寒地型草種と暖地型草種のサイレージ特性に関する比較研究(3)

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Comparative Studies of Ensiling Characteristics between Temperate and Tropical Species

3. The effects of addition of glucose and formic acid on fermentation and proteolysis during ensilage

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Synopsis

KIM, K. H. and S. UCHIDA (1991) : Comparative studies of ensiling characteristics between temperate and tropical species. 3. The effects of addition of glucose and formic acid on fermentation and proteolysis during ensilage. *J. Japan. Grassl. Sci.* **37**, 253-260.

The objective of this study is to compare the differences in the chemical composition and the breakdown of nitrogenous compounds during ensilage between tropical and temperate species treated with and without glucose and formic acid. For this purpose Italian ryegrass (IRG : DM 15.3%, WSC 14.7% DM) and Rhodesgrass (RG ; DM 18.3%, WSC 4.1% DM) were ensiled directly, and after addition with glucose (2.0 and 4.0%) and formic acid (0.3 and 0.6%), fresh basis, respectively.

The addition of glucose produced good quality silages with lactic acid of 4.4-7.5% DM and pH value of 3.9-4.1 in both species and treatments. But there was no effect of formic acid on the quality of RG silage. It would appear that an amount above 0.6% of formic acid, fresh basis, must be needed to promote the effect on the fermentation of RG silages with high moisture conditions.

While the large decrease in total amino acids and increase in the free amino acid contents in both species occurred during the ensilage, the free amino acids was lower considerably in RG silage treated with formic acid 0.6% as against other treatments. The degradation of individual amino acid between species had a tendency for RG silages to be higher in amounts of loss and to be lower in free amino acid contents than IRG silages. These changes were also affected by the treatments in both species. In particular, the more extensive degradation was found in most of the amino acids of RG silage to be no effect of formic acid 0.6%. As in IRG, considerable amounts of Arg and Glu were also catabolised in lactate silage of RG.

The concentration of VBN in each silage was affected by the extent of degradation of the free amino acids. But it cannot be clarified that the degradation of these free amino acids was related to the characteristic of which more extensive changes in the nitrogenous components occur during the fermentation of RG than IRG.

Key Words : Ensilage, Formic acid, Glucose, Italian ryegrass, Proteolysis, Rhodesgrass.

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Introduction

Extensive proteolysis occurs during the ensilage. If a pH value low enough to inhibit clostridial activity has not been achieved, the breakdown of protein is likely to occur considerably due to the enzymes derived from plant and bacteria. Even in unwilted lactate silages, residual protein-N levels usually range from 30 to 45%. But the ammonia-N values are less than 10% total-N and the residual portion is mainly free amino acids. Therefore an ammonia-N value of less than 810% total-N is commonly used to indicate well fermented silages in temperate species[3]. In the first paper of this series[5], however, it was shown that the breakdown of protein during ensilage of tropical grass tended to be greater than that of temperate grass. Several groups also reported the ensiling characteristics of tropical forage species with high ammonia-N contents ranging from 17-36%[12,15].

Glucose and formic acid as silage additives are concerned with fermentation control. The former is applied in order to increase the supply of available energy for the growth of the lactic acid bacteria. The latter is to reduce the pH of crop to a level at which plant and microbial enzymes would be inhibited. Researches relating the effect of addition of either glucose or formic acid have been reported for some forages, but less is known about the differences between tropical and temperate grasses and the changes of nitrogenous components which occur during the ensiling of the tropical forages.

The objectives of this study were i) to investigate the effect of additions of glucose and formic acid in tropical and temperate grasses and ii) to estimate the degree of proteolysis of those which have different ensiling characteristics.

Materials and Methods

At heading stage, the first growth of Italian ryegrass (*Lolium multiflorum* LAM.) grown in an experimental field at Okayama University was harvested with a hand mower on April 28, 1988. After chopping into 1.3 cm, glucose and formic acid were applied to material grass. The amounts of additives mixed with grass before ensiling were 2.0 and 4.0% glucose ; 0.3 and 0.6% formic acid, fresh basis. About 2.0 kg of materials with and without additives were packed by hand into 4 l polyethylene bottle silos duplicately, being careful to seal silos as soon as possible and tightly. All silos were placed in a constant temperature chamber setting at 21 C for 90 days.

In June 17, 1988, Rhodesgrass (*Chloris gayana* KUNTH.) was sown in the same field that Italian ryegrass had been cultivated and cut with a hand mower at early flowering stage of the first growth on August 17, 1988. The ensiling procedure and the treatments for Rhodesgrass were the same as those of Italian ryegrass described above.

The silos were opened after 90 days and then the top and bottom 5 cm of each silage were discarded before sampling. Each sample was collected and frozen until reguired for analyses. Determinations of dry matter (DM), water soluble carbohydrate (WSC), pH, volatile fatty acid (VFA), lactic acid and volatile basic nitrogen (VBN) were carried out using methods decribed previously[5]. Individial amino acid was determined on a JEOL Model JLC-6 AH amino acid analyser using the manufacturer's recommended methodology for protein

hydrolysates. Free amino acids were determined in an aqueous extract of sample with 75% (v/v) boiling ethanol. Total amino acids were determined in a sample of grass or silage after hydrolysis with 6 N hydrochloric acid for 22 hr at 110±1°C.

Results

The chemical composition of the Italian ryegrass (IRG) and silages is shown in Table 1. The DM content of the material grass was 15.3% and which had a high WSC content of 14.7% DM. In spite of the high WSC content, the control without any treatment was very unstable. In general the pH values and the concentrations of lactic acid and VBN of silages showed glucose treatments to be better fermented than the control. The pH values of glucose treated silages were significantly ($p<0.01$) lower than the control silage. The addition of formic acid 0.6% also significantly ($p<0.01$) reduced the pH value and caused the making of

Table 1. Chemical composition of Italian ryegrass silages ensiled for 90 days.

Treatment		Control	Glucose		Formic acid	
	Grass		2.0%(A)	4.0%(B)	0.3%(C)	0.6%(D)
DM (%)	15.34	11.86	15.31**	15.87**	12.57	15.55**
pH	7.07	5.19	4.10**	3.94**	5.00	4.31**
Lactic acid (% DM)	—	.06	4.25**	6.31**	.07	.40
Acetic acid (% DM)	—	4.21	2.84	2.96	4.49	.51*
Propionic acid (% DM)	—	3.46	.42	.38	2.23	.10*
Butyric acid (% DM)	—	6.12	3.92	2.53	5.41	1.19
T-N[a] (% DM)	2.25	—	—	—	—	—
Protein-N (% T-N)	93.03	—	—	—	—	—
VBN[b] (% T-N)	—	34.65	15.34*	13.04*	29.01	5.58**

a) Total nitrogen.
b) Volatile basic nitrogen.
c) Control vs treatments : *($p<0.05$), **($p<0.01$).

Table 2. Chemical composition of Rhodesgrass silages ensiled for 90 days.

Treatment		Control	Glucose		Formic acid	
	Grass		2.0%(A)	4.0%(B)	0.3%(C)	0.6%(D)
DM (%)	18.29	16.46	19.82**	22.02**	16.26	18.34**
pH	7.01	5.23	3.98**	3.88**	5.21	5.05
Lactic acid (% DM)	—	.06	7.06**	7.50**	.11	.19
Acetic acid (% DM)	—	6.89	1.64**	1.16**	5.33	2.40**
Propionic acid (% DM)	—	.59	.10*	.11*	.73	.45
Butyric acid (% DM)	—	4.96	nil	0.32**	5.01	3.52
T-N[a] (% DM)	1.72	—	—	—	—	—
Protein-N (% T-N)	86.66	—	—	—	—	—
VBN[b] (% T-N)	—	40.03	3.84**	5.74**	33.00	23.31

a) Total nitrogen.
b) Volatile basic nitrogen.
c) Control vs treatments : * ($p<0.05$), ** ($p<0.01$).

silages with low levels of butyric acid and VBN.

The NPN concentration per total nitrogen (T-N) in RG material was 6.4% higher than that of IRG. The addition of glucose to Rhodesgrass (RG) reduced the pH values and increased the lactic acid concentrations compared with the control silage (Table 2). These changes were significantly different ($p<0.01$) compared with the control. Formic acid treatments did not affect the decrease in pH value and the inhibition of silage fermentation, hence produced high concentrations of VBN (33.0 and 23.3%). But the higher level (0.6%) slightly reduced the pH value and the VBN concentration.

The percentages of constituents in total amino acid N of the grass materials and silages are given in Fig. 1. These data showed an extensive proteolysis and thus increases in the form of free amino acids during the fermentation. However, extensive catabolism of free amino acids was found in the formic acid treated RG silage having high VBN concentration. Its free amino acid concentration was lower in relative concentration than that of material grass.

The degradation rates of individual amino acid during the ensilage are shown in Figs. 2 and 3. The Glu, Asp, Arg, Tyr and Ser decreased considerably in the IRG silage treated with glucose 2.0%, which had a higher VBN content than the formic acid 0.6% treated silage. Of the all amino acids Arg and Glu had a tendency to decrease considerably in the lactate silage. In an unstable RG silage treated with formic acid, amino acid degradations considerably occurred in most of the amino acids, except for Arg and Glu, compared with lactate silage.

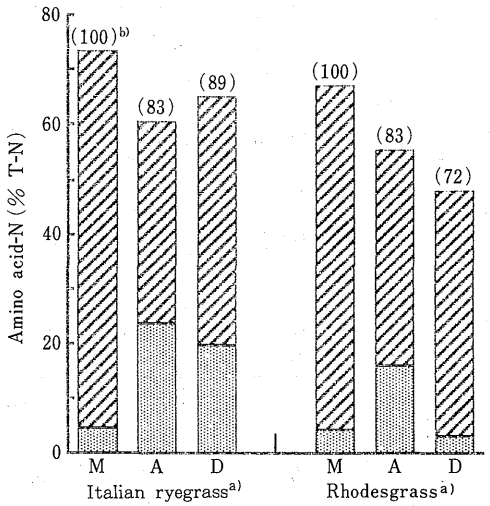

Fig. 1. The percentages of constituents (▨: protein or peptide-N, ▤: free amino acid-N) in total amino acid-N of grasses and silages.
a) M: material grass, A: silages treated with glucose 2.0%, D: silages treated with formic acid 0.6%.
b) Values in parenthesis are the percentages to material grasses.

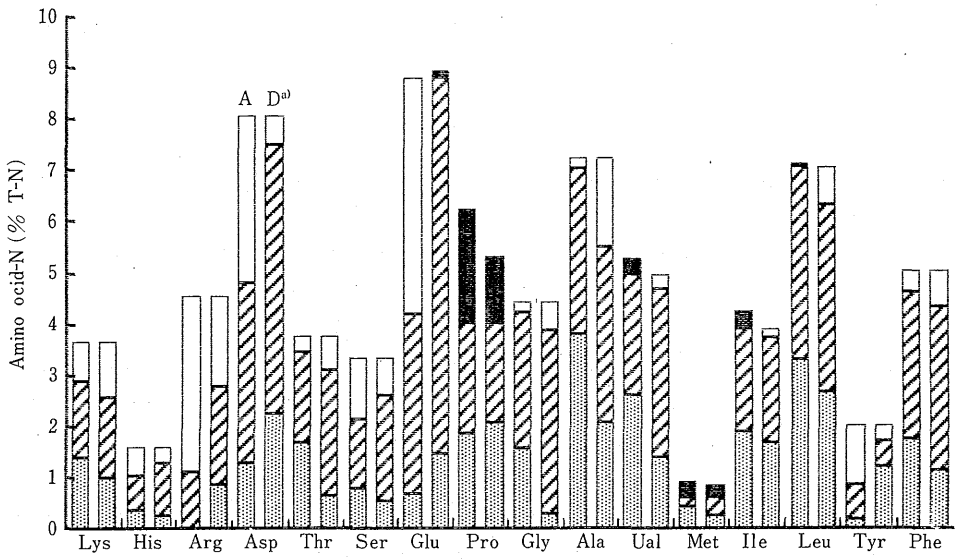

Fig. 2. The changes in individual amino acid-N (AAN : ▨+▩+□) of Italian ryegrass during fermentation.
a) cf. Fig. 1.
▨ : Protein or peptide-N, ▩ : Free AAN, □ : Decreased AAN, ■ : Increased AAN.

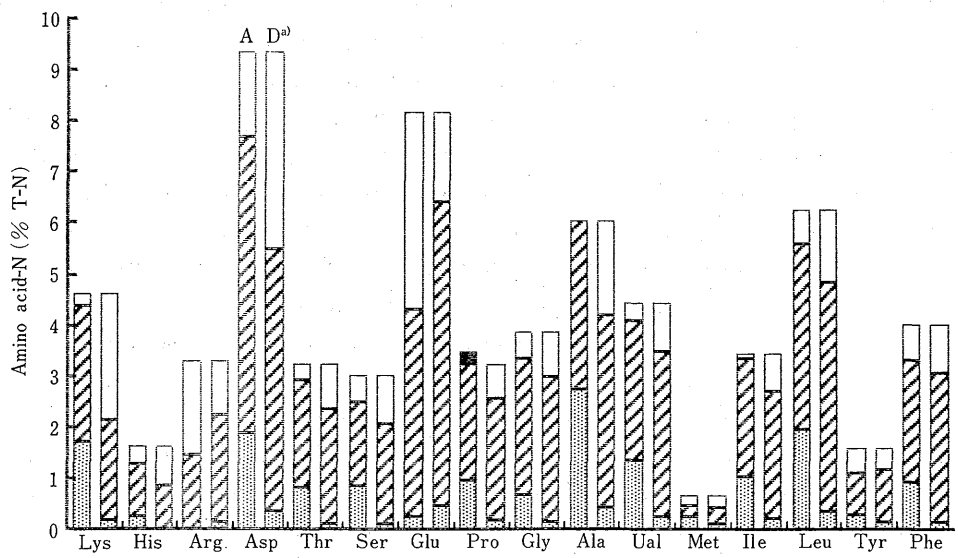

Fig. 3. The changes in individual amino acid-N (AAN : ▨+▩+□) of Rhodesgrass during fermentation.
a) cf. Fig. 1.
▨ : Protein or peptide-N, ▩ : Free AAN, □ : Decreased AAN, ■ : Increased AAN.

Particularly, the breakdowns of Lys, Asp, Ala and Leu were greater than that of stable RG silage treated with glucose. The trends of degradation of Arg and Glu in the lactate silage were similar to those found in the IRG silage.

Discussion

High moisture condition of both grasses in this trial resulted in poor preservation with low lactic acid and high VBN concentration. The disadvantage of ensiling crop with high moisture content has been stressed by many studies through clostridial[7] and water activity[2]. The addition of soluble carbohydrate sources as one of the biological silage additives ensures an adequate supply of sugars for encouraging a rapid decrease in pH, since a lower pH is necessary in order to inhibit the clostridia[8,9]. The chemical compositions of the silages made from RG (Table 2) was consistent with other reports[6,16] which demonstrated that the optimum amount of sugar addition to tropical species encourages to fall pH rapidly and to reduce the VBN content.

Formic acid application does not consistently produce well preserved silages when used with wet crop because of low and incorrect rates of application. HAIGH[3] suggested that formic acid was the most effective in the DM concentration ranged 22–26%. The present data of IRG (Table 1) indicate that at least 0.6% of formic acid should be recommended when DM concentration is less than 20%. In the RG (Table 2), formic acid had no effect on the inhibition of fermentation. But the higher application level (0.6%) reduced slightly the pH value and the VBN concentration to 23.3%. A number of fermentation inhibitors, including formic acid, have apparently never been tested in ensiling trials with tropical species.

Since overall pH optima for proteases are between 5.0 and 7.0, a rapid and extensive decrease in pH inhibits the hydrolysis of plant proteins by the plant enzymes[4,11]. OHYAMA *et al.*[14] reported that glucose addition had a beneficial effect in reducing proteolysis. However, LEIBENSPERGER and PITT[9] showed that even in the case where molasses reduced final pH, the proteolysis was unaffected. They explained that this was because the effect on pH was not seen until late in ensilage. Even at the highest levels of formic acid addition does not necessarily prevent proteolysis[1]. From the results presented in Fig. 1, it is assumed that proteolysis occurs consequently to a protein-N level of 30–45%[13] and the further degradation of free amino acids to ammonia-N is related to the problem of protein catabolism. The RG silage (D) with high VBN content of 23.31% and low free amino acids, lesser than the original RG, supports this assumption.

In the IRG, a high VBN concentration of 15.3% in silage (A) was caused by a greater breakdown of protein and of individual amino acid compared with silage (D), particularly of Glu, Asp, Arg, Tyr and Ser. The ammonia-N is produced by the deamination of these amino acids[10]. In the RG, most of the amino acids in silage (D) were degraded considerably compared with silage (A) during fermentation. These major changes are attributed to clostridial activity, resulting from coupled oxidation-reduction of a pair of amino acids or deamination and decarboxylation of a single amino acid[10]. Arg and Glu are known to be deaminated by certain strains of lactic acid bacteria, including *L. plantarum, L. brevis* and certain *Pediococcus* sp., with the production of ornithine, carbon dioxide and ammonia[13]. In

this trial, the losses of these two amino acids occurred commonly in the lactate silages made from IRG and RG.

KIM and UCHIDA[5] noted more extensive changes in the nitrogenous components during the fermentation of RG than IRG. Although it is evident that ammonia-N concentrations are relatively higher for tropical than for temperate grass silages, it is only in unfavorable conditions to be not achieved low pH values. From all these observations, it is not clear whether these extensive changes were due to the nature of substrate such as solubility or to the specific plant and microbial enzymes. But it seems likely that the NPN concentration of 6.4% higher than IRG and its catabolism were one of the factors responsible for the relatively high increase in the ammonia-N after fermentation of RG silage.

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寒地型草種と暖地型草種のサイレージ特性に関する比較研究

3. 発酵並びに蛋白質分解に対するブドウ糖及びギ酸添加の影響

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要 約

第1報（日草誌 36 巻 3 号）で報告した実験で寒地型牧草と暖地型牧草サイレージの発酵品質並びに VBN 産生量を比較した結果から，両草種がそれぞれサイレージ発酵及びそれに伴う蛋白質分解において異なる特性を有する可能性が示唆された。本報では両草種のこれら特性をさらに詳細に検討するために，ブドウ糖及びギ酸を添加剤に用いて発酵品質を調節してサイレージを調製し，それらの発酵並びに窒素成分組成について調査した。イタリアンライグラス（IRG：DM 15.3%，WSC 14.7% DM）及びローズグラス（RG：DM 18.3%，WSC 4.1% DM）を埋蔵材料とし，添加剤として材料現物当たりブドウ糖 2.0，4.0（%）またはギ酸 0.3，0.6（%）をそれぞれ添加して，実験サイロにより 2 反復でサイレージを調製した。調製されたサイレージについて pH 価の測定と有機酸の分析を行って発酵品質を調査するとともに，材料及び一部サイレージについてはアミノ酸組成を測定し，両草種に対するこれら添加剤の品質改善効果並びにサイレージ調製に伴うアミノ酸画分の変動に及ぼす影響等について比較検討した。

ブドウ糖添加の場合，両草種各区から良質サイレージが調製された。ギ酸添加による場合には，IRG 0.6% 添加区でのみ著しい発酵品質の改善が認められたが，他の区での添加の影響は明確でなかった。アンモニア態 N 比率（$NH_3-N/T-N\times100$）は各区の発酵品質に影響され区ごとで大きく変動したが，IRG ギ酸 0.6% 添加区及び RG ブドウ糖添加区において特に低い値を示した。

両草種材料草及びブドウ糖 2%，ギ酸 0.6% 区サイレージのアミノ態 N 画分について比較した結果，材料草中の総アミノ態 N に対するサイレージ中総アミノ態 N の割合は草種により，発酵品質により影響されること，またアミノ態 N 中に占める遊離アミノ態 N の比率は，一般に材料よりサイレージの方が高くなる傾向にあるが，アンモニア態 N 比率の高い RG ギ酸 0.6% 区においてはむしろ低くなることなどが知られた。材料草からサイレージへの各アミノ酸の含量（N の比率）の変化を調査した結果より，RG サイレージと IRG サイレージを比較した場合，前者は後者に比べサイレージ化に伴う各アミノ酸の消失率が高く，かつ各遊離アミノ酸の含有割合が低いなどの一般的な特徴が認められた。一方，両草サイレージ中の各アミノ酸組成はそれぞれの処理により変動し，発酵品質の劣る RG ギ酸区サイレージでは埋蔵に伴うアミノ酸の消失が総体的に高くなること，また IRG と同様 RG においても乳酸型サイレージで Arg 及び Glu の分解が促進されること等が確認された。

両草種のサイレージ調製に伴う蛋白質分解の特性を解明するためには，今後さらに各種条件下での比較研究を要するものと思われる。

キーワード：イタリアンライグラス，ギ酸，サイレージ調製，蛋白質分解，ブドウ糖，ローズグラス．

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