ISSN 1392-3196
Žemdirbystė=Agriculture, vol. 98, No. 2 (2011), p. 149–156
UDK 633.264:631.526.2/.32:581.192.7

Evaluation of tall fescue (*Festuca arundinacea* Schreb.) varieties and wild ecotypes as feedstock for biogas production

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Abstract

Research was done at the Lithuanian Institute of Agriculture in the experimental fields of the Grass Breeding Department and in the Chemical Research Laboratory during 2008–2010. The genetic collection of tall fescue composed of 4 varieties and 9 wild ecotypes was studied for a complex of agrobiological traits and characters and dry matter (DM) chemical composition.

The varieties 'Navas DS', 'Kora' and 'Bariane' were distinguished for DM yield per plant. The average annual DM yield per plant (1st cut + aftermath) for these varieties was 198, 158, and 156 g, respectively. The average annual DM yield per plant for the variety 'Navas DS' was significantly ($P < 0.05$) or 28.5% higher than the trial average. Tall fescue DM yield per plant of the aftermath (2nd + 3rd cut) accounted for on average 53.7% of the annual yield and ranged from 51.4% (wild ecotype No. 20) to 56.4% (wild ecotype No. 22). At the beginning of heading stage, wild ecotypes Nos 21, 17, 22 and 19 were noted for high nitrogen content in DM – 23.0, 21.9, 21.7 and 20.4 g kg^{-1} DM, respectively. The highest water soluble carbohydrate (WSC) content was measured for wild ecotype No. 25 and the variety 'Bariane' – 210.0 and 197.0 g kg^{-1} DM, respectively. According to DM's neutral detergent fibre (NDF) quality composition, wild ecotype No. 25 and cv. 'Bariane' were noted for low lignin (22.8 and 20.7, respectively), cellulose (261.2 and 293.3) and hemicellulose (265.0 and 281.0) contents g kg^{-1} DM. The DM of productive varieties 'Navas DS' and 'Bariane' at the beginning of heading stage had a C:N ratio of 25.5 and 27.9, which is optimal for biogas production.

Key words: *Festuca arundinacea*, biogas, dry matter yield, C:N ratio, cellulose, hemicellulose, lignin.

Introduction

Political and economic situation of the European Union promotes the use of grasses for energy purposes – biofuel and biogas production. From the economic viewpoint, the most suitable grass species are those that produce high and stable biomass yield, are long-lived and persistent, undemanding for growing conditions, tolerant of cold, drought and other adverse environmental factors. Tall fescue *(Festuca arundinacea* Schreb.) is a plant that meets all these requirements and is distributed over a wide range of climate conditions (Šedys, 1992; Chapman, 1996).

Tall fescue is a widely adapted Eurasian grass species. Natural populations are found from northern Africa to northern Europe, in sites varying from arid to very wet. Tall fescue is better able to avoid drought than other cool-season grasses such as perennial ryegrass (*Lolium perenne* L.) or Kentucky bluegrass (*Poa pratensis* L.), but this notwithstanding, within *F. arundinacea* species cultivars vary in drought resistance (Huang, Gao, 2000). Tall fescue prefers and responds to a high level of nitrogen (N) fertility, but is found on impoverished soils (Easton et al., 1994).

Tall fescue is widely cultivated as a forage and turfgrass species. Recently it has been started to be used for biogas production (Fowler et al., 2003; Noormets et al., 2007). Choice of a cultivar that is matched to growing location conditions is important in biomethane production systems. Biomass production and quality may be improved through genetic selection; however, research suggests no single cultivar is likely to have a yield advantage across all locations (Fike et al., 2006). This may be because location-specific factors, such as soil type

and weather patterns, interact with genotype in determining yield potential, as has been observed in research on biomass production of other species (Fike et al., 2006).

In order to fully meet the requirements for biogas production, tall fescue biomass chemical composition has to secure its high conversion rate. While estimating tall fescue varieties and wild ecotypes' herbage as raw material for biogas production it is critical to know the contents of crude protein, water soluble carbohydrate (WSC), neutral detergent fibre (NDF) and its constituents (cellulose, hemicelluloses and lignin) accumulated in the biomass and carbon to nitrogen ratio (C:N) in it. High content of lignin (a polyphenolic polymer) is especially undesirable in the NDF composition, since it reduces the microbial degradation of other lignocelluloses (fibre) components – cellulose and hemicelluloses (Casler et al., 2008). Cellulose fibres are embedded in a complex lignin-polysaccharide matrix (Saha, 2003). Multiple cross-linking in cell wall limit accessibility of hydrolytic enzymes, consequently, lignin restricts the degradation of structural polysaccharides, thereby limiting the bioconversion of forages and fibrous crops into animal products or into liquid fuels and other industrial products (Clarkson, Xiao, 2000; Vogel, Jung, 2001; Grabber et al., 2004; Grabber, 2005).

Breeding for improved biomass yield and quality in tall fescue has received little attention. The present study was designed to select productive tall fescue varieties and wild ecotypes with well-balanced chemical composition for biogas production.

Materials and methods

The tall fescue genetic collection was explored as spaced plants in the field trials at the Lithuanian Institute of Agriculture over the period of 2008–2010. The tests involved 4 varieties 'Navas DS' (Lithuania), 'Kora' (Czechia), 'Bariane' (Holland), 'Barolex' (Holland) and 9 wild ecotypes collected in natural or semi-natural habitats in Lithuania (No. 17), Latvia (Nos 20–25) and the Ukraine (Nos 18 and 19).

The genetic collection for this study was set up in early spring using the seedlings grown in a greenhouse. At the beginning of June, 30 seedlings per accession (2 replications, 15 x 2) were planted in the field at 50 x 50 cm distances. The soil of the experimental site is *Endocalcari-Epihypogleyic Cambisol* (*CMg-p-w-can*) with the following characteristics of the plough layer (0–25 cm): pH 7.2–7.5, humus content 1.9–2.2%, total nitrogen 0.14–0.16%, mobile P_2O_5 201–270 mg kg^{-1} and mobile K_2O 101–175 mg kg^{-1} soil. In the autumn of each year of use, phosphorus and potassium fertilisers ($P_{60}K_{90}$) were applied. Nitrogenous fertilisers (N_{150}) were applied each year of herbage use in split applications: in spring N_{60} and N_{45} after the first and second cuts.

Average herbage yield per plant (g) for the first, second and third cuts was determined having weighed all plants per plot. Dry matter yield was measured in 500 g herbage samples dried to constant moisture. The first cut was taken at the beginning of heading stage of each variety or wild ecotype. The second and third cuts of all varieties or wild ecotypes were taken simultaneously after regrowth of aftermath (at plant tillering stage, 1.5–2 months after the first or second cut). Samples for biomass chemical composition determination were taken from 13 most productive varieties and wild ecotypes in 2009 and 2010 before the first cut (at the beginning of heading stage).

Methods of sample preparation and chemical analyses. Fresh samples, chopped into particles of 3–5 cm, were fixed at 105°C for 15 min, dried at (65 ± 5)°C and ground in a cyclonic mill with 1 mm sieve. Total C and N contents in herbage samples were determined simultaneously by dry combustion (Dumas method) (LST EN ISO 16634-1:2009[1]) using Vario *EL* III *CNS*-autoanalyser ("Elementar", Germany). The water soluble carbohydrate (WSC) content was determined using anthrone method (Yemm, Willis, 1954). Samples were also subjected to the fibre components analyses: acid detergent fibre (ADF) and neutral detergent fibre (NDF) and acid detergent lignin (ADL) using cell wall detergent fractionation method according to Van Soest (Faithfull, 2002). ADF extraction was done on an ANKOM220 Fibre Analyzer (ANKOM Technology Method 08-16-06[2]) using F57 filter bags (25-µm porosity). NDF was analysed without the use of alpha amylase but with sodium sulphite and the results are presented as ash-free. The content of cell wall structural carbohydrates hemicellulose and cellulose was calculated as the following differences: cellulose = ADF − ADL and hemicellulose = NDF − ADF (Hindrichsen et al., 2006).

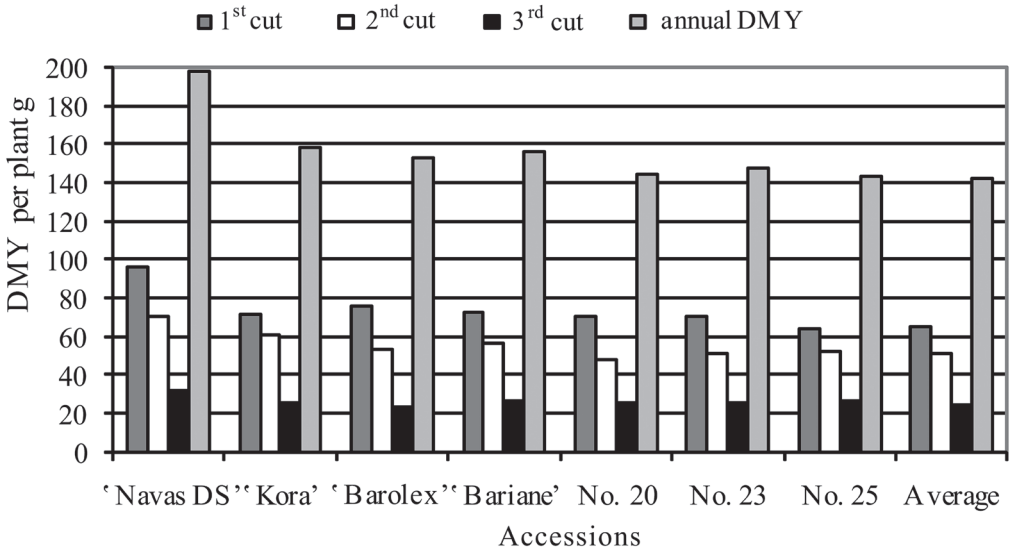
[1] LST EN ISO 16634-1:2009. Maisto produktai. Visuminio azoto kiekio nustatymas pagal Diuma (Dumas) principą ir žalio baltymo kiekio skaičiavimas. 1 dalis. Aliejingosios sėklos ir pašarai (ISO 16634-1:2008)

[2] ANKOM Technology Method 08-16-06 for Acid Detergent Fiber in Feeds Filter Bag Technique. Macedon, NY: ANKOM Technology <http://www.ankom.com>

The air temperature during the growing season of the experimental years exceeded the long-term averages. In 2009, the growing season was characterised by rainy weather, which started at the end of May and lasted throughout the whole summer period. In 2010, abundant rainfall was also characteristic of the entire growing season. As a result, conditions were conducive to herbage growth. The statistical analysis was done using *Stat* software adapted in the *Visual Basic for Application* as macro program to run in the *Excel* (Tarakanovas, Raudonius, 2003).

Results and discussion

Dry matter (DM) yield. The DM yield of plant is an important factor for biogas production. Seeking to identify and select the most promising accessions, 4 tall fescue varieties and 9 wild ecotypes were assessed for agronomically valuable traits. The variation of annual DM yield per plant was high – from 88 to 198 g (Fig. 1). The varieties that stood out in terms of this trait were 'Navas DS', 'Kora' and 'Bariane'. Their annual DM yield per plant (1st cut + aftermath) amounted to 198, 158, 156 g, respectively. The average DM yield per plant for the first, second and third cuts of the variety 'Navas DS' was essentially ($P < 0.05$) higher than the trial mean. The average annual DM yield per plant for the variety 'Navas DS' was 28.5% higher than the trial mean. The average annual DM yield of the most productive wild ecotypes of Latvian origin (catalogue Nos 20, 23, 25) was lower and amounted to 144, 148 and 143 g, respectively. To secure DM yield stability it is important that herbage re-grows well after cuts, namely tall fescue is characterised by good re-growth (Niemelainen et al., 2001). Experimental evidence indicated that DM yield per plant of the aftermath accounted for on average 53.7% of the annual yield, however, this trait of all tested genetic resources was diverse and varied from 51.4% (wild ecotype No. 20) to 56.4% (wild ecotype No. 22).



Note. LSD_{05}: 22.974 g for yield of 1st cut, 11.074 g – for 2nd, 6.982 g – 3rd, 30.809 g – averaged (n = 13).

Figure 1. Dry matter yield (DMY) per plant of most productive tall fescue accessions, 2009–2010

Dry matter quality. In order to estimate tall fescue varieties and wild ecotypes as raw material for biogas production, it is important to know the contents of nitrogen or crude protein, water soluble carbohydrates (WSC), neutral detergent fibre (NDF) and its constituents (cellulose, hemicellulose and lignin) accumulated in the DM and carbon to nitrogen (C:N) ratio.

Chemical research data on DM quality of 4 varieties and 9 wild ecotypes are presented in Table 1. Crude protein is the main nitrogen-containing nutrition component for microbes converting biomass into biogas. High nitrogen contents were noted for Nos 21, 17, 22 and 19 wild ecotypes in which N concentration was 23.0, 21.9, 21.7 and 20.4 g kg^{-1} DM, respectively. Each accession tested in the current trial for chemical analysis was cut at the beginning of heading stage, consequently the differences in N concentration established among the accessions can be basically considered as an attribute of accessions, and the phenological stage has no or only minimal effect on the N concentration variation.

Table 1. Dry matter quality of tall fescue at the beginning of heading stage, 2009–2010

Accessions	N	C	NDF	ADL	WSC	C:N
				g kg^{-1} DM		
'Navas DS'	17.6	450	608	25.1	157	25.5
'Kora'	18.5	446	626	29.5	149	24.1
'Barolex'	18.9	452	641	30.2	173	23.9
'Bariane'	15.9	444	595	20.7	197	27.9
No. 22	21.7	443	588	24.7	150	20.4
No. 20	18.6	446	610	27.8	150	24.0
No. 23	18.7	449	592	24.5	175	24.0
No. 24	19.7	450	610	25.3	169	22.8
No. 17	21.9	444	543	25.7	163	20.3
No. 18	19.7	446	604	29.7	145	22.6
No. 25	18.3	445	549	22.8	210	24.3
No. 21	23.0	447	595	28.4	160	19.4
No. 19	20.4	448	607	28.8	157	21.9
Average	19.5	447	597	26.4	166	23.2

However, N concentration in the DM designed for biogas production has to be optimal. Under anaerobic conditions approximately 76% of the organic nitrogen of protein is mineralized to ammonia (Scharer, Moo-young, 1979). The hydrophobic ammonia molecule may diffuse passively into the cell, causing proton imbalance, and/or potassium deficiency. Among the four types of microorganisms involved in anaerobic digestion, the methanogens are the least tolerant and therefore, the most likely to cease growth due to ammonia inhibition (Braun et al., 2009). Moreover, the methane concentration in biogas decreases with increasing concentrations of protein and carbohydrates. For the anaerobic biomethane process to be optimal, carbon to nitrogen ratio (C:N) in DM is one of the main quality indicators. Literature sources indicate various ranges of C:N values: it is maintained that its optimal value commonly ranges between 20 to 30 (Cotana, Giraldi, 2007; Komatsu et al., 2007), however, some authors indicated this value to range from 15 to 30 (Holliday et al., 2005) or from 25 to 30 (Osman et al., 2006) and other C:N values. In our study, in the DM of tall fescue the range of carbon to nitrogen (C:N) ratio was optimal and varied from 19.4 to 27.9 (Table 1). Of the 13 tested DM samples only in one the value of this ratio was slightly below 20. Thus, according to this quality indicator (C:N), many of the tested accessions of tall fescue genetic resources proved to be suitable for biogas production. The relationship between the C:N ratio in DM and its average yield per plant of the first cut was at 95% probability level (Fig. 2). It is likely that plant productivity is influenced by the compatibility between the intensity of photosynthetic processes (C accumulation) and nitrogen uptake efficiency (N accumulation). Similar relationship between DM yield per plant and C:N values was determined for cocksfoot (Lemežienė et al., 2009). The characteristics of the degradation of cellulose, soluble starch, and glucose in the acidogenic phase and the effects of the substrate loading rate and biological solids retention time on the methanogenic phase of anaerobic digestion are highly different (Noike et al., 1985). The specific rate of substrate utilization decreased in the following order: glucose, soluble starch, acetic acid, and cellulose. Moreover, biomethane potential is higher when WSCs are higher (Nizami et al., 2009). They have a very marked effect on grass DM anaerobic fermentation process, its rate and quality. The microbial conversion of free soluble carbohydrates into lactic acid and the resulting decrease in pH prevents the growth of undesirable microorganisms (Neureiter et al., 2005). The highest WSC contents were established for the wild ecotype No. 25 (Latvia) and cv. 'Bariane' (Holland) – 210.0 and 197.0 g kg^{-1} DM, respectively (Table 1).

At the beginning of heading stage, NDF content in the DM of the tested genetic resources ranged from 543.0 (No. 17) to 641.0 ('Barolex') g kg^{-1} DM (Table 1). Lignin content in the DM of the tested accessions ranged from 20.7 ('Bariane') to 30.2 ('Barolex') g kg^{-1} DM and accounted for 3.5% and 4.7% of the cell wall NDF, respectively. As is seen from the DM quality data, in terms of the high total cell wall (NDF) content and quite considerable lignification, i.e. a fair lignin ADL concentration, the cv. 'Barolex' is less suited for biogas production compared with the other cultivars and wild ecotypes tested. The lowest lignin content was measured for

'Bariane' and wild ecotype No. 25, which were better suited for biogas production according to their biomass quality indicators. In turn, increasing lignin content in the biomass reduces conversion rate of other lignocellulose (fibre) components – cellulose and hemicellulose (Casler et al., 2008). Lignin may form encrustations around degradable fractions and create a physical barrier for the hydrolytic enzymes (Noike et al., 1985; Saha, 2003; Dieterich, 2008). Due to this fact, lignin is one of the most limiting factors of biogas output (Clarkson, Xiao, 2000).

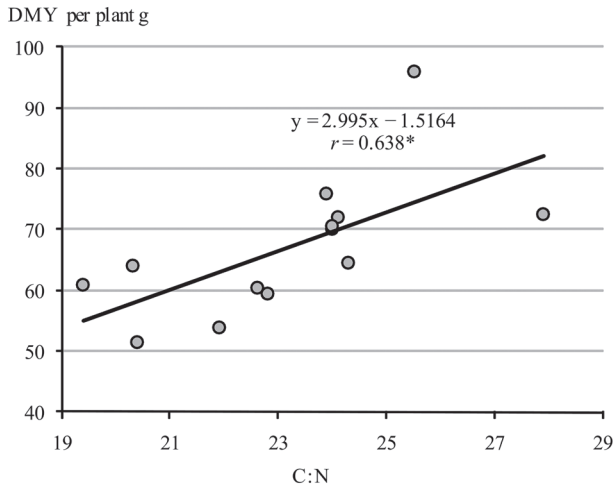


Figure 2. The relationship between DMY per plant of the first cut and C:N value, 2009–2010

When assessing grass biomass as biogas raw material, it is not enough to determine the total amount of cell walls, i.e. NDF and lignin percentage in it. The main cell wall components – structural carbohydrates, i.e. hemicelluloses and cellulose – are potential substrates of anaerobic microbes (Dieterich, 2008). It was determined that anaerobic digestion proceeded through the degradation of the cell solubles and cell wall components, with a total reduction of cellulose and hemicellulose with respect to initial mass of 63% and 58%, respectively (Tambone et al., 2009).

Productive cultivars and wild ecotypes differed in the cell wall structure – cellulose and hemicelullose content and the ratio of these polysaccharides in NDF (Fig. 3). Wild ecotype (No. 25) biomass was found to contain the lowest relative amount of cellulose in fibre – 261 g kg^{-1} DM, or 47.6% NDF and hemicelullose – 265 g kg^{-1} DM, or 48.2% NDF. In the biomass of 'Bariane' containing the lowest amount of non-digestible lignin, the content of cellulose was lower than the trial average – 293 g kg^{-1} DM, or 49.3%. In the biomass fibre of 'Navas DS' the content of cellulose was the highest (323 g kg^{-1} SM) and that of hemicelullose was the lowest (260 g kg^{-1} SM) of all the varieties and wild ecotypes tested, which makes up 53.2% and 42.7% NDF, respectively.

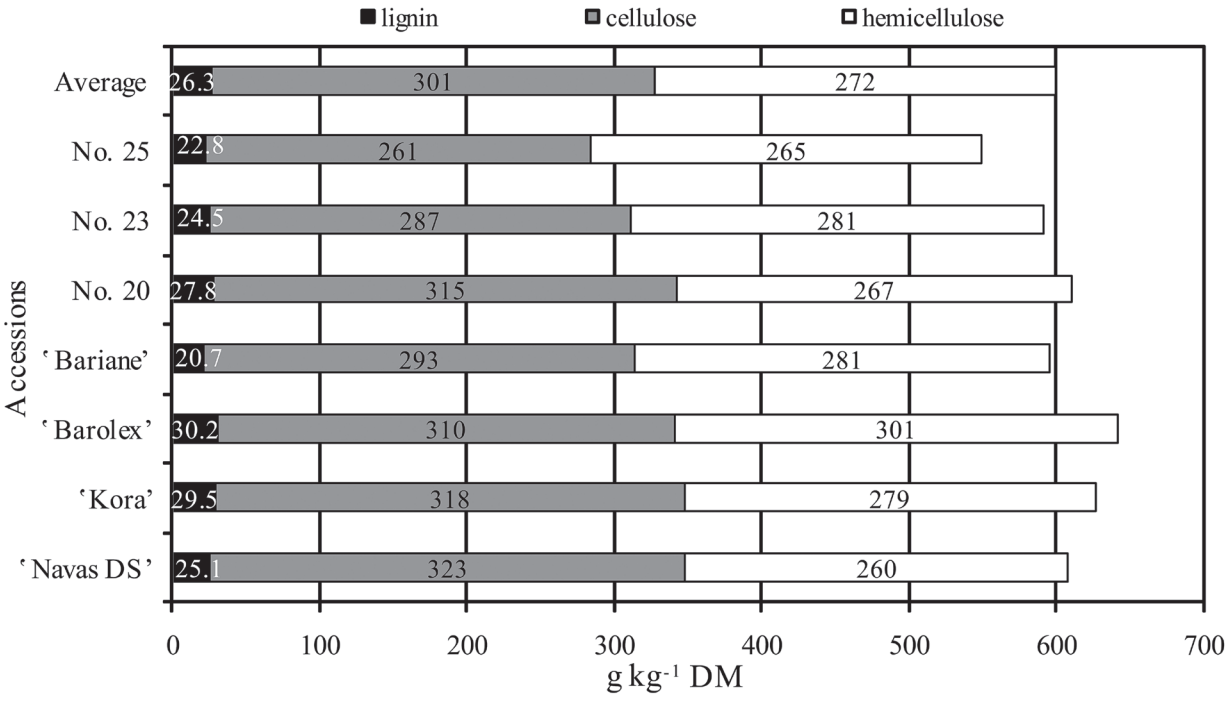


Figure 3. NDF composition of the most productive tall fescue accessions at the beginning of heading, 2009–2010

The digestibility of the cell wall is more closely related to the 1ignin/cellulose ratio than to any other measurement (Van Soest, McQueen, 1973), because lignin is more closely related to cellulose than hemicellulose and has a greater effect on its digestion. Thus, the lignin/cellulose ratio is the critical factor which determines the rate curve of cell-wall fermentation. According to this character, the DM of 'Bariane' and 'Navas DS', whose lignin/cellulose ratio is below 0.08 (Fig. 4) should exhibit the best anaerobic microbe digestion.

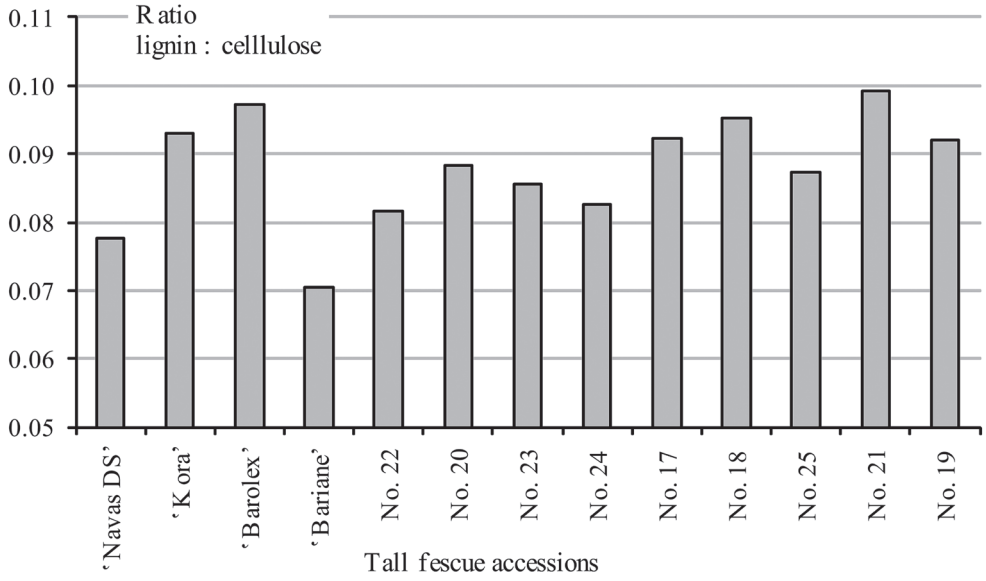


Figure 4. The lignin/cellulose ratio in the DM of tall fescue varieties and wild ecotypes, 2009–2010

Since 'Navas DS' stood out by productivity and its DM had the highest content of cellulose, ADF and crude protein, moderate content of lignin, NDF and WSC, the output of these compounds (g) per plant^{-1} at heading stage was the highest (Table 2). Since WSCs have a considerable effect on improvement of grass anaerobic fermentation process and biomethane potential (Nizami et al., 2009), high WSC yield per plant of 'Navas DS' is a very valuable and evident attribute, sustaining our position to propose current high yielding variety as feasible energy crop for biomethane production in temperate regions. High contents of nitrogenous substances and WSC, optimal C:N ratio, low lignin concentration (compared with the other varieties) in the biomass of this most productive variety shows it to be well-suited for biogas production, irrespective of the fact that cellulose share in cell wall fractions, partly digested by micro-organisms, is also rather high. DM yield of the other 3 varieties and 9 ecotypes

Table 2. The output of tall fescue dry matter quality components per plant (g plant^{-1}) at the beginning of heading, 2009–2010

Accessions	N	C	NDF	ADF	ADL	WSC
'Navas DS'	1.7	43.2	58.4	33.4	2.4	15.1
'Kora'	1.4	32.2	45.1	25.1	2.2	10.8
'Barolex'	1.4	34.5	48.6	26.0	2.2	13.4
'Bariane'	1.2	32.3	43.0	23.0	1.5	14.3
No. 22	1.1	22.9	30.3	17.1	1.3	7.60
No. 20	1.3	31.2	42.4	24.1	1.9	10.6
No. 23	1.4	31.7	41.9	21.9	1.7	12.3
No. 24	1.3	26.9	36.8	19.9	1.6	10.0
No. 17	1.5	28.5	34.8	19.4	1.7	10.4
No. 18	1.3	27.0	36.6	20.3	1.8	8.70
No. 25	1.2	28.7	35.9	24.9	1.5	13.1
No. 21	1.4	27.3	36.3	19.1	1.8	9.70
No. 19	0.7	17.9	23.7	13.7	1.1	6.80
Average	1.30	29.6	39.5	22.1	1.75	11.0

was significantly ($P < 0.05$) lower than that of the most productive variety 'Navas DS'. For this reason the output of dry matter quality components (N, C, NDF, ADF, ADL and WSC) per plant (g plant^{-1}) at the beginning of heading of these accessions was rather low.

Conclusions

1. The average annual dry matter (DM) yield per plant of the variety 'Navas DS' was significantly ($P < 0.05$) or 28.5% higher than the trial average.

2. At the beginning of heading stage, the wild ecotypes Nos 21, 17, 22 and 19 were noted for high nitrogen concentration in DM – 23.0, 21.9, 21.7 and 20.4 g kg^{-1} DM, respectively.

3. The highest water soluble carbohydrate (WSC) concentration – 210.0 and 197.0 g kg^{-1} DM, low lignin – 22.8 and 20.7 g kg^{-1} DM, cellulose – 261.2 and 293.3 g kg^{-1} DM and hemicellulose – 265.0 and 281.0 g kg^{-1} DM was measured for wild ecotype No. 25 and the variety 'Bariane' at the beginning of heading stage.

4. The biogas quality indicator (C:N) ratio (from 20.3 to 27.9) of the tested accessions proved to be suitable for biogas production. The DM of the productive varieties 'Navas DS' and 'Bariane' had the optimal C:N ratio (25.5 and 27.9, respectively) at the beginning of heading stage.

Acknowledgements

The present study was supported by the Lithuanian State Science and Studies Foundation (2008–2009) and the Lithuanian Research Council (2010). Project registration No. N-08004.

Received 07 02 2011
Accepted 09 05 2011

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ISSN 1392-3196
Žemdirbystė=Agriculture, vol. 98, No. 2 (2011), p. 149–156
UDK 633.264:631.526.2/.32:581.192.7

Nendrinio eraičino (*Festuca arundinacea* Schreb.) veislių ir laukinių ekotipų biodujoms gaminti įvertinimas

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Santrauka

Tyrimai atlikti 2008–2010 m. Lietuvos žemdirbystės instituto Žolių selekcijos skyriaus bandymų laukuose ir Cheminių tyrimų laboratorijoje. Pagal agrobiologinių požymių kompleksą ir savybių bei biomasės cheminę sudėtį tirta nendrinio eraičino genetinė kolekcija, sudaryta iš 4 veislių ir 9 laukinių ekotipų.

Vieno augalo sausųjų medžiagų (SM) metiniu derliumi išsiskyrė veislės 'Navas DS' (Lietuva), 'Kora' (Čekija) ir 'Bariane' (Olandija). Jų vieno augalo žolės vidutinis metinis SM derlius (pirma pjūtis + atolas) buvo atitinkamai 198, 158 ir 156 g. Veislės 'Navas DS' vieno augalo vidutinis metinis SM derlius buvo iš esmės ($P < 0,05$), arba 28,5 %, didesnis nei bandymo vidurkis. Nendrinio eraičino vieno augalo atolo SM derlius sudarė vidutiniškai 53,7 % metinio derliaus ir svyravo nuo 51,4 % (laukinis ekotipas Nr. 20) iki 56,4 % (laukinis ekotipas Nr. 22).

Plaukėjimo pradžios tarpsniu laukiniai ekotipai Nr. 21, 17, 22 bei 19 išsiskyrė dideliu kiekiu azoto sausosiose medžiagose (atitinkamai 23,0, 21,9, 21,7 bei 20,4 g kg^{-1} SM). Šiuo augalų vystymosi tarpsniu laukinis ekotipas Nr. 25 (Latvija) ir veislė 'Bariane' (Olandija) sukaupė daugiausia vandenyje tirpių angliavandenių (VTA), atitinkamai 210,0 ir 197,0 g kg^{-1} SM, o pagal neutraliame tirpale išplautos ląstelienos (NDF) kokybinių rodiklių sudėtį pasižymėjo mažu kiekiu lignino (22,8 ir 20,7), celiuliozės (261,2 ir 293,3) ir hemiceliuliozės (265,0 ir 281,0) g kg^{-1} SM. Produktyvių veislių 'Navas DS' ir 'Bariane' augalų sausosiose medžiagose C:N vertės buvo optimalios biodujų gamybai (atitinkamai 25,5 ir 27,9).

Reikšminiai žodžiai: *Festuca arundinacea*, biodujos, sausųjų medžiagų derlius, C:N santykis, celiuliozė, hemiceliuliozė, ligninas.