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# Starch degradation in rumen fluid as influenced by genotype, climatic conditions and maturity stage of maize, grown under controlled conditions

M. Ali[a,b], J.W. Cone[b,*], W.H. Hendriks[b], P.C. Struik[a]

[a] Centre for Crop Systems Analysis, Department of Plant Sciences, Wageningen University, PO Box 430, 6700 AK Wageningen, The Netherlands
[b] Animal Nutrition Group, Department of Animal Sciences, Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands

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ABSTRACT

Starch is the major component of maize kernels, contributing significantly to the feeding value of forage maize when fed to ruminants. The effects of genotype, climatic conditions and maturity stage on starch content in the kernels and on *in vitro* starch degradability in rumen fluid were investigated. Kernels of six maize genotypes, differing in amylose content and vitreousness, grown under three contrasting day/night temperature regimes during grain filling, and harvested at different maturity stages from two greenhouse experiments were investigated. Starch content was measured using an enzymatic method and the gas production technique was used to assess starch degradation in rumen fluid of dairy cows. The extent of starch degradation at different incubation times (6, 12 and 20 h) was calculated from measured gas production data (6, 12 and 20 h, respectively) and a published equation. Gas production (ml gas/g OM) showed a positive linear relationship with starch content in the kernels up to a certain level of starch accumulation. At each maturity stage, whole kernel and starch degradation in rumen fluid depended on the genotype ($P < 0.0001$), growing conditions ($P < 0.0001$), starch content ($P < 0.0001$) and starch amount ($P < 0.0001$) in the kernels. While starch content increased with advancing maturity, starch degradation similarly increased up to a certain level of starch content. *In vitro* starch degradation of the maize kernels in rumen fluid was affected by the starch composition, *e.g.* amylose and amylopectin content. Starch degradation was inversely related to the amylose content and vitreousness. Higher starch degradation was observed in the waxy (no amylose) and non-vitreous genotypes. The highest starch degradation was observed when plants were grown at intermediate temperatures in both experiments. The difference in starch degradability of each genotype at the same accumulated thermal time, *i.e.* maturity stage, was due to differences in grain filling rate, caused by the different temperature regimes. This effect of genotype and climatic conditions was consistent for all incubation times ($P < 0.0001$). Rumen *in vitro* starch degradation is significantly influenced by genotypic, differences in starch content of the maize kernels and their growing conditions.



* Corresponding author. Tel.: +31 317 483 542.
*E-mail address:* john.cone@wur.nl (J.W. Cone).

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## 1. Introduction

The feeding value of forage maize for ruminants largely depends on its starch content and rumen fermentation characteristics (Theurer, 1986; Canizares et al., 2011). Starch degradation of maize kernels in rumen fluid is mainly influenced by starch content, composition of the starch (amylose, amylopectin) and physical properties of the starch (Wolters and Cone, 1992; Stevnebø et al., 2006). Different maize genotypes have different starch structures (*i.e.* amylose or amylopectin) and composition (amylose:amylopectin) (Shannon, 1984). Starch is composed of two distinct polymers, amylose and the higher molecular weight amylopectin. Amylose is a linear polymer of glucose units, with α-1,4-linkages, whereas amylopectin is a highly branched polymer with α-1,6-linkages next to the α-1,4-linkages (Jackson, 2003). Normal maize starch is a mixture of amylose (20–30%) and amylopectin (70–80%), but this can vary among genotypes (Fankhauser et al., 1989). Consequently, genotypes can differ in starch degradation in rumen fluid and, therefore, in feeding value (Frei, 2000; Troyer, 2001; Duvick, 2005; Cone et al., 2008). Maize genotypes can also differ in type of endosperm, *i.e.* floury (dent) *vs.* horny (flint) (Kotarski et al., 1992; Michalet-Doreau and Champion, 1995). Dent maize kernel starch is more loosely bound in a starch-zein protein matrix and becomes indented at maturity (Fox and Manley, 2009). Flint maize kernels mostly have a thick, hard, vitreous endosperm layer surrounding a small, soft granular centre (Ettle et al., 2001). The relative amounts of soft and corneous starch, however, vary among cultivars. The vitreousness is the ratio of vitreous (hard) to floury (soft) endosperm (Fox and Manley, 2009), and is used to assess the type of maize endosperm. The variation in rate and extent of maize starch degradation in the rumen due to genetic variation, therefore, plays an important role in determining the nutritive value of forage maize for ruminants (Cone et al., 2008).

The accumulation of starch in the kernels depends, besides on genotype, on growing conditions, especially temperature as well as maturity stage. Lower starch contents can be the result of either sub- or supra-optimal temperatures during grain filling (Anker-Nilssen et al., 2006). High temperatures during grain filling can impede starch accumulation in combination with an increased growth rate and a reduced grain-filling duration (Muchow, 1990). High temperatures may also impair starch synthesis, with less starch per endosperm and smaller starch granules or change the composition (amylose:amylopectin) (Tester et al., 1991, 1995). Lower temperatures may result in less starch accumulation and lower starch contents because of a slower and limited grain filling, despite the advantage of a longer growth duration (Muchow, 1990; Wilson et al., 1995).

Different maize genotypes show differences in earliness and rate of maturation (Tollenaar, 1989; Rebourg et al., 2003) and, therefore, in their response to growing temperatures. This makes it difficult to understand how genotype and growing conditions interact on starch accumulation and finally on starch degradation (Ettle and Schwarz, 2003). Dry matter (DM) content is a good descriptor of maturity (Jensen et al., 2005) and is an important tool to rank maize genotypes based on their maturity (Schwab et al., 2003; Marton et al., 2007). However, maize genotypes can differ in their nutritive value, even at the same dry matter content (Hetta et al., 2012; Jensen et al., 2005).

The present study aimed to understand how growing conditions and genotype interact at different maturity stages to influence *in vitro* starch degradation of maize kernels in rumen fluid.

## 2. Materials and methods

### 2.1. Maize kernel samples

Maize kernel samples were collected from two glasshouse experiments conducted at Unifarm, Wageningen, The Netherlands. Each experiment included six genotypes and three day/night temperature regimes during grain filling (reproductive phase) with three replications. The genotypes were selected on the basis of variation in amylose content and type of endosperm, *i.e.* vitreousness. Four genotypes (dent, flint, high amylose and waxy) were used in both experiments. In Exp. 1, also the normal counter parts of the high amylose and waxy genotypes were used, while in Exp. 2 also non-vitreous and vitreous endosperm (rumen escaping) types were used. The high amylose counterpart and the waxy counterpart had both similar amylose contents but were from different inbred lines. An overview of the type of maize used in each Exp. is shown in Table 1. The average day/night temperature treatments after pollination were 18/12 °C, 24/18 °C and 30/24 °C with 12 h light and 12 h dark in Exp. 1, and 22/12 °C, 27/17 °C and 32/22 °C with 15 h light and 9 h dark in Exp. 2. Cobs were harvested, the husk leaves were removed and the kernels were manually removed from the cobs on the same day using a sharp knife. The kernels were removed from the middle part of the cob to maintain uniformity. The samples were collected when the starch content in the kernels was between 368 and 633 g/kg OM (350–500 °C d, 24–43 d after pollination) and between 558 and 674 g/kg OM (500–700 °C d, 34–63 d after pollination) in Exp. 1. In Exp. 2, the kernels were harvested at starch contents of 401–618 g/kg OM (300–400 °C d, 22–29 d after pollination) and of 537–695 g/kg OM (550–750 °C d, 37–55 d after pollination). These samples were collected from one plant per replication in Exp. 1 and from two plants per replication in Exp. 2. The different treatment combinations are illustrated in Table 1. The weight of 60 randomly chosen kernels from each cob was recorded and the kernels were subsequently dried at 70 °C for 48 h in a forced ventilation oven, reweighed and ground to pass a 1 mm sieve, using a centrifugal mill (Retsch ZM 100, Haan, Germany), and stored at room temperature.