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Variation for *In Vivo* Digestibility in Two Maize Hybrid Silages

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ABSTRACT

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The experiment was aimed at confirming that silages made of two very similar hybrids could have different *in vivo* digestibility, primarily amylase-treated neutral detergent fibre digestibility (aNDFD), which could strongly influence the result of the calculation of energy value of fodder. Both the stay-green whole-plant types were grown at the same locality during two years and harvested at the same days at two-thirds milk line maturity. In the two subsequent years, silages without preservatives were made of both hybrids tested. All silages were fermented for 90 days. The *in vivo* digestibility of silages was measured in digestion trials with six sheep. All silages had good fermentation quality, and no differences in that regard were found between hybrids or years ($P > 0.05$). Hybrid had stronger effect than year on all indicators of chemical composition and digestibility of nutrients other than dry matter (DM). Hybrid significantly affected all indicators measuring chemical composition and digestibility of nutrients other than DM ($P = 0.18$). The aNDFD was closely correlated with all other measures of nutrients digestibility ($P < 0.01$). The results confirm the importance of breeding hybrids and analyzing silages for aNDFD.

Keywords: ruminant; nutritive value; neutral detergent fibre; fermentation; correlation

Maize (*Zea mays* L.) is considered to have consistent nutritional qualities and a high energy value while being relatively easy to grow and conserve (Carpentier and Cabon 2011). There nevertheless are great differences among hybrids. Hybrids often vary in amylase-treated neutral detergent fibre digestibility (aNDFD), and it is evident that aNDFD is a very important parameter of forage quality. Improving the digestibility of forage maize through plant breeding is important for optimizing the efficiency of ruminant rations, which can be achieved in part by improving the digestibility of stem tissue, a genetically complex and diverse trait that changes during the growing season (Boon et al. 2012).

Enhancing aNDFD in silage significantly increases dry matter (DM) intake (DMI) and milk yield. According to Oba and Allen (1999), a one-unit increase in NDFD *in vitro* or *in situ* is associated with a 0.17 kg increase in DMI, a 0.25 kg increase

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in 4% fat-corrected milk, and a live weight gain (LWG) improvement of 27 g/day. As Oba and Allen (1999) acquired the results "across forages", their conclusions apply in general.

Oba and Allen (1999) studied several forage types, however, they did not focus on maize silage. The objective of a later study by Kramer-Schmid et al. (2016) was therefore to investigate the importance of maize silage aNDFD for DMI, milk production, and LWG. Kramer-Schmid et al. (2016) conducted a literature review across a wide range of studies varying in the ration composition and characteristics of maize silage. The data set compiled for the study comprised 29 experiments with 96 dietary treatments. Enhanced aNDFD was associated with lower starch concentration and higher crude protein (CP) concentration. Nevertheless, the digestibility of DM (DMD) and organic matter (OMD) increased with enhanced aNDFD. Milk yield and LWG also were greater with enhanced aNDFD. A one-unit increase in aNDFD improved daily milk yield by 82 g (P = 0.04) and daily LWG by 12 g (P = 0.03). Their conclusion, therefore, was that aNDFD is an important trait in maize used to produce whole-crop silage for dairy cows and the importance of aNDF and aNDFD has substantially increased.

Numerous studies have evaluated the impact of maize hybrid on chemical composition and digestibility of silage (Andrae et al. 2001; Thomas et al. 2001; Jensen et al. 2005; Filya and Sucu 2010; Hetta et al. 2012). The combined effects of weather on maize chemical composition were considered by Kruse et al. (2008) or Lynch et al. (2012), while Darby and Lauer (2002) investigated its effects on chemical composition development as crop maturity progressed through to harvest time.

The *in vivo* experiment was chosen because of the fact that the data from the variety tests made in the Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ), Brno, Czech Republic, cannot be fully used, as they determine the digestibility under use of spectroscopy in near infrared distance (NIR), based on calibration of the device from *in vitro* or *in situ* experiments after a 48-hour incubation of the samples. In that manner, more accurate, but higher values are achieved. It can be said that the potentially highest performance of the hybrid, not the real one, is assessed, as the fodder usually passes through the dairy cows' rumen within 24 hours, i.e. within half of the time than that calibrated at NIR in ÚKZÚZ. When the aNDFD is measured *in vivo* under production with cows it also includes the combined effect of the ration ingredients and the feed intake of the animals. Balance experiments made with sheep are much less costly than those made with dairy cows. So, those were the main reasons for us to choose the *in vivo* method in sheep to compare the digestibility of two hybrid silages.

The reason for having chosen the methodical concept applied in our study was primarily the comparison from feed specialist perspective, not a comparison from the perspective of breeding of hybrids. We wanted to emphasize that two very similar hybrids could have different digestibility of nutrients, primarily of fibre, which can substantially change the result of calculation of the energy value of fodder. The essential thing is that the feeding practitioners needed to know whether they could use table values (Zeman et al. 1995).

The hypothesis was that the aNDFD effect of the hybrid LGAN250S is greater than that of the hybrid Ronaldinio and the aNDFD correlates with the digestibility of other nutrients. The main objective of our two-year study was to confirm that silages made of two very similar hybrids could have different *in vivo* digestibility, especially in terms of aNDFD in silage, which could strongly influence the result of the calculation of the energy value of the fodder.

MATERIAL AND METHODS

Silages from two maize hybrids were made two years in a row. The first tested hybrid (H1) Ronaldinio (KWS OSIVA CZ), which has been registered in the varieties database of the Czech Republic's Central Institute for Supervising and Testing in Agriculture already since July 5, 2007, excels in terms of its certainty of yield and quality, high performance, and stress tolerance. It is grown and used in many locations and under widely ranging conditions. On the other hand, Ronaldinio's breeder has accepted a medium measure of aNDFD. The second hybrid (H2), LGAN250S (Limagrain Central Europe S.E.), was a newly bred cultivar whose great advantage according to its breeder is high aNDFD.

Both hybrids are early maturing (FAO 250 for silage) stay-green varieties (bred using molecular marker-assisted selection for delayed leaf

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senescence). Both are flint × dent hybrids, three-way (Tc), and excel in their resistance to fungal diseases.

Hybrids were grown on the farm Nový Dvůr located at the village of Červenka (Olomouc Region, Czech Republic) situated at approximately 230 m a.s.l. Both hybrids were planted in the same field and harvested by a cutter Claas Jaguar 690 with a corncracker (Claas, Germany) and chopped to a theoretical length of cut (TLC) of 12 mm when grain was at 2/3 milk line. No biological or chemical additives were used in the ensiling process. The silages were stored in large plastic containers each with a volume of 1.2 m^3 and sealed with a 250 μm thick black and white foil. The methodology of filling, compressing, and sealing was the same for all variants.

After complete fermentation (90 days), the silages were opened and used for an *in vivo* experiment. Three samples of approximately 2 kg each, taken from each container of the final silages, were used for chemical analyses. Fresh silage samples were analyzed for fermentation quality (pH plus concentrations of lactic, acetic, and propionic acids) using an IONOSEP 2001 analyzer (RECMAN – laboratorní technika s.r.o., Czech Republic). DM was obtained by drying chopped fresh material and correcting for volatile components. The dried material was subsequently milled to pass through a 1-mm sieve for laboratory analyses. Dry matter (DM; method No. 934.01), raw ash (method No. 942.05), crude protein (CP; method No. 976.05), and starch (method No. 920.40) were determined as described by AOAC (2005). The acid detergent fibre (ADF) and the amylase-treated neutral detergent fibre (aNDF) were determined according to the procedures of Van Soest et al. (1991), later according to Mertens (2002). The aNDF was determined using a Fibertec™ 2010 analysis system (FOSS Tecator AB, Denmark), under the use of Alpha-Amylase FAA (ANKOM Technology, USA) with 17 400 LU/g activity. Organic matter (OM) was calculated as DM minus ash. Four *in vivo* digestion trials were performed with four silages made from two maize hybrids. Six sheep (wethers of the Romanov breed, live weight 83 ± 9 kg) were used in each trial. The sheep were kept individually in adaptation boxes and subsequently in balance cages and fed at the rate of 1.2 kg DM/animal/day. Prior to the first trial, the animals were fed maize silage for three weeks. The first trial was conducted with a two-week adaptation period (animals were provided experimental silages), followed by a six-day collection period. During the collection period, the fodder intake and amounts of residual feedstuff and faeces were measured on a daily basis. The daily feed ration was offered twice daily in equal portions at 8.00 h and 18.00 h. Silages were offered to the sheep at a body-maintenance feeding level. The animals had free access to drinking water.

Samples of silages, residuals, and faeces were dried and analyzed for DM, ash, CP, aNDF, and ADF as described above. The *in vivo* digestibility coefficients were calculated as follows:

Nutrient digestibility (%) = ((Nutrient consumed (g/day) – Nutrient excreted in faeces (g/day))/(Nutrient consumed (g/day)) × 100

Statistical values were processed using the software package STATISTICA 10 (StatSoft, USA) while utilizing the methods for calculating ANOVA and the modules for analyzing multi-factorial designs and repeated measures designs. The mean differences were separated using Tukey's range test. In these analyses, *P*-values lower than 0.05 were regarded as indicating significance. The correlation coefficients among the variables were calculated using PROC CORR, and multiple regression analyses of the determined variables were computed using the GLM standard procedure.

RESULTS AND DISCUSSION

Chemical composition, fermentation quality, and nutrients digestibility of silages are presented in Table 1.

Our objective was to harvest both hybrids for silage at the 2/3 milk line stage considered optimal for silage-making and which is recommended, for example, by Carpentier and Cabon (2011) and Peyrat et al. (2014). So, we found no differences in DM between hybrids ($P = 0.183$). This distinguishes our approach from others. Most similar research compares hybrids harvested at different DM levels, thereby reflecting different stages of maturity (e.g. Di Marco et al. 2002; Cherney et al. 2004; Cone et al. 2008; Boon et al. 2012; Hetta et al. 2012; Rabelo et al. 2015). Advancing maturity decreases starch, aNDF, and DMD (Jensen et al. 2005). Along with the change in DM, there are changes also in the other indicators of silage's nutritional value. In our study, all measures of

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chemical composition differed between hybrids ($P < 0.05$). Differences between years were not confirmed only in relation to aNDF and starch ($P = 0.083$ and $P = 0.328$, respectively).

The fermentation quality can be characterized by average values of 3.70 ± 0.15 pH, lactic acid (LA) at 71.2 ± 9.75 g/kg DM, and ratio of LA to volatile fatty acids (VFA) at 3.31 ± 0.39. These values indicate good fermentation. The good fermentation quality for all silages reflects the very careful attention we gave to producing the experimental silages. In our silages, no butyric acid was detected. Good quality of fermentation is a major prerequisite for properly evaluating silages. No differences between hybrids or years were found for the silages according to any indicator of fermentation quality. Standard deviations of means were not very large for any parameter.

Our results relating to chemical composition and digestibility of nutrients are comparable to those of Barriere et al. (2004), who tested silages from 478 different maize hybrids. Barriere et al. (2004) in *in vivo* experiments with sheep extending over 34 years showed that the NDFD of silages made using different maize hybrids in the FAO 170–350 range varied from 35.9 to 60.4%.

Our results were also very similar in compare with those of Ferret et al. (1997). They found aNDF of 11 maize silages ranging from 359 to 542 g/kg DM and aNDFD from 34 to 61%.

Digestibility of aNDF of whole maize crop is dependent mainly on the cell wall content. Ivan et al. (2005) showed that feeding silage from maize hybrids with a high cell wall content and high aNDFD resulted in higher DMI and milk yield compared to feeding a hybrid with a lower cell wall content and low aNDFD. Similarly, Boon et al. (2012) analyzed changes during the growing season in the anatomy, chemical composition, and fermentation characteristics of maize stem from a lower internode (internode 7) of two forage maize cultivars differing in whole plant digestibility. The less digestible cultivar had a higher final cell wall thickness than did the cultivar with better digestibility.

For the diverse studies covered in the review by Kramer-Schmid et al. (2016), various methods (*in vivo*, *in situ*, *in vitro*) were used to determine digestibility. Each of these methods has its own advantages and disadvantages. The differences in aNDFD between treatments are greater when measured *in vitro* or *in situ* than when measured

Table 1. Effect of hybrid (H) and year (Y) on chemical composition, fermentation quality, and digestibility of silages

H, Y indicator	H1	H2	Y1	Y2	SEM	*P*-value		
						H	Y	H × Y
DM (g/kg)	327	321	318	331	3.06	0.183	0.008	< 0.001
CP (g/kg DM)	84	90	89	86	0.75	< 0.001	0.023	< 0.001
Ash (g/kg DM)	47	52	47	51	0.58	< 0.001	< 0.001	< 0.001
aNDF (g/kg DM)	460	488	474	475	3.63	< 0.001	0.083	0.004
ADF (g/kg DM)	274	252	270	256	2.51	< 0.001	0.001	0.272
Starch (g/kg DM)	292	298	305	309	5.31	0.031	0.328	0.781
LA (g/kg DM)	72.2	70.8	73.8	69.2	1.66	0.551	0.064	0.003
AA (g/kg DM)	20.1	21.3	21.9	19.5	1.16	0.462	0.168	0.309
LA/VFA	3.50	3.29	3.32	3.47	0.16	0.358	0.508	0.514
pH	3.74	3.77	3.71	3.80	0.03	0.493	0.058	0.520
DMD (%)	67.7	72.0	70.4	69.3	0.72	< 0.001	0.295	0.034
CPD (%)	56.1	61.8	59.7	58.2	1.14	0.002	0.368	0.197
OMD (%)	69.4	73.9	72.2	71.1	0.77	0.001	0.309	0.031
aNDFD (%)	53.4	64.7	61.3	56.8	1.48	< 0.001	0.044	0.053
ADFD (%)	52.2	59.0	60.2	51.0	1.35	0.002	< 0.001	0.001

AA = acetic acid, ADF = acid detergent fibre, ADFD = ADF digestibility, aNDF = amylase-treated neutral detergent fibre, aNDFD = aNDF digestibility, CP = crude protein, CPD = CP digestibility, DM = dry matter, DMD = DM digestibility, LA = lactic acid, OMD = organic matter digestibility, VFA = volatile fatty acids

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in vivo. *In vivo* showed the best results in digestion trials (Di Marco et al. 2002).

Digestibility of ruminant feeds is often determined using sheep rather than cattle as the experimental unit. This reflects the smaller quantities of feed required and the convenience associated with using the smaller species. Opinions differ as to whether or not the digestibility values obtained with sheep should be applied to cattle – as they often are – with the assumption that sheep and cattle have equal digestive abilities (Woods et al. 1999).

Lopes et al. (2009) draw attention to the fact that feed evaluation systems are not internationally standardized, and prediction equations for assessing energy content in the same type of forage often differ dramatically even between neighbouring countries.

For example, in France the UFL (energy units for production of milk) and the DINAG (pepsin-cellulase DMD minus starch and water-soluble sugars) are used to predict feeding value of silage maize hybrids, whereas in Germany the ELOS (enzymatic OMD) is used. The Central Institute for Supervising and Testing in Agriculture, Czech Republic (ÚKZÚZ) stated the following DINAG values for the Ronaldinio hybrid for the years 2010 to 2015: average 48.1%, min. 46.6%, max. 51.3%; the aNDFD in our experiment was 55.0% in the first year and 51.9% in the second year. These are great differences. The resulting values are often used for the calibration of NIR (Montes et al. 2007). There are also differences in the length of incubation. While the incubation of 24 or 30 h is used for the purposes of livestock husbandry, this period is typically 48 h for agronomic purposes. Although the incubation for 48 h is in a sense more accurate, its results say more about the potential capabilities of the hybrid than about the actual feeding value.

In the Feed Catalogue published in the Czech Republic by Zeman et al. (1995), the highest recommended DM content of silage maize is 31%. The values of digestibility from this catalogue have still been used to calculate the energy value of fodder in the Czech Republic. Since then, the hybrids have changed; maize is usually harvested with a higher DM, and the ensilaging technology has changed as well. It is therefore very important for practice to determine the values of digestibility, especially of fibre, instead of copying them from tables, as there is only one table value for all silages.

The CPD and aNDFD values measured in ensilages of the LGAN250S hybrid were higher ($P < 0.001$) than those of the Ronaldinio hybrid. As compared to the table values (Zeman et al. 1995), where the ensilaged maize (harvested with 31% dry matter) shows the digestibility of CP at 57.5% and the digestibility of raw fibre at 63.8%, the CPD and aNDFD values of the LGAN250S hybrid were higher, while those of the Ronaldinio hybrid were lower.

In reality, digestibility may strongly differ from the table values, the values measured by a NIR spectroscopy apparatus calibrated for 48 h, measured *in vitro* or *in situ* with incubation 24 h or 30 h, or analyzed in the experiment *in vivo* with animals. The *in vivo* analysis is the closest to real values.

The correlation coefficients among digestibility values are presented in Table 2. The aNDFD was closely correlated with all the other nutrient digestibility measures ($P < 0.01$). An increase in the digestibility of some nutrients was accompanied by an increase in the digestibility also of other nutrients. Kramer-Schmid et al. (2016) reported a similar conclusion in their review. They reported, for example, that a 1% increase in aNDFD was accompanied by a 0.3% increase in DMD ($P < 0.001$) and a 0.2% gain in OMD ($P = 0.003$). Digestibilities of starch and CP were not related to aNDFD. Correlation coefficients among nutrients and digestibility values are presented in Table 3. P-values lower than 0.01 were found only for DM × ADF digestibility (ADFD), CP × CPD, CP × aNDFD, and aNDF × CPD. The correlation coefficient between aNDF and aNDFD was only 0.46, with $P < 0.05$. The concentration of aNDF in maize silage and aNDFD tended to be positively correlated. Within the same maize genotype, a decreasing aNDF con-

Table 2. Correlation coefficients relating digestibility values ($P < 0.01$)

Indicator	CPD	OMD	aNDFD	ADFD
DMD	0.63	0.99	0.91	0.81
CPD		0.65	0.79	0.48
OMD			0.92	0.82
aNDFD				0.80

ADFD = acid detergent fibre digestibility, aNDFD = amylase-treated neutral detergent fibre digestibility, CPD = crude protein digestibility, DMD = dry matter digestibility, OMD = organic matter digestibility

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Table 3. Correlation coefficients relating chemical composition and digestibility values

Indicator	DMD	CPD	OMD	aNDFD	ADFD
DM	–0.24	0.04	–0.25	–0.20	–0.52[A]
CP	0.31	0.59[A]	0.28	0.55[A]	0.23
Ash	0.23	0.44	0.21	0.30	–0.25
aNDF	0.35	0.60[A]	0.33	0.46[a]	0.06
ADF	–0.39	–0.47[a]	–0.37	–0.42[a]	–0.02
Starch	–0.15	–0.15	–0.13	–0.17	–0.22

ADF = acid detergent fibre, ADFD = ADF digestibility, aNDF = neutral detergent fibre, aNDFD = amylase-treated neutral detergent fibre digestibility, CP = crude protein, CPD = CP digestibility, DM = dry matter, DMD = DM digestibility, OMD = organic matter digestibility

[a]($P < 0.05$), [A]($P < 0.01$)

centration in maize silage as a result of increased maturity has generally been observed together with a decreasing aNDFD (Jensen et al. 2005). Similarly, Andrae et al. (2001) found that the aNDF content of maize silage was poorly correlated with aNDFD. In the review by Kramer-Schmid et al. (2016) an enhanced aNDFD tended to be associated with a higher aNDF concentration ($P = 0.087$) in maize silage and negatively correlated to DM and starch concentration. Furthermore, Andrae et al. (2001) and Kramer-Schmid et al. (2016) noted that starch content in maize silage is not a good predictor of either aNDFD or OMD, which was the case also in our research ($r = –0.17$, $r = –0.13$, respectively).

Therefore, it cannot be excluded that beside aNDF digestibility also one or more of these correlated factors have been responsible for the cow's production responses.

Our current research builds on our previous work (Loucka et al. 2015a, b; Trinacty et al. 2016) in which we evaluated the nutritional values, *in situ* degradability, and *in vitro* digestibility of hybrids according to their FAO maturity group, type of ripening (stay-green vs normal), and type of kernel endosperm (flint vs dent).

CONCLUSION

The aNDFD effect of the hybrid LGAN250S was greater ($P < 0.001$) than that of Ronaldinio. The hybrid (the genotype) had a greater effect upon all indicators of chemical composition and digestibility than the year of cultivation, but not for indicators of the quality of fermentation (pH, amount of acids). The genotype × year interaction effect was significant ($P < 0.01$) in dry matter, crude protein, raw ash and aNDF, ADFD and evaluating the fermentation also in the content of lactic acid.

The aNDFD correlated closely with the digestibility of all other nutrients ($P < 0.01$), but not with the chemical composition, except CP ($P < 0.01$), aNDF, and ADF ($P < 0.05$). Further studies dealt with *in vivo* digestibility need to be conducted with maize silages from different hybrids.

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