Effect of carbohydrates in grass silage-based diets on *in sacco* ruminal degradability of barley (*Hordeum vulgare* L. *cv.* Lomerit) grain ground to different particle sizes

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ABSTRACT: The effect of carbohydrates included in grass silage-based diets on *in sacco* degradability of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and cellulose (CE) of barley (*Hordeum vulgare* L. *cv.* Lomerit) grain ground to different particle sizes was studied using 3 ruminally cannulated cows. The measurements were carried out as a 3 × 2 Latin square design with treatments being carbohydrates in the concentrate portion of diets (starch in diet I vs. pectin in diet II vs. sucrose in diet III) and particle size of ground barley grain (1.0 vs. 5.7 mm). The diet synchronization index between N and energy supply during the day was an average of 0.8. The rumen degradability of barley grain compounds was influenced by the type of carbohydrates included in grass silage-based diets. Higher rumen degradation rates ($P < 0.05$) of barley CP, NDF and CE after 8 h of incubation and of CP and CE after 16 h of incubation were found in the rumen of cows receiving the diet containing sucrose compared with degradation rates found in the rumen of cows receiving the diet containing pectin or starch. The effective degradability of DM, CP, NDF and CE of barley grain in the rumen of cows receiving diets I, II and III was similar (77, 74, 39 and 41%, respectively). Replacing barley starch or beet pulp pectin with molasses sucrose in the grass silage-based diet resulted in the higher rate of barley CP, NDF and CE degradation after 8 h of incubation and of CP and CE after 16 h of incubation. It seems that the energy availability from carbohydrates affected the ruminal microbial activity as a consequence of the degradation rate of substrates. Higher ($P < 0.01$) rumen degradability of CP, NDF and CE after 2, 4, 8, 16 and 24 h of incubation was estimated for 1.0 mm particle size in comparison with that found for grain ground to 5.7 mm particle size, and the degradability of CP after 48 h of incubation was not different ($P > 0.05$). The degradability of barley grain CP, NDF and CE during incubation time was not influenced by the diet composition by particle size interaction ($P > 0.05$). The increase in barley grain particle size from 1.0 to 5.7 mm slowed down the rumen digestion of barley DM, CP, NDF and CE, probably due to restricted access to microbial digestion.

Keywords: cattle; grass silage; nonstructural carbohydrates; rumen degradability

The rate and extent of rumen digestion are influenced by the complementarity of the release of N and energy compounds that has an effect on the ruminal microbial population (Rotger et al., 2006). The diet based on grass silage is characterized by a high level of rapidly degraded crude protein, creating an asynchronous relationship between the availability of protein and energy (Givens and Rulquin, 2004). In the meta-analysis of data reporting the true digestibility of N for the gastrointestinal tract of cattle, Marini et al. (2008) found that the quality and quantity of rapidly fermenting carbohydrates were significant factors in a model used for the prediction of bacterial N passage to the duodenum. Some positive responses to improved synchronization of energy and N in terms of rumen microbial N production have been reported (Charbonneau et al., 2006). However, there is no information about the effects of energy release from carbohydrates of high ruminal availability in a grass silage-based diet on the *in sacco* digestibility of feedstuffs.

The rumen digestion of barley in steers was influenced by the grain processing techniques (Bengochea et al., 2005). Although it is assumed that the extent of processing obtained through mechanical treatments can be used to regulate the rate of digestion of cereal grains in the rumen (Gozho et al., 2008), there are no studies estimating *in sacco* the effects of particle size on the rumen degradability of barley grain depending on the diet composition.

Therefore, the objective of this study was to investigate the effects of including carbohydrates differing in the rate of rumen degradability (starch, pectin or sucrose) in grass silage-based diets and of particle size of ground grains on the degradability of barley nutrients estimated *in sacco*.

MATERIAL AND METHODS

The grain of barley (*Hordeum vulgare* L.) cv. Lomerit, originating from the Research Centre for Cultivar Testing in Słupia Wielka, was used in the experiment. The barley was cultivated according to VCU (1998) under the climatic conditions of northwest Poland.

Experimental design

In sacco degradability of barley grain dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and cellulose (CE) was estimated. The measurements of degradability were carried out as 3 × 2 Latin square design with treatments being carbohydrates included in the concentrate portion (diet I – starch vs. diet II – pectin vs. diet III – sucrose) of diets for cannulated cows based on grass silage and particle size of ground grain (1.0 vs. 5.7 mm).

Animals and feeding

The experimental animals were treated according to guidelines approved by the Polish National Ethics

Table 1. Chemical composition of barley grain, unmolassed dried beet pulp, molasses and grass silage (g/kg DM)

	Barley grain	Unmolassed sugar beet pulp	Molasses	Grass silage
Dry matter	880	884	770	264
Crude protein	114	98	115	117
Ether extract	20	7	20	33
NDF	218	412	1	605
ADF	62	260	1	387
ADL	12	28		42
Sugars			629	
Ash	24	34	123	84
Fermentation characteristics (g/kg of DM)				
Lactic acid				48
Acetic acid				42
Propionic acid				1
Butyric acid				3
PH				4.1
N-nonprotein (g/kg of total N)				520

Table 2. Ingredient and nutrient composition of the experimental diets (g/kg DM)

	Diet		
	I	II	III
Ingredients			
Grass silage	546	545	555
Barley grain	248	45	73
Wheat grain	93	–	–
Unmolassed sugar beet pulp	23	272	69
Wheat bran	22	22	45
Molasses	–	16	141
Rapeseed cake (cold pressed)	23	47	71
Soybean meal	23	33	24
Vitamin-mineral mix	15	15	15
Sodium bicarbonate	6	6	6
Chemical and nutrient composition			
CP	145	147	154
CP-rumen degraded[2]	113	111	126
PDIN[2]	81	81	85
PDIE[2]	91	94	89
UFL[2]	0.96	0.95	0.93
FOM[2]	652	625	614
NDF	313	364	305
ADF	177	230	190
ADL	150	155	155
NFC[3]	335	278	309
Starch	181	30	45
Pectin	33	89	35
Sugar	35	66	116
N-rumen degraded (g/kg FOM)	26	26	30
Synchronization index[4]	0.8	0.8	0.8

[1]chemical composition from laboratory analysis (n = 3)
[2]estimated acc. to IZ-INRA (2001): PDIN – the sum of protein truly digested in the small intestine originated from the rumen-undegraded dietary protein and small intestine-digested microbial protein synthesized from the rumen-degraded dietary protein when energy and other nutrients are not the limiting factor; PDIE – the sum of protein truly digested in the small intestine originated from the rumen-undegraded dietary protein and small intestine-digested microbial protein synthesized from the rumen-degraded dietary protein when the degraded N and other nutrients are not the limiting factor; UFL – net energy value for milk production; FOM – rumen fermentable organic matter
[3]estimated acc. to Cozzi at al. (2002)
[4]estimated acc. to Sinclair equation (Sinclar et al., 1993)

Committee for the Use of Animals in Biomedical Experiments.

Three dry Holstein-Friesian cows (700 ± 27 kg of body weight) fitted with a 10-cm ruminal cannula (Ankom Technology, Fairport, NY) were used in the experiment (12-week trial). Feed was evaluated and the concentrate mixture was formulated according to the IZ-INRA (2001) system. Experimental diets were composed of grass silage (55% of diet DM) and concentrate. The grass silage was prepared from the first cut of mixed grass varieties (*Festuca pratensis* × *Poa pratensis* × *Phleum pratense* × *Dactylis glomerata*) harvested on June 9–12, 2006 at the full-head stage of maturity. Silage was prepared in a silo with *Labacsil* additive (Sano Co., Poland). Concentrate portions were formulated to obtain starch from barley (diet I), pectin from unmolassed dried beet pulp (diet II) and sugar from molasses (diet III) as the primary source of highly digestible carbohydrates. The chemical composition of barley, unmolassed beet pulp, molasses and grass silage is shown in Table 1. The cannulated cows received in daily ration an average of 5.8 kg of DM, 500 g of protein digested in the small intestine originated from the rumen-undegraded dietary protein and protein synthesized from the rumen-degraded dietary protein (PDI) and 5.5 units of the net energy for milk production (UFL). The diets were formulated to be isonitrogenous (149 ± 5 g crude protein in kg of DM) and isoenergetic (0.95 ± 0.2 UFL). Ingredient composition and nutrient content of experimental diets are shown in Table 2. During the experiment cows were fed at a maintenance level. All animals had continuous access to water and block of salt. Feed was offered in two meals at 8.00 and 16.00 (in equal portions) and the concentrates were offered on top of grass silage. The experiment was conducted from October to December 2006. Each of the *in sacco* degradability study periods lasted 28 days: days 1 to 17 were used to adapt the cows to the dietary treatments, and days 18 to 28 were used for *in sacco* degradability studies.

In sacco degradability studies

The rumen degradability of barley grain was determined by the polyester bag technique in agreement with the description by Michalet-Doreau et al. (1987). Samples of barley grain were ground to particle size of 1.0 and 5.7 mm. The ratio of sample size to bag surface area was 20 mg/cm^2. The polyester bags with pore size of about 50 ± 15 μm (Ankom Technology, Fairport, NY), containing 3 g of air-dried grain sample were incubated in the rumen for 2, 4, 8, 16, 24 and 48 h. In the experiment three replications were carried out for each grinding level, each diet and each incubation time. After incubation, all the bags were dried and weighed. The samples of the same particle size and the same incubation time in cows receiving the same diet for each replication were pooled prior to chemical analysis.

Chemical analyses

The analyses of dry matter, crude protein, ether extract, crude fibre and total ash content in grains and components of diets were determined using standard methods (AOAC, 1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were measured according to Goering and Van Soest (1970). Starch was determined using a polarimetric method (ISO 6493:2000) and sugar according to the Luff Schoorl EEC official method (79/786/EEC, Annex II).

Calculation

The content of non-fibrous carbohydrates (NFC) in dry matter was calculated according to Cozzi et al. (2002) as 100 – (crude protein + ether extract + ash + NDF), and cellulose (CE) as ADF-ADL. The content of pectin was calculated according to data in feed tables (NRC, 2001). The nutritive value of cow diets was estimated according to the IZ-INRA (2001) feed evaluation system using the INwar software ver. 1.0 (1993).

Degradability data were analysed statistically by the least-squares Manova procedure of Statistica (Ver. 5.1) in a 3 × 2 factorial arrangement, with diet composition and level of grain grinding as main factors. Their interaction was also evaluated. Throughout, the level of statistical significance was preset at $P < 0.05$.

The rapidly soluble fraction of DM, CP, NDF and cellulose (termed "a"), the insoluble but potentially degradable fraction (termed "b"), and the rate of degradation of "b" (termed "c") were determined for mean degradability of incubation time using the model of Michalet-Doreau et al. (1987) and these coefficients were used to determine effective

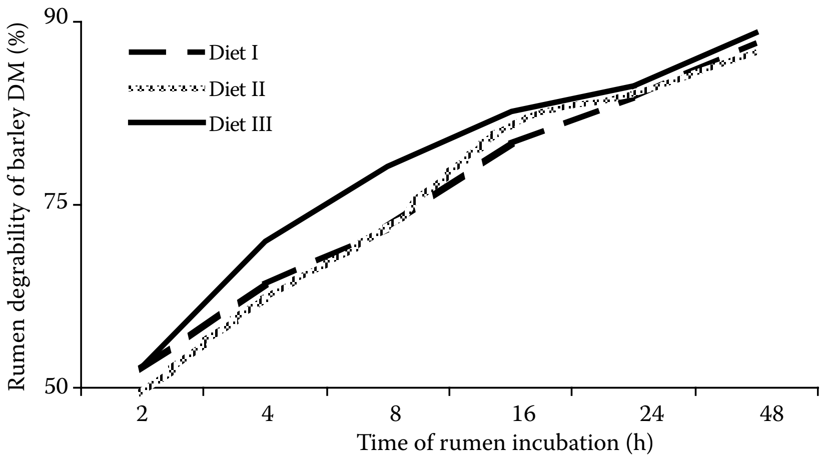

Figure 1. Effect of diet composition on the ruminal degradability of barley dry matter (DM) during incubation periods

degradability according to Ørskov and McDonald (1979) using an outflow rate of 0.06 h.

RESULTS

The data on the chemical analysis of barley grain, unmolassed dried beet pulp and molasses were comparable to those presented in the Tables of Chemical Composition and Nutritive Value of Feedstuffs (BACH, 2003) and of grass silages to the mean silage quality reported by Givens and Rulquin (2004; Table 1).

The nutrient content of the experimental diets reflected the ingredients used in the composition of diets (Table 2). Diets were formulated to have similar CP and ruminally degradable protein levels. The diet contained an average of 148 g of CP and 116 g of rumen degraded CP in 1 kg DM. Diet I was characterized by the highest content of starch (54% of NFC) compared to diet II of pectin (32% of NFC) and diet III of sugar (37% of NFC).

The higher ($P < 0.05$) degradability of barley grain DM was estimated in the rumen of cows receiving diets I and III compared with that found in the rumen of cows receiving diet II after 2 h of incubation (Figure 1). The higher ($P < 0.05$) degradability of DM was estimated in the rumen of cows receiving diet III compared with that found in the rumen of cows receiving diets I and II after 4 and 8 h of incubation and with that found in the rumen of cows receiving diet I after 16 h ($P < 0.05$). The degradability of DM after 24 and 48 h of incubation was not influenced by diet composition ($P > 0.05$).

The higher ($P < 0.01$) rumen degradability of barley grain DM was found for grain ground to particle size of 1.0 mm in comparison with that found for particle size of 5.7 mm for each period of incubation (Figure 2).

The higher ($P < 0.05$) degradability of barley grain CP was estimated in the rumen of cows receiving diets I and II in comparison with that found in the rumen of cows receiving diet III after 2 and 24 h of

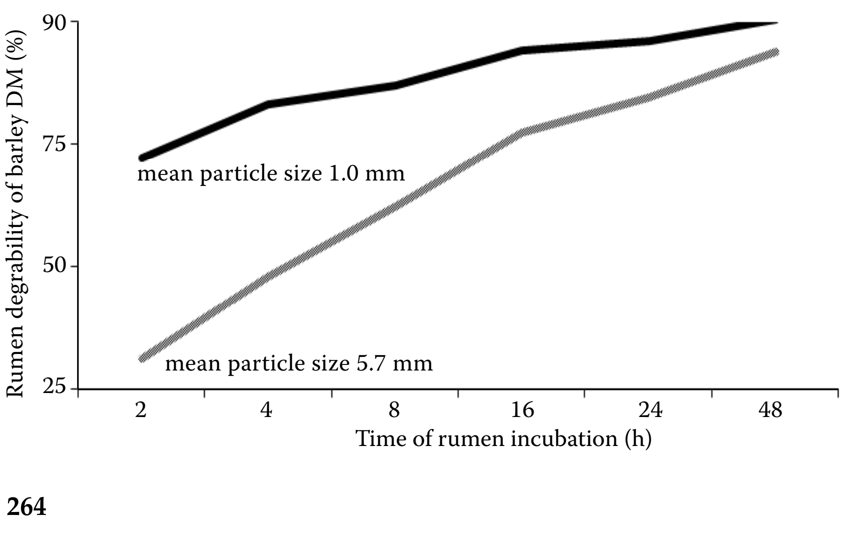

Figure 2. Effect of particle size on the ruminal degradability of barley dry matter (DM) during incubation periods

Table 3. Effects of diet composition (D), particle size (PS) and their interaction (D × PS) on the ruminal degradability of barley crude protein, neutral detergent fibre and cellulose during incubation time (h)

Incubation time (h)	Rumen degradability (%)											Means	SEM	Effects[1]		
	diet (I vs. II vs. III)/particle size (1.0 vs. 5.7 mm)						diet			particle size (mm)						
	I/1.0	II/1.0	III/1.0	I/5.7	II/5.7	III/5.7	I	II	III	1.0	5.7			D	PS	D × PS
Crude protein																
2	66	65	63	29	29	25	48^{a}	47^{a}	44^{b}	65	28	46	4.9	*	**	NS
4	74	74	75	39	40	42	56	57	59	74	40	57	4.3	NS	**	NS
8	75	74	84	53	51	58	64^{B}	62^{B}	71^{A}	78	54	66	2.5	**	**	NS
16	89	91	90	79	81	84	84^{B}	86^{A}	87^{A}	90	81	86	0.9	**	**	NS
24	95	95	94	90	93	89	94^{A}	94^{A}	92^{B}	95	91	93	0.3	**	**	NS
48	97	98	97	97	96	96	97	97	97	97	97	97	0.1	NS	NS	NS
Natural detergent fibre																
2	42	41	41	12	13	12	27	27	26	41	12	27	4.2	NS	**	NS
4	45	47	48	14	16	15	29^{b}	31^{a}	29^{b}	46	15	30	4.3	*	**	NS
8	48	51	51	21	21	24	34^{b}	36^{a}	37^{a}	49	22	36	2.6	*	**	NS
16	53	54	52	25	24	26	39	39	39	53	24	39	2.7	NS	**	NS
24	56	55	58	29	30	32	43	42	45	55	30	43	2.1	NS	**	NS
48	61	60	65	46	44	48	53	53	57	63	46	54	1.8	NS	**	NS
Cellulose																
2	37	33	30	15	13	11	26^{a}	23^{b}	21^{b}	33	13	23	5.9	*	**	NS
4	41	40	41	18	17	16	30	29	28	41	17	29	5.6	NS	**	NS
8	46	45	47	24	25	28	35^{b}	35^{b}	37^{a}	46	26	36	3.6	*	**	NS
16	50	53	58	29	30	34	40^{b}	42^{b}	46^{a}	54	31	42	3.6	*	**	NS
24	66	69	73	40	39	43	53	54	58	69	41	55	2.9	NS	**	NS
48	74	71	78	54	52	57	64	62	67	74	54	64	2.1	NS	**	NS

[1]significant difference found for $^{a,b,*}P < 0.05$; $^{A,B,**}P < 0.01$ and NS $P > 0.05$

incubation (Table 3). The degradability of CP was higher ($P < 0.01$) in the rumen of cows receiving diet III compared with that found in the rumen of cows receiving diet I after 8 and 16 h of incubation. The higher ($P < 0.05$) degradability of barley grain NDF after 4 h of incubation was found in the rumen of cows receiving diet II and after 8 h of incubation in the rumen of cows receiving diet III compared with that found in the rumen of cows receiving diet I. The higher ($P < 0.05$) degradability of barley grain CE was found after 2 h of incubation in the rumen of cows receiving diet I in comparison with that found in the rumen of cows receiving diets II and III ($P < 0.05$). The rumen degradability of barley CP after 4 and 48 h of incubation, of barley NDF after 2, 16, 24 and 48 h and of barley CE after 4, 24 and 48 h was not influenced by the diet composition ($P > 0.05$).

The higher ($P < 0.01$) rumen degradability of CP, NDF and CE after 2, 4, 8, 16 and 24 h of incubation was found for particle size of 1.0 mm in comparison with that found for particle size of 5.7 mm. After 48 h of incubation the rumen degradabilities of CP were similar ($P > 0.05$).

Table 4. Rumen degradability characteristics for barley grain dry matter, crude protein, neutral detergent fibre and cellulose

Item	Diet (I vs. II vs. III)/particle size (1.0 vs. 5.7 mm)						Diet			Particle size (mm)		Mean
	I/1.0	II/1.0	III/1.0	I/5.7	II/5.7	III/5.7	I	II	III	1.0	5.7	
Dry matter												
ED[1]	83	82	84	69	69	72	76	76	78	84	70	76
a[1]	70	68	73	44	39	37	69	43	59	69	43	56
b[1]	19	21	17	40	46	47	30	33	33	20	43	31
c[1]	0.14	0.13	0.13	0.09	0.10	0.16	0.08	0.11	0.17	0.14	0.10	0.11
Crude protein												
ED	82	83	84	64	65	66	74	74	75	83	65	74
a	60	60	52	15	14	8	37	37	32	58	13	35
b	39	40	43	85	86	88	63	63	65	40	86	63
c	0.08	0.08	0.16	0.08	0.09	0.11	0.08	0.08	0.12	0.10	0.09	0.09
Neutral detergent fibre												
ED	51	51	52	24	24	26	37	38	38	51	25	38
a	40	39	41	10	12	12	25	27	25	41	12	26
b	22	20	28	73	73	64	36	37	44	25	88	38
c	0.05	0.09	0.04	0.01	0.01	0.02	0.03	0.03	0.03	0.04	0.01	0.03
Cellulose												
ED	52	52	54	30	30	32	41	40	43	53	30	41
a	34	28	25	12	11	8	23	20	16	29	10	20
b	52	47	56	65	52	56	56	48	55	50	54	51
c	0.03	0.06	0.06	0.02	0.04	0.03	0.03	0.05	0.06	0.05	0.03	0.04

[1]characteristics estimated according to Ørskov and McDonald (1979): ED – the effective degradability using an outflow rate of 0.06/h (%); a – the rapidly soluble fraction (%); b – the insoluble but potentially degradable fraction (%) and c – the rate of degradation of b-fraction

The diet composition by particle size interaction was not influenced ($P > 0.05$) the rumen degradability of barley grain CP, NDF and CE.

The mean effective degradability of DM, CP, NDF and CE of barley grain in the rumen of cows receiving diets I, II and III was similar (Table 4). The mean values for the rate of degradation (c) of insoluble fraction (b) estimated for DM, CP and CE were higher for barley grain digested in the rumen of cows receiving diet III in comparison with the rate of degradation for barley grain digested in the rumen of cows receiving diets I and II. Higher effective degradability, higher content of rapidly soluble fraction and higher rate of degradation of insoluble but potentially degradable fractions were estimated for barley grain ground to particle size of 1.0 mm compared with that estimated for grain ground to particle size of 5.7 mm.

DISCUSSION

The objective of the study was to establish differences in the rumen degradability of barley grain nutrients resulting from the synchronization of energy availability from carbohydrate components of dietary concentrates with specific reference to grass silage protein. The grass silage nitrogen (N) containing compounds is composed of 24–80% of soluble N fraction with degradation rate of about 250%/h (Volden et al., 2002). The experimental diets were composed of grass silage (55% of diet DM) and concentrates included as a primary source of carbohydrates: starch, pectin or sucrose. The selected carbohydrates represent the carbohydrates rapidly fermented by ruminal bacteria but differ in the rates of ruminal degradation. The starch of barley degrades in the rumen at a rate from 0.14 to 0.24/h and is composed of 46–51% of the rapidly disappearing fraction (Offner et al., 2003), pectin degrades at a rate of 0.13/h and 60 to 90% immediately disappears in the rumen (Hall et al., 1998) and the rate of disappearance for sucrose was estimated to be 1.200–1.404%/h (Weisberg et al., 1998). The estimated synchronization index between N and energy supply during the day was similar (0.8 on average).

The results obtained in *in sacco* digestibility studies indicated that the rate and extent of rumen degradability of barley nutrients were influenced by the carbohydrates included in the grass silage-based diet. The highest rumen degradation rates of barley CP, NDF and CE after 8 h of incubation and of CP and CE after 16 h of incubation were found in the rumen of cows receiving the diet containing sucrose compared with the degradation rate estimated in the rumen of cows receiving the diet containing pectin or starch. The improvements in N use and microbial protein synthesis were recorded when sucrose replaced wheat starch in grass silage-based diets fed to sheep (Chamberlain et al., 1993) and when glucose replaced starch, NDF, and a carbohydrate mix in a study conducted with dairy cows (Hristov et al., 2005). However, during the first 2 hours of incubation barley CP, NDF and CE were less intensively degraded in the rumen of cows receiving the diet containing pectin or sucrose compared with those estimated in the rumen of cows receiving starch. Similar effects of altered ruminal fermentation by replacing cornstarch with sucrose during the first hours after feeding were observed in an experiment with a dual-effluent continuous-culture system by Vallimont et al. (2004). These authors found that the butyrate and ammonia N concentrations increased and acetate decreased during the first 2–3 hours after feeding, but during the next period these relationships were reversed. As suggested by Hall and Weimer (2007), the main reason for a delayed time of rumen microbial synthesis was the time-consuming process of glucose utilization from hydrolyzed sucrose; in addition, sucrose itself prolonged the time because of the suppression of fibre disappearance from the rumen and fibre-associated bacteria. These mechanisms could have delayed the onset of growth, enzyme synthesis and the activity of rumen bacteria in the early hours of fermentation. The reduction of microbial synthesis could result from ruminal pH influenced by sucrose supplementation. The lowest pH for glucose compared with starch between 2 and 4 h after carbohydrate administration was observed by Hristov et al. (2005). In our experiment there were no observations of ruminal pH. The present results and results of the previously cited study suggested that synchronizing the availability of N and energy in the diet had different effects on the rumen microbial ecosystem function (Cabrita et al., 2006; Calsamiglia et al., 2008).

The effective degradabilities estimated for barley grain DM, CP, NDF and CE were independent of the carbohydrate type. However, the estimated values of the degradation rate of insoluble fraction were higher for barley grain DM, CP and CE digested in the rumen of cows receiving grass silage-based diet containing sucrose in comparison with the values found in the rumen of cows receiving the diet with pectin or starch. In the present experiment, it seems that by changing the rumen microbial ecosystem the differences in energy availability from carbohydrates included in the diet also influenced the degradation rate of insoluble but potentially degradable fractions of barley grain nutrients.

The increase of barley grain particle size from 1.0 to 5.7 mm slowed down the rumen digestion of barley grain DM, CP, NDF and CE. The rumen fermentation coefficients: effective degradability, content of rapidly soluble fraction and rate of degradation in the rumen of insoluble fraction estimated for barley ground to particle size of 5.7 mm were lower in comparison with analogous coefficients determined for barley ground to particle size of 1.0 mm. Lower effective rumen degradability of organic matter and protein of cracked barley or whole barley compared to finely ground barley was estimated by Horadagoda et al. (2008) in an experiment with cannulated sheep. This is consistent with previous results, which indicated that with a decreasing particle size the ruminal solubility and disappearance rate of barley DM and CP increased (Beauchemin et al., 2001; Bengochea et al., 2005). Gozho et al. (2008) suggested that barley grain processing such as dry-rolling compared with pelleting enhances the microbial protein synthesis in the rumen. The extent of mechanical processing of the grain appears to be the main factor that determined the fermentation characteristics and ruminal degradation of nutrients. The most likely reasons for this effect were the differences in access to microbial digestion.

CONCLUSION

Results from the *in sacco* study showed that the rumen digestibility of barley grain dry matter, crude protein, neutral detergent fibre and cellulose was influenced by the type of carbohydrates included in grass silage-based diets. Replacing starch or pectin with sucrose in the grass silage-based diet resulted in the higher rate of barley CP, NDF and CE degradation after 8 h of incubation and of CP and CE after 16 h of incubation. It seems that the energy availability from carbohydrates affected the degradation rate of substrates as a consequence of increased ruminal microbial activity. The increase in barley grain particle size from 1.0 to 5.7 mm slowed down the rumen digestion of barley DM, CP, NDF and CE and this effect was not related to the diet composition.

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