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Evaluation of quality characteristics in official trials with silage maize varieties in Belgium

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Abstract

In the official Belgian trials for silage maize, qualitative characteristics are incorporated in the evaluation criteria for new varieties since 1992 (digestibility) and from 1995 starch.

To analyse many samples (> 1000/year) the NIRS method based on *in-vitro* with cellulase was proposed by the official Committee for the national variety list for the prediction of digestibility. Comparing NIRS digestibility, based on cellulase, with a NIRS calibration developed at the Plant Breeding Institute based on rumen fluid digestibility, a highly significant correlation has been obtained. It is however necessary that after each new harvest a certain percentage of the samples should be analysed in a classical way to validate the calibration equation against a sample set with new genetic material.

In contrast to literature data there is no clear tendency that NIRS determination over-estimates the digestibility of early varieties or under-estimates late varieties.

The results of the NIRS starch analyses give valuable information about the ear content. The correlation between earliness and starch is not very high but still significant. Since digestibility and starch are not well correlated, the incorporation of starch as a new criterion is valuable and it can give additional information about the net energy content of silage maize varieties.

Keywords: digestibility, starch, silage maize, variety trials, NIRS

Introduction

Up to 1992 the evaluation criteria in Belgium for testing silage maize varieties were: earliness, resistance against lodging and the yield of digestible organic matter (Anonymous, 1979). The latter characteristic was based on the assumption for all varieties that the digestibility of the ears (cob + grains) equals 80% and that of stalk + leaves + husk 60%. This value, calculated from constant coefficients, does not account for varietal differences in digestibility (De Boever *et al.*, 1994 ; Van Waes, 1995). Such an evaluation system further implicates that the ears and the rest of the plant have to be harvested separately, which is labour-intensive and not representative of farm practice.

In 1992 the Committee for the national variety catalogue decided to harvest all maize varieties without separation for ears and stalk + leaves (Anonymous, 1992). At the same time it was proposed to incorporate digestibility of the whole plant into the evaluation criteria. Since the time between harvest (September-October) and the Committee decision for admission of new varieties on the national list (beginning of January) is always limited, a rapid method to analyse many samples (> 1000/year) is necessary. The Near Infrared Reflectance Spectroscopy (NIRS) method of Biston and Dardenne (1988) based on cellulase digestibility was used and gave a good prediction of the *in-vitro* digestibility in general (De Boever *et al.*, 1994). Furthermore, apart from its rapidity, the NIRS technique is non-destructive, needs no chemicals and produces no pollutants.

After four years of using the NIRS method in the official variety tests we have assessed whether this approach is good for variety comparisons or whether we have to search for a reference method more directly linked to the animal.

In an international context, there is not yet an agreement about the most appropriate method for predicting digestibility in maize variety trials: *in-vitro* with rumen fluid Tilley & Terry (The Netherlands); NIRS based on cellulase (in study in Germany and in the United Kingdom); NIRS based on rumen fluid (Switzerland) or chemical analyses (Denmark).

In several countries the *in-vitro* rumen fluid method is used as a reference, which is closely linked to the animal. However it is more difficult to execute and not very practical for variety trials. A NIRS calibration, based on rumen fluid digestibility, was developed in our lab (Carlier, 1993). In this publication we present the results of a comparison of this NIRS digestibility prediction with the NIRS cellulase calibration of Biston and Dardenne (1988). Furthermore we have also examined if NIRS, either based on cellulase or rumen fluid, systematically over- or under-estimates early or late varieties.

In addition to digestibility, another quality characteristic, starch content, was evaluated. Starch has been incorporated as a criterion in Belgium since 1995 onwards (Anonymous, 1995). A higher starch content may lead to a higher energy content (Weissbach, 1993). Weissbach states that from two maize varieties with the same digestibility the one with the highest starch content gives the highest net energy content. Starch content is of further interest for balanced feeding (effects on rumen degradation, glycogenic precursors etc.) and together with the dry matter content it gives a good idea about the maturity stage of the maize plant.

Many authors argue that for the nutritional value of silage maize both digestibility and starch are important (Veen, 1992; Wever, 1993; Weissbach, 1993). According to Weissbach (1993), the convertion of energy from starch (ear) can be utilized more efficiently (64%) when compared with the energy from digestible cell-wall constituents (58%) or sugars (50%). Furthermore Gross and Peschke (1980) have determined a higher net energy from ears in comparison with the rest of the maize plant. However it is evident that rumen degradability and other parameters can have an important influence on the starch value for net energy; these parameters are under discussion.

We have also studied the correlation of starch with earliness, digestibility and ear

content. The information about the ear content is valuable for maize varieties for double use: silage or grain.

Finally a summary of the variation in digestibility and starch for the whole tested gene pool is given with special attention for the influence of the year.

Materials and methods

Plant material – Preparation of samples – Selection of trials

More than 550 varieties, originating from about 30 breeding companies, were evaluated from 1989 to 1995 by the Variety Testing Department (RvP) (Van Waes and De Vliegher, 1989–1995). Harvest always started when the dry matter content of a reference variety with an FAO number of 210 reached at least 27% of dry matter of the whole plant. All varieties were harvested at the same date per trial.

The maize samples, of about 1.5 kg fresh material, were dried at 70°C for 72 hours. At this temperature these large samples dry quickly, thus preventing heating and moulding, so that a good quality is maintained (Van Waes, 1995). The samples were ground with a Gondard mill, fitted with a 1 mm sieve.

In experiments to examine the ear content, ears were harvested and sampled separately from stem, leaves and husks. After drying and grinding we mixed in proportion to produce a composite sample for analysis.

Only trials with a regular emergence and with no drought problems were selected for the analyses of digestibility and starch. After a statistical analysis of the total dry matter yield the coefficient of variation should not be higher than 8%, otherwise the trial is rejected.

Methods for analysis

Four methods were compared for the prediction of the organic matter digestibility.

A. *In-vitro* method with rumen fluid of fistulated sheep – Tilley & Terry (1963) = IVD.
B. *In-vitro* method with cellulase – De Boever et al. (1988) = CED
C. ADF (Van Soest, 1963) (100-ADF is a norm for digestibility) = ADFD
D. NIRS – based on *in-vitro* cellulase (Biston & Dardenne – 1988) = NIRSCED

The analyses for the methods A and C were executed in our Chemical Lab (RvP). Method B was carried out in the Research Station for Animal Nutrition (Melle) while method D refers to the calibration developed at the Research Station of Haute Belgique (Libramont). Furthermore a NIRS calibration, based on *in-vitro* with rumen fluid was developed at the RvP lab (Carlier, 1993 = NIRSIVD).

The analyses of starch were based on the NIRS calibration developed at the Research Station of Haute Belgique (Biston & Dardenne, 1988).

The apparatus (NIRSystems 5000) used for the NIRS analyses was provided with a scanning monochromator covering the wavelength range from 1100 to 2500 nm.

Statistical analyses

The statistical analyses were executed with the programme Data Regression (LOTUS 1.2.3. Release 3.4). The regression line was $y = a + bx$.

Results and discussion

Digestibility

Correlation between NIRS calibrations and relation with the in-vitro rumen fluid method

By comparing both NIRSIVD and NIRSCED for several years, we obtained highly significant correlations (Table 1). For more than 90% of the tested samples, the difference between the two NIRS calibrations was lower than 1% (absolute value). However in a few cases the differences were up to 4%. Therefore it is necessary to update the calibration equations every year with a new set of samples analysed with the reference methods (cellulase and rumen fluid).

Comparison of digestibility methods in one year and over the years

The digestibility of seven varieties was evaluated with four methods (IVD, CED, ADFD, NIRSCED). These varieties belong to different earliness groups (var 3 and 6: early; var 2 and 7: semi-early to semi-late; var 1, 4 and 5: semi-late to late types).

The results in figure 1 are based on the average of the analyses of five locations per year ; each value being the average of three replicates.

For 1989 (Figure 1a) the same variety ranking was recorded for CED, ADFD and

Table 1. Correlation coefficients and statistical significance level between two NIRS calibrations for digestibility: NIRSIVD (x) and NIRSCED (y) – Belgian official silage maize trials 1989–1994.

Testing year	Number of samples	Correlation value (1)	Standard error of b (2)	Standard error of y (estimated)
1989	31	0.71***	0.19	1.28
1990	122	0.75***	0.08	1.60
1991	217	0.91***	0.05	1.61
1992	352	0.92***	0.02	1.48
1993	348	0.85***	0.04	1.21
1994	630	0.85***	0.03	1.80
Average 1989–1994	Total : 1700	0,87***	0.03	1.78

(1) Significance level : *** $P < 0.001$
(2) Regression line: $y = a + bx$

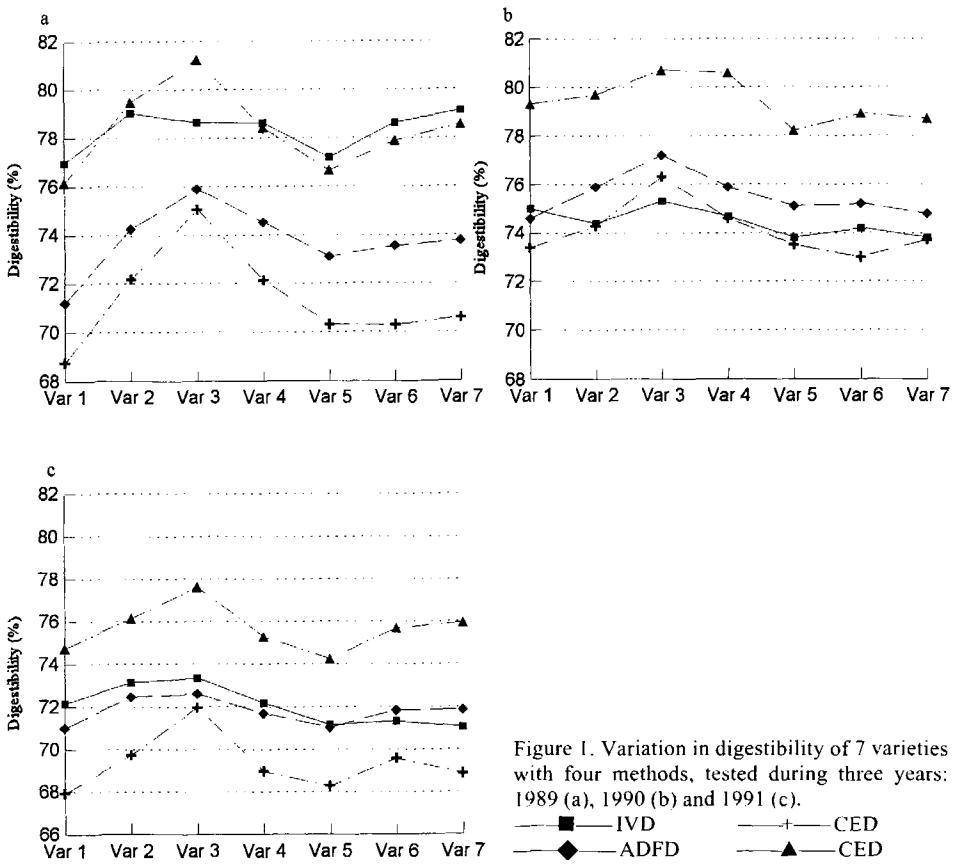

Figure 1. Variation in digestibility of 7 varieties with four methods, tested during three years: 1989 (a), 1990 (b) and 1991 (c).

NIRSCED. The classification of the varieties by IVD deviated from that of the other methods, especially for variety 3. Variety 3 has a high cob content. We see that with NIRSCED the digestibility is somewhat over-estimated for this variety. In contrast with the other three methods, the range in digestibility was very low for IVD (2.8%). Across all varieties the average digestibility level was much higher for IVD and NIRSCED in comparison with ADFD and CED.

The variety ranking in 1990 was nearly the same for CED, ADFD and NIRSCED. Again IVD was somewhat deviant for var 1 and var 3 (Figure 1b). The range in digestibility was low for all four methods. In general NIRSCED scored much higher for digestibility.

For 1991 the variety ranking was the same for the four methods (Figure 1c). The range in digestibility was high for CED and NIRSCED, but low for IVD and ADFD. The average digestibility was much higher for NIRSCED and lower for CED.

Over and across the three years we have calculated the correlation matrix for the four methods for prediction of the digestibility (Table 2).

Table 2. Correlation matrix and significance level between four methods for the prediction of digestibility of silage maize varieties over three years.

(1)	A89	A90	A91	B89	B90	B91	C89	C90	C91	D89	D90	D91
A89	1.00											
A90	0.47*	1.00										
A91	0.60**	0.34 NS	1.00									
B89	0.60**	0.47*	0.66*	1.00								
B90	0.43*	0.55*	0.66**	0.69**	1.00							
B91	0.67**	0.32NS	0.88***	0.66**	0.68**	1.00						
C89	0.59**	0.25 NS	0.60**	0.84***	0.52**	0.67**	1.00					
C90	0.61**	0.51**	0.77***	0.76***	0.92***	0.80***	0.66**	1.00				
C91	0.69***	0.31 NS	0.87***	0.63**	0.65**	0.89***	0.65**	0.81***	1.00			
D89	0.81***	0.73***	0.67**	0.80***	0.69**	0.72***	0.70***	0.75***	0.65**	1.00		
D90	0.41*	0.49*	0.59**	0.65**	0.88***	0.66**	0.54**	0.87***	0.60**	0.64**	1.00	
D91	0.78***	0.42*	0.92***	0.66**	0.65**	0.95***	0.64**	0.81***	0.90***	0.78***	0.63**	1.00

Significance level

*	$P < 0.05$
**	$P < 0.01$
***	$P < 0.001$
NS	not significant

(1)

A: IVD
B: CED
C: ADFD
D: NIRSCED

As expected, the best correlation was detected between CED-NIRSCED, because the latter is derived from the first. For the IVD method the correlation with the three other methods was mostly low.

Comparison of one method across three years resulted in low correlation values for IVD. For the three other methods (CED, NIRSCED, ADFD) the coefficients were higher and in most cases significant at P 0.01 level.

Over- or under-estimation of digestibility with NIRS calibrations in relation to earliness

The digestibility, predicted by the NIRSCED or NIRSIVD, is in general somewhat higher than that based on IVD. The variety ranking is in general similar for all methods (Van Waes, 1995).

We can raise the question: is NIRS systematically over- or under-estimating certain maize types? According to Ebskamp (1993), varieties with a high digestibility and a relatively low ear content are under-estimated with NIRS based on *in-vitro* with cellulase and very early varieties with a dry matter content larger than 34% are appreciably over-estimated.

In the Belgian variety testing system we are comparing all earliness groups harvested on the same date. We have investigated whether the evaluation system based on NIRS (IVD or CED) in comparison with IVD favours early types with a high ear content.

During two years we have compared the digestibility of NIRSCED and NIRSIVD with IVD in relation to the earliness of the varieties.

In 1992 semi-early to very early varieties (A to D) scored better with NIRSIVD and with NIRSCED at location 1 (Table 3). For the categories E to G the values for NIRSIVD in comparison with IVD and NIRSCED were somewhat lower. For the last category (H) NIRSIVD scored about the same but NIRSCED was 2.1% higher for digestibility. For location 2 there was an over-estimation for both NIRS calibrations for all earliness categories, with the greatest differences for the earliness groups B and H.

In 1993 at location 1 semi-early varieties were somewhat over-estimated when NIRSIVD was compared with IVD. The difference for the other earliness groups were very low with the exception of category E. In the comparison with NIRSCED, all varieties were under-estimated with the exception of category C. In both comparisons all varieties were under-estimated in location 2 in 1993.

These results are not in agreement with Ebskamp's findings (1993). We found no clear tendency to over- or under-estimate digestibility with NIRS (IVD and CED) in relation to earliness. However it seems that NIRSIVD performs better than NIRSCED as expected, because the NIRSIVD calibration is derived from IVD-analyses. Furthermore the correlation between earliness and digestibility (NIRSIVD or NIRSCED) is very low ($r = 0.14$ NS for earliness-NIRSIVD; $r = 0.35$ NS for earliness-NIRSCED).

Table 3. Differences in digestibility between IVD and two NIRS calibrations (NIRSCED and NIRSIVD) in relation to earliness (results 1992-two locations and 1993-two locations).

Location 1			Location 2		
Earliness group (1)	Difference in digestibility		Earliness group (1)	Difference in digestibility	
(number of varieties)	NIRSIVD-IVD	NIRSCED-IVD	(number of varieties)	NIRSIVD-IVD	NIRSCED-IVD
1992					
A (3)	+0.8	+0.4	A (3)	+1.2	+0.7
B (5)	+1.0	+1.2	B (4)	+2.8	+2.6
C (3)	+1.8	+2.3	C (4)	+0.5	+0.2
D (3)	+0.3	–0.5	D (4)	+0.2	+0.6
E (5)	–0.6	–0.6	E (5)	+1.4	+1.0
F (5)	–0.5	–0.4	F (5)	+0.6	+0.5
G (4)	–1.2	–1.8	G (3)	+1.3	+1.1
H (4)	–0.3	+2.1	H (4)	+2.2	+2.5
Average	*+0.1*	*+0.3*		*+1.3*	*+1.1*
1993					
A (3)	+0.5	–0.2	A (3)	–1.6	–2.7
B (3)	+0.1	–1.5	B (2)	–1.5	–3.7
C (3)	+1.7	+0.2	C (3)	–0.3	–1.6
D (3)	–0.9	–1.2	D (6)	–1.3	–3.1
E (10)	–1.5	–1.9	E (7)	–1.2	–1.5
F (7)	–0.9	–1.9	F (5)	–1.7	–2.3
G (4)	–0.7	–1.0	G (7)	–1.6	–2.0
H (3)	–0.4	–1.5	H (3)	–1.6	– 2.1
Average	*–0.7*	*–1.5*		*–1.5*	*–2.5*

(1) earliness classification based on the recommended list (Van Waes *et al.*, 1995)
A : > % 34.5% D.M. (early to very early)
B : between 33.4 and 34.5% DM (early)
C : between 32.4 and 33.4% DM (semi-early to early)
D : between 31.4 and 32.4% DM (semi-early)
E : between 30.4 and 31.4% DM (semi-late)
F : between 29.4 and 30.4% DM (semi-late to late)
G : between 28.4 and 29.4% DM (late)
H : < 28.4% DM (late to very late)

Starch content

Correlation with digestibility

The correlation between starch and digestibility was only good in some years (Table 4). The lowest correlations were recorded in 1989. The restricted number of analysed samples in this year limited a clear conclusion. On the average of five years the relationship was good for NIRSCED-starch and moderate for NIRSIVD-starch.

Table 4. Correlation and significance level between NIRS digestibility (x) and NIRS starch (y).

Year (number of analyses)	Correlation NIRSCED-starch	Standard error of b	Standard error of y estimated	Correlation NIRSIVD-starch	Standard error of b	Standard error of y estimated
1989 (31)	0.49*	0.20	1.93	0.18 NS	0.32	2.17
1990 (122)	0.56**	0.11	3.02	0.79***	0.11	2.21
1991 (217)	0.78***	0.04	2.46	0.67**	0.08	2.90
1992 (352)	0.84***	0.04	2.80	0.83***	0.05	2.91
1993 (348)	0.59**	0.06	2.61	0.60**	0.09	2.60
1994 (340)	0.63**	0.03	2.61	0.71***	0.06	3.34
Average 1989-1994 (1410)	0.82***	0.03	2.37	0.63**	0.04	3.59

Significance level : see table 1; Regression line: y = a + bx

Table 5. Variation for digestibility and for starch content (% kg DM) of maize varieties in official trials in Belgium.

	Digestibility (NIRSCED)					Starch content				
	1991	1992	1993	1994	1995	1991	1992	1993	1994	1995
Number of tested varieties (*)	78	108	116	121	125	78	108	116	121	125
Highest value	77.6	82.0	78.1	76.2	80.3	35.2	41.3	36.3	33.9	37.9
Lowest value	71.9	69.7	66.5	71.2	67.5	26.1	23.8	22.5	26.1	25.7
Average	73.8	74.4	72.3	72.7	75.2	29.1	30.5	29.2	29.4	31.5
LSD (P 0.05)	1.9	3.1	2.5	1.8	2.9	3.3	4.1	3.5	2.4	3.1
Difference H-L	5.7	12.3	11.6	5.0	12.8	9.1	17.5	13.8	7.8	12.2

(*) Each value is the average of 9 analyses.

We have studied the variety ranking for NIRS (CED or IVD) digestibility and starch for more than 40 varieties during three years. If digestibility and starch are not well correlated, then it will be worthwhile to have both parameters in our criteria for variety evaluation. The variety ranking for digestibility (NIRSCED) and starch was in general not the same for the 3 years especially for the best or worst varieties (Van Waes, 1995). This was true for both the NIRS calibrations (NIRSCED and NIRSIVD).

Correlation with earliness

The correlation value between starch and earliness was not very high, however it was significant and not greatly influenced by the year (0.61** in 1992; 0.68** in 1993; 0.70*** in 1994). The relationship between earliness and starch for silage maize varieties tested in 1994 is presented in figure 2. The variety ranking for earliness and starch is very different, especially for the early types.

Correlation with ear content

According to Weissbach (1993) the ear content can be derived from the starch content with the following formula : ear content (%) = 1.76 x starch content (%). The basis for his formula is a standardised cob content of 15% and a starch content of the grains of 67% based on the dry matter.

We have studied the relationship between starch and ear content in silage maize variety trials in 1991 (4 locations, 24 varieties, 3 blocs) and in 1992 (4 locations, 26 varieties, 3 blocs). In these two years, there was still a separate harvest of ears and stem + leaves + husks.

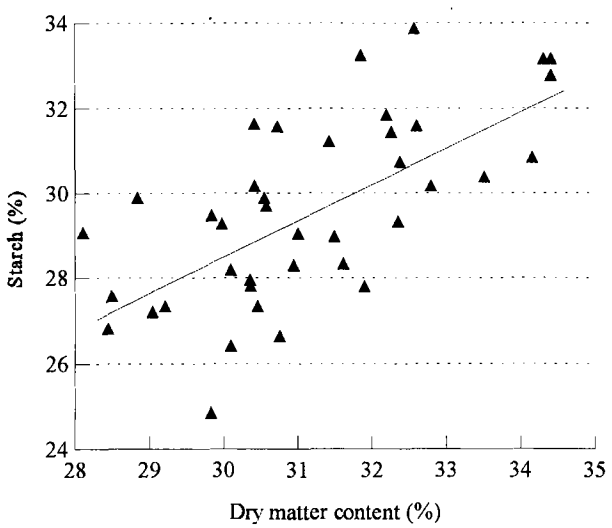

Figure 2. Relation between starch content (y) and earliness (x, expressed as dry matter content) (y = 1.01 + 0.91x, r = 0.70***, SE of y = 1.62). Data from silage maize varieties in 1994.

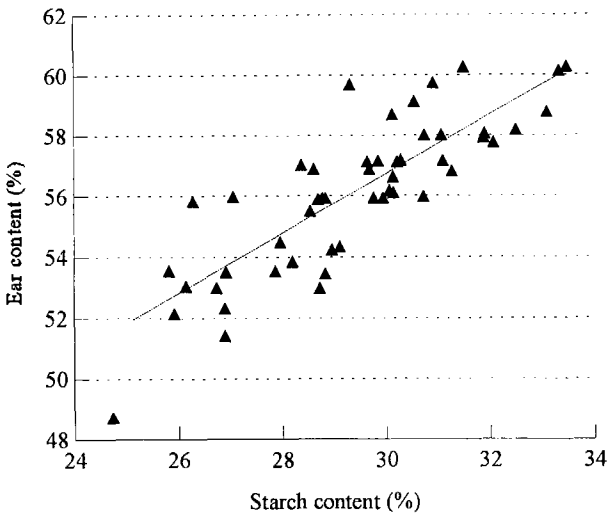

Figure 3. Relation between ear content (y) and starch content (x) (y = 26.45 + 1.01x; r = 0.86***, SE of y = 1.4). Data from silage maize trials 1991–1992.

Our correlation between starch, predicted by NIRS, and the ear content, measured in the field, was good (in 1991: r = 0.82*** and for 1992: r = 0.84***). The regression equation over both years was y = 26.45 + 1.01x (y = prediction of ear content, x = % starch content on dry matter). The correlation value was 0.86*** with a standard error of y = 1.4. In figure 3 the relation between starch and ear content is presented.

On average the difference between the predicted ear content from starch and measured ear content was only 0.1% (abs.) with a standard deviation of 1.1. The difference between our formula and that of Weissbach (1993) may be found in of Weissbach's hypothesis that the cob content is constant at 15%. In our trials with grain maize we have seen differences up to 8% (in absolute value) in the cob content (Van Waes & De Vliegher, 1995).

Variation in digestibility and in starch content

The tested varieties showed a great variation for digestibility and starch in 1992, 1993 and 1995 (Table 5). For 1991 and 1994, the variation was much lower. In these years the levels of the lowest digestibility and starch content were higher compared to the other years.

Conclusions

It can be concluded that digestibility of silage maize varieties may be predicted in a reliable way, based on NIRS analyses (either with *in-vitro* cellulase or rumen fluid as

reference). It is preferable to develop a NIRS calibration on *in-vitro* rumen fluid digestibility for variety testing, because this method refers directly to the animal. It is however necessary to validate the calibration equation against a new sample set with new genetic material every year.

With NIRS (based on cellulase or rumen fluid digestibility) compared to the lab reference method (rumen fluid), there is no clear tendency to over- or under-estimate varieties in relation to earliness.

The analysis of the starch content (NIRS method) gives valuable information about the ear content. The correlation between earliness and starch is not very good. Since digestibility and starch are not very well correlated, the incorporation of starch as a new criterion will be valuable because it can give additional information about the net energy content of a silage maize. Including both characteristics in the criteria for the admission of new varieties will be complementary and not overlapping.

The variation in digestibility and starch content between all tested varieties is large and highly influenced by the year and the genetic origin of new varieties.

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