ローズグラスおよびギニアグラスサイレージに対する予乾および細胞壁分解酵素添加の影響

誌名	日本草地学会誌
ISSN	04475933
著者名	玉,柱 西野,直樹 内田,仙二
発行元	日本草地学会
巻/号	46巻1号
巻号補足	
掲載ページ	p. 22-27
発行年月	2000年4月

Grassland Science 46 (1) : 22-27 (2000)

Fermentation of Rhodesgrass and Guineagrass Silages with or without Wilting and Added Cell Wall Degrading Enzymes

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Received : September 21, 1999/Accepted : February 1, 2000

Synopsis

Yu, Z., N. Nishino and S. Uchida (2000) : Fermentation of rhodesgrass and guineagrass silages with or without wilting and added cell wall degrading enzymes. *Grassland Science* 46, 22-27.

The effects of wilting and added enzymes on fermentation and *in vitro* digestion of rhodesgrass and guineagrass silages were examined. First cuts of the two forages were ensiled in laboratory silos directly or after being wilted for 4 h with or without cell wall degrading enzymes (0.2 g kg^{-1}). Silos were opened after 45 d and the chemical composition and the fermentation quality were determined. The silages were subjected to *in vitro* rumen incubation and the degradation of NDF was determined.

Without enzymes rhodesgrass silage failed to achieve lactate type fermentation, while guineagrass silage had high proportion of lactate even without any of the treatments. Wilting inhibited the organic acid production but decreased the proportion of lactate in rhodesgrass silage. Use of enzymes improved the preservation of both the silages, as evidenced by the increase of lactate and the decrease of pH value and NH_3-N. The contents of NDF and ADF were reduced by enzyme treatment, but the reduction was considerably suppressed when forages were wilted before ensiling.

***In vitro* degradation of NDF was low in direct-cut rather than wilted silage. Addition of enzymes also lowered the degradation, but this effect was restrictedly found in guineagrass silage incubated for 72 h. These results indicate that although added cell wall degrading enzymes can promise an improved fermentation of tropical grass silage, the benefit would be less pronounced when treated with low moisture material.**

Key words : Cell wall degrading enzymes, Guineagrass, Rhodesgrass, Silage, Wilting.

Introduction

The ensiling process may be limited by a lack of fermentable substrates, which are usually expressed as water soluble carbohydrates (WSC), in raw materials. Addition of enzymes that are capable of increasing the substrates could alleviate this problem ; the benefit of cell wall degrading enzymes to increase the lactate has been demonstrated on grass and legume silages[1,9,15,16,17,20,24,25,27]. It has also been suggested that the use of enzymes may enhance the breakdown of structural carbohydrates in the rumen, while claims are still being variable on the digestion of treated silage[10,11,12,17,25,28].

The contents of WSC are generally low in tropical grasses[5,6], indicating that the reduction of pH value may be insufficient in naturally fermented silages. Therefore, the advantage of cell wall degrading enzymes would be more clearly found when used for tropical rather than temperate grasses. In addition, certain differences exist between forages in the composition and structure of cell walls, e.g. tropical grasses usually show higher proportion of hemicellulose than temperate grasses[2]. This may indicate that the effects of enzymes would be plant specific and need to be evaluated on individual species. The efficacy of enzymes may also be influenced by dry matter (DM) content of material crop[27,29], because enzymes would use water as a transport medium. The objective of this study was to investigate the effects of wilting and added enzymes on fermentation and digestion of rhodesgrass and guineagrass silages.

Material and methods

Preparation of silages

Primary growth of rhodesgrass (*Chloris gayana* Kunth) and guineagrass (*Panicum maximum* Jacq.) was manually harvested at heading stage and chopped into approximately 13 mm length using a forage cutter. They were ensiled in laboratory silos directly or after being wilted for 4 h with or without added cell wall degrading enzymes. The amounts of ensiled forages were 540 and 510 g for direct-cut and wilted rhodesgrass, and 550 and 400 g for direct-cut and wilted guineagrass, respectively. The enzymes

(1 : 2 mixture of *Acremonium* and *Trichoderma* cellulase based on avicelase activity), provided by Yukijirushi Shubyo Co. Ltd., were dissolved in least amount of water and added at 0.2 g kg^{-1} just before the ensiling. Triplicate silos for each treatment were stored at 25℃ for 45 d.

Analyses

When the silo was opened, the content was thoroughly mixed and samples were taken for the count of lactic acid bacteria[4] and the analysis of fermentation quality. The pH value by glass electrode pH meter, lactic acid by colorimetry, volatile fatty acids by gas-liquid chromatography (Shimadzu, GC-14 A) and NH_3-N by steam distillation, were determined on cold water extracts[19,20]. Chemical composition of silage was determined on freeze-dried sample that was ground to pass through a 1-mm screen. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the methods of GOERING and VAN SOEST[8]. Total nitrogen was determined by the standard KJELDAHL procedure. Water soluble carbohydrates were determined using anthrone[7] and buffering capacity was measured by titration[23].

Fructose, glucose and sucrose in material crops were determined by high performance liquid chromatography. Freeze-dried samples (500 mg) were extracted with 80% ethanol (30 ml) in an ultrasonic bath (15 min×3 times), and filtered through TOYO No. 5 A paper. The filtrates plus washings were concentrated by using a rotary evaporator, followed by vacuum drying with phosphoric oxide overnight. The dried sample was dissolved with acetonitrile/water (50 : 50, v/v) and passed through a C 18 disposable Sep-pak cartridge (Waters Associates). Isocratic separations were made on Asahipak NH 2 P-50 with acetonitrile/water (75 : 25, v/v) at 40℃. Flow rate was 1 ml min^{-1} and sugars were detected with a differential refractometer (Shimadzu, RID-10 A).

In vitro incubation was carried out using rumen fluid obtained from two goats equipped with permanent rumen cannulae. The animals were maintained on untreated direct-cut silage prepared from the same forage as samples to be incubated. A conventional batch culture was conducted with a mixture (1 : 4) of rumen fluid and artificial saliva. Incubation was terminated after 48 and 72 h, and the disappearance of NDF was determined.

Data were analyzed by two-way analysis of variance with wilting and added enzymes as main factors. Differences were considered significant when probability was less than 0.05.

Results

The DM contents of fresh rhodesgrass and guineagrass were about 180 g kg^{-1}, and then increased by wilting to 291 and 467 g kg^{-1}, respectively (Table 1). The two grasses had similar contents of protein (N×6.25) and ADL, but rhodesgrass was low in NDF and ADF compared with guineagrass. The contents of WSC were less than 70g kg^{-1} DM in both the grasses, suggesting that the substrates may be less than required for good fermentation. Large difference was found in sugar composition ; rhodesgrass was high in sucrose but low in fructose and glucose compared with guineagrass. The numbers of lactic acid bacteria were lower than 10^5 cfu g^{-1} at harvest, but the bacteria slightly multiplied during wilting. Buffering capacity was similar in

Table 1. Chemical composition, lactic acid bacteria count and buffering capacity of material crops.

Item	Rhodesgrass		Guineagrass	
	Fresh	*Wilted*	*Fresh*	*Wilted*
Dry matter (g kg^{-1})	188	291	183	467
Crude protein (g kg^{-1} DM)	116	119	104	118
NDF (g kg^{-1} DM)	561	588	626	617
ADF (g kg^{-1} DM)	344	348	364	342
ADL (g kg^{-1} DM)	42.7	43.8	41.0	38.6
WSC (g kg^{-1} DM)	68.4	64.8	58.4	52.1
Fructose (g kg^{-1} DM)	8.62	7.17	17.5	23.3
Glucose (g kg^{-1} DM)	6.52	5.28	8.21	11.6
Sucrose (g kg^{-1} DM)	34.0	38.0	21.6	7.41
Lactic acid bacteria (log 10 cfu g^{-1})	4.58	5.05	3.78	4.20
Buffering capacity (meq kg^{-1} DM)	250	238	241	230

NDF ; neutral detergent fiber, ADF ; acid detergent fiber, ADL ; acid detergent lignin, WSC ; water soluble carbohydrate (colorimetry by anthrone). Individual sugars were determined by HPLC.

the two grasses, and was not more than 250 meq kg^{-1} DM.

When no treatments were made at ensiling, rhodesgrass silage exhibited butyrate type fermentation (Table 2). Addition of enzymes increased the lactate and decreased the pH value and NH_3-N in both direct-cut and wilted silages. Wilting suppressed lactate production and enhanced acetate fermentation. A high proportion (>120g kg^{-1} N) of NH_3-N was found even when the pH value was reduced to below 4.0, and the proportion was unexpectedly increased by wilting regardless of added enzymes.

Extensive cell wall hydrolysis occurred in enzyme-treated rhodesgrass silage. The reduction of NDF was over 110 g kg^{-1} DM in direct-cut silage and about 70 g kg^{-1} DM in wilted silage. The contents of crude protein and ADL were not affected by either of the treatments. When enzymes were added at ensiling, more WSC remained in the silages.

Even without wilting or added cell wall degrading enzymes, guineagrass silage showed a lactate type fermentation (Table 3). Addition of enzymes enhanced lactate production and decreased the pH value and NH_3-N of direct-cut silage. This effect, however, was only marginal when wilted guineagrass was treated. Wilting lowered organic acid production but failed to inhibit butyrate produc-

Table 2. Fermentation quality and chemical composition of rhodesgrass silage as influenced by wilting and added cell wall degrading enzymes.

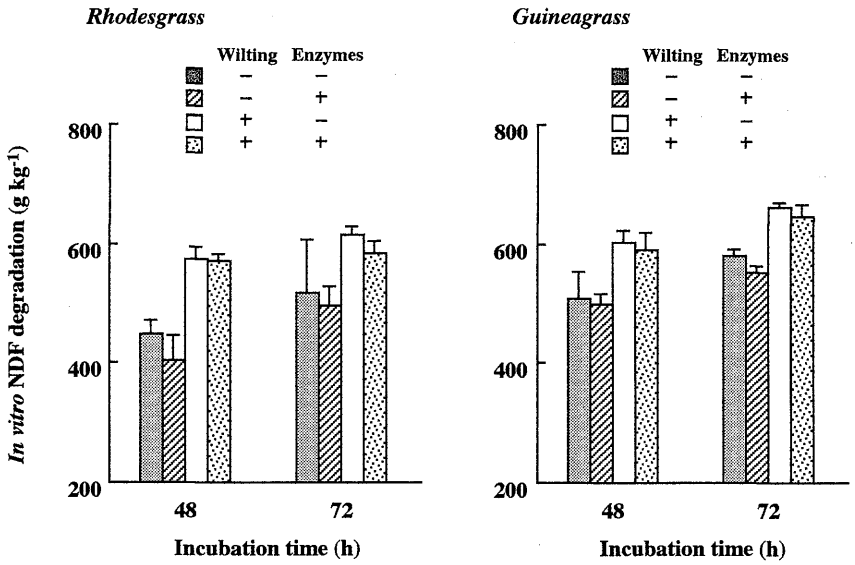
Item	Directcut		Wilted		SE	ANOVA		
	None	*+Enz*	*None*	*+Enz*		*Enzyme*	*Wilting*	*E×W*
Fermentation quality								
pH	5.05	3.73	5.59	4.19	0.19	**	*	NS
Lactic acid (g kg^{-1} DM)	25.6	124	11.0	71.6	10.7	**	*	NS
Acetic acid (g kg^{-1} DM)	10.0	32.9	30.3	36.6	2.90	**	**	*
Propionic acid (g kg^{-1} DM)	3.27	0.00	17.2	12.9	1.85	NS	**	NS
Butyric acid (g kg^{-1} DM)	28.6	10.0	12.8	17.7	5.87	NS	NS	NS
NH_3-N (g kg^{-1} DM)	133	121	178	143	5.16	**	**	NS
Chemical composition								
Dry matter (g kg^{-1})	179	185	279	276	7.12	NS	**	NS
Crude protein (g kg^{-1} DM)	123	116	117	119	2.42	NS	NS	NS
NDF (g kg^{-1} DM)	558	443	589	517	7.30	**	**	*
ADF (g kg^{-1} DM)	359	286	374	327	5.96	**	**	NS
ADL (g kg^{-1} DM)	42.0	40.4	40.4	40.2	1.66	NS	NS	NS
WSC (g kg^{-1} DM)	10.0	18.7	7.40	13.4	0.46	**	**	*

NS ; not significant, * ; $P<0.05$, ** ; $P<0.01$.

Table 3. Fermentation quality and chemical composition of guineagrass silage as influenced by wilting and added cell wall degrading enzymes.

Item	Directcut		Wilted		SE	ANOVA		
	None	*+Enz*	*None*	*+Enz*		*Enzyme*	*Wilting*	*E×W*
Fermentation quality								
pH	4.30	3.75	4.61	4.48	0.02	**	**	**
Lactic acid (g kg^{-1} DM)	88.0	133	49.0	52.9	1.19	**	**	**
Acetic acid (g kg^{-1} DM)	31.6	29.0	14.2	13.1	0.90	NS	**	NS
Propionic acid (g kg^{-1} DM)	2.20	1.03	1.07	1.03	0.41	NS	NS	NS
Butyric acid (g kg^{-1} DM)	1.47	0.93	1.73	1.97	0.22	NS	**	NS
NH_3-N (g kg^{-1} N)	142	94.9	60.2	58.6	3.39	**	**	**
Chemical composition								
Dry matter (g kg^{-1})	187	186	414	409	5.49	NS	**	NS
Curde protein (g kg^{-1} DM)	103	98.5	108	107	2.07	NS	**	NS
NDF (g kg^{-1} DM)	587	524	600	579	4.65	**	**	**
ADF (g kg^{-1} DM)	366	316	359	350	3.81	**	**	**
ADL (g kg^{-1} DM)	44.0	42.1	41.6	41.3	0.42	*	**	NS
WSC (g kg^{-1} DM)	6.37	11.3	8.57	10.4	0.28	**	*	**

NS ; not significant, * ; $P<0.05$, ** ; $P<0.01$.



Time	ANOVA		
	E	W	W×E
48h	NS	**	NS
72h	NS	**	NS

Time	ANOVA		
	E	W	W×E
48h	NS	**	NS
72h	*	**	NS

Fig. 1. *In vitro* NDF degradation of silages prepared from fresh or wilted rhodesgrass and guineagrass with or without added cell wall degrading enzymes.
E ; effect of added enzymes, W ; effect of wilting, NS ; not significant,
* ; $P<0.05$, ** ; $P<0.01$.

tion.

Although the content of NDF was reduced in enzyme-treated silage, the extent was considerably small when compared with rhodesgrass silage. The reduction was about 60g kg^{-1} DM in direct-cut silage and was only 20 g kg^{-1} DM when treated with low moisture material. The content of crude protein was increased by wilting, and that of ADL was decreased by both the treatments. Remaining WSC was also higher in enzyme-treated silages, but the effect of wilting was not consistent. Unlike rhodesgrass silage, the content of NDF was decreased in silage compared with fresh crop even when enzymes were not added.

In vitro degradation of NDF was lowered by added enzymes when guineagrass silage was incubated for 72 h (Fig. 1). The degradation was significantly higher in wilted than direct-cut silages, and the difference appeared greater in rhodesgrass silage.

Discussion

Evidence has shown that lack of fermentable substrates may be primarily responsible for unstable preservation of tropical crop silage[5,6]. Additives to fortify sugars can therefore be a great help to increase the storability[14,26]. The improvement by enzymes was clearly found in rhodesgrass silage, and lactate production was consistently enhanced with or without wilting. Although untreated rhodesgrass silage showed acetate or butyrate fermentation in this study, our previous experiment[20] indicated that lactate silage could be produced from rhodesgrass (255 g DM kg^{-1}) without any additives. The disagreement was difficult to explain, but the previous study also demonstrated that the storage temperature at around 30℃ would be unsuitable for lactate fermentation of rhodesgrass silage.

Guineagrass silage was successfully preserved even without enzymes in this study. Another experiment in our laboratory also produced lactate silage from untreated guineagrass (YU ZHU *et al.* unpublished), whereas a number of reports indicated some difficulty to achieve well preservation without any additives[3,18,21,22].

From the data on DM content, WSC content, lactic acid bacteria count and buffering capacity (Table 1), the two tropical direct-cut silages were expected to show similar fermentation quality. However, rhodesgrass was much inferior to guineagrass to achieve well fermentation during storage. As shown in Table 1, distinct difference was given only in the composition of free sugars between the two grasses. Therefore, plant fructose and glucose might be more favorably utilized than sucrose for

lactate production, although exogenous mono- and disaccharides did not show any differences in promoting lactate fermentation in silage[15].

Effects of enzymes were considerably decreased when added to extensively wilted material. This may be due to less efficient distribution of added enzymes that could be transported with the aid of water. In this study, four times as much as enzymes, compared with manufacturer's recommendation, were added to forages at ensiling. Thus, the effects of enzymes would have disappeared when applied at the recommended level. ZHANG *et al.*[29] added *Acremonium* or *Trichoderma* cellulase to naked barley straw (0.4 g kg^{-1}), and found that the effect actually diminished for either of the enzymes when DM content increased to 500 g kg^{-1}.

The reduced degradation of NDF due to enzymes may indicate that added enzymes could hydrolyze easily digestible components and retained less digestible cell walls in treated silages. However, the differences due to wilting were much more pronounced in this experiment. In tropical grass silages, hemicellulose components would be involved in organic acid production, especially when soluble carbohydrates were less than required in the material crop[13,18]. This hemicellulose fermentation could have similar effects to enzymes on cell wall digestion, and the degradation of NDF would have reduced as the severity of fermentation increased. The sum of organic acids was larger than the content of disappeared WSC in all the silages, suggesting that certain acids were produced from structural carbohydrates in this study.

Wilting can be recommended to inhibit the butyrate fermentation and avoid the risk of effluent production. Use of enzymes could also be encouraged for tropical grasses, because they may have low WSC content and would produce low-density silage due to the coarse and stemmy structures. Although cell wall degrading enzymes could promise well preservation of those tropical grass silages, the benefits may be considerably decreased when extensive wilting was made prior to ensiling.

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* : In Japanese with English summary.

** : In Japanese only. Translated title by the present authors.

要　　旨

玉柱・西野直樹・内田仙二（2000）：ローズグラスおよびギニアグラスサイレージに対する予乾および細胞壁分解酵素添加の影響．日草誌 **46**, 22-27．岡山大学農学部（700-8530 岡山市津島中 1-1-1）

ローズグラスとギニアグラスサイレージの発酵品質ならびに *in vitro* 消化性に対する予乾および細胞壁分解酵素添加の影響について検討した。両草とも一番草を用い，刈り取り後直接あるいは 4 時間予乾してからサイレージ調製に供した。細胞壁分解酵素の添加量は 0.2 g kg^{-1} とし，埋蔵 45 日後に開封して化学成分および発酵品質の変化を調べた。また，各サイレージを *in vitro* で培養して，NDF の分解率を測定した。酵素無添加の場合，ローズグラスサイレージでは酢酸あるいは酪酸が優先したが，ギニアグラスサイレージでは乳酸が最も多かった。予乾は有機酸生成を抑制したが，ローズグラスサイレージでは乳酸の比率も低下させた。両草種とも，酵素を添加すると乳酸量が増大し pH および NH_3-N 比率が低下して発酵品質は改善された。NDF および ADF 含量は酵素添加によって減少したが，その変化は予乾によって大きく減弱された。*in vitro* の NDF 分解率は予乾サイレージより無予乾サイレージの方が低かった。酵素の添加は NDF 分解率を低下させたが，その効果はギニアグラスサイレージを 72 時間培養した場合に限られた。これらのことから，細胞壁分解酵素の使用は暖地型牧草から良質サイレージを調製するうえで非常に有効であるが，低水分の材料草ではその効果が小さくなることが示された。

キーワード：ギニアグラス，細胞壁分解酵素，サイレージ，予乾，ローズグラス．