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Yield and chemical composition of plant parts of silage maize (*Zea mays* L) hybrids and their interest for biogas production

Zsuzsanna Tóthné Zsubori*, János Pintér, Tamás Spitkó, Zsuzsanna Hegyi, Csaba L Marton

Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, H-2462 Martonvásár, Hungary
*Corresponding author: E-mail: zsubori.zsuzsanna@agrar.mta.hu

Abstract

Silage maize (*Zea mays* L) hybrids bred in Martonvásár were tested for morphological traits, yield and chemical composition of the whole plant and the plant parts in three years (2010-2012). Biogas production of the hybrids was investigated in 2009 and 2010. The dry matter, protein, starch, water soluble carbohydrates, neutral detergent fibre, acid detergent fibre, lignin and in vitro digestible organic matter contents were measured by NIR spectroscopy. The ratio of ear attachment height to plant height was smaller, the leaf number above the ear and the proportion of the leaves in the total plant dry matter was greater for leafy hybrids. It was concluded that the differences in the chemical composition of the parts of the same genotype were greater than between the same parts of different genotypes. The leaves had the greatest protein content. The starch and WSC content in the ear was significantly higher than in the other plant parts. The ear had the lowest lignin content and the highest IVDOM content compared to other plant parts. NDF and ADF contents were greatest in the stalk below the ear. Significant differences were found for WSC content of the plant parts of leafy and non-leafy hybrids. Digestible dry matter yield (DDMY) was calculated from individual dry matter production, plant number per hectare and digestible organic matter content. The highest DDMY value was recorded for Siloking (19.18 t ha^{-1}). Biogas yield was highest for Mv Massil (659 l kg^{-1} DM) with 61.38% methane concentration. Leafy hybrids produced significantly more biogas than conventional hybrids. Biogas yield proved to have significant positive correlations with starch and WSC content, and negative correlations with protein and lignin content.

Keywords: silage maize, yield, plant parts, leafy, NIR

Introduction

Silage maize is one of the most important annual forage for ruminant feeding in Hungary. The silage maize hybrids bred in Martonvásár are generally characterized by high yield potential (the ear makes up a large proportion of the total dry matter), valuable chemical components and good digestibility. In addition to conventional silage hybrids, a number of leafy hybrids from Martonvásár have also been registered. The first leafy silage maize hybrid in Europe was developed in Martonvásár and registered under the name of Kámasil in 2002. In these genotypes, the presence of the dominant gene *Leafy1* (*Lfy1)* transforms the plant architecture by increasing the number of leaves above the ear, which are important for photosynthesis (Shaver, 1983). The advantages of this trait have been reported by many authors. The greater ratio of leaves in the total plant dry matter (DM) and the higher carbohydrate content of the leaves above the ear in leafy hybrids (Andrews et al, 2000) have a beneficial effect on silage quality and digestibility (Pinter et al, 2011; Hegyi et al, 2009; Clark et al, 2002; Thomas et al, 2001).

In the breeding program of the Centre for Agricultural Research (CAR) in Martonvásár, biogas production of silage maize hybrids has also been studied. The results of experiments indicated that *leafy* hybrids produce more biogas than conventional hybrids (Hegyi et al, 2009, Hegyi et al, 2011). There are significant correlations between chemical components and biogas production (Hegyi et al, 2011). Biogas production is related to digestibility, since the degradation in the rumen and biogas fermenter proceed similarly at the beginning (Grieder et al, 2012). Moreover, digestibility measurements have also been investigated based on gas production techniques (Tang et al, 2006; Cone et al, 2008; Pell and Schofield, 1993).

The objective of biogas production is to achieve a high concentration of methane in the fermentation end-product. Good quality biogas contains at least 60% methane (Herrmann and Taube, 2006). Grieder et al (2012) found significant positive correlation between in vitro organic matter digestibility (IVDOM) and methane yield (0.73, P=1%), thus indicating the possible use of silage quality traits, particularly IVDOM, for indirect prediction of methane fermentation yield per unit of dry matter (MFY). They advise to use silage maize hybrids with high DM yield and high IVDOM for both feeding and biogas production.

The economic silage biogas production depends on the quantity and quality of the dry matter produced per hectare. Green mass and whole plant DM yield are very important parameters when evaluating

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maize hybrids for silage or gas production uses. In Hungary, only the DM yield and the proportion of the ear (high grain ratio) were analyzed for registration of a new silage maize hybrid up until 2006. Nowadays, the metabolizable energy content (ME) is also investigated and required for registration. This trait is correlated to digestible organic matter content and the chemical composition of the silage.

One way to improve biomass DM yields per hectare is to apply higher plant densities or to grow taller hybrids with larger ears or more leaves. Modifying the morphology of the plants through changes in the ratio of different plant parts does not itself influence the biomass quality. It is thus important to improve the chemical composition and digestibility of each part (Verbič et al, 1995). The ear, particularly grains, is the most energy rich part of plants, so it could be considered favorable to increase the ratio of the ear in the plant DM. For cattle feeding, grains have the best digestibility (Tang et al, 2006; Estrada-Flores et al, 2006), but the extreme increase of the grain ratio induces acidosis due to its high starch content. For biogas production, increasing ear content could be deleterious to whole plant DM yield, due to a premature flowering and growth cessation. The energy content of the leaves is also high (Sun et al, 2009; Tang et al, 2006) and increasing the proportion of this fraction is also favorable for energy yield improvement. Breeding leafy hybrids can thus be a relevant strategy. According to literature data, cows fed on silage made from leafy hybrids produce more milk with better quality compared to those fed conventional non isogenic hybrids (Clark et al, 2002; Thomas et al, 2001). It could than be hypothesized that comparable results could be obtained in biogas production. The stalk has the worst digestibility of all the plant parts, especially the below-ear fractions, since they are the most lignified parts of plants (Boon et al, 2008). Lignins are a non-digestible component of the fiber fraction, play an important part in stalk strength but worsen cell wall digestibility (Barrière et al, 2005) and similarly biogas production. This can be partially compensated for by raising the cutting height, but this is only effective in the case of leafy hybrids, which still have enough dry matter due to the larger number of leaves compared to normal hybrids (Lewis et al, 2004).

The other way of producing more energy per unit area is to improve the cell wall degradability of the forage. This requires improvements not only in the chemical composition of the whole plant, but also in the proportion of the different plant parts and in their digestibility. Degradability can be defined by various indices, measured by various methods. In vitro digestible organic matter content (IVDOM) is usually determined by NIRS, which is an easy, fast and cheap way to measure the chemical composition and digestibility/degradability of many genotypes.

The aim of this work was to study the yield, chemical composition and digestibility/degradability of the whole plant and the different plant parts of silage maize hybrids bred in Martonvásár, and their interest for both cattle feeding and biogas production.

Materials and Methods

The experiments were conducted in three consecutive years (2010-2012) in Martonvásár. Six silage maize hybrids bred in Martonvásár and a standard were tested under irrigated conditions on a cernozem soil. The hybrids were leafy – Limasil, FAO 380; Dunasil, FAO 390; Siloking, FAO 580 – and non-leafy types – Mv NK 333, FAO 390; Mv 437, FAO 480; Maxima, FAO 580; Florencia (standard), FAO 530 – with different maturity periods. Plots were 7.4 m^2 sized with four rows in a randomised complete block design with three replications. The sowing density was 80,000 plants per hectare. The same agrotechnology was applied every year.

The climate of the three years was very different. The quantity of rainfall during vegetation period was twice the 30-year average in 2010 (617.7 mm), while in 2011 and 2012 was the half of it (159.1 and 169.7 mm, respectively). There was no rainfall in August in 2012 and only 5.5 mm in 2011. The average temperature was below the 30-year average in 2010, and above in 2012 (17.1 and 18.7°C, respectively). Meteorological data were recorded in Martonvásár and provided by the National Meteorological Service.

During the vegetation period, data were recorded on the morphological traits of the hybrids (plant height, ear attachment height, leaf number). The crop was harvested 40 days after flowering at the silage maturity stage, at an average dry matter content of 35%. Plants were separated into five fractions: stalk below the ear, stalk above the ear, leaves below the ear, leaves above the ear, ear with husk and cob. The fresh mass of the whole plant and the different plant parts was measured. The dry mass of the whole plant and the plant parts was determined after drying on 105°C to constant weight. The plant parts of five plants per plot were chopped into 1-2 cm pieces. This fresh, chopped material was measured by a Fourier transform NIR spectrometer (Bruker, Germany) with INGOT calibration software to determine their chemical composition, including dry matter, protein, starch, water soluble carbohydrates (WSC), neutral deter-

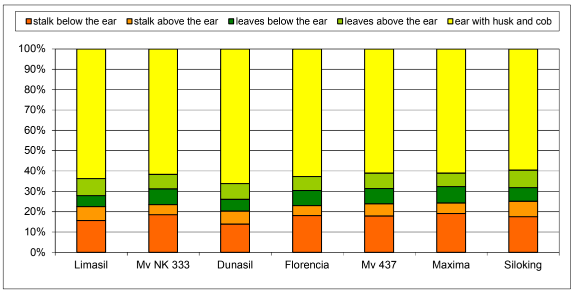

Figure 1 - Proportion of the plant parts in the total plant dry matter of silage maize hybrids (Martonvásár, 2010-2012).

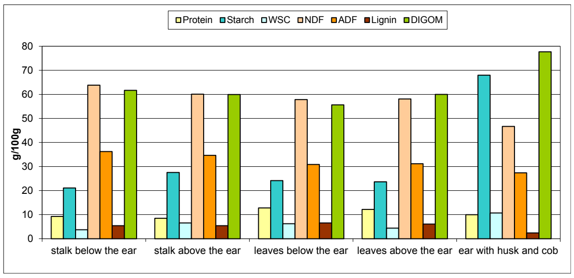

Figure 2 - Chemical composition of the plant parts of silage maize hybrids, averaged over years and genotypes (Martonvásár, 2010-2012). WSC= water soluble carbohydrates; NDF= neutral detergent fibre; ADF= acid detergent fibre: IVDOM= digestible organic matter.

gent fibre (NDF), acid detergent fibre (ADF), lignin and in vitro digestible organic matter (IVDOM) content. Data were statistically analysed using the two-way ANOVA program of the software Agrobase.

Biogas yield was investigated in 2009 and 2010 with four leafy (Limasil, Dunasil, Mv Siloking, Mv Massil) and four non-leafy (Mv Maros, Mv NK 333, Mv TC 437, Maxima) non isogenic hybrids. Plants were harvested at the end of August, at silage maturity stage. Chopped samples were prepared from the whole aboveground part of the plants. The samples were used to analyse biogas yield at the BETA Research Institute in Sopronhorpács. Biogas formation consists fundamentally of two processes, fermentation and methane formation. During the phases of fermentation (hydrolysis, acidic phase) the large-molecule organic matter is decomposed with the help of enzymes and fermentation bacteria. The rate of outgassing, biogas yield (l per 1,000 g dry matter), and methane concentration (%) were analyzed after complete outgassing in the customized 4 litre fermenters with gas meters. Samples containing 12 g DM each were mixed with 4 l of outgassed and filtered anaerob inoculum. The temperature was 37-40°C, and the pH was 7-8 in the fermenters, and controlled daily. During fermentation (2 to 3 weeks), the amount and composition of the gas produced was measured every day. The rate of outgassing of the dry matter was calculated from the DM content of the samples before and after fermentation. Specific biogas yield was determined based on the amount of biogas produced from 1,000 g of outgassed dry matter. Methane concentration was measured by gas chromatography.

Results and Discussion

Morphology and yield

Flowering data were recorded only to determine the time of harvest. Based on literature data (Józsa, 1981; Ma et al, 2006; Giardini et al, 1976; Argillier and Barrière, 1996) and earlier practical experiences, the optimal harvest time for silage maize hybrids is 40 days after flowering, when the dry matter content of the plant is about 35%. The dry matter content of the hybrids was between 30.24% (Mv NK 333) and 34.13% (Siloking), averaged over years (LSD$_{5\%}$=2.09).

Plant height was measured after flowering. Later maturing hybrids were taller. Plant height varied between 219.93 cm (Dunasil) and 280.37 cm (Siloking), averaged over years (LSD$_{5\%}$=20,60). The hybrids were significantly smaller in 2012 than in 2010 and 2011 (228.90 cm, 270.08 cm, 273.75 cm, respectively, LSD$_{5\%}$=13.49). This was probably due to the extremely hot and dry summer. The ratio of ear attachment height to plant height was significantly smaller for leafy hybrids (0.40-0.42) than for non-leafy hybrids (0.44-0.52; LSD$_{5\%}$=0.05). This corresponds to earlier data (Tóthné Zsubori et al, 2010; Shaver, 1983; Subedi and Ma, 2005). Significant differences were also found for above-ear leaf number between leafy (7.59-10.74) and non-leafy hybrids (6.52-7.11; LSD$_{5\%}$=0.48). The leaf area above the ear was found to be greater in earlier work (Hegyi et al, 2009).

The fresh mass of the plants was significantly greater in 2010 and 2011 than in 2012 (890.65 g, 887.36 g, 672.85 g, respectively, LSD$_{5\%}$=80.19). This was not observed for dry matter yield. Dry matter yield per plant varied between 306.76 g (Mv 437) and 353.41 g (Siloking), averaged over years (LSD$_{5\%}$=44.69).

Dry mass of the plant parts was measured to determine their proportion in the total plant dry matter. The proportion of the ear varied between 59.54% (Siloking) and 66.2% (Dunasil), averaged over years (LSD$_{5\%}$=2.56). The proportion of the stalk and the leaves below the ear was smaller, while the proportion of the stalk and the leaves above the ear was greater for leafy hybrids than for the non-leafy ones (Figure 1).

Chemical composition

In general, the differences in the chemical composition of the parts of the same genotype were greater than between the same parts of different genotypes.

Table 1 - Water soluble carbohydrate (WSC) content of the plant parts of leafy and non-leafy silage maize hybrids averaged over years (Martonvásár, 2010-2012).

	Limasil leafy	Mv NK 333 non-leafy	Dunasil leafy	Mv 437 non-leafy	Siloking leafy	Maxima non-leafy	LSD$_{5\%}$
stalk below the ear	3.91	3.58	4.77	2.94	3.47	3.23	0.72
stalk above the ear	6.25	7.36	6.62	5.99	5.91	6.94	0.82
leaves below the ear	6.49	6.80	6.90	5.16	6.04	5.92	0.89
leaves above the ear	4.24	4.66	4.91	3.31	4.36	4.58	0.85
ear with husk and cob	11.00	10.04	11.42	10.75	10.21	10.17	0.75

Table 2 - Green mass, Dry matter yield, Digestible organic matter (IVDOM) content and Digestible dry matter yield of silage maize hybrids averaged over years (Martonvásár, 2010-2012).

	Green mass t ha^{-1}	Dry matter yield t ha^{-1}	Digestible organic matter content %	Digestible dry matter yield t ha^{-1}
Limasil	62.31	26.21	67.43	17.72
Mv NK 333	63.63	25.52	67.37	17.19
Dunasil	59.67	25.8	68.26	17.53
Florencia	63.76	26.69	67.86	18.04
Mv 437	63.13	24.54	67.13	16.49
Maxima	72.62	28.18	67.83	19.09
Siloking	72.39	28.27	67.78	19.18
mean	65.357	26.46	67.66	17.893
$LSD_{5\%}$	9.7993	3.5751	2.2028	2.6073

The same was found by Masoero et al (2006). The effect of the year was highly significant for most of the chemical components for all plant parts.

Studying the chemical composition of the plant parts averaged over hybrids and years, it can be concluded that the leaves had the greatest protein content (12.77 and 12.16%) (Figure 2). According to Bal and Bal (2009) the increased protein content of the leaves is due to the protein synthetizing and transporting activity of the enzymes involved during photosynthesis. The starch (67.98%) and WSC (10.68%) content in the ear was significantly higher than in the other plant parts. The ear had the lowest lignin content (2.40%) and the highest IVDOM content (77.72%) compared to other plant parts. NDF and ADF contents were greatest in the stalk below the ear (63.79 and 36.21%, respectively). These are in accordance with other findings in the literature (Methu et al, 2001; Masoero et al, 2006; Estrada-Flores et al, 2006).

Significant differences were found for WSC content of the plant parts of leafy and non-leafy hybrids (Table 1). In case of Dunasil and Mv 437 the leaves of the leafy hybrid contained more WSC than those of the non-leafy hybrid. In case of Limasil and Mv NK 333 the WSC content of the ear of the leafy hybrid was greater than that of the non-leafy one. Regarding to the other chemical components, no significant differences were observed between the parts of leafy and non-leafy genotypes. The *in vitro* digestible organic matter content (IVDOM) of the whole plant was almost the same for all the hybrids when averaged over years, but there were significant differences between the years. The IVDOM content was lowest (64.28%) in 2010, highest (71,31%) in 2011 and average (67.41%) in 2012 (grand mean=67.66; $LSD_{5\%}$=1.44).

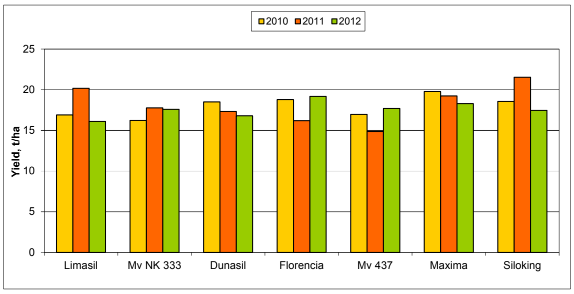

Figure 3 - Digestible dry matter yield per hectare of silage maize hybrids in three years (Martonvásár, 2010-2012). $LSD_{5\%}$ for year=1,7069; $LSD_{5\%}$ for genotype=2,6073; $LSD_{5\%}$ for genotype×year=4,516.

As for the plant parts, the IVDOM content was 61.65% in the stalk below the ear, 59.94% in the stalk above the ear, 55.62% in the leaves below the ear, 59.96% in the leaves above the ear and 77.72% in the ear. The stalk and the leaves above the ear had the same IVDOM content. These plant parts are valuable for silage and biogas production (Adrews et al, 2000; Thomas et al, 2001; Lewis et al, 2004; Herrmann and Taube, 2006). However, no significant differences were found between the IVDOM content of the stalk or leaves above the ear of leafy and non-leafy hybrids.

Lignin has a considerable negative effect on digestibility (Barrière et al, 2005; Boon et al, 2005; Grabber, 2005; Riboulet et al, 2008). The degradation processes in the rumen and biogas fermenter are quite similar (Grieder et al, 2012), so the increased lignin content is considered to decrease biogas yield. Significant negative correlation (-0.6014, p=0.1%) was found for lignin and IVDOM content of the hybrids studied in this experiment.

Digestible dry matter yield

Later maturing hybrids produced significantly greater green mass per hectare than earlier maturing ones. The greatest green mass was recorded for Maxima (72.62 t ha^{-1}) and Siloking (72.39 t ha^{-1}) (Table 2). The effect of the year was highly significant for this trait. Since the green mass per hectare was calculated from green mass per plant, the same tendences were observed. Hybrids produced more green mass per hectare in 2010 and 2011 than in 2012. The dry matter yield per hectare was also calculated from individual production and plant number per hectare. The highest dry matter yield was 28.27 t ha^{-1} (Siloking), and the lowest was 24.54 t ha^{-1} (Mv 437) ($LSD_{5\%}$=3.57). The effect of neither the genotype nor

Table 3 - Specific biogas yield from leafy and non-leafy hybrids (l per 1,000 g dry matter) averaged over the years 2009 and 2010.

Hybrid	Biogas yield (l per 1,000 g DM)			Methane (%)	
	2009	2010	Mean	2009	2010
Mv Maros	628	575	602	62.03	56.00
Mv NK 333	623	469	546	61.88	59.03
Mv TC 437	684	582	633	60.95	63.35
Maxima	719	566	643	59.23	58.23
Limasil	705	554	630	63.13	57.78
Dunasil	637	624	631	61.53	60.10
Mv Siloking	737	544	641	61.95	59.88
Mv Massil	733	585	659	60.43	61.38
Non-leafy mean	664	548	606	61.02	59.15
Leafy mean	703	577	640	61.76	59.79
$LSD_{5\%}$	12.00			0.84	

the year was significant for this trait.

Based on dry matter yield per hectare (DMY) and in vitro digestible organic matter content (IVDOM), the digestible dry matter yield (DDMY) was calculated by the following formula:

DDMY= DMY*IVDOM/100.

This proved to be a suitable method for evaluating silage hybrids because it gives a more precise prediction of the feeding value than dry matter yield or IVDOM content separately.

The digestible dry matter yield of the hybrids was not significantly affected by the year or the genotype, though there were considerable differences between the hybrids in 2011 (but not in 2010 and 2012). Later maturing hybrids did not have significantly greater digestible dry matter yields than earlier maturing ones (Figure 3). The highest DDMY value was recorded for Siloking (19.18 t ha^{-1}) and the lowest for Mv 437 (16.49), while the earliest hybrid Limasil produced 17.72 t ha^{-1} digestible dry matter ($LSD_{5\%}$=2,61).

This emphasizes the importance of studying digestibility, because hybrids with high dry matter yield but poor digestibility may produce lower digestible dry matter yields per hectare than hybrids with lower yields but good digestibility. Since digestibility is related to biogas yield (Grieder et al, 2012), economic biogas production needs silage maize hybrids with high dry matter yields AND good digestibility. Later maturing leafy hybrids bred in Martonvásár are suitable and can be recommended for this purpose.

Biogas production

The biogas production of leafy and conventional hybrids was studied over two years. It was concluded that leafy hybrids produced more biogas during the anaerobic fermentation of silage (640 l per 1,000 g

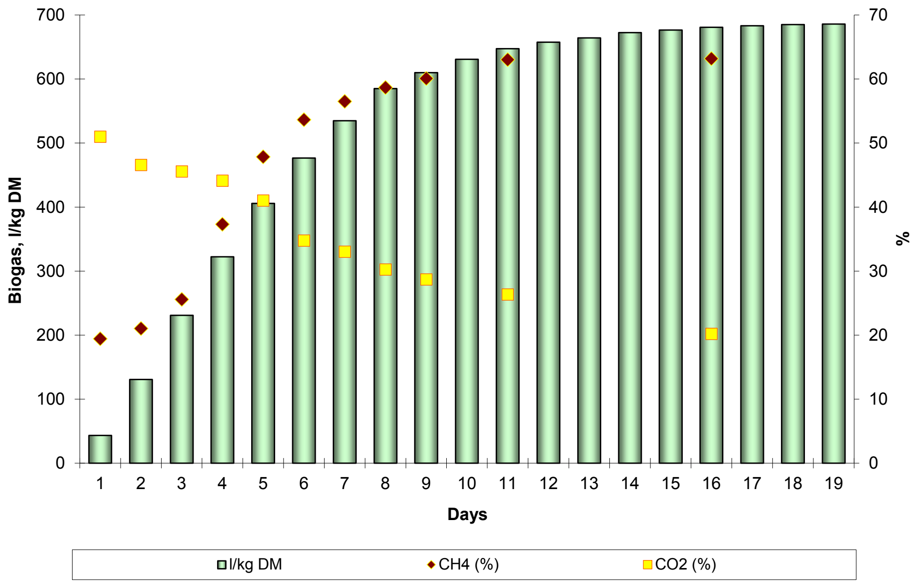

Figure 4 - Specific biogas yield from the maize hybrid Mv Massil, averaged over the years 2009 and 2010.

Table 4 - Chemical composition of the silage and the correlation with biogas yield, averaged over the years 2009 and 2010.

Non-leafy	Biogas	Starch	Protein	Lignin	ADICP	NDF	NDICP	WSC
Mv Maros	601.50	36.56	8.80	4.44	4.13	53.60	2.42	5.41
Mv NK 333	546.00	32.93	9.70	4.24	4.22	53.40	2.40	5.48
Mv TC 437	633.00	34.33	8.50	4.15	4.17	54.48	2.28	5.36
Maxima	642.50	35.60	9.70	4.23	4.20	53.00	2.48	5.75
Mean	605.75	34.86	9.18	4.27	4.18	53.62	2.40	5.50
Correlation		0.59	–0.36	–0.23	–0.34	0.17	–0.04	0.31
Leafy	Biogas	Starch	Protein	Lignin	ADICP	NDF	NDICP	WSC
Limasil	629.50	36.18	8.90	4.27	4.17	54.75	2.35	5.04
Dunasil	630.50	36.50	9.49	4.02	4.07	54.80	2.38	5.57
Mv Siloking	640.50	35.40	8.82	4.21	4.20	53.17	2.51	5.95
Mv Massil	659.00	37.50	8.87	4.07	4.06	55.36	2.28	5.60
Mean	639.88	36.40	9.02	4.14	4.13	54.52	2.38	5.54
Correlation		0.61	–0.48	–0.32	–0.40	0.26	–0.38	0.41

dry matter) than conventional hybrids (606 l per 1,000 g dry matter) (Table 3). The difference was statistically significant. In both years the lowest biogas production was recorded for the same hybrid (Mv NK 333, 546 l per 1,000 g dry matter), while a leafy hybrid (Mv Massil, 659 l per 1,000 g dry matter) produced the greatest biogas yield (Figure 4). The objective of biogas production is to achieve a high concentration of methane in the fermentation end-product. Good quality biogas contains at least 60% methane (Herrmann and Taube, 2006). The highest methane concentrations were recorded for Mv TC 437 in 2010 (63.35%) and Limasil in 2009 (63.13%). There were no significant differences between the methane contents of the leafy and non-leafy hybrids tested in the experiment. The process of outgassing took three weeks and the rate of outgassing was 87%, averaged over years and hybrids.

Biogas yield depends on the chemical composition of the biomass. No significant correlation was found between the chemical composition and specific methane yield of the hybrids by Schittenhelm (2008), but other authors revealed that methane production depends on the composition of the biomass. Oslaj et al (2010) found a significant correlation with crude protein content. In the present experiment, a strong positive correlation was found between the biogas yield and the starch content of the silage, and a moderate positive correlation between the biogas yield and the WSC content (Table 4). The correlation between the biogas yield and the lignin and protein contents was negative, in accordance with data in the literature.

Conclusions

In Western Europe areas removed from cultivation have been utilised for the production of renewable energy sources, which also has the effect of preventing rural migration. Hungary is poor in fossil fuels, but has good agricultural potential. Nevertheless, the number of families living chiefly from agriculture has gradually declined since EU accession. Nearly half a million people have had to give up growing food crops. The production, harvesting and processing of energy crops for the purpose of biogas production could provide jobs for these people, making it unnecessary for them to leave their homes and land. For a number of reasons, biogas production occupies a special place among renewable energy sources. This method of biomass utilisation is able to satisfy consumer demands in a complex manner, being suitable for heating, refrigeration and vehicle fuel purposes, while also resulting in valuable by-products (biofertiliser, carbon dioxide).

Official statistics show that the area sown to silage and fodder maize in Hungary has declined from 350,000 hectares in 1983 to 89,000 ha in 2008. Growing maize for biogas production could open up new prospects for this sector, especially if leafy silage maize hybrids were grown, which produce a larger quantity of biomass per unit area and have good fermentability, resulting in higher biogas yields. In the present experiments the biogas yield of leafy hybrids was significantly higher in both years than that of conventional hybrids. In addition, they produce more digestible dry matter per hectare than the non-leafy hybrids. Thus, they are prosperous for both silage and biogas production.

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