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EFFECTS OF PRESERVATION WITH PRE-FERMENTED GREEN JUICE (FGJ) ON FERMENTATION QUALITY AND ENERGY AND NITROGEN UTILIZATION OF ROUND-BALED ALFALFA SILAGE BY DAIRY CATTLE

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Abstract

This study was conducted to examine the effects of pre-fermented green juice (FGJ) of epiphytic lactic acid bacteria (LAB) on the fermentation quality and animal performance of round-baled alfalfa silage (*Medicago stativa* L., *cv. Dupuits*). Ensiling treatments of wilting and FGJ additives (WFGJ) and direct-cut and FGJ additives (DFGJ) improved the fermentation quality of alfalfa silage more than that of wilting (W). Enhanced fermentation in the WFGJ and DFGJ silage was also associated with the increases of energy and nitrogen utilization of the silage by dry Holstein dairy cattle, as fed on diets formulated with alfalfa silage, oat hay, and oat grains.

Keywords: Bacterial growth, digestibility, epiphytic lactic acid bacteria, pre-fermented inoculant, silage additives, nitrogen retention

Introduction

Alfalfa silage, a nutritious forage with high quantities of proteins and minerals, fail to be preserved well on wet and warm weather in Japan. Commercial inocula of lactic acid bacteria have been proposed to improve the fermentation quality of alfalfa silage. The better fermentation in the silage compared to that of the wilting and commercial inocula was obtained by FGJ additives, which can be simply prepared by farmers (Ohshima et al., 1997). The purpose of this study was to examine whether the enhancement of silage fermentation in alfalfa silage by FGJ additives can alter the digestibility of feed components and nitrogen retention by dairy cattle fed on diets of the W, WFGJ, or DFGJ silage, oat hay, and oat grains.

Material and Methods

Alfalfa was harvested at the early flowering stage in middle May, ensiled with treatments of W, WFGJ, and DFGJ in a round-bale and wrapped to an size of 1 m diameter and 270 kg weight. The FGJ additives was prepared according to the method of Ohshima et al. (1997) and added at a level of 0.1% (v/w) by an auto-sprayer attached to round-baler machine. The viable count of epiphytic LAB in alfalfa tested was $3x10^5$ CFU g^{-1} of fresh weight, and that of the FGJ additives was increased to $3x10^{10}$ CFU ml^{-1}. Five replications were made and kept outside for 70 days before opened.

Twenty-seven samples of alfalfa silage were taken by each of outer, middle, and inner points of upper, middle, and lower layers in three directions of the round-bale. The pH value and ammonia-N concentration in extracts of the silage were determined as described by Ohshima et al. (1997). The concentrations and compositions of volatile fatty acids (VFA) were determined according to a HPLC method (Goto et al., 1993).

A feeding trial of the diets of W, WFGJ, and DFGJ silage was carried out in a three-treatment design, using two dry Holstein dairy cattle (averaged weight: 780 kg), which were

fistulated with a rumen cannulae and maintained in metabolism crates during the experiment. Animals were constantly fed at a level of nutrient requirement for the maintenance on the diet, TDN content of which was 62% of DM basis and was contributed by 50% alfalfa silage, 30% oat hay, and 20% oat grains. Experimental period consisted of 10 days for adaptation and 6 days for measurement, and all feces and urine were collected during the measuring period.

Samples of alfalfa and feces were analyzed to determine the *in vivo* digestibility of feed components and TDN content in the silage, and the urine was to determine the excretion of allantoin (Young and Conway, 1942) and amount of nitrogen retention in animals. The *in situ* DM degradability of the W, WFGJ, and DFGJ alfalfa silage in the rumen was also measured under each of the three diet condition, by suspending ground samples in a 50-ìm pore size nylon bag for 48 h. Rumen fluid collected was used to determine the pH value, ammonia-N and VFA concentrations, and viable counts of rumen microorganisms (bacteria, protozoa, fungal zoo spore).

Results obtained were analyzed with the F-test (Steel and Torrie, 1980).

Results and Discussion

Fermentation of the silage was considerably improved by FGJ additives, irrespective of direct-cut or wilting (Table 1). The WFGJ and DFGJ alfalfa silage had the significantly ($P<0.05$) lower pH value, higher total VFA concentration, and higher composition of lactic acid but the lower of butyric acid than the W alfalfa silage. The ratio of ammonia-N to the total nitrogen content was significantly ($P<0.05$) lower with the WFGJ and DFGJ silage than the other one. The significantly ($P<0.05$) higher content of NSC were also found in the WFGJ and DFGJ alfalfa silage, while no significant differences were with major cell wall components such as NDF and ADF.

The *in situ* DM degradability of alfalfa silage varied depending upon treatments and

diets (Table 2). Within the same diet, the WFGJ and DFGJ alfalfa silage had significantly ($P<0.05$) higher *in situ* DM degradability than the other one. This may be partially due to the higher NSC content and possible weakened cell wall structure, which was attributed to the cell wall hydrolysis with VFA produced during silage fermentation. In comparison of diets, the *in situ* DM degradability of all thee silage was significantly ($P<0.05$) higher in the W diet than in the WFGJ and DFGJ diets. This fact would suggest the higher digestion activity in the rumen of dairy cattle fed on the W diet, in agreement with its higher number of fungal zoospore compared to those of the WFGJ and DFGJ diets. The FGJ additives consequently significantly ($P<0.05$) improved the *in vivo* DM digestibility and TDN content of alfalfa silage.

Urinary excretion of allantoin has been proposed as a non-invasive index for absorbed microbial proteins (Mayes et al., 1995). The FGJ additives had a significantly ($P<0.05$) higher urinary excretion of allantoin, showing enhanced bacterial growth in the rumen of dairy cattle fed on the WFGJ and DFGJ diets. This was reflected in a significantly ($P<0.05$) higher amount and rate of nitrogen retention in the WFGJ and DFGJ diets compared to that of the other one, because the urinary excretion of nitrogen tended to be lower in the WFGJ and DFGJ diets than the W diet.

In conclusion, it was suggested in this study that the FGJ additives of epiphytic LAB can considerably improve the fermentation quality in alfalfa silage and the utilization of energy and nitrogen sources by dairy cattle.

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Table 1 - Effects of wilting and FGJ additives on the fermentation quality and feed composition of alfalfa silage

Item	Treatment		
	W	WFGJ	DFGJ
Moisture %	66.5[a]	65.7[a]	76.6[b]
pH	5.66[a]	4.63[c]	5.04[b]
VFA composition (mol %)			
Lactic acid	50.6[a]	78.0[c]	57.5[b]
Butyric acid	34.5[a]	3.8[c]	17.1[b]
Total VFA (mmol g^{-1}FW)	0.23[a]	0.40[c]	0.34[b]
NH_4-N / T-N	22.4[a]	17.0[b]	14.5[c]
Chemical composition (%, DM)			
NSC	22.4[a]	25.2[b]	25.8[b]
NDF	48.4[a]	46.0[a]	46.1[a]
ADF	39.7[a]	40.6[a]	37.4[a]

1) W, wilting; WFGJ, wilting + FGJ additives; DFGJ, direct-cut + FGJ additives.
2) Means with different superscripts in the same row are significantly different at $P<0.05$.

Table 2 - Effects of wilting and FGJ additives on the utilization of energy and nitrogen sources in alfalfa silage by dairy cattle

Item	Diet		
	W	WFGJ	DFGJ
in situ DM degradability (%)			
W silage	68.2^{aA}	61.7^{bA}	61.4^{bA}
WFGJ silage	72.6^{aB}	69.2^{bB}	68.1^{bB}
DFGJ silage	72.1^{aB}	68.5^{bB}	66.8^{bB}
in vivo DM digestibility of alfalfa silage (%)	61.9^{a}	68.1^{b}	66.5^{b}
TDN content of alfalfa silage (%)	61.3^{a}	66.1^{b}	65.3^{b}
Urinary excretion of allantoin (g d^{-1})	15.1^{a}	18.2^{b}	19.7^{c}
Nitrogen intake (g d^{-1})	145^{a}	139^{b}	152^{c}
Urinary excretion of nitrogen (g d^{-1})	96^{a}	76^{b}	91^{a}
Fecal excretion of nitrogen (g d^{-1})	46^{a}	44^{b}	47^{a}
Nitrogen retention (g d^{-1})	3^{a}	19^{b}	14^{b}
Nitrogen retention (%)	2.1^{a}	13.9^{b}	9.1^{b}

1) W, wilting; WFGJ, wilting + FGJ additives; DFGJ, direct-cut + FGJ additives.

2) Means with different superscripts in the same row (small letter) and column (large letter) are significantly different at $P<0.05$.