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Yield and Quality of Rice under the Effects of Digestate Application

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Abstract: As a major measure to handle livestock manure, digestate is the by-product during biogas production in anaerobic fermentation. Digestate can be returned to cropland as a replacement for chemical fertilizer regarding its cost-effectiveness and rich nutrient content. However, the optimal rates of digestate to substitute chemical fertilizer have not been validated academically. A field study on nine treatments of no fertilizer, chemical fertilizer, and digestate at different rates was conducted to investigate the effects of substituting chemical fertilizer with digestate. The results revealed that replacing chemical fertilizer with liquid digestate did not significantly affect the rice growth regarding the maximum number of seedlings, plant height, tiller numbers, spikelets numbers, ear length, the number of grains per spike, and grain yields. However, improvements were found in the maximum number of seedlings, plant height, tiller number spikelet numbers, the area of the second and third backward leaves, grain yields, and quality when liquid and solid digestate were combined. Furthermore, taking the nutrient inputs, rice growth, grain yield, and quality into consideration, applying liquid digestate of 150 t ha^{-1} and 75 t ha^{-1} of liquid combined with 15 t ha^{-1} solid digestate was suggested for rice production at the study venue.

Keywords: liquid digestate; solid digestate; rice growth; rice grain quality

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1. Introduction

To ensure food security in China, cropland areas with high input of chemical fertilizer and intensive livestock husbandry have grown rapidly in the last four decades, which has broken down the nutrients cycling between crop and livestock systems [1,2]. Conversely, intensive and continuous application of chemical fertilizer causes soil degradation and air and water pollution, including soil acidification [3], soil salinization [4], N_2O emission [5], and nitrate leaching [6].

Meanwhile, intensive livestock farming produced roughly 3800 million tons of fresh weight-based excrement each year [7], causing ecological and environmental deterioration. For example, a previous study revealed that 42% of the nitrogen in water bodies was derived from livestock production in China [8]. Thus, as an important measure to handle livestock excrement, the manure-to-energy method (anaerobic digestion), which can convert livestock and poultry waste into bioenergy (biogas) for power generation, was encouraged by the government [9,10]. However, the large amount of digestate produced from anaerobic digestion is confronted with dire challenges [11]. In many countries and regions, people have been encouraged to apply digestate in cropland instead of chemical fertilizer due to its low cost-effectiveness and rich contents of nitrogen, phosphorus, potassium, and other microelements and microorganisms [12,13]. However, there was no agreement regarding the substitution of chemical fertilizer with digestate on crop yield and grain quality. Rahaman et al., (2021) [1] found that digestate decreased maize yield when used to fully substitute mineral N fertilizer. Whereas, Xu et al. (2019) [11] revealed increased yield and improved quality of Chinese cabbage after liquid digestate substitution of chemical N fertilizer. It seems that climatic factors, soil properties, crop types, and the anaerobic digestion of feedstock-produced digestate can influence the effect of digestate on crop yield and grain quality.

Rice (*Oryza sativa* L.) is a very important food crop in the world and is widely cultivated in Southeast Asia, India, and China. In South and Northeast China, the reuse of digestate as fertilizer is common practice for rice production, where rice cultivation and intensive livestock husbandry are combined. Therefore, in this study, different amounts of digestate were used as a fertilizer substitute in plot experiments. The influence of digestate on rice growth, grain yield, and quality was studied with the specific objectives of the present work including: (i) investigating the effects of digestate application on the agronomic traits and grain quality of rice; (ii) investigating feasible rates of digestate for rice production in the study region.

2. Materials and Methods

2.1. Study Site

The study site is located in Dianjiang, Chongqing City (30°20′ N, 107°25′ E). The region has a warm and subtropical climate with a continental monsoon. The annual average rainfall was 900 mm, and the annual average temperature was 16.8 °C (Figure 1). The soil is paddy soil with a silt loam texture. The physical and chemical characteristics of the soil at a depth of 0–20 cm were as follows: total nitrogen (N) 1.2 g kg^{-1}, total phosphorus (P) 0.3 g kg^{-1}, total potassium (K) 23.1 g kg^{-1}, hydrargyrum (Hg) 0.1 mg kg^{-1}, arsenic (As) 5.9 mg kg^{-1}, copper (Cu) 41.6 mg kg^{-1}, zinc (Zn) 95.9 mg kg^{-1}, lead (Pb) 85.9 mg kg^{-1}, cadmium (Cd) 3.8 mg kg^{-1}, with pH 6.2 and electrical conductivity 125.1 μS cm^{-1}.

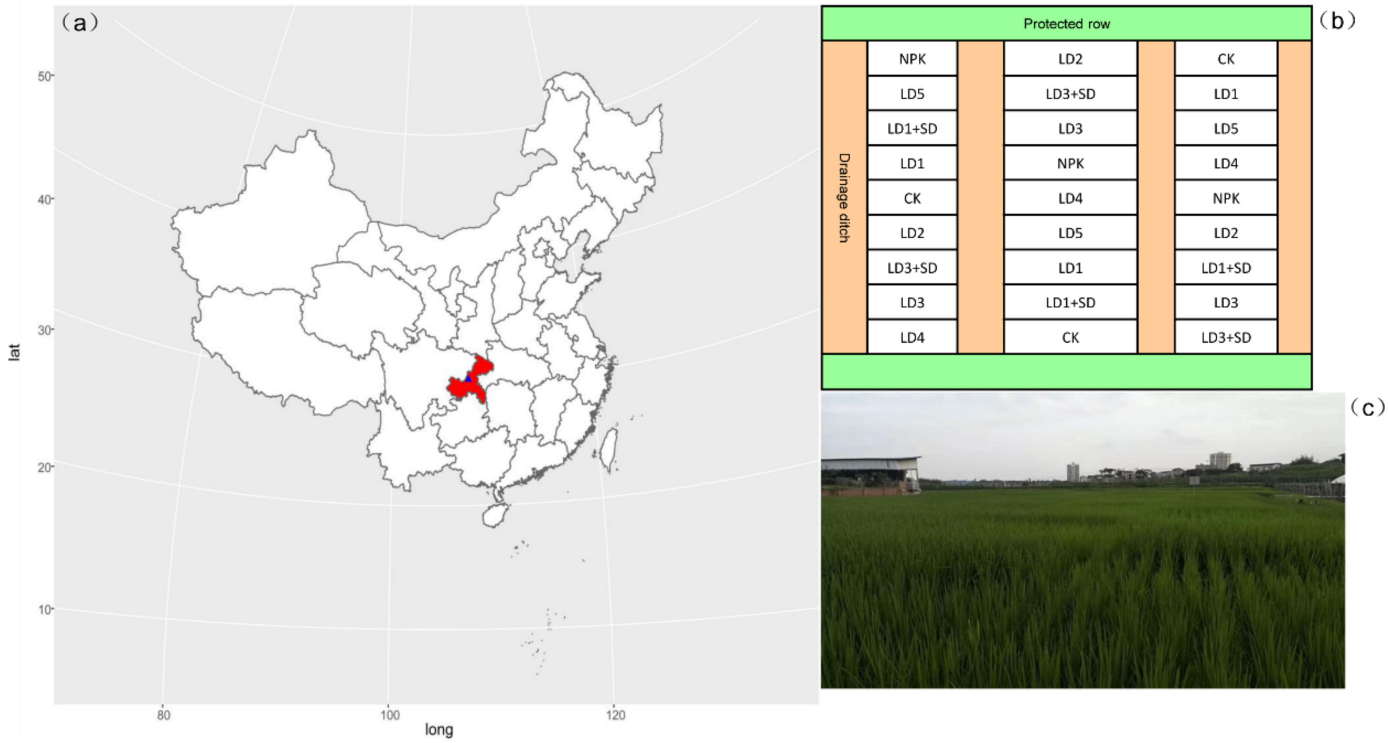

Figure 1. The site (**a**), plot distribution (**b**), and picture (**c**) for the field experiment, respectively. CK, NPK, LD1, LD2, LD3, LD4, LD5, LD1 + SD, and LD3 + SD represent control, chemical fertilizer, liquid digestate (LD1, 150 t ha^{-1}; LD2, 225 t ha^{-1}; LD3, 300 t ha^{-1}; LD4, 375 t ha^{-1}; LD5, 450 t ha^{-1}), and liquid digestate combined with solid digestate, respectively.

2.2. Field Experiment

A total of nine treatments were used. The nine treatments were: (1) no fertilizer (CK); (2) chemical fertilizer with N 200 kg N ha^{-1}, P 80 kg P_2O_5 ha^{-1}, and K 150 kg K_2O ha^{-1} (NPK); (3) liquid digestate 75 t ha^{-1} (LD1); (4) liquid digestate 150 t ha^{-1} (LD2); (5) liquid digestate 225 t ha^{-1} (LD3); (6) liquid digestate 300 t ha^{-1} (LD4); (7) liquid digestate 375 t ha^{-1} (LD5); (8) liquid digestate 75 t ha^{-1} combined with solid digestate 15 t ha^{-1} (LD1 + SD), and; (9) liquid digestate 225 t ha^{-1} combined with solid digestate 15 t ha^{-1} (LD3 + SD), and every treatment had three replications in a randomized complete block design (Figure 1). Each of the plots was 12 m^2 (6 m × 2 m).

Rice was planted on 15 March and harvested on 5 August 2019. Solid digestate was applied as basal fertilizer on 5 March. Liquid digestate and chemical fertilizer was divided into three segments as well as basal, tillering, and earing fertilizer. In the basal stage, tillering stage, and earing stage, 40%, 40%, and 20% of the chemical fertilizer and liquid digestate were used, respectively. The digestate was from a swine farm beside the study site. The properties of digestate are given in Table 1.

Table 1. The properties of digestate.

Terms	Total N (g kg^{-1})	Total P (mg kg^{-1})	Total K (mg kg^{-1})	Ammonium N (mg kg^{-1})	Nitrate N (mg kg^{-1})	DOC (mg kg^{-1})
Liquid digestate	2.0	533	799	1200	10.1	506
Solid digestate	10.1	7200	9272	266	8.5	7466

DOC, dissolved organic carbon.

2.3. Sampling and Analysis

On 18 March and 25 April, an investigation was done on the basic and the highest seedlings, respectively. On 15 June, an investigation was conducted on the plant height, leaf length, leaf width of the flag, and the second backward and third backward leaves by measuring 15 plants in each plot, respectively. Moreover, at the maturity stage, rice (1 m × 1 m) was sampled to investigate tiller numbers, spikelet numbers, ear length, number of grains per spike, seed setting rate, and grain yield, respectively. Then, rice grain was dried (65 °C), finely grounded, and digested with HNO_3 and HF [14] to measure the content of Hg, As, Cu, Pb, and Cd, respectively. The amylose content of rice grain was determined by spectrophotometer (Shimadzu, Kyoto, Japan) [15].

2.4. Statistical Analysis

The original data were analyzed using Microsoft Excel 2007 (Office 2007, Microsoft Corporation, Redmond, WA, USA). The differences in rice growth, yield, and quality among different treatments were analyzed using the Duncan multiple comparison test (SSR) at the 5% level in SPSS 18.0 (SPSS 18.0, IBM Corporation, NY, USA). The analysis of correlation relationships between spikelet numbers, ear length, number of grains per spike, seed setting rate, and rice yields were carried out using Origin Pro 8.0 (Origin Pro 8.0, Origin Lab Corporation, Northampton, MA, USA).

3. Results

3.1. Rice Growth

The basic seedlings ranged from 625 ± 140 thousand plants per hectare to 800 ± 78 thousand plants per hectare, with no significant difference among all the treatments. The highest seedlings significantly increased from 2.5 ± 0.3 million plants to 3.5 ± 0.4 million plants per hectare after chemical fertilizer and liquid digestate application. Moreover, the highest seedlings occurred in liquid digestate combined with solid digestate treatment (LD3 + SD), which were significantly higher than those of chemical fertilizer treatment (NPK) (Figure 2).

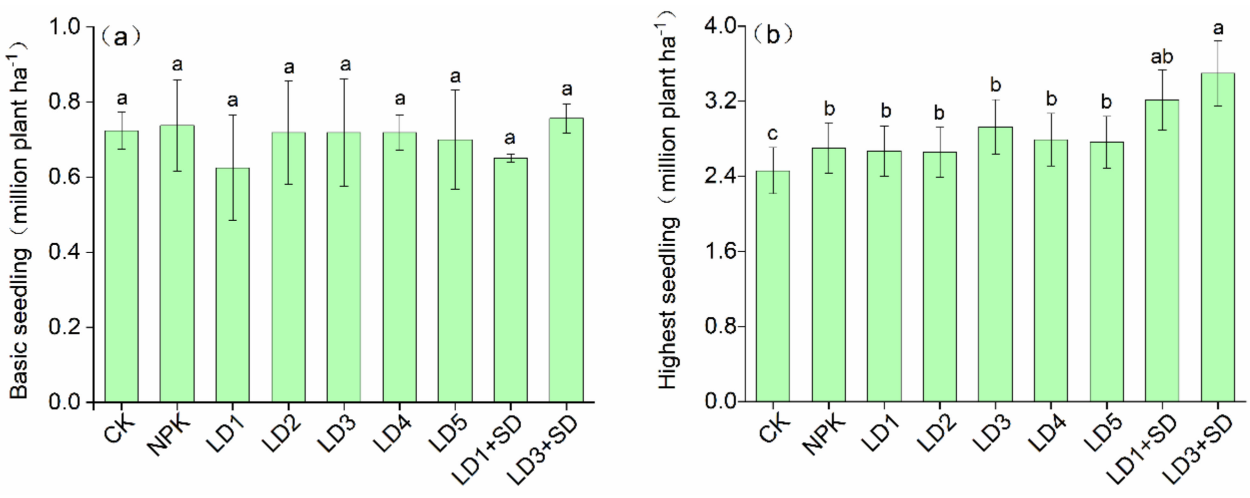

Figure 2. The basic (**a**) and the highest (**b**) seedlings under different treatments, respectively. The values are means with standard error bars. Different letters above the bars indicate significant differences at the 5% level.

The plant height was 143 ± 3–146 ± 3 cm for LD1 + SD and LD3 + SD treatments, followed by liquid digestate treatments (LD1, LD2, LD3, LD4, and LD5) of 127 ± 4–136 ± 7 cm, chemical fertilizer treatment (NPK) of 135 ± 2 cm and no fertilizer treatment (CK) of 123 ± 6 cm, respectively (Figure 3a). In addition, the tiller numbers significantly increased by 8–19% after chemical fertilizer or liquid digestate application, compared with no fertilizer treatment. The tiller numbers further significantly increased by 18–29% after liquid and solid digestate application compared with only chemical fertilizer inputs (Figure 3b).

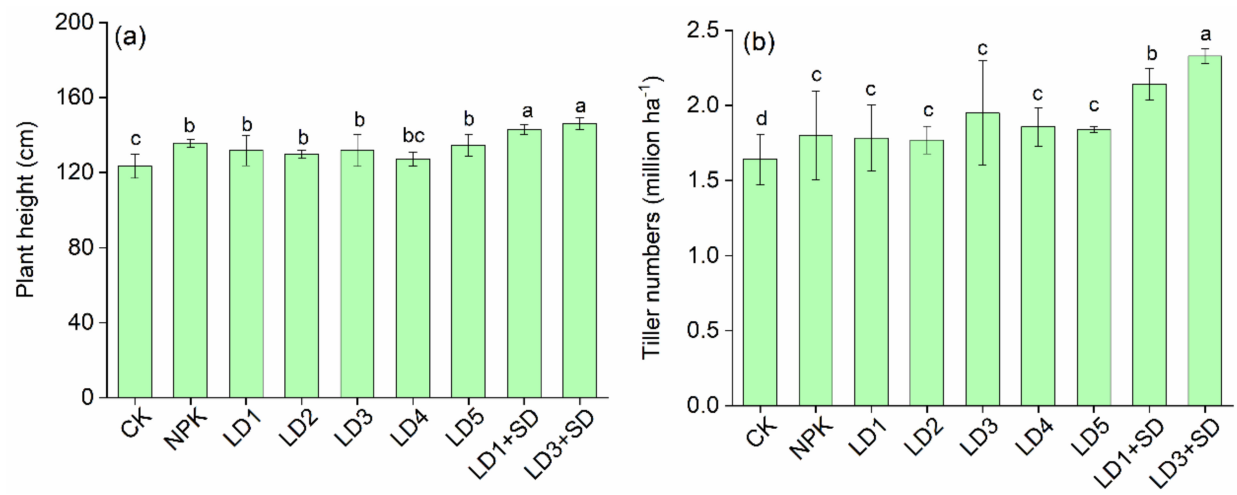

Figure 3. Rice plant height (**a**) and tiller numbers (**b**) under different treatments, respectively. The values are means with standard error bars. Different letters above the bars indicate significant differences at the 5% level.

The leaf length, width, and areas of the flag leaf, second backward leaf, and third backward leaf increased after chemical fertilizer (NPK), liquid and solid digestate (LD1, LD2, LD3, LD4, LD5, LD1 + SD and LD3 + SD) application in comparison with no fertilizer treatment. However, there were no significant differences in leaf length, width, and areas of flag leaf between chemical fertilizer and liquid and solid digestate treatments. The leaf length, width, and areas increased by 3–15%, 11–15%, and 20–36% for the second backward leaf, and 1–16%, 0–26%, and 23–29% for the third backward leaf after liquid and solid digestate application in comparison with chemical fertilizer treatment (Table 2).

Table 2. The leaf length and width and leaf area of rice plants under different treatments, respectively.

Treatments	Flag Leaf			The Second Backward Leaf			The Third Backward Leaf		
	Leaf Length (cm)	Leaf Width (cm)	Leaf Area (cm^2)	Leaf Length (cm)	Leaf Width (cm)	Leaf Area (cm^2)	Leaf Length (cm)	Leaf Width (cm)	Leaf Area (cm^2)
CK	30.3 ± 6.0 c	1.8 ± 0.1 b	72.2 ± 16.8 b	56.7 ± 2.5 c	1.7 ± 0.1 b	97.5 ± 4.4 c	55.3 ± 4.4 b	1.4 ± 0.1 b	79.4 ± 8.2 c
NPK	40.3 ± 4.4 ab	2.2 ± 0.2 a	88.7 ± 17.7 a	59.5 ± 7.2 b	1.7 ± 0.1 b	103.2 ± 18.5 c	56.4 ± 4.3 ab	1.4 ± 0.1 b	82.0 ± 4.1 c
LD1	38.3 ± 4.6 b	2.2 ± 0.3 a	84.3 ± 15.3 a	60.0 ± 4.2 b	1.8 ± 0.0 a	105.7 ± 7.4 c	58.3 ± 3.8 ab	1.5 ± 0.0 b	87.3 ± 4.8 b
LD2	42.3 ± 5.0 ab	2.2 ± 0.2 a	88.4 ± 8.5 a	58.3 ± 2.2 b	1.7 ± 0.1 b	99.8 ± 6.7 c	55.7 ± 2.1 b	1.4 ± 0.1 b	80.3 ± 9.4 c
LD3	39.0 ± 5.2 b	2.1 ± 0.2 a	83.0 ± 14.9 a	59.3 ± 5.7 b	1.8 ± 0.1 a	106.1 ± 15.8 c	59.7 ± 6.2 ab	1.6 ± 0.1 ab	94.0 ± 17.2 b
LD4	41.0 ± 6.1 ab	2.2 ± 0.3 a	96.5 ± 12.3 a	62.1 ± 4.5 b	1.8 ± 0.1 a	110.9 ± 15.1 c	59.1 ± 3.7 ab	1.5 ± 0.1 b	91.5 ± 10.9 b
LD5	44.3 ± 4.3 ab	2.2 ± 0.2 a	89.6 ± 21.1 a	58.3 ± 6.9 b	1.8 ± 0.1 a	102.8 ± 17.2 c	56.7 ± 3.6 ab	1.4 ± 0.0 b	82.0 ± 6.0 c
LD1 + SD	48.3 ± 5.0 a	2.4 ± 0.2 a	106.2 ± 13.3 a	65.1 ± 2.7 ab	1.9 ± 0.2 a	124.1 ± 13.7 b	64.4 ± 1.6 a	1.6 ± 0.1ab	106.1 ± 12.0 ab
LD3 + SD	43.3 ± 6.4 ab	2.3 ± 0.3 a	99.7 ± 20.6 a	68.6 ± 1.6 a	2.0 ± 0.1 a	139.9 ± 3.8 a	61.3 ± 4.3 a	1.9 ± 0.4 a	116.3 ± 7.9 a

CK, no fertilizer; NPK, chemical fertilizer; LD1, liquid digestate 75 t ha^{-1}; LD2, liquid digestate 150 t ha^{-1}; LD3, liquid digestate 225 t ha^{-1}; LD4, liquid digestate 300 t ha^{-1}; LD5, liquid digestate 375 t ha^{-1}; LD1 + SD, liquid digestate 75 t ha^{-1} combined with solid digestate 15 t ha^{-1}; LD3 + SD, liquid digestate 225 t ha^{-1} combined with solid digestate 15 t ha^{-1}. Different letters in the same column indicate significant differences at the 5% level.

3.2. Rice Yield

The rice spikelet numbers were 13 ± 1–19 ± 2 million per hectare, and the highest spikelet numbers occurred in LD1 + SD and LD3 + SD treatments, which were significantly higher than those for CK and NPK treatments (Figure 4a). The number of grains per spike was 167 ± 20 for CK treatment, and the number of grains per spike significantly increased by 8–37% after chemical fertilizer and liquid and solid digestate application (Figure 4c). However, there were no significant differences in rice ear length and seed setting rate among all the treatments (Figure 4b,d).

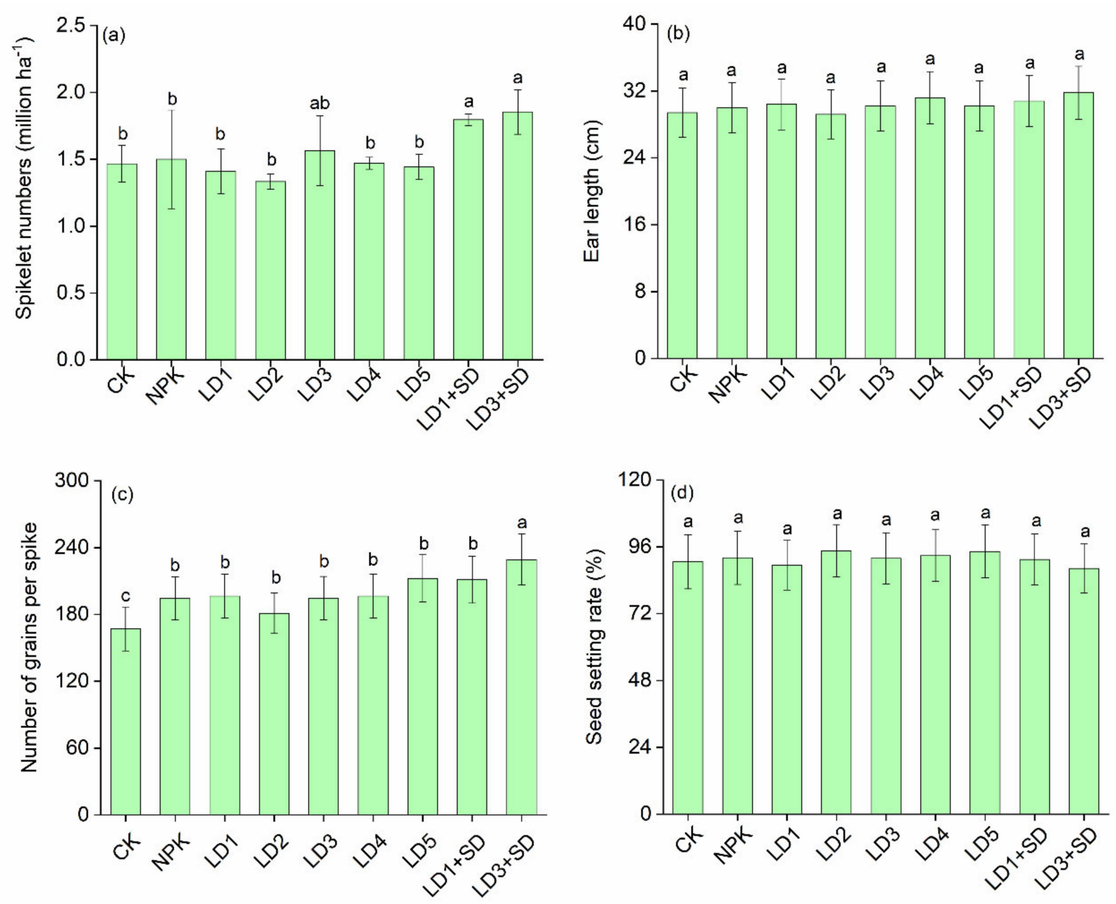

Figure 4. Rice spikelet numbers (**a**), ear length (**b**), numbers of grains per spike (**c**), and seed setting rate (**d**) under different treatments, respectively. The values are means with standard error bars. Different letters above the bars indicate significant differences at the 5% level.

The rice yields were 7.1 ± 0.4 t ha^{-1} for CK treatment and significantly increased by 13–41% with chemical fertilizer or liquid and solid digestate inputs. The highest yields were 9.9 ± 0.2 t ha^{-1} for LD3 + SD treatment (Figure 5a). Moreover, the rice yields were positively correlated with spikelet numbers (R^2 = 0.319, p < 0.01) and numbers of grains per spike (R^2 = 0.584, p < 0.01), respectively (Figure 5b,c).

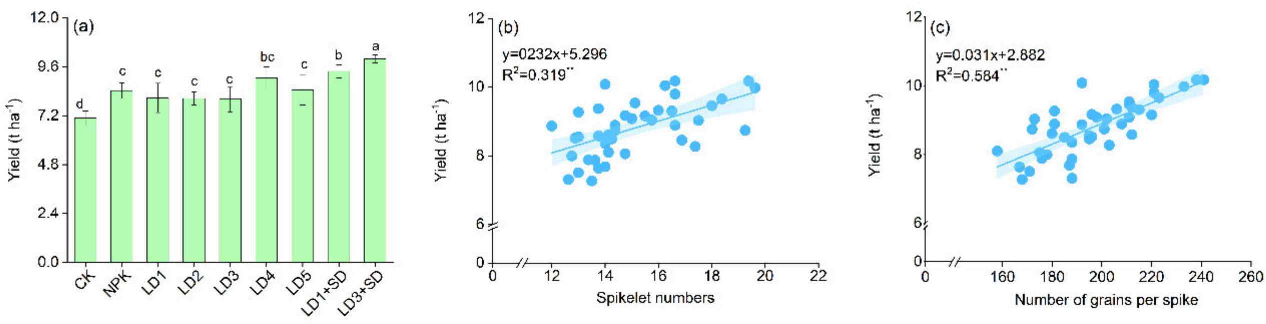

Figure 5. Rice yield (**a**) and the relationships between spikelet numbers (**b**), number of grains per spike (**c**), and rice yield, respectively. The values are means with standard error bars. Different letters above the bars indicate significant differences at the 5% level.

3.3. Rice Quality

The amylose contents of rice grains were 24–26% for CK and NPK treatments, without significant differences between them. The amylose contents significantly decreased to 20–22% with liquid and solid digestate inputs for LD5, LD1 + SD, and LD3 + SD treatments, respectively (Figure 6).

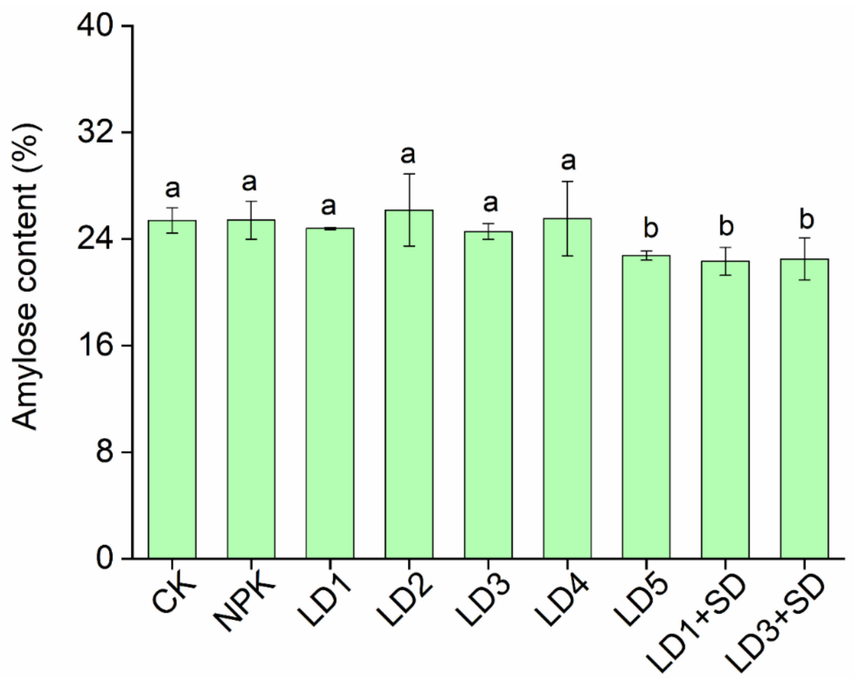

Figure 6. The amylose content of rice grains under different treatments, respectively. The values are means with standard error bars. Different letters above the bars indicate significant differences at the 5% level.

The As, Pb, and Cd contents of rice grains were 0.024 ± 0.000–0.046 ± 0.014 mg kg^{-1}, 0.063 ± 0.001–0.163 ± 0.032 mg kg^{-1}, and 0.072 ± 0.026–0.155 ± 0.045 mg kg^{-1} for all the treatments, respectively. The Hg in rice grains was only detected for LD5 treatment.

However, the As, Hg, Pb, and Cd contents of rice grains were not exceeding the national standard limit for all treatments (Table 3).

Table 3. The arsenic (As), mercury (Hg), lead (Pb), and cadmium (Cd) content in rice grains under different treatments, respectively.

Treatments	As (mg kg^{-1})	Hg (mg kg^{-1})	Pb (mg kg^{-1})	Cd (mg kg^{-1})
CK	ND	ND	0.157 ± 0.044 b	0.145 ± 0.010 a
NPK	0.043 ± 0.001 c	ND	0.063 ± 0.001 d	0.083 ± 0.006 bc
LD1	0.033 ± 0.002 d	ND	0.099 ± 0.041 c	0.106 ± 0.012 b
LD2	0.046 ± 0.014 bc	ND	0.125 ± 0.018 c	0.072 ± 0.026 c
LD3	0.024 ± 0.000 e	ND	0.105 ± 0.030 c	0.153 ± 0.109 a
LD4	0.027 ± 0.003 e	ND	0.095 ± 0.003 c	0.110 ± 0.045 b
LD5	0.027 ± 0.003 e	0.010 ± 0.001	0.163 ± 0.032 a	0.155 ± 0.045 a
LD1 + SD	ND	ND	0.118 ± 0.017 c	0.090 ± 0.045 b
LD3 + SD	ND	ND	0.157 ± 0.010 b	0.109 ± 0.002 b
National standard limit	0.200	0.020	0.200	0.200

CK, no fertilizer; NPK, chemical fertilizer; LD1, liquid digestate 75 t ha^{-1}; LD2, liquid digestate 150 t ha^{-1}; LD3, liquid digestate 225 t ha^{-1}; LD4, liquid digestate 300 t ha^{-1}; LD5, liquid digestate 375 t ha^{-1}; LD1 + SD, liquid digestate 75 t ha^{-1} combined with solid digestate 15 t ha^{-1}; LD3 + SD, liquid digestate 225 t ha^{-1} combined with solid digestate 15 t ha^{-1}. ND, not detected. Different letters in the same column indicate significant differences at the 5% level.

4. Discussion

4.1. Effect of Digestate on Rice Growth

Digestate (including liquid and solid digestate) is known to be a promising source of energy needed for crop growth [16,17]. We applied digestate to rice, and it had positive effects on rice plant height and tiller numbers when liquid digestate and solid digestate combined. Similarly, Haileand Ayalew (2018) [18] found that liquid digestate produced higher plant height and biomass yield. Win et al. (2014) [19] reported that liquid digestate produced more dry matter in rice in comparison to chemical fertilizer. The potential mechanisms related to the following factors: (1) digestate often contains several beneficial substances for plant growth beyond nitrogen (N), phosphorus (P), and potassium (K), including microelements and amino acids, which promote crop metabolism, plant tissue, and organ development [11,20]; (2) digestate increases soil organic carbon content, changes soil microbial community structure and microbial activity [21,22], and thus improves plant growth; (3) application of alkaline digestate (liquid digestate, pH = 7.8; solid digestate, pH = 7.3) can improve soil acidification status and crop growth.

The ammonium and nitrate N contents in liquid and solid digestate were 1210 mg N L^{-1} and 275 mg N kg^{-1}, accounting for 81% and 28% of the total N, respectively. Thus, liquid and solid digestate might be considered available and slow-release nutrients for crop growth. Integrated liquid digestate with solid digestate achieved better crop growth and grain yields than a separated application of liquid digestate or chemical fertilizer, due to the longer and shorter period of nutrient availability for crop growth [23]. Similarly, Rahaman et al., (2008) [24] found that the maximum grain yield is obtained from simultaneous usage of liquid digestate and chemical fertilizer.

4.2. Effect of Digestate on Rice Yield and Quality

In this study, as well as several other studies, it was found that a reasonable application of digestate increased crop yield [11,25]. Our study revealed that the higher spikelet numbers and numbers of grains per spike were responsible for the increased crop yield after digestate input (Figure 5). It is well known that ammonium N promotes tillering [26]. The ammonium N in liquid digestate might be more effective for rice, because of its dissolved state and irrigation application method. Therefore, liquid digestate can increase rice spikelet numbers, and thereby grain yield. In addition, digestate has rich zinc and calcium. Zinc and calcium are beneficial for rice tillering, photosynthesis and grain yield [27].

The amylose content of rice grain is one of the important indexes for evaluating rice quality [28,29]. A lower amylose content indicates a higher quality of rice grain. The amylose content significantly decreased by 15–17% after digestate application, which means that the application of digestate is better for rice quality. Similarly, Wu et al. (2013) [30] found that irrigation with liquid digestate could effectively increase oil-seed yield and quality. Xu et al. (2019) [11] expounded that irrigation with suitable concentrations of liquid digestate significantly increased the soluble sugar and vitamin C content in Chinese cabbage. It seemed that microelements in digestate [31] and multiple auximones such as indoleacetic acid and cytokinin released by microbial pathogens facilitated crop growth and grain quality [32].

Some studies regarded heavy metal residue in crops after digestate application [33]. Fortunately, the heavy metal contents of As, Pb, Cd, and Hg in rice grain did not exceed the maximum permissible level in this study [34]. However, caution should be taken in terms of the result because there was only one season of study. Meanwhile, consecutive monitoring of heavy metal residue in the soil is needed to avoid soil pollution in further studies.

4.3. Implications and Suggestions

The total averages of N, P_2O_5, and K_2O were 150–750 kg N ha^{-1}, 40–200 kg P_2O_5 ha^{-1}, and 60–300 kg K_2O ha^{-1} for liquid digestate treatments; for liquid and solid digestate treatments, they were 150–450 kg N ha^{-1}, 148–188 kg P_2O_5 ha^{-1}, and 152–212 kg K_2O ha^{-1}, respectively. Moreover, in this study, the substitution of chemical fertilizer with digestate did not affect or even significantly increase rice growth, grain yield, and quality. Therefore, taking the nutrient inputs, rice growth, rice yield, and quality into consideration, irrigation with liquid digestate of 150 t ha^{-1} and that of 75 t ha^{-1} combined with solid digestate of 15 t ha^{-1} was suggested in this study.

Namely, the substitution of chemical fertilizer with liquid digestate for 10% of rice could consume 0.5 billion tons of liquid digestate and save 540 thousand tons of N fertilizer, accounting for approximately 50% and 2% of liquid digestate production and N consumption in China, respectively. However, attention should be paid to the negative effects of substituting chemical fertilizer with digestate in rice production. For example, the substitution of chemical fertilizer with digestate may promote CH_4 and CO_2 emissions, owing to the increased available carbon for microorganisms [35]. Moreover, the leaching of dissolved organic carbon must be taken into account, as the total averages of dissolved organic carbon were 38–188 kg C ha^{-1} from digestate.

5. Conclusions

In this study, the substitution of chemical fertilizer with liquid digestate did not affect rice growth and grain yield. Moreover, the rice yield and quality significantly increased when liquid and solid digestate were combined. Taking the nutrient inputs, rice growth, rice yield, and quality into consideration, irrigation with liquid digestate of 150 t ha^{-1} and that of 75 t ha^{-1} combined with solid digestate of 15 t ha^{-1} were suggested for rice production at the study venue. Meanwhile, consecutive monitoring of CH_4 and CO_2 emissions and leaching of dissolved organic carbon is needed to avoid water pollution after digestate application.

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Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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