

## Article

# Does Digestate Dose Affect Fodder Security and Nutritive Value?

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**Abstract:** With the rising interest in digestate use as a fertilizer on permanent cultures, there is a need to examine its effects on food and feed quality. This study is focused on the use of digestate in grassland fertilization and its effects on nutritive value parameters such as mycotoxin contamination (deoxynivalenol, aflatoxin, and T-2 toxin) and nutrient content (crude protein, crude fat, crude fiber, ash, nitrogen-free extract, digestibility of organic matter, acid detergent fiber, and ash-free neutral detergent fiber). The experiment was carried out in the Czech Republic, and the effects of fertilization regime, year, and harvest date (summer and fall cuts) on nutritive value were observed. An effect of the year on DON, AFB1, and T-2 contamination levels was observed. An effect of the harvest or fertilization regime on mycotoxin contamination was not observed. Significant differences were observed in the content of all nutrients, except ash, depending on the year. Differences were found only in the case of ADF levels, depending on the harvest date, as well; however, no differences were found between fertilization regimes. Our findings suggest that digestate does not negatively affect fodder in terms of nutritive value nor safety.

**Keywords:** biogas plant; grass forage; Weende analysis; fertilization; mycotoxin; DON; AFL; T-2 toxin



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## 1. Introduction

Permanent grasslands are frequently underutilized by farmers, abandoned, or managed extensively as a fodder source, due to their special management systems (such as protected landscape areas), often difficult accessibility (steeply sloped terrain), and low economic profitability [1]. Permanent grasslands are agricultural sources that have the attributes of quite stable natural biocenosis, spreading on nearly 1 mil. ha in 2020 in the Czech Republic alone [2]. The nutritive value of forage on extensively managed sites is not high. Nevertheless, may fertilization contribute to improving forage nutritive value? [1,3,4].

The annual forage production from grasslands in the Czech Republic is 0.5–15 t/ha of dry matter, depending on the environment. A low yield is often caused by insufficient fertilization of stands [5]. Digestate produced in biogas plants can be a viable option for sustainable semiliquid fertilizer used on grasslands. Digestate, as a byproduct of anaerobic digestion, comprises small amounts of ammonia, sulphate, and trace elements,

which makes digestate a notable source of nutrients essential for biomass production [6–8]. A study by Walsch suggested that its effect on biomass production is equivalent to that of a mineral fertilizer [9]. The Green Deal, accepted by the European Commission, is supposed to visibly change European agriculture in the following years. Changes are supposed to ensure that Europe becomes the first climate-neutral continent. By the year 2030, approximately  $\frac{1}{4}$  of all agricultural land should become ecologically managed and subsequent use of mineral fertilizers should decrease by 20% [10]. Therefore, the use of digestate seems like a logical option as a substitute for mineral fertilizers.

The quality of feed is affected by fertilization, weather conditions of the site, species abundance in sown mixtures, harvest date, and the antinutritive compound concentration. There is a rising interest in the identification and quantification of antinutritional compounds, due to the increasing pressure on feed and food quality. Mycotoxins are fungal secondary metabolites considered to be undesirable antinutritional compounds in feed. Contamination in forage is closely monitored due to the proven correlation between forage quality at the time of harvest and the quality of produced feed. The number of known mycotoxins is estimated to be 300–500 worldwide [11–14]. Even though mycotoxin producers are present in the forage, mycotoxin contamination may not have occurred [15]. Studies carried out in Poland and Mexico stated that 94–95% of tested feed is contaminated by at least 1–2 mycotoxins [16–20]. Plants may already be contaminated by mycotoxins before harvest, mainly by the species *Fusarium* and *Aspergillus* [21,22]. In cereal as well as forage grass stands, the amounts of mycotoxins depend on the dominant species in the sowed mixture [23]. The most commonly occurring mycotoxins are deoxynivalenol (DON), zearalenone (ZEN), aflatoxins (AF), T-2 toxin, and ochratoxin [24–26]. Additionally, the “Green Deal” and multiple studies point to the fact, that occurrence and amounts of mycotoxins contaminating the biomass are changing due to climate change [27,28].

DON, known also as vomitoxin, is one of the most abundant mycotoxins in the world, produced by the genus *Fusarium* [29]. It causes mainly alimentary disorders, immune function depression, and gastroenteritis [30,31]. The T-2 toxin is known for its anorexiogenic effects on the gastrointestinal tract, as well as disruption of the nervous system of animals [26,29]. Aflatoxins are mycotoxins produced by *Aspergillus flavus* and *A. parasiticus*, which cause acute toxicity in low concentrations, liver necrosis, cancer, and in some cases, the death of humans and animals [30–32]. It is considered one of the most toxic mycotoxins [33,34]. These health concerns in humans as well as animals widely affect animal production in the Czech Republic. It is important to note that, apart from the refusal of contaminated feed by animals, a non-negligible increase in expenses on veterinary treatments occurs as well, due to acute and chronic toxicosis [16,24,35–38]. This shows a high need for field studies focused on mycotoxins in fodder.

This study is focused on the effects of digestate fertilization in four different application variants on the nutritive value of feed, mainly on mycotoxin contamination. We hypothesized that digestate does not negatively affect the nutritive value of forage and feed safety by a positive correlation between increased digestate dose and mycotoxin contamination.

## 2. Materials and Methods

The experimental plots were seminatural grasslands in the Eastern and Central Bohemian regions in the Czech Republic with the following plant groups: 75% grasses, 15% legumes, and 10% forbs. Stands had been fertilized since 2019. Samples were collected during the harvest; representative samples included predominant and codominant species. Predominant species were *Dactylis glomerata* L. and *Lolium perenne* L. Codominant species were *Phleum pratense* L. and *Trifolium repens* L. The experimental plots were 8 m wide; each variant was established on an area of 7000 m<sup>2</sup> (including repetitions). Biomass samples were taken in 2019 and 2020. There were two experimental locations: Skořenice (50.0338 N, 16.1899 E) and Čechtice (49.6317 N, 15.0311 E). The town of Skořenice is situated 294 m a.s.l., with an average yearly precipitation of 600–700 mm and an average temperature of 7–8 °C. The town of Čechtice is situated 496 m a.s.l., with an average yearly precipitation of

650–750 mm and an average temperature of 6–7 °C. Basic soil physical and chemical parameters for both locations are shown in Table 1. The soil type in Skořenice is phaeozem, and in Čechtice, the soil type is cambisol. The soil texture according to the USDA Soil Texture Triangle is silty clay loam in Čechtice and silt loam in Skořenice. The soil characteristics are similar; therefore, we did not take soil type into account. The soil contents of P, K, Ca, and Mg were established according to Schroder et al.; the individual elements were extracted using Mehlich III reagent and then analyzed using atomic emission spectroscopy (55B AA, Agilent, Santa Clara, CA, USA) [39]. Total nitrogen and total carbon were measured using the vario MACRO cube (Elementar Analysensysteme GmbH, Langensfeld, Germany), and pH in CaCl<sub>2</sub> was measured according to ISO 10390:2005 Soil quality—Determination of pH [40]. Soil aggregate stability was measured according to DIN 19683-16 using an M-0813E wet sieving apparatus (Eijkelkamp Soil & Water, Giesbeek, The Netherlands).

**Table 1.** Soil physical and chemical parameters of experimental locations.

| Location                     | Skořenice       | Čechtice         |
|------------------------------|-----------------|------------------|
| pH (-)                       | 6.6 ± 0.36      | 6.69 ± 0.19      |
| Soil aggregate stability (%) | 92.08 ± 3.03    | 92.06 ± 2.34     |
| Total nitrogen (%)           | 0.41 ± 0.05     | 0.41 ± 0.06      |
| Total carbon (%)             | 3.99 ± 0.37     | 4.34 ± 0.68      |
| C/N ratio (-)                | 9.90 ± 0.93     | 10.59 ± 0.57     |
| K (mg/g)                     | 104.00 ± 28.69  | 345.00 ± 95.91   |
| Ca (mg/g)                    | 5231.25 ± 55.35 | 2674.75 ± 525.63 |
| Mg (mg/g)                    | 164.75 ± 14.24  | 321.25 ± 47.23   |
| P (mg/g)                     | 34.00 ± 7.07    | 34.25 ± 9.57     |

Values are expressed as a mean ± standard deviation.

The size of each experimental plot was 7000 m<sup>2</sup> and the width of each plot was 8 m. The effect of location was not evaluated; three biomass samples from each location were taken (88 samples were collected in total).

The factor of digestate dose, the number of its applications, its effects on organic nutrients, and mycotoxin contamination of meadow fodder were investigated. Four variants of fertilization were used:

- D1 (control)—0 m<sup>3</sup>/ha
- D2—80 m<sup>3</sup>/ha applied in one dose (application after 1st harvest)
- D3—40 m<sup>3</sup>/ha applied in one dose (application after 1st harvest)
- D4—80 m<sup>3</sup>/ha divided into two doses (40 m<sup>3</sup>/ha applied after 1st harvest and m<sup>3</sup>/ha after 2nd harvest)

Digestate composition can be found in Table 2; it was produced at an agricultural biogas plant that processes maize silage and cow slurry. The application of digestate was carried out one week after the first and second cuts according to Table 3, using a self-propelled Vredo VT-4556 machine with a Vredo ZB3 8046 injector (P&L Ltd., Biskupice u Luhačovic, Czech Republic), which allows application to the soil (sod). Fields were cut thrice a year and samples were gathered on two harvest dates (Table 3): the second harvest was undertaken in the summer (July/August) and the third harvest was undertaken in the fall (October/November). The first harvest in May/June was not evaluated. Harvests were scheduled according to the buttoning or early flowering stage of the dominant species.

Analyses of the nutritive value of the fodder were conducted at Mendel University in Brno, Czech Republic after drying at 60 °C for 24 h and milling the samples to 1 mm fractions (Pulverisette laboratory cutting mill; Fritsch, Weimar, Germany). Sampling and analyses of the organic nutrient content were conducted according to the methods described in Commission Regulation (EC) No 152/2009 [41]. The following organic nutrients were determined: crude protein (CP), crude fat (CFAT), crude fiber (CF), ash, nitrogen-free extract (NFE), digestibility of organic matter (DOM), acid detergent fiber (ADF), and ash-free neutral detergent fiber (aNDF).

**Table 2.** Average chemical composition of digestate fresh matter depending on the production year.

| Nutrient              | 2019        | 2020        |
|-----------------------|-------------|-------------|
| Dry matter (%)        | 5.08 ± 0.60 | 5.55 ± 0.42 |
| Total nitrogen (g/kg) | 1.60 ± 0.14 | 2.37 ± 0.98 |
| Phosphorus (g/kg)     | 0.36 ± 0.03 | 0.39 ± 0.13 |
| Potassium (g/kg)      | 3.99 ± 0.42 | 3.35 ± 1.01 |

Values are expressed as a mean ± standard deviation.

**Table 3.** Dates of digestate application and biomass harvest each year.

| Location  | Year | Digestate Application |                   | Biomass Sampling |             |
|-----------|------|-----------------------|-------------------|------------------|-------------|
|           |      | After 1st Harvest     | After 2nd Harvest | 2nd Harvest      | 3rd Harvest |
| Skořenice | 2019 | 4 June                | 8 July            | 3 July           | 15 October  |
| Skořenice | 2020 | 27 May                | 11 August         | 1 August         | 26 November |
| Čěchtice  | 2019 | 7 June                | 8 August          | 31 July          | 1 November  |
| Čěchtice  | 2020 | 9 June                | 8 August          | 7 July           | 30 October  |

Mycotoxin analysis was conducted by enzyme-linked immunosorbent assay (ELISA). The sample was processed according to the ELISA test kit manufacturer's directions (MyBioSource, San Diego, CA, USA), and deoxynivalenol (DON), aflatoxin B1 (AFB1), and T-2 toxin (T-2) contents were determined. DON, AFB1, and T-2 toxin concentrations were measured on a Synergy HTX Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA). The absorption wavelength was set to 450 nm.

The data were statistically evaluated with Statistica 12.0 (TIBCO Software, Palo Alto, CA, USA). The normality of the data was tested with the Shapiro–Wilk test; subsequent testing for significant differences was conducted by one-way ANOVA and Kruskal–Wallis ANOVA. Post hoc testing was conducted with the Sheffé test. Results are expressed as a mean ± standard deviation, and differences were regarded as statistically significant at  $p < 0.05$ .

### 3. Results

The evaluation of nutritive value was performed based on nutrient and mycotoxin content, depending on the four fertilization variants and year and month of harvest. Three mycotoxin levels (DON, AFB1, and T-2) and a broad spectrum of nutrients (CP, CFAT, CF, NFE, DOM, ADF, and aNDF) were measured in the fodder samples.

DON levels were not correlated to any level of nutrition; therefore, there was no dependency of DON concentration on the biomass or concentrations of CP, CFAT, CF, ash, NFE, DOM, ADF, or aNDF. A strong correlation was found between AFB1 levels and levels of CP, CF, ash, DOM, ADF, and aNDF. A strong correlation was also found between T-2 levels and the levels of all nutrients.

#### 3.1. Mycotoxin Content

##### 3.1.1. Impact of Year on Mycotoxin Contamination of Biomass

The average concentrations of mycotoxins found in the sampled biomass were DON  $3584.1 \pm 3429.9$  µg/kg of dry matter, AFB1  $31.3 \pm 15.0$  µg/kg of dry matter, and T-2 toxin  $59.1 \pm 61.9$  µg/kg of dry matter. The average mycotoxin concentrations depending on the observed year from both harvests can be found in Table 4.

DON contamination fluctuated between the years; there were significantly higher levels observed in 2019 ( $p < 0.05$ ). AFB1 and T-2 toxin levels in the biomass were highly varied; however, significant differences were found between the observed years. Higher average AFB1 levels were measured in 2020 than in 2019 ( $p < 0.05$ ). T-2 toxin levels were significantly higher in 2019; the measured levels were very low in 2020 ( $p < 0.05$ ).

**Table 4.** Mycotoxin levels ( $\mu\text{g}/\text{kg}$  of dry matter) according to the year of harvest.

| Year | Deoxynivalenol                   | Aflatoxin B1                 | T-2 Toxin                     |
|------|----------------------------------|------------------------------|-------------------------------|
| 2019 | 6370.4 $\pm$ 2733.8 <sup>a</sup> | 25.3 $\pm$ 14.6 <sup>a</sup> | 107.5 $\pm$ 51.6 <sup>a</sup> |
| 2020 | 797.7 $\pm$ 525.5 <sup>b</sup>   | 37.3 $\pm$ 13.3 <sup>b</sup> | 10.7 $\pm$ 16.3 <sup>b</sup>  |

Results are expressed as a mean  $\pm$  standard deviation. Mean values are statistically significant at  $p < 0.05$ . Values in columns are marked with a different letter in the upper index.

### 3.1.2. Impact of Harvest Date on Mycotoxin Contamination of Biomass

The average mycotoxin concentrations depending on the harvest date can be found in Table 5. An effect of harvest on DON, AFB1, and T-2 contamination was not observed; significant differences between summer and fall harvests were not found ( $p < 0.05$ ).

**Table 5.** Mycotoxin levels ( $\mu\text{g}/\text{kg}$  of dry matter) according to the harvest date.

| Harvest | Deoxynivalenol                   | Aflatoxin B1                 | T-2 Toxin                    |
|---------|----------------------------------|------------------------------|------------------------------|
| Summer  | 3441.4 $\pm$ 3365.9 <sup>a</sup> | 34.3 $\pm$ 13.3 <sup>a</sup> | 58.3 $\pm$ 51.0 <sup>a</sup> |
| Fall    | 3726.7 $\pm$ 3597.3 <sup>a</sup> | 28.3 $\pm$ 16.4 <sup>a</sup> | 59.8 $\pm$ 73.0 <sup>a</sup> |

Results are expressed as a mean  $\pm$  standard deviation. Mean values are statistically significant at  $p < 0.05$ . Values in columns are marked with a different letter in the upper index.

### 3.1.3. Impact of Fertilization on Mycotoxin Contamination of Biomass

The average mycotoxin concentrations depending on the fertilization can be found in Table 6. No effect of digestate dose or number of applications was observed in the cases of DON, AFB1, nor T-2.

**Table 6.** Mycotoxin levels ( $\mu\text{g}/\text{kg}$  of dry matter) according to the digestate dose and application.

| Variant | Deoxynivalenol                   | Aflatoxin B1                 | T-2 Toxin                     |
|---------|----------------------------------|------------------------------|-------------------------------|
| D1      | 3875.4 $\pm$ 3883.4 <sup>a</sup> | 36.4 $\pm$ 17.5 <sup>a</sup> | 62.3 $\pm$ 58.2 <sup>a</sup>  |
| D2      | 3698.2 $\pm$ 3873.0 <sup>a</sup> | 27.6 $\pm$ 15.4 <sup>a</sup> | 67.1 $\pm$ 68.4 <sup>a</sup>  |
| D3      | 3798.4 $\pm$ 3711.8 <sup>a</sup> | 31.2 $\pm$ 14.0 <sup>a</sup> | 70.5 $\pm$ 78.5 <sup>a</sup>  |
| D4      | 2964.2 $\pm$ 2763.5 <sup>a</sup> | 30.1 $\pm$ 14.5 <sup>a</sup> | 36.30 $\pm$ 43.8 <sup>a</sup> |

Results are expressed as a mean  $\pm$  standard deviation. Mean values are statistically significant at  $p < 0.05$ . Values in columns are marked with a different letter in the upper index.

A trend of increasing mycotoxin contamination at lower digestate doses was observed. The highest DON levels were found in D1, followed by D3; however, results were not statistically significant ( $p < 0.05$ ). A higher digestate dose (D3) showed a tendency to decrease AFB1 levels in comparison to other fertilization variants. Furthermore, the highest AFB1 levels were observed in the control. However, no significant differences were found ( $p < 0.05$ ). T-2 toxin concentrations were higher in D2 and D3; however, there was no statistical significance ( $p < 0.05$ ). A divided dose of digestate (D4) showed a tendency to lower DON and T-2 levels.

## 3.2. Nutritional Analysis

Levels of CP, CFAT, and DOM were significantly higher in 2019; however, levels of CF, NFE, ADF, and aNDF were significantly higher in 2020. A significant difference between ash values depending on the year was not observed ( $p < 0.05$ ; Table 7).

Significant differences were observed in the case of ADF levels according to the harvest date, with higher levels measured in the second harvest (Table 8). The remaining nutrients were not affected by the harvest date ( $p < 0.05$ ).

**Table 7.** Nutrient levels according to the year of harvest (g/kg of dry matter).

| Nutrient Content (g/kg) | 2019                        | 2020                        |
|-------------------------|-----------------------------|-----------------------------|
| CP                      | 159.37 ± 24.12 <sup>a</sup> | 118.83 ± 30.26 <sup>b</sup> |
| CFAT                    | 38.84 ± 9.00 <sup>a</sup>   | 23.34 ± 4.96 <sup>b</sup>   |
| CF                      | 264.81 ± 28.59 <sup>a</sup> | 302.88 ± 34.11 <sup>b</sup> |
| Ash                     | 110.80 ± 38.04 <sup>a</sup> | 94.80 ± 8.18 <sup>a</sup>   |
| NFE                     | 426.18 ± 53.85 <sup>a</sup> | 460.15 ± 49.53 <sup>b</sup> |
| DOM                     | 65.99 ± 5.62 <sup>a</sup>   | 61.10 ± 5.28 <sup>b</sup>   |
| ADF                     | 337.87 ± 49.12 <sup>a</sup> | 359.04 ± 34.27 <sup>b</sup> |
| aNDF                    | 549.92 ± 74.04 <sup>a</sup> | 643.37 ± 61.00 <sup>b</sup> |

Results are expressed as a mean ± standard deviation. Mean values are statistically significant at  $p < 0.05$ . Values in a row are marked with a different letter in the upper index.

**Table 8.** Nutrient levels according to the harvest date (g/kg of dry matter).

| Nutrient Content (g/kg) | Summer Harvest              | Fall Harvest                |
|-------------------------|-----------------------------|-----------------------------|
| CP                      | 147.08 ± 33.38 <sup>a</sup> | 134.18 ± 33.42 <sup>a</sup> |
| CFAT                    | 31.39 ± 11.76 <sup>a</sup>  | 31.84 ± 9.73 <sup>a</sup>   |
| CF                      | 281.57 ± 28.48 <sup>a</sup> | 283.47 ± 43.10 <sup>a</sup> |
| Ash                     | 91.36 ± 9.16 <sup>a</sup>   | 114.81 ± 36.69 <sup>a</sup> |
| NFE                     | 448.60 ± 47.95 <sup>a</sup> | 435.70 ± 59.65 <sup>a</sup> |
| DOM                     | 63.63 ± 4.95 <sup>a</sup>   | 63.80 ± 6.84 <sup>a</sup>   |
| ADF                     | 359.62 ± 32.13 <sup>a</sup> | 336.38 ± 50.58 <sup>b</sup> |
| aNDF                    | 597.31 ± 76.61 <sup>a</sup> | 589.78 ± 88.49 <sup>a</sup> |

Results are expressed as a mean ± standard deviation. Mean values are statistically significant at  $p < 0.05$ . Values in a row are marked with a different letter in the upper index.

No significant effects on nutrition levels were observed in the case of different fertilization variants ( $p < 0.05$ ; Table 9). CP and CFAT levels were lowest in the control variant (D1). The use of a higher digestate dose (D2, D3, and D4) tended to lead to higher levels of all nutrients except NFE. The trend of higher ash, CF, and DOM levels was observed when the divided digestate application (D4) was used.

**Table 9.** Nutrient levels according to the fertilization regime (g/kg of dry matter).

| Nutrient Content (g/kg) | D1                          | D2                          | D3                          | D4                          |
|-------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| CP                      | 126.58 ± 25.46 <sup>a</sup> | 140.93 ± 35.57 <sup>a</sup> | 139.96 ± 33.20 <sup>a</sup> | 150.94 ± 36.12 <sup>a</sup> |
| CFAT                    | 30.06 ± 11.74 <sup>a</sup>  | 32.72 ± 9.98 <sup>a</sup>   | 30.66 ± 9.23 <sup>a</sup>   | 32.57 ± 12.23 <sup>a</sup>  |
| CF                      | 281.72 ± 32.67 <sup>a</sup> | 284.29 ± 37.88 <sup>a</sup> | 281.74 ± 40.52 <sup>a</sup> | 282.16 ± 36.21 <sup>a</sup> |
| Ash                     | 102.75 ± 34.67 <sup>a</sup> | 102.40 ± 21.52 <sup>a</sup> | 97.82 ± 17.86 <sup>a</sup>  | 109.81 ± 39.22 <sup>a</sup> |
| NFE                     | 458.90 ± 52.47 <sup>a</sup> | 439.67 ± 49.43 <sup>a</sup> | 449.82 ± 52.94 <sup>a</sup> | 424.52 ± 59.33 <sup>a</sup> |
| DOM                     | 62.84 ± 5.99 <sup>a</sup>   | 63.72 ± 5.95 <sup>a</sup>   | 63.46 ± 5.94 <sup>a</sup>   | 64.60 ± 6.21 <sup>a</sup>   |
| ADF                     | 350.15 ± 42.28 <sup>a</sup> | 350.30 ± 41.93 <sup>a</sup> | 352.95 ± 47.76 <sup>a</sup> | 338.58 ± 44.82 <sup>a</sup> |
| aNDF                    | 591.83 ± 83.74 <sup>a</sup> | 597.44 ± 82.87 <sup>a</sup> | 604.52 ± 75.42 <sup>a</sup> | 580.56 ± 90.54 <sup>a</sup> |

Results are expressed as a mean ± standard deviation. Mean values are statistically significant at  $p < 0.05$ . Values in a row are marked with a different letter in the upper index.

#### 4. Discussion

The number of biogas plants in Europe has increased over the period 2007–2017 due to the increased pressure on sustainable and renewable energy production [42]. Digestate can have positive effects on the environment, especially on soil fertility, compared to undigested fertilizers [43]. Therefore, the creation of a digestate as a byproduct produces a need to evaluate this bioresource's effect on forage production and safety, and information on the topic is sparse. Due to the possible presence of pathogens, it may be a source of environmental risks when used as a fertilizer on feed-producing grasslands [44]. Nutritional pathologies have consequent effects on animal susceptibility to diseases and,

therefore, to higher antibiotic usage in livestock breeding. Hence, the research on microbial contamination of animal feed is necessary [41,45]. Schnürer and Schnürer stated that several species of fungi, including mycotoxigenic *Aspergillus* spp., can survive the anaerobic digestion process [46]. Mycotoxin contamination can lead to economic losses, feed safety compromise, and subsequent health problems [47,48]. In our study, there were no increased levels of DON, AFB1, or T-2 in the biomass when compared to the unfertilized control, which suggests that there was no increased contamination by fungal producers of these mycotoxins. Venslovas et al. came to similar conclusions in grass silage [49]. Therefore, the risks of such contamination are not increased using digestate on permanent grasslands, but more research is needed on other agricultural crops.

Some authors have raised a concern about the negative impact of mycotoxin contamination of the biomass on the production of methane during anaerobic digestion [46,47]. However, there was no increase in mycotoxin contamination depending on digestate dose when compared to the control in our experiment. Moreover, Tacconi et al. stated that aflatoxin contamination higher than 100 µg/kg can inhibit the process of anaerobic digestion [50]. The average aflatoxin contamination in our samples did not exceed this limit. De Gelder et al. stated that biogas production was not affected by the presence of DON, ZEN, or AFB1 and, therefore, the contaminated biomass can be safely used for digestate production [51]. This suggests that reuse of digestate for fertilization of the biomass in areas with similar conditions to the ones in our experiment would produce suitable and efficient feeding material for biogas plants. Moreover, the contaminated grass biomass could serve as a sufficient digestion substrate for digestate production in the case of mycotoxin levels exceeding recommended amounts for feed. According to international guidelines, the limits of mycotoxins in feed are 900–12,000 µg/kg DON and 5–50 µg/kg AFB1 [41,52,53]. The grassland biomass harvested in this experiment did not exceed those limits (Table 4) and, therefore, would be suitable for either use—feeding material for a biogas plant or as fodder. T-2 toxin recommended limits for feed were not found in the current European regulations [41,52,53].

Mycotoxin contamination is highly dependent on weather conditions and, therefore, can change both during the vegetation season and throughout the year [16,48,49]. This was partially the case in our study, where significant differences in DON, AFB1, and T-2 were observed between 2019 and 2020, as shown in Table 4 ( $p < 0.05$ ). However, differences in mycotoxin levels between the summer and fall harvests were not found ( $p < 0.05$ ; Table 5). This is in accordance with the results of the Swedish study from 2014, where fungal counts did not increase with a later harvest date [54]. Climate change affects the concentration of mycotoxins in the fodder. However, this factor is not crucial in short-term observations, such as the one in this experiment. In this study, however, the long-term effects of climate change were not the focus of the experiment. In short-term observation, it is more important to consider the factors of weather (such as precipitation and temperature) and harvest date [55].

The study by Karlsson et al. had *Fusarium* spp. and, therefore, potential mycotoxin contamination of the biomass increased with agricultural intensification represented by higher nitrogen fertilization [56]. On the contrary, Krnjaja et al. stated that *Fusarium* sp. and mycotoxin levels did not increase significantly with a higher dose of nitrogen fertilization [57]. However, this effect was significant in the next year of observation; therefore, it is possible to assume that the year has a greater impact on mycotoxin concentration in the biomass than fertilization. Similarly, in the study by Baholet et al., no differences in DON levels were found with an increased digestate dose [58]. This was also demonstrated in our study, where an increased digestate dose did not significantly affect DON, AFB1, or T-2 levels ( $p < 0.05$ ; Table 6). This may point to the safety of its use in fodder production.

The basis of animal nutrition is not dependent on one nutrient, but on a set of integrated components. One of them is CF, comprising ADF and NDF fractions, as an indicator of digestibility and palatability of organic matter, which is correlated with the number of microorganisms in the rumen [59]. According to Kozsel et al., levels of CFAT and CP are

positively correlated with digestate dose [60]. This was not confirmed in our experiment, due to the lack of significant differences in CFAT values (Table 9). However, the trend suggested that the lowest levels of CFAT were indeed found in the unfertilized control, which would be in accordance with the study.

The chemical composition of digestate can influence the fertilization effect on grass stands, which could subsequently be reflected in the nutrient level changes year to year. In 2020, the nitrogen content of digestate used in our experiment was lower than in 2019, which could lead to lower CP and CFAT levels in the biomass (Table 7). Research by Grigatti et al. and Panuccio et al. supports the idea by stating that each sample of digestate can differ in its properties and effects, according to its chemical composition [61,62].

The use of digestate has similar effects on biomass production as the use of mineral fertilizer, especially in lower doses [44,59,63]. However, Tilvikiene states that short-term fertilization of grasslands does not have a significant effect on yield enhancement and increases in yield and nutritive value are attained only after long-term use [64]. Eich-Greatorex et al. add that digestate can reduce bulk density in loamy soil and improve water retention in sandy soil [63]. Chatterjee and Mazumder brought attention to the possibility of structural degradation due to the often-high levels of sodium in digestate [65]. Jasa et al. showed similar views on the topic, with digestate having more positive effects on sandy soils, while on heavy soils, it can be detrimental with respect to porosity and minimal air capacity, which can negatively affect plant fitness [66]. In our experiment, a divided dose of digestate (D4) showed a tendency to lower DON and T-2 levels in comparison to the application of the same amount (80 m<sup>3</sup>/kg) in one dose (D2). The information provided by these authors, combined with our data, suggest that the fertilization of grasslands using digestate in multiple smaller doses could be more beneficial than the application of one large dose per year.

## 5. Conclusions

Our experiment was based on the hypothesis that digestate can be used as a fertilizer on grasslands with no significant effect on nutritive value, and that fodder safety will not be compromised by digestate fertilization in higher doses ( $p < 0.05$ ). The results of our study supported this hypothesis. No significantly negative effect of digestate on DON, AFB1, or T-2 levels was found ( $p < 0.05$ ). Weather conditions throughout the years had a significant effect on mycotoxin levels; however, fertilization and harvest date (summer and fall) did not ( $p < 0.05$ ). The effects of digestate dose and harvest date had no effect on nutrient levels except ADF, which were lower in the fall ( $p < 0.05$ ). Levels of CP, CFAT, and DOM were significantly higher in 2019; levels of CF, NFE, ADF, and aNDF were significantly lower in 2019; and levels of ash did not change between years.

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