SHORT COMMUNICATION

Effects of hybrid and maturity stage on in vitro rumen digestibility of immature corn grain

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Abstract

This study aimed to evaluate the influences of hybrids (HYB) and maturity stage (SAMP) on in vitro rumen digestibility of immature corn grain. Four HYB (Gigantic, Y43, Klips and 9575) from the FAO group 700 were grown under identical agronomic conditions. First sampling (T1) was done after 95 days from seedling and then 4, 8, 13, 18 and 27 days later (T2 to T6). In vitro starch digestibility (STD_7h) and gas production (72 h) were measured. Whole plant and grain dry matter (WP_DM and GR_DM, respectively) and zein content were significantly affected (P<0.01) by HYB and SAMP. Starch content was significantly affected by HYB, SAMP and their interaction. It increased from T1 to T4 (from 67.47 to 72.82% of GR_DM) and then tended to plateau. Concurrently, STD_7h significantly decreased with advancing SAMP and was also affected by HYB. With advancing maturity, total volatile fatty acids (VFA) significantly decreased, with an increase of acetate and a decrease of propionate molar proportion (P<0.01). Gas production rate (GP_c) was significantly affected by HYB, SAMP and HYB×SAMP. Whole plant grain DM correlated (P<0.01) positively with grain starch content (r=0.60 and 0.64) but negatively with STD_7h (r=−0.39 and r=−0.63) and VFA concentration (r=−0.59 and −0.75). Zein percentage in crude protein negatively affected (P<0.01) total DM (r=−0.65), STD_7h (r=−0.73) and GP_c (r=−0.68). Results suggest that genotypes and maturity stages influence DM and rumen starch digestibility of immature corn grain and in this respect zein can play a significant role.

Introduction

Nutritive value of corn silage can be affected by genetic, agronomic, harvesting and preservation practices (Johnson et al., 2002b). In recent years, seed companies have increased the interest in selecting corn hybrids specialised for whole plant corn silage production (Johnson et al., 1997; Kuehn et al., 1999). Rumen starch fermentability can affect the nature and amounts of nutrients delivered to the animal and, in turn, milk yield and composition (Nociek and Tamminga, 1991). The stage of maturity of whole corn plants at harvest may affect starch digestibility (STD_7h) (Harrison et al., 1996) and consequently the energy supply to the animal and its milk yield (Bal et al., 1997).

Ruminal degradability of non-fibrous carbohydrates in corn grain largely determines the energy value of this feed (Allen, 1997). The physical structure of starch granules in corn grain influences digestibility of the stored carbohydrates. The texture of the corn grain is determined by the amount of vitreous endosperm proportionally to the farinaceous endosperm (Watson, 1998). Zein is a major component of the protein matrix that envelopes corn starch granules and largely affects its rumen and total tract digestibility (Philippeau et al., 2000; Correa et al., 2002). Some recent researches evaluated the corn germplasms for the differences in ruminal starch degradability (Philippeau and Michalet-Doreau, 1997; Correa et al., 2002; Johnson et al., 2002b; Taylor and Allen, 2005) to improve the grain and silage utilisation in ruminants; nonetheless, ruminal digestion of the immature grain fraction has not been widely investigated yet (Verbi et al., 1995). Thus, the objective of this study was to evaluate the changes in chemical composition of grain from different corn hybrids at different stage of maturity and the effects on in vitro ruminal STD_7h.

Materials and methods

Cultivation of different corn hybrids were carried out at the experimental farm Vittorio Tedini, Podenzano (PC), Italy (44°57′26.28″ N; 9°41′9.60″ E). The local climate is temperate or type C according to the Köppen-Geiger climate classification (Peel et al., 2007). In terms of rainfall, the area is characterised by typical regime sublittoral with a total annual rainfall equal to 850 to 900 mm distributed on 80 to 85 rainy days. The soil (sand 31.0%, silt 52% and clay 16%) was classified as silt loam (USDA-NC, 1999).

Four corn (Zea mays L.) hybrids belonging to Food and Agriculture Organization (FAO) maturity class 700, 135 CRM group were used: Gigantic (Monsanto, St. Louis, MO, USA), Y43 (Pioneer, Johnston, IA, USA), Klips and 9575 (KWS Saat, Einbeck, Germany). The hybrids were seeded according to a randomised block design with three replicates per hybrid. Parcels were 8 rows large, at a spacing of 70×19 cm, seeded on May 28, 2010 and harvested at six maturity times.

Top dressing fertilisation was made with 200 kg/ha of N after hoeing. For weed control ACETOCLIC (Sipcam Italia S.p.A., Pero, MI, Italy) and MERLIN (Bayer Cropscience Srl, Hyderabad, India) were used at the dosage of 4 L/ha (Acetochlor 1.148 kg/ha + Terbutylazine 0.574 kg/ha + Furlazole 0.384 kg/ha) and 1.2 L/ha (Isoxaflutole 52.8 g/ha), respectively.

First harvest time of grain was planned in order to have a whole plant dry matter (DM) content within the optimal range suggested for ensiling in horizontal silos (Mueller and Green, 2001). Whole plant moisture content was monitored collecting random samples...
from the whole field every other day around the target date. First sampling (T1) was done on August 31, 95 days after the seeding date. This happened four days later than the date foreseen based on field monitoring and due to adverse weather conditions. Subsequent samplings were made after 4, 8, 13, 18 and 27 days (T2, T3, T4, T5 and T6, respectively) from T1.

For each hybrid, at each maturity stage, 4 ears were randomly selected from the central rows excluding the first meter at the front and rear border of each parcel. Grains from the same sampling time were pooled from each parcel and dried at 55°C in a forced air oven until constant weight was achieved. At the same sampling time, 6 whole plants per parcel were also harvested, pooled and cut at a 0.7 cm theoretical length. Dry matter content was measured on representative sample by oven drying at 55°C.

Pre-dried samples were ground to pass through the 1 mm sieve of a knife mill (Wiley mill model 3; Thomas Scientific, Swedesboro, NJ, USA). Ground samples were analysed for: crude protein (CP) (AOAC 990.03), ash (AOAC 942.05), total soluble sugar (phenol-sulfuric acid assay; DuBois et al., 1956) and starch content (AOAC 996.11) (AOAC, 1995). Zein was extracted and the initial DM.

The average GR_DM was 47.64% at T1 and 67.12% at T6. Hybrids had a significant effect (P<0.01) on DM. In particular, Gigantic 9575 had the highest DM throughout all the period, and Gigantic tended to have a lower DM. The interaction HYB×SAMP was not significant, suggesting a similar rate of DM change in all the hybrids. The plant DM increased linearly throughout the experimental period by 0.50 points per day (r=0.88, P<0.01).

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**Results and discussion**

Whole plant and grain dry matter (WP_DM and GR_DM, respectively) contents are shown in Table 1. The mean WP_DM at T1 was 33.05% and at T6 it was 45.59%. There were significant effects of HYB and SAMP (P<0.001) on WP_DM. Hybrid 9575 had the highest DM throughout all the period, and Gigantic tended to have a lower DM. The interaction HYB×SAMP was not significant, suggesting a similar rate of DM change in all the hybrids. The plant DM increased linearly throughout the experimental period by 0.50 points percentage per day (r=0.88, P<0.01).

The average GR_DM was 47.64% at T1 and 67.12% at T6. Hybrids had a significant effect (P<0.01) on DM. In particular, Gigantic showed significantly lower mean DM (53.66%, P<0.05), whereas Klips and 9575 had the highest DM (59.87%, P<0.05). The interaction HYB×SAMP was not significant, suggesting a similar rate of DM change in all the hybrids. The increase of grain DM was linear and as high as 0.71 point percentage per day (r=0.88, P<0.01). Grain dry matter was highly correlated to WP_DM (r=0.83, P<0.01).

For whole plant silage, the optimal harvest point is established based on the achievement of i) maximum DM yield per hectare, reached
between 2/3 milk line and black layer stage of maturity (Ganoe and Roth, 1992), and ii) a DM content high enough to avoid silage effluent production but not too high to allow an efficient packing. These goals are achieved when DM ranges from 30 to 40%, depending on the kind of silo used (Mueller and Green, 2001). The beginning of the controls was conditioned by adverse weather conditions that caused a delay in the date of first control, which had been foreseen on the basis of preliminary tests. Dry matter percentage of whole plant at T1 (33.05%) was almost in the middle of the interval (30 to 35% DM) suggesting that is was suitable for being ensiled in horizontal silos (Mueller and Green, 2001). This also coincides with the time at which the whole corn plant reaches the maximum nutritional value. Extension specialists (Petze and Bertoldo, 2011) suggested that for proper fermentation of corn silage an optimal DM range is between 32 and 38%. In the last sampling time (T6), 27 days after the T1, DM was on average 45.59%, higher than the upper limit of that range. Controls were then extended to monitor changes in grain quality to almost the black layer stage (not reached in this trial), when a significant decrease of digestibility is reported to occur (Bal et al., 1997). The high correlation between WP_DM and GR_DM is in agreement with the observation of Vattikonda and Hunter (1983). The ratio between the changes of DM content in the grain and in the whole plant (0.71/0.50 points percentage per day) was similar to the value usually reported of 1.4 (Vattikonda and Hunter, 1983).

Crude protein, starch, soluble sugars, crude fat, ash and zein contents of the grain of different hybrids are shown in Table 1. The CP content slightly decreased during the sampling period, from 9.23 to 8.81% of DM, and it was significantly influenced by SAMP, HYB and HYB×SAMP. Hybrid Y43 showed the highest (9.62% DM on average) and 9575 the lowest CP content (8.08% DM). Associated with advancing maturation, a slow decline of CP and a much more marked increase of starch contents occurred. Similar decline in CP content is frequently reported in the literature (Zeoula et al., 2003; Xu et al., 2010) and largely attributable to the diluting effect of starch accumulation. On the contrary, Masero et al. (2011) found an increase of CP at black layer, a stage of maturity.

The crude fat content remained almost constant through maturation, and on average it was 3.80% of grain DM. Significant differences were recorded between hybrids, Gigantic and Y43 having a significantly higher (P<0.01; 3.39% of DM, on average) crude fat compared to Klips and 9575 (3.61% of DM, on average).

The starch level was significantly affected by HYB, SAMP and their interaction. Overall, it increased from T1 to T4 (from 67.47 to 72.82% of DM) and then tended to stabilise. Hybrid 9575 showed a more rapid increase across the first 4 sampling dates and the highest mean content (73.77% of DM; P<0.05), whereas the other three genotypes had similar levels (on average 70.52% of DM). The increase of starch content was pronounced and clearly followed a curvilinear pattern tending to plateau at T5 (115 days after sowing), when WP DM was on average 41.34% and GR DM 62.76%. Similar increase of starch content at comparable stages of maturity is reported by other authors for corn grain (Masoero et al., 2011; Thornton et al., 1969) and whole plant (Philippeau and Michalet-Doreau, 1997; Sutton et al., 2000; Andrae et al., 2001; Johnson et al., 2003; Jensen et al., 2005). Differences in chemical composition among corn genotypes are also widely reported in the literature. Similar and even more pronounced differences were found for corn grain obtained from genetically different hybrids (Watson, 2003; Berard et al., 2009), but also for whole corn plant (Johnson et al., 2002a) and its fractions (Masero et al., 2006).

Soluble sugars were significantly affected by HYB, SAMP and their interaction. Sugar content progressively declined in all HYB through maturity from, on average, 10.70% of DM at T1 to 5.49% at T6, and it was negatively correlated with stalk content (r=-0.72, P<0.01; Table 2).

The zein content of corn grain was calculated as zein in % of DM, % of CP and % of starch in the grain. The average zein content was 2.35% GR_DM and it was significantly affected (P<0.01) by both HYB and SAMP. As a percentage of CP, zein increased from 23.77% at T1 to 28.30 at T6 and was also markedly influenced (P<0.01) by both HYB and SAMP. When expressed as percentage of total starch, zein was 3.33% with a slight increase through maturation, from 3.28 (T1) to 3.45% (T6). Among HYB, zein ranged between 2.66% (for 9575) to 3.84% of starch (for Y43) and also differed significantly (P<0.01). Zein content was analysed by an original method that used an extraction procedure derived from the method of Drochioiu et al. (2002). In a preliminary unpublished comparison carried out on 20 samples of immature corn grains, this method appeared to be well correlated (r=0.86) with a line fitted to the data of the laboratory method.

Table 1. Whole plant and corn grain dry matter and chemical composition of corn grain collected at different sampling times from different hybrids.

|          | SAMP          | HYB           | SEM          | P           |
|----------|---------------|---------------|--------------|-------------|
|          | T1            | T2            | T3           | T4          | T5          | T6          | Gigantic    | Klips       | Y43         | 9575        | SAMP         | HYB         | HYB×SAMP    |
| WP_DM,%  | 33.05±        | 34.22±        | 33.15±       | 37.68±      | 41.34±      | 45.59±      | 34.73±      | 38.08±      | 36.06±      | 41.13±      | 0.6159       | 0.0001      | 0.0002      | 0.0675      |
| GR_DM,%  | 47.64±        | 52.33±        | 55.49±       | 60.25±      | 62.76±      | 67.12±      | 53.66±      | 60.06±      | 57.00±      | 59.67±      | 1.0560       | 0.0001      | 0.0003      | 0.1340      |
| CP,%DM   | 9.23±         | 9.20±         | 8.83±        | 8.66±       | 8.74±       | 8.61±       | 9.00±       | 9.08±       | 9.62±       | 8.08±       | 0.04197      | 0.0001      | 0.0001      | 0.0001      |
| Starch,%DM | 67.47±       | 70.33±       | 71.69±       | 72.82±      | 73.94±      | 72.70±      | 71.09±      | 70.23±      | 70.24±      | 73.77±      | 0.2551       | 0.0001      | 0.0001      | 0.0001      |
| Sugar,%DM | 10.70±        | 8.76±         | 7.19±        | 6.99±       | 5.84±       | 5.49±       | 8.01±       | 7.22±       | 8.09±       | 6.63±       | 0.2453       | 0.0001      | 0.0004      | 0.0001      |
| Ash,%DM  | 3.86±         | 3.70±         | 3.82±        | 3.81±       | 3.78±       | 3.83±       | 3.98±       | 3.93±       | 4.00±       | 3.57±       | 0.06154      | 0.0001      | 0.0018      | 0.0001      |
| Zein,%DM | 1.70±         | 1.62±         | 1.51±        | 1.46±       | 1.42±       | 1.41±       | 1.58±       | 1.48±       | 1.52±       | 1.49±       | 0.01411      | 0.0001      | 0.0001      | 0.0001      |
| Zein,C%  | 23.77±        | 24.73±        | 24.28±       | 28.04±      | 28.33±      | 28.30±      | 24.98±      | 27.63±      | 28.08±      | 24.28±      | 0.484±       | 0.0001      | 0.0001      | 0.2200      |
| Zein,St% | 3.28±         | 3.24±         | 3.01±        | 3.44±       | 3.41±       | 3.45±       | 3.15±       | 3.57±       | 3.84±       | 2.68±       | 0.06629      | 0.0001      | 0.0001      | 0.1173      |

SAMP: sampling date; HYB: hybrid; T1: sampling done after 35 days from seeding; T2: sampling done after 4 days from T1; T3: sampling done after 8 days from T1; T4: sampling done after 13 days from T1; T5: sampling done after 18 days from T1; T6: sampling done after 27 days from T1; HYB×SAMP: interaction between SAMP and HYB; WP_DM: whole plant dry matter; GR_DM: grain dry matter; CP: crude protein; CF: crude fat. *Means with different superscripts in the same row differ significantly within SAMP or HYB (P<0.05).
that of Larson and Hoffman (2008) and similar absolute values (2.24 and 2.31% of DM, for this and the Larson and Hoffman method, respectively) were produced. Giuberti et al. (2011) obtained higher zein values for dry corn grains with the method of Larson and Hoffman (2008) (3.30% of DM) compared with that of Drochioiu et al. (2002), with a regression coefficient (R²) as high as 0.60. The same authors reported 8 different samples of dry corn grain, the average zein values ranging from 1.73 to 3.46% of DM, depending on the analytical method used. During the 4-week sampling period, zein increased and the increase was more evident when zein was expressed as a percentage of CP; on the contrary, when zein was expressed on a DM basis these changes were much less pronounced, as the accumulation of starch in the kernel hid this increase.

Little data exists on corn grain zein content at this stage of maturity. Murphy and Dalby (1971) reported an increase in the proportion of zein in corn endosperm protein more marked 30 days after pollination which, in the present research, occurred around T1.

**Seven hour in vitro digestibility trial**

In Table 3, the results from 7 h *in vitro* digestibility trial are presented for total dry matter (DMD_7h) and STD_7h of the corn grain. In both cases, digestibility decreased with advancing maturity and was significantly affected (P<0.01) by SAMP and HYB. Interaction HYB×SAMP was not significant for both parameters suggesting that despite different absolute values, all the hybrids had a similar decline in digestibility. The mean DMD_7h at T1 was 45.42% but at T6 it was 35.18%. The STD_7h behaved similarly to DMD_7h, but it exhibited higher levels at any sampling time, revealing that starch was more digestible than the other components of the grain. At T1 a mean value of 55.22% was recorded, whereas at T6 it decreased to 41.85%. Starch digestibility appeared to have a negative correlation (Table 2) with whole plant (r=-0.41; P<0.01) and grain (r=-0.64, P<0.01) DM content. Starch Kₚ (Table 3) was mathematically derived from STD_7h and consequently it was not statistically evaluated. It was 0.0440/h on average, decreasing from 0.0540 at T1 to 0.0364/h at T6. *In vitro* digestibility of DM and STD_7h followed a similar pattern of variation during maturation among the HYB and these two parameters also highly correlated (r=0.96, P<0.01). This outcome was expected as starch accounted for about 70% of total grain DM and

### Table 2. Correlation between main parameters measured on corn whole plant (only dry matter) and grain collected at different sampling times from different hybrids.

| Item          | GR_DM | CP     | Starch | Sugar | Zein, % DM | Zein, % CP | Zein, % starch | DMD_7h | STD_7h | VFA     | GP_b   | GP_c   |
|---------------|-------|--------|--------|-------|------------|------------|---------------|--------|--------|---------|--------|--------|
| WP_DM, %     | 0.83* | -0.46* | 0.60*  | -0.72* | 0.39*      | -0.41*     | -0.59*        | 0.48*  | -0.67* |
| GR_DM, %     | -0.34* | 0.64*  | -0.89* | 0.33** | 0.61*      | -0.63*     | -0.75*        | 0.55*  | -0.87* |
| CP, % DM     | -0.67* | 0.44*  | 0.65*  | 0.26** | 0.75*      | -0.24**    | -0.49*        | 0.50*  | -0.55* |
| Starch, % DM | 0.72*  | -0.41* | -0.65* | 0.75*  | -0.75*     | -0.46*     | 0.34**        | 0.68*  |
| Sugar, % DM  | -0.45* | 0.62*  | 0.61*  | 0.72*  | 0.54*      | 0.86*      |
| Zein, % DM   | 0.90*  | 0.97*  | -0.60* | -0.64* | -0.25**    | -0.45*     |
| Zein, % CP   | 0.81*  | -0.65* | -0.73* | -0.46* | 0.34**      |
| Zein, % starch | -0.49* | -0.54* | -0.29** |
| DMD_7h, %    | 0.86*  | 0.61*  | 0.80*  |
| STD_7h, %    | 0.55*  | -0.35** |
| Starch_Kₚ/h  | 0.46*  | -0.29** |
| VFA, mmol/L  | 0.77*  |
| GP_b/h       | -0.50* |

**GR_DM**—corn grain dry matter; **CP**—crude protein; **DMD_7h**—dry matter digestibility after 7 h in *in vitro* fermentation; **STD_7h**—grain starch digestibility after 7 h in *in vitro* fermentation; **VFA**—volatile fatty acids; **GP_b**—maximum potential gas production; **GP_c**—gas production rate; **WP_DM**—whole plant dry matter; **starch_Kₚ**—starch digestion rate. *P<0.01, **P<0.005. Only significant values are showed.

### Table 3. Dry matter and starch digestibility, fermentation rate of starch and volatile fatty acids with their molar percentage.

|                      | SAMP | HYB | SEM | P           |
|----------------------|------|-----|-----|-------------|
|                      | T1   | T2  | T3  | T4  | T5  | T6  | Gigantic | Klips | Y43 | 9575 | SAMP | HYB | HYB×SAMP |
| DMD_7h, %            | 45.42| 41.14 | 39.68*| 37.66*| 36.07*| 35.18*| 39.31*| 36.72*| 37.72*| 43.03*| 0.6172| 0.0001| 0.0001| 0.0733|
| STD_7h, %            | 55.22| 51.39 | 48.46*| 45.92*| 45.61*| 41.85*| 48.76*| 45.11*| 45.50*| 52.28*| 0.9591| 0.0001| 0.0009| 0.1648|
| Starch_Kₚ/h          | 0.0540| 0.0483 | 0.0445 | 0.0413 | 0.0397 | 0.036 | 0.0452 | 0.0404 | 0.0408 | 0.0498 |
| VFA, mmol/L          | 36.30| 38.37 | 37.28*| 35.32*| 35.95*| 34.27*| 36.89*| 35.94*| 37.23*| 36.52**| 0.4991| 0.0001| 0.0764| 0.0617|
| Acetate, % mol       | 64.27| 64.48 | 65.05*| 65.20*| 65.42*| 65.53*| 64.82*| 65.17*| 65.30*| 0.1362| 0.0001| 0.0651| 0.0994|
| Propionate, % mol    | 22.83| 22.43 | 21.93*| 21.82*| 21.67*| 21.45*| 22.40*| 21.93*| 21.29*| 21.95*| 0.1138| 0.0001| 0.1181| 0.0750|
| Butyrate, % mol      | 10.61| 10.72 | 10.58 | 10.73 | 10.58 | 10.68 | 10.66*| 10.49*| 11.02*| 10.43*| 0.0923| 0.0001| 0.0638| 0.0720|
| Isovalerate, % mol   | 2.21 | 0.27  | 0.31  | 0.11  | 0.18  | 0.16  | 0.19  | 0.24  | 0.24  | 0.17  | 0.0699| 0.1977| 0.0762| 0.3689|
| Valerate, % mol      | 1.01 | 1.03  | 1.03  | 1.03  | 1.03  | 1.03  | 1.01  | 1.05  | 1.05  | 0.99  | 0.0234| 0.0447| 0.1239| 0.5754|
| Isovalerate, % mol   | 1.07*| 1.06* | 1.09* | 1.12* | 1.11* | 1.15* | 1.07* | 1.19* | 1.13* | 1.08* | 0.0205| 0.0457| 0.0045| 0.6600|

**SAMP**—sampling date; **HYB**—hybrid; **T1**—sampling done after 95 days from seeding; **T2**—sampling done after 4 days from T1; **T3**—sampling done after 8 days from T1; **T4**—sampling done after 13 days from T1; **T5**—sampling done after 18 days from T1; **T6**—sampling done after 27 days from T1. *Means with different superscripts in the same row differ significantly within SAMP or HYB (P<0.05). Measurements were made after 7 h *in vitro* fermentation of corn grain collected at different sampling times from different corn hybrids.
is one of its most digestible components. This results confirms that GR_DM digestibility can represent a good estimate of STD_7h, as already proposed by Correa et al. (2002) for mature grains. The level of STD_7h in this study (47.88% on average) is lower than the values (73.3 to 80.1%) reported for corn silage in the USA by Sniffen et al. (2009). Sniffen and Ward (2011) indicated an average STD_7h of 62.0±9.7% for corn silage. It is known that ensiling increases starch rumen digestibility, and this increase also depends on the length of conservation (Mahanna, 2005). The differences between our results and those reported above can be attributed to using unfermented grain, but laboratory methodological differences may have also played an important role. According to Huhtanen and Svinnebjörnsson (2006), digestibility of starch is a difficult parameter to measure, and every method has pros and cons. In vitro digestibility with enzymes or rumen fluid is suggested to be a valuable method to compare samples of similar origin but not to estimate in vivo ruminal or total tract digestibility (Allen, 2012). Starch digestibility decreased markedly during the 4 week long monitoring period in all the hybrids, but Klips tended to reach the minimum earlier (at T5) and remained almost constant at T6. Similar decreases in STD_7h were reported for whole plant corn, fermented or unfermented, harvested from half milk line to black layer or within a whole plant content range similar to that of the present experiment (Andrae et al., 2001; Johnson et al., 2005; Jensen et al., 2005; Masoero et al., 2011). Others authors, on the contrary, found no significant or very limited effects of maturation on STD_7h. Ngonyamo-Majee et al. (2008) worked on corn grain of 33 inbred lines selected for starch and endosperm characteristics and found a significant but limited decrease from half milk line to black layer. Johnson et al. (2002b) reported no significant changes in starch rumen digestibility on different hybrids from 1/3 milk line to black layer.

Correa et al. (2002) found a decrease of ruminal starch availability after black layer stage of maturity but not between half milk line and black layer. Starch Rs, mathematically linked to STD_7h, obviously presented the same behaviour and differences among HYB. It declined from 0.0553 to 0.0491/h. The low temperature (55°C) adopted to dry the grains in this study should not negatively affect their digestibility. Similar rates of starch digestion in the rumen were reported for unfermented and fermented immature corn grain and whole plant (Philippeau and Michalet-Doreau, 1997; Andrae et al., 2001; Johnson et al., 2003; Jensen et al., 2005). Based on a database of in situ degradation from 48 references Offner et al. (2003) attributed to ground corn a degradation rate of 0.0550/h. Higher digestibility rates of corn silage starch are computed also in the Cornell Net Carbohydrate and Protein System (Lanzas et al., 2007). This discrepancy can partially arise from the techniques adopted to measure the digestibility. Secondary particle loss during the in situ incubation can lead to an overestimation of STD_7h, as already outlined by Huhtanen and Svinnebjörnsson (2006) for neutral detergent fibre.

Concentration of VFA measured in the fermentation liquid at the end of the 7h in vitro digestibility trial (Table 3) decreased with increasing maturity, in agreement with the similar trend of DMD_7h and STD_7h (from 39.60 mmol/L at T1 to 34.27 at T6). Significant differences exist among SAMP (P<0.05), but not in the HYB for VFA. Molar proportions of acetate and propionate (Table 3) in total VFA had opposite trends during maturation, although their variations were quantitatively small. Acetate increased from 64.27% at T1 to 65.53% at T6 and, conversely, propionate reduced from 22.83% at T1 to 21.45% at T6. Significant differences existed among HYB for acetate (P<0.01), but not for propionate. Butyrate was on average 10.63% of total VFA, and this proportion did not change significantly with the advancing stage of maturity, but a significant though quantitatively small effect was evidenced for HYB (P<0.001). Minor VFA isobutyrate, valerate and isovalerate represented on average about 2.5% of total VFA and remained unaffected by SAMP. Valerate concentration was not affected, but isovalerate appeared to be significantly influenced by SAMP (P=0.05) and HYB (P<0.01). The effect of advancing maturity on VFA molar proportions, with the progressive rise of the concentration of acetate and the reduction of propionate is in agreement with the reduction of starch availability and degradation rate. Sutton et al. (2000) reported an almost constant STD_7h for whole plant silages when harvested at 23 or 33% of DM and a marked reduction at 38% of DM. In that case, VFA rumen concentration of dairy cattle fed diets formulated with the different silages showed a non-significant tendency towards an increase of acetate and a decrease of propionate with advancing maturity.

### In vitro gas production

**In vitro gas production**

In vitro gas production was carried out for 72 h in order to allow a good estimation of the parameters describing the kinetics of fermentation (Table 4). In general, GP_b was similar for all the hybrids and increased progressively from T1 to T5, but at T6 a decrease was recorded. The in vitro potential gas yield increased until T5 and then decreased for all the hybrids. If the initial increase can be explained by the increase of starch in grain, the final decrease is justified by the decline of starch fermentability. The declining pathway of rate of gas production from T1 to T6 clearly reflects a progressively reduced availability, mainly attributable to the starch component of GR_DM as maturation progresses. The potential gas yield was significantly correlated with DMD_7h and STD_7h, which is in agreement with Rymer and Givens (1999) and Chai et al. (2004). The GP_c was on average 0.0515/h, and was significantly affected by HYB, SAMP and their interaction. The Klips variety showed

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**Table 4. pH and kinetic parameters of gas production calculated from a 72 h in vitro fermentation of corn grains collected at different sampling times from different hybrids.**

|            | SAMP | HYB | SEM | P      |
|------------|------|-----|-----|--------|
| pH         | 6.34 | 6.34 | 6.34 | 6.33   |
| GP_b, mL/g OM | 380.92 | 381.83 | 387.35 | 386.33 |
| GP_c, h⁻¹    | 0.0553 | 0.0550 | 0.0517 | 0.0503 |

**SAMP, sampling date; HYB, hybrid; T1, sampling done after 95 days from seeding; T2, sampling done after 4 days from T1; T3, sampling done after 8 days from T1; T4, sampling done after 13 days from T1; T5, sampling done after 18 days from T1; T6, sampling done after 27 days from T1; HYB×SAMP, interaction between SAMP and HYB; GP_b, maximum potential gas production; OM, organic matter; GP_c, gas production rate. “Means with different superscripts in the same row differ significantly within SAMP or HYB (P<0.05).**
a lower rate of gas yield that was consistent across sampling times. Hybrid Klips showed the lowest fermentation rate, in agreement with the levels of DMD_7h and STD_7h of this hybrid.

With advancing maturity, the rate of gas production slightly but regularly decreased from 0.0553/h at T1 to 0.0491/h at T6. This behaviours was evident in all hybrids, but between T5 and T6 the decline tended to be reduced. Fermentation rates were very similar to the values of Starch_Kd. These results confirm, by a different technique, Starch_Kd calculated from STD_7h. For corn grain, gas production rate can be assumed to largely reflect starch fermentation rate (Chai et al., 2004), as starch represents the majority of fermentable OM. This assumption was confirmed here by the high correlation found between GP_c and STD_7h (r=0.75, P<0.01). In a previous study (Ahmed et al., 2013), we measured the rates of gas production in a similar range for naturally occurring soluble sugars, except glucose, fructose and sucrose that had a k_d of about 0.10/h.

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