Morphological evaluation of forage degradation *in vitro*

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Introduction

Exogenous fibrolytic enzymes (EFE) as additives in ruminant feeds have been researched worldwide. Promising effects on DMI, digestibility, feed utilization and production in especially dairy cows and feedlot cattle has been demonstrated (Beauchemin, 2003, Eun *et al*., 2007). However, research also points to varied responses of ruminants to EFE. A better understanding of the mode of action of EFE is of importance (Colombatto *et al*., 2003) as responses vary due to many factors, including experimental conditions, dose rate of EFE, method of application and so forth (Beauchemin *et al*., 1995, 2003). The aim of this study was to evaluate the effect of EFE on the morphology of forage sections mounted to microscope slides *in vitro*. It is important to recognize the botanical characteristics of plant material as factor determining its nutritional value (Walters, 1971) as marked interactions exist between the cell wall thickness, lignification and other anatomical characteristics of forages and their digestibility (Wilson, 1993). Therefore investigations on the anatomical build-up of forages are of importance in determining the digestibility potential thereof.

Objectives

1.To determine the effect of EFE on *in vitro* digestibility of Kikuyu hay, *Eragrostis curvula* hay, Lucerne hay and Wheat straw.

2.To qualitatively assess the degradation of plant tissue at histological level when treated with EFE and incubated *in vitro* in rumen fluid.

Materials and Methods

- Buffered rumen fluid collected from sheep was used as the incubation medium and four forage types treated with an exogenous fibrolytic enzyme cocktail or distilled water were used as incubation substrates.
- *In vitro* digestibility were evaluated according to the method for "In vitro true digestibility using the DAISY incubator" as described by ANKOM Technology, Fairport, NY.
- Leaf material of freshly cut *E.curvula* and Kikuyu and stem material from freshly cut Lucerne and Wheat straw were collected and prepared for sectioning by infusion of tissue with tissue freezing medium prior to cryo-freezing in liquid Nitrogen.
- Cross sections (20um) were made on a Cryostat and fixed to microscope slides by means of clear double sided tape, as described by Akin (1982).
- Slides were pretreated with EFE or distilled water prior to incubation in buffered rumen fluid for 6, 12 or 24h *in vitro* in glass tubes sealed with rubber stoppers.
- Upon removal, slides were rinsed in ice cold water and stained with Toluidine blue for 5 min, rinsed and covered with a cover slip.
- Images were acquired by using Olympus 40x (leaf material) and 4x objectives (stem material) and the Cell^{R} imaging software (Soft Imaging Systems).
- *In vitro* digestibility data were subjected to a Main Effects ANOVA, using Statistica 8. Significant forage*treatment interactions were detected and data pertaining to the respective forages were further subjected to a One Way ANOVA. Morphology data were analyzed with either a Bonferroni or Newman-Keuls multifactorial test where significant interactions were observed. Main effects were otherwise interpreted. Significance was declared at P<0.05.

Results

Table 1: Cell wall thickness (CWT) of EFE treated Kikuyu and *E. curvula* tissues after *in vitro* digestion in buffered rumen fluid

Cross sections of leafs	Treatment	Adaxial epidermis CWT, µm	% Reduction in CWT from 0h	Abaxial epidermis CWT, µm	% Reduction in CWT from 0h	Metaxyleme CWT, µm	% Reduction in CWT from 0h	Phloem CWT, µm	% Reduction in CWT from 0h
Kikuyu	Cnt 0h	1.33 ± 0.05	0	1.41 ± 0.07	0	1.49 ± 0.17	0	1.43 ± 0.20	0
	Cnt 6h	1.44 ± 0.07	0	1.68 ± 0.01	0	1.32 ± 0.09	11.7	1.25 ± 0.10	12.7
	EFE 6h	1.53 ± 0.35	0	1.45 ± 0.01	0	1.38 ± 0.13	7.7	1.34 ± 0.08	6.2
	Cnt 12h	1.31 ± 0.11	0.8	1.64 ± 0.14	0	**1.42^{a}** ± 0.05	4.9	**1.41^{a}** ± 0.09	1.3
	EFE 12h	1.38 ± 0.02	0	1.80 ± 0.00	0	**1.03^{b}** ± 0.01	30.7	**1.12^{b}** ± 0.20	21.6
	Cnt 24h	1.34 ± 0.09	0	1.37 ± 0.15	2.5	1.18 ± 0.10	21.1	1.00 ± 0.08	29.7
	EFE 24h	1.11 ± 0.01	16.6	1.32 ± 0.01	5.7	1.35 ± 0.10	9.1	1.02 ± 0.20	28.7
E. Curvula	Cnt 0h	1.61 ± 0.16	0	1.79 ± 0.29	0	1.14 ± 0.09	0	1.17 ± 0.00	0
	Cnt 12h	1.45 ± 0.04	9.9	1.92 ± 0.33	0	**1.12^{a}** ± 0.01	2.0	1.12 ± 0.26	4.1
	EFE 12h	1.54 ± 0.00	4.3	1.39 ± 0.50	22.3	**0.71^{b}** ± 0.00	37.4	0.97 ± 0.16	16.9

Different superscripts within blocks indicate significant effects (P<0.05)

Table 2: Cell wall thickness (CWT) and surface area measurements of EFE treated Lucerne and Wheat straw tissues after *in vitro* digestion in buffered rumen fluid

Cross sections of stems	Treatment	Epidermis CWT, µm	% Reduction in CWT from 0h	Material surface area as % of Total	% Reduction in material surface area from 0h
Lucerne	Cnt 0h	13.0 ± 0.48	0	66.6 ± 2.41	0
	Cnt 6h	9.24 ± 0.83	28.9	52.1 ± 6.98	21.8
	EFE 6h	9.36 ± 0.43	27.9	47.4 ± 1.19	28.9
	Cnt 12h	8.77 ± 0.46	32.5	29.9 ± 3.27	55.1
	EFE 12h	7.39 ± 0.59	43.1	30.9 ± 1.79	53.6
	Cnt 24h	8.48 ± 0.53	34.7	23.7 ± 0.84	64.4
	EFE 24h	7.87 ± 0.17	39.5	23.7 ± 2.17	64.4
Wheat straw	Cnt 0h	13.02 ± 1.22	0	70.9 ± 0.72	0
	Cnt 12h	9.27 ± 0.70	28.8	66.7 ± 1.38	5.9
	EFE 12h	9.11 ± 0.91	29.9	66.7 ± 0.90	6.0
	Cnt 12h			57.9 ± 0.21	18.3
	EFE 12h			58.2 ± 1.66	18.0

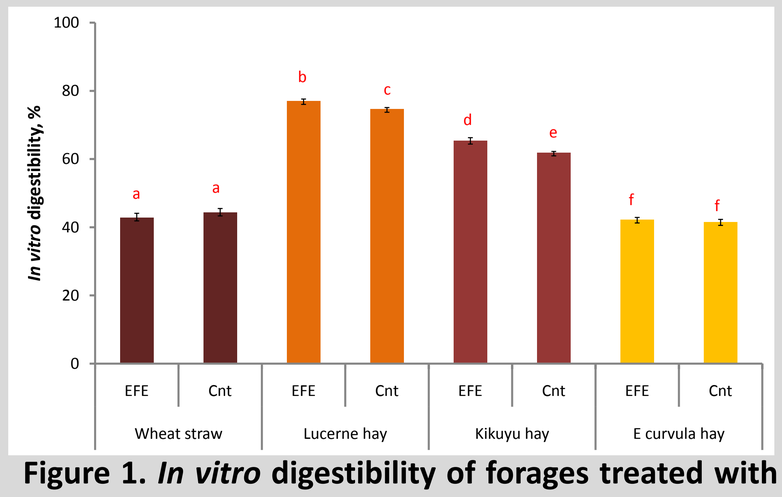

Figure 1. *In vitro* digestibility of forages treated with EFE or distilled water and incubated in buffered rumen fluid for 24h. Error bars represent the SEM. Different alphabetical letters at bars indicate significance at P<0.05.

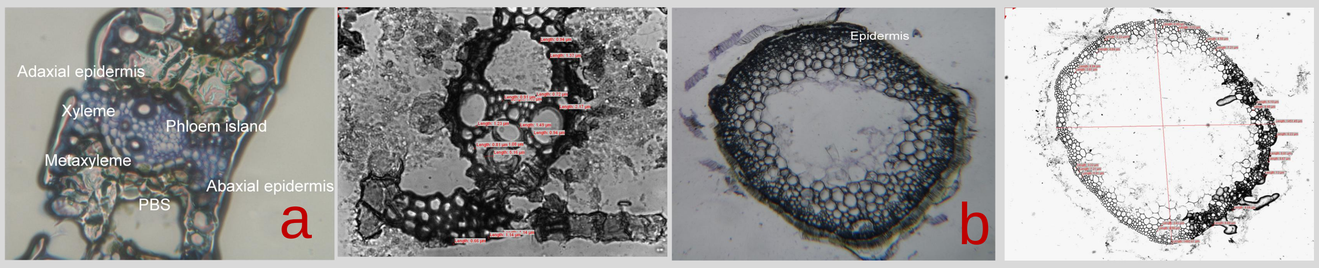

Figure 2. Cross sections of *E.curvula* leaf (a) and Lucerne stem (b) before and after *in vitro* fermentation.

Discussion

- EFE resulted in improved *in vitro* digestibility of Lucerne and Kikuyu hay (P<0.05).
- EFE resulted in thinner metaxyleme and phloem cell walls after 12h for Kikuyu and therefore a higher reduction in cell wall thickness (P<0.05).
- Similar thinning was seen in the metaxyleme cell wall of *E.curvula* after 12h fermentation.
- **There was a tendency of EFE to reduce the cell wall thickness of the tissues studied.**

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