



SZENT ISTVÁN UNIVERSITY

**Improving the chemical quality and digestibility
of silage maize hybrids**

Main points of the PhD Thesis

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1. INTRODUCTION AND OBJECTIVES

Silage maize is a basic forage for ruminants. During digestion the fibre content of the silage is transformed into volatile fatty acids, which are essential for milk production.

In recent decades, both breeders and producers aimed to maximize the fresh and dry matter yield of silage maize and increasing the proportion of the ear in the total plant dry matter. Good silage maize hybrids are considered to have the same quality traits as grain maize hybrids. Many farmers still choose hybrids based on the grain yield, but this is not related to silage quality.

Silage maize hybrids are certified based on fresh and dry matter yield and the proportion of the ear. The metabolizable energy content of the forage is now also tested during the registration process. Testing chemical quality and digestibility is becoming more frequent, but is still not common practice.

The first leafy hybrid in Europe, Kámasil, was produced in Martonvásár, Hungary and registered in 2002. Leafy hybrids produce more dry matter yield, and the greater number of leaves above the ear increases the quantity of carbohydrates formed and stored in the leaves, providing the silage with better chemical quality and digestibility, which is favourable for milk production.

Many research papers have been published all over the world on the topic of silage quality and digestibility, but very few in Hungary.

The objectives of this research were (briefly):

- ❖ a detailed examination of silage maize hybrids;
- ❖ studying the effects of the genotype and the year;
- ❖ studying the correlations between all traits;
- ❖ ranking hybrids based on digestibility;
- ❖ identifying traits responsible for silage quality.

2. MATERIALS AND METHODS

2.1. Genotypes tested in the experiments

In accordance with the objectives listed above, eight hybrids with different type and maturity period were tested in field experiments (*Table 1*). The parental lines of the hybrids were tested in a parallel experiment under the same conditions.

Table 1. Genotypes tested in the experiments

<i>Name</i>	<i>Crossing type</i>	<i>FAO number</i>	<i>Maturity period</i>	<i>Utilisation</i>	<i>Type</i>
Limasil	SC	FAO 380	early	silage	leafy
Mv 328	SC	FAO 330	early	silage	non-leafy
Mv NK 333	MTC	FAO 390	early	silage/grain	non-leafy
Mv 434	TC	FAO 440	medium	silage/grain	non-leafy
Mv 448	MTC	FAO 450	medium	silage	non-leafy
Mv 437	SC	FAO 480	medium	silage	non-leafy
Kámasil	SC	FAO 510	late	silage	leafy
Maxima	TC	FAO 580	late	silage	non-leafy

2.2. Experimental conditions

Field experiments were conducted in three consecutive years (2002-2004) in Martonvásár, in a randomised complete block design with four replications. Plant density was 80,000 plants per hectare. Meteorological data were recorded at a meteorological station near the experimental field. The climate of the three years was very different. The average year of 2002 was followed by a hot and dry summer in 2003 with atmospheric drought, and cooler, humid weather in 2004. The same agrotechnology was applied every year, including the sowing conditions, cultivation, weed control and irrigation.

2.3. Traits studied in the experiments

Agronomic traits: emergence, early vigour, date of 50% anthesis and silking, proterandry, rate of sterile plants, corn borer or common smut infected plants, lodging, root lodging, relative stalk strength, drying rate of leaves and husk.

Morphological traits: plant height, ear attachment height, ratio of ear height to plant height, leaf number below and above the ear, total leaf number.

Yield: fresh mass per plant and per hectare, dry matter yield per plant and per hectare, dry matter content of the whole plant and the plant parts, weight and ratio of different plant parts in the total plant dry matter (stalk below the ear in three fractions, stalk above the ear, leaves below the ear, leaves above the ear, ear with husk and cob).

Chemical composition of the whole plant and the plant parts: crude protein, crude ash, crude fat, crude fibre, acid detergent fibre (ADF), lignin; digestibility of the whole plant.

The digestible dry matter yield per hectare was calculated based on plant density, dry matter yield per plant and digestibility.

2.4. Sampling and chemical analysis

Hybrids were harvested at silage maturity stage, at 35% dry matter content on average. Five plants per plot were separated into seven fractions and measured (plant parts listed above). Dry matter content of the samples was determined after drying at 105°C to constant weight. Another five plants per plot were cut and chopped. Plant parts of three hybrids were cut and chopped separately (*Limasil*, *Mv 328*, *Mv 448*).

Chemical analysis and *in vivo* digestibility trials were conducted in Keszthely, at the Department of Animal Physiology and Feeding, Georgikon Faculty of Agricultural Sciences, Veszprém University. Six ruminally fistulated Texel sheep were used for digestibility trials. The effective and potential degradability of the samples were determined *in sacco* with different incubation periods, based on the method of Ørskov and

McDonald (1979). The chemical composition of the samples was determined by Weendei analysis and by the method of *Van Soest et al. (1991)*, according to the standard MSZ 6830.

2.5. Evaluation of results

The data were analysed using Agrobase software. Two-way analysis of variance was performed to determine the effect of the genotype and the year on the different traits. Digestibility results were analysed separately. Correlation analysis was performed to study the correlations between the traits.

Variation coefficients from ANOVA were compared to examine the stability of the traits over genotypes and over years using the method of *Sváb (1981)*. If the CV value is low, the trait is determined more by the genotype than by the environment.

The heritability of the traits can be estimated based on the rate of heterosis. Traits with high h^2 values show low heterosis. The rate of heterosis was determined based on the performance of the hybrids and their parental lines as a percentage of the parental value.

3. RESULTS

3.1. Agronomic and morphological traits, yield

Among the agronomic traits, emergence and early vigour were strongly affected by the year. *Maxima* had the longest maturity period, and this hybrid produced the greatest dry matter yield despite slow early development. *Kámasil* was proterogynous and the difference between the date of anthesis and silking was the greatest for this hybrid. There was a strong positive correlation between the date of pollination and the date of silking ($r=0.99$). Flowering time had no effect on dry matter content or digestibility of the plants.

Biotic stress factors have a negative effect on yield quantity and quality. Genotype had greater influence on these factors than the environment. *Mv 328* showed the lowest common smut infection rate (0.14% averaged over years), suggesting that this is a resistant genotype. Corn borer infection was similar for the three years, differences were only observed between the genotypes. As a consequence of corn borer damage the plants become susceptible to fusarium infection, resulting in a lower yield with poor quality. The rate of sterile plants was greater in the dry year because the vitality of the pollen decreases intensively at high temperature and low air humidity.

The leaf and husk drying rates were affected more by the genotype than by the year, especially for the stay-green hybrid *Mv 328*. There was no significant correlation between leaf and husk drying rates and digestibility, as reported by other authors (*Schlagheck et al., 2000*). There was a negative correlation between leaf drying rate and lignin content, supporting the hypothesis that lignin content increases during maturation.

Stalk strength was defined by the rate of crushed stalks and by instrumental measurement. No significant correlation was found between the two results. Lignin is the most important factor influencing stalk standability. There was no correlation between lignin content and measured stalk strength, but there was a moderate negative correlation with the rate of crushed stalks.

In the case of plant height and ear height the effects of genotype and year were both significant. The rate of ear height to plant height was only affected by the genotype,

suggesting that this is a genetically determined trait, characteristic of the hybrids. The ear was attached lower in leafy hybrids than in the others, as reported earlier (*Shaver, 1983*). Plant height had a moderate effect on the fresh yield.

The effect of genotype and year and the interaction of the two were significant for leaf number. The latest hybrid (*Maxima*) had the greatest number of leaves below the ear. Leafy hybrids had more leaves above the ear than the other hybrids, in accordance with other results (*Shaver, 1983; Modarres et al., 1997*). Based on data in the literature, the greater number of leaves above the ear results in greater fresh yield, but the correlation was not significant for the present genotypes.

Fresh and dry matter yield depended more on genotype than on the year. Irrigation obscured the differences between the genotypes. Late maturing hybrids produced the greatest dry matter yield, but the differences within the maturity groups were greater than between them. The extreme values were all recorded for the same (early) group. The dry matter content of the plants varied with year and genotype. The grand mean was 36.5%, which is optimal for silage harvest. Digestibility was not influenced by the dry matter content of either the whole plant or the plant parts.

The weight of the different plant parts and their proportion in the dry matter was mainly affected by the genotype. At silage maturity stage the proportion of the ear was over 60% for each hybrid, which is favourable for silage quality (*Józsa, 1981*). The proportion of the ear was highest for *Limasil*, possibly due to a decrease in the proportion of the stalk. There was a strong negative correlation between the proportion of these two plant parts ($r=-0.80$). The ear proportion of dual-purpose hybrids was greater compared to the others. In the humid year (2004) the proportion of the stalk, especially the stalk below the ear, was greater. There was a strong positive correlation between the fresh mass of the stalk below the ear and the total fresh mass of the plant. Dry matter yield was influenced more by the weight of the ear. The ear made up the greatest proportion in the dry matter followed by the stalk. The proportion of the different stalk fractions varied with the genotype. The proportion of the stalk below the ear was lowest for the leafy hybrids, because of the lower attachment height of the ear. The weight and proportion of the leaves above the ear were greatest for the leafy hybrids, corresponding to data in the literature.

3.2. Chemical composition and digestibility

No significant correlations were found between morphological traits and chemical composition, except for a slight negative correlation between leaf number and crude ash content. The weight and proportion of the different plant parts had a greater effect on the chemical composition of the whole plant. There was a moderate positive correlation between the proportion of the stalk above the ear and crude ash content, despite the low crude ash content of the stalk. The fresh weight of the ear was in negative correlation with the crude fat content. Lignin content showed a moderate or strong positive correlation with all the plant parts except the leaves above the ear, where the second lowest lignin content was measured. The ear had the lowest lignin content.

The proportion of the plant parts influenced the chemical composition of the whole plant through their different compositions. The stalk had the highest lignin content, while the ear had the lowest. The crude protein and crude ash content were greatest for the leaves. The crude fibre content was highest for the stalk, as reported in the literature (*Bal and Bal, 2009; Masoero et al., 2006*). *Thomas et al. (2001)* stated that leafy hybrids contain more protein and ash due to the greater number of leaves, but the correlation was not significant for the genotypes tested in the present experiments.

Digestibility was not influenced by the agronomic and morphological traits or yield, but was affected by the dry matter content. Among chemical components, crude fibre and lignin had the greatest influence on digestibility, in accordance with data in the literature. Crude fibre and lignin content were in moderate negative correlation with digestibility ($r = -0.39$ and -0.36 , respectively). If lignin content is defined as the percentage of crude fibre content the correlation becomes very strong. Lignin content does not itself influence digestibility, but its composition and structure are also important, performing a complex effect. The results supported the hypothesis that most of the lignin is located in the stalk, especially in the lower parts of the stalk below the ear (*Boon et al., 2008*). Because of the high lignin content of the stalk below the ear, it is suggested that

silage maize should be harvested with a higher cutting height (*Orosz and Oross, 2007; Lewis et al., 2004*), but this is only effective in the case of leafy hybrids, where the greater mass of leaves compensates for the dry matter loss of the stalk.

There were significant differences between genotypes and years for chemical composition and digestibility. *Verbič et al. (1995)* found no differences in the whole plant digestibility of the genotypes, by contrast to other authors. In the present experiment digestibility depended more on the genotype than on the year, and there were significant differences between the genotypes. Many authors found significant differences in the digestibility of the plant parts. In addition, *Masoero et al. (2006)* found greater differences in the digestibility of the plant parts of the same genotype than between the same parts of different genotypes. It is commonly stated that the stalk has the worst digestibility, and the ear the best.

The digestibility of the leaves is better than that of the stalk. Many authors concluded that leafy hybrids had better digestibility due to the greater number of leaves above the ear, as a consequence of which more nutrients were produced by greater photosynthetic activity, resulting in greater milk production (*Clark et al., 2002; Thomas et al., 2001*). In the present experiment the digestibility of the leafy hybrids was not significantly different from the average. There was no significant correlation between the number or weight of the leaves and digestibility. Nevertheless, the chemical composition of leafy hybrids is favourable, due to the high crude protein and crude ash contents of the leaves. The hybrids with best digestibility had high crude protein and crude ash contents.

Among plant parts the ear, especially kernels, has the best digestibility. It is assumed that digestibility can be improved by increasing the proportion of the ear in the plant dry matter. In contrast, *Phipps and Weller (1979)* reported that the proportion of the ear does not influence digestibility. There was no significant correlation between the proportion or the weight of the ear and digestibility of the present genotypes. The crude fat content was highest for the ear and the crude protein content was the second highest (after the leaves) compared to the other plant parts. The ear contained the least lignin. The proportion of the ear was over 60% for all the hybrids, and this, together with its favourable chemical composition, had a positive effect on the whole plant digestibility.

3.3. Heterosis and stability

Bertoia (2001) found significant positive heterosis for whole plant digestibility and the weight of the stalk, ear and whole plant. In the present experiment the heterosis for fresh and dry matter yield was 164 and 176%, respectively. The hybrids exceeded the parental lines in the weight of the stalk and the ear. The proportion of the ear also increased. The quantities of the chemical components were lower in the hybrids than in the parental lines. In the case of crude fibre, ADF and lignin content this change is favourable. The hybrids contained more crude fat than the parents (heterosis 119%), which could be due to the larger ears and the greater number of kernels.

The heritability of traits can be estimated from the rate of heterosis. Traits with high heritability show less heterosis, while traits with low heritability are influenced more by the environment and show greater heterosis. There was no heterosis for digestibility, as the performance of the hybrids and the parental lines was almost the same (99%). *Moreno-Gonzalez et al. (2000)* also found no heterosis for whole plant digestibility, suggesting that this trait has strong genetic determination. This means that using parental lines with good digestibility may improve the digestibility of the hybrids.

Studies on the variation coefficients revealed that the traits with the best stability were flowering time, the ratio of ear height to plant height and the proportion of the ear in the plant dry matter. Early vigour and dry matter production were influenced most by the environment. The variation between genotypes was greater than between years for the proportion of the different plant parts. Among chemical components, ADF seemed to be influenced least by the year. The variation coefficient was low for digestibility, suggesting that this trait does not depend on environmental effects but only on genotype.

3.4. Novel scientific results

The following novel scientific results were obtained:

- ❖ Silage maize hybrids bred in Martonvásár, including two new leafy hybrids, were characterised in detail.
- ❖ Correlations were found between agronomic and morphological traits, yield, chemical components and digestibility. Digestibility was influenced most by crude fibre and lignin content; moderate negative correlations were found.
- ❖ The proportion of the different plant parts was determined, and was found to be different for each hybrid. The proportion of the ear was more than 60% for all the genotypes. The proportion of the stalk below the ear was lower and the proportion of the leaves above the ear was higher for leafy hybrids than for the others.
- ❖ The chemical composition of the whole plant and the different plant parts was measured. The differences between the plant parts were greater than between the same parts of different genotypes.
- ❖ Digestibility was studied *in vivo*, which is not a commonly used method in Hungary. The hybrids were evaluated and ranked based on the results. *Maxima* and *Mv 434* had the best digestibility.
- ❖ The digestible dry matter yield per hectare was calculated from the experimental data. This is a suitable method for evaluating hybrids.
- ❖ Traits influencing silage quality were determined. A greater proportion of leaves above the ear, a smaller proportion of stalk below the ear, lower lignin content, higher crude protein content, and high digestible dry matter yield are favourable traits for silage production.

4. CONCLUSIONS

4.1. Characterisation of the hybrids

The hybrids were characterised in detail based on the experimental data, including agronomic and morphological traits, yield, chemical composition and digestibility, and digestible dry matter yield. The leafy hybrids were also characterised, complementing earlier results (*Tóthné Zsubori et al., 2003*).

4.2. Effect of genotype and year

Agronomic traits and yield were influenced more by the year, while morphological traits, including the proportion of the different plant parts, yield, chemical composition and digestibility by the genotype. The same results were found in earlier work (*Tóthné Zsubori et al., 2010*).

Proterandry and dry matter yield per plant were influenced most by the year, while the ratio of ear height to plant height the least. This trait proved to have strong genetic determination (*Tóthné Zsubori et al., 2009*). The number of leaves above the ear was also determined by the genotype. Leafy hybrids had more leaves above the ear than the other hybrids every year. The fresh mass per plant and dry matter content were influenced by the year. In the humid year the proportion of the stalk, especially below the ear, was greater, resulting in greater fresh mass. The proportion of the different plant parts was influenced more by the genotype than by the year (*Tóthné Zsubori et al., 2004*). The proportion of the ear was more than 60% for every hybrid.

In the case of chemical composition the differences between the genotypes were greater than between the years. Digestibility was influenced mainly by the genotype.

4.3. Correlations

Digestibility was not directly influenced by the agronomic and morphological traits. There were significant negative correlations between common smut infection and crude fibre content, and between stalk crushing rate and lignin content, in accordance with data in the literature. Morphological traits had the greatest effect on the yield. These correlations confirmed earlier results (*Tóthné Zsubori et al., 2005*).

The proportion of the different plant parts in the dry matter did not directly influence the digestibility of the whole plant, but their weight did (*Tóthné Zsubori et al., 2010*). The weight of the stalk below the ear was in significant positive correlation with ADF and lignin content. The number, weight and proportion of the leaves above the ear had no effect on the chemical composition of the whole plant.

Digestibility was influenced by the chemical composition, though significant correlations were found only with crude fibre and lignin content. Lignin had a negative effect on digestibility. Hybrids with good digestibility had high crude protein content, suggesting that crude protein is favourable for good digestibility.

The chemical composition of the plant parts was different. The differences between the parts were greater than between the hybrids. The stalk contained the most lignin. The crude protein and crude ash content of the leaves were greater than those of the other parts. The ear had the lowest lignin content, the highest crude fat content, and a greater crude protein content than the stalk. Since the chemical composition of the plant parts is different, changes in the proportion of the plant parts can be expected to influence the chemical composition and digestibility of the whole plant. The leaves had the greatest crude protein content, the stalk below the ear contained the most lignin, and the ear had the lowest lignin content. It was concluded that decreasing the proportion of the stalk below the ear and increasing the proportion of the ear and the leaves above the ear in the plant dry matter would result in better digestibility for the whole plant. Changing the ratio of the plant parts does not itself influence digestibility; it is important to improve the quality of each part.

Hybrids with high dry matter yield but poor digestibility produced a lower digestible dry matter yield per hectare than hybrids with lower yields but good digestibility. This means that digestible dry matter production depends on digestibility, rather than dry matter yield.

4.4. Ranking the hybrids

The hybrids were evaluated for all the traits examined. *Maxima* proved to be the best hybrid. The ranking of the hybrids based on digestibility was: 1. *Maxima*; 2. Mv 434; 3. Mv 437; 4. Kámasil; 5. Mv 448; 6. Limasil; 7. Mv NK 333; 8. Mv 328.

4.5. Traits influencing silage quality

Good silage maize has:

- ❖ high dry matter yield;
- ❖ great proportion of the ear in the dry matter;
- ❖ optimal dry matter content at harvest;
- ❖ preferably stay-green type;
- ❖ good chemical composition (high protein, low fibre and lignin content);
- ❖ good digestibility.

The following complementations can be made based on the experimental results:

- ❖ greater proportion of the leaves in the dry matter (more protein);
- ❖ smaller proportion of the stalk below the ear (less lignin);
- ❖ good fertility for more kernel (more crude fat);
- ❖ great dry matter yield and good digestibility together.

4.6. Final conclusion

The final conclusion is that knowledge of the genetic background is important, but not enough to improve traits. Since gene expression is controlled by different molecular mechanisms and environmental effects, and each gene may be involved in the regulation of several metabolic processes, it is not enough to change the gene itself. It is also important to study the whole plant, the final product of breeding. Silage maize hybrids and their parental lines must be tested in field experiments to study the expression, heritability and stability of various traits, and the environmental effects influencing the expression of these traits. The value of silage maize hybrids depends on the efficient utilisation of the forage for milk and meat production. Digestibility and the digestible dry matter yield can give a good estimation of this. Digestibility proved to be a genetically determined trait, which means that using parental lines with good digestibility may improve the digestibility of the hybrids.

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