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THE EVALUATION OF THE BIOMASS QUALITY OF *Lolium perenne* 'MĂGURA' AND *Phleum pratense* 'TIROM' GROWN UNDER THE CONDITIONS OF MOLDOVA

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Abstract

The primary objective of this study was to assess the quality indicators of green biomass harvested from monocultures of the perennial grasses Lolium perenne 'Măgura' and Phleum pratense 'Tirom', grown in the experimental field of the NBGI MSU in Chişinău. The study of the biochemical composition and nutritional value of the dry matter of green mass harvested from the investigated perennial grasses were defined by the following indicators: 86-95 g/kg crude protein, 97-111 g/kg ash, 336-391 g/kg CF, 35.5-41.7 g/kg ADF, 633-734 g/kg NDF, 22-26 g/kg ADL, 65-181g/kg TSS, 9.22-9.92 MJ/kg metabolizable energy, and the net energy for lactation was calculated as 5.24-5.95 MJ/kg. The analyzed green biomass substrates intended for biogas production contained 33.3–39.1% cellulose (Cel), 27.8–31.7% hemicellulose (HC), and a carbon-to-nitrogen (C/N) ratio of 32–36. The estimated biochemical methane potential ranged from 324 to 339 L/kg of dry matter (DM). The Romanian cultivars of perennial grasses — Lolium perenne 'Măgura' and Phleum pratense 'Tirom'— are versatile crops that can serve both as organic forage for livestock and as feedstock for biogas production facilities.

Key words: *biochemical biomethane potential, biochemical composition, green mass, Lolium perenne 'Măgura', nutritional value, Phleum pretense 'Tirom'.*

INTRODUCTION

The Poaceae family, comprising 777 genera and 11,461 recognized species, ranks among the largest and most economically significant plant families worldwide. According to *The Plant List*, the genus *Phleum* L. includes 18 accepted species, while the genus *Lolium* L. includes 11. In the native flora of the Republic of Moldova, four species of *Phleum* and two species of *Lolium* have been recorded (Negru, 2007). In contrast, Romania hosts seven *Phleum* species and four *Lolium* species (Marușca, 1999).
The species *Lolium perenne* L. is characterized by high productivity and economic value, and have been researched in scientific centers as crops with multiple uses (Mut et al., 2017; Chornolata et al., 2018; Janković et al., 2018; Amaleviciute-Volunge et al., 2020; Karbivska et al., 2020; Wang et al., 2020; Olszewska, 2021; Rancāne et al., 2021; Ravindran et al., 2022; Sosnowski et al., 2022; Ţîţei, 2023; Becker et al., 2023; Coşman et al., 2023; Ţîţei et al., 2023; Bozhanska et al., 2024; Bužinskienė, 2024; Czubaszek et al., 2024).
The Catalogue of Plant Varieties of the Republic of Moldova does not include any registered grass cultivars.
However, the Official Catalogue of Agricultural Plant Varieties of Romania lists two cultivars of *Phleum pratense* and twelve cultivars of *Lolium perenne*.
Romanian-developed cultivars yield between 37–65 t/ha of fresh biomass or 9.3–17 t/ha of hay (Marușca et al., 2011).
This research primarily aimed at assessing the quality parameters of *Lolium perenne* and *Phleum pratense* green biomass for use as livestock forage and as feedstock for biogas production in Moldova.

MATERIALS AND METHODS

The cultivars 'Măgura' of perennial ryegrass (*Lolium perenne*) and 'Tirom' of timothy grass (*Phleum pratense*), developed at the Research and Development Institute for Grasslands in Braşov, Romania, and cultivated in monoculture within the experimental plots of the "Al. Ciubotaru" National Botanical Garden (Institute) in Chişinău, Moldova, were used as the subjects of this study. Sampling was conducted during the third growing season, with the first cut performed at the early flowering stage. The harvested biomass was chopped into 1.5–2.0 cm segments using a laboratory forage chopper. Dry matter content was determined by drying the samples at 105 °C, to a constant weight.

For chemical analysis, the chopped samples were further dried in a forced-air oven at 60 °C, then ground using a beater mill equipped with a 1 mm mesh sieve. Key biochemical parameters — crude protein, acid detergent fiber, neutral detergent fiber, acid detergent lignin, total soluble sugars, and ash content were analyzed using near-infrared spectroscopy (NIRS) with a PERTEN DA 7200 NIR analyzer. Cellulose, hemicellulose, digestible dry matter, relative feed value, metabolizable energy, digestible energy and net energy for lactation were calculated using established standard procedures.

The carbon content of the biomass substrates was estimated using an empirical formula proposed by Badger et al. (1979), while the biochemical methane potential (BMP) was calculated based on the methodology outlined by Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

Forage quality is determined by a range of characteristics, with nutrient content influenced by factors such as grass species or cultivars, growth stage, and soil conditions. Based on the analysis of quality indicators for the harvested green biomass of the studied *Lolium perenne* and *Phleum pratense* cultivars (Table 1), it is noteworthy that the dry matter contained 86-95 g/kg of crude protein, 97-111 g/kg of ash, 336-391 g/kg of crude fiber, 35.5-41.7 g/kg of acid detergent fiber, 633-734 g/kg of neutral detergent fiber, 22-26 g/kg of acid detergent lignin, and 65-181 g/kg of total soluble sugars. The green biomass of *Lolium perenne* is characterized by a lower content of minerals and structural carbohydrates, but a higher concentration of total soluble sugars compared to that of *Phleum pratense*. In contrast, *Phleum pratense* green fodder contains a more optimal level of crude protein. The reduced structural carbohydrate content in *Lolium perenne* enhances its digestibility, resulting in higher relative feed value, metabolizable energy, as well as net energy for lactation.

Numerous studies have evaluated the forage quality of *Lolium perenne* and *Phleum pratense*, highlighting considerable variability in their biochemical composition depending on growth conditions, cultivar, and harvest stage.

Burlacu et al. (2002) reported that at the flowering stage, *Lolium perenne* forage contained 250 g/kg dry matter, 8.6% crude protein, 32.7% crude fiber, 7.2% ash, and 18.3 MJ/kg gross energy; in comparison, *Phleum pratense* had 265 g/kg dry matter, 8.7% crude protein, 32.5 crude fiber, 7.8% ash, 18.4 MJ/kg gross energy. Hetta et al. (2003) observed that first-cut *Phleum pratense* contained 182 g/kg dry matter, 12.4% crude protein, 54.5% neutral detergent fiber and 11 MJ/kg metabolizable energy. Tomić et al. (2007) recorded crude protein contents in *Phleum pratense* ranging from 8.36% to 13.95% and crude fiber from 26.92% to 29.36%, while *Lolium perenne* had 9.70-13.40% crude protein and 30.72% crude fiber. Mahnert et al. (2005) reported that perennial ryegrass had 176-256 g/kg dry matter with 11.9-14.7% crude protein, 24.8-29.1% crude fiber and 9.4-9.9% ash. Dewhurst et al. (2009) found early-flowering *Lolium perenne* dry matter to contain 22.9% crude protein, 11.3% ash, 32.0% neutral detergent fiber, 19.3% acid detergent fiber, 80% matter digestibility. Surmen et al. (2013) observed that *Lolium perenne* lines contained 9.43-12.09% crude protein, 55.31-58.52% neutral detergent fiber, 37.24-40.36% acid detergent fiber, 49.24-53.27% total digestible nutrients, and a relative feed value of 92.19-98.57. Under moderate drought, Küchenmeister et al. (2014) found that *Lolium perenne* had 9.0% crude protein, 52.7% neutral detergent fiber, and 28.6% acid detergent fiber, while under severe drought, it had 11.5%

crude protein, 59.0% neutral detergent fiber, and 33.1% acid detergent fiber.

Wang et al. (2014) reported that *Phleum pratense* contained 124-133 g/kg dry matter, 13.6-17.4% crude protein, 58.1% neutral detergent fiber, 32.3-36.0% acid detergent fiber. Tran and Lebas (2015) documented a wide range of *Phleum pratense* dry matter composition: 7.6-22.3% crude protein, 49.5-63.5% neutral detergent fiber, 25.4-44.7% acid detergent fiber 1.4-3.2% lignin, 4.9-11.8% ash, with 58.0-76.9% digestible dry matter and 11.6 MJ/kg digestible energy. Marușca et al. (2016) found *Lolium perenne* forage to contain 14-17% crude protein and 24-28% crude fiber. Mut et al. (2017) noted that genotypes of *Lolium perenne* had 7.20-21.00% crude protein, 50.80-82.70% neutral detergent fiber, 21.58-43.90% acid detergent fiber. Chornolata et al. (2018) characterized *Phleum pratense* as having 16.05% crude protein, 23.10% crude fiber, 50.45% neutral detergent fiber, 24.82% acid detergent fiber, 14.60% cellulose, 1.88% starch, 15.80% hemicellulose, and 6.10% lignin. Janković et al. (2018) reported *Phleum pratense* dry matter had 13.20-14.52% crude protein and 24.30-26.98% crude fiber. Amaleviciute-Volunge et al. (2020) found that *Lolium perenne* fresh biomass contained 10.28% crude protein, 6.67% ash, 32.8% acid detergent fiber, 54.75% neutral detergent fiber, 3.97% acid detergent lignin, and 57.9% digestible dry matter, in comparison, *Festuca arundinacea* had higher crude protein (15.06%), more ash (7.52%), and better digestibility (62.5%). Karbivska et al. (2020) documented the forage quality of *Lolium perenne* dry matter as 11.4% crude protein, 29.6% crude fiber, 58% digestible dry matter., 0.72 fodder units/kg, 8.2 MJ/kg metabolizable energy, and 109 g digestible protein per nutritive unit; for *Phleum pratense* they reported 10.7% crude protein, 28.8% crude fiber, 7.4% ash, 58% digestible dry matter 0.70 fodder units/kg, 8.1 MJ/kg metabolizable energy, 109 g digestible protein per fodder unit. Reiné et al. (2020) compared the species and found *Lolium perenne* to have 371 g/kg dry matter with 6.8% crude protein, 5.8% ash, 63.6% neutral detergent fiber, 2.8% acid detergent lignin, 32.7% acid detergent fiber, and 63.8 digestible dry matter, while *Phleum pratense* had 377 g/kg dry matter, 7.6% crude protein, 3.9% ash, 34.0% acid detergent fiber, 68.5% neutral detergent fiber, 4.0% acid detergent lignin, and 62.4% digestible dry matter. Wang et al. (2020) found *Lolium perenne* herbage had 93.4-94.0% organic matter, 3.20–3.98% nitrogen, 38.8-41.4% neutral detergent fiber, and 784-830 g/kg digestible dry matter. Olszewska (2021) observed that cultivars contained 13.0-14.34% crude protein. Rancāne et al. (2021) evaluated 19 tetraploid genotypes of *Lolium perenne* and reported 6.99-10.68% crude protein, 19.92-25.11% acid detergent fiber, 38.79-46.74% neutral detergent fiber, 693.4-733.8 g/kg digestible dry matter, relative feed value of 138-175, and 6.71-7.02 MJ/kg net energy for lactation.

Table 1. Comparative biochemical and nutritional characteristics of *Lolium perenne* and *Phleum pratense* green mass

Indices	*Lolium perenne* 'Măgura'	*Phleum pratense* 'Tirom'
Crude protein, g/kg DM	86	95
Crude fiber, g/kg DM	336	372
Minerals, g/kg DM	97	111
Acid detergent fiber, g/kg DM	355	417
Neutral detergent fiber, g/kg DM	633	734
Acid detergent lignin, g/kg DM	22	26
Cellulose, g/kg DM	333	391
Hemicellulose, g/kg DM	278	317
Total soluble sugars	181	65
Digestible dry matter, g/kg DM	620	586
Relative feed value	90	72
Digestible energy, MJ/ kg DM	12.09	11.23
Metabolizable energy, MJ/ kg DM	9.92	9.22
Net energy for lactation, MJ/ kg DM	5.95	5.24

According to Ţîţei et al. (2022), the composition of green mass of timothy grass included 10.4-12.4% crude protein, 28.9-35.1% crude fiber, 7.5-8.5% ash, 49.5-58.9% neutral detergent fiber, 31.4-36.8% acid detergent fiber, 3.6-4.1% acid detergent lignin, 56.9-61.4% digestible dry matter, with a relative feed value of 95-121, metabolizable energy of 9.78-10.38 MJ/kg, and 5.81-6.42 MJ/kg net energy for lactation. Sosnowski et al. (2022) reported that the net energy for lactation in *Lolium perenne* forage ranged from 5.80 to 6.12 MJ/kg. According to Becker et al. (2023), the forage value of *Lolium perenne* was characterized by 17.7% crude protein and 6.4 MJ/kg net energy for lactation, while *Phleum pratense* contained 18.7% crude protein and 6.1 MJ/kg net energy for lactation.

Coşman et al. (2023) observed that the nutrient composition of *Lolium perenne* ranged from 212.9 to 298.7g/kg dry matter, with 6.88-10.25% crude protein, 28.49-31.75% crude fiber, and 8.84-11.91% ash. Petkova et al. (2023) found that *Lolium perenne* dry matter contained 10.31% crude protein, 39.66% crude fiber, and 6.04% ash. Similarly, Ţîţei (2023) reported that the dry matter of harvested perennial ryegrass had 10.74% crude protein, 29.95% crude fiber. Bozhanska et al. (2024) highlighted that *Lolium perenne* forage contained 9.06% crude protein, 4.53% ash, 41.30% crude fiber whereas *Phleum pratense* had slightly higher values for crude protein (9.49%) with 4.01% ash, and 40.56% crude fiber.

Table 2. Estimated biochemical methane potential of the analyzed *Lolium perenne* and *Phleum pratense* substrates

Indices	*Lolium perenne* 'Măgura'	*Phleum pratense* 'Tirom'
Crude protein, g/kg DM	86.00	95.00
Nitrogen, g/kg DM	13.76	15.20
Ash, g/kg DM	97.00	111.00
Carbon, g/kg DM	501.67	493.89
Ratio carbon/nitrogen	36.46	32.49
Acid detergent lignin, g/kg DM	22.00	26.00
Hemicellulose, g/kg DM	278.00	317.00
Biogas potential, L/kg VS	686.00	684.00
Biomethane potential, L/kg VS	367.00	364.00
Biomethane potential, L/kg DM	338.74	323.60

Biogas is a renewable energy source that significantly contributes to reducing fossil fuel emissions across sectors such as transportation, heating, and electricity generation. Its utilization plays a key role in climate change mitigation and achieving renewable energy targets. In recent years, the direct production of biogas from harvested and chopped green biomass has garnered considerable interest in Europe.

Our study focused on the quality indices of green biomass substrates from *Lolium perenne* (perennial ryegrass) and *Phleum pratense* (timothy grass), and their potential for biomethane production, as presented in Table 2. The nitrogen content in the analyzed substrates ranged from 13.76 to 15.20 g/kg, while carbon content varied between 493.89 and 501.67 g/kg. The carbon-to-nitrogen (C/N) ratio ranged from 32.49 to 36.46. Acid detergent lignin content was between 22.00 and 26.00 g/kg, hemicellulose content ranged from 278.00 to 317.00 g/kg, and the biochemical methane potential (BMP) varied from 323.6 to 338.74 L/kg DM. *Lolium perenne* exhibited the highest biomethane potential among the tested substrates.

Previous studies have reported varying results. Mähnert et al. (2002) found that *Lolium perenne* fresh mass co-substrate had a dry matter (DM) content of 176 g/kg with 90.1% organic dry matter (ODM), producing 859 L/kg volatile solids (VS) of biogas over 28 days. *Phleum pratense* had a DM content of 148 g/kg, 90.1% ODM, and gas yields between 733-828 L/kg VS. Mähnert et al. (2005) reported a methane yield of 0.36 m^3/kg VS for *Lolium perenne*.

Kaiser and Gronauer (2007) reported specific methane yields for five ryegrass cultivars ranging from 198-443 L/kg VS, or 2,500-5,800 m^3/ha. For timothy grass, yields ranged from 345-375 L/kg VS, or 4,500-4,800 m^3/ha. Ebeling et al. (2013) observed that the methane yield of *Lolium perenne* varied from 320 to 335

L/kg VS depending on harvest timing and fertilization levels.

Żurek and Martyniak (2020) reported a biogas yield of 611.9 L/kg VS with 54.8% methane content for *Lolium perenne*, while according to Amalevičiūtė-Volungė et al. (2021), methane yields were 205.7 L/kg VS.

Ravindran et al. (2022) found that biorefined press cake from perennial ryegrass had a DM content of 390 g/kg, containing 4.67% ash, 682.2 g/kg VS, 2.74 g/kg nitrogen, 47.81% carbon, a C/N ratio of 19, and a biomethane yield of 487 L/kg VS. Bužinskienė (2024) reported that perennial ryegrass biomass substrates yielded 270-410 L/kg or 2,500-6,150 m^3/ha, while timothy grass yielded 151-322 L/kg or 1,362-5,800 m^3/ha. Czubaszek et al. (2024) noted that substrates from grass verges, predominantly composed of perennial ryegrass, contained 380 g/kg DM, 682.2 g/kg VS, 20.76 g/kg nitrogen, and 378.8g/kg total organic carbon, with a biogas yield of 715.05 L/kg VS and methane content of 59.7%.

CONCLUSIONS

The Romanian cultivars *Lolium perenne* 'Măgura' and *Phleum pratense* 'Tirom' demonstrate favorable biochemical composition and nutritional value in their dry matter, with key parameters including 86-95 g/kg crude protein, 97-111 g/kg ash, and 336-391 g/kg crude fiber. Their fiber fractions ranged from 35.5-41.7 g/kg acid detergent fiber, 633-734 g/kg neutral detergent fiber, and 22-26 g/kg acid detergent lignin, while total soluble sugars varied between 65-181 g/kg. Energy values were also substantial, with metabolizable energy of 9.22-9.92 MJ/kg and net energy for lactation of 5.24-5.95 MJ/kg.

The biochemical methane potential of the green biomass from these cultivars ranged from 324 to 339 L/kg dry matter, indicating a solid capacity for renewable energy production. Among the two, *Lolium perenne* 'Măgura' exhibited superior nutritional quality and a higher energy yield.

Both cultivars are well-suited for use in grassland restoration projects, the establishment of temporary pastures (either in monoculture or mixed with other perennial species), and for agroecological purposes such as inter-row planting in orchards and vineyards. Their harvested biomass can serve dual purposes: as valuable forage for livestock and as an efficient substrate for biogas production in the renewable energy sector.

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