Study of wheat (*Triticum aestivum* L.) quality for feeding ruminants using *in vitro* and *in vivo* methods

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ABSTRACT: Nutrient digestibility and parameters of nutritive value for ruminants of two winter wheat (*Triticum aestivum* L.) cultivars were evaluated by means of an *in vivo* balance trial performed by the regression method on two groups of heifers with an increasing proportion of grain in DM (from 6 to 46%). Sulamit and Rapsodia, chosen on the basis of the *in vitro* test from a set of 25 cultivars (grown in 2002–2004) reached significant differences in DM fermentability *in vitro* (by 43.7–78.6 ml/g DM, $P < 0.05$). *In vivo* digestibility of crude protein, nitrogen-free extract, organic matter, parameters of N retention, energy concentrations (metabolisable energy, net energy for lactation and for fattening) and parameters in the PDI system (especially PDIE) increased along with the grain proportion in the diet. At the comparable proportion of grain in the ration the positive differences were significantly higher ($P < 0.05$) for Sulamit than Rapsodia.

Keywords: balance trial; nutrient digestibility; nutritive value of grain; *in vitro* degradation and fermentation

Along with the growing demands for the ruminal productivity and limits of an increase in DM intake due to the rumen fill or satiety, the proportion of the concentrate in feed rations continues to increase (Eastridge, 2006). Due to its high digestible energy content, cereal grain is a suitable feed for highly productive ruminants to maintain their high production. A better description of the nutritive value of cereal grain would identify great opportunities for the higher incorporation of grain into ruminant diets. Carbohydrates make up the largest nutrient component in dairy cow diets, accounting for more than 65% of dietary dry matter (Stokes, 1997). The cereal grain contains a high percentage of non-structural components, mainly starch (40–70% of dry matter, DM). Therefore, an increase in the grain percentage in diets and variation in the rate of starch fermentation in the rumen often lead to an imbalance of the intake of individual nutrients, especially in the balance of non-structural carbohydrates and structural carbohydrates, consequences of which include inefficient ruminal digestion, body weight loss, and animal health complications (Orskov, 1986; Stokes, 1997; Beauchemin, 2007).

The site of starch digestion alters the nature of digestive end products and efficiency of use (Swan et al., 2006). Slower rates of digestion increase the amount of starch bypassing the rumen. Small grains are more rapidly fermented than maize, sorghum or millet, and wheat is considered as one of the most rapidly digested species among the small grain cereals (Herrera-Saldava et al., 1990; Owens et al., 1997). Nevertheless, it has been shown that variation exists also between individual cultivars of the same species (Phillipeau et al., 1999; Bowman et al., 2001; Moss and Givens, 2002).

Winter wheat (*Triticum aestivum* L.) is a leading cereal with the largest growing area in Central Europe. There is little information available about

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the nutritive value of standard wheat cultivars for animal feeding and especially for ruminants.

The objectives of this study were to ascertain if there are considerable differences in grain quality for ruminants between winter wheat cultivars, to select cultivars with significantly different values of DM fermentation in an *in vitro* test, and based on the balance trial performed by the regression method with heifers for two selected, markedly contrasting wheat cultivars, to evaluate *in vivo* digestibility of nutrients, nitrogen retention and parameters of grain nutritive value for cattle when offered a different proportion of grain in the feed ration.

MATERIAL AND METHODS

Plant material: In experiment I, a chosen set of 25 winter wheat (*Triticum aestivum* L.) cultivars was evaluated considering their nutritive value in two *in vitro* tests. Based on the obtained results, two different cultivars were selected and tested *in vivo* in the balance trial with heifers (experiment II).

Experiment I included a set of 25 winter wheat cultivars (Table 2), tested in the Czech Official Trials (A List of Recommended Cultivars) in 2002 to 2004. Grain samples were taken each year (n = 3) from the location Jaroměřice (an experimental station of the Central Institute for Supervising and Testing in Agriculture in the Czech Republic – CISTA), from the trials conducted at an increased level of inputs, according to standard agronomic measures for winter wheat at the experimental stations of the CISTA (Horáková et al., 2005). The cultivars chosen for experiment II, based on the results of *in vitro* testing, Sulamit (S) and Rapsodia (R), were grown in the field in the Kroměříž district (Agricultural Enterprise Ječmínek, Chropyně, Czech Republic) in 2005 using an optimum crop management practice recommended for winter wheat by the CISTA.

Clover-grass silage, used as forage for experiment II, was produced by the Research Institute for Cattle Breeding, Ltd., Rapotín using the silage space at an accredited balance stable.

Chemical analyses of grain and excrements: Wheat grain samples for chemical analyses and *in vitro* test were ground to pass through a 1.0 mm screen in a laboratory mill (Retsch-type SK1). The grain of both selected wheat samples was ground to pass through a 4.0 mm screen for the *in vivo* trial in an experimental mill of the Animal Science Department of the Mendel University of Agriculture and Forestry in Brno (Czech Republic). Nutrients in the grain, silage, faeces and urine were determined according to the Czech State Standard (CSS) 46 7092 "Methods for Feed Testing" (Weenden method). Nitrogen (N) was determined using a Kjeltec 2200 Analyzer Unit, crude fibre (CF) using FIBERTEC-1021, fat (F) after extraction using the Soxtec System HT6 Tecator, and ash (A) according to CSS 46 7092. The starch content was assessed using a polarimetric method according to Ewers, modified by Davídek et al. (1981). The content of crude protein (CP) was assessed by conversion from N content using the factor 6.25. All results were adjusted to g/kg DM.

***In vitro* tests (experiment I)**: To simulate the fermentation of wheat grain samples in the rumen, the device VITROGEST (applied design of the CR, reg. No. PU 6596-97, Pozdíšek, 1996) constructed on the basis of findings by Steingass and Menke (1986) was used. Samples were cultivated at the defined volume of a culture medium (45 ml) that was composed of one portion of rumen fluid (taken at least from two animals using a ruminal cannula) and two portions of "artificial saliva" (McDougall, 1948). The volume of gases produced during fermentation as a marker of DM fermentation activity (DMF) under defined conditions of the temperature and pressure of produced gases on the background of atmospheric pressure was measured. The volume of the produced gases was measured at five times (at 4, 8, 12, 20 and 24 h, each time in six replications) that were verified in experiments and correspond with the course of feed digestion in the rumen (Menke et al., 1979; Gedachew et al., 1998), and was corrected by a gas amount that was determined at the simultaneous culture of the medium with rumen fluid without sample sizes.

***In vivo* trial (experiment II)**: Digestibility and parameters of the nutritive value of the grain of the two winter wheat cultivars (cvs.) S and R were evaluated in an *in vivo* trial with heifers. The balance trial, performed by the regression method (on two groups, e.g. for S and R, with 6 heifers in each group), was conducted at the Research Institute of Cattle Breeding, Ltd., Rapotín, according to Kielanowski (1967) and Vencl (1985). The regression method enables to test the effect of various nutritive parameters and the range of interaction between two components of the feed ration (clover-grass silage as the forage and the tested concentrate). Partial digestibility is expressed by the linear regression equation:

$$y = a + b \times x$$

where:

x = the percentage of the tested grain DM of the feed ration DM

During a preparatory period (14 days), animals' feed intake and adaptation to experimental conditions were observed. The groups of animals for balances were even in live weight (LW, at the mean of 302.2 and 311.8 kg for rations with samples of cvs. S and R, respectively) and in DM intake per kg of LW. The clover-grass silage (forage – to the nearest 50 g) as well as the concentrate (grain – to the nearest 10 g) for individual animals was weighed out into vessels. The samples for laboratory analyses were always taken daily. The aim of the preparatory period was not only the adaptation of animals to the environment and feed ration but also the adjustment of the amount of the weighed out feeds so that the animals take the feeds without refusals, not affecting the accuracy of the results. During the experiment, the intake of drinking water was also measured.

During the main period of the experiment (7 days), faeces and urine of individual animals were collected by a continuous service in the balance stable. After the faeces were weighed at 24 h and homogenized, a relative sample of faeces was made (in the percent amount that was determined on the first day of excrement sampling) at the amount of 2–5% of the weight for a day, and conserved with 5 ml chloroform/kg of faeces. After terminating the main period, samples were taken from the collected and homogenized faeces for a balance period (from individual partial samples per animal) for laboratory analyses. Relative samples of urine were conserved with 20% HCl at the rate of 30 ml/1 000 ml to decrease the pH value below 6 and stored under cold conditions. The mean sample for the main period in each animal was analysed for N content and specific gravity weight.

The differences between ingested and excreted nutrients for each animal were expressed by the amounts of digested nutrients (digested crude protein – dCP, fat – dF, crude fibre – dCF, nitrogen-free extract – dNFE, ash – dA and organic matter – dOM). Based on the nutrient balance for individual animals, regression equations (digestibility partial coefficients) were calculated according to the percentage of DM of the tested wheat samples in the ingested total DM. The energy value of feeds (gross energy – GE, in MJ/kg DM) was calculated using the equation derived from the studies of Schiemann et al. (1971) as reported by Sommer et al. (1994). The determined digestibilities for individual animals were used to predict metabolisable energy (ME, in MJ/kg DM).

The obtained values of GE and ME in individual animals were used to express the values of net energy of lactation (NEL, MJ/kg DM) and net energy of fattening (NEF, MJ/kg DM) according to the equations indicated by Van Es (1978) and others. In addition to the determined values of CP and dCP, the percentage of retained N of digested protein (RetN, in % of DP) and N retention in g per kg of DM (RetN, g/kg DM) were also assessed.

Degradability of CP of feed samples (deg) in the balance trial was determined by the method of *in situ* culture using ruminally cannulated animals. The value of degradability (for cv. S, deg = 79%, for cv. R, deg = 80% and for silage, deg = 72%) was calculated using Neway software, which is based on recommendations by Orskov and Macdonald (1979). Nutritive value parameters in the PDI system (protein digestible in the small intestine – PDIA, allowed by N – PDIN and allowed by energy – PDIE) were expressed according to Jarrige (1989) and Sommer et al. (1994) using the mea-sured CP content, degradability of CP (deg) and digestibility of in rumen non-degraded CP in the small intestine (dsi). The values of dsi were predicted according to the same authors (cv. S, dsi = 90%, cv. R, dsi = 89% and silage, dsi = 67%). Regression equations according to the percentage of DM of the tested samples from ingested rations were calculated and were used to calculate the values of the examined traits and parameters for comparable proportions of grain of the tested samples.

Statistical analyses: All statistical analyses in experiment I and experiment II were accomplished using the GLM procedure of Statistica 7.0 software. Data characterising the results of the *in vitro* test (experiment I) were analysed by one-way analysis of variance (ANOVA) to account for the effects of cultivar and/or experimental year. Differences between the means (2002–2004) of tested cultivars for all the traits assessed were evaluated using post-hoc tests (Fisher LSD and/or Tukey's HSD tests), and the number of statistically different ($P < 0.05$) wheat cultivars (or groups of cultivars) from each other was declared. To measure the variation among the traits, the coefficient of variation (*CV* in %) was calculated.

In experiment I, the rate of the expected *in vitro* DMF depending on the time of culture (for the individual wheat cultivars) was expressed as the slope of linear (or logarithmic) regression. In experiment II, the linear regression procedure was used to predict the equation of the effect of the ration of the tested grain samples (cvs. S and R) in the balance test. In both experiments, regressions for cvs. S and R were mutually compared (after visual inspection of the experimental data for heteroscedasticity) using the Chow test (Chow, 1960), where the test criterion Fc was compared to the tabular value of Fisher-Snedecor F distribution with appropriate degrees of freedom at $P < 0.05$ and 0.01.

RESULTS

Experiment I: The mean values of simulated DMF (Table 1) varied in the whole set of winter wheat cultivars and years, and reached different levels at the incubation times (4, 8, 12, 20 and 24 h). The mean volume of produced gases (PG) ranged from 23.5 ml/g to 273.4 ml/g in the whole set of winter wheat cultivars (in 2002–2004), whereas the mean values increased along with the time of fermentation. The declining *CV* reflected a decrease in the variability level of measured data along with the increasing time of fermentation both in the whole set of winter wheat cultivars examined and in cvs. S and R. The variability in DMF within the whole set of winter wheat cultivars indicated an increasing level of statistical significance of the source of variation "cultivar" depending on the time of sample incubation. In contrast, the source "year" showed significant differences between the results of DMF in individual years (results of ANOVA, no table). Cultivars S and R differed significantly beginning with the 8-h incubation period till the end of the experiment.

Furthermore, the course of *in vitro* fermentation of grain samples was based on the regression of the dependence of the volume of PG on fermentation time. In Table 2, the cultivars are ranked according to the slope of the regression line (linear and/or

Table 1. Mean values and basic statistical characteristics of grain nutritive quality traits in winter wheat cultivars obtained in an *in vitro* test (2002–2004)

Item/trait		Fermentation of DM (in volume of produced gases in ml/g)				
		4 h	8 h	12 h	20 h	24 h
Whole set of wheat samples *n* = 75	mean	57.6	104.4	146.5	197.1	205.2
	SEM	1.64	1.87	2.44	2.66	2.68
	min.	23.5	66.7	102.8	150.6	158.0
	max.	89.0	142.8	191.8	261.4	273.4
	CV (%)	24.6	15.5	14.4	11.7	11.3
	No[1]	11	22	25	25	25
Sulamit *n* = 3	mean[2]	42.5[a]	83.5[a]	115.9[a]	158.3[a]	166.9[a]
	SEM	7.02	3.37	2.89	5.22	7.13
	min.	34.6	77.8	110.3	150.6	158.0
	max.	56.5	89.4	120.1	168.2	181.0
	CV (%)	28.6	7.0	4.3	5.7	7.4
Rapsodia *n* = 3	mean	61.9[a,b,c,d]	127.2[e]	183.1[h]	235.6[h]	245.5[i]
	SEM	9.89	6.72	6.77	1.36	2.86
	min.	45.1	120.4	169.8	234.1	242.2
	max.	79.3	140.7	191.8	238.3	251.2
	CV (%)	27.7	9.1	6.4	1.0	2.0

[1]number of statistically different wheat cultivars (or groups of cultivars) from each other ($P < 0.05$)

[2]means in the same column within cvs. Sulamit and Rapsodia with no common superscripts differ significantly ($P < 0.05$)

Table 2. Parameters of the regression equations of *in vitro* DM fermentability depending on the time of culture (for the individual wheat cultivars in 2002–2004)

Cultivar	$y = b \times x$, $n = 15$		$y = b \times \ln(x) - a$, $n = 15$		
	b	R^2	b	a	R^2
Sulamit	7.83	0.84	71.65	–60.34	0.97
Mladka	8.46	0.77	77.92	–66.32	0.92
Meritto	8.81	0.80	77.37	–59.03	0.95
Ludwig	8.89	0.75	75.04	–51.33	0.94
Rheia	8.90	0.77	75.38	–52.01	0.97
Vlasta	9.00	0.78	76.86	–54.22	0.98
Nela	9.16	0.84	88.33	–83.08	0.92
Samanta	9.24	0.80	86.29	–76.00	0.91
Alana	9.30	0.81	87.04	–77.22	0.90
Ebi	9.31	0.79	81.35	–61.05	0.98
Svitava	9.43	0.79	79.85	–55.30	0.96
Karolinum	9.49	0.77	82.84	–61.92	0.97
Corsaire	9.78	0.85	91.66	–81.74	0.93
Šárka	9.91	0.87	89.45	–85.44	0.98
Clever	9.99	0.82	87.20	–65.82	0.96
Complet	10.09	0.66	82.69	–51.67	0.89
Rialto	10.30	0.69	85.75	–56.02	0.89
Bill	10.40	0.78	89.94	–65.59	0.98
Drifter	10.41	0.69	86.95	–57.44	0.90
Ilias	10.51	0.74	87.94	–58.55	0.94
Alibaba	10.79	0.73	92.89	–66.86	0.93
Globus	10.83	0.73	97.47	–79.23	0.85
Batis	10.85	0.77	93.54	–67.63	0.98
Clarus	10.92	0.76	96.51	–74.21	0.95
Rapsodia	11.72	0.80	106.06	–86.51	0.98

logarithmic) indicating the angle of rapidity of the increase in gas volume at sample culture. The parameters of predicted equations of linear regression demonstrate that the fermentation rate was different in the examined wheat samples. Comparison of the regressions for cvs. S ($y = 7.83\times$, $R^2 = 0.84$) and R ($y = 11.72\times$, $R^2 = 0.80$) using the Chow test shows significant differences in the dependence of gas volume on fermentation time between the tested cultivars (Fc = 17.43, $P < 0.01$).

Experiment II: Table 3 shows the results of the analyses of feeds used in the balance trials. Forage (used for both groups of animals) was clover-grass silage that is suitable, based on its nutritive value parameters, to complete the tested wheat samples. The nutrient structure in ingested feed rations did not markedly differ from the requirements of the animal category tested. In compliance with the principles of conducting balance trials performed by the regression method, the DM percentages of the tested wheat grain of total dietary DM were gradually increased in both groups to the levels of approximately 6, 11, 23, 34 and 46%. The intake of dietary DM per kg of LW was balanced at the level of 16.07 g DM/kg LW in the group with cv. S and 15.73 g DM/kg LW in the group with cv. R (Table 4).

Using the balance digestibilities of basic nutrients in individual animals, regression equations were calculated and expressed as partial coefficients of digestibility of nutrients with gradually increasing proportions of grain of cvs. S and R. Analogical

Table 3. Chemical composition of feeds (g/kg of DM)

Composition	Silage	Wheat grain, cv. Sulamit	Wheat grain, cv. Rapsodia
DM (%)	34.7	86.5	86.7
CP	143.3	150.0	121.2
Fat	28.5	10.5	11.6
CF	273.5	31.8	30.1
NFE	455.1	788.6	818.1
Ash	99.6	19.1	19.0
OM	900.4	980.9	981.0
Starch	N[1]	644.0	681.9

[1]not measured

regression equations were calculated for evaluated nutritive values and N retention. Based on the regression equations of determined digestibilities of main nutrients, N retention, evaluated energy concentration (ME, NEL and NEF), PDIA, PDIN and PDIE content in the DM of ingested rations, the data were predicted at uniform proportions of grain at 10, 20, 30 and 40% of the feed ration for both tested cultivars (Table 5). The table is completed with the results of the Chow test of significance of the differences between the predicted linear regression equations of the course of the above-mentioned traits and parameters depending on the increasing proportions of wheat grain in rations for both cvs. S and R.

The values of dCP and dOM rose at the increasing proportion of wheat grain in feed rations, whereas these differences were larger in cv. S than in cv. R. The tendency similar to dCP and dOM was apparent in dNFE, however, the differences between regressions for cvs. S and R were not statistically significant. The increasing proportion of grain negatively affected dCF and dF, however, significant differences between the regressions in cvs. S and R were found for dCF only. The course of ash digestibility was different. While there was a tendency of

Table 4. Feed intake by animals in the balance trial (in DM)

Wheat cultivars	$x\%_{fact.}$[1]	Silage (kg/day)	Wheat grain (kg/day)	Total (kg/day)	DM intake (g DM/kg LW[2])
Sulamit	6.05	4.96	0.32	5.28	15.43
	11.98	4.16	0.57	4.73	15.41
	23.1	3.89	1.17	5.06	16.15
	33.89	3.23	1.65	4.88	16.22
	44.54	2.67	2.15	4.82	16.39
	46.77	1.77	1.56	3.33	16.80
Rapsodia	5.97	5.00	0.32	5.32	15.54
	11.77	4.69	0.63	5.31	15.90
	23.21	2.95	0.89	3.84	15.94
	33.94	3.54	1.82	5.36	16.29
	34.69	2.88	1.53	4.41	14.09
	46.48	2.78	2.41	5.19	16.63

[1]$x\%_{fact.}$ = factual percentage of the grain DM of the total DM intake
[2]LW = live weight

Table 5. Results of the balance trial at a comparable percentage of grain of tested winter wheat cultivars in diets

Cultivar	$x\%_{calc.}$[1]	Digestibility and N retention						
		dCP (%[2])	dF (%)	dCF (%)	dNFE (%)	dA (%)	dOM (%)	Ret N (%) of DP[3]
Sulamit	10	64.18	65.79	68.21	75.67	48.57	71.79	21.04
	20	65.23	63.54	66.94	77.89	49.52	73.46	22.89
	30	66.27	61.28	65.68	80.12	50.46	75.13	24.73
	40	67.31	59.03	64.41	82.34	51.41	76.80	26.57
Rapsodia	10	60.93	68.51	68.21	76.12	52.80	71.55	19.64
	20	61.26	65.77	65.12	77.93	51.69	72.25	20.39
	30	61.59	63.04	62.02	79.74	50.59	72.94	21.14
	40	61.92	60.31	58.92	81.55	49.48	73.63	21.89
Chow test	Fc[4]	20.9**	2.26	6.06**	0.99	2.88	6.7**	0.97
		nutritive value and N retention (in DM)						
Cultivar	$x\%_{calc.}$	ME (MJ/kg)	NEL (MJ/kg)	NEF (MJ/kg)	PDIA (g/kg)	PDIN (g/kg)	PDIE (g/kg)	Ret N (g/kg)
Sulamit	10	10.34	6.19	6.18	30.25	88.04	83.98	3.07
	20	10.86	6.57	6.64	30.53	89.25	86.25	3.45
	30	11.39	6.94	7.10	30.81	90.46	88.52	3.83
	40	11.92	7.32	7.55	31.10	91.66	90.79	4.21
Rapsodia	10	10.11	6.05	6.02	29.49	86.23	83.04	2.72
	20	10.28	6.17	6.18	29.01	85.55	83.70	2.78
	30	10.46	6.30	6.33	28.54	84.87	84.35	2.84
	40	10.63	6.42	6.49	28.06	84.19	85.00	2.91
Chow test	Fc	53.28**	46.16**	39.77**	9 959.61**	41 677.67**	31.15**	3.29

[1]$x\%_{calc}$. = calculated percentage of the wheat grain DM of the total DM intake (on the same levels in Sulamit and Rapsodia)
[2]see Material and Methods
[3]DP = digested protein; [4]Fc = Chow test criterion: *Fc > Ftab.(= 3.37) at $P < 0.05$ and **Fc > Ftab.(= 5.53) at $P < 0.01$; n1 = 2, n2 = 26

an increase in dA in cv. S, in contrast, the higher grain proportion in feed rations decreased the dA in cv. R.

All parameters of N retention increased along with the increase in the grain proportion. A tendency of the higher values of RetN (in % of DP) and RetN (in g/kg DM) was found in rations with cv. S, but no significant differences were determined between the predicted courses of regressions for both cultivars.

In relation to the determined parameters obtained in the balance trial, higher energy concentrations (ME, NEL and NEF in MJ/kg of DM) were noticed at the increasing proportion of wheat in rations. The increase in energy concentrations was significantly higher in cv. S as compared with that in cv. R. The nutritive value parameters expressed in the PDI system, given in Table 5, document that the differences in the content of PDIA, PDIN and PDIE (in g/kg of DM) are significant in favour of cv. S.

At the comparable proportion of grain in the ration (40%), the inclusion of cv. S led to an increase in dCP, dNFE, dOM, ME, NEL, NEF, parameters PDIN and PDIE ($P < 0.05$), RetN (g/kg DM, $P = 0.55$), RetN (% of DP) and to a decrease in dCF ($P < 0.05$), compared to cv. R.

DISCUSSION

A high proportion of wheat on arable land in Central Europe and increasing proportion of its grain for feeding out of the total production also affect the approach of nutritionists to the function of this species in ruminant nutrition. Opinions of the wheat grain proportion in cattle diets differ. Doepel et al. (2006) found that up to 20% of wheat could be included in the diet of dairy cows without causing milk fat depression, Hoover and Miller (1995) recommended that non-structural carbohydrates could be limited to 35 to 40% in high starch rations, and other authors (Eastridge, 2006) stated that the grain proportion in diets of highly productive dairy cows generally has about 40–60% concentrates. Among small grain cereals, wheat is considered as a species with the high rate of degradation in rumen, which is associated with increased risks of health problems in cattle. In the present study the metabolic tests, performed at the end of the main period of experiment II (results are not a part of this paper), were found to be within the reference range. Nevertheless, the short-term trials may be too short to observe the adverse effects on ruminal and animal health that may occur with long-term feeding of excessive concentrate (Broderick, 2006).

Nutritionists have identified that ruminants require wheat but it has low rumen degradability and high whole tract digestibility. Therefore, the assumption that the rate of DM fermentability can influence the digestibility and utilisation of wheat in the *in vivo* experiment was an original hypothesis used in this study. A number of papers devoted to the study of wheat grain quality document differences between cultivars that are caused by environmental effects (location in combination with temperatures and precipitation) and agronomic management (level of N fertilizer, preceding crop), by the method of grain processing and treatment, and last but not least, by combination with other feeds and supplements in animal diets (Owens et al., 1986; Stokes, 1997; Rowe et al., 1999; Black, 2001; Tománková and Homolka, 2004; De Campaneere et al., 2005; and others). Moss and Givens (2002) found that the rumen degradable starch disappearance, which is an important characteristic of the nutritive value of wheat for ruminants, is influenced by the year of harvest, site of growth, agronomic management and cultivar. The chosen cultivars were grown in three years (2002–2004) at one location using identical agronomic management and grain treatment for *in vitro* testing. Although seasonal differences in the values of DMF were found, similarly like in the above-mentioned study, they did not overlap cultivar variability, particularly after a longer incubation time. The largest differences were confirmed between cvs. S and R that significantly differed in the volume of produced gases as a measure of simulated fermentation of DM in the rumen ($P < 0.05$, at 8, 12, 20 and 24-h cultivation with rumen fluid by 43.7, 67.2, 77.3 and 78.6 ml/g, respectively). The results of the *in vitro* test demonstrated their different sensitivity in testing feeds for ruminant nutrition.

Gas produced by fermentation comes from the carbohydrate fraction of the feed to the largest extent since ash does not ferment, fat produces no gas, and protein produces very little gas (Robinson and Getachew, 2002). Gas production techniques have a good potential to predict rumen OM degradation, in particular by the provision of kinetic information, and could be widely used to evaluate the energy values and rumen fermentation characteristics of feeds (Umucalilar et al., 2002), but the potential to provide parameters of degradation of OM components seems limited (Dijkstra et al., 2005). If the amount of gas is recorded frequently, then the gas generation curve can be constructed which shows differences between feeds in the rate and extent of digestion of carbohydrates.

Parameters of the regression equations of the course of *in vitro* DM fermentability (according to the volume of produced gases) depending on the time of culture were calculated and they revealed differences between the individual wheat cultivars. Though the increase in the volume of produced gases over time fitted rather a logarithmic curve (which was confirmed by determined R^2 coefficients ranging from 0.85 to 0.98 vs. $R^2 = 0.66–0.87$ for the linear relationship, assumed intercept = 0), the differences between regressions for the two chosen cultivars were calculated, because of simplification, by comparing linear relationships. The Chow test confirmed significantly different ($P < 0.01$) regressions, i.e. kinetics of the volume of produced gases between cv. S and R.

Rohr et al. (1986) compared the results from *in vivo* and *in vitro* methods using concentrated feeds and showed that the correlation between the values determined by these methods was very high ($R^2 = 0.90$). Some researchers also showed significant correlations between the energy levels obtained by *in vitro* gas test and *in vivo* trial. Although the gas production technique only determines the rate and level of OM fermentation, it may be assumed that a high rate of fermentation is an indication of a low amount of rumen bypassing starch in the animal (Hindle et al., 2005). In the conducted balance trial with heifers, performed by the regression method, percentages of the tested wheat grain of total dietary DM were gradually increased (from 6 to 46% of the DM) in both groups with cv. S and R. Disregarding the lower expected DMF rate in cv. S compared to R, it could seem that due to the higher mean content of starch in cv. R (681.9 g per kg vs. 644 g/kg in cv. S), the differences in total tract digestibility of some nutrients will be at least insignificant. Nevertheless, the factual measured values of nutrient digestibility and parameters of N retention and, based on them, calculated energy concentrations and parameters in the PDI system corresponded with our hypothesis that cultivars with a low fermentation rate are more suitable for the nutrition of ruminants. The increased percentage of grain of both cultivars in diet resulted in increased digestibility (d) of CP, OM, NFE, higher parameters of N retention, higher energy concentrations (ME, NEL and NEF) and values of PDIE, while cv. S was better than R in all cases. At the comparable proportion of grain in the ration (40%), expressed on the basis of calculated partial regression coefficients, the inclusion of cv. S resulted in a significant increase ($P < 0.05$) in dCP, dNFE and dOM (by 5.39, 0.79 and 3.17% DM, respectively), N retention (by 4.68 % of DP and by 1.31 g/kg of DM), ME, NEL and NEF (by 1.29, 0.89 and 1.06 MJ per kg DM, respectively), and also in all parameters calculated in the PDI system, especially PDIN and PDIE (by 7.47 and 5.79, respectively) compared to cv. R.

However, the comparison of results obtained in experiment I (*in vitro* test) and experiment II (*in vivo* trial) with literature showed that although conclusions drawn from both experiments were consistent, they did not fully elucidate the existing differences between the two cultivars. Swan et al. (2006) reported that wheat cultivars of different grain hardness had different proportions of a fine milling fraction and that starch granules of hard cultivars were damaged more than those of soft ones, which was reflected both in the level of starch degradation and its rate. Both examined cultivars are classified into different quality categories based on bread-making quality (Horáková et al., 2005) – cv. S ranks to elite wheat (category E) and cv. R is a cultivar unacceptable for leavened dough (category C). The cultivars of quality category E always have harder grain compared to cultivars of quality category C. Thus, in accordance with data reported by Moss and Givens (2002) or Swan et al. (2006), they should have exhibited the highest degradation rate and in contrast, cultivars of category C having soft grain should have behaved conversely. However, it did not apply to any of the three examined years of both cultivars tested.

The results of DMF in the *in vitro* test can be associated with different content and structure of CP and non-starch polysaccharides (NSP) in the tested wheat cultivars (Vaculová et al., 2005). MC Allister et al. (1993) concluded that the structure and linkages among nutrients were often more important than the degradation of starch itself within one species. The pericarp of cereal grains is resistant to microbial attachment and penetration. This structure must be fractured by mechanical processing or mastication for digestion to proceed (MC Allister and Cheng, 1996). On the contrary, Hindle et al. (2005) stated that although the layers of protein and lipids around starch molecules may be present in some starch sources, this is not determinative for the degree of degradation. It was found that cv. S was characterised not only by a higher content of CP but also by a statistically higher content of NSP, especially pentosans, than cv. R (Vaculová and Horáčková, 2007). Though the content and structure of NSP (predominately occurring in the cell walls) have a low effect on the availability of energy from cereal grains for ruminants (Black, 2001), together with the increased protein content they may probably result in the different proportion of coarse and fine milling particles and influence the OM degradability in rumen in a different way.

Although the values of apparent digestibility in the small intestine were obtained from the conversion of factual values of nutrient digestibility and degradability *in situ*, partial regression coefficients for the increasing proportion of grain in the ration show differences in the values of the parameters PDIA and PDIN between cvs. S and R. While in cv. S the increase in PDIA and PDIN values was determined at a higher proportion of grain, cv. R showed opposite results. The Chow test confirmed a significant difference for regressions of these parameters between cvs. S and R.

The probable explanation is that the increase in dietary CP with cv. S (by 28.8 g/kg DM compared to cv. R) resulted in a stimulatory effect of protein on the intestinal disappearance of starch or non-structural carbohydrates that was observed in experiments with steers (Richards et al., 2002) and dairy cows (Abramson et al., 2002). This explanation was in accordance with Ipharraguerre et al. (2005), who concluded that cows fed high CP diets tended to have higher postruminal digestibility of OM. In their experiment the percentage of dietary CP profoundly influenced the intake, ruminal fermentation, intestinal supply and digestion of N and starch. The proportion of starch consumed by the cows that was apparently digested in the small intestine increased in response to higher inputs of dietary CP.

Broderick (2006) indicated that the inefficient utilisation of CP in ruminant diets led to excessive feed costs and environmental N losses in dairy production. Therefore, the choice of suitable wheat cultivars can influence the feeding efficiency and meet the still increasing requirements to reduce pollution caused by livestock animals.

In spite of that the range of the study was too narrow to provide an exact picture of all factors affecting estimated differences between both winter wheat cultivars, it was found that the results of the *in vitro* expected ruminal fermentation, measured by a simple gas production technique, had a good potential for the prediction of DM degradation, and could lead to significantly different digestibility of nutrients and parameters of nutritive value *in vivo*. The selection of winter wheat cultivars can positively affect these traits at a higher proportion of wheat grain in the ration.

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