Effects of ensiling maize and sample conditioning on in situ ruminal degradation of dry matter, starch and fibre

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\section*{Abstract}

High-production ruminants are commonly fed maize silage, which makes accurate evaluation of its nutritive value a key economic issue. However, evaluations of the rate and extent of ruminal degradation of starch and cell wall fractions from maize silage carry uncertainty due to the lack of a standardized method. Here, we investigated the effects of ensiling and sample conditioning on in situ-measured degradation of maize forage. Eight series of maize samples (two hybrids × two maturity stages × two methods of conservation [non-ensiled or fresh and ensiled]) were nylon-bagged in three conditionings: dried and ground to 1 mm (D1), dried and ground to 4 mm (D4), frozen and coarse-ground (FG). Disappearance of dry matter (DM), starch and fibre (aNDF) was measured in situ in cow rumen after different incubation times (2, 4, 8, 16, 24, 48 and 96 h). Effects of ensiling, sample conditioning, genotype, maturity and their interactions on DM, starch and aNDF degradation were analyzed using the SAS MIXED procedure. Effective dry matter degradability (ED\textsubscript{DM}) was significantly higher (P<0.001) in silage than in fresh maize due to a significantly higher rapidly degradable fraction (a) (P<0.001). Effective starch degradability (ED\textsubscript{S}starch) followed the same trend due to a higher silage degradation rate (P<0.01). Conversely, effective aNDF degradability (ED\textsubscript{ANDF}) was lower in silage (P<0.001) than fresh maize due to the longer lag-time to degradation (P<0.001) and lower hemicellulose ((aNDF − AD\textsubscript{F})/aNDF) fraction in silage. Effective DM degradability was higher (P<0.001) for D1 samples than D4 and FG samples, mainly due to the higher rapidly degradable fraction (a) (P<0.001) in D1 than D4 or FG samples. In relation to high degradation rate, starch degradability was significantly higher (P<0.001) in FG than D1 and D4 samples, whereas aNDF degradation was lowest in the FG samples. This study shows that ensiling maize increases starch degradability and decreases aNDF degradability compared to fresh plant. Alongside conservation method, fine-grinding samples (as D1) led to high losses through the pores of the nylon bags, and these losses were correlated with high starch degradability, whereas coarse grinding (as FG) led to low aNDF degradability, probably due to insufficiently reduced particle sizes. In conclusion, using ensiled samples dried and ground to 4 mm (D4) emerges as the appropriate method for in situ studies of starch-rich forages used as silages.

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\textit{Abbreviations:} AD\textsubscript{F}, acid detergent fibre; aNDF, neutral detergent fibre; D1, dried and ground to 1 mm; D4, dried and ground to 4 mm; DM, dry matter; ED\textsubscript{2\text{\textsubscript{AD\textsubscript{F}}}}, effective aNDF degradability; ED\textsubscript{4\text{\textsubscript{DM}}}, effective dry matter degradability; ED\textsubscript{6\text{\textsubscript{S}starch}}, effective starch degradability; F, flint grain; FD, flint-dent grain; FG, frozen and coarse-ground; N, nitrogen.
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1. Introduction

Maize silage is currently used as forage in the diet of high-yielding ruminants (dairy cows and fattening animals) to ensure a high energy supply. Accurate evaluation of maize silage feed value is a key economic issue due to its impact on animal health and production. The rate and extent of ruminal degradation of maize silage can vary strongly with stage of maturity (Johnson et al., 2002; Jensen et al., 2005) and hybrid (Verbic et al., 1995; Philippeau and Michalet-Doreau, 1997; Ngonyamo-Majee et al., 2009), but these variations cannot be properly understood until there is a standardized method to evaluate rate and extent of ruminal degradation of maize silage starch and fibre. In situ measurement of ruminal degradation is the reference method in many feed evaluation systems for assessing ruminal nitrogen (N) degradation (Vérité and Peyraud, 1989; NRC, 2001; NorFor, 2011). The forage samples used for in situ measurements are routinely oven- or freeze-dried then fine-ground, but it has been shown that using finely-ground samples overestimates the ruminal degradability of DM and N in grass samples (Dulphy et al., 1999) and starch in maize samples (Philippeau and Michalet-Doreau, 1997) due to the amount of insoluble particles that get lost through the bag pores and confounded with soluble and rapidly degradable fractions. Furthermore, although measuring degradability on samples collected from fresh forage before conservation makes it easy to form representative samples and screen large forage samples, there are issues over whether measurements on fresh forage can be reliably extrapolated to estimate the degradability of the corresponding conserved forage. Recent studies show that length of ensiling can modify maize degradation in the rumen (Hoffman et al., 2011; Der Bedrosian et al., 2012). Thus, it is important to evaluate these differences in ruminal degradability between fresh and conserved forage for the different nutrient fractions. The aim of this study was to assess the effect of ensiling and sample conditioning procedure on the ruminal degradability of whole-plant maize DM, starch and fibre fractions measured in situ.

2. Materials and methods

The experiment was conducted in compliance with national legislation on animal care (French Ministry of Agriculture-issued Certificates of Authorization to Experiment on Living Animals, Nos. 63-30 and 63-158). The experimental protocol was approved by the Auvergne-region institutional review board for animal experimentation, under No. CE12-09.

2.1. Maize hybrids and sample conditioning

Two maize hybrids chosen for this study were grown and harvested in 2011 at the ARVALIS—Institut du Vegetal experimental farm in northwest France. These hybrids were selected for their contrasted grain texture determining rate of starch degradation, i.e. flint grain (F) (considered “low starch availability”) and flint-dent grain (FD) (considered “high starch availability”). The two hybrids were harvested at two stages of maturity—early and late. Maturity at harvest was characterized by whole-plant DM content, proportion of ears in the whole plant, and DM content of ear and stem-leaf fractions (Table 1). At harvest, representative samples of the fresh non-ensiled material were snap-frozen and stored at −20 °C, and the rest was ensiled in bags (closed under anaerobic conditions). After 17 weeks to ensure even fermentation, silage pH was measured as 3.66 for hybrid F stage 1, 3.81 for hybrid F stage 2, 3.71 for hybrid FD stage 1 and 3.86 for hybrid FD stage 2. Then, samples of silages and corresponding fresh material were conditioned in three ways ready for nylon-bagging: (i) dried samples (72 h at 60 °C) ground through a 4 mm screen (Retsch Mill, SM100) (D4); (ii) dried samples (72 h at 60 °C) ground through 1 mm screen (Rotary Mill, Brabender GmbH, Duisburg, Germany) (D1); (iii) frozen samples chopped through a coarse screen (Mincer–Grinder–Law S3) and directly bagged without drying (FG). Around 50% of the DM of FG samples were retained on a 4 mm sieve by wet sieving. The FG bags were kept frozen before incubation in the rumen. The FG conditioning is designed to simulate chewed maize reaching the rumen.

For each of the 24 maize samples (2 hybrids × 2 stages of maturity × 2 types of forage conservation × 3 methods of sample conditioning), a series of 56 nitrogen-free polyester bags (3 × 10 cm, given porosity 50 μm, R510 Ankom® Technology, Macedon, NY) was prepared with approximately 3 g DM of weighed samples placed into each bag. Small bags were used to enable simultaneous incubation of one variety, one stage of maturity, two types of conservation (fresh or silage) and three conditioning methods (D1, D4 and FG).

<table>
<thead>
<tr>
<th>Hybrid (H)</th>
<th>Flint (F)</th>
<th>Flint-dent (FD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maturity (M)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dry matter of whole plant (g/kg fresh matter)</td>
<td>275</td>
<td>304</td>
</tr>
<tr>
<td>Ear/whole plant</td>
<td>0.58</td>
<td>0.61</td>
</tr>
<tr>
<td>Dry matter of ear fraction</td>
<td>0.38</td>
<td>0.41</td>
</tr>
<tr>
<td>Dry matter of stem-leaf fraction</td>
<td>0.19</td>
<td>0.22</td>
</tr>
</tbody>
</table>

A Data expressed as proportion of dry matter.
2.2. Ruminal incubations and experimental design

Three non-lactating rumen-cannulated Holstein cows were used for in situ incubations at the National Institute for Agricultural Research (INRA) experimental farm in Saint-Genès-Champanelle, France. The animals were fed twice daily (half the diet at 8 a.m. and the other half at 4 p.m.) with a limited quantity (78 g DM/kg W0.75 covering maintenance requirements) of a mixed diet composed of 2/3 grass hay and 1/3 concentrates (230 g/kg wheat, 200 g/kg barley, 11 g/kg molasses, 300 g/kg dried sugar beet pulp, 71 g/kg soybean meal, 150 g/kg rapeseed cake, 11 g/kg minerals, 6 g/kg salt). The cows were also supplemented with 200 g/day of vitamins and minerals mixture. This supplement pellet was sprinkled daily on the ration. The in situ incubations were performed on three cows over two four-week periods. During one week, one cow received the bags of one of the four combinations of hybrids and stages of maturity. For that combination and that cow, samples of the two conservations (fresh and ensiled) and the three sample preparations were simultaneously incubated. At the end of the four weeks, each cow received the bags of the four combinations of hybrids and stages of maturity (and thus all conservations and sample conditionings). This design was then repeated in full for another four-week period, reproducing the two repetitions or ‘runs’ according to Udén et al. (2012).

The nylon bags were inserted into the rumen before the morning meal and removed after 2, 4, 8, 16, 24, 48, and 96 h of incubation. Once removed from the rumen, the bags were rinsed in cold water, washed for 10 min four times over in a washing machine, then dried at 60 °C and weighed to determine DM content. After that, for each of the 24 treatment combinations and each incubation time, the 6 remaining bags (three cows × two replicates) were pooled, ground through a 1 mm sieve for aNDF analysis, and then through a 0.5 mm sieve for starch analysis.

2.3. Chemical analysis

The DM content of fresh maize and silages was determined by drying at 80 °C for 48 h, and the DM content of silages was corrected for losses of volatile fermentation products (Dulphy et al., 1975). Samples used for fibre content determination were dried at only 60 °C for 48 h. Starch content was determined by enzymatic method (ISO, 2004). aNDF and ADF content were determined according to Van Soest and Wine (1967) using an Ankom system (Ankom® Technology, Macedon, NY). aNDF determination used a heat-stable amylase without addition of sodium sulphite. ADF residues were obtained after boiling with ADF reagent and expressed inclusive of residual ash. For the 192 nylon bag residues, aNDF content was estimated using near-infrared reflectance spectroscopy (NIRS) according to the following procedure. All bag residues were scanned in a NIRSystems 6500 monochromator (Foss NIRSystems, Silver Spring, MD), and laboratory determinations were performed on a subset of 83 residues selected based on their spectra. These laboratory analyses were then used with the NIR spectra to expand the prediction equations for routine analysis. The statistical parameters of the prediction model (standard error of calibration, standard error of cross-validation and coefficient of determination) were SEc = 1.385 (g/kg DM), SEcv = 1.724 (g/kg DM) and r² = 0.987.

2.4. Data analyses

DM, starch and aNDF disappearance rate (in g/g) was calculated for each cow, each replicate per cow, each maize sample, and each incubation time. The disappearance kinetics of DM, starch and aNDF were fitted to an exponential model according to the method described by Dhanoa (1988):

\[ D(t) = a + b \times (1 - e^{-c(t-L)}) \]

where \( D(t) \) is proportion (g/g) disappeared at time \( t \) (h), \( a \) is proportion of rapidly degradable fraction (g/g), \( b \) is proportion of potentially degradable fraction (g/g), \( c \) is the digestion rate constant (h⁻¹) of the fraction \( b \) and \( L \) (h) is the lag time. The undegradable fraction was calculated as \( 1 - (a+b) \). Data were fitted using the non-linear least squares regression procedure (NLIN) of SAS (9.1 version, 2003) with the Marquardt parameter in order to obtain the smallest residual sum of squared deviations from the model. From adjustment results of this model, as the L of DM and starch was not significantly different to 0, it was assumed to equal 0 as per Jensen et al. (2005) and Van Duinkerken et al. (2011). For starch, data were fitted using the linear regression on log-transformed residues according to incubation time until 24 h (Mertens et al., 1993), and assuming \( a+b=1 \), since no residual starch was observed in 48 h residues. The effective degradability of DM (ED4DM), aNDF (ED2aNDF) and starch (ED6Starch) was calculated as:

\[ ED = a + \left( b \times e^{-k_p \times L} \right) \left( c + k_p \right) \]

where \( a \), \( b \), \( c \) and \( L \) are as described above, and \( k_p \) (h⁻¹) is the ruminal outflow rate of particles. In accordance with Mertens et al. (1993), \( k_p \) is assumed to be 0.04 for DM (moderate time delay during passage in the rumen and a slow degradation rate), 0.06 for starch (short time delay during passage in the rumen and a fast degradation rate), and 0.02 for aNDF (long time delay during passage in the rumen and a slow degradation rate).
2.5. Statistical analyses

Effects of conservation method (C), hybrid (H), maturity (M) and their interactions (C × H, C × M, H × M, C × H × M) on the chemical composition of maize samples were analyzed as fixed effects using the GLM procedure of SAS (9.1 version, 2003). After averaging repetitions, the 72 observations (2 hybrids × 2 stages × 2 conservations × 3 sample preparations × 3 cows) were subjected to analysis of variance. Effects of conservation method (C), sample conditioning (S), hybrid (H), maturity (M) and their interactions (H × C, H × M, H × S, M × C, M × S, C × S, C × [H × M], S × [H × M × C]) on degradation parameters were analyzed as fixed effects and animal was analysed as random effect using the MIXED procedure of SAS. The degradation rate (c) of aNDF was log-transformed to get a normal distribution of statistical residues. Significant differences were set at P<0.05.

3. Results

3.1. Chemical composition of the maize samples

There were no interactions between conservation and maturity effects (C × M), between conservation and hybrid effects (C × H) or between hybrid and maturity (H × M) effects on starch, aNDF, ADF contents or hemicellulose ((aNDF – ADF)/aNDF) fraction (Table 2). Both maturity and hybrid had significant effects on starch, aNDF, ADF contents and hemicellulose fraction. Starch content increased with stage of maturity from 278 to 392 g/kg DM whereas aNDF content decreased from 406 to 369 g/kg DM and ADF content decreased from 210 to 175 g/kg DM. Hybrid FD was richer in starch (P<0.01) than hybrid F (385 and 333 g/kg DM, respectively), probably due to the higher proportion of ears in the whole plant for hybrid FD than hybrid F, regardless of DM content (see Table 1). Hybrid FD had lower aNDF, ADF contents and hemicellulose fraction than hybrid F (P<0.05, P<0.01 and P<0.05, respectively).

Total fibre content was lower in silage than fresh plant, i.e. aNDF content decreased from 398 to 377 g/kg DM. The conservation effect was even more significant (P<0.01) on hemicellulose content expressed as a fraction of aNDF (P<0.01). However, there was no significant conservation effect on starch and ADF content (Table 2).

3.2. Disappearance of dry matter (DM)

Effective DM degradability (ED4DM) was higher (P<0.01) for silage than for fresh maize (Table 3). This was mainly related to the differences in fraction (a) that was higher (P<0.01) in ensiled maize. Conversely, DM fraction (b) was lower (P<0.01) for ensiled than fresh maize and degradation rate (c) tended to be lower (P=0.07) for ensiled maize.

For fresh maize, ED4DM was higher for D1 than for FG samples (P<0.01) and for FG than D4 samples (P<0.01), largely due to differences between fractions (a). Fraction (b) of fresh maize was higher (P<0.01) for D4 samples than for the two other conditionings. The corresponding degradation rate (c) of fresh maize was higher (P<0.01) for FG samples than for the two other conditionings. For maize silage, the same effects of sample conditioning were observed on ED4DM and on parameters (a), (b) and (c), as there was no interaction between the effects of conservation and sample conditioning.

Hybrid and stage of maturity also had a significant (P<0.01) effect on ruminal DM degradation (Fig. 1A). ED4DM was higher (P<0.01) for hybrid FD than hybrid F, whatever the stage of maturity. This was mainly related to the potentially degradable fraction (b) which was higher (P<0.01) for hybrid FD than hybrid F. Conversely, fraction (a) was higher (P<0.05) for hybrid F than for hybrid FD. Hybrid had no effect (P=0.33) on DM degradation rate (c). Furthermore, ED4DM decreased significantly with stage of maturity (P<0.01), mainly due to the fact that rapidly-degradable fraction (a) decreased significantly with stage of maturity (P<0.01). The opposite trend was observed for parameters b and c which increased (P<0.01) with stage of maturity.

3.3. Disappearance of starch

Like for DM, the effective degradability of starch (ED5Starch) was higher (P<0.01) for ensiled than fresh maize (Table 3). This result was mainly related to rapidly-degradable fraction (a) and degradation rate (c) which were higher (P<0.01) for ensiled than fresh maize.

For fresh maize, ED5Starch was not different (P=0.99) between D1 and FG samples but was higher (P<0.05) for both conditioning methods than for D4 samples. Fraction (a) and degradation rate followed the same trend as ED5Starch: the highest values were observed for FG and D1 samples (P<0.01) without differences between these two conditionings. For maize silage, ED5Starch was highest for FG samples, followed by D1 samples, and was lowest for D4 samples (P<0.01). This was mainly related to the higher degradation rate (c) (P<0.01) for FG samples than for D1 and D4 which were similar. Fraction (a) was higher in D1 samples than FG samples (P<0.01) and higher in FG samples than D4 samples (P<0.01).

Contrary to DM, starch degradation (ED5Starch) was lower for hybrid FD (P<0.01) than hybrid F at maturity stage 1 but was higher for hybrid FD (P<0.01) than hybrid F at maturity stage 2 (Fig. 1B). This was mainly related to rapidly-degradable fraction (a) which was lower for hybrid FD (P<0.01) than for hybrid F at maturity stage 1. Like for DM, ED5Starch decreased
Table 2  
Effect of conservation, hybrid variety and stage of maturity on chemical composition of the whole-plant maize (g/kg DM) (n = 24).

<table>
<thead>
<tr>
<th>Conservation (C)</th>
<th>Fresh</th>
<th>Silage</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrid (H)</td>
<td>F</td>
<td>F</td>
<td>FD</td>
<td>FD</td>
</tr>
<tr>
<td>Maturity (M)</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Starch</td>
<td>286&lt;sup&gt;b&lt;/sup&gt;</td>
<td>378&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>348&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>427&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>aNDF&lt;sup&gt;A&lt;/sup&gt;</td>
<td>436&lt;sup&gt;a&lt;/sup&gt;</td>
<td>397&lt;sup&gt;b&lt;/sup&gt;</td>
<td>403&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>355&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADF&lt;sup&gt;B&lt;/sup&gt;</td>
<td>205&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180&lt;sup&gt;b&lt;/sup&gt;</td>
<td>183&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>155&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hemicellulose&lt;sup&gt;C&lt;/sup&gt; (as a fraction of aNDF)</td>
<td>0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

For each line, means with different superscript letters are significantly different at P<0.05.

<sup>A</sup> Neutral detergent fibre assayed with a heat-stable amylase and expressed inclusive of residual ash.

<sup>B</sup> Acid detergent fibre expressed inclusive of residual ash.

<sup>C</sup> Hemicellulose = (aNDF – ADF)/aNDF.
Table 3

Effect of maize conservation and sample conditioning on the ruminal in situ degradation of dry matter (DM), starch and NDF (n = 72).

<table>
<thead>
<tr>
<th>Variable of the kinetic model</th>
<th>Fresh Dried and ground to 4 mm (D4)</th>
<th>Dried and ground to 1 mm (D1)</th>
<th>Fresh-ground (FG)</th>
<th>Dried and ground to 4 mm (D4)</th>
<th>Dried and ground to 1 mm (D1)</th>
<th>Fresh-ground (FG)</th>
<th>SEM</th>
<th>P Conservation (C)</th>
<th>Sample conditioning (S)</th>
<th>Conservation × sample conditioning (C × S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM degradation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED4_Dm</td>
<td>0.55f</td>
<td>0.60b</td>
<td>0.57c</td>
<td>0.58c</td>
<td>0.64a</td>
<td>0.63a</td>
<td>0.006</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(a)</td>
<td>0.29f</td>
<td>0.37c</td>
<td>0.28e</td>
<td>0.33d</td>
<td>0.46e</td>
<td>0.41b</td>
<td>0.007</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>(b)</td>
<td>0.67a</td>
<td>0.58b</td>
<td>0.59b</td>
<td>0.59b</td>
<td>0.49c</td>
<td>0.46d</td>
<td>0.014</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(c)</td>
<td>0.033b</td>
<td>0.026b</td>
<td>0.040a</td>
<td>0.030b</td>
<td>0.026b</td>
<td>0.037a</td>
<td>0.002</td>
<td>0.07</td>
<td>&lt;0.01</td>
<td>0.54</td>
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<tr>
<td>Starch degradation</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED6_Starch</td>
<td>0.51e</td>
<td>0.68c</td>
<td>0.68c</td>
<td>0.66d</td>
<td>0.78b</td>
<td>0.83a</td>
<td>0.005</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(a)</td>
<td>0.09f</td>
<td>0.36b</td>
<td>0.33b</td>
<td>0.29c</td>
<td>0.56a</td>
<td>0.59a</td>
<td>0.012</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>(b)</td>
<td>0.91a</td>
<td>0.64c</td>
<td>0.67bc</td>
<td>0.71b</td>
<td>0.44d</td>
<td>0.41d</td>
<td>0.012</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>(c)</td>
<td>0.054c</td>
<td>0.062b</td>
<td>0.067b</td>
<td>0.067b</td>
<td>0.083a</td>
<td>0.002</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>aNDF degradation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED2_aNDF</td>
<td>0.50a</td>
<td>0.52a</td>
<td>0.41b</td>
<td>0.42b</td>
<td>0.50a</td>
<td>0.42b</td>
<td>0.012</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(a)</td>
<td>0.09b</td>
<td>0.22a</td>
<td>0.02c</td>
<td>0.09b</td>
<td>0.19a</td>
<td>0.10b</td>
<td>0.009</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(b)</td>
<td>0.72a</td>
<td>0.66ab</td>
<td>0.69ab</td>
<td>0.72a</td>
<td>0.64b</td>
<td>0.63b</td>
<td>0.020</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>0.17</td>
</tr>
<tr>
<td>(c)</td>
<td>0.030</td>
<td>0.025</td>
<td>0.039</td>
<td>0.030</td>
<td>0.031</td>
<td>0.033</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Log (c)</td>
<td>–1.32                  ab</td>
<td>–1.83b</td>
<td>–1.44a</td>
<td>–1.57b</td>
<td>–1.54ab</td>
<td>–1.49b</td>
<td>0.001</td>
<td>0.95</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>L1</td>
<td>3.01b</td>
<td>9.24a</td>
<td>7.08ab</td>
<td>10.90a</td>
<td>11.94a</td>
<td>8.97a</td>
<td>0.014</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>1 – [(a) + (b)]</td>
<td>0.19b</td>
<td>0.12c</td>
<td>0.28a</td>
<td>0.19b</td>
<td>0.17a</td>
<td>0.27a</td>
<td>0.020</td>
<td>0.26</td>
<td>&lt;0.01</td>
<td>0.12</td>
</tr>
</tbody>
</table>

For conservation effect (fresh and silage), means with different superscript letters in the same row are significantly different at P<0.05 (Tukey test).

A: ED4_Dm: Effective degradability of DM calculated by the model of Ørskov and Mc Donald (1979) (assuming a particle outflow rate of 0.04 h⁻¹).
B: Rapidly degradable fraction calculated by the model of Ørskov and Mc Donald (1979).
C: Potentially degradable fraction calculated by the model of Ørskov and Mc Donald (1979).
D: Rate of degradation (h⁻¹) calculated by the model of Ørskov and Mc Donald (1979).
E: ED6_Starch: Effective degradability of starch calculated by the model of Ørskov and Mc Donald (1979) (assuming a particle outflow rate of 0.06 h⁻¹).
F: Neutral detergent fibre assayed with a heat-stable amylase and expressed inclusive of residual ash.
G: ED2_aNDF: Effective degradability of aNDF calculated by the model of Dhanoa (1988) (assuming a particle outflow rate of 0.02 h⁻¹).
H: L: lag-phase time to degradation (h) calculated by the model of Dhanoa (1988).
I: 100 – [(a) + (b)]: Undegradable fraction calculated from parameters of the model of Dhanoa (1988).
as maturity stage increased (P<0.01), regardless of hybrid, due to the lower (P<0.01) fraction (a) and degradation rate of maturity stage 2 than maturity stage 1.

3.4. Disappearance of fibre (aNDF)

Unlike DM and starch, the effective degradability of aNDF (ED\textsubscript{2aNDF}) was higher (P<0.01) for fresh than ensiled maize, but only in D4 samples (Table 3). The opposite trend was observed for lag time (L) and fraction (a), which were higher for ensiled than fresh maize (P<0.01 but only for D4 conditioning and P<0.01 but only for FG samples, respectively). Potentially degradable fraction (b), degradation rate (c) or undegradable fraction (1 - [(a) + (b)]) did not significantly differ between fresh and ensiled maize whatever the conditioning.

For fresh maize, ED\textsubscript{2aNDF} was lower in FG samples than for D1 samples (P<0.01) and D4 samples (both similar, P=0.43). This was mainly related to fraction (a) which was lower for FG samples than D1 samples (P<0.01) and lower for D1 samples than D4 samples (P<0.01). Parameter (c) of fresh maize showed the opposite trend: (c) was higher (P<0.01) for FG samples than for D1 samples (with no difference between FG and D4 samples). For fresh maize, the same effects of sample conditioning methods were observed on fraction (b) and on undegradable fraction, as there was no interaction between the effects of conservation and sample conditioning. For ensiled maize, ED\textsubscript{2aNDF} was lower (P<0.01) in FG and D4 samples (both similar, P=1.00) than D1 samples (P<0.01). The same ranking between conditionings was observed for fraction (a). Fraction (c) was similar between D1 samples and D4 samples (P=0.99) and between D1 samples and FG samples (P=0.98). Ensiled maize showed the same effects of sample conditioning methods on parameters (b) and L and on fraction (a), as there was no interaction between the effects of conservation and sample conditioning.

Like with DM, aNDF degradation (ED\textsubscript{2aNDF}) was lower (P<0.01) for hybrid F (which had the highest average aNDF content) than hybrid FD regardless of maturity stage (Fig. 1C). This was mainly related to fraction (b), which was lower (P<0.01) for hybrid F than hybrid FD.
hybrid F than hybrid FD regardless of maturity stage. Degradation rate (c) followed the same trend (only for maturity stage 1) whereas undegradable fraction showed the opposite trend (only for maturity stage 2). Unlike DM and starch, maturity stage had no effect on aNDF degradation (P=0.26).

4. Discussion

The main objective of this work was to assess the effects of conservation and sample conditioning on the in situ degradability of DM, starch and aNDF in maize forage. The discussion of the results is therefore mainly focused on differences in degradation parameters between ensiled and fresh maize and between sample conditionings.

4.1. Effect of silage conservation on ruminal degradation

This study found strong differences in degradability between fresh and ensiled maize. Like DM degradation, starch degradability was higher than ensiled than fresh maize whereas DM and starch content were unaffected by ensiling. In agreement with other studies (Philippeau and Michalet-Doreau, 1998 on grain with an in situ method; Jurjanz and Monteils, 2005 on whole plant with an in situ method; Opsi et al., 2013 on whole plant with an in vitro method), the starch was degraded more rapidly (higher rapidly-degradable fraction (a) and higher degradation rate) in ensiled maize than fresh maize. The higher starch degradability with silage than fresh plant can be explained by partial hydrolysis of the protein matrix encapsulating starch granules (Gibbons et al., 2003) during the silage-induced fermentation process (Rooney and Pflugfelder, 1986; Jurjanz and Monteils, 2005; Hoffman et al., 2011). Indeed, studies (Baron et al., 1986; McAllister et al., 1993) have reported a partial hydrolysis of endosperm proteins in ensiled corn grain that involved the protein matrix losing protective ability.

Conversely, fibre degradability (aNDF) was lower in ensiled than fresh maize: ensiled maize led to a significantly longer lag to aNDF degradation than fresh maize. In vitro, Der Bedrossian et al. (2012) and Weinberg and Chen (2013) also found a lower ruminal degradability of fibre (aNDF) in ensiled than in fresh plant. In situ, Jurjanz and Monteils (2005) found the opposite trend. With the partial hydrolysis of hemicellulose in silage, the remaining aNDF in silage was probably less degradable than the aNDF in fresh plant, which could explain the longer lag time to degradation for silage. Moreover, bags have already been shown to be a micro-environment where pH and microbial activity depend more on the incubated feedstuffs than on ruminal conditions (Nozière and Michalet-Doreau, 1996; Nozière and Michalet-Doreau, 2000). It could thus be expected that pH would be lower in bags containing maize silage than bags containing fresh maize, which could further contribute to the lower aNDF degradation for silage than fresh plant.

4.2. Effect of the sample conditioning method on degradation in the rumen

The in situ method using undried and unground samples incubated in bigger (10 × 20 cm) bags has been employed by some teams (Priegge et al., 1984; Nocek, 1988; Andrae et al., 2001) but was not applied here as it limits the number of bags simultaneously present in the rumen.

Conditioning method also had significant effects on ruminal degradation of fresh and maize silage measured in situ. Fine-grinding samples have been shown to increase the rapidly-degradable fraction, partly due to an increase in soluble and insoluble particle losses through the pores of the bags. Other studies (Cerneau and Michalet-Doreau, 1991; Philippeau and Michalet-Doreau, 1998; Whadwa et al., 1998) suggest that finely-grinding samples could increase rapidly-degradable fraction (a), part of which is lost through the pores of the bags. This explains why DM, starch and aNDF degradability were significantly higher in dried samples ground to 1 mm than in the other two conditionings. Coarse-grinding (FG samples) also enhances starch degradability due to a higher subsequent degradation rate. One explanation for the faster degradation rate (c) of starch in FG samples was that the FG samples were not dried. Indeed, it cannot be ruled out that in dried samples (D1 and D4), a proportion of the protein from the matrix protecting the starch granule could have coagulated in response to heating at 60 °C for 72 h. This protein matrix coagulation could have limited the accessibility of starch to microbial enzymes and thus explain the lower starch degradation of dried samples (D1 and D4).

In contrast, FG conditioning was associated with the lowest aNDF degradability. The FG conditioning simulated soaked chewed particles during ingestion (Fernandez and Michalet-Doreau, 2002) but failed to take into account the essential chewing during rumination that reduces particle size to facilitate aNDF degradability by microbial enzymes (Beauchemin and Yang, 2005). Contrary to D1 and D4, the coarse grinding of the FG conditioning is likely insufficient to make aNDF accessible to microbial enzymes, which would explain the lower aNDF degradation of FG samples. As highlighted by Andrae et al. (2001), particle size has a variable effect on aNDF degradation measured in situ. For example, Johnson et al. (2002) found that ruminal NDF disappearance tended to increase when corn silage was mechanically processed whereas Bal et al. (2000) showed that NDF disappearance was unaffected by processing.

4.3. Effect of hybrid and maturity stage on ruminal degradation

In line with previous reports, hybrid (Verbic et al., 1995; Ngonyamo-Majee et al., 2009) and maturity stage (Correa et al., 2002; Johnson et al., 2002; Ramos et al., 2009) both had significant effects on the ruminal degradation of maize (especially starch). The significantly higher DM degradability of hybrid FD can be related to its higher starch content due to a higher
proportion of ears in the whole plant. Hybrid FD, characterized by flint-dent grains and richest in starch, also showed significantly higher starch degradability than hybrid F (but only at maturity stage 2). One hypothesis is that grain structure (determining the starch degradation rate) has an effect on the ruminal starch degradability. At equal proportions of corn grain DM, flint-dent-grain hybrid is more starch-degradable than flint-grain hybrid (according to Philippeau and Michalet-Doreau, 1997). Flint-grain starch granules are wrapped in protein aggregates and embedded in a dense matrix that limits the action of hydrolytic enzymes (Philippeau and Michalet-Doreau, 1997). This could be the reason why flint corn showed lower degradability than flint-dent corn. aNDF showed the same trend, with hybrid FD (less rich in aNDF than hybrid F) posting the highest degradability.

This study clearly confirmed the decrease in whole-plant DM degradation with increasing maturity stage, regardless of hybrid, in agreement with Philippeau and Michalet-Doreau (1997) and Jensen et al. (2005). Starch degradation reproduced this hybrid-independent decrease, whereas aNDF degradation showed no effect of maturity stage. The greater rapidly-degradable fraction means that the higher ruminal starch degradability of maize harvested at early stage could be explained by a low core grain vitreousness. Philippeau and Michalet-Doreau (1998) effectively showed that immature (stage 1) grain with lower vitreousness has higher starch degradability than mature (stage 2) grain. The vitreous endosperm characterizing flint corn grows with stage of maturity and will thus limit the action of hydrolytic enzymes as soon as the plant becomes relatively mature (Philippeau and Michalet-Doreau, 1997).

5. Conclusions

Ensiling increased the ruminal degradation of maize DM. This difference in favour of silage seemed to be due to a higher degradability and degradation rate of starch, since fibre degradation was lower in ensiled than fresh maize. This may be explained by proteolysis during the ensilage process that promotes the solubilization of starch granules, and by silage acidity that enhances the hydrolysis of hemicellulose in the aNDF fraction. The insights reported here make it clear that using fresh-plant samples to evaluate maize silage degradability will underestimate starch degradability, although fresh samples are far more convenient than silage samples for large screening trials on multiple varieties. This study confirms that sample conditioning method has a significant effect on starch and aNDF degradability measurements. Drying at 60 °C and grinding samples at 4 mm (the ‘D4’ conditioning here) looks to be a good compromise for the in situ study of maize forage, as it limits early-degradation-process starch losses and ensures sufficient availability of cell walls for reliably measuring anD degradability. Furthermore, this method reproduces differences in degradability between hybrids and maturity stages that are consistent with the literature, and it could thus be proposed as a benchmark method for in situ studies of starch-rich forages.

Conflict of interest statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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