Journal of Animal and Feed Sciences, 26, 2017, 303–310 https://doi.org/10.22358/jafs/80039/2017
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

The effect of rolled barley, sodium hydroxide-treated wheat or maize cob silage on digestive enzymes activity in the alimentary tract of dairy cows

A. Moharrery[1,3], L. Hymøller[2] and M.R. Weisbjerg[2]

[1] Shahrekord University, Animal Science Department, Agricultural College, P.O. Box 115, 8818634141 Shahrekord, Iran
[2] Aarhus University, Department of Animal Science, AU-Foulum, DK-8830 Tjele, Denmark

KEY WORDS: dairy cows, digestive tract, starch, digestion, enzyme activity

Received: 21 April 2016
Revised: 25 July 2017
Accepted: 16 November 2017

[3] Corresponding author:
e-mail: moharrery@agr.sku.ac.ir

ABSTRACT. In the present study digestive enzyme activities were studied in the rumen, intestine and faeces of dairy cows fed rations differing in starch source. Three total mixed rations were prepared for dairy cows with maize cob silage (MCS), sodium hydroxide-treated wheat (SHW) or rolled barley as starch source. The study was arranged as 3 × 3 Latin square design with 3 cows fistulated in the rumen, duodenum and ileum. The rations consisted of grass-clover silage and maize silage (~60% of dry matter (DM)), rapeseed cake, soyabean meal, sugar beet pulp and 1 of 3 different starch sources MCS, SHW or rolled barley (~25% of DM). Samples from different parts of the digestive tract (rumen, duodenum, ileum) and faeces were collected and enzymatic activities of α-amylase, protease and lipase as well as their products content in fresh samples were estimated. When MCS replaced barley or SHW, it resulted in lower DM (2.61 vs 2.91 and 3.15%) and a higher ash content (30.99 vs 29.24 and 24.31%) in the ruminal fluid without affecting enzyme activities. Positive correlation between lipolytic and amylolitic activities in ruminal fluid was stated, which supported the hypothesis that amylolytic bacteria provide energy for lipolytic bacteria. So, the enzymes activities in the different parts of the digestive tract were not affected by the different starch sources.

Introduction

Studies on nutritional regulation of digestive enzymes in ruminants are scarce. The proportion of feed digested ruminally vs post-ruminally is of considerable interest, as the end products, and thereby implications for the animal performance, differ. Significant progress has been made in developing quantitative relations among the chemical composition of ruminant feeds, dynamic aspects of digestion in the rumen, products of digestion absorbed by the ruminant, and, the most importantly, the manner in which these products can be manipulated to improve animal productivity (Moharrery and Das, 2001). Feed quality is essential for the availability of energy and nutrients. Animal responses to a feed are, therefore, dependent upon a complex interaction among the composition of the diet, its preparation and as a consequence nutritive value. Dietary factors influence the rumen ecosystem, which may exert positive or negative effects on microbial enzymes activity and consequently on animal production. The exocrine secretions of pancreas contain α-amylase, protease and lipase, which play a key role in the digestion of

the microbial biomass and other rumen escape nutrients. Small intestinal protein flow stimulates pancreatic α-amylase production (Richards et al., 2003).

Sodium hydroxide-treated wheat (SHW) is characterised by a lower rumen digestibility than rolled barley (Moharrery et al., 2014b), and therefore rumen digestion and microbial synthesis might be lower when cows are fed SHW in comparison to barley, whereas maize cob silage (MCS) might be more comparable to barley in terms of rumen digestibility. Also, such immature and ensiled maize starch as MCS is characterised by higher ruminal degradation than mature maize starch (Jensen et al., 2005).

The objectives of the present study were: 1. to study the effect of different sources of starch in dairy cow ration on the combined microbial and host animal α-amylase, protease and lipase activities in rumen, duodenal and ileal digesta, and faeces, and 2. to examine whether enzymes activities are correlated.

Material and methods

Cow management, experimental design and sampling

Three rumen-, duodenum- (simple T-cannula placed 60 cm caudal to the pylorus) and ileum- (simple T-cannula placed near to ileocaecal valve) fistulated Danish Holstein dairy cows were fed 1 of 3 rations during 3 periods according to a 3 × 3 Latin square design; each period lasted 3 weeks. Treatments consisted of 3 different mixed rations in which the main energy supplement was 25% of total ration dry matter (DM) from either rolled barley, SHW or MCS (Table 1). The mixed rations were vacuum packed with ~15 kg in each bag and stored at −20 °C. Before feeding, the bags were thawed and the cows were fed twice a day at 05:30 and 17:30 according to the *ad libitum* feed intake of previous day. Refusals were kept at ~5% of the daily intake. Further details are described in the study of Hymøller et al. (2014). About 500 g samples were collected from the rumen, duodenum, ileum and faeces 4 h after morning feeding (09:30) on the last day in each experimental period. Rumen samples were collected from the ventral and the central rumen sacs. All samples were immediately transferred to the laboratory and divided into 2 parts. One part was used for DM and ash contents determination in milled samples. The second part was filtrated through 1 layer of 45 μm Dacron mesh, and the filtrate was centrifuged at 15 000 *g* for 15 min at 4 °C to precipitate particulates matter.

Table 1. Composition of mixed rations containing rolled barley, maize cob silage (MCS) or sodium hydroxide-treated wheat (SHW)

Indices	Rations		
	barley	MCS	SHW
Feedstuff, g · kg^{-1} DM			
rolled barley	246	–	–
MCS	–	246	–
SHW	–	–	246
rapeseed cake	74	74	74
soyabean meal	25	25	25
sugar beet pulp	99	99	99
grass-clover silage	272	272	272
maize silage	271	271	271
vitamin-mineral mixture[1]	13	13	13
Chemical and nutritional composition, g · kg^{-1} DM			
DM, g · kg^{-1}	545	427	526
ash	69	69	76
CP (N × 6.25)	155	150	154
crude fat	27	26	25
starch	210	164	227
NDF	328	365	303
TDN	698	673	728
metabolizable energy, MJ · kg^{-1}	10.54	10.16	10.98

DM – dry matter; TDN – total digestible nutrients; CP – crude protein; NDF – neutral detergent fibre; [1] one kg DM of vitamin-mineral mixture contained: IU: vit. A 566 000, vit. D_3 102 000, vit. E 3.388; g: NaCl 246, Ca 290, Mg 62, S 39; mg: Mn 4, Zn 7, Cu 1.385, Co 31, Se 28, I 198

The supernatant was analysed for enzymes activities within 30 min after collection.

Chemical analysis and determination of enzymes activities

Dry matter and ash content. Samples from each part of the alimentary tract were dried at 60 °C overnight to reach constant weight for determination of DM residue and then ground through a 1-mm screen for further analyses. Ash was determined by ignition to a constant weight at 525 °C.

Proteases. The assay for determination of proteases activity was based on the Blackburn (1968) method. A unit of proteolytic activity was defined as the amount of enzyme that would release the equivalent of 1.0 μg tyrosine in 1 min. The tyrosine equivalent in the background was determined, and enzyme activity was reported as unit per ml of sample.

α-amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1). The activity of α-amylase was determined by measuring the rate of reducing sugars release during incubation of the sample with maize starch. For this purpose, 0.25 ml sample, 0.25 ml starch solution (1 g starch in 100 ml distilled water) and 0.5 ml phosphate buffer were mixed in a tube

and incubated for 15 min at 39 °C. Then, for colour development, a dinitrosalicylic acid (DNS) solution was used as described by Moharrery and Das (2001). Enzymes activity was expressed as μg of reducing sugars (R-sugar) released per min per ml of sample. Total reducing sugar in the background was determined by the DNS method, and reported in μg per ml of sample (Moharrery and Das, 2001).

Lipase (triacylglycerol lipase, EC 3.1.1.3). Lipase activity was determined by titration of the fatty acids produced by hydrolysis according to the method of Cherry and Crandall, as described by Oser (1965). Samples were incubated with an olive oil emulsion and lipase-liberated fatty acids were titrated with 0.05 N sodium hydroxide with an auto-titrator (Mettler Toledo®, Columbus, OH, USA). Units of lipase activity per ml of enzyme sample were calculated as ml of NaOH used for titration. Free fatty acids (FFA) in the background of samples were determined as described by Oser (1965), and reported as units per ml of sample.

Statistical analysis

All measurements of concentrations and activities were performed at least in duplicate; however, data analysis was performed on mean results per sample. The statistical analysis was performed with the use of GLM procedure of SAS® software (SAS Institute Inc., Cary, NC, USA). If a significant ($P < 0.05$) main effect was detected, the main effect least square means were separated by the simulate option of SAS®. The correlations between examined parameters were estimated using the CORR procedure of SAS®, and correlation coefficients were tested using the Student's t-test.

Results

Analysis of digesta

The mean concentration of total R-sugar and tyrosine equivalent increased gradually from rumen digesta to faeces (Table 2). The total R-sugar concentration in faeces was 6.7 times higher than in rumen fluid, and tyrosine equivalents in faeces were about 4 times higher in comparison to rumen fluid. For enzyme activities only α-amylase activity showed a gradual increase from rumen digesta to faeces. Protease and lipase activity in duodenum and ileum chyme were higher in comparison to rumen digesta and faeces. Free fatty acids concentration did not fluctuate between different parts of the digestive tract. Except ash, the lowest concentrations of all other biochemical parameters (total R-sugar, tyrosine-E and DM content) were found in the rumen as compared to other parts of the digestive tract.

Table 2. Descriptive statistics of parameters in alimentary tract of dairy cows

Indices	n	Mean	SD	Minimum	Maximum
Rumen					
total R-sugar	9	355	167	183	611
amylase	9	55	20	31	97
tyrosine-E	9	67	5.8	56	73
protease	9	0.322	0.071	0.184	0.432
free fatty acids	9	2.17	0.29	1.74	2.67
lipase	9	0.239	0.150	0.082	0.478
ash in total digesta	9	8.72	1.06	7.36	10.75
ash in fluid	9	28.18	3.06	23.94	31.96
DM in total digesta	9	14.30	1.68	11.68	17.60
DM in fluid	9	2.89	0.24	2.51	3.22
Duodenum					
total R-sugar	9	1191	1514	335	4930
amylase	9	106	54	37	236
tyrosine-E	9	159	187	53	618
protease	9	13.47	4.77	9.17	23.43
free fatty acids	9	2.54	0.19	2.30	2.95
lipase	9	1.204	0.904	0.396	3.157
ash	9	19.64	1.31	17.97	21.59
DM	9	4.09	0.16	3.86	4.29
Ileum					
total R-sugar	9	1602	319	1016	1960
amylase	9	139	80	74	259
tyrosine-E	9	148	27	88	169
protease	9	12.16	2.64	10.03	18.40
free fatty acids	9	2.23	0.23	1.89	2.60
lipase	9	3.487	1.317	2.337	6.813
ash	9	17.70	0.78	16.36	18.90
DM	9	6.81	0.30	6.34	7.32
Faeces					
total R-sugar	9	2392	618	1048	2917
amylase	9	191	116	33	410
tyrosine-E	9	261	48	180	355
protease	9	1.736	0.772	0.825	2.939
free fatty acids	9	2.00	0.08	1.91	2.16
lipase	9	1.00	0.92	0.03	2.59
ash	9	12.36	1.56	9.65	14.34
DM	9	13.11	2.01	10.04	15.41

SD – standard deviation; DM – dry matter; units: total R-sugar (μg · ml^{-1}); amylase (μg R-sugar · ml^{-1} · min^{-1}), tyrosine-E (μg · ml^{-1}), protease (U · ml^{-1}), free fatty acids (U · ml^{-1}), lipase (U · ml^{-1}), ash (% of DM), DM (%)

The total R-sugar content was highly variable in duodenal chyme and ranged from 335 to 4930 μg · ml^{-1} indicating a wide variation among cows. In faeces, the highest α-amylase activities were 12.4 times

higher than the lowest activities, but the highest lipase activities were 86.3 times higher than the lowest activities. The concentrations of FFA, ash and DM in digesta and chyme in the different parts of the digestive tract showed less variation (Table 2).

Effect of treatment on digestive enzymes activities and some biochemical parameters

Total R-sugar concentration in the rumen fluid was affected by dietary treatment ($P = 0.03$) with higher values stated in dairy cows fed diet with SHW than in cows fed diet with barley (Table 3). In other parts of the digestive tract and faeces no such effect was observed ($P > 0.05$). Ash ($P = 0.005$) and DM ($P = 0.04$) concentrations in the rumen fluid were affected by dietary treatment. Feeding diet based on SHW caused the highest DM content and the lowest ash content in the rumen fluid in comparison to other treatments. The ration with rolled barley tended to decrease the DM content ($P = 0.09$) and increase the ash content ($P = 0.09$) in the duodenal chyme. No other measured parameters (digestive enzymes activities, FFA and tyrosine-E contents) were affected by the examined feeding treatments ($P > 0.05$).

Correlation between enzyme activities

In rumen (Table 4) and in duodenum (Table 5) α-amylase activity was positively correlated with both protease and lipase activities. In duodenal chyme also positive correlation between protease and α-amylase activity was stated. In ileal chyme (Table 5) no correlations between digestive enzymes activities were observed.

In ruminal fluid the correlation between total R-sugar and tyrosine-E or FFA content was negative whereas the correlation between tyrosine-E and FFA levels was positive. In duodenal and ileal chyme the products of digestive enzymes activities were positively correlated (R-sugar vs FFA, R-sugar vs tyrosine-E and FFA vs tyrosine-E).

Ash content in ruminal digesta or fluid was negatively correlated with DM in digesta or fluid, respectively. Besides a positive correlation between ash concentration and total R-sugar or tyrosine-E contents in ileal chyme, the ash concentration was not correlated with either enzymes activities or digestive products in rumen and duodenum. Also, no correlation was found between DM concentration and digestive enzyme activities regardless gastrointestinal tract localization.

Table 3. Influence of different rations on enzymatic parameters in different parts of alimentary tract of dairy cows

Indices	Rations			SE	*P*-value
	barley	MCS	SHW		
Rumen					
total R-sugar	339.0^{b}	324.9^{ab}	400.9^{a}	12.56	0.032
amylase	62.9	46.4	56.2	7.67	0.459
tyrosine-E	64.9	67.8	69.1	2.13	0.500
protease	0.357	0.282	0.329	0.026	0.323
free fatty acids	2.281	2.101	2.139	0.104	0.544
lipase	0.240	0.174	0.303	0.071	0.550
DM in digesta	13.99	14.51	14.39	0.929	0.922
DM in fluid	2.91^{ab}	2.61^{b}	3.15^{a}	0.055	0.041
ash in digesta	8.31	8.52	9.32	0.463	0.428
ash in fluid	29.24^{a}	30.99^{a}	24.31^{b}	0.255	0.005
Duodenum					
total R-sugar	565.4	2511.8	496.7	784.4	0.320
amylase	147.8	79.7	90.3	23.8	0.296
tyrosine-E	77.0	329.0	72.0	97.5	0.306
protease	15.45	14.11	10.84	1.86	0.330
free fatty acids	2.409	2.679	2.546	0.115	0.420
lipase	1.771	1.216	0.625	0.407	0.331
DM	3.90	4.19	4.18	0.052	0.090
ash	20.29	19.65	18.98	0.212	0.091
Ileum					
total R-sugar	1499.2	1712.7	1593.3	45.23	0.152
amylase	147.5	132.2	140.2	4.72	0.298
tyrosine-E	137.8	152.0	155.5	8.19	0.378
protease	14.57	11.20	10.73	1.15	0.233
free fatty acids	2.168	2.217	2.300	0.057	0.418
lipase	3.434	4.178	2.850	0.667	0.501
DM	6.72	6.68	7.02	0.188	0.500
ash	17.86	17.95	17.30	0.326	0.457
Faeces					
total R-sugar	2298.9	1988.8	2603.5	120.2	0.267
amylase	274.8	147.7	225.2	38.41	0.431
tyrosine-E	232.2	251.1	298.5	25.36	0.500
protease	2.06	1.99	1.37	0.38	0.600
free fatty acids	1.993	1.945	2.055	0.038	0.314
lipase	1.259	0.642	1.108	0.293	0.453
DM	12.83	11.87	14.63	0.531	0.126
ash	11.91	11.69	13.49	0.525	0.222

MCS – maize cob silage; SHW – sodium hydroxide-treated wheat; SE – standard error; DM – dry matter; units: total R-sugar (μg · ml^{-1}), amylase (μg R-sugar · ml^{-1} · min^{-1}), tyrosine-E (μg · ml^{-1}), protease (U · ml^{-1}), free fatty acids (U · ml^{-1}), lipase (U · ml^{-1}), DM (%), ash (% of DM); ^{ab} – values within a row with different superscripts are significantly different at $P < 0.05$

In faeces (Table 6) the positive correlations were stated between: lipase and α-amylase activities, lipase activity and tyrosine-E content, total R-sugar and FFA contents, and ash and DM content.

Table 4. Correlations between enzymatic parameters in ruminal fluid of dairy cows

Indices	Amylase	Tyrosine-E	Protease	FFA	Lipase	Ash C	Ash F	DM C	DM F
Total R-Sugar	−0.15[a]	−0.747	−0.498	−0.838	−0.559	−0.551	−0.091	0.638	0.135
	0.686[b]	0.021	0.899	0.005	0.124	0.124	0.816	0.064	0.729
Amylase		0.239	0.914	0.152	0.718	0.348	−0.210	−0.425	0.213
		0.535	0.001	0.697	0.029	0.359	0.587	0.254	0.582
Tyrosine-E			0.099	0.710	0.627	0.509	−0.298	−0.273	0.198
			0.799	0.032	0.071	0.162	0.437	0.477	0.610
Protease				0.212	0.591	0.339	−0.264	−0.439	0.301
				0.584	0.094	0.372	0.493	0.237	0.431
FFA					0.433	0.429	−0.101	−0.493	0.167
					0.244	0.249	0.795	0.178	0.668
Lipase						0.600	−0.443	−0.498	0.384
						0.088	0.232	0.172	0.308
Ash in digesta							−0.512	−0.849	0.296
							0.158	0.004	0.439
Ash in fluid								0.111	−0.920
								0.777	0.001
DM in digesta									0.003
									0.995

FFA – free fatty acids; DM – dry matter; C – in digesta; F – in fluid; [a] – coefficient of correlation; [b] – level of probability

Table 5. Correlations between enzymatic parameters in duodenal and ileal chymes in dairy cows

Indices	Amylase	Tyrosine-E	Protease	FFA	Lipase	Ash	DM
Duodenal chyme							
total R-sugar	−0.205[a]	0.992	−0.171	0.845	−0.138	0.372	0.187
	0.598[b]	<0.000	0.661	0.004	0.724	0.324	0.630
amylase		−0.158	0.821	−0.179	0.879	0.339	−0.571
		0.684	0.007	0.645	0.002	0.372	0.109
tyrosine-E			−0.096	0.846	−0.083	0.374	0.204
			0.806	0.004	0.832	0.322	0.599
protease				−0.187	0.969	0.210	−0.332
				0.631	<0.000	0.588	0.383
FFA					−0.188	0.127	0.513
					0.629	0.745	0.158
lipase						0.362	−0.501
						0.339	0.170
ash content							−0.585
							0.098
Ileal chyme							
total R-sugar	0.452	0.922	0.040	0.847	0.323	0.795	0.346
	0.222	0.000	0.919	0.004	0.397	0.010	0.361
amylase		0.563	0.537	0.670	0.585	0.300	0.134
		0.115	0.136	0.049	0.098	0.433	0.730
tyrosine-E			−0.012	0.876	0.232	0.725	0.591
			0.976	0.002	0.548	0.027	0.094
protease				0.062	0.300	0.128	−0.218
				0.873	0.432	0.743	0.574
FFA					0.206	0.563	0.377
					0.595	0.114	0.317
lipase						0.243	−0.150
						0.529	0.700
ash content							0.287
							0.455

FFA, DM – see Table 4; [a] – coefficient of correlation; [b] – level of probability

Table 6. Correlations between enzymatic parameters in feacal material in dairy cows

Indices	Amylase	Tyrosine-E	Protease	FFA	Lipase	Ash	DM
Faecal material							
total R−sugar	−0.407	−0.074	−0.434	0.707	−0.380	0.298	0.349
	0.317	0.863	0.283	0.050	0.353	0.474	0.397
amylase		0.642	0.189	−0.159	0.967	0.461	0.480
		0.086	0.553	0.708	<0.000	0.251	0.209
tyrosine−E			−0.297	0.039	0.737	0.644	0.581
			0.475	0.927	0.037	0.085	0.126
protease				−0.167	0.163	0.199	0.200
				0.693	0.700	0.637	0.635
FFA					−0.338	0.495	0.577
					0.374	0.176	0.104
lipase						0.495	0.577
						0.176	0.104
ash content							0.966
							<0.000

FFA , DM – see Table 4

Discussion

Treatment effects on enzyme activity. The present study was based on samples obtained from a digestibility trial comparing rolled barley, maize cob silage (MCS) and sodium hydroxide-treated wheat (SHW) as starch rich energy concentrates for dairy cows (Hymøller et al., 2014). Ration composition of the MCS was characterized by 25% lower starch content in comparison to other treatments and had influence on α-amylase activity in the ruminal fluid (22% lower) and in the duodenal chyme (33% lower). Starch content in all diets was not high enough to cause a strong amylolytic activity in the rumen and lower in the alimentary tract, and so it might have also affected lipase and protease activities in the rumen. Additionally, lower enzymatic activities throughout the gastrointestinal tract, particularly in the rumen were caused by MCS diet. Freezing the MCS may artificially decrease soluble nutrient digestibility, especially soluble protein, due to the condensation of soluble protein with other compounds. On the other hand, lipid oxidation, enzymatic esterification, protein degradation and change may occur along with the freezing of foods (Fennema and Powrie, 1964). The obtained results showed that the activity of α-amylase was stable in duodenum and ileum digesta when cows were fed rolled barley (147.8 and 147.5 μg R-sugar · ml^{-1} · min^{-1}). When animals were fed MCS and SHW diets, α-amylase activity in duodenum was lower (79.7 and 90.3 μg R-sugar · ml^{-1} · min^{-1}) than in ileum (132.2 and 140.2 μg R-sugar · ml^{-1} · min^{-1}). However, treatment effects were far from significant. Crude protein and crude fat concentrations in all 3 rations were similar and resulted in similar enzymes activities in all examined parts of the alimentary tract. Moharrery et al. (2014a) reported that the lipase activity in the duodenal chyme can increase with increased fat concentration in the ration, and Romo et al. (2000) stated that the total fat digestibility increase by abomasal infusion of fat mixtures. Lahaie (1984) found that fatty acids are effective in increasing pancreatic lipase activity when administered both in the diet and intravenously. In the present study it was shown that the enzyme activity in the ruminal fluid of dairy cows is much lower than the corresponding enzyme activity in the intestine. However, due to the procedure used for preparation of rumen fluid, microbes attached to the solid part of the rumen digesta have been removed from the rumen sample, which could have affected enzyme activity in this media (Moharrery and Das, 2001). In this manner, α-amylase activity was 2 times higher, the protease activity was 42 times higher and the lipase activity was 5 times higher in duodenal chyme than in the ruminal fluid. Obviously, it can be extended to enzyme products but, because of some enzyme products possibly absorbed *via* intestinal tissue, it was not measureable. However, enzymes activity has been measured in ruminal fluid and probably it is a main microbial activity in this media. Bacterial proteases are associated predominantly with the cell surface and a significant intracellular proteolytic activity is present in rumen bacteria (Kopecny and Wallace, 1982). For protein hydrolization, bacteria should be attached to the protein substrate and this probably reduces enzyme activity in ruminal fluid. This might be the reason why the protease activity in the intestinal fluid was 42 times higher than in ruminal fluid.

Data comparing enzymes activities in the rumen and intestine in ruminants are lacking. In the present study it was found that α-amylase in the intestinal chyme can be accumulated and did not reduce its activity during passage through the digestive tract. In this manner, the α-amylase activity in faecal material was much higher than in ileal or duodenal chyme.

Correlations between enzymes. In ruminal fluid, positive correlations between α-amylase and protease activity, and between α-amylase and lipase were found. At least 30–35% of protease activity in the rumen is due to bacterial action (Agarwal et al., 1991). However, the rumen protozoa and fungi are known to be proteolytic. The main proteolytic activity of the protozoa is likely to be in the hydrolysis of the particle proteins of a certain particle size, whereas fungal protease made a minor contribution to ruminal proteolysis (Hobson and Stewart, 1997). Amylolytic bacteria in the rumen are usually proteolytic as well (Wallace et al., 1997); thus any factor that fosters amylolytic populations will be also associated with enhanced proteolytic activity in ruminal digesta. On the other hand, most of the proteolytic bacteria in the rumen are starch degrading bacteria, e.g., *Ruminobacter amylophilus* (Hobson et al., 1968) and *Prevotella ruminicola* (Blackburn and Hobson, 1962; Wallace and Brammall, 1985). Strong positive correlations indicated a very close cooperative action between proteolytic and amylolytic enzymes, or that a presence of some species of bacteria with both activities such as *Streptococcus bovis*, *Prevotella ruminicola* and *Ruminobacter amylophilus* is possible. Lipolytic bacteria need energy and nitrogenous substrate, which could explain the significant correlations between lipase, α-amylase and protease in the rumen. Another explanation is that long chain fatty acids liberated by lipase activity exert an energy-sparing effect because of direct incorporation into membrane lipids, which spares energy needed for *de novo* fatty acid biosynthesis (Hobson and Stewart, 1997). In the present study lipase and α-amylase activities were correlated, and lipase and protease activities tended to correlate in ruminal fluid. This finding is in agreement with the data of Moharrery and Das (2001) who found strong correlation between lipase and α-amylase and protease activities in the whole rumen fluid in sheep fed diets of different composition.

Free fatty acids as a product of lipase activity showed strong negative correlation with total R-sugar content in the rumen fluid. Latham et al. (1972) stated that the inclusion of sugar in the diet of a sheep or of glucose *in vitro* reduced lipolysis.

In ruminal fluid ash and DM contents were negatively correlated, which might be explained by the microbial production in the rumen. Microbial mass has lesser ash content than digesta in the rumen. In ruminal fluid microbial mass including microbial metabolites, such as sugar is the main source of DM. In our study, α-amylase activity was positively correlated with protease and lipase activities in the duodenum chyme. In this part of the digestive tract, mainly the pancreas and intestinal tissue produce enzymes. It has been reported that protein in the small intestine released 2 hormones – cholecystokinin and secretin, of which cholecystokinin increases the production of protease and lipase, while secretin increases α-amylase release (Magee and Hong, 1959; Mabjeesh et al., 2003). In other study it was presented that colipase in rats varies directly with protein and fat intake (Saraux et al., 1982). According to 'parallel' secretion of enzymes (Babkin, 1950), a positive correlation between protease and α-amylase activities was expected, because the pancreas exocrine secretion contains both α-amylase and protease.

The results of the present study are in agreement with the data of Moharrery et al. (2014a) who found a positive correlation between protease and α-amylase activity in duodenal chyme of dairy cows fed rations with different fat content. Because the major site of enzyme production and secretion is the duodenum, the activity of enzymes in the ileal chyme originated from the former part which passed to ileum *via* digesta passage. In the present study protease activity decreased from duodenum to ileum (from 13.47 to 12.16), whereas α-amylase activity was more stable. Enzymes activity in ileal chyme referred to resistance of their structure against degradation and rate of water absorption, which can affect their concentration in the ileal chyme. Total R-sugar as the end product of α-amylase activity and tyrosine-E as the product of protease activity and FFA as the product from lipase activity were strongly correlated.

In faecal material digestive enzymes activity refer to residual of enzymes activity from host animal and enzymes from microbial activity during the passage of digesta *via* caecum. The obtained results showed that protease was low in activity, whereas α-amylase had high and lipase intermediate activity. In this manner only lipase and α-amylase activities have shown strong positive correlation.

Conclusions

When maize cob silage was replaced by barley or sodium hydroxide-treated wheat as the major starch source in mixed ration for dairy cows, a lower dry matter and higher ash content in ruminal fluid without affecting digestive enzymes activities in different parts of the digestive tract were found. Depending upon the type of enzyme in each part of the digestive tract, correlations were seen. Proteolytic and amylolytic activities were highly correlated. Strong positive correlation between lipolytic and amylolitic activities in ruminal fluid supported the hypothesis that energy for lipolytic bacterium can be provided by the action of amylolytic bacteria. Digestive enzymes activities in the ruminal fluid of dairy cows are much lower than in the intestinal chyme.

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