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Methane yield of perennial grasses as affected by the chemical composition of their biomass

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Abstract

The aim of the research work was to study the chemical properties of most cultivated perennial Fabaceae and Poaceae plants: common cocksfoot (*Dactylis glomerata* L.), reed canary grass (*Phalaris arundinacea* L.), tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), common lucerne (*Medicago sativa* L.), common sainfoin (*Onobrychis viciifolia* Scop.) and switchgrass (*Panicum virgatum* L.), and to link them to methane (CH_4) and biogas yields under laboratory conditions. The results of chemical analyses showed that the biomass of legumes was most suitable for biogas production: high digestibility: 78.4–73.5% dry matter (DM) and low cellulose concentration 20.8–24.4% DM, were determined to be most suitable for anaerobic digestion. The biomass of *L. perenne* and *F. arundinacea* was best-suited for anaerobic digestion due to the highest content of water-soluble carbohydrates (WSC) 16.4–20.3% DM, and the lowest content of cellulose 27.3–28.9 % DM and acid detergent fibre (ADF) 31.3–32.8% DM. The best yields of methane were obtained from *O. viciifolia* – 277.7 L CH_4 kg^{-1}, *D. glomerata* – 213.9 L CH_4 kg^{-1} and *L. perenne* – 205.7 L CH_4 kg^{-1}. The correlations corroborated that the methane yield depended on the chemical composition of the biomass. Methane yield positively and significantly correlated with WSC – 0.761** and digestibility – 0.744** ($P \leq 0.01$), and negatively correlated with cellulose – 0.793**and ADF – 0.762**. The best results of specific methane yields were demonstrated by *O. viciifolia* (1453 m^3 CH_4 ha^{-1} DM), *M. sativa* (1326 m^3 CH_4 ha^{-1} DM) and *L. perenne* (1060 m^3 CH_4 ha^{-1} DM).
This study with seven species of perennial grasses could serve as a basis for more advanced experiments on how to choose grass species for the best methane yield.

Key words: anaerobic digestion, biomass, biogas, methane, perennial plants.

Introduction

Due to the growing demand for energy and concerns about the increasing greenhouse gas emissions, perennial grasses have attracted worldwide attention as renewable energy sources offering several advantages over annual crops, such as lower establishment costs, higher biomass productivity, improved soil health, increased water quality and reduced soil erosion (Nazli, Tansi, 2019).

Europe is the world's leading producer of biomethane with 459 plants in 2015 producing an estimated 1.23 billion m^3 annually and 414 plants in the European Union (EU) and 1.2 billion m^3 annually, in comparison with the total biogas production of about 18 billion m^3 in the same year. Biogas upgrading to biomethane takes place in 15 European countries and is injected into the natural gas grid in 10 countries (Wellinger et al., 2013; Scarlat et al., 2018). The leading countries in the biogas production in the EU are Germany, United Kingdom, Italy, Czech Republic and France. Germany is the European leader with a biogas production of 329 petajoule (PJ) and a share of 50% of the total biogas production in the EU in 2015. Lithuania produces 981 terajoule (TJ), Latvia – 3674 TJ, Poland – 9581 TJ of biogas. Most biomethane production plants are in Germany (185), United Kingdom (80) and Sweden (61). In the other countries the biomethane production volumes are still marginal (EBA, 2018; Scarlat et al., 2018).

Perennial grasses are attractive sources of biomass for Northern Europe and North America, as they meet agronomic, environmental and societal requirements for successful deployment as energy grass crops (Price et al., 2004; Monti et al., 2009; Rösch et al., 2009; Allison et al., 2012). Perennial grasses will likely be a dominant feedstock for on-farm anaerobic digestion in Northeast Europe. Sustainable development is a current notion strictly related to the concept of a circular economy. In this respect, the production of renewable energy by the valorisation of wastes or by-products is considered as one of the most dominant future renewable energy sources (Appels et al., 2011; Coppolecchia et al., 2015; Chiumenti et al., 2018).

The use of energy crops has increased in several countries, particularly in Germany and Austria, due to their exceptionally high methane yields which increase the profitability of biogas production. Co-digestion of various substrates also contributes significantly to the improvement of the digestion process, the improvement of biogas yield and biogas plant performance. However, the sustainability debated on the use of energy crops and their impact on land use changes and on food security has led to limitations for the share of energy crops used for biogas production in Germany, Austria and Denmark. Thus, it is expected that the use of energy crops and their potential in the future biogas production in the EU will be increasingly limited due to sustainability considerations and support directed only to the use of waste and residues. Alternatively, landscape grass, consisting of herbaceous plant composite, could represent one of the most promising feedstocks to improve the sustainability of the biogas sector (Scarlat et al., 2018).

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In Lithuania, particular attention has been paid to the productivity of local and introduced plant species with high energy value and the ability to use them as a biofuel in recent years (Slepetys et al., 2012; Jasinskas et al., 2014; Slepetiene et al., 2016). Native, naturally growing perennial grasses, such as *Festuca arundinacea*, *Dactylis glomerata* and *Phalaris arundinacea*, have already been studied in Lithuania (Tilvikienė et al., 2012; Nekrošius et al., 2014; Butkutė et al., 2014; Pocienė, Kadžiulienė, 2016; Slepetiene et al., 2016; Tilvikiene et al., 2016) as well as *Panicum virgatum*, which is an introduced grass (Norkevičienė et al., 2016). Agricultural researchers also show interest in other perennial plants: *Medicago sativa*, *Onobrychis viciifolia* and *Lolium perenne* (Slepetys et al., 2012; Slepetiene et al., 2016; Kemesyte et al., 2017).

The stability and productivity of anaerobic digestion of biomass are mostly influenced by its chemical composition (Slepetiene et al., 2016; Tilvikiene et al., 2016). Higher concentrations of fibre components (cellulose, hemicellulose and lignin) are a disruptive factor in the process of methane production (Seppälä et al., 2009; Triolo et al., 2011; McEniry, O'Kiely, 2013; Yang et al., 2015; Tilvikiene et al., 2016). Each fibre component in biomass has specificity and all components are tightly interdependent (Klimiuk et al., 2010; Butkutė et al., 2014). Hemicellulose is most susceptible to anaerobic conversion or to pre-treatment effects, whereas cellulose is not readily degradable and needs more energy or pre-treatment for the digestion (Yang et al., 2015). Therefore, cellulose, hemicellulose and lignin levels and their ratio are important in grass biomass composition (Kupryś-Caruk et al., 2019).

Anaerobic digestion is a biological process, wherein diverse group of microorganisms decompose the complex organic matter in the absence of oxygen. Thermochemical conversion destroys every component in biomass converting it to carbon dioxide, carbon monoxide, hydrogen, methane, nitrogen oxides and water in various amounts. Methane fermentation technologies exhibit higher specificity: lignin is not converted, 34–92% of proteins are hydrolysed and fermented depending on various conditions, 70–95% of lipids, 65–70% of polymerised sugars and ~95% of sugar oligomers are destroyed (Bentsen, Felby, 2012). High biomass yield and high specific methane yield are parameters important when choosing the most appropriate crops for biogas production (Kupryś-Caruk et al., 2019). However, there is a lack of knowledge on the use of fresh perennial herbaceous plants for methane production and which biomass would be the most efficient and rational to use as a renewable source.

The aim of the research was to study the chemical properties of perennial herbaceous plants and to link them to methane (CH_4) and biogas yields under laboratory conditions.

Materials and methods

Experimental plots. Seven common grass species: common cocksfoot (*Dactylis glomerata* L.), reed canary grass (*Phalaris arundinacea* L.), tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), common lucerne (*Medicago sativa* L.), common sainfoin (*Onobrychis viciifolia* Scop.) and switchgrass (*Panicum virgatum* L.), were grown in the field plots (0.5 m^2) within three replicate blocks in 2018 at the Institute of Agriculture (55°23′49″ N, 23°51′40″ E), Lithuanian Research Centre for Agriculture and Forestry. Herbage samples were taken in May–June (1st cut – heading / inflorescence emergence, the first harvest year) of 2018 and were cut to 1 cm pieces and stored at −18°C temperature for biogas analyses. Biomass yield was measured at the same time.

Chemical analyses were done at Chemical Research Laboratory of Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. For chemical analyses and biogas experiment, plant samples were fixed at 105 ± 5°C temperature for 20 min and dried at 65 ± 5°C temperature for 24 hours, and after that ground in a laboratory mill. Chemical composition of the plant samples was determined according to the standard methods: for ash and organic matter (OM) content the dried samples were incinerated at 550°C temperature. Before testing, the samples for biogas production and chemical composition were ground by an ultra-centrifugal mill ZM 200 (Retch, Germany) using 1 mm mesh size. Total organic carbon (C) content was determined by a spectrophotometric procedure at a wavelength of 590 nm using glucose as a standard after wet combustion according to Tyurin method modified by Nikitin (1999). Nitrogen (N) content was determined by the Kjeldahl method using a spectrophotometric procedure at the wavelength of 655 nm.

Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) in plant biomass were determined using a cell wall detergent fractionation method according to Van Soest et al. (1991). NDF and ADF extraction was done on a fibre analyser ANKOM 220 (ANKOM Technology, USA) using F57 filter bags (25-μm porosity). Lignin was determined in beakers on the remaining material from the ADF procedure as a residue insoluble in sulfuric acid (72% w/w). Cellulose (Cel) was determined as the difference between ADF and ADL and hemicellulose (Hcel): Hcel = NDF − ADF. The concentrations of water-soluble carbohydrates (WSC) were determined by anthrone method. Digestion of plant biomass and crude proteins (CP) was measured by a NIRS 6500 system (Foss, Denmark).

The feedstocks (biomass + inoculum) before anaerobic digestion were analysed for total solids (TS), volatile solids (VS) and mineral solids (MS) by a gravimetric method. This method includes evaporation of the sample, drying of the residue at 105°C temperature to a constant weight, and then repeating the steps taking into account the burning of the dry residue at 550°C temperature (Dudek et al., 2017). The anaerobic process indicator, the chemical oxygen demand (COD), which indicates the decomposition of OM and total nitrogen (N) of feedstock, was measured using spectrophotometer Lange DR3900 (Hach Lange GmbH, Germany). Acidity (pH) was determined in 1 M KCl (soil to solution ratio 1:2.5, w:v) using a potentiometric method. Organic acid to alkalinity ratio (FOS:TAC) was measured by titration with sulfuric acid 0.1% solution.

Anaerobic digestion experiments. The investigation of biogas production in this paper is based on the results of laboratory experiments conducted in the Chemical Research Laboratory of Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. The experiment was performed by the manometric control system OxiTop (WTW, Germany).

Biogas production was continuously measured using a glass vessel of 250 mL volume. The manometric device consists of a glass vessel provided with a pressure transducer located in a measuring head. During anaerobic tests, the vessels were mixed by a magnetic stirrer. The pressure due to biogas accumulation in the headspace was automatically registered by the measuring heads. Each bottle was filled with 0.05 L of inoculum (pH 8.4, FOS:TAC 0.10, TS 2.29%, MS 53.12% TS, VS 46.87% TS, N 2.33 g L^{-1} and COD 4.68 g L^{-1}) and 0.25 g OM of biomass, with a starting organic load 5 g L (Dudek et al., 2017; Zielinski et al., 2017). A sufficient headspace volume was provided not to exceed the maximum pressure value of 400 hPa. Before starting, the vessel headspace was flushed with N_2 for 120 s. The biogas production was accompanied by daily automatic measurements with an interval of 4 min. The maximum of each replicate and average gas production rates in hPa were calculated. To evaluate the repeatability of the method, the tests were conducted in triplicate. OxiTop vessels were incubated at 37 ± 1°C temperature under mesophilic conditions for 35 days in a thermostat. The composition of biogas was measured by a gas data analyser GFM406 (Gas Data, UK).

Calculations. Biogas production was calculated by the formula:

n (mol) = p × V / R × T, where p is headspace pressure (hPa), V – bottle volume (ml), R – ideal gas constant (8.314 J / (mol × K), T – incubation temperature (K).

Biogas volume was calculated by the formula: L biogas = n (mol) × 22.4; under normal conditions (t = 0°C; P = 1.013 × 10^5 Pa) one mol of gas takes 22.4 L.

The specific methane (CH_4) yield was calculated as the cumulative sum of the methane volume produced over a 35-day incubation period relative to the substrate by dry matter (DM) concentration added to the test. In addition, the area-specific methane yield (m^3 CH_4 ha^{-1}) was calculated using values for specific methane yield and the total solids yield per hectare (McEniry, O'Kiely, 2013; Chiumenti et al., 2018).

Statistical analysis. The data structuring analysis and processing were conducted using analysis of variance (ANOVA) software SAS, version 9.4 (SAS Institute Inc., USA); *P* value <0.05 was considered statistically significant. The different letters a–e in the column indicate significant differences ($P < 0.05$) between the components. The correlations between chemical composition and methane yield were estimated by Pearson method: ** – significant at $P \leq 0.01$, * – significant at $P \leq 0.05$ level.

Results and discussion

Different plant species produced different biomass yields. Biomass production per surface of land area amounted to 0.54–7.9 t ha^{-1} DM (Table 1). *Medicago sativa* produced the highest biomass yield of 7.9 t ha^{-1} DM. Biomass yields of *Onobrychis viciifolia* and *Lolium perenne* were 5.32 and 5.15 t ha^{-1} DM, respectively. The lowest biomass yields were produced by *Festuca arundinacea* and *Phalaris arundinacea* – 0.54 and 2.37 t ha^{-1} DM, respectively. Biomass yields of *Dactylis glomerata* and *Panicum virgatum* were 3.96 and 3.63 t ha^{-1} DM.

The chemical characteristics of the investigated perennial grasses are summarised in Table 1. Each perennial herbaceous plant species had different chemical composition. Results of our research are similar to other researchers' data on the chemical composition of the plants: NDF 49.6–74.15 (Allison et al., 2012; McEniry, O'Kiely, 2013; Butkutė et al., 2014), ADF 35.5–37.5 (Butkutė et al., 2014), ADL 1.2–31 (McEniry, O'Kiely, 2013; Butkutė et al., 2014), WSC 4.9–18.8 (McEniry, O'Kiely, 2013; Butkutė et al., 2014; Kupryś-Caruk et al., 2019), Cel 21.4–35.0 (Chiumenti et al., 2018; Kupryś-Caruk et al., 2019), HCel 21.9–24.6 (Butkutė et al., 2014; Kupryś-Caruk et al., 2019) CP 13.8–17.7 (Slepetys et al., 2012; McEniry, O'Kiely, 2013) and C:N 18.8–40.3 (Butkutė et al., 2014; Kupryś-Caruk et al., 2019). But there are some differences in chemical composition of plants biomass, they are presented in the discussion. *P. virgatum* had significantly ($P < 0.05$) the highest NDF, ADF, cellulose, hemicellulose, C content, carbon to nitrogen ratio (C:N) and the lowest values of WSC, crude proteins and digestibility.

Table 1. Biomass yield and chemical composition of the seven perennial herbaceous plant species

Grass species	Yield t ha^{-1} DM	Ash	NDF	ADF	ADL	WSC	Cel	HCel	C	N	Digestibility	CP	C:N
		% DM											
Dactylis glomerata	3.96	7.63 b	55.26 e	33.2 d	4.18 c	13.5 c	29.0 c	22.04 c	45.8 abc	2.25bc	58.4 d	13.39 d	20.3 c
Phalaris arundinacea	2.37	7.43 bc	62.71 d	37.8 b	4.67bc	6.75e	33.01 b	24.96 b	46.6 ab	2.20 bc	45.3 e	12.59 d	21.2 c
Festuca arundinacea	0.54	7.52 bc	52.19 d	31.3 e	4.02c	16.4 b	27.3 d	20.88 c	44.7 c	2.46 b	62.5 c	15.05 c	18.2 dc
Lolium perenne	5.15	6.67 d	54.75 c	32.8 d	3.97c	20.3 a	28.9 c	21.93 c	45.9 abc	1.63cd	57.9 d	10.28 e	28.1 b
Medicago sativa	7.9	9.17 a	37.38 e	35.3 c	10.9a	11.1 d	24.4 e	2.07 d	45.8 abc	3.45 a	73.5 b	20.93 a	13.3 d
Onobrychis viciifolia	5.32	6.92 cd	32.66 f	32.1 d	11.3a	16.9 b	20.8 f	0.59 d	45.4 bc	2.59 b	78.4 a	19.45 b	17.5 dc
Panicum virgatum	3.63	5.80 e	70.49 a	39.9 a	5.70b	5.91 e	34.2 a	30.54 a	46.8 a	1.11 d	42.8 e	8.70 f	42.1 a

Note. DM – dry matter; NDF – neutral detergent fibre, ADF – acid detergent fibre, ADL – acid detergent lignin, WSC – water-soluble carbohydrates, Cel – cellulose, HCel – hemicellulose, C – carbon, N – nitrogen, CP – crude proteins, C:N – carbon to nitrogen ratio; different letters a–e in the column indicate significant differences ($P < 0.05$) in concentration of respective biomass component.

P. arundinacea had high values of ADF, cellulose and hemicellulose and low values of digestibility. McEniry and O'Kiely (2013), Dandikas et al. (2014) and Conga et al. (2018) have reported that biogas potential of different plants correlates negatively with ADF, cellulose, hemicellulose and NDF. Significantly the lowest ADF concentrations were determined in the biomass of *F. arundinacea* 31.3% DM, *O. viciifolia* 32.1% DM and *L. perenne* 32.8% DM, while the highest amount of ADF 39.9% DM was found in the biomass of *P. virgatum*. The biomass of *O. viciifolia* and *M. sativa* had the highest amounts of ADL – 11.3% and 10.9% DM, respectively, whereas the lowest ADL content was found in the biomass of *L. perenne* 3.97% DM. The biomass of *L. perenne* had the highest value of WSC 20.3%; higher amount of WSC was determined in the biomass of *O. viciifolia* 16.9% DM and *F. arundinacea* 16.4% DM. The biomass of *P. virgatum* had the highest amount of cellulose 34.2% DM and hemicellulose 30.54% DM compared with other treatments. The carbon amount of the investigated biomass varied within 44.7–46.8% DM range.

Biomass composition of legume plants was more appropriate for methane production compared with other treatments. One of the most important biomass indicators for methane production is digestibility. The highest digestibility was determined for the biomass of *O. viciifolia* 78.4% DM and *M. sativa* 73.5% DM. The lowest digestibility was determined for the biomass of *P. virgatum*.

M. sativa and *O. viciifolia* had high amount of crude proteins – 20.93% and 19.45%, respectively. According to the chemical composition, *O. viciifolia* had the best qualities for biogas production, because its biomass had higher digestibility, a higher amount of WSC and the lowest amount of cellulose compared with other treatments. Biomasses of *M. sativa* with the highest digestibility and *L. perenne* with the highest amount of WSC are suitable for biogas production.

The optimal C:N in plant substrates for the biogas production process is from 20:1 to 30:1 (Lehtomäki et al., 2008; Klimiuk et al., 2010). Optimization of the anaerobic process, biological degradability of biomass and methane yield depend on this ratio. In our investigated grass biomass the C:N ranged from 13.3 to 42.1. According to Lehtomäki et al. (2008) and Klimiuk et al. (2010), *P. virgatum* had too high C:N – 42.1, *M. sativa* and *O. vicifolia* had too low C:N – 13.3 and 17.5 for the biogas production process, but *L. perenne*, *D. glomerata* and *P. arundinacea* had optimal C:N.

In order to obtain a high methane yield, plant substrate has to have optimal C:N, low lignin content but high content of readily degradable non-structural carbohydrates and soluble cell components (Kupryś-Caruk et al., 2019). Before the anaerobic digestion, chemical analysis was performed on the biomass of each perennial grass species tested (Table 2). For all of the studied feedstock, the pH ranged from 8.4 to 8.5. According to Orhorhoro et al. (2017), for microbiological activity the most favourable environment is neutral or slightly alkaline ($6.5 < pH < 8.5$) and optimal range is from 7.0 to 8.0.

At pH values below 7, the activity of the microorganisms that degrade volatile fatty acids is lower, which reduces biogas production. Stable biogas production usually takes place at a FOS:TAC value below 0.4; the optimal FOS:TAC value is close to 0.36. At pH values below 7, the activity of the microorganisms that degrade volatile fatty acids is lower, which reduces biogas production (Zielinski et al., 2017).

All the studied biomasses had the correct FOS:TAC value below 0.36 (Dudek et al., 2017; Zielinski et al., 2017). The volatile FOS:TAC value ranged from 0.10 to 0.13 (Table 2), biomasses of *P. arundinacea* and

Table 2. Properties of feedstock (plant biomass and inoculum) for anaerobic digestion

Feedstock (biomass + inoculum)	pH	FOS:TAC	TS	MS	VS	N	COD
			%	% TS		g L^{-1}	
Dactylis glomerata	8.5 ± 0.01	0.10 ± 0.001	2.94 ± 0.001	37.0 ± 0.01	63.0 ± 0.01	2.33 ± 0.001	6.0 ± 0.01
Phalaris arundinacea	8.4 ± 0.01	0.13 ± 0.001	3.50 ± 0.001	36.4 ± 0.01	63.6 ± 0.01	1.68 ± 0.001	6.6 ± 0.01
Festuca arundinacea	8.4 ± 0.02	0.10 ± 0.001	4.04 ± 0.003	44.5 ± 0.01	55.5 ± 0.01	2.34 ± 0.001	6.4 ± 0.01
Lolium perenne	8.4 ± 0.02	0.10 ± 0.001	4.14 ± 0.002	38.1 ± 0.01	61.9 ± 0.02	2.25 ± 0.001	6.9 ± 0.01
Medicago sativa	8.4 ± 0.01	0.11 ± 0.001	3.02 ± 0.003	54.6 ± 0.03	45.4 ± 0.03	2.20 ± 0.001	6.7 ± 0.01
Onobrychis viciifolia	8.4 ± 0.02	0.12 ± 0.001	3.76 ± 0.004	36.3 ± 0.04	63.7 ± 0.04	2.22 ± 0.001	7.1 ± 0.01
Panicum virgatum	8.4 ± 0.01	0.11 ± 0.001	3.09 ± 0.005	45.1 ± 0.03	54.9 ± 0.03	2.33 ± 0.001	5.5 ± 0.01

FOS:TAC – organic acid to alkalinity ratio, TS – total solids, MS – mineral solids, VS – volatile solids, N – total nitrogen, COD – chemical oxygen demand; ±SE – standard error from average

O. viciifolia had the most favourable FOS:TAC value for the anaerobic process. The content of total solids (TS) of the feedstock for anaerobic digestion was 2.94–4.14%, mineral solids (MS) – 36.3–54.6 (% TS) and volatile solids (VS) – 45.4–63.7 (% TS). Feedstock of *F. arundinacea*, *P. virgatum* and *D. glomerata* had the highest concentration of total nitrogen (N) 2.33–2.34 g L^{-1}. In contrast, the feedstock of *P. arundinacea* had less N – 1.68 g L^{-1}. The highest chemical oxygen demand (COD) was found when using biomass of *O. viciifolia* – 7.1 g L^{-1} and *L. perenne* – 6.9 g L^{-1}. The COD value of other biomasses varied between 6.0–6.7 g L^{-1}. The lowest COD 5.5 value was determined for the feedstock of *P.virgatum*. Zielinski et al. (2017) indicated COD range of feedstock 2.8–12.7 g L^{-1}.

The data of Table 3 show the results of biogas production for 35 days in anaerobic conditions by system OxiTop. Biogas release from the biomass of seven plant species having the same organic matter content slightly differed until day 30 of the anaerobic experiment, but later the treatments differentiated and produced different amounts of biogas. During this investigation, 0.076–0.096 L of biogas was produced. Biomass of *L. perenne* produced the highest amount of biogas – 0.096 L. Lower biogas yields were generated from the biomass of *F. arundinacea* – 0.087 L, *O. viciifolia* – 0.086 L and *D. glomerata* – 0.084 L. The lowest biogas production was determined for *P. virgatum* – 0.076 L and *P. arundinacea* – 0.077 L.

According to the results of the biogas analysis, biogas and methane yield in dry (DM) and in fresh (FM) mass was calculated (Table 3). Biogas yield ranged from 63.2 to 114.3 NL kg^{-1} FM and from 210.0 to 435.3 NL kg^{-1} DM.

Legumes and grasses were compared according to biogas production. Assessment of the production of biogas from the biomass of different perennial grasses (in DM) showed that the highest biogas yield was produced by *O. viciifolia* – 435.3 NL kg^{-1} (63.2 NL kg^{-1} FM), *D. glomerata* – 329.0 NL kg^{-1} (80.0 NL kg^{-1} FM) and *F. arundinacea* – 307.5 NL kg^{-1} (90.6 NL kg^{-1} FM) compared with the other treatments. Lower amount of biogas was produced by *L. perenne* – 304.3 NL kg^{-1} (114.3 NL kg^{-1} FM) and *M. sativa* – 266.9 NL kg^{-1} (90.0 NL kg^{-1} FM). The least-suited biomass for biogas production was that of *P. virgatum* and *P. arundinacea*.

Chiumenti et al. (2018), who studied the perennial grasses, reported biogas yield of 164.6 NL kg^{-1} FM and 507.5 NL kg^{-1} DM, and methane yield – 87.4 L CH_4 kg^{-1} FM and 269.5 L CH_4 kg^{-1} DM. Scarlat et al. (2018) suggest that methane yields from grass were 55–128 L CH_4 kg^{-1} FM and 300–450 L CH_4 kg^{-1} DM. Nekrošius et al. (2014) have documented 345–448 L CH_4 kg^{-1} DM, Seppälä et al. (2009) – 264–310 L CH_4 kg^{-1} DM. The biogas and methane content of grass biomass ranged between 214.8–435.3 NL kg^{-1} DM and 40.3–77.3 L CH_4 kg^{-1} FM and 123.1–277.7 L CH_4 kg^{-1} DM.

Table 3. Yield of biogas (NL kg^{-1}) and methane (L CH_4 kg^{-1}) in fresh (FM) and in dry matter (DM)

Grass species	Biogas volume	NL kg^{-1}	L CH_4 kg^{-1}	NL kg^{-1}	L CH_4 kg^{-1}
		FM		DM	
Dactylis glomerata	0.084 b	80.0 d	52.0 d	329.0 b	213.9 b
Phalaris arundinacea	0.077 c	110 ab	63.0 c	214.8 c	123.1 c
Festuca arundinacea	0.087 b	90.6 c	56.4 d	307.5 b	191.2 b
Lolium perenne	0.096 a	114.3 a	77.3 a	304.3 b	205.7 b
Medicago sativa	0.081 cb	90.0 c	56.6 d	266.9 b	167.9 b
Onobrychis viciifolia	0.086 b	63.2 e	40.3 e	435.3 a	277.7 a
Panicum virgatum	0.076 c	102.7 b	70.1b	210.0 c	143.4 c

Note. Different letters a–e in the column indicate significant differences ($P < 0.05$) in concentration of respective biomass component.

The correlations between the chemical composition and methane yield were determined; the results are presented in Table 4.

From the Table 4 one can see that the methane yield was most dependent on the amount of WSC in the biomass and digestibility. Positive and significant correlations were established for WSC 0.761** and digestibility 0.744** ($P \leq 0.01$). Cell wall component ADF and cellulose in the biomass had a negative impact on the methane production. Negative significant correlation of cellulose −0.793** and ADF −0.762** ($P \leq 0.01$) with methane yield was determined.

The correlations between other chemical composition indicators were also found (Table 4). ADF positively correlated with cellulose 0.711** and carbon 0.681** ($P \leq 0.01$), but negatively – with WSC −0.873**, digestibility −0.693** ($P \leq 0.01$) and CP −0.428* ($P \leq 0.05$). ADL negatively correlated with hemicellulose −0.878** and cellulose −0.724** ($P \leq 0.01$), but positively – with CP 0.792** and digestibility 0.721** ($P \leq 0.01$).

WSC positively correlated with digestibility 0.570** too, but negatively – with cellulose −0.592** ($P \leq 0.01$). Strong correlation was determined between cellulose and hemicellulose 0.921** ($P \leq 0.01$). The strongest negative correlation was determined of cellulose with digestibility −0.986** ($P \leq 0.01$) as well as with crude proteins −0.853** ($P \leq 0.01$). Hemicellulose negatively correlated with digestibility −0.935** ($P \leq 0.01$), crude proteins −0.933** ($P \leq 0.01$) and nitrogen −0.748** ($P \leq 0.01$). Carbon positively correlated with C:N

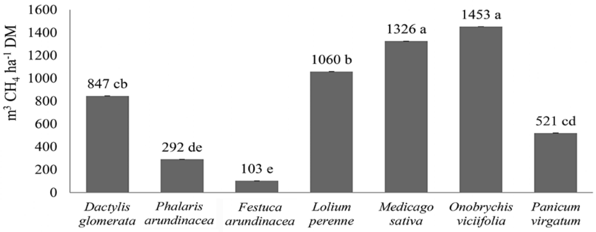
Table 4. Pearson correlations of biomass chemical composition parameters and methane (CH_4) yield

	ADF	ADL	WSC	Cel	HCel	C	N	Digestibility	CP	C:N
ADF										
ADL	–0.029									
WSC	–0.873**	–0.015								
Cel	0.711**	–0.724**	–0.592**							
Hcel	0.440*	–0.878**	–0.340	0.921**						
C	0.681**	–0.078	–0.499	0.525*	0.366					
N	–0.396	0.542	0.145	–0.655*	–0.748**	–0.347				
Digestibility	–0.693**	0.721**	0.570**	–0.986**	–0.935**	–0.529*	0.690**			
CP	–0.428**	0.792**	0.197	–0.853**	–0.933**	–0.431	0.873**	0.876**		
C:N	0.594	–0.322	–0.312	0.637*	0.650*	0.483*	–0.926**	–0.657*	–0.790**	
CH_4	–0.762**	0.379	0.761**	–0.793**	–0.582	–0.462*	0.206	0.744**	0.427	–0.285

Note. ADF – acid detergent fibre, ADL – acid detergent lignin, WSC – water-soluble carbohydrates, Cel – cellulose, HCel – hemicellulose, C – carbon, N – nitrogen, CP – crude proteins, C:N – carbon to nitrogen ratio; **, * – significant at $P \leq 0.01$ and $P \leq 0 .05$ level.

0.483* ($P \le 0.05$), but negatively with digestibility −0.529* ($P \le 0.05$). Nitrogen positively correlated with crude proteins 0.873** and digestibility 0.690** ($P \le 0.01$). Strong negative correlation was determined between nitrogen and C:N –0.926** ($P \le 0.01$). Crude proteins positively correlated with digestibility 0.876** ($P \le 0.01$), but negatively with C:N −0.790**($P \le 0.01$).

After assessing the biomass yield of seven perennial grass species produced in 2018, specific methane yield was calculated (Fig.).



Note. Different letters a–e in the column indicate significant differences ($P < 0.05$) in area-specific methane yield in DM of biomass of seven grass species.

Figure. Area-specific methane (CH_4) yield in dry matter (DM) of biomass of seven grass species

The results revealed a methane yield of 103 to 1453 m^3 CH_4 ha^{-1} DM. Chiumenti et al. (2018) have documented methane yields of 263–1181 m^3 CH_4 ha^{-1} DM, McEniry and O'Kiely (2013) received higher values – 1157–2252 m^3 CH_4 ha^{-1} DM.

According to the biomass and methane yields, the results of specific methane yield were obtained: *O. viciifolia* biomass produced the highest amount of 1453 m^3 CH_4 ha^{-1} DM, *M. sativa* – 1326 m^3 CH_4 ha^{-1} DM and *L. perenne* – 1060 m^3 CH_4 ha^{-1} DM. Less suitable for methane production were *F. arundinacea* (103 m^3 CH_4 ha^{-1} DM), *P. arundinacea* (292 m^3 CH_4 ha^{-1} DM) and *P. virgatum* (521 m^3 CH_4 ha^{-1} DM).

The highest methane yield was generated from the biomass of *O. viciifolia* – 277.7 L CH_4 kg^{-1} DM; its biomass contained the highest amount of WSC and digestibility, while less ADF and cellulose.

High content of ADF, cellulose and the lowest WSC amount and digestibility of the biomass of *P. arundinacea* and *P. virgatum* led to the lowest methane yields – 123.1 L CH_4 kg^{-1} DM and 143.4 L CH_4 kg^{-1} DM, respectively. Based on the correlations, it can be inferred that methane yield depended on the chemical composition of plant biomass. High content of WSC and low content of ADF and cellulose in plant biomass favoured methane production.

Assessment of grass biomass and methane yields suggested that the biomass of Fabaceae (*O. viciifolia* and *M. sativa*) and *L. perenne* from Poaceae families was most suitable for methane production. Current study provided valuable information on the suitability of different grass species, grown under the same or similar management, for biogas production. Only limited number of such studies can be found in scientific literature.

Conclusions

1. Chemical composition of the biomass of the Fabaceae species tested, specifically due to the high digestibility (73.5–78.4% DM) and low cellulose (20.8–24.4% DM) content, was determined most suitable for the anaerobic digestion process. The biomass of *Lolium perenne* and *Festuca arundinacea* was found to be appropriate for anaerobic digestion compared with that of the other investigated Poaceae plants, because it had the highest content of water-soluble carbohydrates (WSC) (20.3–16.4% DM) and the lowest content of cellulose (28.9–27.3% DM) and acid detergent fibre ADF (32.8–31.3% DM).

2. The results of anaerobic digestion analysis showed that the highest methane (CH_4) yields were obtained from *O. viciifolia* – 277.7 L CH_4 kg^{-1} DM (40.3 L CH_4 kg^{-1} FM), *Dactylis glomerata* – 213.9 L CH_4 kg^{-1}DM (52.0 L CH_4 kg^{-1} FM) and *L. perenne* – 205.7 L CH_4 kg^{-1} DM (77.3 L CH_4 kg^{-1} FM). The correlations corroborated that the methane yield depended on the chemical composition of the biomass. Methane positively and significantly correlated with WSC 0.761** and digestibility 0.744** ($P \le 0.01$). Cellulose and ADF had negative impact on the methane production, significant correlations of cellulose −0.793** and ADF −0.762** ($P \le 0.01$) were found.

3. After assessing the biomass yield of the first cut and methane yield of seven perennial grasses, specific methane yields were calculated: *O. viciifolia* – 1453 m^3 CH_4 ha^{-1} DM, *Medicago sativa* – 1326 m^3 CH_4 ha^{-1} DM and *L. perenne* – 1060 m^3 CH_4 ha^{-1} DM, gave the best results. *F. arundinacea* and *Phalaris arundinacea* were found to be less suitable for methane production in terms of specific methane yields.

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Metano išeigos iš daugiamečių žolių rūšių sąsajos su jų biomasės chemine sudėtimi

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Santrauka

Tyrimo tikslas – nustatyti dažniausiai auginamų daugiamečių miglinių ir pupinių šeimų žolinių augalų: paprastosios šunažolės (*Dactylis glomerata* L.), nendrinio dryžučio (*Phalaris arundinacea* L.), nendrinio eraičino (*Festuca arundinacea* Schreb.), daugiametės svidrės (*Lolium perenne* L.), mėlynosios liucernos (*Medicago sativa* L.), sėjamojo esparceto (*Onobrychis viciifolia* Scop.) ir rykštėtosios soros (*Panicum virgatum* L.), chemines savybes ir jas susieti su metano (CH_4) ir biodujų išeigomis, gautomis laboratorinėmis sąlygomis. Biomasės cheminės sudėties tyrimo duomenimis, biodujoms išgauti tinkamiausia buvo pupinių šeimos augalų sudėtis. Nustatyti aukšti virškinamumo rodikliai 78,4–73,5 % sausoje masėje (SM) ir maži celiuliozės 20,8–24,4 % SM kiekiai, tinkamiausi anaerobiniam procesui. Iš tirtų miglinių šeimos augalų anaerobiniam procesui tinkamiausi yra *L. perenne* ir *F. arundinacea*. Jų biomasėje nustatyti didžiausi kiekiai vandenyje tirpių angliavandenių – 20,3–16,4 % SM ir mažiausi kiekiai celiuliozės – 28,9–27,3 % SM bei netirpios ląstelienos – 32,8–31,3% SM. Geriausios metano išeigos gautos iš *O. viciifolia* – 277,7 L CH_4 kg^{-1}, *D. glomerata* – 213,9 L CH_4 kg^{-1} ir *L. perenne* – 205,7 L CH_4 kg^{-1}. Koreliacijos patvirtino metano išeigos priklausomumą nuo augalų biomasės cheminės sudėties. Metano išeiga teigiamai koreliavo su vandenyje tirpių angliavandenių kiekiu biomasėje 0,761** bei virškinamumu 0,744** ($P \leq 0{,}01$) ir neigiamai su celiulioze −0,793** bei netirpia ląsteliena −0,762**. *O. viciifolia* (1453 m^3 CH_4 ha^{-1} SM), *M. sativa* (1326 m^3 CH_4 ha^{-1} SM) ir *L. perenne* (1060 m^3 CH_4 ha^{-1} SM) biomasėse nustatytos geriausios metano išeigos pagal derlių.
Tyrimo rezultatai galėtų būti pagrindas tolesniems eksperimentams, siekiant pasirinkti geriausios metano išeigos žolių rūšis.

Reikšminiai žodžiai: anaerobinis skaidymas, biomasė, biodujos, daugiamečiai augalai, metanas.