EFFECT OF SORGHUM SILAGE IN DIET OF DAIRY COWS ON EIGHTEEN-CARBON FATTY ACIDS IN MILK FAT

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ABSTRACT

The aim of the study was to evaluate the effect of inclusion of sorghum silage into dairy cow diets on the eighteen-carbon fatty acids (FA) in milk fat. The on-farm experiment was carried out on mid-lactating Czech Fleckvieh cows (Agrospol a.d. Knínice, farm Vanovice) and was divided into two consecutive periods of 3 months each. In the first period cows were fed a total mixed ration (TMR) based on maize silage and grass haylage (Control) while in the second period grass haylage was partially replaced by sorghum silage (Sorghum). In each period samples of evening and morning milk were taken from ten cows and were analysed for basic constituents and FA profile. The basic milk components were analysed in an accredited laboratory (LRM Brno-Tuřany). The FA profiles were analysed using gas chromatography with flame ionization detection. The total content of C18 acids was on average 34.6% of all FAs in Sorghum and 29.4% in Control ($P < 0.05$). Similarly, a higher content of stearic (C18:0), oleic (C18:1n9c), linoleic (C18:2n6c) and α-linolenic (C18:3n3) acid was

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found in Sorghum compared to Control ($P < 0.05$). Content of linolelaidic acid (C18:2n6t) was not affected by the treatment ($P > 0.05$). The inclusion of sorghum silage into the ration had a positive effect on the content of polyunsaturated FAs and n-3 FAs ($P < 0.05$) and tended to increase n-6 FAs ($P = 0.064$).

Keywords: α-linolenic acid; linoleic acid; n-3 fatty acids; n-6 fatty acids; saturated fatty acids; unsaturated fatty acids

INTRODUCTION

Milk and dairy products are very important sources of nutrients in the human diet providing energy, high-quality proteins, essential minerals and vitamins (Lock and Bauman, 2004). The primary energy source in milk is fat, which is also a key component contributing to its technological properties. It determines the physical and sensory characteristics and organoleptic properties of dairy products (Lock and Bauman, 2004).

A considerable part of the human population relies on milk as an important source of fat. Milk fat contains various different fatty acids (FAs). From a nutritional standpoint, it is beneficial to reduce the proportion of saturated FAs (SFAs) while increasing the proportion of unsaturated FAs, with a special emphasis on polyunsaturated FAs (PUFAs) in milk (Hanuš et al., 2018). PUFAs, particularly n-3 and n-6 are essential FAs acknowledged to exert pronounced beneficial effects on human health (Angulo et al., 2012). Additionally, monounsaturated FAs (MUFAs) have numerous biological functions that provide health benefits when their intake is increased, particularly when they replace common SFAs in the diet (Calder, 2015). Oleic acid (C18:1n9c) and α-linolenic acid (C18:3n3) exhibit anti-cancer and anti-atherogenic properties, have positive effect on cholesterol level and improve immune response, linoleic acid (C18:2n6c) and linolelaidic acid (C18:2n6t) improve insulin sensitivity and can therefore help prevent type 2 diabetes (Hanuš et al., 2018).

Dewhurst et al. (2006) recommended that total fat in human diet should contribute 15-30%, SFAs less than 10%, n−6 PUFAs less than 5-8%, n−3 PUFAs less than 1-2% and trans FAs should contribute less than 1% of total energy intake. Samková et al. (2014) outlined that in bovine milk fat, the typical proportions of total FAs are 70-75% SFAs, 20-25% MUFAs, and around 5% PUFAs. The concentration of fat in milk is affected by numerous factors. The most significant are genotype, age, health, stage and number of lactations. Crucially, the concentration of fat in milk and the FA profile of milk can be modified by the dairy cow diet as up to 44% of milk fat can originate from it (Hanuš et al. 2018; Shingfield and Griinari 2007). Despite the extensive metabolism of dietary unsaturated FAs to stearic acid (C18:0), in vitro and in vivo studies have demonstrated that a variety of intermediates, including C18:1, C18:2 and C18:3 are formed during biohydrogenation. The specific profile of these biohydrogenation intermediates produced in the rumen is influenced by the composition of the diet (Shingfield & Griinari, 2007).

Sorghum, a globally significant crop, is, unlike maize, well-adapted to diverse agronomic and environmental conditions, particularly in regions with low rainfall or limited irrigation water. Forage sorghum can produce yields comparable to maize, indicating its potential as a substitute in areas with constrained water supplies. However, there is a trade-off, as maize silage, due to its high grain content, typically offers

superior digestible energy content compared to sorghum forages (Getachew et al., 2016).
Khosravi et al. (2018) observed that substituting maize silage with sorghum silage did not affect milk production, feed efficiency, or the concentrations of milk fat, protein, lactose, and solids-not-fat. However, cows fed the maize silage diet produced higher yields of milk fat, protein, and lactose compared to those fed the sorghum silage diet. Furthermore, cows fed the sorghum silage had a higher percentage of PUFAs in fat compared to the cows fed maize silage.
Research by Cattani et al. (2017) demonstrated that total replacement of maize silage with sorghum silage reduced milk yield, increased the concentration of milk fat and lowered the percentage of PUFAs, but did not negatively influence milk coagulation properties and maintained milk composition. Thus, adding sorghum silage to daily ration can be done without a negative effect on animal performance.
The objective of this research was to assess the concentration of eighteen-carbon FAs and specific FA groups in the milk fat of Czech Fleckvieh cows, in relation to the inclusion of sorghum silage into their diet.

MATERIAL AND METHODS

Design of experiment, animals and feeding

The on-farm feeding experiment was carried out on a group of 120 mid-lactating Czech Fleckvieh cows (Agrospol a.d. Knínice, farm Vanovice) from which ten representative cows were selected for detailed study of milk composition. The trial was divided into two consecutive periods of 3 months each. Each period consisted of an adaptation period (3 weeks) followed by a collection period (60-70 days) in which feed intake and milk yield was monitored and samples of milk were taken three times in monthly interval.

Cows were fed a total mixed ration once daily; during the day the feed was pushed regularly six times per day using an automatic feed pusher. In the first period the TMR diet was based on maize silage, grass haylage and a commercial supplemental feeding mixture (Control) while in the second period grass haylage was partially replaced by sorghum silage (Sorghum). Composition of diets is given in Tables 1 and 2. During the collection period the amount of feed offered as well as the amount of feed refusals was recorded on 3 consecutive days on the same term as milk sampling. Cows were milked twice daily (at 4am and 3pm) and milk yield was recorded.

Table 1. Composition of feed rations (in kg/d, on as fed basis)

Component (kg/d, as fed)	Control	Sorghum
maize silage	20	21.5
grass haylage	10.5	5
sorghum silage	-	5
sugar beet pulp	7	7
molasses	1.2	1.2
supplemental mixture	9.8	9.7
rapeseed meal	0.85	1
sum of diets	49.35	50.4

Table 2. Composition of supplemental feeding mixture (%)

Component	Content (%)
wheat	29.5
barley	12.5
maize	19
soybean meal	17.5
rapeseed cake	14
SM Production U[1]	3.5
limestone	1.5
salt	0.5
sodium bicarbonate	2

[1]SM Production U – vitamin and mineral mixture

Collection and analyses of samples

Samples of feed and feed refusals were taken on 3 consecutive days and were analysed for dry matter content.

Samples of evening and morning milk were taken from ten cows once per month during the regular milk recording. Samples for analysis of basic milk constituents were cooled (6 °C) while samples for FA analysis were kept frozen (-20 °C) until analysis.

The basic milk components (fat, protein, casein, lactose, urea, somatic cell count) were analysed in an accredited laboratory (LRM Brno-Tuřany).

Prior FA analysis evening and morning milk was pooled into one sample relative to milk yield. Milk fat was extracted using a modified Folch et al. (1957) extraction procedure. A mixture of chloroform and methanol was used as a solvent, and to wash out non-lipid components salt solution was used (Eggers and Schwudke, 2016). Transesterification of triglycerides in extracted fat to FA methyl esters (FAMEs) was done by adding hexane and 1.5M methanolic sodium hydroxide solution in a volumetric ratio of 100:3. FAMEs were analysed by gas chromatography with flame ionization detection using GC Agilent 8860 (Agilent, United States) under optimised conditions (oven program: 40 °C for 1 min to 150 °C @ 25 °C/min to 240 °C @ 2 °C/min, detector: FID @ 250 °C) using the ZB-FAME column (Phenomenex, United States) with dimensions 30m x 0.25mm x 0.20µm. The identification of FAs was carried out using the analytical standards (Restek, United States). In total, 37 FAs were observed, out of which 30 were identified. The FA profile was determined by calculating the ratio of each FA's peak area to the total peak area of all detected FAs.

Statistical evaluation of data

Statistical analysis was performed using Microsoft Excel (Microsoft) and Statistica software (TIBCO Software Inc.). For testing of significance between the groups (Control; Sorghum) T-test for individual FAs and Mann-Whitney U test for specific FA groups were used.

RESULTS AND DISCUSSION

The results presented here are preliminary as the entire experiment has not yet been evaluated and include results from one sampling term in each experimental group (n=10).

The effect of inclusion of sorghum silage into the diet of mid-lactating dairy cows on dry matter intake (DMI) and milk performance is shown in Table 3. The DMI in Sorghum was higher compared to Control ($P < 0.05$). This is in contrast with Cattani et al. (2017) and Khosravi et al. (2018) that found no differences in DMI between cows fed maize silage- or sorghum silage-based diets. On the other hand, Yang et al. (2019) documented significantly lowered DMI when feeding sorghum silage-based diet. However, it should be noted that in the above-mentioned studies, the sorghum silage completely replaced the maize silage while in our study there was only partial replacement of grass silage. Inclusion of 5 kg of sorghum silage at the expense of grass silage could improve palatability of the diet and positively influence DMI. Further, the contrast between results from literature and our findings can be attributed to the variations in diet composition and use of a different breed.

In our study milk yield and composition was not influenced by the diet ($P > 0.05$) except for the content of urea that was higher in Sorghum compared to Control (Table 3, $P < 0.01$). Some authors (Cattani et al., 2017; Yang et al., 2019), in contrast to our results, reported, that sorghum silage in daily ration, lowered milk yield. However, as mentioned above, in these studies sorghum silage was used as a complete replacement of maize silage. Thus, it can be expected that low amount of sorghum silage in the diet will have a negligible effect on milk yield. In terms of milk components, our study is in agreement with Khosravi et al. (2018), Yang et al. (2019) or Cattani et al. (2017) that also observed no significant differences in the percentage of fat, protein, lactose, somatic cell count or urea concentration when feeding sorghum silage, respectively. Although the content of urea in milk was higher in Sorghum group, still the value was within the physiological range (15 – 30 mg/100 ml, Zadražil, 2002). Except for dietary factors (Roseler et al., 1993) milk urea concentration can be influenced by production and environmental factors such as herd, parity, stage of lactation, individuality of animals, milk yield, time of milking etc. (e.g.

Jílek et al., 2006; Dhali et al., 2005). Thus, the effect of sorghum silage on the milk urea in our study is not unambiguous.

Table 3. Milk yield and composition from cows fed two diets

Item	Control[1]	Sorghum[2]	P
Days in milk	237±69	226±52.5	0.696
Dry matter intake (kg/d)	21.5±0.7	23.98±0.25	0.004
Milk yield (kg/d)	22.8±5.3	23±6.1	0.942
Fat (%)	4.32±0.92	4.2±0.55	0.728
Protein (%)	4.03±0.37	4±0.18	0.822
Casein (%)	3.02±0.25	3.11±0.16	0.354
Lactose (%)	4.62±0.29	4.78±0.18	0.168
Urea (mg/100mL)	15.1±3.66	22.12±3.81	0.001
Somatic cell count (1000/mL)	205.9±136.3	313.1±301.1	0.324

[1] diet based on maize silage and grass haylage; [2] diet based on maize silage and grass haylage that was partially replaced by sorghum silage

Changes in the concentration of observed C18 FAs and specific FA groups are shown in Table 4. Concentrations of C18:0, C18:1n9c, C18:2n6c and C18:3n3 were higher in Sorghum in comparison with Control ($P < 0.05$). Concentration of C18:2n6t did not differ between the two diets ($P > 0.05$). In contrast to this, Khosravi et al. (2018) observed no concentration shifts between cows fed maize diet and cows fed sorghum diet for C18:0 and C18:1n9c. Nevertheless, in accordance with our results, Yang et al. (2019) showed that milk from cows that received sorghum silage contained higher levels of C18:0, C18:1n9c, C18:2n6c and C18:3n3 compared to cows fed maize silage. The same results are reported in research by Cattani et al. (2017) for C18:0 levels.

Table 4. Levels of identified C18 FAs and specific FA groups (% of

total FA) in milk fat of lactating Czech Fleckvieh cows as related to inclusion of sorghum silage into feeding ration

FA	Control[1]		Sorghum[2]		P
	mean	SD	mean	SD	
C18:0	6.55	0.44	8.36	1.34	0.002
C18:1n9c	19.80	1.97	22.71	3.30	0.028
C18:2n6t	0.34	0.05	0.33	0.11	0.870
C18:2n6c	2.33	0.26	2.70	0.37	0.018
C18:3n3	0.41	0.04	0.49	0.08	0.010
SFA	72.65	2.26	69.73	3.59	0.076
MUFA	23.77	2.12	26.20	3.38	0.104
PUFA	3.22	0.33	3.70	0.48	0.021

SD – standard deviation; [1] diet based on maize silage and grass haylage; [2] diet based on maize silage and grass haylage that was partially replaced by sorghum silage

It is important to note that these studies involved different diet compositions and a complete substitution of maize silage with sorghum silage, which contrasts with the methodology employed in our research. Incorporating sorghum silage into the cows' diet can also influence the proportion of SFAs, MUFAs and PUFAs. The concentration of SFAs tended to lower with the inclusion of sorghum silage into the cow's diet ($P = 0.076$). PUFAs concentration has raised for Sorghum in comparison with Control ($P < 0.05$). This can be explained by sorghum affecting rumen fermentation, potentially leading to incomplete biohydrogenation of unsaturated FAs. These changes in concentrations of SFAs and PUFAs are very important and positive. PUFAs, as mentioned previously, play a crucial role in maintaining various bodily functions and have been associated with numerous health benefits, making them an important component of a healthy diet and a marker of high-quality food products.

Feeding with sorghum silage naturally affected also the total amount of n-3 PUFAs and n-6 PUFAs as showed in Figure 1. The concentration of n-3 PUFAs was higher in Sorghum than in Control representing 17.97% and 17.16% of all PUFAs, respectively ($P<0.05$). The inclusion of sorghum silage into the diet also tended to increase levels of n-6 PUFAs ($P=0.064$). This contrasts with the findings of Cattani et al. (2017) who reported a decline in both, n-6 PUFAs and n-3 PUFAs concentrations in milk after changing from maize silage to sorghum silage. As mentioned earlier, direct comparison with literature is not possible owing to the fact that the majority of research focuses on the total replacement of maize silage by sorghum silage, while our research replaced 5 kg of grass silage with the same amount of sorghum silage. It is noteworthy that the cow breed used in research by Cattani et al. (2017) was Holstein-Friesian dairy cows, which can be another explanation for the differences in the results as cow breed also affects the concentrations of n-3 and n-6 PUFAs (Samková et al., 2014). Moreover, higher levels of n-3 and n-6 PUFAs found in sorghum silage compared to grass haylage (Maggioni et al. 2009) could also partly explain differences between our results and those in literature.

According to Farková et al. (2024) n-6 PUFA intake should be between 5 to 20% of the total energy intake to reduce the risk of chronic diseases, lower blood low-density lipoprotein (LDL)-cholesterol levels and decrease the risk of coronary heart disease. Furthermore, n-3 PUFAs have anti-inflammatory and antioxidant properties (Liput et al., 2021). For these reasons, higher levels of n-3 and n-6 PUFAs after the partial inclusion of sorghum silage instead of grass silage are perceived positively.

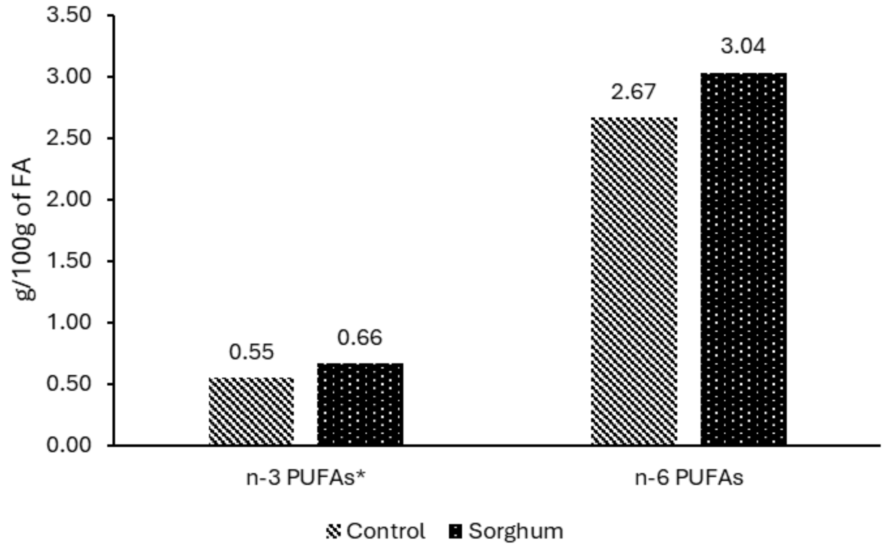

Figure 1. Proportion of n-3 PUFAs[1] and n-6 PUFAs[2] as related to addition of sorghum into feeding ration (Control[3], Sorghum[4]) in milk fat of Czech Fleckvieh cows.

*[1]n-3 PUFAs – sum of C18:3n3 and C20:3n3; [2]n-6 PUFAs – sum of C18:2n6t and C18:2n6c; [3] diet based on maize silage and grass haylage; [4] diet based on maize silage and grass haylage that was partially replaced by sorghum silage; * means statistical significance (P < 0.05)*

The total proportion of all C18 FAs was higher in Sorghum compared to Control (Figure 2, $P < 0.05$). Fat yield was not influenced by the diet ($P > 0.05$). In terms of fat yield, our observations contradict those of Yang et al. (2019) who reported a significant increase of fat yield in milk from cows fed sorghum silage than from those fed maize silage and Khosravi et al. (2018) who observed a decrease in fat yield after changing from maize silage to sorghum silage. The differences in findings can be explained by the interaction of the extent of the change of diet (total vs. partial replacement), the genetic predisposition of different cow breeds, and other dietary and environmental conditions (Adediran et al., 2010; Hanuš et al., 2018; Sinclair et al., 2015).

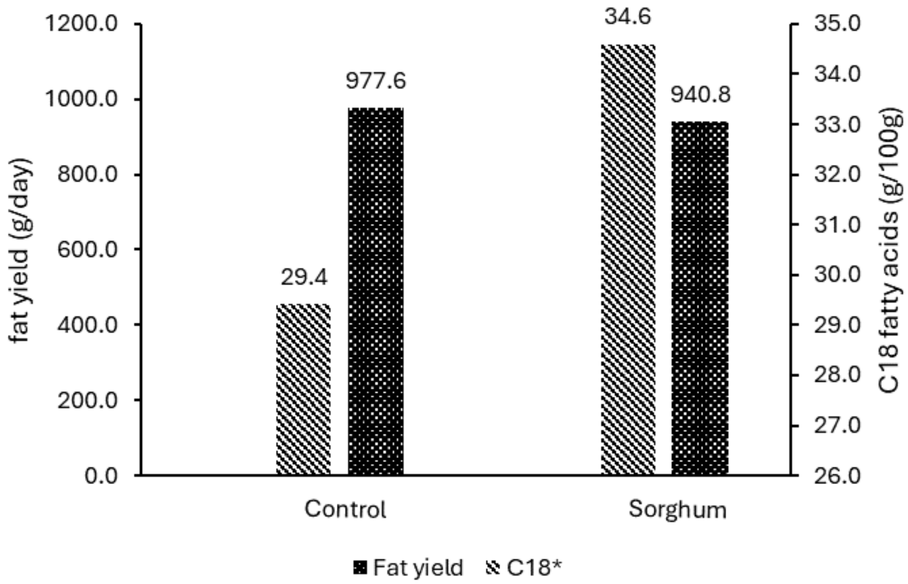

Figure 2. Fat yield[1] (g/day) and total C18[2] FAs content (g/100 g of total FA) comparison related to addition of sorghum silage into feeding ration (Control[3], Sorghum[4])

*[1]Fat yield = fat content (g.kg-1) x milk yield (kg.d^{-1}); [2]C18 – sum of C18:0, C18:1n9c, C18:2n6c, C18:2n6t and C18:3n3; [3] diet based on maize silage and grass haylage; [4] diet based on maize silage and grass haylage that was partially replaced by sorghum silage; * means statistical significance ($P < 0.05$)*

CONCLUSION

Under the above-described conditions, feeding a sorghum silage as a partial replacement of grass silage in diets of mid-lactating dairy cows positively influenced dry matter intake and maintained milk yield and content of basic milk constituents. This partial substitution of grass silage with sorghum silage resulted in positive changes in C18 fatty acids, namely in increased concentrations of stearic (C18:0), oleic

(C18:1n9c), linoleic (C18:2n6c) and α-linolenic (C18:3n3) acids and a sum of C18 ($P < 0.05$). In addition, content of n-3 fatty acids and polyunsaturated fatty acids was higher ($P < 0.05$), and content of n-6 fatty acids tended to be higher ($P = 0.064$) in Sorghum group compared to Control. These preliminary results suggest that sorghum silages could have a potential as substitute of grass silages in dairy cow diets.

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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%.

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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 (Mitrík 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 andcalculated 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

Table 1, 30 – hour NDF digestibility

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%**

Table 2, Average 30-hour NDF digestibility		
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

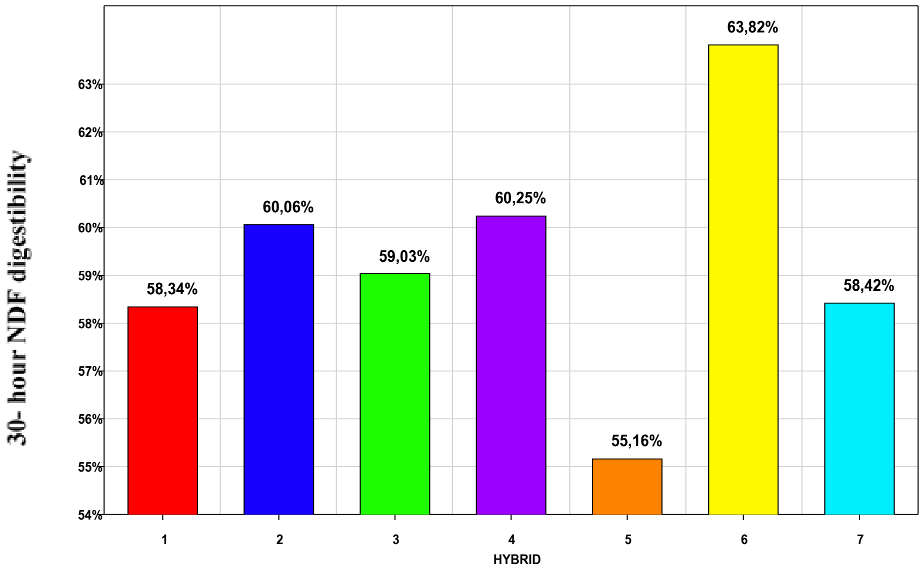

Chart 1, Average 30-hour NDF digestibility from 4 sample collection points maximum 63,82% (hybrid 6).

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%

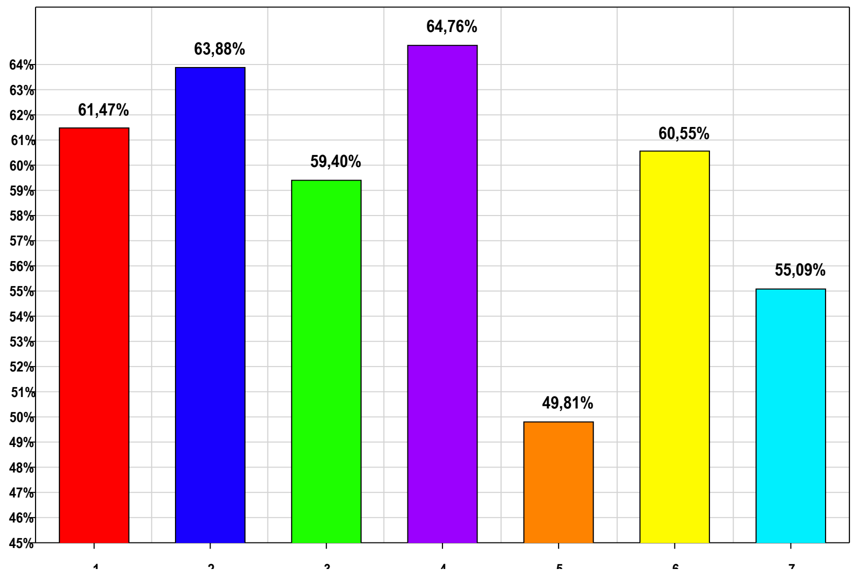

Chart 3 – 30-hour NDF digestibility with DM range 27 – 35%

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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