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Influence of the Fibrolytic Enzymes of Cellulase and Xylanase on *in Vitro* Gas Production Kinetics of Five Diets of Different Concentrate and Forage Ratios

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Abstract

This study was carried out to assess the effects of cellulase, xylanase, and their mixture (1:1, v/v) on in vitro ruminal fermentation of five mixed rations with different silage (F) to concentrate (C) ratios (0F:100C, 25F:75C, 50F:50C, 75F:25C, 100F:0C). Samples were incubated using rumen inoculum collected from Brown Swiss cows fed concentrate and alfalfa hay (1:1). Interaction effects ($P<0.0001$) were observed between diet and enzyme for discrete lag time (L) prior to GP. Diet type affected asymptotic GP ($P<0.0001$) and L values ($P=0.0003$). However, in vitro GP at 24 to GP at 72 were affected ($P<0.0001$) by diets type. It could be concluded that the most effective diets for in vitro GP during different time were differed between different measured parameters. Enzyme administration was more affective when the ratio of silage was increased in the diet.

Keywords: Cellulase, Silage: Concentrate Ratio, Xylanase

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Introduction

Fibrous feeds are characterize with its high cellulose and hemicellulose content that can create an insoluble complex network of cellulose, hemicellulose and lignin causing a reduced nutrients digestibilities and reduced efficient utilization of forages by ruminants. Stimulating rumen digestive microorganism's activities using cell wall degrading enzymes, can improve degradation of fibrous feeds (Khattab *et al.* 2011; Salem *et al.* 2013). Exogenous fibrolytic enzymes can be used to affect insoluble complex network of cellulose, hemicellulose and lignin (Giraldo *et al.* 2007). It also can work synergistically with rumen microbial enzymes and thus could increase the digestion and nutritive value of fibrous diet (Morgavi *et al*. 2000).

The efficient utilization of dietary nutrients for ruminant production depends on many factors; the roughage to concentrate ratio is one of the most important (Kumari *et al.* 2012). Balancing roughage to concentrate ratio should improve the efficiency of microbial crude protein synthesis in the rumen than do the high concentrates diets (Verbic 2002). The aim of this study was to assess the effects of different exogenous fibrolytic enzymes on *in vitro* ruminal fermentation of total mixed rations of different silage to concentrate ratios.

Material and Methods

Substrate and Treatments

Three samples of each total mixed ration (TMR) of different silage concentrate rations (0R:100C, 25R:75C, 50R:50C, 75R:25C, 100R:0C) were prepared, dried at 60°C for 48 h, ground and stored in plastic bags for subsequent determination of chemical composition and *in vitro* gas production. Five types of diets were used in the absence (control) or presence of 1 μL/g DM of cellulase (C), xylanase (X) or XC (1:1, v/v) as an exogenous fibrolytic commercial enzymes (Dyadic® PLUS, Dyadic international, Inc., Jupiter, FL, USA) in liquid form. Activities of the exogenous fibrolytic enzymes were provided by the manufacturers, and were cellulase 30,000 to 36,000 units/g and beta-glucanase from 7,500 to 10,000 units/g for cellulase plus, and xylanase from 34,000 to 41,000 units/g, beta-glucanase from 12,000 to 15,000 units/g and cellulose at 45,000 to 55,000 units/g for xylanase plus.

In vitro Incubations

Rumen inoculum was collected from three Brown Swiss cows fed 1:1 commercial and alfalfa hay with all times fresh water. Exogenous fibrolytic enzymes of C, X or CX were added on bottle (180) immediately before closing and incubated for 72 h in an incubator at 39°C. Gas production was recorded at 2, 4, 6, 8, 10, 12, 24, 36, 48 and 72 h of incubation using the gas production technique (Extech instruments, Waltham, USA) of Theodorou *et al.* (1994). Samples of the TMR were analyzed according to AOAC (1997).

Statistical Analyses

Data were analyzed as a factorial experiment using the PROC GLM option of SAS (2002).

Results

An interaction effect (P<0.0001) was observed between diet and enzyme for the L value. Diet affected (P<0.05) the asymptotic GP and L value. However, increasing the ratios of silage to concentrate caused a lowered in vitro GP volume when no enzymes were added (Table 1). In vitro GP after 36, 48 and 72 h of incubation were affected linearly (P<0.0001) with diets type. Different enzyme types also were affected GP24 (P=0.0005) and (P<0.0001), GP36, GP48, and GP72 (Table 2).

Table 1. *Chemical Composition[1] of the Diet with Different Silage and Concentrate Ratio (g/kg DM)*

Diet	OM	CP	NDF	ADF	ADL
0F:100C	927.4[b]	172.0[a]	145.1[e]	70.3[e]	8.1[e]
25F:75C	932.6[ab]	133.2[b]	217.7[d]	88.2[d]	10.3[d]
50F:50C	939.6[a]	138.7[b]	302.2[c]	127.0[c]	12.6[c]
75F:25C	943.7[a]	92.0[c]	371.7[b]	149.0[b]	15.0[b]
100F:0C	944.2[a]	85.0[c]	499.4[a]	229.3[a]	20.4[a]
SEM	14.82	9.34	12.56	10.98	1.42

[1]OM: organic matter, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber, ADL: acid detergent lignin, SEM: stander error of the mean.

[a,b,c,d,e]Different superscripts following means within column indicate differences at *P*<0.05.

Discussion

Lowered *in vitro* gas production with increasing the ratios of silage is related to decrease microbial fermentation in the higher roughage proportions in the rations. High cell wall and lignin present, may cause suppressing effect resulting in decreased attachment of ruminal microbes to feed particles (Paya *et al.* 2007). Reddy (2003) found a decrease in gas volume as the red gram straw level increased in the complete ration by replacing the concentrate proportion.

Combination of the two enzymes cockatiels of cellulase and xylanase improved GP than the single administration of cellulase and xylanase. Exogenous fibrolytic enzymes may improve the nutritive value of fibrous feeds due to enhanced attachment by rumen microorganisms (Nsereko *et al.* 2002), and stimulate microbial colonization (Giraldo *et al.* 2004). It has been reported that some commercial fibrolytic enzymes increase total gas production and rates of *in vitro* fermentation of feed (Wallace *et al.* 2001). Both of cellulase and xylanase were not able to degrade the crystalline complex of cellulose and hemicellulose with other cell wall complexes and confirm the synergistic effect of cellulase and xylanase (Bhasker *et al.* 2012).

Table 2. *In vitro rumen gas kinetics of five different mixture ratios of forage with concentrate as affected by cellulase (C) and xylanase (X) or their combination (CX, 1:1, v/v)*

Diet	**Enzyme (EZ, 1µ/g DM of substrate)**	**Gas production parameters**[1]			***In vitro* gas production (mL/g DM)**										
		b	***c***	***L***	**Gas2**	**Gas4**	**Gas6**	**Gas8**	**Gas10**	**Gas12**	**Gas24**	**Gas36**	**Gas48**	**Gas72**
0F:100C	0	146.7	0.083	1.75	22.57	41.66	57.79	71.44	82.98	92.74	126.72	139.25	143.89	146.27
	C	140.7	0.077	1.92	20.17	37.43	52.20	64.85	75.68	84.96	118.49	131.82	137.14	140.14
	X	145.6	0.073	1.93	19.78	36.88	51.65	64.42	75.45	84.98	120.36	135.09	141.22	144.84
	XC	152.1	0.072	1.40	20.28	37.83	53.02	66.17	77.56	87.42	124.35	140.09	146.85	151.07
	P value	0.4114	0.3238	0.0650	0.4039	0.4186	0.4342	0.4502	0.4664	0.4824	0.5459	0.5276	0.4824	0.4315
25F:75C	0	145.0	0.079	1.98	21.45	39.74	55.33	68.62	79.96	89.62	124.04	137.28	142.37	145.09
	C	150.3	0.069	1.96	19.48	36.41	51.14	63.95	75.09	84.78	121.58	137.65	144.70	149.19
	X	161.7	0.08	1.76	25.51	46.85	64.73	79.73	92.32	102.92	139.47	153.02	158.22	161.09
	XC	144.9	0.075	2.04	20.09	37.36	52.23	65.02	76.03	85.52	120.37	134.69	140.62	144.14
	P value	0.2818	0.5381	0.2845	0.4516	0.4457	0.4396	0.4331	0.4263	0.4192	0.3734	0.3324	0.3058	0.2859
50F:50C	0	271.3	0.031	2.32	16.52	32.03	46.59	60.26	73.10	85.15	143.5	183.5	210.9	242.7
	C	268.8	0.032	2.57	16.62	32.21	46.84	60.56	73.43	85.50	143.8	183.5	210.6	241.6
	X	264.4	0.031	2.26	15.98	30.99	45.10	58.35	70.10	82.4	139.2	178.2	205.0	236.2
	XC	270.4	0.034	2.47	18.23	35.21	51.01	65.74	79.45	92.22	152.5	192.1	218.2	247.0
	P value	0.9026	0.6408	0.6149	0.6365	0.6391	0.6418	0.6444	0.6472	0.6502	0.6702	0.6951	0.7251	0.7914
75F:25C	0	193.5	0.038	1.69	14.1	27.26	39.40	50.66	61.10	70.77	115.62	144.05	162.09	180.80
	C	183.8	0.036	2.62	12.93	24.95	36.12	46.50	56.15	65.12	107.12	134.23	151.74	170.37
	X	185.9	0.369	2.43	60.85	75.34	83.56	90.55	96.99	102.99	131.35	150.01	162.28	175.66
	XC	186.0	0.036	2.29	12.87	24.84	35.99	46.36	56.02	65.01	107.29	134.79	152.68	171.89
	P value	0.3742	0.4453	0.1404	0.4503	0.4571	0.4643	0.4713	0.4779	0.4841	0.5041	0.4749	0.3796	0.2192
100F:0C	0	316.5	0.026	6.69	16.00	31.18	45.60	59.29	72.29	84.63	146.62	192.02	225.29	267.51
	C	284.9	0.024	3.71	13.47	26.31	38.53	50.17	61.27	71.8	125.49	165.59	195.57	234.76
	X	299.4	0.032	3.46	18.27	35.39	51.43	66.47	80.58	93.80	157.57	201.12	230.97	265.78
	XC	320.5	0.025	3.32	15.49	30.22	44.25	57.60	70.30	82.39	143.59	189.06	222.85	266.60
	P value	0.4924	0.0574	0.0007	0.0366	0.0381	0.0399	0.0419	0.0442	0.0468	0.0705	0.1112	0.1683	0.2946
[2]LSD pooled		18.797	0.4679	21.939	23.537	22.784	21.864	21.009	20.227	16.801	15.23	14.892	15.686	28.858
Interactions (*P* value)															
Diet															
Linear		<.0001	0.4082	0.0003	0.6177	0.4827	0.4356	0.4772	0.6155	0.8682	0.0005	<.0001	<.0001	<.0001
Quadratic		<.0001	0.6218	0.3197	0.6696	0.5265	0.4450	0.4239	0.4558	0.5414	0.1629	0.0001	<.0001	<.0001
EZ		0.3820	0.3797	0.0013	0.3093	0.2561	0.2112	0.1765	0.1500	0.1296	0.0736	0.0693	0.0902	0.1692
Diet × EZ		0.6183	0.4890	<.0001	0.5388	0.5845	0.6229	0.6490	0.6625	0.6648	0.5254	0.3554	0.3003	0.3722

[1] *b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); *L* is the initial delay before gas production begins (h).
[2] LSD, Least significant difference.

Conclusion

Administration of cellulase or/ and xylanase with total mixed ration could affect the in vitro rumen gas kinetics and cumulative GP, which may enhance the productive performance of ruminants in some further in vivo experiments in ruminants. The most effective silage: concentrates ratio varied between different measured parameters. However, enzymes administration was more affective when the ratio of silage was increased in the diet.

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