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By-Product from Livestock Waste Recovery System Used as Fertilizer: Bioactive Compounds and Antioxidant Activity of Tomato Fruit as Affected by Fertilization under Field and Greenhouse Conditions

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Abstract: Conversion of livestock manure into organic fertilizer is a sustainable strategy in crop production. In contrast to composted manure, the agronomic characteristics of an anaerobic digestion by-product, digestate, have not been well characterized. This study aimed to investigate the effects of digestate and compost, derived from a pilot-scale livestock waste recycling system, on bioactive compounds in tomato fruits. Both field and greenhouse experiments were conducted to compare the effects of these two organic fertilizers with the application of chemical fertilizer. These comparisons were made by evaluating their influence on tomato yield and bioactive compound contents and antioxidant activity of fruits. The experiment included a control (no fertilizer) and three fertilization treatments with the same nitrogen dose: chemical fertilizer, digestate, and compost. The results revealed that the application of digestate and compost yielded similar results in terms of tomato production, surpassing both the chemical fertilizer application and the control group under both field and greenhouse conditions. Fertilization exhibited a significant influence on the bioactive compound contents and antioxidant capacity of the fruits. Furthermore, the application of digestate and compost led to an increase in the concentration of sugars, phenolic compounds, and several organic acids in the fruits while simultaneously reducing the citric acid levels in comparison to the chemical fertilizer treatment. Moreover, the application of both organic fertilizers improved the total phenol and total flavonoid contents in tomato fruits, and the antioxidant capacity in fruits was significantly higher than that of the chemical fertilizer treatment. In conclusion, the application of digestate or compost derived from the livestock waste recycling system reduced use of chemical fertilizers and resulted in higher tomato yields and fruit with considerably superior bioactive compounds. The results suggested that using digestate or compost as an alternative to inorganic fertilizers for tomato cultivation could assist farmers in increasing productivity, improving the content of bioactive compounds in tomato fruit, and promoting agricultural waste management.

Keywords: digestate; compost; phenolic compounds; tomato; antioxidant capacity; value added products

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1. Introduction

In modern agricultural production, livestock manure recycling systems have emerged as a sustainable approach to agricultural waste management. Livestock manure recycling systems convert livestock manure into organic fertilizer by effectively utilizing waste resources while reducing environmental pollution and chemical fertilizer use [1]. Composting is a natural aerobic process that stabilizes organic matter and livestock manure. In livestock manure recycling systems, composting is an important way of effectively recycling livestock manure. Approximately 50% of solid waste from livestock manure is composted [2]. Compost is known to provide abundantly available nutrients to plants as organic fertilizer, improve soil physicochemical properties, and promote the proliferation of beneficial microorganisms, thereby promoting increased crop yields and reducing the application of chemical fertilizers [3–5]. However, the livestock manure recycling system comprises a large semi-solid or liquid fraction, including urine and water cleanup collection, which is difficult to compost. Therefore, anaerobic biomass digestion for gas production is used as an additional important waste management strategy in livestock manure recovery systems [6,7]. It not only helps to reduce greenhouse gas emissions from the agricultural sector but also produces biogas, a biofuel that can be used to generate heat and electricity [8]. An important environmental aspect of biogas production is that anaerobic digestion installations generate large amounts of a by-product termed digestate [9]. Excess digestate is predicted to be generated because of the proliferation of biogas plants in certain areas of intensive livestock farming [10]. If the application of digestate as fertilizer is applied, it could lead to a greater efficacy within the realm of circular agriculture, thereby attaining enhanced environmental benefits. Therefore, it is necessary to provide directions for the utilization of digestate for value addition.

Recently, the utilization of digestate as a fertilizer has garnered significant attention [11]. Several studies have highlighted the sustainable viability of digestate as an alternative to chemical fertilizers [12]. Digestate also contains large amounts of microelements, organic matter, phenolic compounds, and phytohormones, in addition to being rich in nitrogen and potassium. These substances have multiple benefits for plant growth but are not usually incorporated into chemical fertilizers [13,14]. Long-term use of chemical fertilizers in crop production tends to reduce soil fertility and decreases protein content in the fruits [15]. Hence, the utilization of digestate may provide a high-quality fertilizer potential.

Tomato (*Solanum lycopersicum* L.) is widely grown and consumed as a vegetable crop. Tomato fruits are rich in various bioactive substances, such as antioxidants, flavonoids, and organic acids, that are important for human health [16]. Several studies have shown that organic fertilization is a good substitute for chemical fertilizers and potentially minimizes the adverse impacts of chemical fertilization [17,18]. As natural nutrient sources, organic fertilizers generally increase the nutritional properties of plants [19,20]. Previous studies have shown that tomato yield and fruit quality are influenced by fertilization practices. For example, Hernández et al. reported that the application of organic fertilizer could reduce mineral N by approximately 40% while achieving similar tomato fruit yields comparing to chemical fertilizer [21]. Bilalis et al. reported a higher sugar-to-acid ratio (SAR) in tomato fruits under organic fertilization compared with that under conventional inorganic fertilization [22]. Anton et al. found that organic fertilization increased the ascorbic acid and phenolic compound contents in the fruit compared with those under conventional fertilization [23]. However, research on the effects of applying digestate as an organic fertilizer on bioactive compounds in tomato production is still an understudied area. In addition, to the best of our knowledge, the present study is the first to compare the effects of different fertilization strategies (digestate and compost as by-products of livestock manure recycling systems from the same pilot-scale cattle farm and chemical fertilizer) on the bioactive substances and antioxidant activity of tomato fruit, as well as fruit yield, sugar, and organic acid contents.

Based on the above background, this study hypothesized that the utilization of digestate or compost from livestock manure recycling systems could serve as a viable fertilizer and augment the presence of bioactive compounds in tomato fruits. Therefore, the objective of this study was to evaluate the effect of the bioactive substances and the antioxidant activity of tomato fruit after the application of digestate or compost as organic fertilizers in tomato production. In addition, we evaluated the changes in tomato yields and sugars and acids of fruits under different fertilization practices. By systematically analyzing the effects of by-products of livestock manure recycling systems as organic fertilizers on the content of bioactive substances in tomato fruits, we provided a scientific basis for the optimal use of agricultural wastes and fertilization strategies in tomato production.

2. Materials and Methods

2.1. Fertilizer Sources

The NPK fertilizer used in the current work was purchased from the Hokuren Fertilizer Co. (Sapporo, Japan). Digestate and compost were collected from a pilot-scale cattle farm waste recycling system located on the campus of Hokkaido University, Hokkaido, Japan. This farm produces livestock manure (approximately 98% cattle manure). There are grooves in the floor of the livestock farm where the manure can fall and accumulate in the grooves. Three to four times a day, the manure is dumped into a cistern, rinsed with water, and agitated with a large blade. It is then piped to a digester. Then, it is digested into an 80–120 m^3 biogas containing 60–65% methane, thereby producing digestate as a by-product of anaerobic digestion. A remainder portion of the solid livestock manure is made into compost (Figure 1). The digestate and compost were used directly in the current study. The physicochemical properties of the digestate and compost are summarized in Table 1.

(a) Digestate (b) Compost

Figure 1. Organic fertilizers used in this study.

Table 1. Characteristics of digestate and compost used in this study.

	Moisture	C	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	%	% dm	% dm	% dm	% dm	% dm	% dm	% dm	ppm dm	ppm dm	ppm dm
Digestate	94.93	40.45	2.133	1.463	4.469	2.306	0.963	0.195	183.6	337.0	55.4
Compost	65.10	40.51	2.502	0.673	3.055	1.496	0.552	0.574	349.4	136.5	21.5

2.2. Field Experiment

The field experiment was conducted from 20 June 2021 to 24 October 2021 in a field on the campus of Hokkaido University (43°4′ N, 141°20′ E; 20 m above sea level), Hokkaido, Japan (Figure S1a). Prior to the experiments, soil samples collected from a depth of 0–20 cm in the experimental field were characterized (Table 2). The experiment was performed using a completely randomized factorial design, with 3 replications per treatment, for a total of 12 plots. The amount of nitrogen applied in each treatment was approximately 180 kg ha^{-1}. Three fertilization treatments and a control were applied: (1) CK, no fertilizer after transplanting; (2) NPK, fertilized with 514 kg ha^{-1} of 14–14–14 NPK fertilizer as the basic fertilizer, then fertigated with 386 kg ha^{-1} of 14–14–14 NPK fertilizer during flowering, and fertigated with 386 kg ha^{-1} of 14–14–14 NPK fertilizer during fruit swelling; (3) digestate, fertilized with 66.67 t ha^{-1} of digestate as the basic fertilizer, then fertigated with 50 t ha^{-1} of digestate during flowering, and fertigated with 50 t ha^{-1} of digestate during fruit swelling; (4) compost, fertilized with 8.25 t ha^{-1} of compost as the basic fertilizer, then fertigated with 6.18 t ha^{-1} of compost during flowering, and fertigated with 6.18 t ha^{-1} of compost during fruit swelling. At the four-leaf stage, uniform healthy tomato seedlings were transplanted into the experimental plots. Each plot was 2.2 m long and 1.3 m wide, comprising a total area of 2.86 m^2 and consisting of 2 rows. Plants were spaced 35 cm apart, with 50 cm between rows, for an average planting density of 3.5 plants m^2. The shortest spacing between tomato plants in each plot and neighboring plots was 80 cm. The meteorological conditions from transplanting to harvest are listed in Table S1. The determinate tomato cultivar used in this experiment, 'Medium Matina', is popular with local growers and was purchased from Greenfield Project Corporation (Nagano, Japan). Agronomic management was identical for all CK and fertilization treatments, including fertilization, de-worming, and de-leafing. Irrigation under field conditions was normally every 3 days according to the water-holding capacity in the field, with less irrigation or even no irrigation required during the rainy season. In addition, tomato plants were staked with canes and covered with bird-proof nets in the field (Figure S2a).

Table 2. Characteristics of the soil used in the field and greenhouse experiments.

Parameter	Unit	Field	Greenhouse
Attributes		Sandy soil	Loamy soil
P-absorption coefficient		480	1099
CEC	cmol kg^{-1}	0.97	2.93
TN	g kg^{-1}	0.89	1.982
Olsen-P	mg 100 g^{-1}	6.4	38.1
K exchangeable	mg 100 g^{-1}	22	61.0
Ca exchangeable	mg 100 g^{-1}	157.8	401.1
Mg exchangeable	mg 100 g^{-1}	15.8	39.4
Cu	ppm	3.93	2.86
Zn	ppm	3.17	25.75
Mn	ppm	19.18	156.11
B	ppm	0.30	0.73

CEC, cation exchange capacity.

2.3. Greenhouse Experiment

The greenhouse experiment was conducted from 29 June 2021 to 23 November 2021 in a greenhouse on the campus of Hokkaido University (43°4′ N, 141°20′ E; 20 m above sea level), Hokkaido, Japan (Figure S1b). Prior to the experiments, soil samples collected from a depth of 0–20 cm in the experimental greenhouse were characterized (Table 2). The experimental set-up and cultivation management in the greenhouse were identical to those in the field experiment. The tomato plants were trellised using vertical strings in the greenhouse (Figure S2b). The only difference was that the greenhouse was irrigated regularly every 3 days according to the water-holding capacity in the greenhouse, whereas the field was irrigated less frequently during the rainy season, for a total of 24 times during the growing season.

2.4. Sampling and Analytical Methods

2.4.1. Tomato Fruit Yield

Red-ripened tomato fruits were harvested until the end of crop production. The fruits were harvested in sequence, according to the order of fruit ripening. After weighing each picked fruit with an electronic balance, the sum in each experimental plot was recorded and fruit yield was defined as the total weight of fruits per m^2 of plants.

During the fruiting period, at least 30 fruits were collected from 10 plants per plot to generate a representative pooled fruit sample. Prior to further analysis, the fruits were washed and sterilized. Tomato fruits were sliced and then homogenized in a blender for analysis of physicochemical parameters and contents of sugars, acids, and phenolic compounds.

2.4.2. Analysis of Sugars

Sugar in the fruits was extracted in accordance with S–A1 using the described method of Xi et al. [24]. Monosaccharide (fructose and glucose) contents were detected by high performance liquid chromatography (HPLC) with an RI detector on an Agilent 1260 series instrument (Agilent Technologies, Santa Clara, CA, USA) fitted with a Sugar SH–1821 column and an SH–G guard column (Shodex, Tokyo, Japan). The separation of sugars was conducted using 2 mM H_2SO_4 as the mobile phase with an injection volume of 50 μL and a flow rate of 0.6 mL min^{-1}.

2.4.3. Analysis of Organic Acids

Acid in the fruits was extracted in accordance with S–A1 using the described method of Xi et al. [24]. Organic acids (citric acid, fumaric acid, malic acid, oxalic acid, succinic acid, and tartaric acid) were detected by HPLC with a UV detector at a wavelength of 210 nm on an Agilent 1260 series instrument equipped with an RSpak KC–811 column and a KC–G guard column (Shodex, Tokyo, Japan). The separation of acids was conducted using 1 mM $HClO_4$ as the mobile phase with a flow rate of 0.7 mL min^{-1} and an injection volume of 50 μL.

2.4.4. Analysis of Phenolic Compounds

Phenolic compounds were extracted from the fruits in accordance with S–A2 as described by Anton et al. [23]. Phenols (caffeic acid, chlorogenic acid, ρ-coumaric acid, ferulic acid, gallic acid, syringic acid, kaempferol, naringenin, catechin, quercetin, and rutin) were detected by HPLC with a UV detector at a wavelength of 290 nm with a C18M 4E column (Shodex, Tokyo, Japan). The separation of phenolic compounds was achieved by gradient elution using methanol (solvent A) and 0.5% acetic acid (solvent B) as the mobile phase at a flow rate of 0.8 mL min^{-1} and an injection volume of 20 μL. The protocol was from 30% solvent A up to 90% and from 70% solvent B down to 10% over 25 min. The percentage of solvent A was then reduced to the initial conditions, and the column was re-equilibrated for 10 min. The run time for one sample was 35 min.

2.4.5. Analysis of Chemical Parameters

Soluble sugar content of tomato fruits was determined using the described method by Wang et al. [25]. Total phenolic content (TPC) was measured by the Folin–Ciocalteu method [26]. Total flavonoid content (TFC) was detected using the colorimetric method described by Yuan et al. [27]. A modified method was used to detect the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and the antioxidant capacity of tomato fruits was expressed as μg trolox equivalents per g fresh weight (μg g^{-1}) [28].

2.5. Statistical Analysis

All data are presented as the mean ± standard error (SE) of three replicates (n = 3). The data were subjected to analysis of variance (ANOVA), and differences between the individual means were compared using Duncan's post hoc test at a probability of 95%. Statistical analyses were conducted using IBM SPSS Statistics 24.0 (IBM Corporation, Armonk, NY, USA). All figures were generated using Origin 2022.

3. Results and Discussion

3.1. Tomato Yield

Fruit yields of tomato plants subjected to fertilization with digestate or compost under field or greenhouse cultivation were markedly higher than those in the CK and NPK treatments (Figure 2). In both cultivation environments, digestate treatment produced the highest fruit yield, which was 159.10%, in the field, and 63.01%, in the greenhouse, higher than that of CK. No significant difference in fruit yield was observed between the digestate and compost treatments in the field, and the same was true in the greenhouse; however, both organic fertilization treatments achieved relatively higher fruit yields compared with the NPK treatment. Fruit yields of digestate treatment under the field and greenhouse conditions were 19.30% and 7.98% higher than those of NPK, respectively. The fruit yields of the compost treatment were 17.47% (field) and 7.87% (greenhouse) higher than those of NPK under the corresponding condition. The current results are consistent with the findings of Zhao et al., who reported that different organic fertilizers increase crop yield [29]. Cristina et al. observed a potential positive effect of digestate on tomato growth, and Wu et al. reported that compost application could increase tomato fruit yield [30,31]. Tomato yield was significantly affected by fertilization ($p < 0.001$) and cultivation environments ($p < 0.001$) and their interaction ($p < 0.01$).

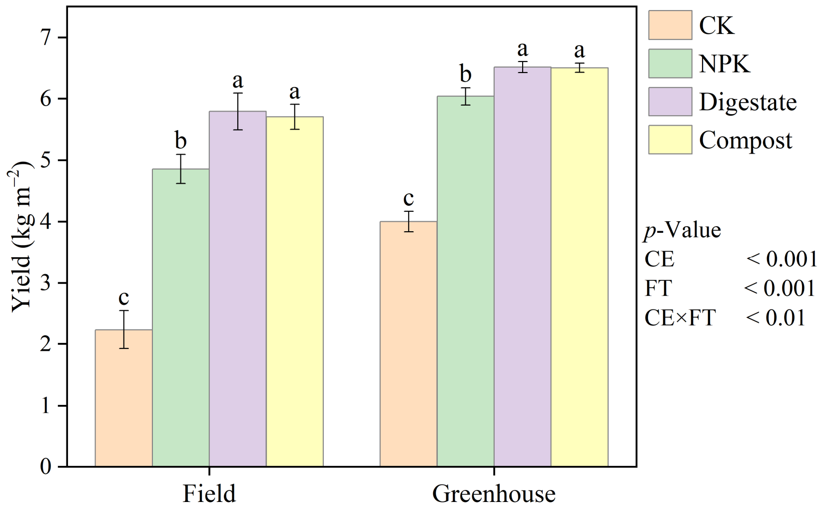

Figure 2. Effects of fertilization treatments on tomato yield in field and greenhouse conditions. CE: cultivation environment. FT: fertilization treatment. The values presented in the figures are given as mean ± SD ($n = 3$). Different lowercase letters indicate significant differences at $p < 0.05$ under the same cultivation experiment.

Livestock waste can be recycled using many methods to address rising energy prices, achieve sustainable agricultural production, and reduce the environmental impacts of traditional livestock waste management practices [6]. Appropriate use of livestock manure for biogas, composting, and vermicompost manufacturing is useful to improve crop yield and sustainability in agricultural production [32]. Recovery of digestate as an organic fertilizer after biogas fermentation is considered a suitable use because it recovers plant nutrients and reduces the application of chemical fertilizers [7]. The present study confirmed that the application of digestate and compost can reduce the necessity for chemical fertilizers while maintaining satisfactory tomato yields, even up to 2.59-fold in digestate treatment of fruit yield compared with CK under field conditions. Organic fertilizers are increasingly recommended for crop production as an alternative to inorganic fertilizers [21,30]. In the present work, the application of digestate or compost as an alternative to chemical fertilizers significantly increased tomato yield. More precisely, the highest yields were achieved with digestate treatment followed by compost treatment. The application of organic fertilizer emphasized the accumulation of soil organic matter and fertility through the use of organic sources such as composts, and it relies on the activity of a diverse soil ecosystem to make N and other nutrients available to plants [33]. The increase in tomato yield in the treatment of digestate or compost may also be due to the increased availability of potassium, calcium, and magnesium as well as some micronutrients at the same amount of nitrogen applied, which resulted in better growth of tomatoes [31]. However, the slight (nonsignificant) increase in yield under digestate treatment compared with compost application was negligible.

3.2. Sugar Contents

To investigate how the fertilization treatment affected fruit sweetness, we measured the contents of soluble sugar, glucose, fructose, and sucrose in the fruit (Figure 3). Digestate- and compost-fertilized plants had higher amounts of saccharides, including soluble sugar, monosaccharides (fructose and glucose), and sucrose compared with those of the CK under both field and greenhouse conditions. These contents were even significantly higher than those of the NPK treatments. Soluble sugar was 24.51% and 21.27% higher in the digestate treatment than in the NPK treatment under the field and greenhouse conditions, respectively, and was 18.18% and 15.79% higher in the compost treatment than in NPK under the field and greenhouse conditions, respectively. Similar results to the present work, showing that different organic fertilizers increased the soluble sugar content of tomato fruit, were reported by Ma et al. [34]. However, the various sugars in tomato fruits did not differ significantly between the digestate and compost treatments under both cultivation conditions. Fructose and glucose were the main saccharides, and sucrose was detected in small amounts from red-ripened tomato fruit under all treatments. Li et al. reported similar results, where organic fertilizer application promoted the glucose and fructose content of tomato fruit [35]. Riahi et al. reported that organic farming systems substantially enhanced the accumulation of sugars in organic tomato fruit and that these differences were statistically significant [33]. Two-way ANOVA revealed a significant interaction effect between fertilization and cultivation environment only for fructose content, and no significant interaction was detected for soluble sugar, glucose, and sucrose contents. The present results showed that organic fertilizers (both digestate and compost) significantly promoted the accumulation of sugars in tomato fruit. Hu et al. presented similar evidence [36].

ANOVA				
	Glucose	Fructose	Sucrose	Soluble sugar
CE	**	**	***	***
FT	**	**	***	***
CE×FT	ns	**	ns	ns

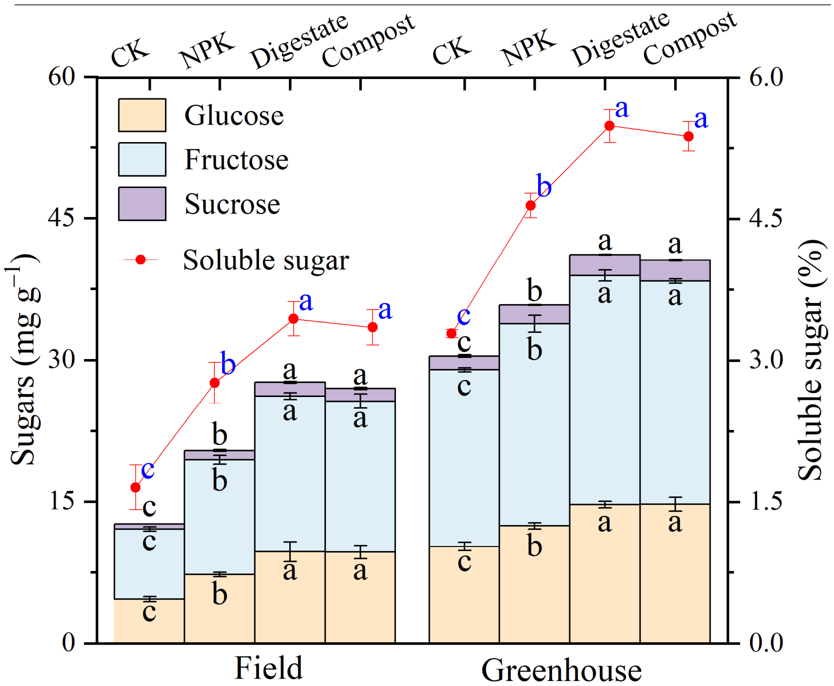

Figure 3. Effects of fertilization treatments on tomato fruit sugars (glucose, fructose, and sucrose) in the field and greenhouse environments. CE: cultivation environments. FT: fertilization treatment. The values presented in the figures are given as mean ± SD (n = 3). Different lowercase letters indicate significant differences at $p < 0.05$ under the same cultivation experiment. Student's t-test (*** $p < 0.001$, **, $p < 0.01$; ns $p > 0.05$).

3.3. Organic Acid Contents

The contents in the fruit of all six organic acids analyzed were affected by the fertilization treatment (Table 3). The citric acid content was highest, and the oxalic acid content was lowest under the field and greenhouse environments. Citric acid contents were higher in the NPK treatment than in the other treatments under the field and greenhouse environments. The citric acid content in the NPK treatment was significantly enhanced by 27.07% and 14.54% in the field and greenhouse, respectively, compared with those of the digestate treatment. Correspondingly, the citric acid content in the NPK treatment was increased by 20.48% and 13.46% in the field and greenhouse, respectively, compared with those of the compost treatment. Organic fertilizer application may also affect the acidity of tomato fruit, with chemical fertilizer-applied crops containing higher contents of organic acids compared with those treated with organic fertilizer [37]. Different results were reported by Pieper et al., who showed that organic fertilizer application resulted in higher organic acid content in tomato fruit, but the results were not statistically different from those of tomatoes under inorganic fertilization [38]. These findings may be due to the difference in the amount of nitrogen applied in those studies. The most prominent effect on the fruit citric acid content is observed with the application of a reasonable amount of nitrogen [39]. Much of the acidity in tomato fruit is attributed to citric acid, which is the predominant organic acid in tomato fruit and is responsible for the sour taste [40]. Many studies have shown that the application of organic fertilizers reduces the titratable acidity in tomato fruit compared with that under chemical fertilizer applications [21,22]. Almost all of these reports measured the titratable acidity with citric acid as a conversion factor, which is consistent with the present results. In contrast, the contents of fumaric acid, malic acid, and oxalic acid in the digestate and compost treatments were significantly higher than those in the NPK treatment under both cultivation conditions. The succinic acid and tartaric acid contents did not differ significantly among the digestate, compost, and NPK treatments in the field and greenhouse.

In the present study, the application of digestate and compost promoted an increase in the contents of certain organic acids, such as malic, fumaric, and oxalic acids; however, the citric acid content of the fruit was significantly lower in the digestate and compost treatments than in the chemical fertilizer treatments. In addition, all organic acid contents in the fertilization treatments were significantly improved compared with those of the CK. Li et al. reported similar results, in that organic fertilizer application reduced the citric acid content but increased the malic acid content [35]. In the current study, organic fertilizer treatment did reduce the fruit citric acid content and increased the malic acid content compared to chemical fertilizer-treated tomatoes. Many factors, such as cultivation conditions, temperature, light, and humidity, could affect the flavor of tomato fruit. However, fertilizer availability is the most important factor affecting fruit flavor [41]. We found a significant interaction effect between fertilization and cultivation environment for citric acid, malic acid, and oxalic acid, but no significant interaction for fumaric acid, succinic acid, and tartaric acid in tomato fruit. Organic fertilizers play an important role in the organic acids and sugars in tomato fruit. In addition, the significantly higher sugar content in the plant may be due to loss of citric acid as a result of sugar synthesis [37]. The SAR is an important indicator that affects the taste of tomato fruit. In the present study, higher fruit SAR was detected in the digestate and compost treatments compared to the NPK treatment; however, the SAR of fruit in the NPK treatment was not significantly different from that of the control (Table S2). This observation is consistent with previous studies [22,25,30]. The lack of flavor in fruit treated with inorganic fertilizers may be due to a low SAR [35].

Table 3. Contents of organic acids in tomato fruits under different fertilization treatments in two cultivation environments.

Treatment	Citric Acid	Fumaric Acid	Malic Acid	Oxalic Acid	Succinic Acid	Tartaric Acid
Field	(mg 100 g^{-1})	(mg 100 g^{-1})	(mg 100 g^{-1})	(mg 100 g^{-1})	(mg 100 g^{-1})	(mg 100 g^{-1})
CK	404.87 ± 17.51 c	0.64 ± 0.07 c	25.94 ± 1.27 c	0.36 ± 0.05 c	22.73 ± 3.34 b	22.94 ± 3.68 b
NPK	621.73 ± 31.67 a	2.33 ± 0.39 b	53.56 ± 3.05 b	0.85 ± 0.04 b	50.90 ± 5.75 a	49.63 ± 8.42 a
Digestate	489.29 ± 20.60 b	4.10 ± 0.47 a	72.52 ± 2.45 a	1.08 ± 0.05 a	54.14 ± 4.98 a	53.51 ± 8.65 a
Compost	476.5 ± 16.74 b	3.56 ± 0.42 a	68.20 ± 2.91 a	1.06 ± 0.04 a	51.86 ± 6.74 a	51.60 ± 7.44 a
Greenhouse						
CK	626.48 ± 7.81 c	1.29 ± 0.17 c	41.10 ± 1.40 c	0.65 ± 0.01 c	43.66 ± 4.04 b	32.69 ± 7.11 b
NPK	747.30 ± 17.16 a	3.17 ± 0.41 b	63.98 ± 1.42 b	1.36 ± 0.01 b	66.45 ± 7.05 a	64.36 ± 4.20 a
Digestate	652.42 ± 9.34 bc	4.52 ± 0.28 a	75.76 ± 2.80 a	1.48 ± 0.02 a	66.09 ± 5.21 a	66.93 ± 7.09 a
Compost	658.63 ± 18.95 b	4.22 ± 0.32 a	75.79 ± 1.06 a	1.49 ± 0.01 a	62.81 ± 5.15 a	65.31 ± 8.85 a
F-value						
CE	510.80 ***	21.46 **	103.77 ***	974.54 ***	45.16 ***	19.42 ***
FT	86.43 ***	115.08 ***	436.75 ***	778.90 ***	33.62 ***	27.77 ***
CE × FT	6.81 *	0.40 ns	7.83 *	12.33 **	1.04 ns	0.14 ns

CE: cultivation environment; FT: fertilization treatment. The different lowercase letters indicate a significant difference between the samples of the same cultivation experiment ($p < 0.05$). Student's *t*-test (*** $p < 0.001$, **, $p < 0.01$; *, $p < 0.05$, ns $p > 0.05$).

3.4. Phenolic Compound Contents

The contents of 11 phenolic compounds in the fruit were analyzed under the field and greenhouse conditions (Tables 4 and S3). The contents of the phenolic compounds varied significantly with fertilization treatment under the field and greenhouse conditions. Fruit from the digestate and compost treatments had higher phenolic compounds content compared with those of the CK and NPK treatments under both cultivation conditions, except for ferulic acid and syringic acid in the greenhouse environment. All phenolic compounds in the digestate and compost treatments did not differ significantly under the field and greenhouse conditions. Chlorogenic acid was the predominant phenolic compound in the fruit in both cultivation environments. The highest chlorogenic acid content was in the digestate treatment in the field (34.90 mg kg^{-1}) and compost treatment in the greenhouse (43.48 mg kg^{-1}). Moreover, the flavonoid with the highest content presented in tomato fruits was rutin. The current findings regarding the major phenolic compounds are similar to previous findings, in that the application of organic fertilizers increased the synthesis of predominant phenolic compounds, such as chlorogenic acid and rutin, in tomato fruit [42]. In addition, the contents of all phenolic compounds in the digestate, compost, and NPK treatments were significantly enhanced compared with those of the CK. Regarding fertilizer application, in most scenarios, variations in fertilization treatments resulted in marked differences in phenolic compound values of tomato fruits [43]. Two-way ANOVA revealed that fertilization and cultivation condition had significant interactions for most phenolic compounds but catechin, ferulic acid, and quercetin did not.

The bioactive compounds in tomato fruit form a complex system with multiple interactions. According to previous studies, tomato fruit is rich in bioactive compounds, including secondary metabolites (e.g., phenolics and flavonoids) [23]. The current study confirmed that the application of digestate and compost had a positive impact on the synthesis of phenolic compounds. The application of digestate improved the contents of most phenolic compounds in tomato fruit under field and greenhouse cultivation. The beneficial effects of digestate or compost have been observed in cucumber, kale, and lettuce [19,44,45]. Organic fertilizers increase the synthesis of flavonoids in plants [16,23]. The present results confirm this finding, as treatments with digestate or compost resulted in higher contents of flavonoids, such as rutin, quercetin, and naringenin, compared with those of fruit in the NPK and CK treatments. In addition, organic fertilizers contain high amounts of organic matter and phytohormones, which could significantly enhance the synthesis of bioactive substances in crops [12,44]. This can be explained by the fact that some organic substances provided in organic fertilizers are able to promote the synthesis of phenolic compounds in plants [46].

Table 4. Contents of phenolic compounds in tomato fruits under different fertilization treatments in two cultivation environments.

Treatment	Chlorogenic Acid	Gallic Acid	Rutin	Quercetin	Syringic Acid
Field	(ug g^{-1})	(ug g^{-1})	(ug g^{-1})	(ug g^{-1})	(ug g^{-1})
CK	9.36 ± 1.35 c	4.18 ± 0.25 c	2.62 ± 0.14 c	0.92 ± 0.08 c	4.18 ± 0.21 c
NPK	14.57 ± 1.63 b	6.33 ± 0.36 b	4.15 ± 0.13 b	1.46 ± 0.06 b	5.55 ± 0.27 b
Digestate	34.90 ± 1.72 a	9.88 ± 1.00 a	5.40 ± 0.11 a	1.83 ± 0.07 a	8.61 ± 0.33 a
Compost	33.24 ± 1.38 a	9.58 ± 0.81 a	5.45 ± 0.06 a	1.82 ± 0.04 a	8.52 ± 0.22 a
Greenhouse					
CK	18.36 ± 1.30 c	6.87 ± 0.35 c	9.28 ± 0.61 c	2.30 ± 0.04 c	9.15 ± 0.17 b
NPK	29.83 ± 1.54 b	10.84 ± 0.38 b	11.18 ± 0.75 b	2.73 ± 0.10 b	9.63 ± 0.15 ab
Digestate	41.04 ± 2.92 a	14.06 ± 0.45 a	14.76 ± 0.94 a	3.16 ± 0.11 a	9.92 ± 0.17 a
Compost	43.48 ± 1.54 a	13.68 ± 0.37 a	13.68 ± 0.65 a	3.21 ± 0.06 a	9.91 ± 0.55 a
F-value					
CE	204.05 ***	292.15 ***	1282.77 ***	1970.24 ***	630.76 ***
FT	289.91 ***	177.20 ***	74.74 ***	197.97 ***	119.348 ***
CE × FT	7.156 *	3.21 ns	7.91 **	0.79 ns	63.79 ***

CE: cultivation environment; FT: fertilization treatment. The different lowercase letters indicate a significant difference between the samples of the same cultivation environment ($p < 0.05$). Student's t-test (*** $p < 0.001$, **, $p < 0.01$; *, $p < 0.05$, ns $p > 0.05$).

3.5. Total Phenolic and Flavonoid Contents

Phenolic compounds including flavonoids are present in almost all fruits and vegetables at varying levels [47]. The present study found the TPC and TFC of tomato fruit in the digestate and compost treatments were significantly higher than those of the CK and NPK treatments under both the field and greenhouse conditions (Figure 4). The highest TPC (595.9 ug g^{-1}) was found in the greenhouse condition fertilized with compost, and the highest TFC (104.85 ug g^{-1}) was found in the greenhouse condition fertilized with digestate. The range of values reported in this research is similar to those found by Hallmann [37]. By contrast, a number of earlier studies reported TPC to be lower than that found in the current study [48]. Differences in findings among studies may be attributed to differences in genotype, growing status, and analytical methods [49]. The TPC in the digestate and compost treatments was 33.29% and 25.84% higher, respectively, than those of the NPK treatment under the field condition and 20.77% and 22.72% higher, respectively, than those of the NPK treatment under the greenhouse condition. The TFC in the digestate and compost treatments was 25.38% and 23.17% higher, respectively, than those of the NPK treatment under the field condition and 28.49% and 21.06% higher, respectively, than those of the NPK treatment under the greenhouse condition. These results are also in agreement with a previous work showing the stimulatory effects of digestates on flavonoid and phenol synthesis in cucumber fruits, suggesting that the digestates, rich in carbon and organic matters, stimulated plant resource reallocation to secondary metabolites production [50]. Many studies have focused mainly on the improvement of crop quality or yield by organic fertilizers [17,20] but have not systematically focused on the effects on the bioactive substances and antioxidant activity of the fruit, and the effects of digestate application on these parameters has received even less attention. In current study, the application of digestate improved the TPC and TFC in tomato fruit under field and greenhouse cultivation. In general, it is considered that the main factors responsible for chemical differences in bioactive compounds, such as phenolic compounds, sugars, and organic acids, are differences in nutrient content and plant uptake of these elements under different fertilization treatments. In addition, the biochemical differences observed by many researchers are partly attributed to other environmental conditions [46,48].

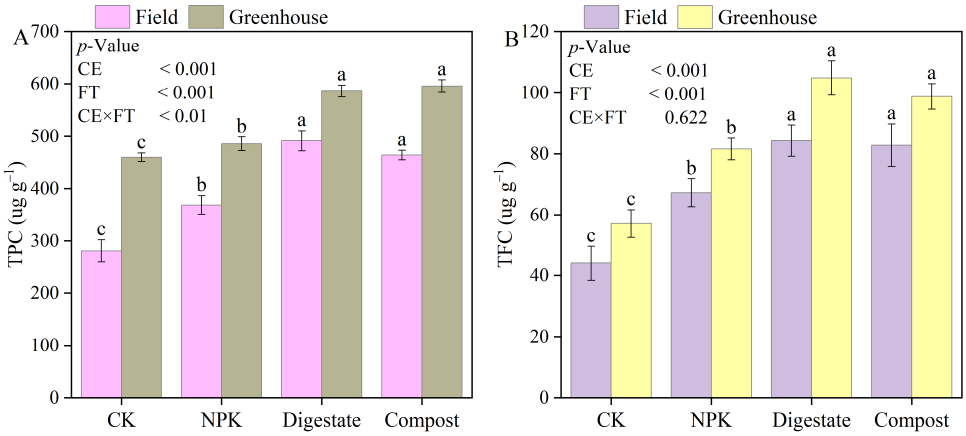

Figure 4. Effects of fertilization treatments on total phenolic content (TPC) and total flavonoid content (TFC) in the field and greenhouse environments. (**A**): TPC. (**B**): TFC. CE: cultivation environment. FT: fertilization treatment. The values presented in the figures are given as mean ± SD (n = 3). Different lowercase letters indicate significant differences at $p < 0.05$ under the same cultivation experiment.

3.6. Antioxidant Capacity

Tomato is considered a nutritional indicator of good dietary habits and a healthy lifestyle because the fruit contains high quantities of antioxidants [51]. Oxidation plays an important role in the emergence of certain diseases and in human aging. The antioxidant capacity that helps to limit the oxidative process is a highly desirable property in foods [52]. In the present study, high antioxidant capacity was observed in tomato fruit treated with digestate or compost under the field and greenhouse conditions (Figure 5). The antioxidant capacity in the digestate and compost treatments was 52.44% and 28.08% higher, respectively, than that of the NPK treatment in the field and 62.69% and 50.37% higher, respectively, than that of the NPK treatment in the greenhouse. The highest antioxidant capacity in the fruit was detected in the digestate treatment in the field and greenhouse conditions. The antioxidant capacity did not differ significantly between the digestate and compost treatments under both conditions, yet the antioxidant capacity in the digestate treatment was 19.02% and 8.19% higher than that of the compost treatment in the field and greenhouse environments, respectively. The presence of caffeic, chlorogenic, ferulic, and p-coumaric acid have been widely reported to increase plant antioxidant levels [53]. The current study obtained similar findings. The present study also found that the antioxidant capacity of the fruit was significantly affected by fertilization ($p < 0.001$) and cultivation condition ($p < 0.001$), but their interaction had no significant effect ($p > 0.05$). The antioxidant capacity and TPC differed significantly among the different fertilization treatments. Organic fertilization has a stimulating effect on the synthesis of phenolics that possess high potential activity as antioxidants compared with control and bio-organic treatments [46]. Our study found these similar results. A strong correlation between antioxidant activity and lycopene content was reported by Zanfini et al. [54]. Ilahy et al. stated that tomato fruit containing high amounts of lycopene has high antioxidant activity [16]. Although the fruit lycopene content was not examined in the current study, we did observe enhanced antioxidant capacity of tomato fruit under organic fertilization. Recent studies have reported the antioxidant and free radical scavenging properties of polyphenolic compounds in several plant extracts, suggesting a possible protective role in reducing the risk of cardiovascular diseases in humans [55]. Overall, tomatoes are considered to be antioxidant-rich foods [51,52], and the consumption of tomatoes and tomato products is therefore considered a nutritional indicator of good dietary habits and a healthy lifestyle [42]. Anton et al. conducted a three-year field study applying organic fertilizers to fertilize tomatoes and found that changes in tomato phenolic compounds were more affected in organic fertilizer treatments compared to chemical fertilizers [23]. However, neither sugars nor acids were reported. Long-term field trials are needed to understand the effects of repeated applications of digestate or compost from the same livestock waste recycling system on tomato growth and bioactive compounds of fruits.

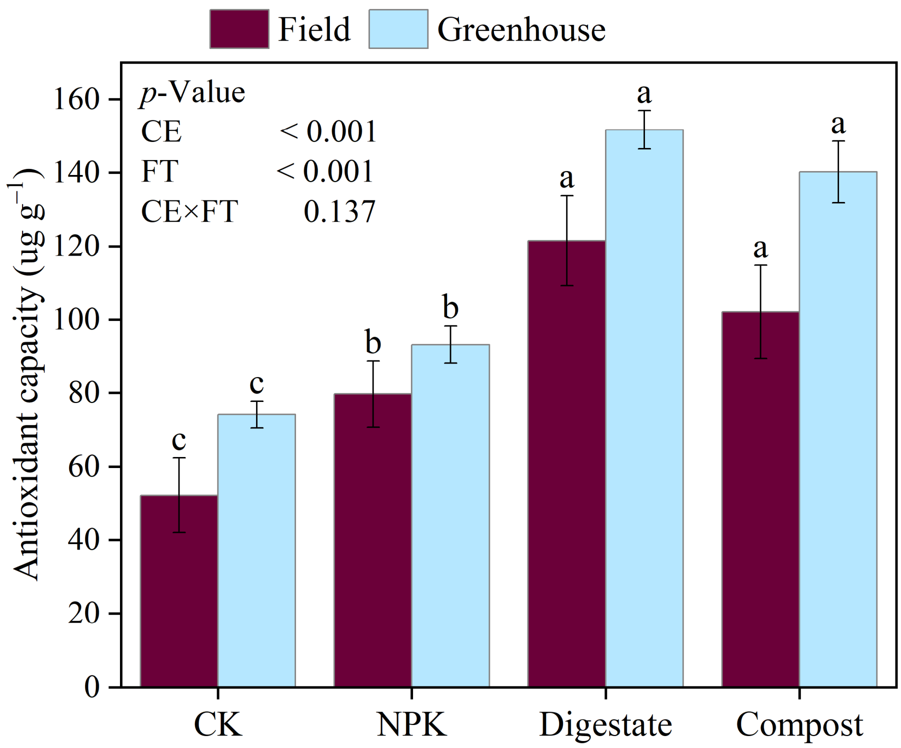

Figure 5. Effects of fertilization treatments on antioxidant capacity in the field and greenhouse environments. CE: cultivation condition. FT: fertilization treatment. The values presented in the figures are given as mean ± SD (n = 3). Different lowercase letters indicate significant differences at $p < 0.05$ under the same cultivation experiment.

4. Conclusions

In the present study, tomato plants did obtain a better growth potential under the same nitrogen dose following application of digestate or compost instead of chemical fertilizer because yields in tomato fruits were enhanced under the field and greenhouse conditions. However, the yield was not significantly different between the application of digestate and compost. In this study, the content of most bioactive compounds in tomato fruits was significantly increased when the same nitrogen dose of digestate or compost was applied compared to the chemical fertilizer application. Specifically, the increase in fruit sugar compounds under treatment with digestate or compost was accompanied by a decrease in the citric acid content of the fruit. Both digestate and compost applications resulted in the higher synthesis of phenolic compounds compared to chemical fertilizer and control. We found that TPC and TFC of tomato fruits from either digestate or compost application were significantly higher than those from chemical fertilizer application under both field and greenhouse conditions. The antioxidant capacity of the fruits in digestate fertilization even increased by 52.44% and 62.69% compared to chemical fertilization under the field and greenhouse cultivation conditions, respectively. In conclusion, our results confirm that the application of digestate or compost from livestock manure recycling systems may benefit tomato production and improve the synthesis of bioactive compounds in its fruits. The application of digestate or compost instead of chemical fertilizers offers the potential for tomato cultivation. This practice not only enhances the content of bioactive compounds in tomato fruits, but it also facilitates agricultural waste management. Further studies will be conducted in the future to investigate long-term effects and optimize application methods for maximum production benefits.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9080714/s1, Figure S1: Experimental sites at First Farm of Hokkaido University; Figure S2: Experimental sites used in the study. (a) Field; (b) greenhouse. Table S1: Climatic conditions of experimental sites during production season; Table S2: Effect of fertilization on sugar-acid ratio (SAR) in the field and greenhouse; Table S3: Effect of fertilization on phenolic compounds in the field and greenhouse.

Author Contributions: F.L. designed the experiment and wrote the manuscript; F.L. and Y.Y. determined the experimental indicators and analyzed the data; F.L., J.M. and N.S. revised the manuscript; F.L., Y.Y., N.H., X.L. and R.B. were involved in the field and greenhouse experiments; F.L. and N.S. provided funding; N.S. supervised. All authors have read and agreed to the published version of the manuscript.

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