Influence of Genotype and Ensiling of Corn Grain on In Situ Degradation of Starch in the Rumen

C. PHILIPPEAU and B. MICHALET-DOREAU[1]
Station de Recherches sur la Nutrition des Herbivores,
Institut National de la Recherche Agronomique,
63122 St Genès Champanelle, France

ABSTRACT

This trial was conducted to determine the influence of genotype and ensiling of corn grain on the rate and extent of ruminal starch degradation. Two cultivars of corn that differed in texture of the endosperm, dent (*Zea mays* ssp. *indentata*) or flint (*Zea mays* ssp. *indentura*) were harvested at 30% whole-plant dry matter (DM). After separation from stover and cob, the kernels were coarsely chopped and ensiled or not ensiled. Grains were oven-dried at 40°C and either ground through a 3-mm sieve or left unground. Ruminal DM and starch degradabilities were determined using the in situ technique. The proportion of starch lost through the pores of the bag without degradation was also determined. Mean ruminal DM and starch degradabilities were higher for ground grains than for chopped grains, which could be related to the proportion of DM and starch lost through the pores of the bag. For unensiled, chopped grain, ruminal starch degradability was higher for dent corn than for flint corn (72.3% vs. 61.6%). The ensiling process increased ruminal starch degradability, averaging 5.8 percentage units. The difference in ruminal starch degradability between dent corn and flint corn remained constant whether the corn was unensiled or ensiled (10.7 vs. 11.6 percentage units).
(**Key words**: corn, silage, rumen, starch digestion)

INTRODUCTION

Corn that is harvested for silage is currently a major forage component in the diets of cattle; more than 3,500,000 ha of corn are grown for this purpose in the European Union. Corn that is harvested for silage offers numerous advantages as a forage source. Agronomically, corn silage is easy to produce and store, and, nutritionally, it is readily accepted by cattle and provides a basic diet that is rich in energy. Corn that is harvested for silage differs from other forage sources because of the presence of grain, which represents about 45% of the whole-plant DM. The rate and site of digestion of each of the two fractions, starch in the grain and cell wall in the stover, are different. Although digestion of the cell-wall fraction takes place mainly in the rumen, that of the starch fraction occurs at different segments within the gastrointestinal tract. The site of digestion of dietary starch strongly influences the nature of the end products of digestion and how starch is utilized by the animal (13, 21, 28). One way to modify the site of starch digestion is to select corn cultivars with different rates of starch degradation in the rumen. Choice of corn cultivars has been shown to affect in situ ruminal digestion of corn silage DM (1) and animal performance (3, 4). The effects of genetic variability on ruminal cell-wall digestion of corn stover have prompted numerous studies of corn hybrids (9, 12, 30, 32). In situ ruminal cell-wall degradation studies were used to estimate the physical fill of feedstuffs in the rumen (16, 29). In contrast, few studies have reported on the ruminal starch digestion of corn silage. At the silage stage, Verbic et al. (32) and Philippeau and Michalet-Doreau (25) have reported a large variation in the ruminal degradation of grains between dent and flint genotypes. Those studies (25, 32) were conducted before ensiling; therefore, whether the differences observed before ensiling are the same as those after ensiling is questionable. The objective of this trial was to determine the effects of genotype and ensiling on the rate and extent of ruminal starch digestion of corn grain harvested at the one-half milkline stage of maturity.

MATERIALS AND METHODS

Plant Material

The trial used two experimental hydrids of corn that differed in texture of the endosperm, dent (*Zea mays* ssp. *indentata*) or flint (*Zea mays* ssp. *indentura*). These two hybrids were sown in 1996 in Limagne, France in the same experimental field at a corn population density of 77,500 plants/ha. Dent

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[1]To whom correspondence should be addressed.

corn is characterized by the presence of a vitreous endosperm at the sides and back of the kernel; the central core extends to the crown of the kernel and is floury. Upon drying, the center part collapses to produce a distinct indentation. Flint corn has a vitreous endosperm surrounding a small proportion of the floury endosperm. A kernel of flint corn is rounded with no denting. Vitreousness, which was determined using a mass method (25), was 40.3 and 55.4% for the dent and flint corns.

Twenty whole plants (30% DM) and 40 ears of corn were harvested by hand at the one-half milkline stage of maturity. Whole-plant DM content was determined using 10 chopped plants after oven-drying at 80°C for 48 h. For each sample, ears were immediately stored at –20°C, and kernels were removed individually from the median ring of frozen ears. The grain content of the whole plant was determined on two samples of five whole plants by removing kernels from the plant. The two fractions were weighed fresh and oven-dried at 80°C for 48 h to obtain the respective DM proportions of the two fractions. Grains comprised 41.0 and 32.2% of the whole-plant DM for the dent and flint genotypes, respectively. Dry matter content of the grain was 46.4 and 52.3% for dent and flint corns, respectively. The starch content (8) in the grain fraction was 71.1 and 68.6%, respectively, for dent and flint corns.

Ensiling Method

To simulate the action of a forage harvester, grains were coarsely chopped in a blender for approximatly 1 min. The particle size distribution of corn was determined by dry-sieving (type 3D; F. Kurt Retsch Gmbh & Co. KG, Haan, Germany) with seven sieves of nominal aperture sizes between 4500 and 500 μm. The arithmetic mean particle size was 4133 and 3941 μm for dent and flint corn, respectively. The proportion of DM particles <52 μm (i.e., roughly the pore size of the nylon bag) was nil. Each genotype was divided into two fractions, unensiled and ensiled. Unensiled grains were stored at –20°C. Grains (170 g) were put in a net (20 × 20 cm) with a 1-mm aperture and were packed alternatively (four for each genotype and each silo) between two 15-cm layers of whole-plant chopped corn. The corn was harvested with a precision chop harvester at 30% whole-plant DM. Silos (35 dm^3) were sealed with rubber stoppers and equipped with a Bunsen valve for the release of gas. The silos were kept at a temperature between 4 and 10°C for at least 90 d.

In Situ Digestion Trial

Unensiled and ensiled grains were dried in a ventilated oven at 40°C for 72 h and then ground in a hammer mill through a 3-mm screen or left unground. The particle size distribution of unensiled ground corn was determined by laser diffraction (Malvern Mastersizer; Malvern Instruments SA, Orsay, France) according to de Monredon et al. (6) (range of measurements, 4 to 2000 μm). The arithmetic mean particle size was 222 and 369 μm for dent and flint corns, respectively. The proportion of DM mass found in particles <52 μm was 25 and 11% for dent and flint corns, respectively.

In situ measurements of ruminal starch degradation were carried out using three ruminally cannulated nonlactating Jersey cows weighing 522 kg. The cows were housed in metabolism stalls in an air-conditioned room (maximum daily temperature = 20 ± 2.7°C; minimum daily temperature = 14 ± 2.7°C) and received 6 kg of corn silage DM supplemented with 180 g of a urea premix (Uresil; Ucanor, Caen France) in two equal meals (0800 and 1700 h). An adaptation period of 3 wk was allowed before measurements were taken. Approximately 3 g of grain DM were placed into nylon bags (pore size, 53 μm; internal dimensions, 5 × 10 cm; Ankom Co, Fairport, NY), yielding a ratio of sample mass per bag area of 27 mg/cm^2. Bags were introduced in the rumen at the same time, just before the morning meal, and were removed after 3, 6, 9, 15, 24, and 48 h of incubation. Six measurements (three cows × two repetitions) were made for each incubation time. After removal, bags were washed in a washing machine for three successive 5-min washings, dried at 80°C for 48 h, and weighed. Within each cow two bags of each feed at each incubation time were pooled and ground in a ball mill before the starch content was determined (8).

The degradation kinetics of DM and starch obtained for each feed and for each cow were fitted with an exponential model: disappearance (t) = a + b (1 – e^{-ct}). This model assumes two degradable fractions: a rapidly degradable fraction in the rumen (a) and a slower degradable fraction (b). The rate of degradation (c) of fraction b was reduced exponentially over time (t). The three parameters a, b, c were estimated by an iterative least squares procedure of SAS (27), and best fit values were chosen using the smallest sum of squares after convergence. Degradabilities of DM and starch were calculated according to the equation of Ørskov and McDonald (24) at a ruminal outflow rate of 0.05/h (26).

To study the suitability of the in situ sample preparation (chopping vs. grinding), the proportion of particulate starch that passed through the pores of the bag without degradation was determined for each

genotype and each sample preparation method according to the calculations of Philippeau and Michalet-Doreau (25). Three grams of DM were placed in nylon bags, immersed in 250 ml of a buffer solution at pH 6.9 (33), and agitated for 2 h in a 39°C water bath. After removal, the bags were rapidly washed with distilled water. Lost particles were recovered from the solution by filtration (6 μm). The filters were dried at 80°C for 48 h and weighed, and the starch content was determined (8). Duplicate samples underwent the complete procedure.

Statistical Analysis

Dry matter and starch degradation parameters were subjected to an analysis of variance using the general linear models procedure of SAS (27). The model had four main factors (cow, sample preparation, genotype, and conservation) and two-way interactions between factors. All of the factors and the two-way interactions between factors were tested using the residual error of the model. The sums of squares were further partitioned with the orthogonal contrasts to compare the effect of conservation (unensiled vs. ensiled) and genotype (dent vs. flint) on chopped grain. Means were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Ruminal DM degradability was lower for chopped grain than for ground grain ($\overline{X}$ = 67.9 and 80.4%, respectively; Table 1) across all other factors. The same trend was observed for ruminal starch degradability, which was 69.9 and 82.8%, respectively, for chopped and ground grains (Table 2; Figure 1). Differences in ruminal DM and starch degradabilities were mainly due to differences in the rapidly degrad-

TABLE 1. Influence of sample preparation, genotype, and conservation method on the rate and extent of in situ ruminal DM degradation of corn grain.

	Degradation characteristics				
Preparation, genotype, and conservation	Undegradable fraction	Rapidly degradable fraction (a)	Slowly degradable fraction (b)	Degradation constant rate (c)	Effective degradability[1]
	(%)			(/h)	(%)
Chopped corn					
Dent					
Unensiled	0.7	25.5	73.8	0.0660	67.0
Ensiled	1.0	35.0	64.0	0.0870	75.0
Flint					
Unensiled	0.1	15.1	84.8	0.0504	63.2
Ensiled	0	32.9	67.1	0.0584	66.4
Ground corn					
Dent					
Unensiled	0	57.1	42.9	0.0584	80.0
Ensiled	0	71.0	29.0	0.0623	86.9
Flint					
Unensiled	0	47.9	52.1	0.0549	73.6
Ensiled	0	56.0	44.0	0.0667	80.9
SEM	0.3	2.6	2.5	0.0094	1.6
	P				
Preparation	*	***	***	NS[2]	***
Genotype	*	***	***	NS	***
Conservation method	NS	***	***	NS	***
Preparation × genotype	*	NS	NS	NS	NS
Preparation × conservation method	NS	NS	NS	NS	NS
Genotype × conservation method	NS	NS	NS	NS	NS
Chopped corn					
Unensiled vs. ensiled	NS	***	***	NS	**
Unensiled dent vs. unensiled flint	NS	**	***	NS	NS
Ensiled dent vs. ensiled flint	NS	NS	NS	*	**

[1]Effective degradability was calculated using the equation a + bc/(c + k), where k = ruminal outflow rate (assumed to be 0.05/h).

[2]$P \geq 0.05$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

able fraction (27.7 vs. 58.4%, respectively, for chopped and ground samples) (Tables 1 and 2). The increase in in situ starch degradability because of grinding agrees with previous results (5, 20, 22). Different hypotheses can be suggested to explain the responses to grinding. The accessibility of starch granules to ruminal microorganisms could be increased because of the fineness of the particles. However, in this study, the degradation rate of the slowly degradable starch in the rumen remained constant regardless of sample preparation. Another hypothesis is that the proportion of particles that escaped through pores of the bag without degradation was increased by grinding. Indeed, the difference in rapidly degradable DM and starch fractions between chopped and ground samples, which averaged 30.7 percentage units, was of the same percentage as the difference in the proportions of DM and starch lost through the pores of the nylon bag (31.5 and 37.7 percentage units, respectively) (Figure 2). Particulate losses (averaged across genotype and conservation method) were much lower for chopped samples than for ground samples (8.7% vs. 40.1% of DM and 11.0% vs. 48.7% of starch initially introduced in the bag; Figures 2, a and b, respectively). This difference in particulate losses can be partly related to the difference in the proportion of particles <52 μm, which was negligible for chopped samples and, on average, 18.3% for ground samples. In this trial, for 3-mm ground grains that were not ensiled, the proportion of particulate starch losses was much greater (47.3%) than that determined in a previous study (10.4%) (25) in which grains were not chopped before grinding. Chopping damages cells and might have increased the particle fineness upon grinding.

Genotype and ensiling effects on the degradabilities of DM and starch in the rumen were similar,

TABLE 2. Influence of sample preparation, genotype, and conservation method on the rate and extent of in situ ruminal starch degradation of corn grain.

Preparation, genotype, and conservation	Degradation characteristics				
	Undegradable fraction	Rapidly degradable fraction (a)	Slowly degradable fraction (b)	Degradation constant rate (c)	Effective degradability[1]
	(%)			(/h)	(%)
Chopped corn					
Dent					
Unensiled	0	34.8	65.2	0.069	72.3
Ensiled	0.4	37.0	62.6	0.102	78.6
Flint					
Unensiled	0	9.9	90.1	0.068	61.6
Ensiled	0	31.1	68.6	0.055	67.0
Ground corn					
Dent					
Unensiled	0.2	58.0	41.8	0.088	84.4
Ensiled	0	77.6	22.4	0.076	91.1
Flint					
Unensiled	0	43.5	56.5	0.059	73.7
Ensiled	0	55.8	44.2	0.074	82.0
SEM	0.2	3.6	3.5	0.011	0.8
	P				
Preparation	NS[2]	***	***	NS	***
Genotype	NS	***	***	*	***
Conservation method	NS	***	***	NS	***
Preparation × genotype	NS	NS	NS	NS	NS
Preparation × conservation method	NS	NS	NS	NS	NS
Genotype × conservation method	NS	NS	NS	NS	NS
Chopped corn					
Unensiled vs. ensiled	NS	***	***	NS	**
Unensiled dent vs. unensiled flint	NS	***	***	NS	***
Ensiled dent vs. ensiled flint	NS	*	*	**	***

[1]Effective degradability was calculated using the equation a + bc/(c + k), where k = ruminal outflow rate (assumed to be 0.05/h).

[2]$P \geq 0.05$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

regardless of sample preparation of the grain (i.e., grinding or chopping). Thus, in the following discussion, we only consider chopped grains, which were characterized by a lower proportion of particulate losses through the pores of the bag. For unensiled samples, ruminal DM degradability was not significantly different for dent and flint corns (Table 1). The difference between genotypes was more marked for ruminal starch degradability (72.3 and 61.6% for dent and flint genotypes, respectively; Table 2). This difference was mainly due to a difference in the rapidly degradable fraction (34.8 and 9.9%, respectively, for dent and flint genotypes; Table 2). The variation in ruminal starch degradability between the two genotypes agreed with previous results obtained for immature (25, 32) and mature grains (15, 19, 23). Unlike floury endosperm, starch granules in vitreous endosperm are enclosed in a continuous protein matrix that contains protein bodies. These nonstarchy components limit the accessibility of starch to ruminal microorganisms (17). The lower degradability of starch in flint corns that was reported in in situ studies was caused by a lower proportion in the rapidly degradable fraction, a lower constant rate of degradation, or both (19, 25, 32). The difference in ruminal starch degradability (10.7 percentage units) could be related to the difference in the proportion of vitreous endosperm in the grain (i.e., the vitreousness) (15.1 percentage units) as we showed in a previous study (25). The difference in ruminal starch degradability between these two corns also could be explained by the difference in the grain DM content (46.4 and 52.3% for dent and flint genotypes, respectively). However, it is not possible to differentiate between the effects of these two factors, because vitre-

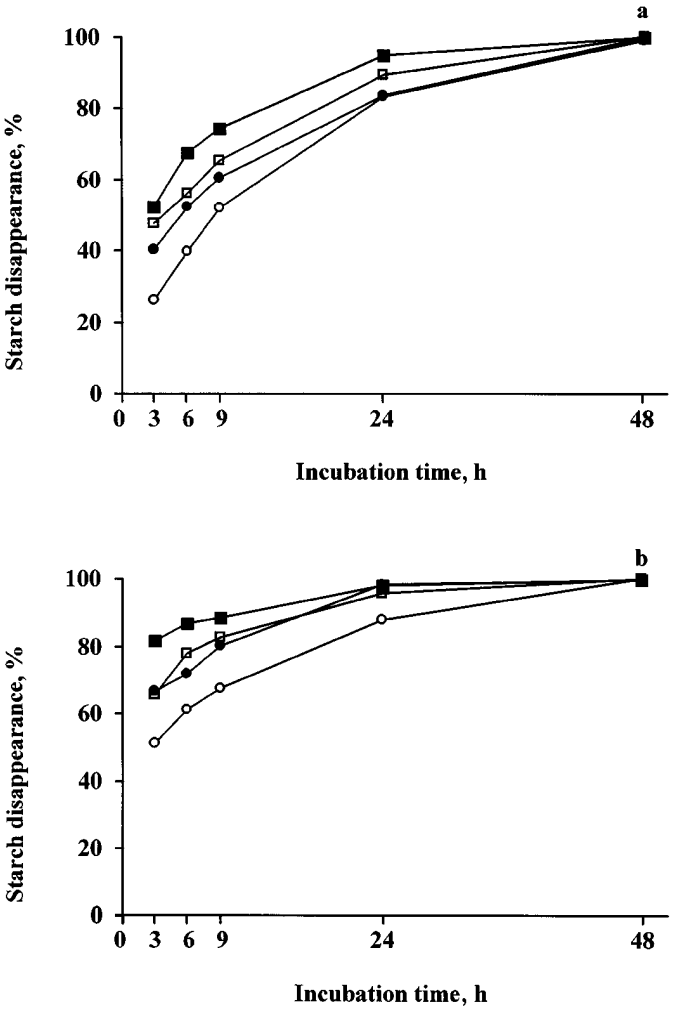

Figure 1. Effects of genotype and conservation method on in situ starch disappearance of chopped (a) and ground (b) corn grains. Values represent the means of six measurements (three cows × two repetitions). Legend: ensiled dent corn grain (■), unensiled dent corn grain (□), ensiled flint corn grain (●), and unensiled flint corn grain (○).

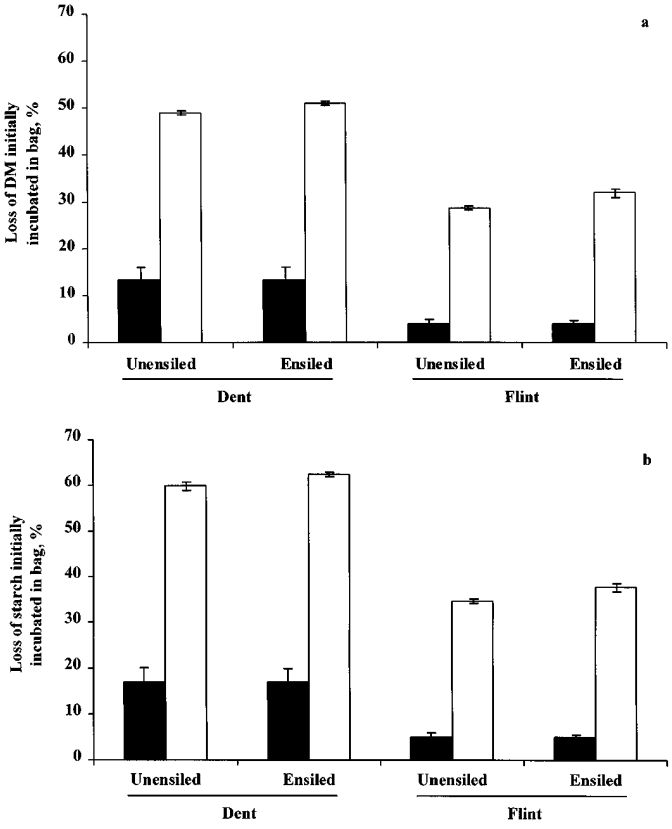

Figure 2. Influence of sample preparation of corn grain [chopped (solid bar) vs. ground (open bar)] on particulate DM (a) and starch (b) losses through pores of the nylon bag. Data are given as means (±SEM) of duplicate incubations.

ousness and DM content of grain are strongly correlated (25).

Ensiling increased mean ruminal DM and starch degradabilities by +5.6 and +5.9 percentage units, respectively. The increase in DM and starch degradabilities was mainly due to the increase in the rapidly degradable fraction (Tables 1 and 2). Ruminal digestion of high moisture ensiled grain was also higher than that of mature grain as was shown in in vitro (14), in situ (7, 11, 20), and in vivo (10, 14, 18) studies. However, this comparison is tenuous because the difference in ruminal DM or starch degradabilities between high moisture and mature grains may be due to the effects of both stage of maturity and the ensiling process. In this trial, for grains harvested at the same stage of maturity, we could study the effect of ensiling (i.e., an increase in ruminal starch degradability). Ensiling induces a partial solubilization of proteins of corn grains (2), and accessibility of starch granules to ruminal microorganisms could be determined mainly by the proteins of the endosperm (17). The increase in ruminal starch degradability after ensiling could be partly derived from the solubilization of endosperm proteins during silage fermentation. The extent of the increase in ruminal starch degradability was the same regardless of corn genotype (+6.3 and +5.4 percentage units, respectively, for dent and flint corn grains). The difference between genotypes in ruminal starch degradability remained constant before and after ensiling (10.7 and 11.6 percentage units, respectively). The genetic variability in ruminal starch degradability of corn silage was in agreement with the results of Verbic et al. (31) who used whole-plant corn silage.

CONCLUSIONS

When corn kernels were harvested at the one-half milkline stage of maturity, ruminal starch degradability was found to be higher for dent corn than for flint corn before and after the ensiling processing. Ensiling induced an increase in in situ ruminal starch degradability, and the extent of this increase was the same regardless of the genotype studied. Ruminal starch degradability of unensiled corn grain was closely related to that of ensiled corn grain.

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