

# IN VITRO DIGESTIBILITY OF SILAGE MAIZE HYBRIDS

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#### **ABSTRACT**

The aim of this study was to determine in vitro NDF digestibility of different silage maize hybrids using upgraded method in vitro gas production. Digestibility was measured up to 48 hours and the focus of our work was on 30-hour digestibility. Based on our latest work we focused on 2 evaluations: 1. evaluation of NDF digestibility to find correlation between dry matter of silage maize hybrids and NDF digestibility in 30-hour point and 2. Differences of NDF digestibility between silage maize hybrids in 30-hour point. We found highest NDF digestibility 62,80% in dry matter range from 30-35% which correlates to our latest work to determine the best silage window. Differences in NDF digestibility of silage maize hybrids in 30-hour point were not significant (P = 0.580) and the range was from 55,16% - 63,82%. In dry matter range 27-35% we found 30-hour NDF digestibility from 49,81-64,76%.





**Keywords:** silage maize hybrid; NDF digestibility; in vitro gas production

### INTRODUCTION

Maize silage is very often used as a basis of TMR, and it is important to determine the best time for harvesting and create the best feed from the perspective of nutrients, silage fermentation and digestibility. Neutral detergent fiber (NDF) digestibility is important factor which has impact on the milk production (Krämer-Schmid et al., 2016).

Our latest works showed that FAO groups are not a good prediction model for silage maturity prediction (Mitrík T., Mitrík A., 2022; Mitrík T., Mitrík A., 2023) and to determine silage maturity we need better model as described (Mitrik T., 2023). Based on the results and nutrients we found the best silage maturity level at 30% of dry matter (DM). The aim of this work is to evaluate silage maturity from side of the digestibility. Attempts to predict and describe in vivo digestibility using in vitro digestibility fermentation started in twentieth century but due to poor technique which requires anaerobic environment and inadequate buffers were results using in vitro technique lower than using in vivo method. In 1963 had been developed two-stage Tilley and Terry method which is still widely used today with some modifications. In 1970 Goering and Van Soest developed in vitro NDF digestibility which requires standardization to ensure reproducible results. The newer method developed by Ankom is still used and it requires filter bags with sample and all bags share a common environment with sample weight 0,25 - 0,50 g per sample (William and Hall, 2020). Digestibility is measured after the given incubation time. The latest method is measurement of gas production from in vitro fermentation



which is indirect method to determine digestibility kinetics based on gases production and final sample weight difference before and after the incubation (Tedeschi and Fox, 2020) This method was improved by Pell and Schofiled (1993, 1995), Schofield (2000) and Williams (2000). Based on pitfalls such as particle size, small sample weight, closed bags which floats, small fermentation flasks we developed the new digestibility method.

## **MATERIAL AND METHODS**

An experiment was performed with 7 different maize silage hybrids FAO 200 – 530 and the sample collection was performed in the interval of 34 days at 4 different terms (12.8.2021, 19.8.2021, 2.9.2021, 13.9.2021). Samples (500 – 750 g) were dried at MEMMERT UFE 500 and UFE 700 with < 60 °C 16 - 24 hours. Dried samples were milled by SM-100 (RETCH) to pass a 2 mm sieve and subsequently by TWISTER (RETSCH) with 1 mm sieve. All nutrients were analysed by NIRS Antaris II FT-NIR Analyzer (Thermo Fisher Scientific) on samples with 1 mm grinding using calibrations from FEEDLAB s.r.o. company. Amount of dry matter was evaluated from laboratory dry matter and dry matter analysed by NIRS method. 1,5 g sample after 2 mm mill sieve was taken to the large (8 x 10 cm) open bags handy made from PET mash with mash-opening 36 µm (PET 1500 140/355-31W). Samples for measuring NDF digestibility had chemically isolated NDF ANKOM NDF Method 13 as principally described Van Soest et al. (1991), NRC 2021 and updated by temporarily sealing bag. After NDF determination, bags were placed into ultrasonic water bath to clean detergent from the samples. After that bags were reopened and prepared for isolated NDF digestibility using IN VITRO Ankom Gas Production system. Digestibility was determined as describe Ankom



Gas Production Operator 's Manual Appendix C with some modifications. Due to larger bags, we used 1000 ml flasks, and every flask contains 2 sample open bags of isolated NDF with 560 ml of buffer and 140 ml of filtered inoculum. Plastic stick was placed into open bags to kept bag open and preventing to blow the bag. Opening of the bag was set up above the inoculum surface. Data collection was set for every 5 minutes with pressure 1,5 psi. Every sample run included blank flask without sample. After incubation time 48 hours, samples were flushed with hot water, resealed and placed into ultrasonic water cleaner. Cleaned samples were dried at 103 °C and weighed. Final % NDF digestibility was determined as weight difference after incubation using gas production kinetics and calculated for every hour till 48-hour point using mathematical methods. Statistical evaluation was performer by NCSS 12 (64 bit) – version 12.0.18 – NCSS LLC with ANOVA method.

# RESULTS AND DISCUSSION

<b>Table 1, 30</b> –	hour NDF digestib	ility					
sample collection	DRY MATTER (g/kg)	200 - 250	250 - 300	300 - 350	350 - 400	400 - 450	Total
1 12.8.2021	Count	3	2	2			7
	Mean	59,80%	54,90%	61,40%			58,80%
	Min	50,60%	53,80%	61,30%			50,60%
	Max	69,50%	56,00%	61,50%			69,50%
2 19.8.2021	Count		5	1	1		7
	Mean		64,00%	66,50%	59,90%		63,70%
	Min		59,40%	66,50%	59,90%		59,40%
	Max		67,20%	66,50%	59,90%		67,20%
3 2.9.2021	Count		4		1	2	7
	Mean		54,50%		61,50%	51,70%	54,70%
	Min		48,40%		61,50%	48,30%	48,30%
	Max		62,30%		61,50%	55,00%	62,30%
4 13.9.2021	Count			1	1	3	5
	Mean			61,80%	64,70%	57,10%	59,90%
	Min			61,80%	64,70%	55,00%	55,00%
	Max			61,80%	64,70%	59,30%	64,70%
average	Count	3	11	4	3	5	26
	NDF DIGESTIBILITY (%)	59,80%	58,50%	62,80%	62,00%	54,90%	59,30%



HYBRID	COUNT	30 h. IV NDF DIGEST. (%)
1	4	58,34%
2	4	60,06%
3	4	59,03%
4	4	60,25%
5	4	55,16%
6	4	63,82%
7	4	58,42%
average	4	59,29%

We found NDF digestibility at 30-hour level with average from 54,90% - 62,80% (Table 1). The highest NDF digestibility was at range 300-350 g/kg dry matter (DM) content -62,80%. We found increasing digestibility till 300-350 g/kg DM and decreasing NDF digestibility with rising DM content. The lowest 30 hours NDF digestibility was 48,30% and the highest was 69,50%. Differences in NDF digestibility on the level of hybrids were not significant with P=0.580 (Table 2 and Chart 1). The average of 30 hours NDF digestibility of all maize silage hybrids was 59,29% with minimum of 55,16% (hybrid 5) and

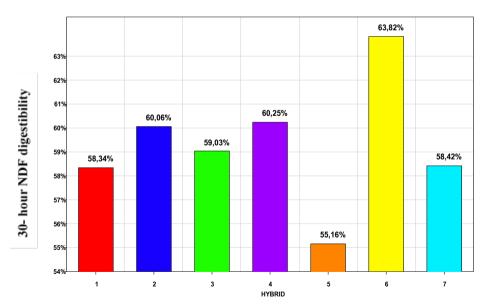


Chart 1, Average 30-hour NDF digestibility from 4 sample collection points



maximum 63,82% (hybrid 6).

These results confirm our hypothesis about silage maturity determination that the best dry matter window for highest NDF digestibility is from 300 - 350 g/kg which correlates with Mitrík T. et al. (2022). NRC 2001 evaluate 30 h. NDF digestibility of maize silage

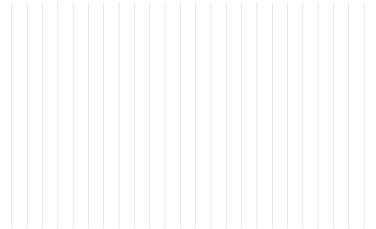


Chart 2 – Average NDF digestibility from 4 sample collection points with DM 27 – 35%

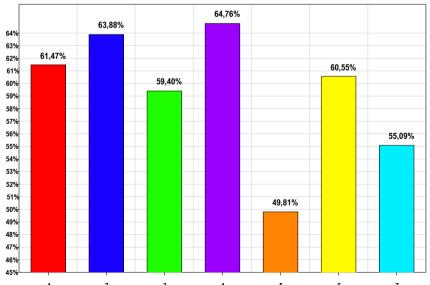


Chart 3 – 30-hour NDF digestibility with DM range 27 – 35%



from 32,5 - 61,2%. Increased maturity of maize is bonded with lowering NDF digestibility (Jensen et al., 2004) and our results support these findings. As describes Mitrík (2023), in this work we choosed silage maturity range from 27% - 35% as range which is the best for ensiling and also with culmination of 30-hour NDF digestibility in that range. In that range we compared average NDF digestibility on the level of hybrids from 0 – 48 hour as show Chart 2. Differences of dynamics of NDF digestibility between hybrids are high.

On the level of hybrid and at 30-hour NDF digestibility point with DM range 27 - 35% we found differences with P = 0.476 (Chart 3) In that DM range we can see 30-hour NDF digestibility from 49.81 - 64.76%. These results confirm that differences between hybrids are not strong, but they are present, and they can vary with different maize silage hybrids.

## **CONCLUSION**

The new updated model of determination proved good and reliable results. New model with grinding on 2 mm sieve, higher sample weight, open bigger bags without floating proved repeatability and solved pitfalls described by Weiss et al. (2020) or NRC 2021. On the other hand, this method is more expensive and more difficult for preparation and need more repetitions. Results showed that differences in 30-hour NDF digestibility on the level of hybrid are not statistically significant, but values have wide range from 55,16–63,82%. On the other hand, we also can see differences of 30-hour NDF digestibility with DM range 27 - 35%. For better evaluation, it is necessary to obtain more data in silage maturity dry matter range 27 - 35%. However, these results also confirm differences between NDF



digestibility of maize silage hybrids and different kinetics of their digestibility.

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