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Impact of Digestate Application as a Fertilizer on the Yield and Quality of Winter Rape Seed

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Abstract: The operation of an agricultural biogas plant is associated with the formation of a large amount of digestate. The basic trend in digestate management, after taking into account its physicochemical properties, should be its use as a fertilizer. The possibility of the agricultural use of digestate as a fertilizer was determined. Digestate obtained from the agricultural biogas plant was tested for the content of macronutrients and heavy metals. The content of macronutrients was also determined in soil for winter rape cultivation. The analysis showed an increase in the yield depending on the digestate dose applied. In addition, an increase in the fat and protein content was found in winter rape seeds. The best results of mean values were obtained during the application of the 50,000 L ha^{-1} dose. The average diameter of the stem base was 1.48 cm. The average height was 36.20 cm. The seed yield was 3.44 t ha^{-1}. The thousand seed weight was—5.34 g. The fat and protein contents were 43.62% and 22.95%, respectively. By applying a digestate dose of 50,000 L ha^{-1}, the highest content of macronutrients, as well as polyunsaturated fatty acids (PUFA) (31.17%), monounsaturated fatty acids (MUFA) (61.89%) and saturated fatty acids (SFA) (6.87%), was recorded.

Keywords: digestate; winter rape; sustainability agriculture; fertilization

1. Introduction

Agricultural production, which aims at the production of food with good quality parameters, must be carried out in accordance with the environmental protection requirements. It is important to maintain the safe use of fertilizers in the agricultural environment, with particular regard to nitrogen fertilizers. Fertilizers used in a liquid form get into the soil solution faster and are better absorbed by plants.

Organic and mineral components in soil determine aggregation of particles with water and polyvalent cations. The aggregation degree affects the soil porosity, as well as the movement of water and air. To improve the soil structure stability, the most important procedure is to apply the organic matter. The fertilization of fields with organic matter affects the chemical and physical fertility of the soil [1,2].

Anaerobic fermentation is a promising process in the agricultural practice. It can reduce the negative impact of waste on the environment. During anaerobic fermentation, the total nitrogen is preserved, and complex organic nitrogen compounds are converted to the ammonium form (this is a pre-sowing form of nitrogen, is well retained in soil, is absorbed more slowly by plants, and works well at low temperatures). [3]. It is considered a sustainable and environmentally friendly process [4]. One of the by-products generated during anaerobic fermentation is digestate [5,6]. Depending on the content of metals and other compounds, it can be considered as waste that should be treated or used as a potential source of raw materials for the chemical industry, biotechnology, or agriculture [7].

As waste, digestate may be subjected to a disposal process, but it is generally recommended to carry out a recovery process. The most commonly used method of digestate pulp treatment is recovery using the R 10 method—"surface treatment for the benefit of agriculture or improvement of the state of environment" [8]. Therefore, the digestate may also be used as a soil quality improvement agent [9].

After taking into account its physicochemical properties, one should strive to use digestate as a bio-fertilizer [10,11]. The beneficial effect of digestate is manifested by improving the soil nutrients [12]. A biogas plant located in agricultural areas should receive from farmers organic products generated on their farms. In contrast, farmers, taking care of the soil quality on their farms, should receive digestate produced from the biogas plant and use it for fertilization purposes [10,13]. The digestate used as a fertilizer improves the soil fertility, quality, and resistance of plants to biotic and abiotic factors [11,14]. The digestate can be directly spread in the field or treated to separate solids and liquids [15].

In terms of the action rate (components uptake by plants), it is similar to mineral fertilizers, because the N, P and K components are easily available to plants. The digestate also contains a portion of organic matter that has a positive effect on the physicochemical properties of the fertilized soils [10,16].

The aim of the study is to select a digestate dose and analyze the response of "Artoga" winter rape to its different doses.

2. Materials and Methods

The object of the study included winter rape plants and seeds obtained from them, grown in the field conditions in the growing seasons 2016/2017, 2017/2018 and 2018/2019. A soil sample was also tested determining its pH and macronutrient content, both before sowing and after harvesting. Digestate samples for the heavy metal content were also tested. The tests were carried out at the District Chemical and Agricultural Station in Lublin according to PN-EN ISO/IEC 17025: 2005.

The winter rape seeds (*Brassica napus spp oleifera* var. *biennis*) of the "Artoga" cv. obtained from Limagrain company were used in the experiments. Soil samples originated from the arable land of the Experimental Farm (University of Life Sciences in Lublin) in Czesławice (Lubelskie Voivodeship, 51°18′23″ N, 22°16′2″ E). The soil samples were taken with Egner Riehm soil sticks from the 0–20 cm soil layer from 12 experimental plots, and then mixed to obtain representative samples.

The field experiment was established on medium soil of sandy dust granulation. The area of the experimental plots was 75 m^2. In the experiment, seeds of winter rape "Artoga" (Limagrain)—a hybrid cultivar, which is characterized by a very high seed yield, were sown. The glucosinolate content is average, the seed fat content is quite high, the protein content in dry non-fat matter is medium, and rosettes after winter are large. Plants are tall with medium lodging resistance. This information is prepared by the breeder.

The average (out of 10 measurements) plant density per 1 m^2 was 35 plants (2017), 48 plants (2018) and 30 plants (2019). The average plants density was measured in spring.

Polifoska 6 mineral fertilizer (with 6% nitrogen (N) content as ammonium, 20% phosphorus (P_2O_5), 30% potassium (K_2O)), was applied before sowing. The following doses of Polifoska 6 were used: 2017—350 kg ha^{-1}; 2018—350 kg ha^{-1}; and 2019—350 kg ha^{-1}.

Plant protection treatments were performed using a Pilmet 312 LM suspended field sprayer. The dose of liquid during the treatments was 270 L ha^{-1}, the working speed 5.4 km h^{-1}, and the working pressure 3.0 bar. The height of the boom was 50 cm above the sprayed surface.

Winter rape seeds were sown in the field after wheat, where stubble cultivation was carried out to the depth of 12 cm, then plowing at the depth of 20 cm. The field was leveled with a tilling set consisting of a light tine cultivator and a string roller. Seeds were sown with a seedbed consisting of a cultivator, packer tine rollers and a seeder. The seed drill was equipped with disc coulters. The sowing depth was 2 cm. The sowing date in 2016 and 2017 was 24 August, and in 2018 it was 23 August.

The scope of the field tests covered 4 variants of the experiment, on which digestate has been spilled (spilled using a slurry tanker with an adapter with hoses on the soil surface):

variant I—the total digestate dose 25,000 L ha^{-1} (I dose—12,500 L ha^{-1}, II dose—12, 500 L ha^{-1});
variant II—the total digestate dose 37,500 L ha^{-1} (I dose—18,750 L ha^{-1}, II dose—18, 750 L ha^{-1});
variant III—the total digestate dose 50,000 L ha^{-1} (I dose—25,000 L ha^{-1}, II dose—25, 000 L ha^{-1});
variant IV—a control object, which was sown with seeds that were not fertilized with digestate.

The above doses were established based on the soil samples and other authors' studies [3,17].

Fertilization using digestate was carried out in spring and two doses were applied:

- the first dose at BBCH 30 phase (main shoot elongation—beginning of shoot elongation, no internodes (rosette),
- the second dose at BBCH 33 phase (main shoot elongation—three internodes visible).

The digestate was taken from Piaski biogas plant (Lubelskie Voivodeship, 51°8′16″ N, 22°53′39″ E). The following raw materials were used in the production of digestate: maize silage (70%), sugar beet pulp (15%), fruit pomace (5%), dairy waste (5%) and manure (5%).

At the turn of the second and third week of July, rape was harvested with a single-stage Wintersteiger plotter combine-harvester of KM-ELITE type. The harvested seeds were purified on PETKUS K212 faucet. After cleaning, the seeds were stored in jute bags for two weeks in laboratory rooms at the temperature of 20 °C, mixing each day to equalize humidity throughout the seed mass, and then analyzed.

Tests of the seed quality after harvesting of winter rape were based on the determination of the seed moisture, fat, protein, and macronutrient content (N, P, K, Ca, Mg), and the fatty acid profile. The above tests were carried out at the Central Agroecological Laboratory of the University of Life Sciences in Lublin.

The fat content was determined using Soxtec 8000 apparatus. The Soxhlet method involves the repeated continuous extraction of a fatty substance from the crushed and previously dried product, the use of an organic solvent that is removed and determination of a fatty substance by the weight method.

The nitrogen content was determined by titration (Kjeldahl). This method involves the sample mineralization in the concentrated sulfuric acid with the addition of selenium as a catalyst, hydrogen peroxide as an oxidant (if necessary) and potassium sulfate to increase the boiling point of sulfuric acid. After sample mineralization, the nitrogen contained occurs in the form of ammonium sulfate.

The phosphorus content was determined based on the spectrophotometric method. Spectrophotometric methods for the determination of elements are based on the absorption of visible radiation and are one of the most precise methods of analysis. The basis of spectrophotometric determinations is the relationship between the absorbance of the solution and the concentration of the colored substance in the solution. For the spectrophotometric determination of an element, it is converted into a colored complex, and sometimes the color of the ions of the element itself is also used.

Contents of potassium, calcium and magnesium were determined based on the method of flame atomic absorption spectrometry (FAAS). Atomic absorption spectrometry (AAS) is one of the most widely used techniques.

The determination of fatty acid composition was carried out using gas chromatography with flame ionization detection (GC-FID). Fatty acid separation and determination were carried out on a Varian 45-GC gas chromatograph. To control the chromatograph, collect, integrate and convert the results, the Galaxie™ Chromatography Data System software was applied using a flame ionization detector (FID), a 30 m capillary column (internal diameter 0.32 mm, Supelcowax 10 liquid phase, film thickness 0.25 m) and injector (1177 Split/Splitless) at 250 °C injector and 300 °C detector temperature, Select TM Biodiesel for FAME (Agilent Technologies) capillary column with dimensions L × ID × OD respectively: 30 m × 0.32 mm × 0.45 mm. The carrier gas was helium (flow rate 1.0 cm^3 min^{-1}).

The plant height was measured from the ground to the end of the main shoot (BBCH 30 ÷ BBCH 39 phase). The diameter was measured at the base of the stem (BBCH 30 ÷ BBCH 39 phase). The chlorophyll concentration in rapeseed was determined as the SPAD value using a manual chlorophyll-meter based on the absorption measurement (SPAD 502 Plus from Konica Minolta) (BBCH 30 ÷ BBCH 39 phase). The values indicated by the meter are proportional to the chlorophyll content of the leaf. They are calculated based on the amount of radiation passed through the leaf in two ranges of radiation differently absorbed by chlorophyll. The maximum absorption of radiation is on blue and red waves while the minimum absorption on green and infrared waves. The meter emits 650 and 94 nm wavelength light from the LED light source. The waves, after passing through the leaf blade, reach the receptor that converts them into an electrical signal. The amplified signal is converted into an electric signal and is used to calculate the SPAD value.

Measurements of a diameter, height of plant and chlorophyll were made before digestate application and after the first and second dose of digestate for a period of 14 days.

The economic effect of digestate application was computed based on the value of the yield increase resulting from the use of extracts and costs of their application. The income growth resulting from the use of extracts (O_{sb}) was calculated from the following formula:

$$O_{sb} = W_{pp} - K_{sb}, \left(\text{EUR}\cdot\text{ha}^{-1}\right) \tag{1}$$

where W_{pp} is the value of yield increase, (EUR·ha^{-1}), and K_{sb} is the cost of extracts use, (EUR·ha^{-1}).

The value of yield increase (W_{pp}) was computed acc. to the following formula:

$$W_{pp} = (P_{nb} - P_{nk})\cdot C_n, \left(\text{EUR}\cdot\text{ha}^{-1}\right) \tag{2}$$

where P_{nb} is the seed yield from the combination with extracts application, (t·ha^{-1}), P_{nk} is the seed yield from the control combination, (t·ha^{-1}), and C_n is the average price of seeds in a given study year, (EUR·t^{-1}).

Costs of the use of extracts (K_{sb}) were computed acc. to the following formula:

$$K_{sb} = k_b + k_z, \left(\text{EUR}\cdot\text{ha}^{-1}\right) \tag{3}$$

where k_b is the cost of extracts purchase, (EUR·ha^{-1}), and k_z is the cost of performing the treatment, (EUR·ha^{-1}).

Data used to compute income growth resulting from the application of digestate show in Table 1. The average purchase price was established based on information from wholesale markets. The cost of purchasing digestate was calculated as a mean price from 3 wholesale companies supplying farms. The water use cost was calculated based on the price of 1 m^3 of tap water in a village community in Lubelskie Province. The cost of treatment performance was calculated as an average price of the manure spreading with a 10000-L tank slurry tanker with ramp with dragged hoses.

Table 1. Data used to compute income growth resulting from the application of digestate.

Specification	Unit	Value
Price of winter rape seeds	EUR t^{-1}	376.55
Price digestate	EUR m^{-3}	1.15
Price of the application	EUR ha^{-1}	1.15

The obtained test results were subjected to statistical analysis. The Shapiro–Wilk test was used to check the normality of the variable distribution. The average values were determined for all variables and the Tukey method was applied for multiple comparisons. The analysis was performed at the significance level $p < 0.05$. All calculations were made using Statistica 13 software from Statsoft.

3. Results

Meteorological conditions prevailing during the cultivation of winter rape from Lublin—Radawiec station were analyzed. Table 2 presents weather conditions such as: parameters of atmospheric pressure, air temperature and amount of precipitation during the cultivation of winter rape.

Table 2. Weather conditions in 2016–2018.

Month	Average Monthly Temperature (°C)	Average Monthly Atmospheric Pressure (hPa)	Total Rainfall (mm)
		2016	
08	17.7	1019.0	45.3
09	15.1	1019.3	12.4
10	6.7	1021.7	116.3
11	2.2	1017.6	47.6
12	−2.0	1025.4	63.9
		2017	
03	5.4	1016.2	33.8
04	7.0	1016.1	55.8
05	13.5	1016.8	40.6
06	17.6	1013.3	30.2
07	18.1	1013.5	86.7
08	19.1	1017.7	55.1
09	13.6	1015.3	87.6
10	8.8	1015.6	92.4
11	3.7	1016.0	46.2
12	1.6	1013.1	29.3
		2018	
03	−6	1010.0	32.5
04	13.0	1015.1	46.7
05	16.7	1017.5	44.1
06	18.3	1014.4	65.4
07	19.9	1012.0	62.0
08	20.2	1017.4	25.6
09	15.3	1020.1	52.0
10	9.8	1019.4	36.5
11	3.5	1025.4	8.6
12	1.0	1018.0	68.5
		2019	
03	4.9	1015.1	22.1
04	9.4	1018.7	32.3
05	12.8	1012.1	101.5
06	21.3	1017.7	19.5
07	18.3	1012.5	29.7

Due to the high fat content, rape seed needs significantly more water than cereals to start germination, i.e., about 7–10 g of water, which is almost twice its weight. By imposing these amounts on the usually average field water content (60–70%) and not always favorable soil structure, the soil water dose needed for rape emergence is as high as 20–30 mm. In the analyzed period, the sum of rainfall was 45.3 mm (August) in the first year of study, 55.1 mm in the second year (August), and 25.6 mm (August) in the third year. Based on the above meteorological data, the optimal soil water content can be assumed. Adequate moisture is crucial because water activates a number of biochemical changes that lead to the formation of the germ and cotyledons. Their appearance above the soil surface means the correct start of the plant growth.

Depending on the soil moisture, rape seeds are saturated with water for 12 to 36 h. About 90 C-days (degree days—the sum of daily temperatures) is needed for the field emergence. In the first growing season 2016/2017, until rape emergence (about 14 days after sowing), the daily temperature value was 232.2 C-days. However, in the second growing season 2017/2018, the sum of daily temperatures was 234.4 C-days, and the third was 238.6 C-days. The above data indicate good emergence of winter rape.

Prior to the use, digestate was tested for the content of macronutrients and heavy metals (Table 3). The pH of digestate used for winter rape cultivation is equal to 8.70, which is close to the value of bovine manure (7.90).

Analyzing the obtained test results, the heavy metal content was below the detection limit of the measuring apparatus. In addition, digestate contains significant amounts of macronutrients, therefore it has been found possible to use digestate as a fertilizer.

Table 3. Comparison of selected macronutrients and heavy metals in digestate.

Trait Tested	Digestate for Winter Rape			
	2016/2017	2017/2018	2018/2019	Mean $\bar{x}$
Phosphorus [g L^{-1}]	0.09	0.13	0.12	0.11
Potassium [g L^{-1}]	5.25	4.20	5.18	4.88
Calcium [g L^{-1}]	0.25	0.34	0.31	0.3
Magnesium [g L^{-1}]	0.04	0.08	0.05	0.06
Cadmium [mg L^{-1}]	<0.43	<0.43	<0.43	<0.43
Lead [mg L^{-1}]	<0.43	<0.43	<0.43	<0.43
Nickel [mg L^{-1}]	<0.43	<0.43	<0.43	<0.43
Chromium [mg L^{-1}]	<0.43	<0.43	<0.43	<0.43
Copper [mg L^{-1}]	0.43	0.49	0.44	0.45
Zinc [mg L^{-1}]	2.01	1.90	1.92	1.94
Manganese [mg L^{-1}]	2.20	1.80	2.14	2.05
Iron [mg L^{-1}]	70.70	78.70	73.66	74.35

Before sowing and after harvesting of winter rape, the soil analysis for acidity and macronutrient content was carried out (Tables 4 and 5).

Table 4. Tests for pH and macronutrient content in soil for winter rape cultivation.

Years	Acidity	Phosphorus	Potassium	Magnesium
	(pH)	(mg 100 g^{-1} Soil)	(mg 100 g^{-1} Soil)	(mg 100 g^{-1} Soil)
2016	5.89	43.60	40.60	10.09
2017	5.34	41.90	46.90	9.80
2018	5.63	47.30	48.50	7.23
Mean $\bar{x}$	5.62	44.27	45.33	9.04

Table 5. Tests for pH and macronutrient content in soil after winter rape harvest.

Years	Acidity	Phosphorus	Potassium	Magnesium
	(pH)	(mg 100 g^{-1} Soil)	(mg 100 g^{-1} Soil)	(mg 100 g^{-1} Soil)
2017	5.84	19.50	23.40	12.10
2018	5.20	17.96	26.14	11.80
2019	5.60	18.20	25.20	11.60
Mean $\bar{x}$	5.55	18.55	24.91	11.83

The acidity (pH) of soil from the control (variant IV) after harvesting of winter rape was 5.80 (2017), 5.16 (2018), 5.58 (2019). The content of macronutrients was as follows:

– Phosphorus—18.47 mg 100 g^{-1} soil (2017), 17.52 mg 100 g^{-1} soil (2018) and 17.87 mg 100 g^{-1} soil (2019),
– Potassium—22.94 mg 100 g^{-1} soil (2017), 25.39 mg 100 g^{-1} soil (2018) and 24.79 mg 100 g^{-1} (2019),

– Magnesium—11.20 mg 100 g^{-1} soil (2017), 10.90 mg 100 g^{-1} soil (2018) and 11.30 mg 100 g^{-1} soil (2019).

The average acidity (pH) of soil before sowing from three years of research was 5.62 (acidic). After harvesting, the soil pH was 5.55 (acidic). The average pH value before sowing is within the lower limit of optimal soil requirements for rape development. The average phosphorus content in soil after harvest decreased by 25.72 pp (percentage point), whereas potassium increased by 20.42 pp. In turn, the magnesium content increased by 2.79 pp. As for the relative percentage difference, for the studied macronutrients the phosphorus content decreased by 41.90%, the potassium content decreased by 54.95%, and the magnesium content increased by 30.86%.

3.1. Impact of the Fertilization Dose on Physical Parameters of Winter Rape Plants

Before using digestate, the diameter at the stem base was measured in the BBCH 30 phase. The diameter (before using the digestate from all experimental plots) in the first growing season 2016/2017 was 0.96 cm (average of 10 measurements), in the second growing season 2017/2018 it was 0.98 cm (average of 10 measurements), and in the third growing season it was 0.95 cm (average of 10 measurements). Measurements were repeated 14 days after the first and second dose of digestate. The results are shown in Table 6.

Table 6. Average diameter at the base of winter rape stem in individual experiment variants.

Variant	Mean Diameter (cm)							
	After the First Dose				After The Second Dose			
	2017	2018	2019	Mean $\bar{x}$	2017	2018	2019	Mean $\bar{x}$
I	1.25	1.27 a,b	1.26 a,b	1.26 a,b	1.43	1.43	1.42	1.43 a,b
II	1.27	1.28 a,b	1.27 a,b	1.27 b,c	1.46	1.44	1.45	1.46 b,c
III	1.28	1.30 b	1.29 b	1.29 c	1.48	1.49	1.47	1.48 c
IV	1.24	1.25 a	1.23 a	1.24 a	1.42	1.43	1.41	1.42 a

a, b, c—different letters in the same year of study indicate statistically significant difference ($p < 0.05$).

The highest average diameter at the base of the stem after the first dose of digestate was observed in plants from the third variant of the experiment in all vegetation periods, although in 2017 the differences were insignificant. After the first dose of digestate, the average diameter at the stem base in the first growing season, 2016/2017, ranged from 1.25 to 1.28 cm, and for the control object the average diameter was 1.24 cm. In the second growing season, 2017/2018, the average diameter at the stem base ranged from 1.27 to 1.30 cm, and for the control object it was 1.25 cm, whereas, in the third vegetation period, the values were as follows: 1.26 to 1.29 cm, and for the control object the average diameter 1.23 cm.

After the application of the second digestate dose, an increase in the mean diameter was found at the stem base. In all vegetation periods, the largest average diameter at the stem base was observed in the third variant of the experiment—1.48 cm (2017), 1.49 cm (2018) and 1.47 cm (2019). For the control object, the average diameter at the stem base in the first growing season 2016/2017 was 1.42 cm, in the second growing season 2017/2018 it was 1.43 cm, and in the third growing season 2018/2019 it was 1.41 cm. However, the statistical analysis showed no statistically significant differences.

Tukey's confidence intervals confirmed that differences between the mean diameter at the base of the winter rape stem after the first dose of digestate, depending on the dose used, were significantly differentiated between the dose of digestate in variant II and variant III of the experiment and for the average of three years of research.

After the second digestate dose, Tukey's confidence intervals confirmed that the differences between the mean diameter at the base of the winter rape stem, depending on the digestate dose used, were significant, but only for the average of three years of research.

The average plant height (in the BBCH 30 phase) before digestate application was 13.98 cm in the first growing season, 13.91 cm in the second growing season and 14.03 cm in the third growing season. Table 7 shows the change in the height of a winter rape plant after the first and second digestate doses.

Table 7. Average height of winter rape in individual experiment variants.

Variant	Mean Height (cm)							
	After the First Dose				After the Second Dose			
	2017	2018	2019	Mean $\bar{x}$	2017	2018	2019	Mean $\bar{x}$
I	14.72	14.85	14.69	14.75 a	31.88 a	33.02 a,b	30.78 a	31.89 a
II	14.86	14.96	14.76	14.86 a,b	34.30 a,b	35.20 a,b	33.20 a,b	34.23 b
III	15.00	15.10	14.98	15.03 b	36.20 b	37.20 b	35.20 b	36.20 c
IV	14.69	14.78	14.71	14.73 a	30.80 a	31.80 a	29.90 a	30.83 a

a, b, c—different letters in the same year of study indicate statistically significant difference ($p < 0.05$).

The average plant height, after the first digestate dose, in the first growing season ranged from 14.72 to 15.00 cm. In the second growing season, the average plant height ranged from 14.85 to 15.10 cm. Meanwhile, in the third growing season, the average plant height ranged from 14.69 to 14.98 cm. For the control object, the average height in all years of the study was 14.73 cm.

After the application of the second digestate dose, the average plant height in the first growing season, 2016/2017, ranged from 31.88 to 36.20 cm. In the growing season 2017/2018, the average plant height ranged from 33.02 to 37.20 cm. In the third growing season, 2018/2019, the average height ranged from 30.78 to 35.20 cm. For the control object, the average height of winter rape plant from three growing periods was 30.83 cm.

Tukey's confidence intervals confirmed that differences between the average height of winter rape after the first digestate dose, depending on the dose used, were significantly differentiated, but only for the average values from three years of research.

After the second digestate dose, Tukey's confidence intervals also confirmed that the differences between the average height of winter rape plant, depending on the digestate dose used, were significantly different.

Changes in chlorophyll concentration in winter rape leaves were also assessed. Before using digestate, the chlorophyll concentration values were as follows: in the first growing season 2016/2017 the chlorophyll concentration was56.24 SPAD units, in the second growing season 2017/2018 it was 64.14 SPAD units, and in the third growing season it was62.57 SPAD units. The chlorophyll concentration changes are shown in Table 8.

Table 8. Average chlorophyll concentration in winter rape leaves in individual experiments variants.

Variant	Mean Chlorophyll Concentration (SPAD)							
	After the First Dose				After the Second Dose			
	2017	2018	2019	Mean $\bar{x}$	2017	2018	2019	Mean $\bar{x}$
I	64.06	64.24	63.86	64.05 a,b	64.88	65.07	64.71	64.89 a,b
II	64.25	64.44	64.04	64.24 b	65.33	65.52	65.11	65.32 b
III	64.18	64.38	64.02	64.19 b	64.14	64.57	64.19	64.30 b
IV	64.64	63.99	63.29	63.64 a	63.77	64.06	63.66	63.83 a

a, b—different letters in the same year of study indicate statistically significant difference ($p < 0.05$).

Based on the obtained results, the average concentration of chlorophyll in winter rape leaves was found to be over 62 SPAD units. Higher chlorophyll concentration was recorded in the second growing season. For the control object, these values were: vegetation period 2016/2017—64.64 SPAD units, vegetation period 2017/2018—63.99 SPAD units and 63.29 SPAD units (vegetation period 2018/2019).

After the second dose of digestate for the control object, the average concentration of chlorophyll was in the vegetative period 2016/2017—63.77 SPAD units, in the vegetative period 2017/2018—64.06 SPAD units, and in the third vegetation period—63.66 SPAD units.

Tukey's confidence intervals confirmed that differences between the average chlorophyll concentration after the first digestate dose, depending on the digestate dose used, were significantly differentiated, but only for the average values from three years of research for variants I and II, I and III, I and IV, II and IV, III and IV.

After the second digestate dose, Tukey's confidence intervals confirmed that the differences between the average chlorophyll concentration after the second digestate dose were significantly differentiated between experiment variants I and II, I and III, I and IV, II and IV, III and IV for the mean values from the three years of research.

3.2. The Amount of Digestate Dose vs. Rape Yield

Table 9 presents changes in the yields obtained and the thousand seed weight in individual experiment variants.

Table 9. Changes in yield thousand seed weight in individual experiment variants.

Variant	Yield (t ha^{-1})				1000-Seed Weight (g)			
	2017	2018	2019	Mean $\bar{x}$	2017	2018	2019	Mean $\bar{x}$
I	3.32 b	3.35 b	3.28 b	3.32 b	5.25 b	5.27 a	5.21 a,b	5.24 b
II	3.38 b,c	3.40 b,c	3.34 b,c	3.37 c	5.29 b	5.32 b	5.24 b	5.28 c
III	3.45 c	3.47 c	3.41 c	3.44 d	5.35 c	5.36 b	5.30 c	5.34 d
IV	3.14 a	3.15 a	3.09 a	3.13 a	5.19 a	5.23 a	5.17 a	5.20 a

a, b, c, d—different letters in the same year of study indicate statistically significant difference ($p < 0.05$).

Analyzing the obtained test results, a discrepancy in yields was obtained in the first growing season, 2016/2017, from 3.32 to 3.45 t ha^{-1}. In the second growing season, 2017/2018, the sizes were similar and ranged from 3.35 to 3.47 t ha^{-1}. However, in the third growing season, 2018/2019, depending on the applied dose, from 3.28 to 3.41 t ha^{-1} were collected. The control plot produced 3.14 t ha^{-1} in the growing season 2016/2017, 3.15 t ha^{-1} in the second growing season 2017/2018, and 3.09 t ha^{-1} in the third growing season 2018/2019. The obtained thousand seed weight for three growing periods was similar, and on average from three years of research it was 5.24 to 5.34 g. For the control object, the average thousand seed weight from three years of the experiment was 5.20 g.

Tukey's confidence intervals confirmed that differences between the obtained yield, depending on the applied digestate dose, were significantly different. Based on the Tukey's test, it was found that the differences between the thousand seed weight, depending on the digestate dose used, were significantly different.

3.3. The Effect of Digestet Dose on Seed Quality after Winter Rape Harvest

The moisture content of winter rape seeds collected in 2017 was 9.10% for the control object (critical humidity exceeded by 0.10%); in 2018 it was 5.00% and in 2019 it was 5.10%. In the first growing period, the humidity ranged from 5.40% in the third variant to 8.70% in the second variant of the experiment. In the second growing season, the humidity level was more even and ranged from 4.29% in the third variant to 6.19% in the first variant of the experiment. The seeds collected after the third growing season contained from 5.49% moisture in the third variant to 6.02% in the fourth variant of experiment.

Table 10 presents the results of research on the fat and protein content in winter rape in individual experiment variants.

Table 10. Changes in fat and protein contents in winter rape seeds in individual experiment variants.

Variant	Fat Content (%)				Protein Content (%)			
	2017	2018	2019	Mean $\bar{x}$	2017	2018	2019	Mean $\bar{x}$
I	42.44 b	42.47 b	42.42 a,b	42.44 b	22.63 b	22.71 b	22.60 b	22.65 b
II	42.52 b	42.56 b	42.50 b	42.53 b	22.77 b,c	23.00 c	22.83 c	22.87 c
III	43.63 c	43.67 c	43.57 c	43.62 c	22.89 c	23.04 c	22.93 c	22.95 d
IV	42.20 a	42.21 a	42.18 a	42.20 a	22.40 a	22.42 a	22.36 a	22.39 a

a, b, c, d—different letters in the same year of study indicate statistically significant difference ($p < 0.05$).

Analyzing the obtained research results, the average fat content of winter rape seeds was increased depending on the applied digestate dose—it ranged from 42.44% to 43.62%. In the control object, the average fat content from three harvest years was 42.20%. Such fat content indicates a good quality of the collected seeds.

Tukey's confidence intervals confirmed that differences between the fat content, depending on the digestate dose used, were significantly different for the II and III variants in 2017 and 2018 and for the whole 2019 year. However, for the average values from three years of research, they were significantly differentiated for variants I and III, I and IV, II and III, II and IV, III and IV.

The average protein content in the winter rape seeds collected from the control plot was 22.39% from three years of research. In the remaining variants of the experiment, the average protein content increased with the increase in the digestate dose and ranged from 22.65% to 22.95%.

Tukey's confidence intervals confirmed that the differences between the protein content, depending on the digestate dose, were significantly different in the seeds collected in 2017 and for variants I and II, I and IV, II and IV, III and IV in 2018 and 2019, as well as for the average values from three years of research.

Table 11 presents the results of research on changes in the content of macronutrients in winter rape seeds collected in 2017, 2018 and 2019, respectively. Based on the obtained results, a similar level of the macronutrient content in winter rape seeds was found.

The obtained results indicate an increase in the content of macronutrients depending on the amount of the digestate dose applied. The average nitrogen content from three years of research ranged from 3.10% to 3.14%, and phosphorus from 7.69% to 7.85%. In turn, the potassium content ranged from 6.24[1] to 6.29 g kg^{-1}, calcium ranged from 3.30 to 3.39 g kg^{-1}, and magnesium ranged from 2.21 to 2.24 g kg^{-1}.

Tukey's confidence intervals confirmed that the differences between the content of macronutrients, depending on the applied digestate dose, were significantly different. However, in the case of potassium, statistically significant differences occurred in the second and third year of the study. No statistically significant differences were found in the case of calcium analysis in individual study years, and only average values for the entire study varied significantly.

Table 12 presents changes regarding the fatty acids content in individual experiment variants. Analyzing the obtained test results from individual experiment variants, similar values of the tested parameters were found in three vegetation periods. For the control plot, the average value from three years of saturated fatty acid testing for three years was 6.62%, with monounsaturated fatty acids comprising 61.69% and polyunsaturated fatty acids comprising 30.71% of the sum of acids.

The average values of saturated fatty acid tests from three growing periods, depending on the applied digestate dose, ranged from 6.70% to 6.87%, monounsaturated fatty acids ranged from 61.75% to 61.89%, and polyunsaturated fatty acids ranged from 30.81% to 31.17% of the sum of acids.

Tukey's confidence intervals confirmed that differences between the content of saturated fatty acids, depending on the dose of digestate used, were significantly different. In case of monounsaturated fatty acids, Tukey's confidence intervals confirmed that the differences were significantly differentiated only for average values of three years of research. In contrast, the content of polyunsaturated fatty acids varied significantly, depending on the digestate dose used, based on Tukey's multiple comparison test, only during the vegetative periods 2017/2018 and 2018/2019. There were no statistically significant differences for variants I and II.

Table 11. Changes in the content of macronutrients in individual experiment variants.

Variant	Nitrogen (%)				Phosphorus (%)				Potassium (g kg^{-1})				Calcium (g kg^{-1})				Magnesium (g kg^{-1})			
	2017	2018	2019	$\bar{x}$	2017	2018	2019	$\bar{x}$	2017	2018	2019	$\bar{x}$	2017	2018	2019	$\bar{x}$	2017	2018	2019	$\bar{x}$
I	3.11 b	3.12 b	3.08 b	3.10 b	7.69 a,b	7.72 a,b	7.65 a,b	7.69 b	6.24	6.28 a,b	6.21 a,b	6.24 b	3.31	3.32	3.26	3.30 a, b	2.22 a,b	2.23 a,b	2.17 a, b	2.21 b
II	3.12 b, c	3.14 b,c	3.07 b.c	3.11 b	7.74 a,b	7.77 a,b	7.70 a,b	7.74 b,c	6.28	6.31 a,b	6.25 a,b	6.28 b, c	3.35	3.35	3.29	3.33 a, b	2.25 b	2.25 a,b	2.18 a, b	2.23 b
III	3.15 c	3.17 c	3.11 c	3.14 c	7.87 b	7.87 b	7.80 b	7.85 c	6.27	6.32 b	6.29 b	6.29 c	3.40	3.41	3.36	3.39 b	2.25 b	2.26 b	2.20 b	2.24 b
IV	3.06 a	3.08 a	3.02 a	3.05 a	7.50 a	7.51 a	7.46 a	7.49 a	6.18	6.21 a	6.15 a	6.18 a	3.24	3.28	3.20	3.24 a	2.15 a	2.15 a	2.09 a	2.13 a

a, b, c—different letters in the same year of study indicate statistically significant difference ($p < 0.05$) $\bar{x}$—mean.

Table 12. Changes in fatty acid contents in individual experiment variants.

Variant	Saturated Fatty Acids (% of the Sum of Acids)				Monounsaturated Fatty Acids (% of the Sum of Acids)				Polyunsaturated Fatty Acids (% of the Sum of Acids)			
	2017	2018	2019	$\bar{x}$	2017	2018	2019	$\bar{x}$	2017	2018	2019	$\bar{x}$
I	6.71 a,b	6.73 a,b	6.67 a,b	6.70 b	61.75	61.79	61.71	61.75 a	30.82	30.84 a,b	30.78 a,b	30.81 a
II	6.80 b,c	6.82 b,c	6.72 b,c	6.78 c	61.83	61.84	61.77	61.81 a,b	31.11	31.18 a,b	31.07 a,b	31.12 b
III	6.86 c	6.91 c	6.83 c	6.87 d	61.92	61.95	61.81	61.89 b	31.16	31.22 b	31.14 b	31.17 b
IV	6.61 a	6.65 a	6.60 a	6.62 a	61.69	61.78	61.68	61.69 a	30.71	30.75 a	30.66 a	30.71 a

a, b, c, d—different letters in the same year of study indicate statistically significant difference ($p < 0.05$), $\bar{x}$—mean.

The collected winter rape seeds from all experiment variants were also tested for the contents of omega-3, omega-6, and omega-9. For the control object, the average content of omega-3 acids from three years of research was 9.33%, while the content of omega-6 was 21.29% and that of omega-9 was 61.48% of the sum of acids. The average content of omega-3 acids (Figure 1) in winter rape seeds harvested from three growing periods ranged from 9.38% to 9.48% of the sum of acids. In turn, the average content of omega-6 (Figure 2) ranged from 21.30% to 21.37% of the sum of acids. As for omega-9 (Figure 3), the average content ranged from 61.53% to 61.63% of the sum of acids.

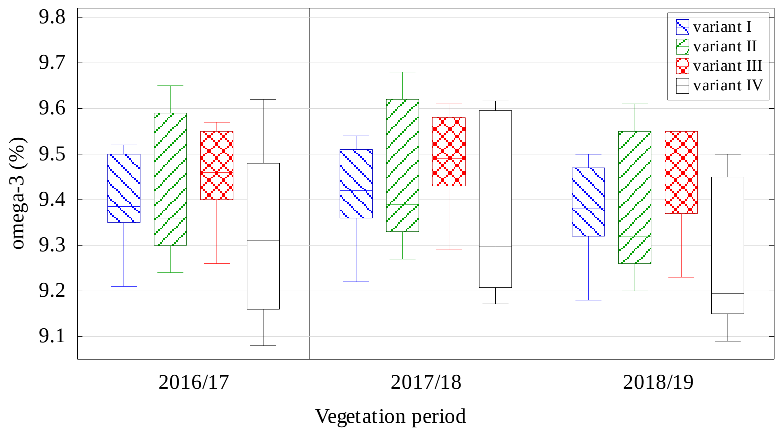

Figure 1. Box-plot chart for omega-3 content.

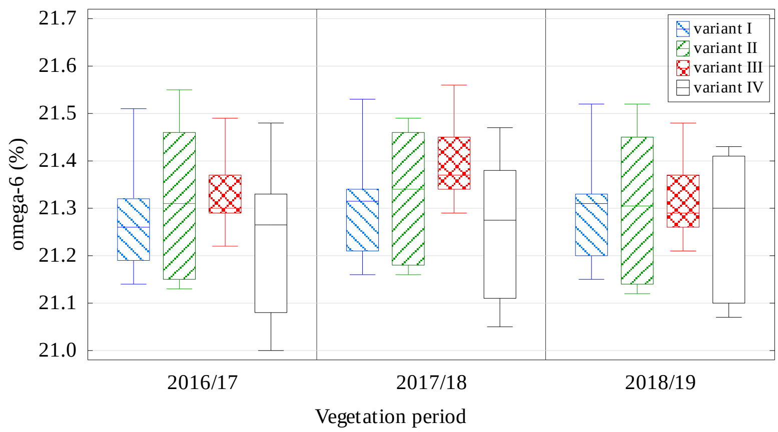

Figure 2. Box-plot chart for omega-6 content.

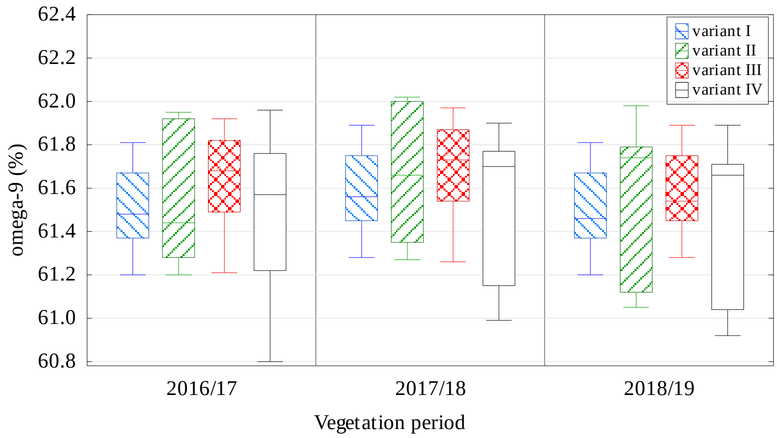

Figure 3. Box-plot chart for omega-9 content.

Tukey's confidence intervals did not confirm statistically significant differences between omega-3, omega-6 and omega-9 contents depending on the digestate dose used.

3.4. Economic Analysisose on Seed Quality after Winter Rape Harvest

A comparative analysis of the economic effects of digestate use and modes of application in winter rape seed cultivation should underlie decision about its use. The conducted analyses confirmed the seed yield increase caused by the applied digestate to be the factor improving the profitability of rape production. In all combinations tested, the application of the studied digestate had a positive effect on the winter rape seed yield and increased the cost-effectiveness of the cultivation. On average, the profitability of digestate use ranged from 1.79 to 14.07 EUR·ha^{-1} (Figure 4). The most cost-effective is the lowest dose (25,000 L ha^{-1}); however, the highest dose (50,000 L ha^{-1}) best affects the quality of winter rapeseed.

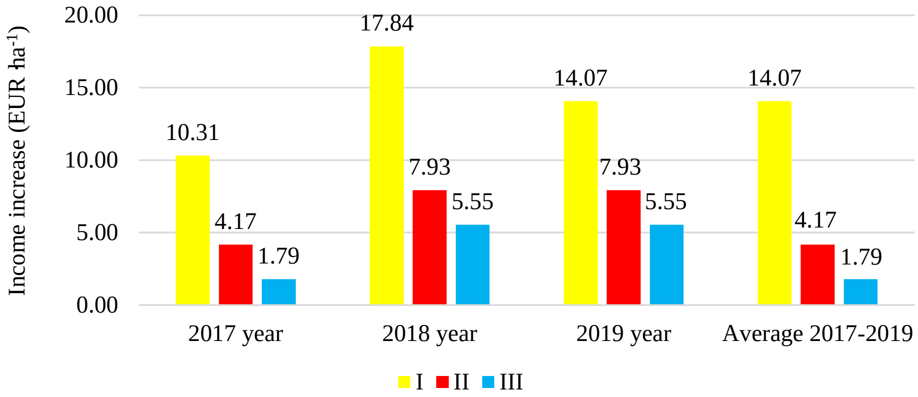

Figure 4. Economic effects of digestate use.

4. Discussion

In the presented paper, an attempt was made to assess the effect of the digestate dose on the yield and quality of winter rape seeds.

The tests used to determine the quality of seeds were: 1000-seed weight, fat content, protein content, fatty acid profile and macronutrient content. Czuba and Mazur [18], among basic quality characteristics of rapeseed shaped by fertilization, also included the content of fats, fatty acids, protein, glucosinolates, organic compounds in meal and oilcake as well as mineral components.

Winter rape performs particularly well in areas with high humidity. Higher yields can be obtained in regions with annual rainfall exceeding 525 mm than in regions with less rainfall [19]. The meteorological data shown enable a conclusion to be drawn that the annual rainfall was above the given value. Rape yields are positively affected by more frequent, but fine rainfall, especially during flowering at relatively low temperatures. Spring rape is particularly sensitive to rainfall deficiency. Winter rape from emergence to inhibition of vegetation before winter is insensitive or not very sensitive to water shortages in the soil. Even 3–6-week periods of drought at this stage of development do not negatively affect the crop. Only a lack of rainfall during the rosette forming phase causes a decrease in the yield, by approximately 15%. Although rape needs a small amount of water (48–52% of the seed mass) to start germination, due to the low suction power of seeds, its content in soil should be at least 32–35% of the field water capacity. Good soil moisture in the germination and emergence phase determines even plant development in autumn. Drying of the soil during sowing and lack of rainfall during seed germination delays emergence and leads to uneven development of plants in autumn, which can affect the rape seed yielding [19,20].

However, winter rape is much more sensitive to drought during spring and summer development. The critical period falls on the flowering and then ripening stages. The direct reason for lowering the yield in this phase is flower fall or seed failure. The number of pods with seeds thus decreases. The decrease in the yield due to the decreasing number of pods is mitigated by the increasing thousand seed weight, but cannot compensate for it. On the other hand, with soil water scarcity, the thousand seed weight drops strongly during ripening and is the main reason for the decreasing crop. Drought lasting longer than one development phase has a particularly negative impact on yielding. Rape reacts more strongly to air humidity than to rainfall, when the total sum exceeds 500–600 mm. In the years with a cooler ripening period, and thus also a higher relative humidity of the atmosphere, it gives higher yields, even in years with lower rainfall [19,20].

Winter rape is particularly sensitive to low temperatures at the end of winter, when winter thaws lead to significant tempering. However, it tolerates, after good hardening in autumn, even greater temperature drops at the beginning of winter (December). Critical temperatures for its above-ground part are then below −20 °C. Much more sensitive to frost is its root system, for which critical temperatures are below −5 to −6 °C. Rape with damaged leaf rosette yields up to 11% less, and that with damaged buds has a yield which is as much as 26% worse [19,20]. During the test, temperatures below −6 °C were not observed, which is beneficial for the development of winter rape.

Hołubowicz-Kliza and Wałkowski [20] and Muśnicki [19] reported that rape does not tolerate an acidified, damaged structure, poor in humus and abundant in weeds. The soil reaction optimal for the development of rape is neutral or slightly acidic, with a pH in KCl 5.1–6.5. The pH value tested in soil analyses from experimental plots was in the above-mentioned range and, in the vegetation periods 2016/2017, 2017/2018 and 2018/2019, amounted from 5.34 to 5.89. This proves that the position for winter rape was well selected.

Hołubowicz-Kliza and Wałkowski [20] stated that rape does not tolerate an acidified, damaged structure, poor in humus and weeds. The soil reaction optimal for rape development is neutral or slightly acidic, with a pH in KCl 5.1–6.5. The pH value tested in soil analyses from experimental plots was in the above-mentioned range from 5.34 to 5.89 in vegetation periods 2016/2017, 2017/2018 and 2018/2019. This proves that the position for winter rape plants was well selected.

Das et al. [21] and Francisco et al. [22] noted that changes in the environment, such as changing soil pH levels, have a significant impact on ammonia emissions. As a result, NH_3 emissions from agricultural activities play a significant role in the global atmospheric budget. An organic fertilizer can reduce nitrogen losses by the reduction in emissions of nitric oxide and ammonia [23].

Based on his own research, Muśnicki [24] stated that the height of rape plants decreased along with their advancement in development. This variability was primarily determined by habitat conditions with a significant share of agrotechnical factors; however, the share of these factors at the beginning of generative development was greater than during flower formation and flowering. At the beginning of spring vegetation, the rape plants were small. Their intensity increased with the passage of time and took on the maximum dimensions at the stage of full budding. This dynamic rape growth lasted until full flowering. After this time, the plant height increments suddenly collapsed, and even a slight decrease in the plant height due to drying took place during the ripening phase.

A similar rhythm of growth was observed in the conducted studies. After the second dose, two-fold growth of winter rape plants was observed.

Muśnicki [24] characterized ripening rape plants in terms of the stem base diameter. The average diameter of the stem base in Muśnicki's [24] studies was 1.03 cm.

In the tests carried out, the average diameter at the base of the winter rape plant stems before digestate was similar. After the first dose of digestate, the average diameter at the base of the stem increased to about 1.25 cm, and after the second increased to about 1.45 cm.

The average chlorophyll concentration in winter rape leaves during the vegetative periods 2016/2017, 2017/2018 and 2018/2019 was from 60 to 66 μg cm^{-1}. A similar concentration of chlorophyll was also indicated by Ghassemi-Golezani et al. [25].

Depending on the raw materials used for the biogas production, digestate may differ in the content of micro and macronutrients. The research conducted by Różyło et al. [17] showed that digestate contains a disproportionately large amount of potassium (2.69 g kg^{-1} DM) and sodium (0.29 g kg^{-1} DM) relative to other elements. A high potassium content was also found in digestate samples used in our study. Chiew et al. [26] stated that the use of digestate as a fertilizer increases the content of macro and microelements in soil and plants. Vázquez-Rowe et al. [27] and Wysocka-Czubaszek [3] also paid attention to the use of digestate as a fertilizer instead of mineral fertilizers.

Physical and chemical analysis of winter rape seeds in the conducted research included, among others, the yield and weight of one thousand seeds. The obtained yield averaged over three years of the research from 3.13 to 3.44 t ha^{-1}. The obtained thousand seed weight was, on average, for three years of the research from 5.20 to 5.34 g. Similar results were obtained by Różyło et al. [28] who, based on the conducted research, found an increase in the seed yield after digestate application in comparison to the control. Additionally, the thousand seed weight increased significantly compared to the thousand seed weight collected from the control plot. In the third year of research, there was a reduction in the thousand seed weight after fertilizing with mineral fertilizers.

The fat and protein content, as well as fatty acids composition are, among others, important parameters in assessing the quality of winter rape seeds intended for the food industry. These parameters depend to a different degree on the agrotechnical and climatic factors and on the cultivar [29]. The fat and protein content as a function of the content of these components in seeds and seed yield largely depends on the weather, especially on the amount of precipitation [19]. The average fat content from three years of research ranged from 42.20% to 43.62%. Murawa et al. [30] reported that after using various herbicides in the protection of spring rape, the fat content, depending on the cultivar, ranged from 47.9% (Star cv.) to 50.0% (Lisonne cv.). In turn, Banaszkiewicz and Borkowska [31] stated that basic components of rape seeds are fat and protein, the content of which was more than 40% in case of fat and about 20% in case of protein. Różyło et al. [28] found that the fat content of rape seeds depends on the type of fertilization. In the second year of research, digestate fertilization reduced the fat content in seeds, while in the third year of research, the fat content was higher than in seeds harvested from the field fertilized with mineral fertilizers. Fertilizing with mineral fertilizers significantly reduced the stearin content as compared to digestate. Digestate fertilization increased the percentage of arachidic, arachidonic and behenic acids in relation to the control object, and sometimes also to plots fertilized with mineral fertilizers.

In the conducted tests, a significant effect of digestate fertilization dose on fat and protein content in winter rape seeds was found.

Montemurro et al. [2] and Pan et al. [4] reported that using digestate, macronutrients are supplied to the soil, in particular nitrogen, phosphorus and potassium. The results of tests conducted by Panuccio et al. [12] indicate an alternative use of digestate instead of mineral fertilizers. Rape seed oil had the most favorable percentage in the amount of energy SFA, PUFA, omega-3, and omega-6 vegetable oils referring to the recommended daily fat intake (ERDI-37.7 kJ g^{-1}) [32].

In our own research, the content of saturated fatty acids averaged over three years of research from 6.62% to 6.87% of the sum of acids. The average content monounsaturated fatty acids over the three years of the research was from 61.69% to 61.89% of the sum of acids. The content of polyunsaturated fatty acids averaged over the three years of the research from 30.71% to 31.17% of the sum of acids. Omega-3 fatty acids averaged over three years of the research from 9.33% to 9.45%, omega-6—21.29–21.37%, and omega-9—61.48–61.63% of the sum of acids.

Różyło et al. [28] stated that the content of omega-3 and omega-6 acids in seeds harvested from digestate-fertilized plots decreased compared to those from the harvested plots fertilized with mineral fertilizers. Fertilization with digestate reduced the share of omega-3 and omega-6 in comparison to the control object and fertilized with mineral fertilizers.

5. Conclusions

Changing economic conditions and social preferences cause a reorientation in farming systems. Production systems that provide socially acceptable quality raw materials while maintaining ecological safety are preferred.

Based on the conducted research, the following conclusions can be drawn:

1. The amount of the digestate dose affects the amount of yield obtained and the mass of one thousand seeds.
2. The amount of digestate dose determines the fat and protein content in winter rape.
3. The amount of digestate dose affects the content of macronutrients and saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids.
4. The content of omega-3, omega-6 and omega-9 acids does not depend on the digestate dose.
5. Of the three digestate doses used, the highest one is recommended.

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