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DEGRADATION OF STARCH AND CRUDE PROTEIN IN DENT AND DENT X FLINT MAIZE HYBRIDS IN DIFFERENT STAGES OF MATURITY

Z. MLYNEKOVÁ*, Z. ČEREŠŇÁKOVÁ

Animal Production Research Centre Nitra , Nitra, Slovak Republic

ABSTRACT

In situ experiment was conducted to determine degradation of starch and crude protein in maize hybrids with dent and dent x flint type of grains. The samples of grains were harvested in four stages of maturity. The first harvesting was done in milk-waxy stage and than fornightly at intervals until full grain ripeness. Used dent x flint hybrids were Mesnil, Chambord, Queen, while Aude, Meridien, KX 13 93, Omero belonged to dent.
In sacco experiment was carried out on three rumen canullated cows of live weight 630 kg. The cows were fed a ration at maintenance level twice daily.
The effective degradability of crude protein and starch of maize grains have decreasing tendency with increasing maturity stage. The maize hybrids of dent type had higher effective starch degradability (60.2 %) than hybrids dent x flint (59.6 %). The degree of maturity had a significant ($P < 0.01$) effect on effective degradability of starch and crude protein. The maize hybrids KX 1393 and Meridien had the highest rate of starch degradability (parameter c: 0.075 $\%.h^{-1}$ and $0.074.h^{-1}$, respectively). Protein and starch in the hybrids dent x flint were less rapidly degraded than dent hybrids. This means that the starch and crude protein of these hybrids are more efficiently used by ruminants.

Key words: dent; dent x flint hybrids; maize grain; starch; crude protein; rumen degradability; *in sacco* method

INTRODUCTION

Maize grain is a primary energy supplement in dairy diets and can contribute up to 30, 60, and 98 % of the diet's protein, net energy, and starch, respectively. Specialty maize hybrids are one result of efforts to select corn based on nutrient content (Dado, 1999). The default form for farming based on kernel characteristics, maize are divided into five types: flint, popcorn, floury, dent and sweet corn (Corona *et al*., 2006). The endosperm of flint corn consists of hard-textured vitreous starch and has a greater proportion of vitreous endosperm than dent maize (Philippeau *et al.*, 1999, Kotarski *et al*, 1992). With advancing maturity, kernel vitreousness and density increases while ruminal starch availability and total tract starch digestibility decreases (Philippeau and Michalet-Doureau, 1997; Correa *et al.*, 2002; Johnson *et al.*, 2002; Pereira *et al*., 2004; Szasz *et al.*, 2007). Starch in vitreous dry corn is more extensively encapsulated by prolamins and is less degradable in the rumen as compared to floury or opaque maize grains (Kotarski *et al*, 1992). Phillipeau *et al.* (1999) compared 8 dent and 6 flint varieties and reported that dent maize averaged 51 % vitreousness vs. 72 % for flint maize and effective ruminal degradability was on average 62 % for dent and 46 % for flint maize varietiess.

Since maize grain often accounts for a large part of the diet of beef cattle, information on the pattern of degradation of maize protein in rumen contents is important in order to improve the precision with which

***Correspondence:** E-mail: mlynekova@cvzv.sk
Zuzana Mlyneková, Animal Production Research Centre Nitra ,
Hlohovecká 2, 949 92 Nitra, Slovak Republic
Tel.: +421 37 6546 214

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diets are compounded (Níkolić and Filipović, 1981).

In Slovakia maize hybrids are nowadays imported from other countries and it is important to determine their nutritional quality. The rate and extent of degradability of crude protein and starch are important characteristics for their effective utilization by ruminants. Therefore, the aim of our study was to determine the effect of grain type and maturity stage on ruminal crude protein and starch degradability *in situ* from selected corn hybrids of dent and flint x dent types.

MATERIAL AND METHODS

The grains of maize hybrids of the type dent (Aude, Meridien, KX 1393, Omero) and dent x flint (Mesnil, Chambord, Queen) were used in the experiment.

The maize grains were sampled at fornightly intervals, first at the time of milk-waxy maturity (13.8., at harvest maize for silage), until full ripeness, i.e. 26.8, 10.9 and 23.9. Maize grains were shelled from collected cobs, than dried at room temperature, and ground to pass through a 3 mm sieve.

Starch and crude protein degradability in the maize grains were determined *in sacco* (Harazim and Pavelek, 1999). *In sacco* experiments were carried out in three nonlactating cows with large rumen cannulae (an average of 10 cm). The animals were fed twice a day with a diet consisting of 70 % forage and 30 % concentrate on a dry matter basis at maintenance level. The ration consisted of maize silage, alfalfa hay, wheat, barley meal (1:1) and Vitamix S1. Access to water was *ad libitum.*

Ground samples were weighed (approx. 2.50 g dry matter) into bags made of Uhelon 130T (HEDVA, "Moravská Třebová", the Czech Republic) with pore size of 47 μm. Minimum of three separate bags for hybrids, incubation time and animals were used. The bags with samples were incubated for 2, 3, 4, 6, 9, 16, 24, and 48 hours. The 0 h time bags were only washed in water to determine washing losses.

The bags containing 15 mg samples were inserted into the rumen just before morning feeding. The content of starch and crude protein was determined in the maize hybrids and in the residues after all incubation times. The content of starch was determined acoording to modified enzymatic method (Salomonsson *et al.*, 1984) and crude protein according to Decree MP 2145/2004-100. The parameters of degradability (a, b, c, and "effective degradability") were calculated using the equations by Ørskov and McDonald (1979) with outflow rate of $0.06.h^{-1}$.

The obtained data on nutrients and degradability of crude protein and starch in maize hybrids were evaluated statistically using models in statistical package Statistix 8.0.

RESULTS AND DISCUSSION

Due to lack of information on the grain quality of maize hybrids grown in Slovakia this study emphasized on chemical composition and in situ ruminal degradability of crude protein and starch in dent and dent x flint hybrids. Dent x flint maize (*Zea mays L.*) hybrids are commonly planted in the early maize growing regions of Europe (Moreno-Gonzales *et al*., 1997; Moreno-Gonzales *et al*., 2000). Table 1 shows the results of starch and protein content, on the grounds that these two nutrients could most affect grain degradability in the rumen. The differences among maize hybrids as well as between the stages of maturity in concentration of nutrients and extent of degradability were observed in many studies (Lebzien *et al*., 1997; Loose *et al*., 1998; Pereira *et al.*, 2004). The endosperm contains primarily starch and abundant storage proteins. The main maize proteins are prolamins, named zein, that comprise 50-60 % of total protein in maize grain.

Results from the tested hybrids showed that the content of starch depends on the type of grain (Table 1). The mean starch content was higher in dent hybrids, and kept on changing with ripening of grain. The maximum starch concentration was determined in hybrid KX 1393 (716.6 $g.kg^{-1}$ DM) and the lowest in hybrid Queen (669.3 $g.kg^{-1}$ DM). The differences between individual hybrids were significant ($P<0.05$), and while between KX1393 and Queen were highly significant ($P<0.01$). The results also confirm the changes in the starch content in corn grain during the ripening process. Highly significant differences were noted between 1st and 2nd, 3rd and 4th samplings ($P<0.01$) and between 3rd and 4th samplings ($P<0.05$) respectively in the content of crude protein (CP), which was also different among hybrids and kept on changing with the date of sampling. Average (average of all hybrids and harvestings by type of grain) concentration was lower (102.8 $g.kg^{-1}$ DM) in the dent x flint hybrids than in dent hybrids (108.4 $g.kg^{-1}$ DM). Among the hybrids differences were statistically significant, although dent hybrids had 2 % higher content.

Maize grain differs from other cereals in terms of degradation in the rumen and has high content of starch and unique structure and characteristics of grain (Kopčeková *et al.*, 2008). Degradation of maize starch is about 60 %, while for barley and wheat it is more than 90 %. Kopčeková *et al.* (2010) and Šimko *et al.* (2006) showed resistance to degraded maize starch in the rumen. Similar results were also documents by Lebzien *et al.* (1997), who found the effective degradability of

Table 1: Content of starch and crude protein in grains of maize hybrids (g.kg^{-1} DM)

Nutrients	Sampling	Hybrids						
		Dent x flint			Dent			
		Mesnil	Chambord	Queen	Aude	KX 1393	Meridien	Omero
Crude protein	1.	98.3	97.3	112.4	115.7	108.2	117.5	115.2
	2.	106.6	88.9	107.4	101.9	102.6	101.5	114.6
	3.	109.4	94.9	116.5	96.2	109.5	106.9	102.7
	4.	107.3	90.7	108.5	112.0	108.9	106.7	112.5
	$\bar{x}$	102.4a	93.0a,b	109.6a,b	106.2b,c	106.0b,c	108.0a,b	111.2a,b,c
	SEM	6.73	3.96	2.39	8.90	8.56	2.11	5.48
Starch	1.	689.4	680.8	676.5	651.2	726.2	671.8	673,6
	2.	693.9	715.7	689.7	718.7	742.7	717.9	679.4
	3.	658.3	659.6	632.8	692.3	708.4	681.2	675.8
	4.	702.4	722.5	678.3	694.3	688.9	722.7	721.5
	$\bar{x}$	686.0a*	694.7	669.3a,b	689.1a	716.6a*,	698.4b	687.6a
	SEM	17.80	27.47	23.18	25.93	21.41	23.74	21.05

Means with the same letters in the same row are significantly different at P<0.01 for CP content
Means with the same letters in the same row are significantly different at P<0.05 and P<0.01*
SEM = Standard Error of Mean

maize starch at 55 % and wheat starch at 95 %.

According to Kotarski *et al.* (1992) better understanding of chemical factors that potentially influence starch digestibility in ruminants required understanding of anatomy and physiology of maize seed. From three basic morphological parts, the endosperm represents approximately 75 % to 80 % of the corn kernel by weight and is the morphological structure which contains starch. Prolamin proteins cross-link encapsulating starch into a water tight (hydrophobic) matrix. Starch kernels in vitreous maize are more extensively encapsulated by prolamins (zein) and therfore are less degraded in the rumen as compared to floury maize.

Also, according to Michalet-Doreau *et al*. (1995) differences in the degradation of starch in maize grain among hybrids are primarily linked to the structure of the endosperm. The vitreous endosperm is hard, and abundant in protein matrix, with larger and more numerous protein bodies, and compact and polygonal starch granules. In the floury endosperm, the protein matrix is discontinuous and has few protein bodies, and the starch granules are spherical, larger, less aggregated and surrounded by air spaces (Robutti *et al*., 1974; Pratt *et al.*, 1995; Gibbon *et al.*, 2003).

Proportions of vitreous and floury endosperm vary among corn hybrids and maturity at harvest. Corn hybrids with kernels containing high proportions of vitreous (or horney) endosperm are flinty and those containing high proportions of floury endosperm are called floury, or dent (Kotarski *et al.*, 1992).

Dent type hybrids (Aude, KX 1393, Meridien and Omero) showed higher effective degradability of starch than type dent x flint in all harvestings. Averages of degradability parameters for individual hybrids from all four harvesting times confirmed lower starch degradation rate in dent x flint maize grains than dent maize grains (Table 2). Effective degradation of starch (EDS) was lower for crossbred dent x flint varieties with properties closer to dent than for flint hybrids. The differences between them were not high even though differences among hybrids were significant. The hybrids Mesnil (62.7 %) and Aude (57.7 %) were beyond the average. The rate of starch degradability was the highest in dent hybrids KX 1393 (0.075 %.h^{-1}) and Meridien (0.074 %.h^{-1}). Philipeau *et al.* (1999) reported a significant effect of the properties of the protein matrix of endosperm, especially on the rate of starch and crude protein degradation. Flint corn has a greater proportion of vitreous endosperm than dent corn. Philippeau and Michalet-Doureau (1997) observed that increased kernel vitreousness was associated with decreased ruminal starch degradation.

Average effective degradability of starch declined from 64.5 % (1st sampling) to 55.9 % (3rd sampling) but in the last sampling it increased again (60.1 %).

Table 2: Parameters of effective degradability of starch in grains of dent x flint and dent type maize hybrids

Nutrients	Sampling	Hybrids						
		Dent x flint			Dent			
		Mesnil	Chambord	Queen	Aude	KX 1393	Meridien	Omero
a (%)	1.	21.2	19.0	22.6	18.0	33.0	31.1	33.0
	2.	22.4	22.8	23.0	18.8	17.0	18.2	17.0
	3.	30.7	22.4	27.2	23.4	14.4	15.3	14.4
	4.	27.1	27.2	21.6	22.4	26.9	27.3	26.9
	$\bar{x}$	26.9	22.8	23.6	20.7	26.2	23.0	22.8
	SEM	4.01	4.17	3.14	2.40	4.45	6.93	8.64
b (%)	1.	72.5	81.0	77.4	82.0	66.9	67.5	66.9
	2.	76.8	77.2	77.0	81.2	83.0	81.8	83.0
	3.	65.7	74.1	70.4	76.6	85.6	84.7	85.6
	4.	72.9	27.2	77.7	77.6	73.1	71.1	73.1
	$\bar{x}$	72.0	75.6	75.6	79.4	73.2	76.3	77.1
	SEM	4.75	6.41	3.78	2.40	4.47	7.86	8.75
c (% h^{-1})	1.	0.082	0.058	0.072	0.069	0.061	0.091	0.061
	2.	0.071	0.037	0.046	0.053	0.065	0.068	0.065
	3.	0.071	0.052	0.052	0.044	0.063	0.064	0.063
	4.	0.038	0.054	0.058	0.056	0.059	0.072	0.059
	$\bar{x}$	0.066g	0.051cfg	0.057be	0.056ad	0.074def	0.075abc	0.062
	SEM	0.020	0.015	0.016	0.014	0.021	0.015	0.014
ED starch (%)	1.	69.4	60.0	63.2	60.9	58.7	70.5	58.7
	2.	63.2	56.4	58.0	57.5	60.4	58.7	60.4
	3.	59.8	49.9	56.5	53.4	53.9	47.4	53.9
	4.	58.4	60.8	59.7	59.2	62.2	61.0	62.2
	$\bar{x}$	62.7f	56.8ef	59.3b	57.7a	65.0abcde	59.4d	58.8e
	SEM	4.77	5.05	3.96	3.61	5.57	9.27	4.86

Means with the same letters in the same row are significantly different at $P<0.05$ and $P<0.01$
SEM = Standard Error of Mean

The parameters of degradation (a, b, c) varied with grain ripening (Table 2). It was found that the effective degradability of starch as well as the rate of degradation of fraction „b“ (parameter „c“) had decreasing trend with the stage of maturity. The rate of degradation in the hybrid Mesnil dropped from 0.082 %.h^{-1} in 1st sampling to 0.038 %.h^{-1} in 4th sampling. The decline the parameter “c” was more marked in dent x flint hybrids than in dent type hybrids. The parameter “a“ was the highest in dent type hybrids (Meridien and Omero 33.0 %) from the 1st harvesting. Dent grain is not so hard as compared to dent x flint type hybrids. The starch in dent grain is more soluble. Similar results were obtained by Bal *et al.* (2000). According to Philippeau and Michalet-Doreau (1997) increasing maturity at harvest resulted in increased vitreousness and decreased *in situ* ruminal starch degradation for both flint and dent hybrids. Starch degradability by ruminal microbes was much greater for dent hybrids compared to the flint hybrid at similar DM concentrations from ~ 30 % to ~ 40 % for whole plant DM.

Prolamins define differences in the chemical composition between vitreous dry corn (glassy, translucent) and floury or opaque corns although the relationship is not absolute. Prolamins are characterised by a highly hydrophobic glutamine and proline contents that develop tertiary structure localized on exterior of starch granules Philippeau and Michalet-Doreau (1997).

Table 3: Parameters of effective degradability of crude protein in grains of dent x flint and dent type maize hybrids

Nutrients	Sampling	Hybrids						
		Dent x flint			Dent			
		Mesnil	Chambord	Queen	Aude	KX 1393	Meridien	Omero
a (%)	1.	24.7	34.4	30.0	27.2	26.9	23.4	25.5
	2.	21.4	24.3	20.3	23.6	20.4	19.6	21.3
	3.	24.8	13.5	21.1	15.8	23.6	23.6	15.1
	4.	13.1	14.4	15.7	13.2	16.9	19.3	19.9
	$\bar{x}$	21.0	21.7	21.8	20.0	22.0	21.5	20.5
	SEM	4.99	8.89	5.87	5.92	4.82	2.30	4.66
b (%)	1.	74.8	65.6	70.0	73.4	73.1	76.1	73.0
	2.	78.6	74.2	78.4	76.4	79.3	80.4	78.7
	3.	75.2	83.0	72.4	84.2	76.4	76.4	84.9
	4.	86.9	79.7	84.3	86.8	77.8	80.7	80.1
	$\bar{x}$	78.9	75.6	76.3	80.2	76.6	78.4	79.2
	SEM	5.14	8.21	6.03	5.73	3.96	2.58	5.79
c (% h^{-1})	1.	0.064	0.035	0.039	0.040	0.038	0.057	0.029
	2.	0.034	0.033	0.030	0.027	0.041	0.035	0.040
	3.	0.029	0.026	0.036	0.030	0.035	0.037	0.040
	4.	0.014	0.031	0.023	0.018	0.040	0.027	0.019
	$\bar{x}$	0.035	0.031	0.032	0.029	0.039	0.039	0.032
	SEM	0.022	0.018	0.016	0.013	0.020	0.019	0.019
EDCP starch (%)	1.	61.2	61.1	59.4	57.8	58.3	59.0	50.0
	2.	52.0	53.9	49.9	51.8	55.1	52.0	54.6
	3.	52.3	43.1	49.8	45.3	53.2	53.2	48.6
	4.	42.2	43.3	45.5	41.4	48.3	49.1	47.9
	$\bar{x}$	51.9	50.3	51.1	49.0a	53.7a	53.3	50.2
	SEM	7.53	8.83	6.26	7.72	5.57	5.79	4.65

Means with the same letters in the same row are significantly different at $P<0.05$
SEM = Standard Error of Mean

Potentially, starch digestion requires rumen bacteria to first degrade prolamin-zein before amylolitic activity in the rumen to actively hydrolyze starch (Cotta, 1988).

Starch in vitreous dry corn is more extensively encapsulated by prolamins and is less degradable in the rumen as compared to floury or opaque corns Philippeau and Michalet-Doreau (1997).

In individual stage of maturity small differences in degradability of CP (Table 3) was noted among hybrids. The degradability was higher in the dent type than dent x flint hybrids but differences were not signifficant. Effective degradability of CP was statistically different ($P <0.05$) only between hybrids Meridien and Aude both of which are dent type. The effective degradability is affected by the rate of degradation "c" of the insoluble fraction "b". This parameter showed a decreasing tendency with ripening of grain (for Aude form 0.040 $\%.h^{-1}$ in the 1st sampling to 0.018 $\%.h^{-1}$ in the 4th sampling, Mesnil from 0.064 $\%.h^{-1}$ to 0.014 $\%.h^{-1}$). Not every hybrid had such a sharp decline rate with maturity.

According to Pereira *et al.* (2004) vitreousness of the hard and soft texture grain increased linearly with advancing maturity. It is important that effective crude protein degradability (ECPD) declined with grain ripening grain (Table 3) in both types of grains. The most significant changes were in hybrids Mesnil and Chambord, where the differences between the first and fourth samplings in EDCP were until

18 % units. For hybrid Omero (dent) a slight decline in degradation rate was noted with ripening (from 50.0 % to 47.9 %). Comparing the degradation of crude protein in all four samplings, it was found that the parameters degradability and ECPD were highly statistically significant (P <0.01). By reducing the degradation of the protein degradability of starch was also reduced.

Resistance to degradation by ruminal microbes for starch in vitreous endosperm compared with floury endosperm is primarily because of the distribution of proteins in the endosperm. Concentrations of zein proteins increase and glutelin proteins decrease with increasing vitreousness (Philippeau *et al.*, 2000). The insoluble zein proteins limit accessibility of the starch granules to ruminal microbes compared with the soluble glutelin proteins (Philippeau *et al*., 2000). The protein matrix seems to limit the enzymatic digestion of starch in cereals (Kotarski *et al*., 1992) and is responsible for differences in ruminal degradability of grains (McAllister *et al*., 1993; Rooney and Pflugfelder, 1986). There is evidence that hard-textured maize grain is less degraded in the rumen than soft grains (Philippeau and Michalet-Doreau, 1997).

CONCLUSION

There were differences in starch content between dent and dent x flint maize hybrids during ripening process. Dent type maize hybrids are higher in starch content. With advancing maturity in maize grain of dent x flint type hybrids starch and CP degradability decrease was more than in dent hybrids. The effective degradability of crude protein and starch in dent type hybrids was higher than in dent x flint type hybrids. In practical terms this means that the use of protein and starch as a source of energy from maize grains of dent x flint type hybrids is more effective in ruminants as starch and CP are less extensively degraded in the rumen to glucose and ammonia in comparison to dent type hybrids grains.

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