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The effect of different sources of xylanase on *in vitro* dry matter digestibility measured by the filter bag method*

J. Ludwin and Z.M. Kowalski[1]

Agricultural University of Kraków,
Department of Animal Nutrition
Al. Mickiewicza 24/28, 30-059 Kraków, Poland

ABSTRACT

The effect of including two sources of xylanase (produced by *Trichoderma viride* or rumen microorganisms, Megazyme, Ireland)) into the incubation solution on *in vitro* DM digestibility (IVDMD) determined by the cellulase-pepsin method using filter bags and a Daisy[II] Incubator (DI; Ankom Co, Fairport, NY) was studied. The source of xylanase did not significantly affect IVDMD. Moreover, irrespective of the concentration used, none of the xylanases improved IVDMD determination.

KEY WORDS: *in vitro* digestibility, cellulase-pepsin, xylanase, filter bag

INTRODUCTION

Every year advances in biotechnology make new enzymes available for animal nutrition, including enzymes that can be used effectively in determining *in vitro* DM digestibility (IVDMD) of feedstuffs. Compared to the traditional Tilley-Terry method (1963), the enzymatic ones do not require cannulated animals as rumen fluid donors. They are also rapid and economical (Adesogan et al., 2000). Cellulase is the most widely and successfully used enzyme in *in vitro* studies (De Boever et al., 1988; Aufrère and Guérin, 1996). However, since the enzymatic activity of the rumen ecosystem is very complex, it seems probable that the introduction of other fibrolytic enzymes, such as xylanase, could improve the precision of the *in vitro* method (better prediction and reproducibility). Xylanases belong to the glucanase enzyme family that breaks down various xylans to produce short-chain xylo-oligosaccharides. The aim of this study was to determine the effect of including xylanase from two sources in the incubation solution on IVDMD determined by the

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[1] Corresponding author: email: rzkowals@cyf-kr.edu.pl

cellulase-pepsin method using filter bags and a Daisy[II] Incubator (DI; Ankom Co, Fairport, NY).

MATERIAL AND METHODS

In vitro DM digestibility (IVDMD) was determined on 5 forage samples (3 grass hays, 1 grass silage and 1 maize silage) ground to pass through a 1.5 mm screen. The silages were dried in a forced-air oven for 48 h at 50°C. Their chemical composition was determined by standard methods (AOAC, 1995). About 0.5 g of a feed sample was placed in a filter bag (Ankom F57; 50 × 55 mm) made from polyester-polyethylene extruded filaments of pore size about 25 µm. There were 4 bags for each feed. IVDMD was determined by three methods: cellulase-pepsin (C-P), cellulase+xylanaseA-pepsin (CXA-P), and cellulase+xylanaseB-pepsin (CXB-P). In each method the samples were first incubated for 48 h in cellulase (Onozuka R10, *Trichoderma viride*; Yakult Honskha LTD, Tokyo, Japan) solution and then for 24 h in pepsin-HCl (Merck 2000 FIP U/g art. 7190; 0.2% solution (P/V) in 0.1 N HCl) solution. Both incubations were performed in DI jars at 39.5°C. The solutions were prepared according to Aufrère and Graviou (1996). In the CXA-P and CXB-P methods the cellulase solution was supplemented with either M1 (Endo- Beta Xylanase, *Trichoderma* sp., Megazyme, Ireland) or M6 (Beta Xylanase from rumen microorganisms, Megazyme, Ireland) at a rate of 700, 1400 and 450, 900, 1350, 5400 U/L, for M1 and M6, respectively. According to the producer, the specific activity of xylanase M1 and M6 was 157 and 230 U/mg, respectively. After the pepsin stage the bags were rinsed with tap water and dried at 80°C in an oven to a constant weight. The results of IVDMD were subjected to two-way (feed x xylanase concentration) analysis of variance and the Scheffe range test (SAS, 1996), separately for each source of xylanase. The results of the C-P method were used as the xylanase 0 level.

RESULTS AND DISCUSSION

The chemical composition of the feeds is presented in Table 1. The wide range of crude protein (7.58-16.50% in DM) and NDF (36.71-70.04%) concentrations makes the comparison reliable. None of the xylanases under study, irrespective of the concentration used, increased IVDMD over the values obtained for cellulase alone (Tables 2 and 3). When used in the highest concentration (5400 U/L), the xylanase of rumen microorganisms (M6) even decreased IVDMD ($P<0.001$).

TABLE 1

Chemical composition of the feedstuffs, % DM

Feed	DM, %	Ash	Crude protein	Crude fibre	NDF
Grass hay 1	89.1	9.33	9.48	32.97	67.30
Grass hay 2	88.3	16.52	12.68	28.05	60.06
Grass hay 3	88.4	6.24	7.58	35.72	70.04
Grass silage	29.3	16.19	16.50	24.82	43.32
Maize silage	38.8	3.49	8.64	18.99	36.71

TABLE 2

The effect of xylanase M1 on IVDMD (%) determined by the filter bag method

Feed	Method			P[1]	SE[2]
	C-P	CX_A-P; xylanase concentration, U/L			
		700 U/L	1400 U/L		
Grass hay 1	34.33	34.93	31.85		
Grass hay 2	44.81	41.72	43.76		
Grass hay 3	33.19	34.43	34.56		
Grass silage	57.79	55.42	59.90		
Maize silage	41.04	37.52	37.78		
Mean	42.3	40.8	41.8	0.41	1.23
SD[3]	9.8	8.5	10.6		

[1] P - probability, [2] SE - standard error of the mean, [3] SD - standard deviation

TABLE 3

The effect of xylanase M6 on IVDMD (%) determined by the filter bag method

Feed	Method					P	SE
	C-P	CX_B-P; xylanase concentration, U/L					
		450	900	1350	5400		
Grass hay 1	34.78	38.93	38.37	35.11	33.48		
Grass hay 2	47.50	47.53	46.85	47.1	46.61		
Grass hay 3	37.82	38.34	38.65	37.72	34.08		
Grass silage	58.82	58.29	58.63	56.33	55.68		
Maize silage	52.97	49.58	50.92	49.92	45.11		
Mean	46.4[A]	46.5[A]	46.9[A]	45.2[A]	43.0[B]	P<0.001	0.86
SD	9.5	7.7	8.2	8.4	8.8		

[A,B] - significant differences at P<0.001

Neither of the xylanase sources showed a significant interaction between feeds and the concentration of the xylanase in the incubation medium. The reason for the lack of effect of xylanase on IVDMD is not clear since both xylanases are supposed to break down the hemicellulose fraction of the feeds. The enzymes cellulase and xylanase do not compete for the substrate, and addition of the latter should have increased IVDMD. It is possible that the pH range of the buffer used

for cellulase is not suitable for the xylanases studied here. A destructive effect of cellulase on xylanase seems improbable.

The IVDMD values obtained in the study are lower than *in vivo* dry matter digestibility coefficients (data not presented). However, when the same feed samples where incubated in water only, dry matter disappearance was in the range of about 20%, indicating the presence of cellulase and pepsin activities inside the bags.

CONCLUSIONS

It is concluded that the source and concentration of xylanase did not significantly affect *in vitro* DM digestibility (IVDMD). Moreover, using a mixture of enzymes (cellulase + xylanase) has no advantage over the use of cellulase alone in IVDMD determination.

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STRESZCZENIE

Wpływ różnych źródeł ksylanazy na strawność *in vitro* suchej masy oznaczanej metodą woreczków filtracyjnych

Badano wpływ dodatku dwóch ksylanaz (produkowanej przez *Trichoderma viride* lub przez mikroorganizmy żwaczowe. Megazyme, Irlandia) do roztworu inkubacyjnego na strawność *in vitro* suchej masy pasz objętościowych oznaczaną metodą celulaza-pepsyna, z zastosowaniem woreczków filtracyjnych oraz inkubatora Daisy II (Ankom Co, Fairport, NY). Dodatek ksylanazy, bez względu na pochodzenie oraz koncentrację, nie miał wpływu na strawność *in vitro* suchej masy.