INFLUENCE OF ADDITION OF PREVIOUSLY FERMENTED JUICE TO ALFALFA ENSILED AT DIFFERENT MOISTURE CONTENTS

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ABSTRACT
Alfalfa (*Medicago sativa* L.)was ensiled with or without previously fermented juice (PFJ) after wilting for 0, 3, 6 and 24 h. They were ensiled in 900ml glass bottles at 400g/800cm^3 and preserved at 25°C for 45 d. The moisture content of the silages were 810, 730, 690 and 580g/kg, respectively. The PFJ was prepared by macerating 200g of freshly harvested alfalfa with 1,000ml of water, filtering through double cheese cloth, adding 20g/l sucrose and incubating anaerobically at 30°C for 2 d. They were added 2.5ml/kg of chopped alfalfa. Lactic acid fermentation of silage was enhanced bAlfalfa (*Medicago sativa* L.) was ensiled with or without previously fermented juice (PFy the addition of PFJ at every moisture content studied. Without additive, the pH of silages containing 810, 730, 690 and 580g moisture/kg were 4.95, 4.67, 5.26 and 5.50, respectively, while they were 4.42, 4.45, 4.53 and 4.72, respectively, in PFJ silages. This suggests that a high pH observed in wilted silage partially due to a lack of lactic acid bacteria (LAB) and addition of PFJ at ensiling provides enough LAB to lower it. PFJ was also effective in depressing clostridial fermentation at the moisture contents where butyric acid was found without it.

KEYWORDS
Alfalfa, silage, fermentation quality, previously fermented juice, silage, additive, wilting

INTRODUCTION
Until recently, alfalfa was regarded as being difficult to ensile without wilting to 500 -600g moisture/kg (McDonald *et al*, 1991). But we found that silages of good fermentation quality can be made at any ensiling temperature from direct cut alfalfa harvested in different stages of maturity by adding previously fermented juice (PFJ), a novel additive, even when the alfalfa ensiled without any additives or with *L. casei*, a conventional additive, results in poor quality silage (Ohshima *et al*., submitted to AFST). Although wilted alfalfa is known to be a good source of silage, more improvement of the quality was expected by treating it with PFJ. Therefore, the effect of treatment of wilted alfalfa with PFJ on silage quality was studied at different moisture contents.

MATERIALS AND METHODS
A small amount of alfalfa was harvested from which a 200g sample was taken and macerated with 1,000ml of water using a blender. This mixture was filtered through double cheese cloth, and 500ml of the filtrate was collected to a 900ml glass bottle to which 10g of sucrose was added. This bottle was fitted with a gas trap and maintained at 30°C for 2 days (PFJ). At this time 1st growth of alfalfa, 20kg was harvested on April 24th at the vegetative stage, spread on a concrete pavement, collected on 0, 3, 6 and 24 h after spreading, cut with a knife into 1cm and treated before ensiling with 2.5ml of water or PFJ/kg of chopped alfalfa. The number of lactic acid bacteria in PFJ was counted with GYP-$CaCO_3$ agar plate. Three replicate glass bottle silios of 900ml capacity were prepared for each treatment. The silos were fitted with gas traps and preserved at 25°C.

After 45 days, all the silos were opened and 20g of each silage was macerated with 200ml of water. The macerate was filtered through double cheese cloth and the filtrate used to determine the pH with a pH meter, the lactic acid content calorimatrically, the VFA levels by gaschromatography and the ammonia-N level by steam distillation. A portion of the silage was freeze dried and the weight lost during drying was considered to be the moisture content.

Statistical analysis was made using two way layout design (Snedecor and Cochran, 1967) and mean values were compared by Fisher's least significant difference test.

RESULTS AND DISCUSSION
The lactic acid bacteria count in PFJ was $1.29x10^8$/ml and it was calculated that $3.23x10^5$ was inoculated per 1kg of chopped alfalfa by adding 2.5ml PFJ/kg.

The effect of moisture content on the fermentation quality of silage is shown in Table 1. Silages containing 810, 730, 690 and 580g moisture/kg were obtained by wilting for 0, 3, 6 and 24 h, and they were referred to as 810, 730, 690 and 580 silage, respectively.

Without additive (control), 810 and 730 silages contained around 100g/kgDM of lactic acid but their pH were as high as 4.95 and 4.67, respectively. As the lactic acid content was considered to be too high, the analysis was repeated three times. All the repetitions showed the same results suggesting the high pH is correct and may be derived from a high buffering capacity of the material alfalfa. PFJ silage contained more lactic acid and showed lower pH compared with control silage at every moisture content studied. Reduction of moisture content lower than 700g/kg accompanied a decrease of lactic acid content and an increase of pH in both control and PFJ silages and the rate was significantly greater in control silage than in PFJ silage. Addition of PFJ had a slight but significant effects on acetic and propionic acid concentrations. But the significant negative effect of PFJ on acetic acid content is negligible when compared with the great increase of lactic acid content. Although no significant differences were obtained because of the wide variations in 810 control silage, it was interestingly observed that 810 and 690 control silages contained butyric acid at high concentrations while 730 control silage contained only a small amount. The clostridial acitivity found in 810 control silage was due to the high moisture content and in 680 might be due to the longer wilting time compared with 730. The lower moisture content than 600g/kg depressed the activity of clostridia. Even in the silages where some clostridial activities were found, ammonia concentrations were around 100g/kgTN suggesting that they were not deteriorated. PFJ depressed clostridial activity of silage in all the moisture contents studied and reduced the ammonia formation of silages at the moisture contents where butyric acid was formed without it.

The effectiveness of commercial inoculant containing a few strains of lactic acid bacteria in improving silage quality at different moisture contents was also reported (Jones et al., 1992), but the PFJ used in the present study is a novel additive which can be easily made by farmers without paying any money. Furthermore, we made it clear that addition of PFJ to direct cut alfalfa improves silage quality independently of harvest seasons, maturity stages, and storage

temperatures even when inoculation of a commercial LAB is ineffective (Ohshima et al., submitted to AFST). Direct cut alfalfa will not be used practically because of effluent loss and the intention of the previous study was to prove the effectiveness of PFJ in improving silage quality at the best condition for clostridial fermentation. The present study showed that PFJ was also effective in improving silage quality at the best moisture contents for high and low moisture silages which are 700 and 600g/kg, respectively.

Aerobic stability of PFJ treated silage should be studied before the practical application of the technique because PFJ contains many kinds of microorganisms other than lactic acid bacteria.

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Table 1
Effect of addition of previously fermented juice (PFJ) at different moisture contents on the fermentation quality of silage

		Moisturecontent(g/kg)[1]				Mean[2]	Analysis of variance[3]			RMS[4]
		810	730	690	580		Moisture	PFJ	Interaction	(d.f.=16)
pH	None	4.95^{bc}	4.67^{c}	5.26^{ab}	5.50^{a}	5.10^{A}	**	**	*	0.02
	PFJ	4.42^{c}	4.45^{c}	4.53^{b}	4.72^{a}	4.53^{B}				
Lactic acid(g/kgDM)	None	110.0^{a}	96.7^{a}	69.9^{b}	41.2^{c}	79.5^{B}	**	**	*	76.1
	PFJ	140.5^{a}	146.1^{a}	125.8^{b}	100.6^{c}	128.3^{A}				
Acetic acid(g/kgDM)	None	14.9^{a}	11.4^{b}	12.6^{b}	4.8^{c}	10.9^{B}	**	*	*	0.76
	PFJ	16.4^{a}	13.6^{b}	11.4^{c}	5.6^{d}	11.8^{A}				
Propionic acid(g/kgDM)	None	0.32	0.17	0.27	0.05	0.20^{A}	**	*	NS	0.01
	PFJ	0.25	0.11	0.12	0.04	0.13^{B}				
	Mean	0.29^{a}	0.14^{bc}	0.20^{ab}	0.05^{c}					
Butyric acid(g/kgDM)	None	5.73	0.08	0.56	0.00	1.59	NS	NS	NS	4.43
	PFJ	0.09	0.04	0.00	0.00	0.03				
	Mean	2.91	0.06	0.28	0.00					
NH_3-N(g/kgTN)	None	103.7^{ab}	88.7^{b}	110.3^{a}	58.6^{c}	90.3^{A}	**	**	*	77.6
	PFJ	87.7^{a}	85.1^{a}	79.0^{ab}	65.3^{b}	79.3^{B}				

1. Each value is mean of three replicate silos. a, b, and c on the same row are significantly different.
2. A and B show the significant effect of PFJ addition.
3. N.S., not significant; *significant at P<0.05; and **significant at P<0.01.