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THE IMPACT OF DIGESTATE ON THE CHEMICAL PROPERTIES OF MAIZE (*Zea mays* L.) GRAINS

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Abstract

Maize grains accumulate nitrogen (N), especially in the form of storage proteins, correlated to zeins and starch content. Digestate, a new bio-based fertilizer rich in essential nutrients, is an effective alternative for mineral fertilizers. The aim of this study was to examine the effect of digestate application on chemical properties and maize grain yield. Field experiment was established during year 2018 on eight different fertilization treatments: control treatment without fertilization, mineral fertilizer (MF), liquid cattle manure, solid fraction of digestate (SFD), liquid fraction of digestate (LFD), digestate, a mixture of MF+SFD, a mixture of MF+LFD. Nitrogen content in grains was positively correlated with proteins (99.93%) and zein (73.91%) content. In addition, the results showed that only the LFD and digestate treatments had a positive correlation between the N content in the grains and the starch content. Statistically higher grain yield was observed on MF (13.1 t ha^{-1}), MF+SFD (11.2 t ha^{-1}) and MF+LFD (12.2 t ha^{-1}) treatments compared to others. Digestate showed positive effect on chemical properties of the maize grain.

Key words: *chemical properties, crop, digestate fractions, grain, maize, nitrogen.*

INTRODUCTION

In recent decades, sustainability has become an indispensable factor for survival, and the agricultural sector plays a crucial role in achieving multiple sustainable development goals by ensuring access to nutritious and healthy food, promoting sustainable agricultural practices and upholding human rights and fair working conditions (FAO, 2023). As the population grows, the demand for food also increases, leading to higher energy consumption and the use of non-renewable energy sources. The growing amount of agricultural waste (farmyard manure and various types of green waste) poses a major environmental challenge for the agricultural industry (European Directive, 2006; Brancoli et al., 2017; Tur-Cardona et al., 2018). In view of the fact that nowadays sustainability is being sought, loops are being closed and a circular economy is being pursued, an effective solution to this problem is processing it by anaerobic digestion in biogas plants. The production of biogas and the by-product digestate are an important source of renewable energy based on raw materials such as liquid manure, straw, hay, maize silage, food industry waste and energy crops (Holm-Nielsen et al., 2009; Wallace, 2011; Makadi et al., 2012; Nkoa, 2014).

Digestate is a high-quality fertilizer product and rich in plant-available nutrients such as nitrogen (N), phosphorus (P), potassium (K) and other macro- and micronutrients, making it a promising and sustainable alternative to mineral fertilizers (Vaneeckhaute et al., 2013; Risberg et al., 2017; Đurđević et al., 2018; Przygocka-Cyna & Grzebisz, 2020; Šatvar Vrbančić et al., 2024).

Maize (*Zea mays* L.) is the second most important crop in the world in terms of agricultural production and yield. In 2023, 1.2 billion tonnes of maize was produced - an increase of 46% since 2010. It is used in all areas, as food, biofuel, animal feed, etc. The largest producers are North and South America, led by the USA and Brazil (42% of global production), followed by China (23%) in Asia. In Croatia, it serves as an important raw material for various industries and is the most widely used of all cereals (Ranum et al., 2014; Mesterházy et al., 2020; FAO, 2023; Jukić et al., 2024).

Grains accumulate considerable amounts of N, especially in the form of storage proteins. These proteins play a crucial role in seed germination and serve as a vital food source for humans and animals (Duvnjak et al., 2020). The protein content ranges from 6% to 20% of the dry matter (DM). This range is influenced by several factors, such as the type of grain, the specific variety, agrotechnical processes, etc. In addition, the storage proteins of maize are classified according to their solubility in different solvents. About 70% of the proteins in maize grains are storage proteins, with zeins (prolamins) accounting for over 60% of this fraction. In addition to zeins, albumins, globulins and glutelins also belong to the storage proteins in maize (Wu et al., 2014). Grain endosperm is mainly composed of protein and starch (about 70% of the composition and consists of two types of carbohydrate chains: amylose and amylopectin) (Martinez et al., 2017).

The most important factors influencing zein and proteins are genotype and environment. Genotype is the most important determinant of zein properties and can contribute to 50-60% of the variability in hybrids, especially those resistant to abiotic and biotic stresses. Environmental factors such as N fertilization, irrigation and high temperatures have less influence, but still cause a small amount of variation within the same cultivar. The remaining 40-50% of the variability is determined by agricultural practices such as fertilization, disease control and pest management carried out by humans. This underscores the critical role of both genetic traits and advances in agricultural practices in optimizing maize quality (Duvnjak et al., 2020; Duvick, 2005).

The aim of this study is to determine the effect of digestate as fertilizer product on chemical properties of maize grain. In this case, the effect of different fertilization treatments on the content of protein, zein and starch in the maize grain.

MATERIALS AND METHODS

Field experiment were conducted on the experimental field Maksimir of the University of Zagreb Faculty of Agriculture, Croatia. Eight different fertilization treatments were carried out: 1-control without fertilization (C), 2-mineral fertilizers NPK 15-15-15+CAN (MF), 3-liquid cattle manure (LCM), 4-solid fraction of digestate (SFD), 5-liquid fraction of digestate (LFD), 6-mixture of solid and liquid fractions of digestate (SFD+LFD), 7-mixture of mineral fertilizer NPK 15-15-15 and solid fraction of digestate digestate (MF+SFD), and 8-mixture of mineral fertilizer NPK 15-15-15 and liquid fraction of digestate (MF+LFD).

The treatments were arranged in a completely randomized block design with four replicates (Figure 1). In addition, the average nitrogen (N) content in the soil was determined using the N_{min} method and amounted to 37 kg N_{min} ha^{-1}. In accordance with the Nitrates Directive (91/676/EEC), which sets a limit of 170 kg N ha^{-1}, 140 kg N ha^{-1} was applied in the field for each treatment.

As a test crop, maize hybrid Pioneer PR 0725 was sown on April 27, 2018 and harvested on September 28, 2018. Maize grain yield was adjusted to a moisture content of 14%.

Figure 1. Field experiment during fertilization

A soil sample was taken in the field from a depth of 0-30 cm, air-dried and sieved through a 2 mm sieve. It was then classified as a silt loam soil with an acid reaction and low humus content (Table 1).

Table 1. Soil characterization of field experiment

Depth cm	pH		Humus %	N_{min} kg ha^{-1} of soil	P_2O_5 mg 100 g^{-1} of soil	K_2O mg 100 g^{-1} of soil
	H_2O	KCl				
0-30	5.47	4.21	1.65	37.34	16.68	21.63

Table 2 presents the characteristics of the organic fertilizer products used at the beginning of the experiment. All materials were analyzed according to standard procedures: dry matter (DM) was measured by determining the residual mass after drying at 105 °C for 48 hours. The pH value was measured with a Mettler Toledo EL20/EL2 pH meter. Total N was determined using Kjeldahl destruction (HRN ISO 11261:2004), phosphorus (P_2O_5) was determined spectrophotometrically, and potassium (K_2O) was analyzed using a flame photometer (AOAC, 2015).

The digestate fractions were collected from the biogas plant Bojana in Čazma in northwest Croatia, where it was produced from a mixture of maize silage and LCM. The LCM was obtained from a near cattle farm.

Table 2. Fertilizer products characterization at the beginning of the experiment

Fertilizer products	pH H_2O	% N FW	% P_2O_5 DM	% K_2O DM
LCM	6.6	0.4	2.5	4.3
SFD	8.7	1.3	1.7	1.4
LFD	7.7	0.8	2.3	7.1

Note. LCM-liquid cattle manure; SFD-solid fraction of digestate; LFD-liquid fraction of digestate; DM: on dry matter; FW: on fresh weight.

The data of average air temperature and precipitation were taken from the Croatian Meteorological and Hydrological Service for the Maksimir station (DHMZ, 2018).

Table 3 shows the average values of temperature and precipitation for each month during maize vegetation growth from May to September 2018 as well as the optimal temperature and precipitation for normal maize growth according to Pucarić et al. (1997).

According to Walters climate diagram (Figure 2), based on the course of the precipitation and temperature curve, it can be concluded that the precipitation curve crosses the temperature curve in August, so there is a dry period on average during August 2018.

Table 3. Average air temperature and precipitation from May to September 2018 with optimal conditions according to Pucarić et al., 1997

	Optimal (Pucarić et al., 1997)		2018.	
Month	AT, °C	P, mm	AT, °C	P, mm
May	18.3	87.5	19.5	68.7
June	21.7	87.5	21.4	127.8
July	22.8	112.5	22.5	85.2
August	22.8	112.5	23.7	40.7
September	warmer and droughter		17.7	59.0

Note. AT: average air temperature; P: precipitation

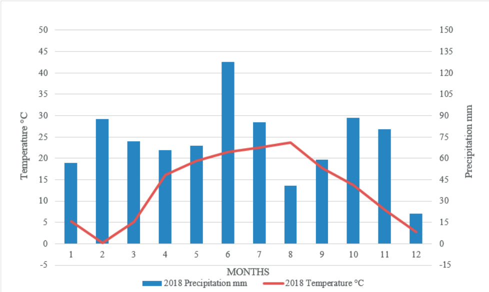


Figure 2. Walters climate diagram for Maksimir 2018

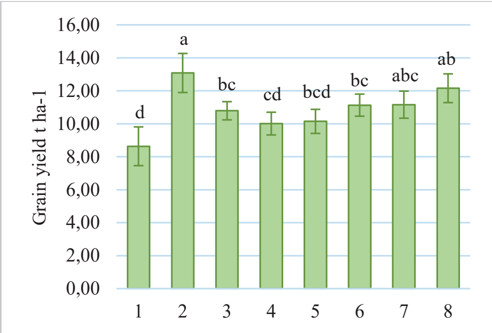
The proteins were calculated according to the following formula: % N x 6.25 (Vajić, 1964). Also, the zeins present in the vitreous and floury endosperm were extracted according to the protocol described by Wallace et al. (1990). In addition, starch and non-starch lipids were isolated from the floury and vitreous endosperm using the sequential extraction method described by Gayral et al. (2015). Both methods are explained in more detail in the work by Zurak et al. (2020).

Statistical analysis was performed using the Statistical Analysis System (SAS) 9.3. Analysis of variance (ANOVA) and mean comparisons (Tukey test, $p<0.05$) were performed to evaluate differences in corn yield between treatments. Additionally, correlation was performed between grain yield and chemical properties of the maize grain (protein, zein and starch content).

RESULTS AND DISCUSSIONS

The highest grain yield (Figure 3) was recorded on MF treatment (13.1 t ha^{-1}), a mixture of MF+SFD (11.2 t ha^{-1}) and a mixture of

MF+LFD (12.2 t ha^{-1}) treatments, while the lowest on C treatment (8.63 t ha^{-1}). It is also worth mentioning that the MF+SFD treatment gave statistically comparable results with MF+LFD treatment.



Note. Letters indicate significant differences between treatments ($p < 0.001$). 1-unfertilized control (C); 2-mineral fertilizers (MF); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-solid fraction of digestate+liquid fraction of digestate (SFD+LFD); 7-mixture of mineral fertilizer with solid fraction of digestate (MF+SFD); 8-mixture of mineral fertilizer with liquid fraction of digestate (MF+LFD).

Figure 3. Grain yield on different fertilization treatments

According to the Statistical Yearbook of the Republic of Croatia (DZS) (2023), the data for maize production and yield in the last five years (2018-2022) fluctuated between 6.1 and 9.1 t ha^{-1}. Yield variability could also be influenced by annual climatic conditions, as the total yields reported in the Statistical Yearbook of the DZS show. The weather conditions in 2018 contributed to higher yields, which is consistent with the results of this study. Although there was a dry period, precipitation that fell months earlier helped the maize overcome the drought.
Similar results were reported by Chantigny et al. (2008) in a three-year trial with treatments such as control, mineral fertilization, raw manure and digestate. They applied a total N dose of 130 kg N ha^{-1}every year. Over the three-year period, the average maize grain yield was 8.4 t ha^{-1} for the control, 9.6 t ha^{-1} for the mineral fertilizer, 9.7 t ha^{-1} for the raw manure and 9.5 t ha^{-1} for the digestate. On average, all treatments resulted in higher grain yields compared to the control. Przygocka-Cyna & Grzebisz (2020) also carried out a three-year trial with digestate at application rates of 0.2, 0.4 and 0.8 t ha^{-1}. These values correspond to a grain yield of 11.5, 10.8 and 9.2 t ha^{-1} maize grain in 2014, 2015 and 2016, respectively.
Yields can be influenced by many factors such as weather conditions, soil properties, suitable agrotechnical practices, human and many other factors.
Table 4 shows the chemical properties of maize grain (protein, zein and starch content) in different fertilizer treatments. The statistically highest protein content was found in the MF and MF+SFD treatments and the lowest concentration in the C treatment. In addition, the statistically highest zein content was found in MF, LCM and MF+SFD. The starch content was also statistically highest in the MF, LCM, LFD and MF+LFD treatments.

Table 4. Chemical properties of maize grain

Treatment	Protein %	Zein g kg^{-1} DM	Starch g kg^{-1} DM
1	6.8^{b}	28.8^{b}	718.4^{c}
2	8.3^{a}	48.2^{a}	732.1^{abc}
3	7.3^{b}	44.7^{a}	732.8^{abc}
4	7.1^{b}	40.1^{ab}	721.4^{bc}
5	7.3^{b}	38.6^{ab}	746.8^{a}
6	7.2^{b}	39.8^{ab}	719.7^{bc}
7	7.6^{ab}	42.4^{a}	725.5^{bc}
8	7.4^{b}	41.1^{ab}	736.0^{ab}

Note. Letters indicate significant differences between treatments ($p < 0.001$). 1-unfertilized control (C); 2-mineral fertilizers (MF); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-solid fraction of digestate+liquid fraction of digestate (SFD+LFD); 7-mixture of mineral fertilizer with solid fraction of digestate (MF+SFD); 8-mixture of mineral fertilizer with liquid fraction of digestate (MF+LFD); DM: on dry matter.

The maize grain yield was positively correlated with the zein concentration (67.9 %) in all treatments. Table 5 shows the correlation for each fertilization treatment separately. Additionally, it shows which of the treatments had the strongest correlation between maize grain yield and zein. The MF+LFD (99.6 %), MF (90.7 %) and LFD (88.0%) treatments showed the strongest and highest positive correlation between maize grain yield and zein, while the lowest correlation was found for C treatment (2.9 %).

Table 5. Correlation of maize grain yield and zein between treatments

Treatment	Grain yield and zein correlation %
1	2.9
2	90.7
3	42.8
4	34.6
5	88.0
6	60.6
7	32.8
8	99.6

Note. 1-unfertilized control (C); 2-mineral fertilizers (MF); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-solid fraction of digestate+liquid fraction of digestate (SFD+LFD); 7-mixture of mineral fertilizer with solid fraction of digestate (MF+SFD); 8-mixture of mineral fertilizer with liquid fraction of digestate (MF+LFD).

Since N directly influences yield, grain protein content (Tsai et al., 1978; Pearson & Jacobs, 1987; Uhart & Andrade, 1995) and grain hardness (Tamago et al., 2016), it also affects the zein concentration in the endosperm. In addition to this study, in which it can be clearly seen that N content is an important part for the zein concentration, this effect was also observed under field (Gerde et al., 2016; Tsai et al., 1978; Tsai et al., 1984; Ahmadi et al., 1995) and in vitro conditions (Singletary et al., 1990). As shown in Figure 4 N content was strongly and positively (<.0001) correlated with the concentration of proteins (99.93 %) and zeins (73.91 %) in the grains in all treatments, while starch gave negative correlation with N content.

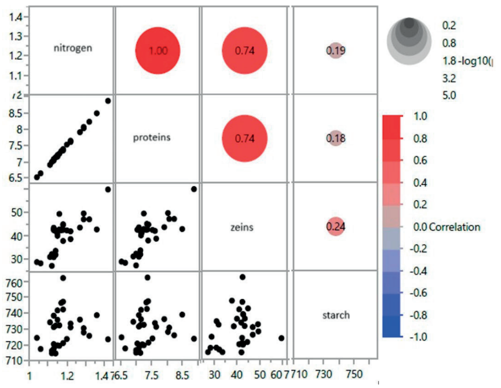


Figure 4. Correlation between N, proteins, zeins and starch as scatterplot matrix

From June to August 2018 there was a drought that could lead to a reduced of the starch content. Further, heat stress can also lead to a reduction in the starch content of the grains, while the protein content can increase (Tester & Karkalas, 2001; Wang & Frei, 2011; Thitisaksakul et al., 2012), what was also the case in this research.

CONCLUSIONS

In conclusion, this study emphasizes the important role of N accumulation in maize grains, especially in the form of storage proteins, which are strongly correlated with the zein and protein content. Digestate as a bio-based fertilizer product, rich in essential nutrients has proven its potential as an effective alternative to mineral fertilizers. The field experiment revealed that the N content in the maize grains was strongly associated with protein and zein content. The highest grain yields were obtained in treatment with mineral fertilizer (MF) and combined treatments (MF+SFD and MF+LFD), highlighting the synergistic effects of integrating digestate with mineral fertilizers.

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