1 2	Starch degradability of maize kernels as influenced by type of endosperm and soil in rumen fluid at different maturity stages
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Abstract

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56 57 Starch is considered a major nutritional factor of maize (Zea mays L.) kernels, and can be influenced by the type of endosperm. The effects of endosperm type (vitreous and non-vitreous) and type of soil (clay and sand) on the starch content of kernels of maize, and on the in vitro degradation of starch were investigated in the rumen fluid after harvesting at 6 different maturity stages during 2008 and five different maturity stages in 2009. Starch degradation, in rumen fluid, was determined after 6 h, 12 h and 20 h of incubation, using the technique of gas production. A positive linear relationship was observed during gas production (ml gas/g organic matter) and starch degradation (g kg<sup>-1</sup> starch) at all incubation times, with starch contents of maize kernels to a certain limit of starch accumulation (i.e. at starch contents 451-519 g/kg OM) and negative relationship afterwards. This suggests significant effects of maturity on ruminal starch degradation of maize kernels. At each harvest date, ruminal starch degradation of maize kernels was affected by crop genotype as well as soil type. The *in vitro* ruminal degradation potential of starch in maize kernels was influenced by the nature of the endosperm, with a higher degradation of non-vitreous kernels than of vitreous kernels. The rumen starch degradation was also influenced by type of soil, with better degradation on clay than sandy soil. For all the incubation times and maturity stages the effects of genotype, soil type and maturity stage were consistent in rumen fluid.

Key Words: Maize genotypes, starch degradation, soil, in vitro, ruminants

### 1. Introduction

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The percentage and digestibility of starch contents are two important factors influencing the nutritional motives of maize silage-based rations for ruminants. Starch is a very vital part of maize kernels and provides the energy to ruminants. Starch is mainly comprised of amylopectin amylose. Amylopectin is branched and more complex for digestion than amylose (Santacruz 2004). Starch diversity in maize is also associated with types of endosperm i.e. dent vs. flint (Kotarski et al. 1992; Michalet-Doreau and Champion 1995). In a starch-zein protein matrix the floury starch (dent) is not bound tightly, and hence, becomes indented at maturity level (Fox and Manley 2009) and hence the kernel type is called dent. Whereas in horny or flint starch a thick, hard, vitreous endosperm layer surrounding a small, soft granular center is present(T Ettle et al. 2001). Vitreous to floury endosperm ratio is called vitreousness (Fox and Manley 2009), thus is used to determine the endosperm type of maize. Maize genotypes exhibit variations in their vitreousness, and hence may differ in their nutritional value. Compared to kernels of flint maize, dent maize kernels have relatively lesser proportion of vitreous-ness (C Philippeau et al. 1999). These genetic variations may result in differences in the rate and extent of ruminal starch degradability of maize, thus has a vital role in assessing nutritional values of maize kernels in maize fodder for ruminants (J. Cone et al. 2008).

Crop growth can be influenced by type of soil or its composition. Although soil composition is not directly a crop yield limiting factor, it significantly affects the final yield of a crop. This is because it plays a vital role in mineral and nutrient availability, and water and air supply to the roots. An ideal soil for plant growth can be with optimum numbers of pores for storage of water, transmission of air, and pores in which roots of crop can grow(Veiga et al. 2009). FAO (2007) classified soils, based on particle size, as sandy which is not tightly packed (Miles 2015), and has bigger particles (0.05–2 mm in diameter), silt has particles with medium size (0.002–0.05 mm in diameter), clay which has very small and packed particles (<0.002 mm). Clayey soils have higher water holding capacity and nutrient use efficiency as compared to sandy soils. As clay particles have more plant nutrients carrying capacity than those of sand or silt (FAO, 2007). Therefore, different soils types can significantly influence the crop growth and yield.

Genotypes of maize show differences in maturation rate(Ali et al. 2014; Rebourg et al. 2003) and, hence, in their responses to growing conditions. It creates a problem in understanding

the interaction of genotype and growing conditions on accumulation of starch content and its ruminal degradation (Thomas Ettle and Schwarz 2003). Normally, based on their maturity, the maize genotypes could be ranked using their dry matter content (DM) as a tool (Marton et al. 2007; Schwab et al. 2003). But these maize genotypes may differ in their nutritive value, even at the same dry matter content(Hetta et al. 2012; Jensen et al. 2005). Moreover, vitreousness can increase with advancing maturity, which may result in reduced starch degradability at later stages (Ali et al. 2014; LM Johnson et al. 2002). Therefore, it is imperative to understand the relationship between starch degradability and vitreousness at different maturity stages to assess the optimum maturity stage for both vitreous and non-vitreous maize genotypes. The afore-mentioned understanding may allow for improved selection of maize genotypes, resulting in better feeding values for ruminants.

Therefore, the aims of current study were understanding of how soil type and genotypes interact at different maturity levels to affect the *in vitro* ruminal starch degradation of maize kernels.

# 2. Materials and methods

## 2.1. Field experiment

Four field studies, Exps 1-4, were carried out on clay and sandy soils in the vicinity of Wageningen in 2008 and 2009. Recommended doses of nutrients were applied to plants on the basis of soil analysis in all four field studies. Weeds were controlled by the application of herbicides.

The plant density was  $9.5 \text{ m}^{-2}$  (Exp. 1 on clay soil and Exp. 2 on sandy during 2008) or 10.5 (Exp. 3 on clay soil and Exp. 4 on sandy during 2009) with a row to row distance of 75 cm in all cases. In all experiments, two varieties (non-vitreous and vitreous) were sown using randomized complete block design (RCBD) having four replications. Sowing dates were  $6^{th}$  of May in 2008 and May 8 in 2009. The crop husbandry followed the recommended agronomic practices. Individual plots had a net plot size of 15 m x 9 m in Exps 1 and 2, and  $13 \text{ m} \times 9 \text{ m}$  in Exps 3 and 4. The time of silking and pollination was recorded as these two growth stages formed the basis for the crop harvesting.

Temperature data was obtained from the Weather Station of Wageningen University to calculate accumulated thermal time. A base temperature of 8 °C was used to calculate accumulated thermal time(Wilkens and Singh 2003). The temperature below which kernel growth is assumed to be zero is the base temperature. Accumulated thermal time was calculated using formula given below:

- 121 Accumulated Thermal Time (in °Cd) =  $\Sigma$  [(Tmax. + Tmin.)/2 Tbase]
- Whereas, Tmax and Tmin are the daily maximum and minimum temperatures, respectively and
- 123 Thase is the base temperature in °C.

Sampling was done at six different times fom Exps 1 and 2, every fortnight during grain filling (starting 14 days after silking) by the harvesting of 2 rows/plot of 1m length (1.5 m²) for the intermediate harvests, keeping two guard rows between the samplings. Final harvesting was carried out on two rows of 5 m length (7.5 m²). From the crops of experiments 3 and 4 (Exp 3 & Exp 4) samples were collected five times, fortnightly during grain filling stage, starting 14 days after silking by harvesting single row/plot of 1 m (0.75 m²) for the intermediate harvests, keeping two guard rows between samplings. The final harvest was carried out on single row of 4m length (3.0 m²). Manual harvesting was done in all the field experiments, by removing ears from the plants, counting, shelling the cobs, and subsampling the shelled kernels to assess the dry matter content (DM). Maize kernel samples used for present investigation were collected after 4,8,12 weeks (Exps. 1 and 2) and 2,6 and 10 weeks (Exps. 3 and 4). Dry matter (DM) contents were recorded after drying the samples at 70 °C temperature for 48 hours. A centrifugal mill was used to ground the oven-dried samples over a 1 mm sieve (Retsch ZM 100, Haan, Germany).

### 2.2. Chemical analysis

Samples were oven dried for 4 hours at 103 °C temperature to calculate the dry matter content of maize kernels, gravimetrically (Standardization 2002), the ash contents were determined by incineration of the samples at 550 °C for 3 h (ISO 2002). Starch contents were measured using amylo-glucosidase method described by Keppler and Decker (1970).

### 2.3. In vitro rumen fermentation and starch degradation

Maize kernel samples were ground through 1 mm sieve and to measure gas produced from incubated samples in buffered rumen fluid the gas production technique, described by J. W. Cone et al. (1996), was used. Rumen fluid was collected from two rumen fistulated lactating cows, two hours after the morning feeding. These cows were fed with a ration of maize, grass silage and concentrate. Kernel samples (0.5 g of substrate) were incubated in 250 ml bottles, containing 60 ml of buffered rumen fluid. Samples were placed in a shaking water bath in duplicate, and temperature was maintained at 39 °C as per described by J. W. Cone et al. (1996). Gas production was determined for 72 hours. For blank gas productions, the results were corrected, i.e. production of gas in buffered rumen fluid without any substrate. In each run, consisting of 40 samples in duplicate, to allow standardization a pure starch (control) and two standard maize samples i.e. normal and gelatinized accompanied the test maize samples. T-tests showed non-significant differences in the gas production between the runs for control (P > 0.53) and standard samples (P > 0.21 and 0.48 for normal and gelatinized starch, respectively), and thus were not corrected for the differences between runs.

Ruminal starch degradation was calculated for 6, 12 and 20 h of incubation times, based on the volume of gas production at 6 h, 12 h and 20 h of incubation and the starch content of the samples, using the equation of Chai et al. (2004) given below:

160 Starch degradation at time t (g/kg OM)

161 = 
$$-191.6(\pm 14.6) + 0.303 (\pm 0.025) \times \text{starch content(g / kg OM)}$$
  
162 +  $1.648 (\pm 0.053) \times \text{gas produced at t (ml / g OM)}$ 

Degradation of starch per gram of Organic Matter (OM) was calculated as

Starch degradation 
$$(g/kg \ starch) = \left[ \frac{starch \ degradation \ (g/kg \ OM)}{starch \ content \ (g/kg \ OM)} \right] \times 1000$$

165 2.4. Statistical analysis

The general analysis of variance procedure in Statistix (1985-2005) and the following model was used to determine the effects of genotype and soil interaction on starch contents, calculated ruminal starch degradation (after 6 h, 12 h or 20 h of incubation):

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$$Y_{ijk} = \mu + G_i + S_j + R_k + G_i \times S_j + G_i \times R_k + S_j \times R_k + \varepsilon_{ijk}$$

Whereas,

- 172  $Y_{ijk}$  = starch content or calculated rumen starch degradation at 6, 12 and 20 h;
- 173  $\mu$  = overall mean;
- 174  $G_i$  = genotype;
- 175  $S_i = \text{soil type}$ ;
- 176  $R_k$  = replication;
- 177  $\varepsilon_{ijk}$  = general error term.

## **3. Results**

- 179 3.1. Starch Contents
- Starch contents (g/kg OM) in maize kernels were significantly affected by the maize genotype (p values ranging from < 0.0002 to 0.008) and soil (p values ranging from 0.001 to 0.008) at six maturity stages of the maize crop in Exp. 1 and 2 (Table 1) and Exp. 3 and 4 (Table 2) during the years 2008 and 2009, respectively. The maximum starch content was recorded in vitreous maize genotypes, and minimum starch contents were noted in non-vitreous genotypes in all four experiments (Tables 1 and 2; Fig. 1). Regarding the soil type, the highest starch contents were
- recorded for clay soil in both years (Tables 1 and 2) while the lowest starch content was recorded
- for sandy soil in both years. An irregular trend of differences in starch content and calculated
- rumen degradation of starch between clay and sandy soil was found for non-vitreous and vitreous
- genotypes for both years (Tables 1 and 2).

## 3.2. Starch degradation

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The *in vitro* ruminal starch degradation (g/kg starch) of the ground maize kernels was significantly different (P<0.05) in the genotypes and soil types, while it progressed with incubation duration (Tables 1 and 2). The *in vitro* degradation of starch is plotted against the starch contents (g/kg OM), which is a proxy for maturity in Figs 2 and 4, and against accumulated thermal time (°Cd) in Figs 3 and 5 for all experiments. Non-vitreous genotypes, grown on clay soil at all three incubation durations and maturity stages (thermal time) showed highest calculated degradation of starch in all four experiments. The relationship of ruminal starch degradation of maize genotypes against their starch contents (Figs 2 and 4) showed that the vitreous genotypes gave the lowest starch degradation (Exps 1-4) across all starch content levels, even at relatively higher starch

contents (Figs 2 and 4), Also Tables 1 and 2 demonstrated that vitreous genotypes showed lower values of starch degradation compared to the non-vitreous genotypes.

Thermal times (growing degree days, °Cd) also significantly affected the starch degradation of both genotypes, on both soil types and incubation durations in Exps 1 and 2 (Fig. 3) and Exps 3 and 4 (Fig. 5). The highest starch degradation (g/kg starch) was noted at an intermediate thermal time (500-600 °Cd), but the lowest starch degradation was noted at a minimum thermal time of 150 °Cd in all four experiments at all incubation durations, soil types and genotypes (Figs 3 and 5). The year comparison of ruminal degradation of starch of both genotypes against their relative starch content percentages (Fig. 4), and the accumulated thermal time (Fig. 5) showed similar trends for genotypes and soil types. Non-vitreous genotypes on clay soil produced the highest starch degradation at intermediate thermal times, but its degradation decreased during a further increase in thermal time in all experiments.

The *in vitro* ruminal starch degradation was significantly affected by maturity stage. Ruminal degradation of starch declined as the maturation progressed, also at higher contents of starch (Tables 1–2, Figs 2-5). The *in vitro* ruminal degradation of starch exhibited a linear relationship, up to a certain maturity stage i.e. the starch degradation increased, with increasing starch content. However, a negative effect was observed between rumen starch degradation and starch content after a certain level of maturity, i.e. the rumen starch degradation started to decrease as starch contents further increased. This trend was observed at all three incubation durations during all experiments (Figs 2 and 4).

## 4. Discussion

In vitro ruminal degradation of starch in maize kernels (calculated from the gas produced) was significantly affectsed by the starch content of maize kernels and endosperm types (i.e. vitreous-ness) at all the incubation times in all the four experiments. All the variables strongly depended on the type of endosperm in the different maize genotypes, types of soil and maturity stages. Further, a higher starch degradability in rumen fluid can be associated with the starch contents and also type of endosperm within a specific genotype (vitreous or non-vitreous)(Ali et al. 2014; Kotarski et al. 1992; Christelle Philippeau et al. 1998). The types of endosperm (dent and flint) are also genotype-specific. Accumulation of starch in maize kernels also depends upon the soil type and the maturity stage of crop, that also determines the starch contents, hence affecting

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the ruminal starch degradation. There was no significant interaction observed among soil type and genotype.

Accumulation of starch was significantly influenced by the type of soil. Higher starch contents were observed on clay than on sand in both years. This could be due to the better water holding capacity and availability of water and nutrients for the maize crop (Mitchell et al. 2007; Reddy et al. 2007). Other reason could be that sandy soil is loose and not tightly packed like clay, which negatively influences its fertility due to lesser activities of microbes and higher susceptibility to wind erosion and drought. This makes it less favorable for crop production and consequently negatively influences maize crop growth, resulting in reduced starch accumulation on sandy soil.

The results of the present study indicated that with increasing starch contents of the kernels at all incubation times, the calculated ruminal starch degradation has increased to a specific stage of maturity in both genotypes of maize. In the current study the lower ruminal starch degradation in the vitreous maize genotype (Zea mays indurata) could be associated with its endosperm type with higher vitreous-ness (Ali et al. 2014; Christelle Philippeau et al. 1998) than the non-vitreous genotype. This could be because of the dense packing of starch granules in vitreous and also the starch molecules are very close, hence making them less degradable than non-vitreous genotypes. This dense network gets denser with increasing vitreous-ness, which results in less accessibility of the granules to the degrading enzymes(Cui and Oates 1999; Svihus et al. 2005; Vesterinen et al. 2002). Moreover, vitreous (horny) maize kernels have a higher proportion of vitreous endosperm (C Philippeau et al. 1999) and show a very low degradability and a slower rate of degradation in the digestive track of ruminants, mainly due to very hard and thick starch content(Fox and Manley 2009). In contrast, non-vitreous (floury) maize kernel endosperms have a fast degradation ability in ruminants, due to the loose, soft and less complex structure of the starch particles(T Ettle et al. 2001). Actually, the nature of endosperms are genetic characteristics of each cultivar and varies among cultivars(Benefield et al. 2006), and used to determine the digestibility and nutritional values of silage of maize kernels, therefore, different endosperm type maize kernels differ in their energy compositions(Schwarz et al. 1996). So, it can be concluded from the present study that at the same maturity stage, the non-vitreous starch structure results in a higher ruminal starch degradability than that of the vitreous type of maize endosperm.

The starch contents and calculated ruminal starch degradation also depend upon the stage of maturity at which the maize kernels are harvested. The DM concentration of maize kernels strongly depends on plant development and maturation whereas the relative contribution of the various plant fractions to whole plant biomass could be determined by development and maturation(Marton et al. 2007; Phipps and Weller 1979). These plant phenological variations may influence the accumulation of starch and fiber (Jensen et al. 2005), hence influence the ruminal degradation of starch. The maximum value of starch contents was calculated at the latest maturity stage in both years (Hetta et al. 2012), but ruminal starch degradation of maize kernels showed different trends at different maturity stages. Ruminal degradation of starch of the maize kernels in both genotypes sown on clay and sandy soils showed similar trends, i.e. the non-vitreous genotype gave higher values regarding rumen starch degradation than vitreous type of genotype. Ali et al. (2014) concluded that rumen starch degradation of kernels of different maize genotypes has a linear relationship with advancing maturity, up to a specific level, after which it reduces the rumen degradability. The lower ruminal starch degradation, of both genotypes, at later maturity stages could be due to the increased virtuousness of the maize kernels (T Ettle et al. 2001; Pereira et al. 2004; Tolera et al. 1998; Tolera and Sundstøl 1999). It suggested that lower the degree of vitreousness, higher will be the ruminal starch degradation and vice versa. The chemical composition of maize kernels changes up to a certain maturity stage (Struik 1983), like an increased dry matter content and reduced fiber content(L Johnson et al. 1999), which negatively influences the ruminal starch degradation of maize kernels(Ali et al. 2014; T Ettle et al. 2001). It might be the reason of the significant effect of maturity stage on ruminal starch degradation of maize kernels to a specific maturity stage (i.e. at starch contents 451-519 g/kg OM). Therefore, harvesting maize crops at a proper maturity stage is vital for a better feeding value of maize kernels.

# 5. Conclusions

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In vitro starch degradation of maize kernels in rumen fluid is affected by the maize genotypes (type of endosperm; vitreous and non-vitreous), type of soil (clay and sandy) and maturity stage of the plant (in terms of either starch content or thermal time). The vitreous genotypes with hard endosperm showed a lower ruminal starch degradation at all the incubation times (6 h, 12 h and 20 h), as compared to the non-vitreous type of genotypes (soft endosperms). Growing maize on clay soil showed a positive effect, compared to sandy soil, with increased starch

(Wageningen, The Netherlands).

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contents and increased degradation at all incubation times in rumen fluid. Crop maturation stage 290 can have both positive and negative effects on the ruminal degradation of starch of maize kernels, 291 depending on its range. This study also clearly showed that too early or too late crop harvesting 292 results in less ruminal degradation of starch, thus a lower nutritional value of kernels. 293 **Conflict of interest statement** 294 There are no conflicts of interest in this study. 295 Acknowledgements 296 297 This research was financially supported by the Higher Education Commission of Pakistan through a PhD scholarship to M. Ali, and by the Dutch Dairy Product Board (Zoetermeer, The 298 Netherlands), Blgg AgroeXpertus (Wageningen, The Netherlands) and Wageningen University 299

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Table 1 Starch content, calculated ruminal starch degradation at different incubation times (6, 12 and 20 h) as influenced by type of endosperm (non-vitreous and vitreous) and types of soil (sandy and clay) when harvested at three maturity stages (starch contents ranging from 185-729 g/kg OM) in Exp. 1 and 2.

			(4 weeks a	after 50% sil	king)		(8 weeks after 50% silking)				(12 weeks after 50% silking)			
		Starch contents (g/kg OM)	Ruminal starch degradation (g/kg starch)			Starch contents (g/Kg OM)	Ruminal starch degradation (g/kg starch)			Starch contents (g/Kg OM)	Ruminal starch degradation (g/kg starch)			
			(Starch co OM)	ontents 185-3	09 g/kg		(Starch co OM)	ntents 416-5	43 g/kg		(Starch contents 587-729 g/kg OM)			
			6h	12h	20h		6h	12h	20h		6h	12h	20h	
Genotype	Vitreous	266a	164 <sup>b</sup>	334 <sup>b</sup>	471 <sup>b</sup>	519a	288 <sup>b</sup>	644 <sup>b</sup>	768 <sup>b</sup>	700 <sup>a</sup>	135 <sup>b</sup>	531 <sup>b</sup>	612 <sup>b</sup>	
	Non-vitreous	218 <sup>b</sup>	246 <sup>a</sup>	435a	618 <sup>a</sup>	451 <sup>b</sup>	401 <sup>a</sup>	756 <sup>a</sup>	860 <sup>a</sup>	611 <sup>b</sup>	215 <sup>a</sup>	634 <sup>a</sup>	687ª	
	P	0.002	0.004	0.001	0.005	0.0002	0.005	0.001	0.0001	0.0002	0.004	0.001	0.003	
Soil	Clay	267ª	220 <sup>a</sup>	418 <sup>a</sup>	606 <sup>a</sup>	501 <sup>a</sup>	377 <sup>a</sup>	735 <sup>a</sup>	838 <sup>a</sup>	677 <sup>a</sup>	220 <sup>a</sup>	617 <sup>a</sup>	693ª	
	Sandy	217 <sup>b</sup>	190 <sup>b</sup>	351 <sup>b</sup>	483 <sup>b</sup>	469 <sup>b</sup>	312 <sup>b</sup>	665 <sup>b</sup>	$790^{\rm b}$	634 <sup>b</sup>	131 <sup>b</sup>	548 <sup>b</sup>	$607^{\rm b}$	
	P	0.002	0.03	0.002	0.007	0.003	0.02	0.003	0.002	0.004	0.003	0.003	0.002	
Genotype*Soil		0.78	0.89	0.10	0.21	0.90	0.23	0.64	0.45	1.00	0.99	0.06	0.94	
Genotype*Rep		0.22	0.23	0.11	0.80	0.13	0.63	0.21	0.75	0.84	0.19	0.67	0.29	
		Clay-	Clay-	Clay-	Clay-	Clay-	Clay-	Clay-	Clay-	Clay-	Clay-	Clay-	Clay-Sandy	
		Sandy <sup>1</sup>	Sandy	Sandy	Sandy	Sandy	Sandy	Sandy	Sandy	Sandy	Sandy	Sandy		
	Vitreous	14	30	59	141	32	79	72	51	42	88	55	87	
	Non-vitreous	16	30	77	105	33	50	68	46	30	90	83	86	

Means followed by different letters are significantly different (P<0.05).

OM: Organic matter.

<sup>&</sup>lt;sup>1</sup> Difference between clay soil and sandy soil for specific genotype

Table 2 Starch content, calculated ruminal starch degradation at different incubation times (6, 12 and 20 h) as influenced by type of endosperm (non-vitreous and vitreous) and types of soil (sandy and clay) when harvested at three maturity stages (starch contents ranging from 71-632 g/kg OM) in Exp. 3 and 4.

			(2 weeks after 50% silking)					after 50% silk	king)	(10 weeks after 50% silking)				
		Starch contents (g/kg OM)	contents (g/kg starch) (g/kg			Starch contents (g/Kg OM)	Ruminal starch degradation (g/kg starch)			Starch contents (g/Kg OM)	Ruminal starch degradation (g/kg starch)			
			(Starch co	ntents 71-107	g/kg OM)		(Starch co OM)	ntents 369-4	71 g/kg		(Starch contents 530-632 g/kg (			
			6h	12h	20h		6h	12h	20h		6h	12h	20h	
Genotype	Vitreous	97ª	87 <sup>b</sup>	185 <sup>b</sup>	283 <sup>b</sup>	435a	270 <sup>b</sup>	467 <sup>b</sup>	681 <sup>b</sup>	611ª	175 <sup>b</sup>	592 <sup>b</sup>	658 <sup>b</sup>	
	Non-vitreous	84 <sup>b</sup>	129 <sup>a</sup>	347 <sup>a</sup>	418 <sup>a</sup>	398 <sup>b</sup>	323 <sup>a</sup>	585 <sup>a</sup>	764 <sup>a</sup>	558 <sup>b</sup>	221 <sup>a</sup>	646 <sup>a</sup>	709 <sup>a</sup>	
	P	0.008	0.008	0.0005	0.006	0.002	0.008	0.004	0.001	0.003	0.004	0.004	0.005	
Soil	Clay	98 <sup>a</sup>	119 <sup>a</sup>	297ª	493a	445a	328a	574a	747 <sup>a</sup>	601a	241a	641ª	730 <sup>a</sup>	
	Sandy	83 <sup>b</sup>	96 <sup>b</sup>	236 <sup>b</sup>	299 <sup>b</sup>	389 <sup>b</sup>	265 <sup>b</sup>	478 <sup>b</sup>	$697^{\rm b}$	568 <sup>b</sup>	156 <sup>b</sup>	596 <sup>b</sup>	637 <sup>b</sup>	
	P	0.006	0.025	0.003	0.009	0.001	0.006	0.005	0.004	0.008	0.001	0.005	0.002	
Genotype*Soil		0.43	0.73	0.07	0.70	0.08	0.33	0.16	0.15	0.16	0.80	0.79	0.49	
Genotype*Rep		0.25	0.16	0.21	0.08	0.16	0.57	0.54	0.53	0.30	0.10	0.80	0.53	
		Clay- Sandy <sup>1</sup>	Clay- Sandy	Clay- Sandy	Clay- Sandy	Clay- Sandy	Clay- Sandy	Clay- Sandy	Clay- Sandy	Clay- Sandy	Clay- Sandy	Clay- Sandy	Clay-Sandy	
	Vitreous	14	25	49	100	62	69	112	57	26	84	62	96	
	Non-vitreous	16	22	74	109	50	57	80	43	46	90	46	90	

Means followed by different letters are significantly different (P<0.05). OM: Organic matter.

<sup>&</sup>lt;sup>1</sup> Difference between clay soil and sandy soil for specific genotype

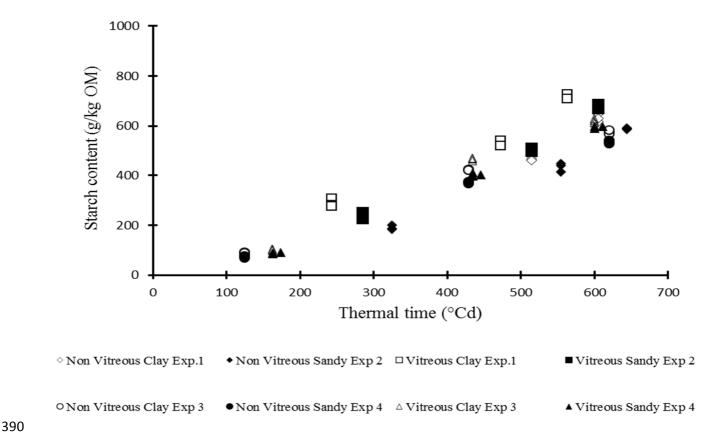


Fig. 1. Relationship between starch content and accumulated growth thermal time during grain filling. Open markers are for clay and closed markers are for sandy soils.

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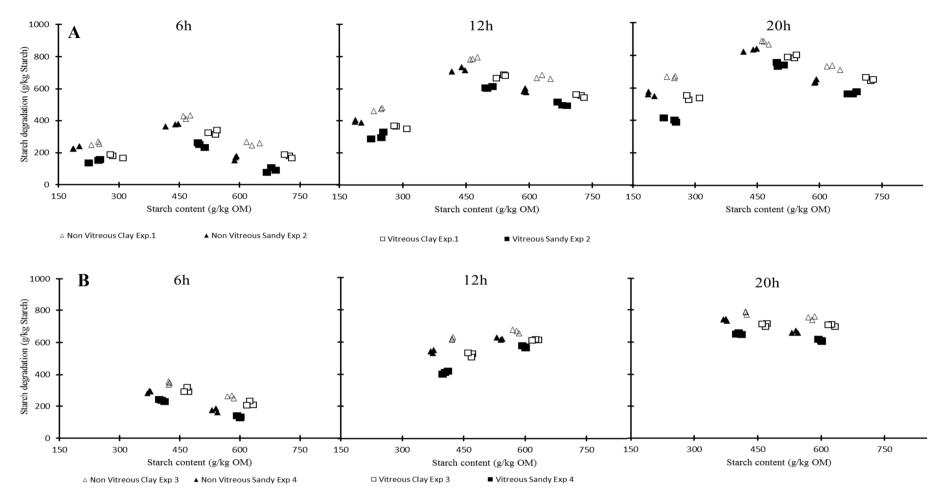


Fig. 2. Relationship between rumen starch degradation (g/kg starch) of 6, 12 and 20 h incubated maize kernels against their starch content (g/kg) at two different types of soils; panel A (clay Exp. 1 and sandy Exp. 2) and panel B (clay Exp. 3 and sandy Exp. 4). Open markers are for clay (Exps 1 and 3), and closed markers are for sandy soil (Exps 2 and 4).

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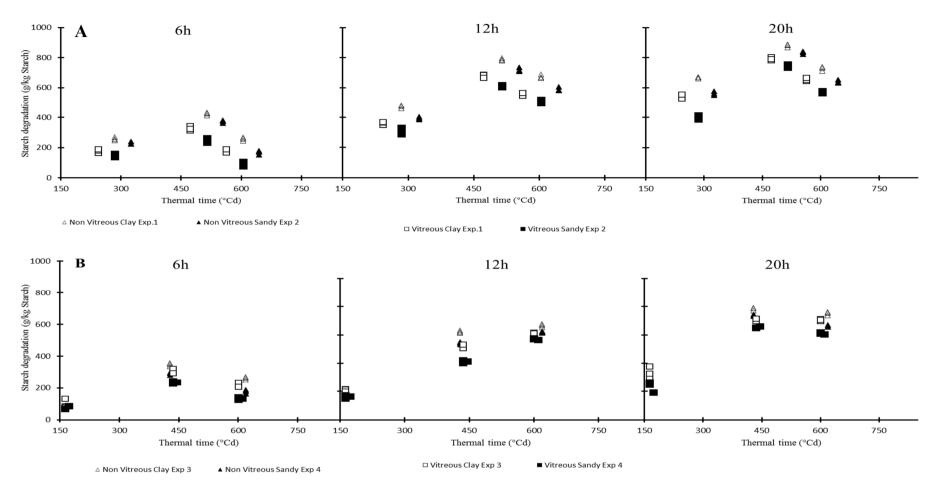


Fig. 3. Relationship between rumen starch degradation (g/kg starch) of 6, 12 and 20 h incubated maize kernels against their relative accumulated thermal time (°Cd) at two different types of soil; panel A (clay Exp. 1 and sandy Exp. 2) and panel B (clay Exp. 3 and sandy Exp. 4). Open markers are for clay (Exps 1 and 3), and closed markers are for sandy soil (Exps 2 and 4).

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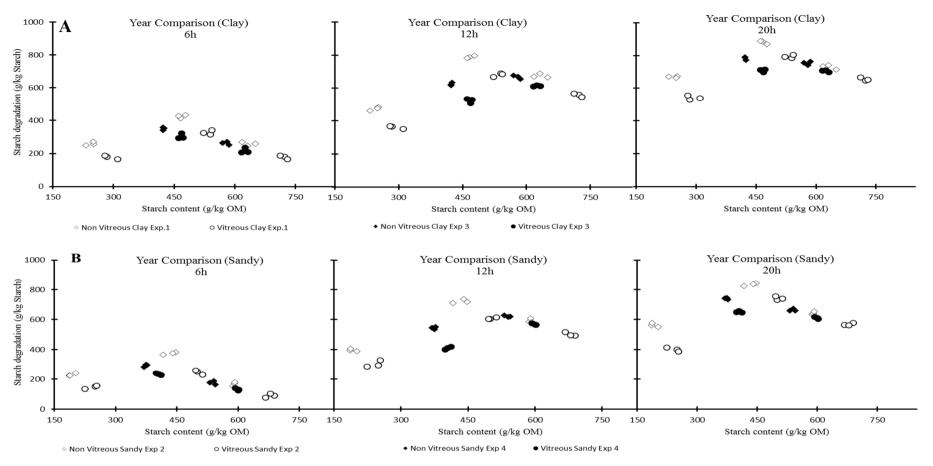


Fig. 4. Year wise relationship between rumen starch degradation (g/kg starch) of 6, 12 and 20 h incubated maize kernels against their starch contents (g/kg OM) at two different types of soils; panel A (clay Exp. 1 and sandy Exp. 3) and panel B (clay Exp. 2 and sandy Exp. 4). The markers are for specific genotypes and soils as explained below the graphs respectively.

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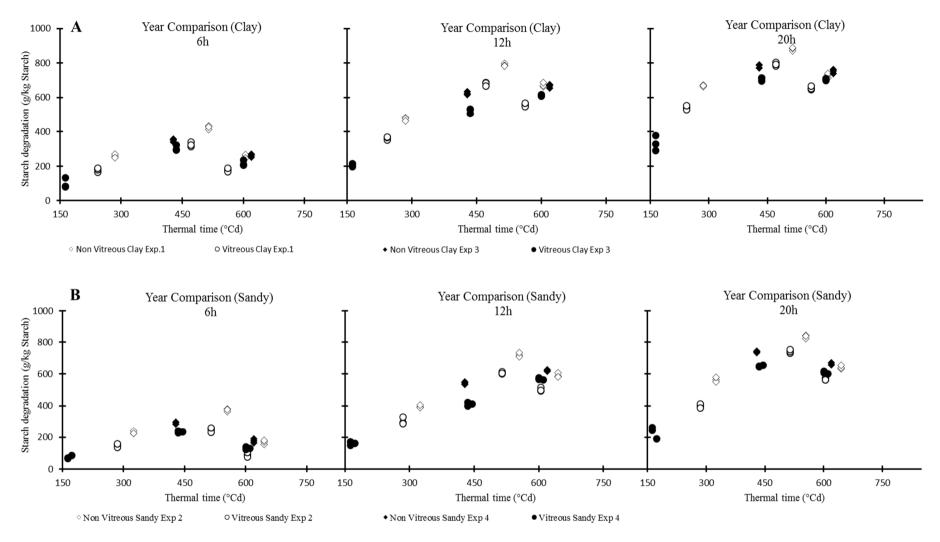


Fig. 5. Year wise relationship between rumen starch degradation (g/kg starch) of 6, 12 and 20 h incubated maize kernels against their relative accumulated thermal time (°Cd) at two different types of soils; panel A (clay Exp. 1 and sandy Exp. 3) and panel B (clay Exp. 2 and sandy Exp. 4). The markers are for specific genotype and soil are explained below the graphs respectively.