

Effect of maize grain treatment on ruminal fermentation and the site and extent of starch digestion in cows

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ABSTRACT

Four non lactating cows with rumen and duodenal T-cannules were used in the 2 × 2 Latin square experiment to study the effect of crushed or ground maize grain on ruminal fermentation and on starch outflow from the rumen, ruminal and postruminal starch digestibility. Cows were fed diets consisting of, %: forage 70, maize grain crushed or ground 27, soyabean meal 2 and Vitamix S 1, on dry matter basis. Maize treatment did not affect ruminal fermentation. Total VFA concentration was somewhat increased when the animals were fed ground maize ($P>0.1$). Effective starch degradability was higher ($P<0.01$) for ground (70.7%) than for crushed maize (65.1%). Starch passage into the duodenum was higher ($P<0.01$) when crushed than ground maize was fed (33.5 vs 21.2%). Postruminal digestibility of starch entering the duodenum was high for both diets (82.2 vs 85.5%; $P<0.01$). Apparent total GIT digestibility of starch was affected by grain processing ($P<0.05$).

KEY WORDS: maize grain, grain processing, starch, *in sacco* degradability, intestinal digestibility, rumen fermentation

INTRODUCTION

The chemical composition and structure of starch in the individual grain types are primary factors which affect the rate and extent of starch digestion in the rumen (French, 1973; Kotarski et al., 1992; Mills et al., 1999a). The proportion of starch escaping rumen digestion can be altered by changing the source of starch or grain processing method. Maize starch is less degradable in the rumen than wheat, barley or oats starch, up to 40% of maize starch can be found to escape ruminal fermentation (Lebzien et al., 1997; Philippeau et al., 1999a). Physical processing of grain such as grinding, increases the ruminal degradability of starch compared with dry-rolling, chopping or crushing grains (Ørskov, 1986; Nocek

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and Tamminga, 1991; Phippeau and Michalet-Doreau, 1998). Type and degree of grain processing have altered the site of starch digestion and the use of nutrients by the animal (Galyean et al., 1981).

The quantity of starch digested in the intestines has been discussed by many authors (Nocek and Tamminga, 1991; Huntington, 1997; Mills et al., 1999b; Matthé, 2001; Hindle et al., 2004; Kowalik et al., 2004). The higher resistance of starch against rumen degradation decreased starch digestion in the small intestine (Nocek and Tamminga, 1991; Mills et al., 1999b). Starch digested in the small intestine improves the availability of the energy from the grain (Owens et al., 1986). Mills et al. (1999b) showed a mean total tract starch digestibility of 93% which was positively correlated with both ruminal and postruminal starch digestion. Type and degree of grain processing have altered the site of starch digestion and the use of nutrients by the animal (Galyean et al., 1981).

The objective of our experiment was to examine the effect of maize grain processing - crushing or grinding - on digestibility of starch in the rumen and total digestive tract, and the digestibility of starch postruminally and ruminal fermentation parameters. To complete this study, *in sacco* method was used to investigate the effect of processing on starch effective degradability.

MATERIAL AND METHODS

Maize grain (hybrid Svetlana) was processed in a crushing equipment Murska 350 S2 (Kemira, Finland) or in a hammer mill UH 50/18 (Germany). The samples of crushed (CM) and ground maize (GM) were separated in a set of sieves. Particles with size >3 mm (78%), with 3-1 mm (12%) and with size <1 mm (10%) were in crushed maize. Particles with size >2 mm (10%) with 1-2 mm (67%) and with size <1 mm (23%) were in ground maize.

Four non lactating Holstein-Frisien cows were used in a 2 × 2 Latin square design experiment. The animals with a mean liveweight of 550 kg were fitted with large rumen fistulae and T cannulae (Bar Diamond, Inc., USA) in the proximal duodenum (about 20 cm distal to the pylorus). The cows were housed in tie stalls with free access to drinking water.

The cows were fed twice daily (at 6.30 and 18.30). The amount of CM and/or GM (2.7 kg DM) in the experimental diets was set. The amounts of the other components were balanced at 1.25 × maintenance ME requirements (Sommer et al., 1994) as follows, kg/DM: maize silage 2.9, lucerne hay 3.9, soyabean meal 0.19, Vitamix S - Super 0.09, and fed as a mixed diets. Feed consumption was recorded daily; refusals were collected before morning feeding. The chemical composition of experimental feeds is given in Table 1.

TABLE 1

Chemical composition of feeds, g/kg DM

Nutrients n=4	Crushed maize	Ground maize	Soyabean meal	Maize silage	Lucerne hay
DM g/kg	857.3	865.7	893.5	361.7	888.7
Crude protein	94.8	97.6	509.3	78.6	168.8
Crude fibre	27.0	27.7	43.8	197.9	378.3
Ether extract	45.2	47.1	17.8	38.8	17.6
Starch	716.1	726.3	65.3	375.7	52.3
NFE ¹	823.1	823.6	356.1	640.8	364.2
Organic matter	984.1	985.7	927.0	955.8	928.6

¹ nitrogen-free extractives

A 14-day adaptation period was followed by a pre-experimental period (two days) during which duodenal chymus and faeces were collected for the determination of Cr blank levels. In the following 10 days and during the sampling of duodenal digesta and faeces the animals received daily 100 g of Cr₂O₃, as a nutrients flow marker, in four portions (25 g of Cr₂O₃ - marker wrapped in filter paper was placed in the rumen *via* the rumen fistulae at 6 and, 12 a.m., 6 and 12 p.m). Preparation of Cr - marker was similar to those reported by Rohr et al. (1979). Cr content was 129.2±0.3 mg in 1 g of Cr₂O₃ - marker.

In the sampling period (on day 1, 2, 4 and 5) duodenal samples were taken every 2 h to obtain a composite sample from 12 subsamples of a 24-h interval (Rohr et al., 1979). Faeces grab samples were collected quantitatively in the same days like duodenal digesta into a daily samples. Every total daily sample was homogenized and 4% were taken for chemical analyses of mean daily samples. The mean faeces and duodenal digesta samples were stored at - 20°C prior to analysis. The aliquots of the mean faeces and duodenal digesta samples were freeze-dried and used for Cr and nutrient determinations.

Rumen fluid was collected just before feeding (0 h) and 1, 3, 6 and 8 h after morning feeding on d 6 and 7 of the sampling period.

Effective degradation of maize starch and degradation parameters (a,b,c) were determined by *in sacco* method (Harazim and Pavelek, 1999). Three rumen fistulated cows (fed twice a day by experimental diet consist of 70% forage and 30% concentrate on dry matter basis) were used for 2, 3, 6, 9, 16, 24 and 48 h of incubation time of CM and GM samples (with a minimum of three bags per animal, incubation and feed). The parameters of starch degradation and effective degradation were calculated using the Neway programme based on the equations described by Ørskov and McDonald (1979). In the calculation of effective starch degradation an outflow rate of 0.06·h⁻¹ was used.

Chemical composition of feeds (Table 1), refusals, duodenal and faecal freeze dried samples were determined by the Wende system analysis (STN, 1985).

Starch was determined by the enzymatic method according to Salomonsson et al. (1984).

Ruminal fluid pH was measured immediately after sampling, VFA concentration was determined using gas chromatography on a 1.8 m column with 10% SP1200 and 1% H₃PO₄ on Chromosorbe WAW 80/100 mesh with isocaproic acid as an internal standard (GC Carlo Erba). Ammonia concentration was measured by the Conway method (Voigt and Steger, 1967). Concentration of Cr in faecal and duodenal samples was determined by AAS (Solar 9000 Unicam Cambridge, UK) according to the procedure of Williams et al. (1962).

The data of *in vivo* experiment were evaluated by the analysis of variance with *m* observations (in one experiment *m* = 2) by the linear model (Gill, 1978):

$$y_{ijkl} = \mu + \rho_i + \gamma_j + \alpha_k + (\rho\gamma)_{ij} + e_{ijk}$$

where *y* is the dependent variable, μ is the overall mean, ρ_i is the fixed effect of animals, γ_j is the fixed effect of period, α_k is the fixed effect of treatment, $(\rho\gamma)_{ij}$ is the fixed effect of interaction animal \times period and e_{ijk} is the random residual effects distributed $N(0, \sigma^2)$. The significance of differences between periods or treatments were tested on the basis of significance F-test.

The effective degradability of starch in CM or GM and the parameters of degradation (*a*, *b*, *c*) were evaluated by using t-test.

RESULTS

In sacco starch degradation

Crushing compared with grinding decreased the effective degradation (*edg*) of maize starch by 5-6% units (Table 2). Soluble fraction *a* in GM was higher than in CM whereas that of the insoluble but degradable fraction *b* was lower ($P < 0.01$). There were no differences between CM and GM in the degradation rate of fraction *b*.

TABLE 2

Characteristics of maize grain starch degradation in the rumen

Indices	Crushed maize	Ground maize
<i>a</i> , %	29.9 ^a	39.1 ^b
<i>b</i> , %	70.1 ^a	60.9 ^b
<i>c</i> , %	0.060	0.062
Effective degradability, % ¹	65.1 ^a	70.7 ^b

means within a row with different letters significantly differ ($P < 0.01$)

¹ at $k = 0.06 \cdot h^{-1}$

Composition of the diets and nutrient intake

Intake of nutrients was not influenced by the physical treatment of maize although the animals consumed slightly more the GM diet which resulted in the higher consumption of starch (Table 3).

TABLE 3

Mean daily intake of nutrients, g

Nutrients	Diets	
	CM ¹	GM ²
Dry matter	9353 ± 339	9636 ± 105
Organic matter	8818 ± 314	9118 ± 56
Crude protein	1178 ± 40	1187 ± 36
Crude fibre	2077 ± 115	2100 ± 53
Ether extract	297 ± 17	313 ± 17
Starch	2917 ± 99	3250 ± 129
N-free extractives	5239 ± 194	5481 ± 34

¹ crushed maize diet, ² ground maize diet

Rumen fermentation

The characteristics of rumen fermentation are given in Table 4. Processing of maize did not cause any pronounced changes in rumen fermentation parameters. The changes of pH between 0 to 8 h after feeding are given in Figure 1. No effects of maize particle size upon pH values could be noted.

The differences in the mean molar proportion of VFA between CM and GM diets were not significant ($P > 0.05$; Table 4). The changes in acetic and propionic acid concentration are illustrated in Figures 2a and 2b throughout the observation period where the maximum for CM and GM occurred at different times after feeding. An increasing tendency of concentration of butyric acid was recorded with GM diet in comparison with CM diet (Figure 2c). Lactic acid

TABLE 4

Effect of crushed or ground maize grain on rumen fermentation products

Indices	Diets		Significance of differences
	CM	GM	
Total VFA, mmol/L	78 ± 1.90	75 ± 1.76	n.s.
Acetic acid	65.8 ± 2.22	65.4 ± 2.62	n.s.
Propionic acid	17.8 ± 1.84	17.8 ± 1.61	n.s.
Butyric acid	11.6 ± 0.81	11.0 ± 1.72	n.s.
Acetate:propionate ratio	3.69	3.65	n.s.
pH	6.7 ± 0.34	6.7 ± 0.34	n.s.
Ammonia N, mmol/L	8.29 ± 4.02	8.25 ± 4.33	n.s.

¹ the data represent the mean values of five sampling time; n.s. ($P > 0.05$)

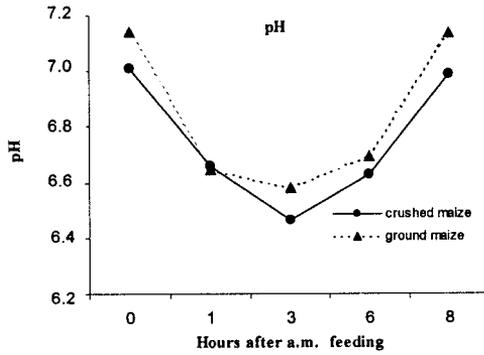


Figure 1. Rumen fluid pH when feeding diets containing crushed or ground maize

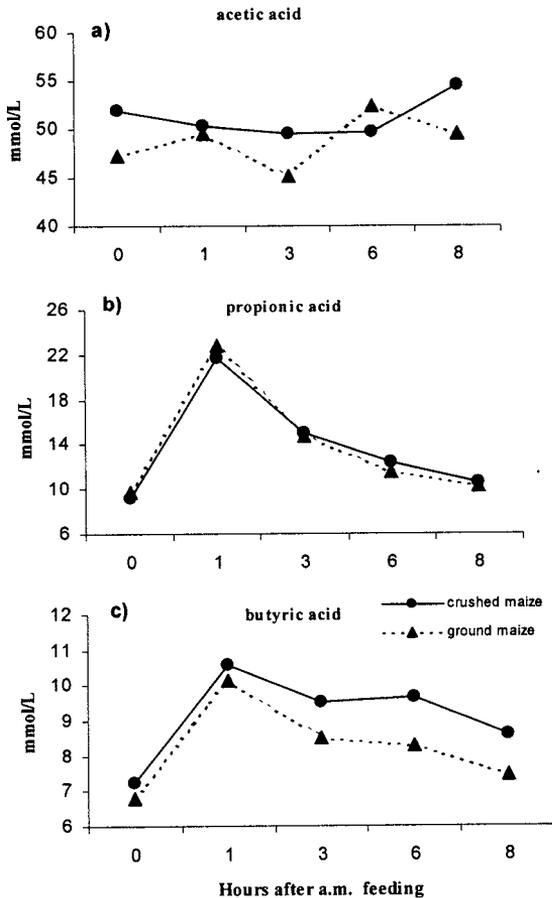


Figure 2. Changes of acetic (a), propionic (b) and butyric (c) acid concentration in the rumen fluid when feeding diets containing crushed or ground maize

concentration was slightly increased only 1 h after feeding (Figure 3). Total VFA concentration tended to be higher in cows fed CM, however differences between CM and GM were non significant ($P>0.05$). One h after feeding expressive increasing in molar percentage of propionic acid in the rumen fluid resulted in decreasing of acetate:propionate ratio (Figure 4). The latter was seen to increase up to the value of 5.14 and 4.84 (8 h after feeding CM and GM, respectively). Maize grain processing did not effect concentrations of ruminal ammonia in ruminal fluid (Figure 5).

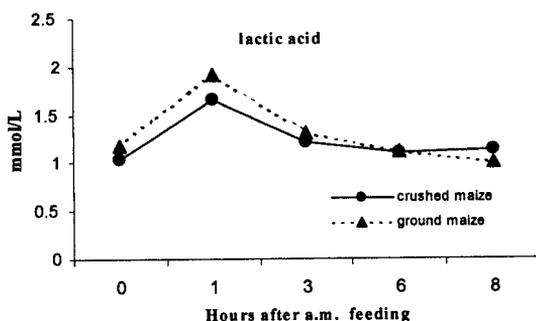


Figure 3. Changes in lactic acid concentration in the rumen fluid when feeding diets containing crushed or ground maize

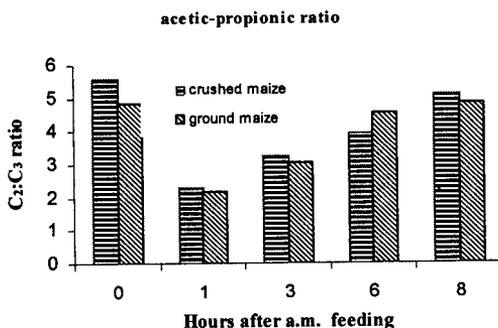


Figure 4. Changes in the acetate-to-propionate ratio in the rumen fluid after the morning feeding

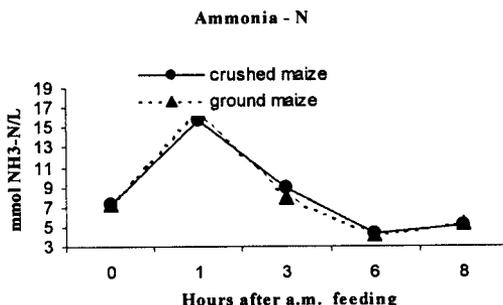


Figure 5. Changes in the ammonia N concentration in the rumen fluid when feeding diets containing crushed or ground maize

Ruminal and postruminal nutrient digestibility

Neither flow to the duodenum nor postruminal DM and OM digestion were influenced significantly by maize treatment (Table 5). Of the DM passing to the duodenum were digested in the intestine, with total tract digestion that was more than 70%. Rumen digestion of organic matter was similar to that of dry matter with non significant differences between CM and GM diets ($P>0.05$). Almost identical amounts of organic matter from the CM and GM diets were digested postruminally.

TABLE 5
Passage of DM and OM and their apparent digestion in various sites of the digestive tract of cows

Parameter	Diets		Significance of differences
	CM	GM	
<i>Dry matter</i>			
intake g/day	9353	9636	n.s.
passage to duodenum, g/24 h	6340	6379	
% of intake	67.7	66.2	n.s.
digested in rumen % of intake	32.3	33.8	n.s.
digested postruminally, g/24 h	3786	3715	
% of passage to duodenum	59.4	58.0	n.s.
total GIT apparent digestibility, %	72.6	72.3	n.s.
<i>Organic matter</i>			
intake g/day	8818	9118	n.s.
passage to duodenum, g/24 h	5239	5192	
% of intake	59.3	56.9	n.s.
digested in rumen % of intake	40.7	43.1	n.s.
digested postruminally, g/24 h	2980	2830	
% of passage to duodenum	56.5	54.1	n.s.
total GIT apparent digestibility, %	74.3	74.1	n.s.

n.s. at $P>0.05$

Starch intake, ruminal and post-ruminal digestion are shown in Table 6. Passage of starch of CM diet starch to the duodenum was significantly higher than with the GM diet ($P<0.01$). With the GM diet, as much as 78.8% of the ingested amount of starch were degraded in the rumen. With the CM diet of the ingested amount of starch as much as 33.5% passed to the duodenum and 82.2% was digested postruminally. The postruminal digestion of starch from GM diet was a slightly higher. Total apparent GIT digestion of starch was high with the significant difference between the CM and the GM diet ($P<0.05$).

TABLE 6

Passage and apparent digestibility of starch in various sites of the digestive tract of cows

Starch	Diet		Significance of differences
	CM	GM	
Intake, g/day	2917	3250	n.s.
Passage to duodenum, g/24 h	970	686	
% of intake	33.5	21.1	**
Digested in the rumen, % of intake	66.5	78.8	**
Digested postruminally, g/24 h	798	582	
% of passage to the duodenum	82.2	84.8	n.s.
Total GIT apparent digestibility, %	94.0	96.8	*

*P<0.05; ** P<0.01; n.s. at P>0.05

DISCUSSION

When animals are fed high doses of grain (wheat, barley), rapid degradation and fermentation of starch and thus an abrupt increase in VFA levels and decrease in pH occur which cause serious disturbances (De Visser and De Groot, 1981). For this reason increased attention has been paid to the use of maize or maize starch, the ruminal degradation of which is slower than that of other cereal starch (Žebrowska et al., 1997; Philippeau et al., 1999b). This phenomenon is a consequence of the different physical and chemical structure as well as the properties of proteins that encoat maize starch particles (Kotarski et al., 1992; McAllister et al., 1993).

The chemical composition and structure of starch in maize grain primary affect the rate and extent of starch *in sacco* disappearance and effective degradability; they are lower than for other grain species (Tamminga et al., 1990). Particles size in ground maize enhanced the soluble fraction, the rate of degradation and also effective degradation of starch. By Galyean et al. (1981) maize particles size had little effect on starch disappearance in 6,000 3,000 and 1,500 µm size (average of 9.2%), but starch disappearance of 750 µm maize was higher (21.7%) than for other fractions. The processing method that producing smaller particles effects on the disruption of the protein matrix of the endosperm and permits easier access of bacterial or animal enzymes to the starch granules (Hale, 1973). In the present experiment larger particles in crushed maize (78% of size >3 mm and 12% with 3-1 mm) decreased the surface area, starch *in sacco* disappearance and effective degradability in comparison with ground maize, where 23% particles were smaller than 1 mm and a size of 67% of particles was 1-2 mm.

Forages presented 70% and maize grains 27% of the dry matter of the diets with the daily intake of starch making up 31.2% and only slightly but not

significantly higher, 33.7% of the DM of the respective rations. Loose et al. (1998) reported that the proportion of maize grain higher than 50% in diet did not cause any negative effects on the cows.

Processing of maize grains did not cause any pronounced changes in the parameters of ruminal fermentation. With both diets the mean pH values were 6.7 and revealed an identical course throughout the experimental period (Figure 1).

Ammonia and VFA concentrations in the rumen fluid were similar for both ground or crushed maize treatments. The similar results were found by Knowlton et al. (1998) with dry ground and rolled maize when mean particle size was 618 μm and/or 1725 μm . On the other hand *in vitro* VFA production (Belyea et al., 1997) at 24 h was greater for maize with the 1 mm (97 mmol/L) than 5 mm particle size (89 mmol/L). In maize-fed animals the levels of acetic acid which is the main fermentation product of bacteria digesting cellulose and hemicelluloses increased as late as 6 and 8 h after feeding, respectively (Figure 2a). Propionic acid which is produced by starch-fermenting bacteria (Ørskov, 1986) and lactic acid reached maximum concentrations in 1 h after feeding (Figures 2b and 3). The abrupt increase in propionic and lactic acid levels shortly after feeding rations containing high level of starch is often reported as a cause of digestive malfunctions, decreased production and health problems (De Visser and De Groot, 1981; Sutton et al., 1987). A marked increase of propionic acid concentration evidently decreased the acetate:propionate ratio one h after feeding (Figure 4).

Nocek and Tamminga (1991) reported that a site of starch digestion and digestion within site could be manipulated as processing and feeding management strategies largely influence site digestion. Feeding larger particle size of maize to cattle reduced digestibility (Waldo, 1973). Maximum maize digestibility by cattle occurred with a modulus of fineness between 3.0 and 4.8 mm (Adeeb et al., 1971). In our experiment, ground maize significantly increased (66.5 vs 78.8%; $P < 0.05$) *in vivo* ruminal starch digestion compared with crushed maize (Table 6). Similar digestibility of ground maize in the rumen was found by Waldo (1973). Feeding of finely ground maize increased rumen starch digestibility, compared with coarsely cracked maize.

The proportion of digested starch entering the small intestine depends on enzymatic activity, starch source, passage rate and particle size of the digesta (Mills et al., 1999b). The capacity for the enzymatic hydrolysis of starch entering the small intestine is important when high starch intake leads to significant quantities of starch escaping fermentation in the rumen (Mills et al., 1999b). With the CM diet 970 g starch passed to the duodenum within 24 h which was by 284 g more than with the GM diet. Of these amounts as much as 798 and 582 g/24 h, respectively, were digested postruminally. Postruminal digestion of starch was high in both cases (over 80%), and the difference between CM and GM diets was

significant ($P < 0.01$). There was a tendency of higher digestibility for GM diet in the intestines, where more smaller particles with larger surface area were present. The smaller maize particles are more susceptible to enzymatic digestion in the small intestine. Adequate access of enzymes to starch granules is one of the factors limiting intestinal starch digestion (Owens et al., 1986).

The grinding of maize decreased faecal starch flow and increased starch digestion in the total digestive tract because of increased starch disappearance from the large intestine (Knowlton et al., 1998). They reported starch digestion in the total tract 88.9% for dry ground maize and 98.2% for high moisture maize. Mills et al. (1999b) showed a mean total tract starch digestibility of 93% and Waldo (1973) higher, more than 99% for ground maize. In our experiment the total tract digestibility of starch from CM diet was significantly lower ($P < 0.05$) than from GM diet (Table 6). However, the differences in total digestion were rather low (3.4%) with GM revealing a tendency to higher digestibility.

Concluding it can be stated that maize grain processing influences the sites of starch digestion. Larger particles in crushed maize caused the lower ruminal starch degradability and increased passage of starch to the duodenum than ground maize. The crushing process decreases post-ruminal and total tract starch digestion. Neither crushing nor grinding affect ruminal VFA, and ammonia concentration or ruminal pH.

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STRESZCZENIE

Wpływ obróbki ziarna kukurydzy na przebieg fermentacji w żwaczu oraz rozmiar i miejsce trawienia skrobi przez krowy

W doświadczeniu przeprowadzonym w układzie kwadratu łacińskiego 2×2 , na 4 zasuszonych krowach z przetokami do żwacza i dwunastnicy, badano wpływ gniecenia bądź śrutowania ziarna kukurydzy na fermentację i wypływ skrobi ze żwacza oraz jej strawność w żwaczu i dalszych odcinkach przewodu pokarmowego. Krowom podawano diety złożone (% s.m.) z paszy objętościowej, 70; ziarna kukurydzy gniecionej lub śrutowanej, 27; śrutu sojowej, 2 i preparatu witaminowego S, 1. Sposób traktowania kukurydzy nie wpłynął na przebieg fermentacji w żwaczu, stężenie LKT w żwaczu było jednak nieco większe, ale nieistotnie, u zwierząt otrzymujących kukurydzę śrutowaną. Efektywny rozkład w żwaczu skrobi kukurydzy śrutowanej był większy ($P < 0,01$) niż gniecionej, odpowiednio 70,7 vs 65,1%; do dwunastnicy przechodziło więcej skrobi gniecionej, 33,5% niż śrutowanej, 21,2% ($P < 0,01$). Skrobia kukurydzy śrutowanej przechodząca do dwunastnicy była trawiona w mniejszym stopniu ($P < 0,01$) w dalszym odcinku przewodu pokarmowego (82,2%) niż kukurydzy gniecionej (85,5%). Strawność w całym przewodzie pokarmowym skrobi śrutowanej była większa ($P < 0,05$) niż gniecionej (94,0 vs 96,8%).