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# Origin of starch and its effect on fermentation in the rumen and amino acids passage to the intestinum of cows

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## ABSTRACT

Two origin of starch – maize and wheat were tested by using four non lactating cows with rumen and duodenal T- cannules in the experiment. $Cr_2O_3$ was used as a marker of nutrient flow to the duodenum. Cows were fed diets consisting of, %: forage 70, maize and/or wheat meal 27 and/or 29, soyabeanmeal 2 and Vitamix S 1, on dry matter basis. Starch origin did not affect ruminal fermentation significantly. Concentration of all VFA was higher with wheat than with maize meal. The mean of acetate:propionate ratio was significantly higher ($P<0.05$) when wheat was fed. Maize in the diet significantly increased the flow of starch to the duodenum (21.4 % vs. 10.2 % from the daily intake). With both diets higher amounts of crude protein and amino acids passed to the duodenum than were ingested (101.8 % with wheat and 130.4 % with maize). In comparison with intake, flow of essential lysine (176.6% and 140.8 %) and nonessential glycin (289.7 % and 186.4 %) were the highest. It means that availability of energy and nitrogen by microbes in the rumen are more effective from maize than wheat as the starch origin.

**Keywords:** wheat, maize, starch, rumen fermentation, nutrients passage, amino acids

## INTRODUCTION

Carbohydrates of grains and forages are an important source of energy for ruminants. Since grains contain high leveles of starch they influence the effectivity of use of the whole feeding ration of ruminants (Tamminga et al., 1990). Rate and site of starch digestion are important in terms of nutrient availability in ruminants (Offner and Sauvant, 2004). There are considerable differences in the ruminal degradation of starch and crude protein from grains, maize, and other concentrate (Zebrowska et al., 1997). Rate and extent of starch digestion in the rumen are affected with the structure of starch in the individual grain types (French, 1973; Kotarski et al., 1992). Maize starch is less degradable in the rumen than wheat, barley or oat starch. Up to 40 % of maize starch can be found to escape ruminal fermentation (Lebzien et al. 1997; Kotarski et al.,1992).

The extent of microbial proteosynthesis and supply of the animal not only with microbial protein but amino acids as such depend upon the amount of fermentable energy in the rumen (Owens et al., 1986; Huntington, 1997). For this reason synchronization of carbohydrate fermentation and release of nitrogen has great importance for the synthesis of ruminal microbial proteins and their passage into the small intestine (Aldrich et al., 1993 and Overton et al., 1998).

Our investigation was focused on the determination of ruminal digestibility of nutrients in nonlactating cows fed balanced diets that contained ground maize and wheat, fermentation in the rumen, and simultaneously passage of amino acids to the duodenum.

## MATERIAL AND METHODS

Four non lactating cows with a mean live weight of 550 kg were used in the experiment; the animals were fitted with large rumen fistulae and T cannulae in the proximal duodenum. The cows were fed with the experimental diets twice a day. The diets contained 2.57 or 2.59 kg of dry matter of ground maize and ground wheat. The amounts of the other components were balanced. Water was always available.

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**Table 1: Composition of experimental diets**

| Feeds | Wheat | | Maize | |
|---|---|---|---|---|
| | (kg DM) | % | (kg DM) | % |
| Maize silage | 3.00 | 33 | 2.93 | 31 |
| Lucerne hay | 3.31 | 37 | 3.87 | 40 |
| Maize meal | - | | 2.59 | 27 |
| Wheat meal | 2.64 | 29 | - | |
| Soyabean meal | - | | 0.19 | 2 |
| Vitamix S-Super | 0.097 | 1 | 0.09 | 1 |
| Total | 9.047 | | 9.67 | |

The experimental design was replicated Latin square with 2 treatments. Chromic oxide was used as a marker of nutrient flow to the duodenum, apparent digestion of nutrients in the rumen. Preparation of Cr - marker and sampling of duodenal digesta was similar to those reported by Rohr et al. (1979). In the main experimental period the animals received daily 100g of $Cr_2O_3$ - marker in four portions (25 g of $Cr_2O_3$ - marker wrapped in filter paper was placed in the rumen via the rumen fistulae at 6 a.m.,12 a.m., 6 p.m. 12 p.m). Chromium content was 129.2 ± 0.3 mg in 1 g of $Cr_2O_3$ – marker.

Samples of duodenal chymus were obtained via the duodenal T cannula by the method of Rohr et al. (1979) every two hours and collected into a bulk 24 hour sample. After sampling of duodenal contents was finished, ruminal fluid was collected for two days by means of a ruminal fistula prior to the morning feeding and 1, 3, 6 and 8 hours after feeding.

Chemical composition of feeds (Table 2), rests of feeds, duodenal freeze dried samples were determined by the Wende analysis (CSN 1977). Starch was determined by the enzymatic method according to Salomonsson et al. (1984).

**Table 2: Mean chemical composition of feeds (g/kg DM)**

| Nutrients (n=4) | Wheat meal | Maize meal | Soyabean meal | Maize silage | Lucerne hay |
|---|---|---|---|---|---|
| Dry matter g/kg | 875.8 | 865.7 | 893.5 | 361.7 | 888.7 |
| Crude protein | 153.0 | 87.6 | 509.3 | 78.6 | 168.8 |
| NDF | 140.6 | 45.2 | 83.5 | 441.6 | 490.3 |
| ADF | 50.1 | 34.7 | 76.7 | 231.1 | 431.1 |
| Ether extract | 17.9 | 47.1 | 17.8 | 38.8 | 17.6 |
| Starch | 618.7 | 655.3 | 65.3 | 304.6 | 51.3 |
| N-free extractives | 774.3 | 823.3 | 356.1 | 640.8 | 364.2 |
| Organic matter | 977.3 | 985.7 | 927.0 | 955.8 | 928.6 |

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% $H_3PO_4$ on Chromosorbe WAW 80/100 mesh with isokaprylic acid as an internal standard (GC Carlo Erba). Ammonia concentration was measured by the Conway method (Voigt and Steger, 1967). Concentration of Cr duodenal samples was determined by AAS (Solar 9000 Unicam Cambridge, UK) according to the procedure of Williams et al. (1962).

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

$$y_{ijkl} = \mu + \rho_i + \gamma_j + \alpha_k + (\rho\gamma)_{ij} + e_{ijk}.$$ (Gill, 1978).

In the linear model y is the dependent variable, $\mu$ is the overall mean, $\rho_i$ is the fixed effect of animals, $\gamma_j$ is the fixed effect of period, $\alpha_k$ is the fixed effect of treatment, $(\rho\gamma)_{ij}$ is the fixed effect of interaction animal x 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.

## RESULTS AND DISCUSION

In our experiment proportion of wheat grain or maize grain in the dry matter of the feed rations was 29 % and 27 % (Table 1) and mean daily intake of wheat starch was 2396 g (27 % of DM intake) and/or 3250 g maize starch (33.7 % of DM intake). Lower N content in maize meal (Table 2) have to be balanced by using soybean meal supplementation of the ration There are differences in amino acids content and amino acids patern between wheat and maize (Table 3).

Large amounts of starch and sugars included in feed rations fed dairy cows, could dramatically reduce ruminal pH and rise the levels of propionic and lactic acid ( de Visser et al., 1980). This led to the recommendation not to include more than 25 % starch in concentrates fed to dairy cows. Research showed that the negative influence of non-structural carbohydrates on rumen fermentation is caused by soluble sugars, and rapidly degraded starch (Taminga et al., 1990). There are knowledges of differences in ruminal behaviour between starches of various origin (Lebzien et al., 1983;Tamminga et al., 1990; Zebrowska et al., 1997; Matthé, 2001; Kopčeková et al., 2003; Offner and Sauvant, 2004) and its effect on fermentation in the rumen. We observed differences in rumen fermentation when cows were fed with wheat or maize meal in feed rations (Fig. 1 –6). In spite of nonsignificant differences in means of individual parameters (Table 4), we found lower ruminal pH with wheat meal than maize meal in all sampling times. Lower rumen pH is affected with higher concentration of VFA originated from higher acetic acid,

**Table 3: Mean content of amino acids of feeds (g AS/kg DM) (n=3; *n=4)**

| Amino acids | Wheat meal | Maize meal | Soyabean meal |
|---|---|---|---|
| Thr | 4.33 | 2.90 | 19.84 |
| Val | 6.48 | 5.52 | 21.47 |
| Ileu | 5.08 | 2.43 | 21.10 |
| Leu | 10.13 | 8.98 | 38.50 |
| Tyr | 3.85 | 3.29 | 17.28 |
| Phe | 6.09 | 3.54 | 24.53 |
| His | 3.76 | 2.16 | 12.93 |
| Lys | 5.57 | 2.76 | 32.50 |
| Arg | 9.21 | 4.58 | 44.18 |
| Met | 1.73 | 1.41 | 6.20 |
| ΣEAA | 56.21 | 35.59 | 237.00 |
| Cys | 3.38 | 1.40 | 5,87 |
| Asp | 10.13 | 5.39 | 59,96 |
| Ser | 6.74 | 3.82 | 26,34 |
| Glu | 37.50 | 14.60 | 91,27 |
| Pro | 12.75 | 7.27 | 26,70 |
| Gly | 6.22 | 3.36 | 21,81 |
| Ala | 5.58 | 5.52 | 21,47 |
| ΣneAA | 82.28 | 41.37 | 253,42 |
| ΣAA | 138.49 | 76.96 | 490.43 |

**Table 4: Effects of feeding on the $NH_3$-N-concentration (mmol/l) in rumen liquid before and after morning feeding**

| Time after feeding (min) | Ration | | | | | |
|---|---|---|---|---|---|---|
| | Lucerne hay* | | Maize silage* | | | |
| | H 70 | HLO 70 | H 30 | HLO 30 | PSEM |
| Before | 3.1 | 3.8 | 6.5 | 5.5 | 1.3 |
| 30 | 3.8 | 4.9 | 8.1 | 7.1 | 1.8 |
| 60 | 7.4 | 6.2 | 11.5 | 9.7 | 1.7 |
| 90 | 8.9 | 7.9 | 12.1 | 10.1 | 1.6 |
| 120 | 8.8 | 8.2 | 12.2 | 10.7 | 2.5 |
| 180 | 6.9 | 7.7 | 4.9 | 7.2 | 2.0 |
| 300 | 2.8 | 3.2 | 1.2 | 1.7 | 0.8 |

1) see Table 3

**IV. Effect of starch origin on rumen fermentation products (n =30 )**

| Indices | Wheat meal | Maize meal | Signif. of differences |
|---|---|---|---|
| ∑VFA (mM/100 ml) | 11.6 ± 0.36 | 7.5 ± 1.76 | ** |
| UMK (mol %) | | | |
| Aceti acid | 65.1 ± 1.83 | 65.4 ± 2.62 | n.s. |
| Propionic acid | 17.5 ± 1.03 | 17. ± 1.61 | n.s. |
| Butyric acid | 13.2 ± 1.06 | 11.0 ± 1.72 | n.s. |
| Ammonia -N (mg/100 ml) | 16.3 ± 1.20 | 11.5 ± 6.41 | n.s. |
| PH | 6.4± 0.06 | 6.7 ± 0.34 | n.s. |
| Acetát:propionate ratio | 4.25 | 3.65 | * |

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

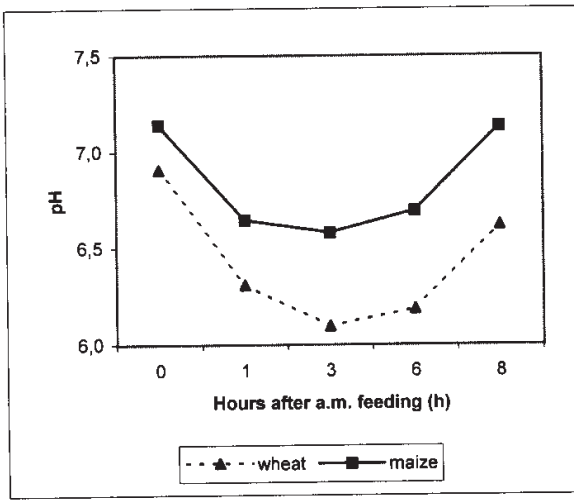


**Fig. 1: Rumen fluid pH when feeding diets containing maize or wheat meal**

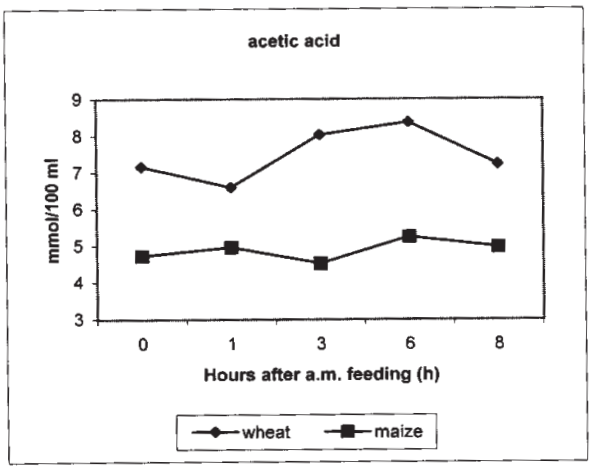


**Fig. 2: Changes of acetic acid concentration in the rumen fluid when feeding diets containing maize or wheat meal**

| Effects[1)] | | |
|---|---|---|
| Hay | Linseed oil | HxLO |
| 0.036 | 0.771 | 0.407 |
| 0.050 | 0.993 | 0.511 |
| 0.019 | 0.300 | 0.851 |
| 0.105 | 0.392 | 0.353 |
| 0.167 | 0.621 | 0.830 |
| 0.485 | 0.380 | 0.664 |
| 0.056 | 0.504 | 0.929 |

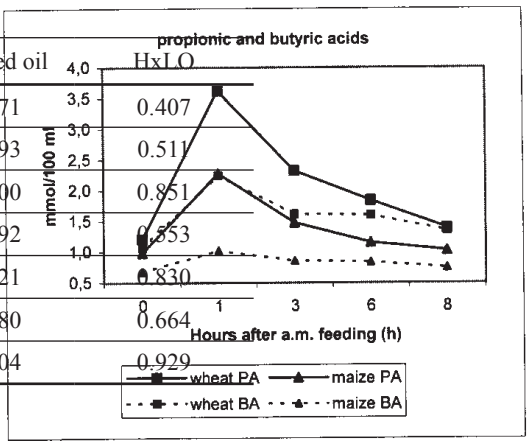


**Fig. 3: Changes of propionic and butyric acid concentrations in the rumen fluid when feeding diets containing maize or wheat meal**

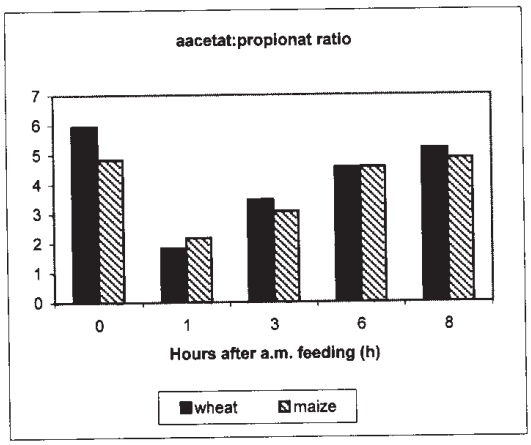


**Fig. 4: Changes in the acetate-to-propionate ratio in the rumen fluid after the morning feeding**

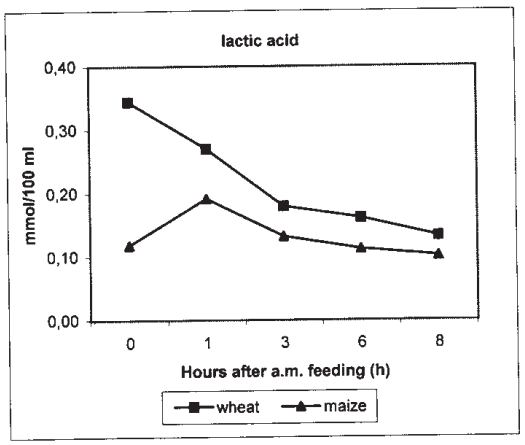


**Fig. 5: Changes of lactic acid concentration in the rumen fluid when feeding diets containing maize or wheat meal**

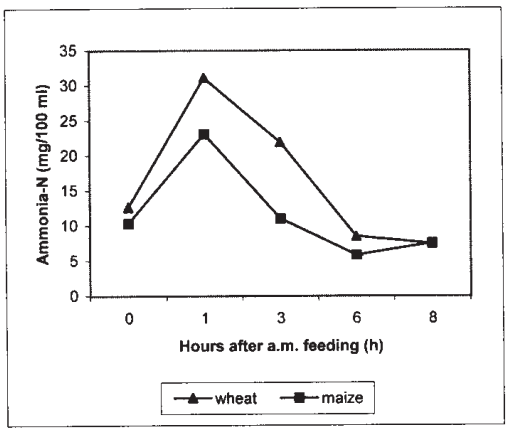


**Fig. 6: Changes in the ammonia N concentration in the rumen fluid when feeding diets containing maize or wheat meal**

propionic, butyric acids (Fig. 2 and 3) and lactic acid. Propionic acid which is produced by starch fermenting bacteria (Ørskov, 1986) reached maximum concentration in one hour after a.m. feeding (Fig. 3). A marked increase of propionic acid concentration decreased the acetat:propionat ratio one hour after a.m. feeding (Fig. 4). Because the level of acetic acid which is the main product of activity of cellulolytic bacteria is more stable during the day, acetic: propionic ratio is affected mainly by the kinetic of propionic acid production. Consuming the diet with wheat meal increased concentration of lactic acid (Fig. 5). The mean concentration of lactic acid was higher (2.17 mmol/l) with wheat meal than with maize meal (1.31 mmol/l).

In sacco results with maize grain, showed a more slower degradation of the CP as compared to other cereals (Tamminga et al., 1990; Zebrowska et al. 1997; Čerešňáková et al., 2000). The increased disappearance of N from wheat meal supported increasing N-Nh3 concentration in the rumen fluid in the first hours after a.m. feeding (Fig. 6).

Starch intake and ruminal digestion are shown in Table 5. Passage of starch of maize ration was significantly higher than with wheat diet ($P<0.01$). With the wheat meal as much as 89.8 % of the ingested amount of starch were degradraded in the rumen and only 11.2 % passed to the duodenum. If maize was origin of starch in the diet, degradation was significantly lower (78.6 %) in the rumen.

Disappearance of organic matter from the rumen was significantly lower with the maize meal (43.1 %) than with wheat meal (50.8 %). The same effect of starch origin on organic matter digestibility observed Lebzien et al. (1983) but difference between maize and wheat diets were bigger. The differences in organic matter digestion can have an impact on microbial proteosynthesis in the rumen. In our experiment this difference was only 7 % units but microorganisms have sufficient energie for utilization of ammonia –N.

**Table 5: Passage of organic matter, starch and crude protein to the duodenum of cows and apparent digestibility in the rumen**

| Indices | Diets | | Signif. of differences |
|---|---|---|---|
| | Wheat meal | Maize meal | |
| **Organic matter** | | | |
| Intake g/24 h | 8323 | 9118 | ** |
| Passage to duodenum g/24 h | 4092 | 5192 | ** |
| % of intake | 49.2 | 56.9 | * |
| Digested in the rumen, % of intake | 50.8 | 43.1 | * |
| **Starch** | | | |
| Intake g/24 h | 2396 | 3205 | ** |
| Passage to duodenum g/24 h | 268 | 686 | ** |
| % of intake | 11.2 | 21.4 | ** |
| Digested in the rumen, % of intake | 89.8 | 78.6 | ** |
| **Crude protein (N x 6,25)** | | | |
| Intake g/24 h | 1242 | 1164 | n.s. |
| Passage to duodenum g/24 h | 1263 | 1518 | * |
| % of intake | 101,8 | 130,4 | ** |

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

We observed much higher passage of nitrogen (101.8 % vs. 130,4 %) and amino acids (96.3 % vs.128.2 %) from the rumen than was their intake (Table 5 and 6). These data documented the utilization of nitrogen released by degradation feed´s protein and urea nitrogen from the rumeno-hepatic cycle in microbial proteosynthesis. We found differnces (P<0.01) between N- and amino acids flow to the duodenum with maize diet than wheat diet. Some authors reported higher flow of AAN (amino acids nitrogen) as a percentage of non ammonia-N in duodenal contents with maize diet than with wheat diet (Lebzien et al.,1983; Ørskov et al., 1971; Tamminga, 1973). In our experiment the flow of essential and nonessential amino acids was not affected to the same degree (Table 6). With regard to individual essential amino acids - lysine, threonine, valine, isoleucine, leucine, phenylalanine were significantly higher (P<0.01) with maize diet than wheat diet. Mainly flow of lysine from intake was 140.8 % vs. 176.6 %. This result do not egree with result by Lebzien et al. (1983). With regard to nonessetial amino acids for glycine was difference between maize and wheat diets the biggest (289.7 % vs. 186.6).

Finally it can be stated that the feeding of maize meal more positively affected starch flow to the duodenum than wheat meal. Maize content in the feed ration increased flow of nitrogen and essential amino acids (mainly lysine) from the rumen towards nitrogen and amino acids intake. The ration has to be well balanced.

**Table 6: Mean daily intake and passage of amino acids to the duodenum of cows**

| Amino acids | Wheat meal | | | Maize meal | | | Signif. of differences | |
|---|---|---|---|---|---|---|---|---|
| | intake (g AK/ 24 h) | passage g/24h | % of intake | intake (g AK/ 24 h) | passage g/24h | % of intake | passage g/24h | % of intake |
| Thr | 42.3 | 54.2 | 128.1 | 36.5 | 61.1 | 167.3 | * | ** |
| Val | 58.6 | 51.7 | 88.2 | 46.7 | 54.3 | 116.3 | n.s. | ** |
| Ileu | 44.6 | 44.7 | 100.4 | 36.0 | 47.1 | 130.8 | n.s. | ** |
| Leu | 84.8 | 75.3 | 88.8 | 80.5 | 93.5 | 116.1 | ** | ** |
| Tyr | 46.1 | 59.6 | 129.3 | 35.2 | 44.4 | 126.1 | ** | n.s. |
| Phe | 49.0 | 46.7 | 95.4 | 44.6 | 50.0 | 112.1 | n.s. | * |
| His | 32.4 | 27.2 | 83.8 | 26.3 | 22.8 | 86.7 | * | n.s. |
| Lys | 51.4 | 72.2 | 140.8 | 40.2 | 71.0 | 176.6 | n.s. | ** |
| Arg | 53.0 | 52.7 | 99.87 | 39.2 | 51.1 | 130.4 | n.s. | n.s. |
| Met | 16.6 | 15.5 | 93.4 | 17.3 | 16.7 | 96.5 | n.s. | n.s. |
| ΣEAA | 478.8 | 499.9 | 104.5 | 402.5 | 512.0 | 127.2 | n.s. | * |
| Cys | 16.8 | 14.9 | 88.4 | 13.6 | 17.9 | 131.6 | n.s. | ** |
| Asp | 130.1 | 112.0 | 86.1 | 102.6 | 123.4 | 120.3 | n.s. | ** |
| Ser | 49.4 | 53.5 | 108.3 | 42.7 | 62.6 | 146.6 | n.s. | ** |
| Glu | 168.2 | 120.6 | 72.0 | 119 | 153.9 | 129.3 | ** | ** |
| Pro | 93.6 | 45.2 | 48.4 | 75.3 | 60.9 | 80.9 | ** | ** |
| Gly | 51.3 | 95.5 | 186.4 | 35.7 | 109.5 | 289.7 | n.s | ** |
| Ala | 60.6 | 66.8 | 110.4 | 57.2 | 75.7 | 132.3 | * | ** |
| ΣNEAA | 570.0 | 508.5 | 89.2 | 446.8 | 603.8 | 135.1 | ** | ** |
| ΣAA | 1048.8 | 1008.4 | 96.1 | 818.0 | 1115.8 | 136.4 | n.s | ** |

* p <0,05; ** p <0,01; n.s. pre P>0,05

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