In vitro ruminal degradability of cereal grain starch

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ABSTRACT: An *in vitro* method was used to determine ruminal degradability of starch (IVRDS) in a set of cereal grains. The set included 9 feed samples, including 2 samples of ground wheat, 2 samples of wheat treated with sodium hydroxide, ground barley, barley treated with sodium hydroxide, 2 samples of ground oats and ground maize. Ruminal degradability of starch was assayed by the feed fermentation for 2, 4, 6, 16 and 24 hours. A significant difference in starch degradability was found between treated and untreated ground samples after 2-hour fermentation (13.73 ± 3.12 vs. 32.77 ± 8.17; $P < 0.001$), 4-hour fermentation (33.44 ± 7.31 vs. 60.30 ± 16.71; $P < 0.001$) and 6-hour fermentation (42.63 ± 7.13 vs. 74.20 ± 6.38; $P < 0.001$). On the basis of the rate of ruminal degradability of starch the order of cereal grains was as follows (from the highest to the lowest value): ground oats, ground wheat, ground barley, ground maize, wheat and barley treated with sodium hydroxide.

Keywords: cereals; starch; ruminal degradability; *in vitro* method

Ruminants obtain most of their energy from cereal grains with starch as the principal nutrient. The amount of starch in feeds for ruminants may vary from zero in green fodder to almost 100% in potatoes and maize (Sommer, 2000). Cereal grains contain a relatively high amount of starch (40–70% in dry matter), with the highest percentage in maize grain (Sommer, 2000). Maize grain is different in comparison with others cereal grains as regards ruminal starch degradation. Differences in the chemical and physical structure of starch granules determine the quality of starch in feed and its availability and degradability. In maize, for example, the rate of starch degradability is lower than in other cereals (Philippeau *et al*., 1999). Except other factors the chemical composition and physical structure of the maize starch chain, which is more resistant to the attack of chemical bonds by bacterial amylase, play and important role.

The most important site of cereal grain starch digestion is the rumen (Theurer, 1986). The rate and extent of starch digestion in the rumen are determined by intricate interrelations between several factors, including source of dietary starch, diet composition, mechanical and chemical processing, and degree of adaptation of ruminal microbiota to the diet (Huntington, 1997). Studies comparing the ruminal starch degradability in different feeds (Richards *et al*., 1995) confirmed that from the aspect of starch degradation, the maize grain is a highly concentrated feed with high content of lower degradable starch in the rumen (Philippeau *et al*., 1999). For this reason the maize is ideal for the feeding of high-yielding dairy cows. The process of starch degradation in the rumen may be affected by mechanical alterations of the grain (Cooper *et al*., 2002). Herrera-Saldana *et al*. (1990) described the starch degradability for five (dry and steam rolled) cereal grains: maize, sorghum, wheat, barley and oats. The investigation led to a ranking of the five grains for starch degradation *in situ* and *in vitro*. Both the *in situ* and *in vitro* data gave the same ranking of the grains in terms of starch degradability, which indicates that *in vitro* data can represent starch degradability as it occurs in the rumen. The objective of the study was the application of *in vitro* enzymatic method to determine starch degradability in the rumen for wheat, barley, oats and maize. The trial also included a comparison of the effect of alkali treatment of wheat and barley grain with sodium

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hydroxide (NaOH) on the rate of starch degradability in the rumen.

MATERIAL AND METHOD

Animals

Two non-lactating cows (Black Pied) fitted large rumen cannulas were used for our experiments. The diets consisted of 4 kg hay, 10 kg of maize silage and 1 kg of ground barley with a mix of vitamins and minerals. The diet was administered in two equal rations daily. Non-lactating cows were used as donors of ruminal fluid for an *in vitro* method.

Feeds

Common types of feeds for ruminants were tested (Table 1): 2 samples of ground wheat (UW1 and UW2), 2 samples of treated whole wheat grain (TW3 and TW4), ground barley (UB1), treated whole barley grain (TB2), 2 samples of ground oats (UO1 and UO2) and ground maize (M). Dry barley and wheat grains were treated with sodium hydroxide (3 kg NaOH per 100 kg barley or wheat) with added water. The final dry matter for TW3 was 62.85%, for TW4 65.61% and for TB2 64.92%. The samples UW1 and TW3, UB1 and TB2 were identical, UW2 and TW4 were randomly selected. The treatment was performed in a mixer feed wagon. The contents of total starch and dry matter are shown in Table 2.

Ruminal starch degradability (IVRDS)

The total starch content in the samples was estimated as total α-linked glucosides, using the method described by MacRae and Armstrong (1968). Each feed was subject to triple analysis. Glucose was assayed enzymatically using Bio-la-test, GLU GOD 250, (Lachema, Brno, Czech Republic). The starch content was calculated as 90% of the total glucose content and recalculated per sample dry matter.

Degradable starch content was determined via fermentation of the samples in a fermentation solution (buffer mixed with ruminal fluid) for 2, 4, 6, 16 and 24 hours. The buffer was prepared using the modified Goering and Van Soest (1970) method. The ruminal fluid was taken from two cannulated cows, mixed, filtered, tempered to 39°C and saturated with CO_2. The fermentation started by mixing the feed sample (0.5 g) with 50 ml of fermentation solution and saturation of the soaked sample with CO_2. The trial included a blank for each time interval, a glucose standard and a standard sample for elimination of the variability of ruminal fluid. The fermentation was performed at 39°C and finished after 2, 4, 6, 16 and 24 hours with addition of 1ml 6 N H_2SO_4. After that the samples were autoclaved for 1 hour at 130°C. After cooling 25 ml of acetate buffer containing 0.2 g of amyloglucosidase (A7255, Sigma Chemical, St. Louis, MO) was added. The fermented feed samples were incubated at 55°C for the period of 24 hours, then cooled and transferred into volumetric flasks. To obtain the volume of 100 ml, distilled water was added to the samples. A 15 ml aliquot was centrifuged at 2 000 × g for 10 minutes, filtered and 10 ml of supernatant was mixed with distilled water to obtain the volume of 100 ml. Glucose measurement was the same as described above.

Statistical analyses were performed using the GLM procedure of SAS (SAS Institute Inc., 2001). The model included treated feed as the fixed effect.

Statistical significance of differences in measured parameters was evaluated by the following model equation:

Table 1. The values of ruminal degradability of starch (%) determined by an *in vitro* method

Incubation time (h)	Feeds								
	UW1	UW 2	TW3	TW 4	UB1	TB2	UO1	UO2	M
2	25.67	47.34	10.07	16.96	26.00	14.16	29.69	34.28	21.62
4	58.56	65.16	23.76	37.63	44.15	38.93	76.67	76.95	34.32
6	72.02	75.27	33.29	45.79	63.58	48.81	79.80	80.32	40.62
16	83.37	91.70	79.41	86.13	84.14	83.16	94.56	95.31	79.37
24	96.35	96.66	97.37	96.35	96.13	97.15	98.93	98.89	97.48

Table 2. List of feeds, contents of dry matter and starch

Feed	DM (g/kg)	Starch (g/kg DM)
Wheat 1 U	894.9	678.2
Wheat 2 U	894.5	599.1
Wheat 3 T	917.5	604.1
Wheat 4 T	918.5	594.2
Barley 1 U	912.1	609.5
Barley 2 T	917.5	595.8
Oat 1 U	903.1	452.7
Oat 2 U	898.2	481.8
Maize	898.7	699.4

U – untreated feeds, T – alkali-treated feeds

$$y_{ij} = \mu + A_i + e_{ij}$$

where: y_{ij} = observed variable
μ = mean value
A_i = fixed effect of feed treatment
e_{ij} = random error

RESULTS AND DISCUSSION

For total starch contents in the tested feeds see Table 2. While oats showed the lowest content of starch, the highest value was found in maize. Grains treated with NaOH showed a slightly lower starch concentration than the untreated grains. Marked differences between the individual cereals were observed in incubation intervals. The IVRDS values were measured in intervals of 2, 4, 6, 16 and 24 hours of fermentation. For the calculations of IVRDS from the total starch contents see Table 1. It follows from Table 3 and Figures 1 and 2 that the most significant differences between feeds treated with NaOH and the untreated feed samples were found after 2, 4 and 6-hour fermentation. The differences in IVRDS values decreased after 16 and 24-hour fermentation *in vitro*. While after 16-hour fermentation the differences were still statistically significant at $P < 0.01$, after 24-hour fermentation the differences were statistically insignificant (Table 4). This finding corresponds well with that of Richards *et al*. (1995), who used the *in vitro* method of Wester *et al*. (1992) for specification of starch loss in peas, maize and wheat. A trial with two different ratios of ruminal fluid to artificial saliva (1 : 1, 1 : 2) resulted in the values of IVRDS reaching the limits of 80% in maize and 90% in wheat after 16 and 20-hour fermentation. In a trial presented by Boss and Bowman (1996), the starch loss determined by an *in situ* method in different varieties of barley amounted to more than 80% as early as after 6-hour fermentation in the rumen, which is more than the values found in our study.

Table 3. Mean ± standard deviation of *in vitro* ruminal degradability of starch in 2, 4, 6, 16 and 24 h incubation of treated and untreated feeds

Incubation time (h)	Feeds	
	[a] untreated	[b] treated
2	32.77 ± 8.17	13.73 ± 3.12
4	60.30 ±16.71	33.44 ± 7.31
6	74.20 ± 6.37	42.63 ± 7.13
16	89.79 ± 5.34	82.90 ± 3.01
24	97.39 ± 1.37	96.95 ± 0.53

[a]mean ± SD of IVRDS of untreated feeds (UW1, UW2, UB1, UO1 and UO 2)
[b]mean ± SD of IVRDS of treated feeds (TW3, TW4 and TB2)

Our statistical evaluation did not include the IVRDS value of maize among the untreated feeds, as ruminal degradability of maize is specific due to the low starch degradation in comparison with wheat, barley and oats. The IVRDS value in maize after 6-hour fermentation reached 40.62%. This corresponds with results of Dado and Beek (1998), who found IVRDS of 39.1% after the same fermentation time in maize grain obtained from a normal lysine maize hybrid harvested in the stage of physiological maturity. The IVRDS value of maize grain after 16-hour fermentation measured in our trial reached 82.9%. In their experiments Dado and Beek (1998) measured the IVRDS value 89.2% even after 12-hour fermentation. The authors also pointed out the importance of sample preparation and ground grain particles for the resulting value of ruminal starch degradability.

No correlation was found between total starch content and ruminal degradability of starch. The values of the correlation coefficient showed a low degree of correlation. The comparison of *in vitro* results of ruminal starch degradability with the values resulting from an *in sacco* trial with identical feeds (Homolka, unpublished) in cannulated cow rumen after 4-hour incubation showed slightly higher val-

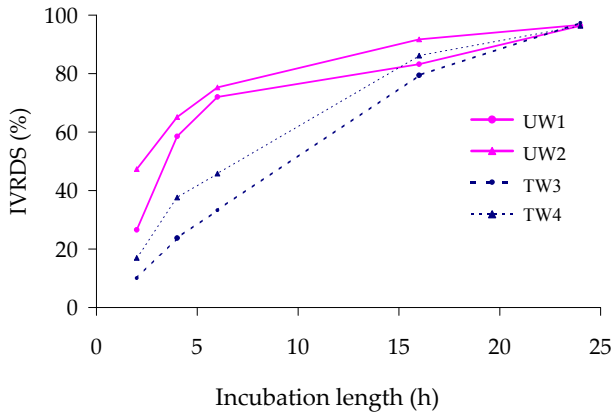

Figure 1. *In vitro* ruminal degradability of starch of untreated and alkali-treated wheat

IVRDS – *in vitro* ruminal degradability of starch
UW – untreated wheat, TW – alkali-treated wheat

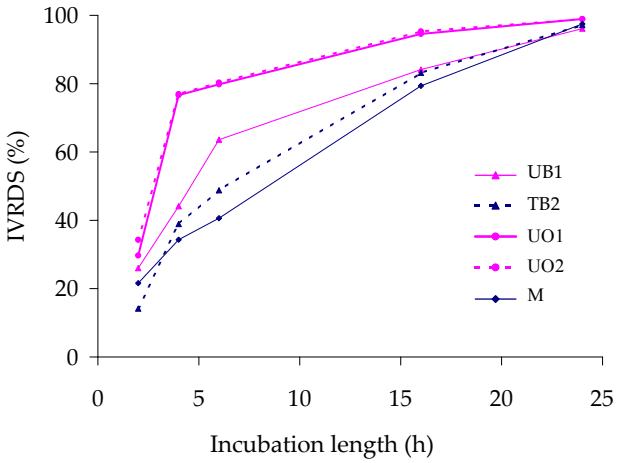

Figure 2. *In vitro* ruminal degradability of starch of untreated barley, oat and maize and alkali-treated barley

IVRDS – *in vitro* ruminal degradability of starch
UB – untreated barley, TB – alkali–treated barley,
UO – untreated oat, M – untreated maize

ues reached by the former method in treated feed samples, while the opposite was true for untreated samples, especially of oats, where the values were lower than those in the *in sacco* trial.

The advantages of *in vitro* methods include higher reproducibility in comparison with the other methods. The IVRDS technique is rather demanding and time-consuming, but offers the instruction to study and explain more precisely differences in the utilization of cereal grain energy by animals.

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Table 4. Statistical analysis of IVRDS values in 2, 4, 6, 16 and 24 h incubation of treated and untreated feeds

Incubation time (h)		Feed		*F*-value	Significance
		Treated	Untreated		
2	mean	13.73	32.77	44.56	***
	SE	2.258	1.749		
4	mean	33.44	60.30	20.59	***
	SE	4.679	3.624		
6	mean	42.63	74.20	126.36	***
	SE	2.220	1.720		
16	mean	82.90	89.79	12.46	**
	SE	1.543	1.195		
24	mean	96.95	97.39	0.83	NS
	SE	0.380	0.294		

$^{**}P < 0.01$, $^{***}P < 0.001$, NS = not significant, SE = standard error

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ABSTRAKT

Degradace škrobu zrnin v bachoru stanovená metodou *in vitro*

Metodou *in vitro* byla stanovena bachorová degradovatelnost škrobu (IVRDS) u souboru zrnin. Soubor zahrnoval 9 krmiv: 2 vzorky pšeničného šrotu, 2 vzorky louhované pšenice, ječný šrot, louhovaný ječmen, 2 vzorky ovesného šrotu a kukuřičný šrot. Bachorová degradovatelnost škrobu byla stanovena fermentací krmiva v časech 2, 4, 6, 16 a 24 hodin. Výrazný rozdíl v intenzitě degradace škrobu byl zjištěn mezi vzorky ošetřenými louhováním a vzorky neošetřených šrotů ve dvouhodinové fermentaci (13,73 ± 3,12 a 32,77 ± 8,17; $P < 0,001$), čtyřhodinové fermentaci (33,44 ± 7,31 a 60,30 ± 16,71; $P < 0,001$) a šestihodinové fermentaci (42,63 ± 7,13 a 74,20 ± 6,38; $P < 0,001$). Získáno bylo následující pořadí obilnin podle rychlosti degradace škrobu v bachoru (od nejvyšší po nejnižší hodnotu): ovesný šrot, pšeničný šrot, ječný šrot, kukuřičný šrot, louhovaná pšenice a ječmen.

Klíčová slova: zrniny; škrob; bachorová degradovatelnost; *in vitro* metoda

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