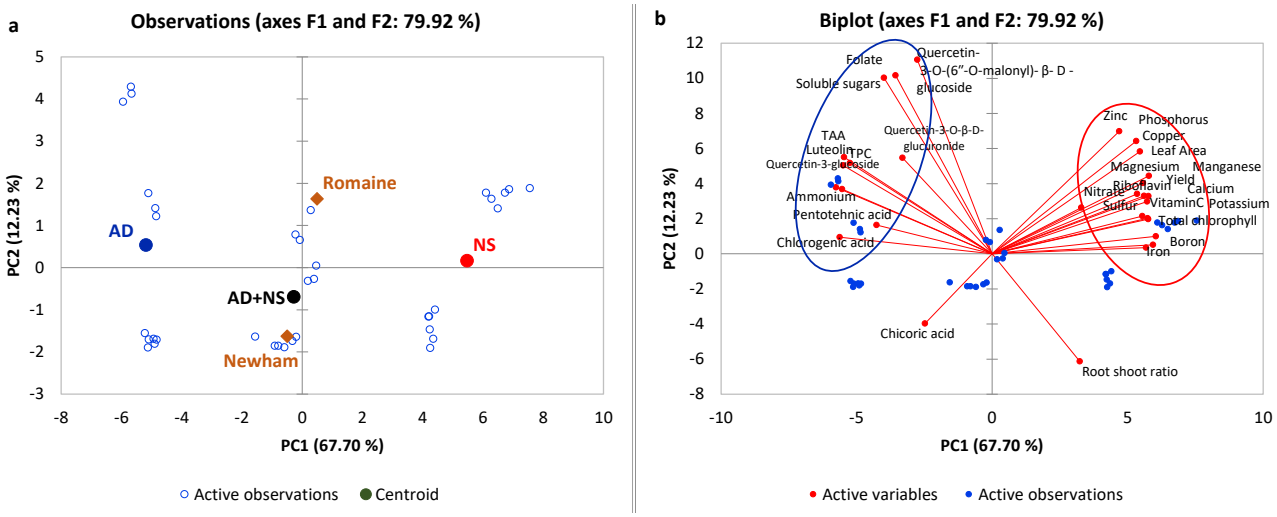


**Figure 1.** Principal component analysis (PCA) showing segregation of nutrient feed solutions (AD, NS, and AD+NS) and lettuce cultivars (Romaine and Newham) in observation plot (**a**). Biplot showing the association between growth parameters, yield, phytonutrients, heavy metals, and bioactive compounds in lettuce cultivars grown in different nutrient solutions (**b**). Anerobic dairy digestate (AD), inorganic nutrient solution (NS), and their combination (AD+NS). Total antioxidant activity (TAA) and total phenolic content (TPC).

The AD-grown lettuce accumulated significantly higher folate, soluble sugars, TPC, TAA, pantothenic acid, luteolin, chlorogenic acid, chicoroic acid, and quercetin derivates (Figure 1a,b). Contrarily, the lettuce grown in NS was associated with higher LA, chlorophyll content, fresh biomass, a root–shoot ratio, riboflavin, vitamin C, and phytominerals (Figure 1a,b). The phytonutrients were higher in NS; however, AD-grown lettuce expressed an optimum range of Fe, Zn, B, and Mn, and was slightly lower than the optimum range of P, K, Ca, Mg, and Cu (Tables 2 and 3). Nevertheless, growth, yield, and minerals were higher in NS-grown lettuce, whereas polyphenols and antioxidants (associated with AD) are known to enhance the immunity, detoxify various carcinogens, reduce chronic diseases [7,10]. Therefore, AD could be used as a biofertilizer and an organic nutrient source to enhance the phytonutrient profile of lettuce.

## 4. Discussion

The valorization of AD is important not only to reduce environmental risks but also in order to use valuable nutrients available in the nutrient laden dairy stream. Herein, we tested the potential of AD as a biofertilizer in the hydroponics greenhouse and determined its effects on the growth, biomass production, and phytonutrients of two lettuce cultivars. Previous studies have investigated the effects of different organic and inorganic feed nutrient sources on the growth, fresh biomass, minerals, vitamins, and dietary polyphenol composition of lettuce under both field and greenhouse settings. However, the impacts of cultivating lettuce in high $NH_4^+$ AD and the combination of AD and NS on the growth, fresh biomass, mineral, vitamin, polyphenol, antioxidant, heavy metal bioaccumulation, and sugar content in hydroponics remained unexplored.

### *4.1. Growth and Fresh Biomass*

The leaf area and chlorophyll play pivotal roles in light interception, photosynthesis, and other physiological processes that lead to enhanced plant growth and final biomass [31]. Zandvakili et al. [19] reported higher LA and chlorophyll content in lettuce grown in commercial inorganic fertilizers than organic fertilizers, similar to the results observed in present study (Table 1). Higher LA and chlorophyll content in NS treatment enhanced fresh biomass of lettuce compared to lettuce crop grown in AD solution produced lowest LA and chlorophyll content (Table 1). Such increments in LA and chlorophyll content

might be associated with adequate $NH_4^+$-N/$NO_3^-$-N supply and the availability of other nutrients in NS versus the reduced uptake of cations in AD+NS- and AD-grown plants (Supplementary Table S1 and Tables 1–3). Sapkota et al. [32] reported that biomass production depends on several factors such as nutrient composition, cultivars, and growing conditions. Li et al. [33] observed reduced chlorophyll content and shoot fresh biomass in *Arabidopsis thaliana* due to $NH_4^+$ stress. They observed that the AMOS1/EGY1 gene (plastid metalloprotease), involved in chlorophyll synthesis, required for the expression of $NH_4^+$-N-responsive genes and the maintenance of chloroplast functionality. Such functioning is also required for chloroplast development, the formation of thylakoid grana, the lamella system, and the accumulation of chlorophyll and chlorophyll a/b-binding proteins in chloroplast membranes.

Garbin and Dillenburg [34] reported higher root growth, higher root biomass accumulation, and higher root branches in *Araucaria angustifolia* plants grown in a balanced $NH_4^+$-N/$NO_3^-$-N solution, while we observed higher root growth in NS-grown lettuce (Table 1). Similarly, we witnessed an 83% lower root–shoot ratio in AD-grown plants than NS-grown plants, possibly due to excessive $NH_4^+$-N in the nutrient solution (Table 1). At the cellular level, $NH_4^+$-N is a fundamental substrate for amino acid and protein synthesis, but it becomes toxic to root cells when present in excess amounts in the growth medium [35,36]. Britto and Kronzucker [37] reviewed stunted root growth as a phenotypic indicator of high $NH_4^+$-N concentrations in the growth medium. Recent research has advanced our understandings on $NH_4^+$-N stress and its effects on root growth inhibition in growing plants [33,38–43]. $NH_4^+$-N affects the primary root elongation, root cell division, cell expansion, root gravitropism, and lateral root formation downregulation of auxin transporters and enzyme activities [43,44]. Auxin is associated with root development and many of its biosynthesis genes are expressed in the growing root tip and root apex [38,45]. Auxin is biosynthesized in the shoots; however under $NH_4^+$ stress, the downregulations of two important auxin transporters (AUX1 and PIN2) occur for shoot-derived auxin which cause root inhibition [33,41,46]. Additionally, the activity of GDP-mannose pyrophosphorylase (GMPase) which regulates the synthesis of GDP-mannose is essential for the N-glycosylation of proteins and is sensitive to $NH_4^+$ toxicity in the roots, resulting in growth inhibition [41,43,47]. Our results corroborate the findings of an earlier researcher [39,43] who reported a significant reduction in root growth due to higher $NH_4^+$-N in AD (Supplementary Table S1 and Table 1). AD solution in the present study contains excessive $NH_4^+$-N that might have reduced root growth, chlorophyll synthesis, LA, and eventually final biomass production, as noted by other researchers [33,48].

### *4.2. Phytonutrient Profile of Lettuce in Response to NFSs*

Leafy vegetables are major sources of macro and microminerals required for human health; however, mineral concentrations in leafy vegetables are affected by different factors such as different nutrient feed solutions [10,49]. For example, [32,50] reported a higher mineral concentration in lettuce grown with inorganic nutrient feed than organic sources in soil or soilless systems. In the current study, we observed significant effects of NFSs on mineral concentrations in order of AD < AD+NS < NS (Tables 2 and 3). The lettuce grown with NS and AD+NS showed a sufficient range of minerals required for optimum plant growth, as reported by Timothy et al. [51]. However, AD-treated lettuce showed slightly lower macro-nutrients (Tables 2 and 3), although no visible deficiency symptoms were observed during the entire growing period (Supplementary Figure S1). Reduced macro mineral concentrations in the lettuce fed with AD could be attributed to the presence of higher $NH_4^+$-N, which caused an ionic imbalance and a reduced uptake of essential cations (such as $K^+$, $Ca^{2+}$, and $Mg^{2+}$) [36,52]. Furthermore, Hoopen et al. [53] demonstrated a negative correlation between $NH_4^+$-N and $K^+$ uptake in barley and Arabidopsis plant tissues, showing $NH_4^+$ uptake preferences and a direct competition with $K^+$ under higher $NH_4^+$ availabilities. In present study, we observed similar pattern between $NH_4^+$ and other cations where high $NH_4^+$-N concentrations in AD showed negative correlations with

$K^+$, $Ca^{2+}$, and $Mg^{2+}$ uptake in lettuce plants in the hydroponic system (Supplementary Figure S2a–c). Szczerba et al. [54], Szczerba et al. [55], and Hoopen et al. [43] observed and suggested that the reduced $K^+$ uptake due to high $NH_4^+$-N could be corrected by increasing $K^+$ concentrations in the growth medium. Though macro nutrients, such as P, $K^+$ $Ca^{2+}$, and $Mg^{2+}$ concentrations, were significantly lower in AD-grown plants (Table 2), we did not observe any visible nutrient deficiency disorders in lettuce, suggesting that the $K^+$ transport mechanism might have been activated to enable the efficient transportation of $K^+$ from root to shoot to sustain $K^+$ concentrations, thereby securing $K^+$-dependent metabolic processes [53,56]. It is important to note that AD-grown lettuce exhibited optimum micronutrient concentrations for optimum plant growth (Table 3).

Mou and Ryder [57] and Zandvakili et al. [19] reported significant differences in mineral concentration in different lettuce cultivars and are associated with differential head morphologies. In the present study, Romaine lettuce showed a higher mineral uptake due to lose head morphology than Newham which exhibited lower minerals due to tighter head morphology (Tables 2 and 3; Figure S1). Further investigations are required to unravel the mechanisms associated with a higher mineral uptake in loose and tighter head morphologies in lettuce cultivars.

Plants can uptake N either in $NH_4^+$ or $NO_3^-$ form; however, $NH_4^+$-N is the preferred uptake form due to low energy costs [54,58]. In $NH_4^+$-rich growth media, aquaporin (AQP) appeared as a good candidate to facilitate $NH_4^+$ uptake compared to ammonium transporters (AMTs) or non-selective cation channels (NSCCs) in root membranes [35,36,42]. Such a higher uptake in $NH_4^+$-rich feeds such as AD results in higher $NH_4^+$ concentrations in lettuce leaves (Table 2). Esteban et al. [36] reported that $NH_4^+$ is translocated to plant leaves when roots become fully saturated and such higher translocations produce a variety of amino acids. Marino and Moran [52] suggested that plants have several strategies to handle such excessive $NH_4^+$ in cells including $NH_4^+$ efflux to apoplast, $NH_4^+$ storage in the vacuole, and $NH_4^+$ assimilation to organic compounds; the latter is responsible to enhance the produce quality. $NH_4^+$ concentrations do not impose harmful effects, whereas high $NO_3^-$ in leafy vegetables is detrimental and poses serious threats to human health [57]. Vegetables are considered as a major dietary source of $NH_4^+$, ranging from 300 to 940 mg $g^{-1}$ of daily intake, whereas accumulation of $NO_3^-$ was higher > 2500 mg $kg^{-1}$ fresh biomass [57,59]. Leafy vegetable production with inorganic N fertilizers constitutes a group of foods which maximally contributes to $NO_3^-$ content and is eventually consumed by human beings [60], whereas organically grown lettuce could provide an alternative way of reducing $NO_3^-$ concentrations. In the current study, we observed a significant negative correlation between $NH_4^+$ and $NO_3^-$ uptake in lettuce (Figure S2d), suggesting that lettuce cultivation in AD could reduce $NO_3^-$ concentrations in leaves and enhance production quality. Hence, hydroponically grown lettuce in AD not only reduces $NO_3^-$ uptake but also provides an opportunity for sustainable vegetable production to significantly reduce nutrient loads in the environment.

Several studies have reported that polyphenol biosynthesis by plants offers anti-inflammatory and therapeutic properties that may reduce cardiovascular disease, neurodegenerative disorders, cancer, and obesity [12,61–64]. The biosynthesis of these polyphenols is highly associated with plant nutrient status and nutrient fertilization practices in soil or soilless growth medium [65,66]. Nitrogen deficiency or higher $NH_4^+$-N concentrations in growth media can trigger the biosynthesis of polyphenols in growing plants [62,67,68]. Kusano et al. [69] and Caretto et al. [40] reported that phenolic biosynthesis is affected by carbon nitrogen metabolism and nitrogen deficiency directly alters such metabolism in the growing vegetables. Zhou et al. [65] also observed carbon metabolism, amino acid metabolism, and phenolic biosynthesis metabolism in lettuce were significantly affected by low nitrogen supply (LN). The phenolic content was significantly increased in LN-treated lettuce, means that phenolic biosynthesis was triggered by the LN treatment. The reduced citrate cycle and enhanced glucose and sucrose content suggested there is a relative excess of carbon resources in LN-treated lettuce. These findings suggest that LN treatment may

increase the phenolic accumulation in lettuce by effectively redirecting more carbon and nitrogen resources to the phenolic biosynthesis pathway. Deng et al. [62] also reported a shift in primary and secondary metabolites due to plant's internal carbon nitrogen balance. Similarly, higher $NH_4^+$-N might have altered carbon nitrogen metabolism by enhancing carbon availability for polyphenol biosynthesis in the current study and AD-grown plants displayed significantly higher polyphenols than the inorganic feed solution (Table 4). Hence, plants recycle $NH_4^+$-N in cells involving phenylalanine ammonia-lyase (PAL), the first enzyme of the phenylpropanoid biosynthetic pathway that converts phenylalanine to cinnamic acid, a substrate required for the biosynthesis of diverse polyphenols including chlorogenic acid, chicoric acid, luteolin, and quercetin derivatives [70]. Previous studies have reported enhanced PAL activities during increased $NH_4^+$-N concentrations in growing plants [62]. Therefore, increased $NH_4^+$ triggers PAL activities, which is known to synthesize a wide variety of amino acids and polyphenols through the shikimate pathway using phenylalanine as the substrate [71]. For instance, a recent study conducted by Mollavali et al. [72] reported the increased concentration of quercetin-4′-O-β-D-glucoside and quercetin-3,4′-di-O-β-D-glucoside in the onion bulbs under $NH_4^+$-N stress. Similarly, other researchers also reported enhanced polyphenol concentrations in organically grown lettuce compared to conventionally grown lettuce in soil media in greenhouse settings [73,74]. In the current study, we observed higher chlorogenic acid, chicoric acid, luteolin, quercetin-3-glucoside, quercetin-3-O-β-D-glucuronide, and quercetin-3-O-(6″-O-malonyl)-β-D–glucoside in hydroponically grown lettuce in AD (organics) solution compared to NS (Table 5). Another study conducted by Zhang et al. [75] reported the higher biosynthesis of polyphenols as a result of increased glutamine synthetase (GS) and glutamate synthase (GOGAT) enzymes, as well as the higher expression of relative genes in bell pepper when fed with $NH_4^+$-N-rich feed solution. Zhou et al. [65] reported increased GOGAT activities in lettuce plants due to reduced N supplies and observed that GOGAT is partially related to $NH_4^+$-N recycling from phenylalanine which is involved in polyphenol biosynthesis. In the current study, we observed significant and positive correlations between $NH_4^+$ concentrations and the polyphenol concentrations in lettuce plants, indicating the enhanced biosynthesis of such bioactive compounds under higher $NH_4^+$-N feed in the hydroponic system (Figure S3a–f). Therefore, increased $NH_4^+$-N concentrations in the growth medium resulted in enhanced polyphenol biosynthesis that could improve the nutritional quality and production of health-promoting dietary polyphenols in lettuce and other leafy vegetables [13,76].

Vitamins are naturally occurring organic compounds biosynthesized in leafy vegetables and are required for proper metabolism [9]. Vitamins are good food additives and are considered as important medical therapeutic agents [13]. Vitamin C, riboflavin, folate, and pantothenic acid are important vitamins biosynthesized by lettuce, as reviewed by Beiquan [77]. Previous studies reported increased vitamin C in bok choy and cucumber when grown in biogas slurry [78,79]. Likewise, Wei-Sheng [80] reported increased vitamin C in lettuce cultivated in biogas fertilizer compared to inorganic fertilizers in a soilless medium. In contrast, Ibrahim et al. [81] observed lower vitamin C concentrations in lettuce and Labisia pumila Benth when cultivated in organic nutrient sources compared to inorganic fertilized lettuce. A study conducted by Alhaj Alali et al. [82] observed higher $NH_4^+$ concentrations that caused a significant decrease in vitamin C in apple. In the present study, we also observed a similar pattern in lettuce and a noted decrease in vitamin C in AD-grown (high $NH_4^+$) lettuce compared to inorganic fertilized lettuce (Table 5). El-Nakhel et al. [10] reported a positive correlation between $NO_3^-$ and $K^+$ content in lettuce and an increased $K^+$ in nutrient solution resulted in higher vitamin C. In the present study, $NH_4^+$-N-rich AD reduced $K^+$ uptake and vitamin C biosynthesis; however, higher K uptake along with increased vitamin C was observed in NS-grown lettuce (Table 5). Additionally, we observed a significant and positive correlation of vitamin C with $K^+$ ($r = 0.95$ ***), and a significant and negative correlation with $NH_4^+$ concentrations ($r = -0.78$ ***) in lettuce (Figure S3a,b). Riboflavin, pantothenic acid, and folates are also important vitamins required for normal human metabolic functions. Watson and Noggle [83] investigated the effect of different

NFSs (deficient in one or more nutrients) on riboflavin content in oat plant and observed that mineral deficiencies affected riboflavin synthesis and found a positive relationship between the N uptake and riboflavin synthesis in oat leaves. In the present study, we observed lower riboflavin content and higher pantothenic acid and folates in AD-grown lettuce (Table 5), probably due to $NO_3^-$-N deficiency or a lower $NO_3^-$-N/$NH_4^+$-N ratio, as explained by Watson and Noggle [82].

Kim et al. [13] reviewed the composition and nutritional values of lettuce and reported variations in vitamins such as folate varies in lettuce cultivars. Likewise, Beiquan [77] observed higher concentrations of riboflavin, pantothenic acid, and folates varies in different lettuce cultivars. Previous studies reported riboflavin concentrations ranging from 0.25 µg to 0.77 µg $g^{-1}$, pantothenic acid concentrations ranging from 0. 91 µg to 1.50 µg $g^{-1}$, and folate concentrations ranging from 0.29 to 1.36 µg $g^{-1}$ in five lettuce cultivars (including Romain, crisphead, butterhead, red leaf, and green leaf) [77]. In the present study, we observed significantly higher concentrations of riboflavin (Newham: 2.98 ± 0.21 µg $g^{-1}$ FW; Romain: 3.83 ± 0.35 µg $g^{-1}$ FW), pantothenic acids (Newham: 20.90 ± 1.59 µg $g^{-1}$ FW: Romain: 22.73 ± 3.17 µg $g^{-1}$ FW), and folate (Newham: 211.83 ± 11.28 µg $g^{-1}$ FW; Romain: 398.67 ± 66.88 µg $g^{-1}$ FW) to what was observed by Beiquan [77].

Polyphenols and antioxidants possess redox properties to scavenge reactive oxygen species (ROS) and reduce oxidative damage to plant cells [78,84,85]. Higher antioxidants, polyphenols, and anti-proliferative properties in vegetables have significant value in human nutrition to reduce cardiovascular and cancer disease risks and are therefore important nutritional components in diet [82,83,86,87]. Ibrahim et al. [81] reported higher TPC and TAA values in *Labisia pumila* L. cultivated with organic nutrient sources (chicken dung @ 10:10:10 NPK) than inorganic fertilizer (15:15:15 NPK) in a soilless growth medium. Another study conducted by Prinsi et al. [66] reported higher TPC and TAA values with a higher $NH_4^+$-N feed solution compared to $NO_3^-$N-fed basil plants in the hydroponic system. In the present study, AD contains high $NH_4^+$-N and low $NO_3^-$-N supplies which most likely trigger the synthesis of TPC and TAA due to $NH_4^+$-N stress (Table 5). Additionally, higher $NH_4^+$-N availability in feed solution might have triggered PAL activity that is highly correlated with TPC and TAA biosynthesis (Figure S4a,b) [63,81]. Mampholo et al. [88] and Liu et al. [78] reported significant differences in TAA and TPC among lettuce cultivars. We also observed a similar pattern in lettuce cultivars in the present study where Romaine produced 8% higher TAA than Newham (Table 5), which could be due to genetic make-up and a higher potential of phytochemical accumulation than Newham lettuce.

Soluble sugars play an important part in osmotic adjustment during abiotic stresses in plants cells [89]. However, sugar synthesis in plants may vary under different mineral nutrient composition; for example, higher $NH_4^+$-N in a nutrient solution affects the synthesis of soluble sugars in lettuce due to an internal C:N imbalance [62,65]. Such sugar synthesis further triggers the biosynthesis of TPC and TAA, which play an important role in osmotic adjustments [89]. In the present study, we observed higher soluble sugars, TPC, and TAA in AD-grown lettuce compared to NS (Table 5), and AD-grown Romaine plants expressed 253% higher soluble sugar compared to NS (Table 5). Similarly, significant positive correlations between $NH_4^+$-N and TPC, TAA, chicoric acid, chlorogenic acid, luteolin, quercetin-3-O-β-D-glucuronide, quercetin-3-glucoside, and quercetin-3-O-(6″-O-malonyl)-β-D–glucoside were observed (Figures S4a,b and S5a–f). Such associations suggest that AD could have the potential to produce lettuce with high phytonutrients in hydroponic systems; however, further research is needed to reduce $NH_4^+$ in AD without diluting other macro and micronutrients.

### *4.3. Effect of AD on Heavy Metal Bioaccumulation*

The AD can be used as an alternative to synthetic fertilizers in hydroponic greenhouse vegetable production. However, the different organic nutrient sources could retain high levels of heavy metals which can be toxic to plants, animals, and humans. Different authors have documented the absorption and phytotoxic effects of heavy metals on several

crops [90–93]. Our results show the lower bioaccumulation of As, Cd, Co, Cu, Pb, Mo, and Ni in AD-grown lettuce (Table 6) and are within the permissible limits set by the European Union and the FAO of the United Nations. Our results are in line with Ezzidine et al. [92] who reported lower metal concentrations in the produce. In another study, Wang et al. [94] noticed higher concentrations of heavy metals in lettuce plants grown in biogas slurry solution and lower in Hoagland solution. However, in the present study, we observed significantly lower heavy metals in lettuce grown with AD, suggesting a safe eatable produce (Table 6).

## 5. Conclusions

It is evident based on the results reported in this study that NFSs had significant effects on the growth parameters, yield, minerals, vitamins, TPC, TAA, soluble sugars, heavy metals, and polyphenols. This research demonstrates that lettuce grown with AD produced a lower growth and yield; however, enhanced TPC, TAA, soluble sugars, vitamins (folate, pantothenic acid), and polyphenols were also found, as well as lower heavy metal accumulation in hydroponically grown lettuce. This research further demonstrates that the Romaine cultivar has a higher production potential; a lower bioaccumulation of heavy metals; and a higher tendency of phytonutrients, vitamins, and other bioactive compounds compared to Newham. We may conclude that AD has the potential to be used as a biofertilizer in hydroponic settings in a greenhouse for the production of high-quality leafy vegetables and can offer sustainable solutions to minimize nutrient losses and mitigate the challenges of food security associated with climate change and diminishing land resources. Further research is needed to understand the $NH_4^+$-N variation in partially digestate AD to obtain a $NH_4^+/NO_3^-$ -N balance without diluting and loosing essential nutrients used in the hydroponic system.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13010182/s1. Table S1: Assessment of heavy metal concentrations in anerobic dairy digestate used as nutrient sources in hydroponics under controlled growth conditions. Values are mean of three replications ± standard error. Table S2: Mineral analyses of undiluted anaerobic dairy digestate (AD) and three nutrient feed solutions used in current study including inorganic nutrient solution (NS), diluted liquid AD, and a mixture of inorganic nutrient solution and dairy digestate (50% AD + 50% NS). All values are mean ± standard error for three analysis reports. Figure S1: Effect of nutrient feed solutions on the growth of Newham (a) and Romaine (b) lettuce in floating hydroponic system in greenhouse settings. DD: dairy digestate; NS: inorganic nutrient solution; inorganic nutrient solution mix as NS+DD: 50% NS + 50% DD mixture. Figure S2: Pearson correlation showing relationship between potassium (a), calcium (b), and magnesium (c), and nitrate (d) with ammonium ($NH_4^+$) concentrations in lettuce leaves grown with different nutrient feed solutions in hydroponics. *, **, *** represent significant differences at alpha values of 0.5, 0.01, and 0.001. Figure S3: Pearson correlation showing the relationship between vitamin C (a) and potassium (b) with ammonium ($NH_4^+$) concentrations in lettuce plants grown with different feed solutions in hydroponics. *** represent significant differences at an alpha value of 0.001. Figure S4: Pearson correlation showing the positive association between ammonium ($NH_4^+$) concentrations and total phenolic content (a) and total antioxidants (b) in lettuce plants grown with different feed solutions in hydroponics. **, *** represent significant differences at alpha values of 0.01 and 0.001. Figure S5: Pearson correlation showing positive association between the ammonium ($NH_4^+$) and chicoric acid (a), chlorogenic acid (b), luteolin (c), quercetin-3-O-β-D-glucuronide (d), quercetin-3-glucoside (e), and quercitin-3-O-(6′′O-malonyl)-β-glucoside (f) in lettuce plants grown with different feed solutions in hydroponics. *, ** represent significant differences at alpha values of 0.5 and 0.01.

**Author Contributions:** M.C., L.G. and M.N.: conceptualization, supervision, methodology, writing, and reviewing. M.F.: conducted experiment, data curation, analytical analysis, and first draft formulation. C.F.M. and R.H.T.: conceptualization, administration, and interpretation of biochemical analyses. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research work was supported by research grants from Atlantic Canada Opportunities Agency (ACOA # 208281); Tourism, Culture, Industry, and Innovation (TCII # 5404.1727.102); Canadian Agricultural Partnership (CAP 2018 # 1819-0043; CAP 2019 # 1920-0241); and Grenfell Campus Memorial University of Newfoundland and Labrador, Canada. Authors are thankful to New World Dairy Inc., Newfoundland for providing the logistics to conduct this experiment.

**Conflicts of Interest:** The authors declare no competing financial interests or personal relationships that could affect the work reported in this manuscript.

## References

1. Won, S.; Shim, S.-M.; You, B.-G.; Choi, Y.-S.; Ra, C. Nutrient production from dairy cattle manure and loading on arable land. *Asian-Australas. J. Anim. Sci.* **2017**, *30*, 125–132. [CrossRef] [PubMed]
2. Zhang, N.; Bai, Z.; Luo, J.; Ledgard, S.; Wu, Z.; Ma, L. Nutrient losses and greenhouse gas emissions from dairy production in China: Lessons learned from historical changes and regional differences. *Sci. Total Environ.* **2017**, *598*, 1095–1105. [CrossRef] [PubMed]
3. Chastain, J.P.; Camberato, J.J. Dairy manure production and nutrient content. In *Confined Animal Manure Managers Certification Program Manual B Dairy Version 1*; Clemson University Cooperative Extension Service: Clemson, SC, USA, 2004; pp. 1–16.
4. Alburquerque, J.A.; de la Fuente, C.; Ferrer-Costa, A.; Carrasco, L.; Cegarra, J.; Abad, M.; Bernal, M.P. Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. *Biomass Bioenergy* **2012**, *40*, 181–189. [CrossRef]
5. Jovicich, E.; Cantliffe, D.J.; Simonne, E.H.; Stoffella, P.J. Comparative water and fertilizer use efficiencies of two production systems for cucumbers. *Acta Hortic.* **2007**, *731*, 235–241. [CrossRef]
6. Correia ECS. *Reação de Cultivares de Alface do Grupo Americano a Meloidoygine Incognita, M. Javanica e M. Enterolobii (55f. Dissertação (Mestrado em Agronomia)*; Universidade Estadual Paulista "Júlio de Mesquita Filho": Botucatu, Brazil, 2013.
7. Sacks, F.M.; Willett, W.C.; Smith, A.; Brown, L.E.; Rosner, B.; Moore, T.J. Effect on Blood Pressure of Potassium, Calcium, and Magnesium in Women with Low Habitual Intake. *Hypertension* **1998**, *31*, 131–138. [CrossRef] [PubMed]
8. Roberts, S. What Is the Main Function of Phosphorus in the Body? 2018. Available online: https://healthyeating.sfgate.com/main-function-phosphorus-body-5789.html (accessed on 6 March 2020).
9. Survase, S.; Bajaj, I.; Singhal, R. Biotechnological production of vitamins. *Food Technol. Biotechnol.* **2006**, *44*, 381–396.
10. El-Nakhel, C.; Petropoulos, S.A.; Pannico, A.; Kyriacou, M.C.; Giordano, M.; Colla, G.; Troise, A.D.; Vitaglione, P.; De Pascale, S.; Rouphael, Y. The bioactive profile of lettuce produced in a closed soilless system as configured by combinatorial effects of genotype and macrocation supply composition. *Food Chem.* **2020**, *309*, 125713. [CrossRef]
11. Herrera, E.; Jiménez, R.; Aruoma, O.I.; Hercberg, S.; Sánchez-García, I.; Fraga, C. Aspects of antioxidant foods and supplements in health and disease. *Nutr. Rev.* **2009**, *67* (Suppl. 1), S140–S144. [CrossRef]
12. Davis, J.M.; Murphy, E.A.; Carmichael, M.D. Effects of the dietary flavonoid quercetin upon performance and health. *Curr. Sport. Med. Rep.* **2009**, *8*, 206–213. [CrossRef]
13. Kim, M.J.; Moon, Y.; Tou, J.C.; Mou, B.; Waterland, N.L. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). *J. Food Compos. Anal.* **2016**, *49*, 19–34. [CrossRef]
14. Sulaiman, S.F.; Yusoff, N.A.; Eldeen, I.M.S.; Seow, E.M.; Sajak, A.A.B.; Supriatno; Ooi, K.L. Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (*Musa* sp.). *J. Food Compos. Anal.* **2011**, *24*, 1–10. [CrossRef]
15. CCME. *Recommended Canadian Soil Quality Guidelines*; Canadian Council of Ministers of the Environment (CCME): Winnipeg, MB, Canada, 1997.
16. CCME. *Recommended Canadian Soil Quality Guidelines*; Canadian Council of Ministers of the Environment (CCME): Winnipeg, MB, Canada, 2015.
17. CCME. *Recommended Canadian Soil Quality Guidelines*; Canadian Council of Ministers of the Environment (CCME): Winnipeg, MB, Canada, 2009.
18. Spehia, R.S.; Devi, M.; Singh, J.; Sharma, S.; Negi, A.; Singh, S.; Chauhan, N.; Sharma, D.; Sharma, J.C. Lettuce growth and yield in Hoagland solution with an organic concoction. *Int. J. Veg. Sci.* **2018**, *24*, 557–566. [CrossRef]
19. Zandvakili, O.R.; Barker, A.V.; Hashemi, M.; Etemadi, F.; Autio, W.R. Comparisons of commercial organic and chemical fertilizer solutions on growth and composition of lettuce. *J. Plant Nutr.* **2019**, *42*, 990–1000. [CrossRef]
20. Chapman, H.D.; Pratt, P.F. Methods of Analysis for Soils, Plants and Waters. *Soil Sci.* **1962**, *93*, 68. [CrossRef]
21. Bottoms, T.G.; Smith, R.F.; Cahn, M.D.; Hartz, T.K. Nitrogen requirements and N status determination of lettuce. *HortScience* **2012**, *47*, 1768–1774. [CrossRef]
22. Fishman, M.J.; Friedman, L.C. *Methods for Determination of Inorganic Substances in Water and Fluvial Sediments*; US Department of the Interior, Geological Survey: Washington, DC, USA, 1989.
23. Akhavan, H.-R.; Barzegar, M. Determination of water-soluble vitamins in 15 Iranian pomegranate cultivars and their variation after pasteurization and cold storage. *Int. Food Res. J.* **2017**, *24*, 1429–1436.
24. Boonpangrak, S.; Lalitmanat, S.; Suwanwong, Y.; Prachayasittikul, S.; Prachayasittikul, V. Analysis of ascorbic acid and isoascorbic acid in orange and guava fruit juices distributed in Thailand by LC-IT-MS/MS. *Food Anal. Methods* **2016**, *9*, 1616–1626. [CrossRef]

25. Gavrilova, V.; Kajdzanoska, M.; Gjamovski, V.; Stefova, M. Separation, characterization and quantification of phenolic compounds in blueberries and red and black currants by HPLC-DAD-ESI-MSn. *J. Agric. Food Chem.* **2011**, *59*, 4009–4018. [CrossRef]
26. Manful, C.F.; Vidal, N.P.; Pham, T.H.; Nadeem, M.; Wheeler, E.; Hamilton, M.C.; Doody, K.M.; Thomas, R.H. Unfiltered beer based marinades reduced exposure to carcinogens and suppressed conjugated fatty acid oxidation in grilled meats. *Food Control* **2020**, *111*, 107040. [CrossRef]
27. Vidal, N.P.; Manful, C.; Pham, T.H.; Wheeler, E.; Stewart, P.; Keough, D.; Thomas, R. Novel unfiltered beer-based marinades to improve the nutritional quality, safety, and sensory perception of grilled ruminant meats. *Food Chem.* **2020**, *302*, 125326. [CrossRef] [PubMed]
28. Cano, A.; Acosta, M.; Arnao, M.B. Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (*Lycopersicon esculentum* Mill.). *Postharvest Biol. Technol.* **2003**, *28*, 59–65. [CrossRef]
29. Benzie, I.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef] [PubMed]
30. Shao, Y.; Lin, A.H.-M. Improvement in the quantification of reducing sugars by miniaturizing the Somogyi-Nelson assay using a microtiter plate. *Food Chem.* **2018**, *240*, 898–903. [CrossRef] [PubMed]
31. Slamet, W.; Purbajanti, E.D.; Darmawati, A.; Fuskhah, E. Leaf area index, chlorophyll, photosynthesis rate of lettuce (*Lactuca sativa* L) under N-organic fertilizer. *Indian J. Agric. Res.* **2017**, *51*, 365–369. [CrossRef]
32. Sapkota, S.; Sapkota, S.; Liu, Z. Effects of nutrient composition and lettuce cultivar on crop production in hydroponic culture. *Horticulturae* **2019**, *5*, 72. [CrossRef]
33. Li, B.; Li, Q.; Xiong, L.; Kronzucker, H.J.; Krämer, U.; Shi, W. Arabidopsis plastid AMOS1/EGY1 integrates abscisic acid signaling to regulate global gene expression response to ammonium stress. *Plant Physiol.* **2012**, *160*, 2040–2051. [CrossRef]
34. Garbin, M.L.; Dillenburg, L.R. Effects of different nitrogen sources on growth, chlorophyll concentration, nitrate reductase activity and carbon and nitrogen distribution in Araucaria angustifolia. *Braz. J. Plant Physiol.* **2008**, *20*, 295–303. [CrossRef]
35. Bittsánszky, A.; Pilinszky, K.; Gyulai, G.; Komives, T. Overcoming ammonium toxicity. *Plant Sci.* **2015**, *231*, 184–190. [CrossRef]
36. Esteban, R.; Ariz, I.; Cruz, C.; Moran, J.F. Review: Mechanisms of ammonium toxicity and the quest for tolerance. *Plant Sci.* **2016**, *248*, 92–101. [CrossRef]
37. Britto, D.T.; Kronzucker, H.J. NH4+ toxicity in higher plants: A critical review. *J. Plant Physiol.* **2002**, *159*, 567–584. [CrossRef]
38. Overvoorde, P.; Fukaki, H.; Beeckman, T. Auxin control of root development. *Cold Spring Harb. Perspect. Biol.* **2010**, *2*, a001537. [CrossRef] [PubMed]
39. Li, Q.; Li, B.-H.; Kronzucker, H.J.; Shi, W.-M. Root growth inhibition by NH4+ in Arabidopsis is mediated by the root tip and is linked to NH4+ efflux and GMPase activity. *Plant Cell Environ.* **2010**, *33*, 1529–1542. [CrossRef] [PubMed]
40. Caretto, S.; Linsalata, V.; Colella, G.; Mita, G.; Lattanzio, V. Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. *Int. J. Mol. Sci.* **2015**, *16*, 26378–26394. [CrossRef] [PubMed]
41. Qin, C.; Qian, W.; Wang, W.; Wu, Y.; Yu, C.; Jiang, X.; Wang, D.; Wu, P. GDP-mannose pyrophosphorylase is a genetic determinant of ammonium sensitivity in Arabidopsis thaliana. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 18308–18313. [CrossRef]
42. Pantoja, O. High affinity ammonium transporters: Molecular mechanism of action. *Front. Plant Sci.* **2012**, *3*, 34. [CrossRef]
43. Liu, Y.; Lai, N.; Gao, K.; Chen, F.; Yuan, L.; Mi, G. Ammonium inhibits primary root growth by reducing the length of meristem and elongation zone and decreasing elemental expansion rate in the root apex in Arabidopsis thaliana. *PLoS ONE* **2013**, *8*, e61031. [CrossRef]
44. Li, B.; Li, G.; Kronzucker, H.J.; Baluška, F.; Shi, W. Ammonium stress in Arabidopsis: Signaling, genetic loci, and physiological targets. *Trends Plant Sci.* **2014**, *19*, 107–114. [CrossRef]
45. Petersson, S.V.; Johansson, A.I.; Kowalczyk, M.; Makoveychuk, A.; Wang, J.Y.; Moritz, T.; Grebe, M.; Benfey, P.N.; Sandberg, G.; Ljung, K. An auxin gradient and maximum in the Arabidopsis root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. *Plant Cell* **2009**, *21*, 1659–1668. [CrossRef]
46. Kempinski, C.F.; Haffar, R.; Barth, C. Toward the mechanism of NH4+ sensitivity mediated by Arabidopsis GDP-mannose pyrophosphorylase. *Plant Cell Environ.* **2011**, *34*, 847–858. [CrossRef]
47. Conklin, P.L.; Norris, S.R.; Wheeler, G.L.; Williams, E.H.; Smirnoff, N.; Last, R.L. Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 4198–4203. [CrossRef]
48. Loqué, D.; Mora, S.I.; Andrade, S.L.; Pantoja, O.; Frommer, W.B. Pore mutations in ammonium transporter AMT1 with increased electrogenic ammonium transport activity. *J. Biol. Chem.* **2009**, *284*, 24988–24995. [CrossRef] [PubMed]
49. Neocleous, D.; Savvas, D. The effects of phosphorus supply limitation on photosynthesis, biomass production, nutritional quality, and mineral nutrition in lettuce grown in a recirculating nutrient solution. *Sci. Hortic.* **2019**, *252*, 379–387. [CrossRef]
50. Kapoulas, N.; Koukounaras, A.; Ilić, Z.S. Nutritional quality of lettuce and onion as companion plants from organic and conventional production in north Greece. *Sci. Hortic.* **2017**, *219*, 310–318. [CrossRef]
51. Timothy, K.H.; Johnstone, P.R.; Williams, E.; Smith, R.F. Establishing lettuce leaf nutrient optimum ranges through DRIS analysis. *HortScience* **2007**, *42*, 143–146.
52. Marino, D.; Moran, J.F. Can ammonium stress be positive for plant performance? *Front. Plant Sci.* **2019**, *10*, 1103. [CrossRef]
53. Hoopen, F.T.; Cuin, T.A.; Pedas, P.; Hegelund, J.N.; Shabala, S.; Schjoerring, J.K.; Jahn, T.P. Competition between uptake of ammonium and potassium in barley and Arabidopsis roots: Molecular mechanisms and physiological consequences. *J. Exp. Bot.* **2010**, *61*, 2303–2315. [CrossRef] [PubMed]

54. Szczerba, M.W.; Britto, D.T.; Balkos, K.D.; Kronzucker, H.J. Alleviation of rapid, futile ammonium cycling at the plasma membrane by potassium reveals K+-sensitive and -insensitive components of NH4+ transport. *J. Exp. Bot.* **2008**, *59*, 303–313. [CrossRef] [PubMed]
55. Szczerba, M.W.; Britto, D.T.; Ali, S.A.; Balkos, K.D.; Kronzucker, H.J. NH4+-stimulated and inhibited components of K+ transport in rice (*Oryza sativa* L.). *J. Exp. Bot.* **2008**, *59*, 3415–3423. [CrossRef]
56. Britto, D.T.; Kronzucker, H.J. Futile cycling at the plasma membrane: A hallmark of low-affinity nutrient transport. *Trends Plant Sci.* **2006**, *11*, 529–534. [CrossRef]
57. Mou, B.; Ryder, E.J. Relationship between the nutritional value and the head structure of lettuce. *Int. Hortic. Congr. Adv. Veg. Breed.* **2002**, *637*, 361–367. [CrossRef]
58. Piwpuan, N.; Zhai, X.; Brix, H. Nitrogen nutrition of Cyperus laevigatus and Phormium tenax: Effects of ammonium versus nitrate on growth, nitrate reductase activity and N uptake kinetics. *Aquat. Bot.* **2013**, *106*, 42–51. [CrossRef]
59. Santamaria, P. Nitrate in vegetables: Toxicity, content, intake and EC regulation. *J. Sci. Food Agric.* **2006**, *86*, 10–17. [CrossRef]
60. Gorenjak, A.H.; Cencič, A. Nitrate in vegetables and their impact on human health. A review. *Acta Aliment.* **2013**, *42*, 158–172. [CrossRef]
61. André, C.M.; Oufir, M.; Hoffmann, L.; Hausman, J.-F.; Rogez, H.; Larondelle, Y.; Evers, D. Influence of environment and genotype on polyphenol compounds and in vitro antioxidant capacity of native Andean potatoes (*Solanum tuberosum* L.). *J. Food Compos. Anal.* **2009**, *22*, 517–524. [CrossRef]
62. Deng, B.; Li, Y.; Xu, D.; Ye, Q.; Liu, G. Nitrogen availability alters flavonoid accumulation in Cyclocarya paliurus via the effects on the internal carbon/nitrogen balance. *Sci. Rep.* **2019**, *9*, 2370. [CrossRef] [PubMed]
63. Taleon, V.; Dykes, L.; Rooney, W.L.; Rooney, L.W. Effect of genotype and environment on flavonoid concentration and profile of black sorghum grains. *J. Cereal Sci.* **2012**, *56*, 470–475. [CrossRef]
64. Healy, E.F.; Sanders, J.; King, P.J.; Robinson, W.E., Jr. A docking study of L-chicoric acid with HIV-1 integrase. *J. Mol. Graph. Model.* **2009**, *27*, 584–589. [CrossRef] [PubMed]
65. Zhou, W.; Liang, X.; Li, K.; Dai, P.; Li, J.; Liang, B.; Sun, C.; Lin, X. Metabolomics analysis reveals potential mechanisms of phenolic accumulation in lettuce (*Lactuca sativa* L.) induced by low nitrogen supply. *Plant Physiol. Biochem.* **2021**, *158*, 446–453. [CrossRef]
66. Prinsi, B.; Negrini, N.; Morgutti, S.; Espen, L. Nitrogen starvation and nitrate or ammonium availability differently affect phenolic composition in green and purple basil. *Agronomy* **2020**, *10*, 498. [CrossRef]
67. Toscano, S.; Ferrante, A.; Leonardi, C.; Romano, D. PAL activities in asparagus spears during storage after ammonium sulfate treatments. *Postharvest Biol. Technol.* **2018**, *140*, 34–41. [CrossRef]
68. Naguib, A.E.-M.M.; El-Baz, F.K.; Salama, Z.A.; Abd El Baky Hanaa, H.; Ali, H.F.; Gaafar, A.A. Enhancement of phenolics, flavonoids and glucosinolates of broccoli (Brassica olaracea, var. Italica) as antioxidants in response to organic and bio-organic fertilizers. *J. Saudi Soc. Agric. Sci.* **2012**, *11*, 135–142. [CrossRef]
69. Kusano, M.; Fukushima, A.; Redestig, H.; Saito, K. Metabolomic approaches toward understanding nitrogen metabolism in plants. *J. Exp. Bot.* **2011**, *62*, 1439–1453. [CrossRef] [PubMed]
70. Kováčik, J.; Klejdus, B.; Bačkor, M.; Repčák, M. Phenylalanine ammonia-lyase activity and phenolic compounds accumulation in nitrogen-deficient Matricaria chamomilla leaf rosettes. *Plant Sci.* **2007**, *172*, 393–399. [CrossRef]
71. Abbate, C.; Toscano, S.; Arcidiacono, R.; Romano, D.; Russo, A.; Mazzeo, G. Induced responses of Bougainvillea glabra Choisy (Nyctaginaceae) against Phenacoccus peruvianus Granara de Willink (Hemiptera: Pseudococcidae) attack: Preliminary results. *Arthropod-Plant Interact.* **2017**, *12*, 41–48. [CrossRef]
72. Mollavali, M.; Perner, H.; Rohn, S.; Riehle, P.; Hanschen, F.S.; Schwarz, D. Nitrogen form and mycorrhizal inoculation amount and timing affect flavonol biosynthesis in onion (*Allium cepa* L.). *Mycorrhiza* **2018**, *28*, 59–70. [CrossRef] [PubMed]
73. Durazzo, A.; Azzini, E.; Lazzé, M.C.; Raguzzini, A.; Pizzala, R.; Maiani, G.; Palomba, L.; Maiani, G. Antioxidants in Italian head lettuce (*Lactuca sativa* var. *capitata* L.) grown in organic and conventional systems under greenhouse conditions. *J. Food Biochem.* **2014**, *38*, 56–61. [CrossRef]
74. Rajaashekar, C.B.; Oh, M.-M.; Carey, E.E. Organic crop management enhances chicoric acid content in lettuce. *Food Nutr. Sci.* **2012**, *3*, 1296–1302. [CrossRef]
75. Zhang, J.; Lv, J.; Dawuda, M.M.; Xie, J.; Yu, J.; Li, J.; Zhang, X.; Tang, C.; Wang, C.; Gan, Y. Appropriate ammonium-nitrate ratio improves nutrient accumulation and fruit quality in pepper (*Capsicum annuum* L.). *Agronomy* **2019**, *9*, 683. [CrossRef]
76. Zhou, W.; Liang, X.; Dai, P.; Chen, Y.; Zhang, Y.; Zhang, M.; Lu, L.; Jin, C.; Lin, X. Alteration of phenolic composition in lettuce (*Lactuca sativa* L.) by reducing nitrogen supply enhances its anti-proliferative effects on colorectal cancer cells. *Int. J. Mol. Sci.* **2019**, *20*, 4205. [CrossRef]
77. Beiquan, M. Nutrient content of lettuce and its improvement. *Curr. Nutr. Food Sci.* **2009**, *5*, 242–248.
78. Liu, X.; Ardo, S.; Bunning, M.; Parry, J.; Zhou, K.; Stushnoff, C.; Stoniker, F.; Yu, L.; Kendall, P. Total phenolic content and DPPH radical scavenging activity of lettuce (*Lactuca sativa* L.) grown in Colorado. *LWT Food Sci. Technol.* **2007**, *40*, 552–557. [CrossRef]
79. Wen-jun, G. Effect of biogas slurry and biogas residues on quality of greenhouse mini cucumber. *Soil Fertil. Sci. China* **2007**, *5*, 40–43.
80. Wei-Sheng, S. Effect of biogas fertilizer on yield and quality of lettuce in soilless culture. *Soil Fertil. Sci. China* **2008**, *1*, 60–62.
81. Ibrahim, M.H.; Jaafar, H.Z.E.; Karimi, E.; Ghasemzadeh, A. Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (*Labisia pumila* Benth). *Molecules* **2013**, *18*, 10973–10988. [CrossRef] [PubMed]

82. Alhaj Alali, F.; Askari Sarcheshmeh, M.A.; Babalar, M. Effect of ammonium content in nutrition solution on vitamin C, phenols, and antioxidant capacity of three apple cultivars during cold storage. *DYSONA Appl. Sci.* **2020**, *1*, 64–72.
83. Watson, S.A.; Noggle, G.R. Effect of mineral deficinecies upon the synthesis of riboflavin and ascorbic acid by the oat plant. *Plant Physiol.* **1947**, *22*, 228–243. [CrossRef] [PubMed]
84. Caldwell, C.R. Alkylperoxyl radical scavenging activity of red leaf lettuce (*Lactuca sativa* L.) phenolics. *J. Agricutlural Food Chem.* **2003**, *51*, 4589–4595. [CrossRef] [PubMed]
85. Jakovljević, D.; Topuzović, M.; Stanković, M. Nutrient limitation as a tool for the induction of secondary metabolites with antioxidant activity in basil cultivars. *Ind. Crops Prod.* **2019**, *138*, 111462. [CrossRef]
86. Cheng, L.; Han, M.; Yang, L.-m.; Li, Y.; Sun, Z.; Zhang, T. Changes in the physiological characteristics and baicalin biosynthesis metabolism of Scutellaria baicalensis Georgi under drought stress. *Ind. Crops Prod.* **2018**, *122*, 473–482. [CrossRef]
87. Pott, D.M.; Osorio, S.; Vallarino, J.G. From central to specialized metabolism: An overview of some secondary compounds derived from the primary metabolism for their role in conferring nutritional and organoleptic characteristics to fruit. *Front. Plant Sci.* **2019**, *10*, 835. [CrossRef]
88. Mampholo, B.M.; Maboko, M.M.; Soundy, P.; Sivakumar, D. Variety-specific responses of lettuce grown in a gravel-film technique closed hydroponic system to N supply on yield, morphology, phytochemicals, mineral content and safety. *J. Integr. Agric.* **2018**, *17*, 2447–2457. [CrossRef]
89. Anjana, S.U.; Iqbal, M. Nitrate accumulation in plants, factors affecting the process, and human health implications. A review. *Agron. Sustain. Dev.* **2007**, *27*, 45–57. [CrossRef]
90. Jordan, R.A.; Riberio, E.F.; de Oliveira, F.C.; Geisenhoff, L.O.; Martins, E.A.S. Yield of lettuce grown in hydroponic and aquaponic systems using different substrates. *Rev. Bras. Eng. Agric. Ambient* **2018**, *22*, 525–529. [CrossRef]
91. Eissa, M.A.; Negim, O.E. Heavy metals uptake and translocation by lettuce and spinach grown on a metal-contaminated soil. *J. Soil Sci. Plant Nutr* **2018**, *18*, 1097–1107. [CrossRef]
92. Ezziddine, M.; Liltved, H.; Seljåsen, R. Hydroponic Lettuce Cultivation Using Organic Nutrient Solution from Aerobic Digested Aquacultural Sludge. *Agronomy* **2021**, *11*, 1484. [CrossRef]
93. Lira, R.M.D.; Silva, Ê.F.D.F.; Silva, G.F.D.; Santos, A.N.D.; Rolim, M.M. Production, water consumption and nutrient content of Chinese cabbage grown hydroponically in brackish water. *Rev. Ciência Agronômica* **2015**, *46*, 497–505. [CrossRef]
94. Wang, L.; Guo, S.; Wang, Y.; Yi, D.; Wang, J. Poultry biogas slurry can partially substitute for mineral fertilizers in hydroponic lettuce production. *Environ. Sci. Pollut. Res.* **2019**, *26*, 659–671. [CrossRef]