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Quality of reed canary-grass (*Phalaris arundinacea* L.) silage produced using glucose, formic acid, and tannic acid

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ABSTRACT

We determined the effects of adding glucose, formic acid, and tannic acid on the quality of reed canary-grass (RCG) silage. For each of the silage types, we measured chemical components, degradability in the rumen, and in vitro digestibility in sheep. The RCG was harvested at the pre-blooming stage of regrowth and stored in a plastic bag for 39 days. All of the additions inhibited ammonium-nitrogen production, but only formic acid lowered silage pH. Although all of the additions decreased acetic acid production in the silage, addition of glucose stimulated butyric acid production. The lactic acid content was lower in the silage produced with tannic acid than the other silages. Addition of tannic acid reduced the degradability of silage protein in the rumen of sheep. The in vitro dry matter digestibility of silage was improved by the addition of glucose.

Key words: Digestibility, degradability, volatile fatty acid, reed canary-grass, silage.

INTRODUCTION

Reed canary-grass (*Phalaris arundinacea* L.)(RCG), a temperate grass species used for domestic ruminant feed, is cultivated in many European countries and temperate regions in the northern hemisphere [1]. In Japan, RCG is grown at low altitudes in various regions in Hokkaido, Hokuriku, and Chubu [2, 3]. Throughout its growth period, RCG is tolerant to a wide variety of environmental conditions, including hot and dry conditions and cold and humid conditions, and it is usable for harvesting or grazing animals [4]. RCG can also grow in wet and volcanic soils and shows stable yields without having to compete with weeds. The dry matter yield of RCG in the first crop reaches its maximum at the heading stage, but the amounts of crude protein and digestible dry matter peak at the pre-heading stage [5].

Compared with silage produced from other temperate grasses such as timothy grass, orchard grass, and Italian ryegrass, less is known about RCG silage as a stock feed- additives can promote organic acid production from grass materials and improve the nutritive value for domestic ruminants. For example, addition of application of carbohydrates (e.g., glucose) to the silage material can promote microbial activities during fermentation and increase the production of acetic and lactic acids in silage. Addition of formic acid silage materials can rapidly decrease the pH of the silage in a silo, and inhibit undesirable microbial activities in the fermentation process. Such techniques and knowledge are fundamental points for making good-quality silage from grass materials. Therefore, we aimed to study the quality of reed canary-grass silage produced with or without addition of glucose, formic acid, and tannic

acid. To measure silage quality, we analyzed chemical components, degradability in the rumen of sheep, and *in vitro* dry matter digestibility of the various silages.

MATERIALS AND METHODS

Cultivation and sampling of experimental grass

RCG was cultivated in the experimental field of the Fuji Animal Research Farm, Nippon Veterinary and Life Science University, Yamanashi, Japan, and was harvested at the second regrowth pre-blooming stage (mid-July) for use in these experiments. The grass was cut into small pieces (3 - 4 cm long), then packed in a plastic bag. The total grass weight was 2 kg per bag. The grass material in each bag was de-aerated using a vacuum machine for 1 min, and then the bag was sealed. Glucose, formic acid, and tannic acid were added at 2%, 0.5%, and 2.5% (w/w), respectively, on a fresh weight basis. Each treatment had three replicates. All bags were stored in the dark room at 25°C for 39 days.

Chemical analysis

For each sample, moisture was measured as described by Morimoto [6] using toluene solution (Wako Chemical Co. Ltd., Tokyo, Japan). Crude protein was determined by the Kjeldahl method following Association of official analytical chemists (AOAC) recommendations [7] and plant fiber was measured using the detergent fiber fractionation system [8]. A 100 g subsample was mixed with 1000 ml of water in an Erlenmeyer flask, then kept overnight at 4°C. The solution was filtered through No. 5 paper (Toyo Co. Ltd., Tokyo, Japan) and used for volatile fatty acids (VFA) [9] and ammonium-nitrogen analyses [6]. The VFA were analyzed by high-pressure gas-chromatography (HPGC). Chromatographic analyses were performed using a Hitachi HPGC (Hitachi G-3000) instrument. A fused Hitachi ultra-alloy 8H column of length (30 m long, 0.8 mm I.D., and 1 µm film thickness) was used as the stationary phase. Nitrogen gas (flow rate, 25 ml/min) was used as the carrier phase. For samples and standards, (VFA standard solution, Wako Chemical Industries, Ltd., Tokyo, Japan), 5 µl samples were injected for analysis.

Measurement of degradability and in vitro dry matter digestibility

Two sheep (castrated Suffolk, average body weight 69.5kg) fitted with rumen cannula were used in these experiments. Each sheep was kept in a pen and was fed good-quality alfalfa (65 g DM/body weight$^{0.75}$/day). The sheep had free access to fresh water and a salt-mineral block. The nylon-bag technique [10] was used to measure the degradability of each silage sample in the rumen of sheep. A nylon bag (120 µm mesh, 90 mm wide × 120 mm long) containing 5 g DM of each silage sample was immersed in the rumen for 48 hours. The bag was then washed with tap water, the residues collected, and the crude protein and fiber components analyzed. To measure the *in vitro* dry matter digestibility (IVDMD) of the silage samples, rumen contents were collected via the rumen cannula and then filtered through double gauze. An aliquot of rumen liquor was used to prepare the incubation medium. The *in vitro* dry matter digestibility of all samples was measured using the two-stage technique described by Tilley and Terry [11]. The digestion of pepsin-pancreatin was measured by the method of Akeson and Stahmann [12].

Statistical analyses

Data were subjected to analysis of variance and statistical differences between control and treatments were determined by student's t-test. The difference between mean values and among variants was calculated using the least significant difference (LSD) method with a 5% level of significance [13].

RESULTS AND DISCUSSION

Chemical composition and fermentation quality of silage

Table 1 shows chemical composition of reed canary-grass cut at the pre-blooming stage. The proportion of crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) was 20.1%, 57.0% and 32.5%, respectively. These values were not significantly different from their respective values in the various silages (Table 2).

Table 1. Chemical composition (% on a dry matter basis) of reed canary-grass harvested at pre-blooming stage

Dry matter	CP[1)]	NDF[2)]	ADF[3)]	Cellulose	HC[4)]	ADL[5)]
11.6	20.1	57.0	32.5	27.3	24.5	2.7

1) Crude protein. 2) Neutral detergent fiber. 3) Acid detergent fiber. 4) Hemicellulose. 5) Acid detergent lignin.

The pH value was significantly lower in the formic acid-treated silage than in the other silages (Table 2). Even when 2.5% glucose was added to silage, the pH was not significantly decreased. Although glucose is a good substrate for the growth of microorganisms involved in silage fermentation, addition of 2.5% glucose to the silage material did not significantly lower the silage pH in this experiment. In contrast, addition of formic acid resulted in a lower pH of silage. Therefore, compared with glucose and tannic acid, formic acid is a more effective addition for producing silage with desirable attributes (e.g., with abundant lactic acid bacteria) from reed canary-grass. Cho *et al*. [14] showed that addition of formic acid to silage material inhibited protein break-down during the fermentation process.

Table 2. Chemical composition[1)] of various reed canary-grass silages

	Control	Glucose	Formic acid	Tannic acid
pH	5.7[a]	5.4[a]	4.7[b]	5.5[a]
Moisture	85.6	85.1	86.0	84.4
Crude protein	20.7	22.6	24.3	20.6
NDF	60.2	52.9	52.3	49.0
ADF	34.0	29.1	29.9	27.4
Cellulose	26.8	24.2	25.6	23.1
HC	26.5	23.9	22.4	21.6
ADL	3.2	2.6	2.5	2.5
Ammonium-N[2)]	22.9[a]	15.6[b]	17.0[b]	14.3[b]

1)See footnote of Table 1. 2) Percentage of total nitrogen.
a,b: Within a column, different superscript letters indicate significant differences (P<0.05).

The ammonium-nitrogen content in silage was higher in the control than in all of the treatments. A higher ammonium-nitrogen content indicates that crude protein in the grass was degraded by undesirable microorganisms during fermentation. A single material, in this case, reed canary-grass, is often not sufficient for good fermentation. Compared with the control and the formic acid- and tannic acid-treated silages, the glucose-treated silage produces less acetic acid but more butyric acid (Table 3).

Table 3. Volatile fatty acid composition[1)] of reed canary-grass silages

	Control	Glucose	Formic acid	Tannic acid
Acetic acid	42.6[a]	36.9[b]	39.5[ab]	46.6[a]
Propionic acid	6.7	5.4	6.6	6.3
Isobutyric acid	3.8	3.3	4.3	4.1
Butyric acid	23.7[b]	31.1[a]	23.5[b]	24.2[b]
Isovaleric acid	6.9	5.6	7.1	6.8
Valeric acid	1.4	1.2	1.3	1.4
Isocaproic acid	1.2	0.8	1.3	0.5
Caproic acid	3.4	5.8	3.4	3.9
Lactic acid	10.5[a]	9.5[a]	12.8[a]	6.6[b]
Acetic/Lactic acid	4.06[b]	3.88[b]	3.09[b]	7.06[a]
Butyric/Lactic acid	2.26[b]	3.27[a]	1.84[b]	3.67[a]

1) Percentage of total volatile fatty acids.
a,b: Within each column, different superscript letters indicate significant difference (P<0.05).

Lactic acid production in the silage was significantly decreased by adding tannic acid. The acetic to lactic acid ratio was higher in tannic acid-treated silage than in the other silages. In a previous study, addition of glucose to the silage resulted in increased acetic acid production, which lowered the pH [15]. In our silage, 2.0% glucose was not sufficient to promote acetic acid production, and did not result in a lower pH than that of the control. However, addition of 2.5% tannic acid stimulated acetic acid production in the silage.

Degradability and in vitro digestibility of silage

The dry matter and CP degradabilities of the silage in the rumen of sheep were significantly lower in the silage produced with tannic acid than in the other silages (Table 4). Cho [14] and Takano [16] noted that addition of formic acid to silage inhibited the degradation of crude protein by enzymes and microorganisms in the rumen. In our experiment, the addition of tannic acid to silage decreased the degradation of crude protein in the silage. This may mean that the addition of 0.5% formic acid was insufficient, or that the effect on crude protein degradation was stronger in the 2.5% tannic acid treatment than in the 0.5% formic acid treatment. All of the treatments lowered the degradability of plant fibers in the sheep rumen, as reflected by the NDF and ADF data.

Table 4. Degradability and *in vitro* digestibility of experimental silages

	Control	Glucose	Formic acid	Tannic acid
		Degradability		
Dry matter	69.1[1)a]	70.0[a]	69.4[a]	61.4[b]
CP	87.4[a]	88.9[a]	90.9[a]	76.4[b]
NDF	57.0[a]	51.8[b]	48.8[b]	41.9[c]
Cellulose	62.3[a]	56.4[b]	54.4[b]	47.4[c]
ADF	55.5[a]	51.9[b]	49.4[b]	43.6[c]
Digestibility as determined by the method of rumen-pepsin method				
Dry matter	58.7[b]	66.3[a]	67.2[a]	57.6[b]
Digestibility as determined by the method of pepsin-pancreatin method				
Dry matter	5.3[b]	1.9[c]	2.6[c]	7.6[a]
CP	23.4[a]	22.1[a]	10.2[b]	13.9[b]

1) Dry matter basis.

a,b,c: Within each column, different superscript letters indicate significant difference (P<0.05).

The *in vitro* dry matter digestibility of silage was improved by the addition of 2.0% glucose and 0.5% formic acid (Table 4). Digestibility, as measured by the pepsin-pancreatin method, was lower in the formic acid- and tannic acid-treated silages than in the other silages. This means that the digestibility of crude protein in silage material was decreased in the fore gut (rumen) and the hind (posterior) part in the ruminant intestine by the addition of 0.5% formic acid and 2.5% tannic acid. Driedger [17] demonstrated tannic acid inhibited soybean protein digestion in the lower gut of ruminants. In contrast, Cho [14] reported that addition of tannic acid did not inhibit the pepsin-pancreatin digestibility of red-clover silage. Nishimuta [18] showed that the ruminal bypass of dietary soybean protein was not improved by addition of tannic acid to silage. Comparing the results of those studies with our results, we conclude that a tannic acid treatment may have different effects on silage proteins in those materials.

CONCLUSION

We investigated the quality of reed canary-grass silages produced with glucose, formic acid, and tannic acid additions. We measured chemical components, degradability in the rumen, and *in vitro* digestibility in sheep. RCG was harvested at the pre-blooming stage of regrowth, cut into pieces, and stored in a plastic bag for 39 days. All treatments resulted in lower ammonium-production, but only formic acid lowered silage pH. All treatments resulted in lower acetic acid production in the silage, but addition of glucose stimulated butyric acid production. The lactic acid content was lower in silage produced with tannic acid than in the other silages, including the control. Addition of tannic acid lowered the degradability of silage proteins in the sheep rumen. The *in vitro* dry matter digestibility of silage was improved by addition of 2.5% glucose. Although these results illustrate the characteristics of RCG silage, further research is required to determine the optimum rates of addition for each additive, and to analyze the effects of each additive on rumen microbes, in both the fore (rumen) and the hind intestines. It would be also interesting to compare our results, which were obtained in a laboratory experiment, with those of silages produced in commercial RCG silage facilities.

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