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Additives Effects of Fermented Juice of Epiphytic Lactic Acid Bacteria and Acetic Acid on Silo Fermentation and Ruminal Degradability of Tropical Elephant Grass

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Abstract: This laboratory scale study examined on silo fermentation of Elephant grass (*Pennisetum purpuereum*) without additive (Control). And with additives of fermented juice of epiphytic lactic acid bacteria (FJLB) and commercial acetic acid (ACE) powder. The subsequent silages and fresh matter were used to determine their *in situ* rumen degradability. Duplicate of 3.0g milled silages and fresh matter were weighed in Nylon bags (140 x 75 mm, pore size 20 to 40 um) and incubated in the rumen of dry lactating cow fed on maintenance requirement. Nylon bags were withdrawn at 8, 16, 24, 48 and 72 hours after insertion. The 0-h measurement was obtained by soaking the two bags of each sample in warm water (37 °C) for 1 h. Nylon bags were then washed with cold water for 20 min in a washing machine and dried for 48 h at 60°C. To estimate the rate and extent of DM and NDF degradability and their degradation characteristics data were fitted to the exponential equation of $P = a+b(1-e^{-ct})$. The results in this study revealed that FJLB treatment increased ($P<0.0$) lactate concentration and decreased ($P<0.0$) losses of nutrients in silages compared to Control and ACE treatments. Similarly, the digestibility of DM and NDF were higher ($P<0.05$) in the FJLB silage than in the Control or ACE silages. The rate at which (b) was degraded was considerably higher for the FJLB silage [(c) = 0.091 for DM and (c) = 0.34 for NDF] than for the Control [(c) = 0.06 for DM and (c) = 0.28 for NDF] and the ACE silage [(c) = 0.08 for DM and (c) = 0.31 for NDF]. Similarly, the total or cumulative rate (a+b) of DM (65.91%) and NDF (53.78%) digestibility was higher ($P<0.05$) in the FJLB silage, but that for ACE and Control treatments appeared similar. The additive of ACE at ensiling did not improve the fermentation of Elephant grass and its *in situ* rumen degradability of silage. More research is needed to evaluate the type and number of the of epiphytic LAB in the forage extract before incubation and in the FJLB after incubation with a view to identify their effectiveness during ensiling and *in vivo* digestibility of tropical crops silage.

Key words: Elephant grass, FJLB, ACE, silage fermentation, ruminal degradability

Introduction

Poor fermentation is a major problem of most of the Tropical legumes and grasses. The inherent characteristics of higher buffering capacity, lower fermentable substrates and extensive lignifications in most tropical legumes and grasses required the addition of additives at ensiling. Although a lots of commercial additives of lactic acid bacteria (LAB) inocula have been employed during their ensiling of forages, previous studies with temperate crops indicated that the use of epiphytic LAB improved fermentation quality of silages more than commercial additives of LAB inocula (Ohshima *et al.*, 1997a, 1997b and Li-man *et al.*, 2002). Many techniques have been employed and appreciable results obtained towards improving the nutritive value of forages, however, information on the utilization of epiphytic LAB as additives during fermentation of tropical forages is scarce relative to that available for temperate forage crops.

Also digestibility experiments with grasses and silage have considerable value in the estimation of their nutritive value to the ruminant, but these experiments are tedious and require large quantities of forages. Consequently, rapid techniques such as *in vitro* and *in situ or in sacco* have attracted wide recognition. These techniques satisfactory provide a rapid guide for measuring in situ nutrient disappearance as well as offer a quantitative estimate of the degradable feed available for rumen microbes and the un-degradable feed which may be available for post rumen microbial-enzyme digestion.

This laboratory scale study examined on silo fermentation of Elephant grass (*Pennisetum purpuereum*) without additive (Control) and with additives of fermented juice of epiphytic lactic acid bacteria (FJLB) and commercial acetic acid (ACE) powder, as well as determined its *in situ* rumen degradability.

Materials and Methods

Preparation of Fermented Juice of Epiphytic Lactic Acid Bacteria (FJLB): The Elephant grass (*Pennisetum purpuereum*) was cut at fifth growth stage in September from Ryukyus University Experimental Research Farm, Okinawa Japan. To prepare the FJLB, the harvested fresh matter was immediately cut into 2 to 3 cm lengths using a mechanical forage cutter, thoroughly mixed and 200g of fresh matter was immediately macerated with a liter of sterilized, distilled water. This was filtered through a sterilized double layer of cheesecloth and the juice transferred into a liter bottle. 5% (w/v) sugar (glucose) was added into the extract solution and incubated for 2 days at 30°C. At the end of 48 h, the pH value of the extracts (FJLB) was determined using a pH meter and then used as silage additives (i.e., added to the fresh crop at ensiling). The numbers of the of epiphytic LAB in the forage extract before incubation and in the FJLB after incubation were determined by counting the colony-forming unit (cfu) with GYP-$CaCo_3$ agar plate (Masuko *et al.*, 1992).

Silage Preparation: The fresh matter from Elephant grass (*Pennisetum purpuereum*) (24.6% of DM) was cut into 2-3cm length. The entire lot was thoroughly mixed and approximately 500g fresh Elephant grass was ensiled into three silos of 1000ml capacity with the three treatments being T1 (Control i.e., without any additive), and T2 and T3 with the addition of 5% (w/v) of FJLB and commercial acetic acid (ACE), respectively. Three silos from each treatment (in total, 9 silos) were opened after 40 days and weighed to determine the extent of DM and structural carbohydrate loss. A representative sample from each silo was mixed and frozen at -15°C for further chemical analysis. Silage extracts was prepared immediately by macerating a 50 g silage sample with 300ml of distilled water. This was filtered through double layer of cheesecloth and used to determine pH value and concentrations of volatile fatty acids and volatile basic nitrogen.

Chemical Analyses: Dry matter (DM) content of fresh materials and resultant silage from the three treatments was determined by freeze drying for a minimum of 24 h. Crude protein (CP) content was determined as described previously (Yahaya *et al.*, 2001a). Water-soluble carbohydrates (WSC) content was determined according to the method of Deriaz (1961). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined as described by Goering and Van Soest (1970) and as modified by Van Soest *et al.* (1991). Cellulose and hemicellulose contents were calculated by subtracting ADL from ADF and ADF from NDF, respectively. The concentrations of organic acids (i.e., formate, lactate, acetate, propionate, n- and iso-butyrate, n- and iso-valerate) and ethanol in the silage extracts were determined by HPLC analysis (Jasco co, Tokyo Japan) using an ion exchange column (Shimadzu SCR-102 (H), 12 mm ID X 30 cm, Shimadzu Co., Japan). Silage pH was immediately determined from the prepared silage extracts using a pH meter (a glass electrode), while ammonia, as % total N, was analyzed according to Conway and O' Malley (1942). The buffering capacity of the Elephant grass was determined according to the methods described by Playne and McDonald (1966).

In Situ Digestibility: Nylon bags (140 x 75 mm, pore size 20 to 40 um) containing 3 g of dry feed sample (milled through a 2-mm screen) were incubated in the rumen of dry lactating cow. The cow was fed on maintenance requirement of 5 kg Oat hay, 1 kg each of lucerne and beet pulp hays plus 2 kg concentrate at 13.00 h daily. Cow had free excess to water and mineral/vitamin licks. Nylon bags were withdrawn at 8, 16, 24, 48 and 72 hours after insertion. The 0-h measurement was obtained by soaking the two bags of each sample in warm water (37°C) for 1 h. Nylon bags were then washed with cold water for 20 min in a washing machine and dried for 48 h at 60°C. The DM degradation data were fitted to the exponential equation $P = a + b\ (1 - e^{-ct})$ (Ørskov and McDonald, 1979; McDonald, 1981) to determine the degradation characteristics (a, b, A, B, A+B, c, ED).
Where
P = is DM degradation at time t,
A = washing loss (representing the soluble fraction of the feed);
B = (a+b) - A. i.e. insoluble but fermentable fraction;
c = the rate of degradation of B;
a = zero intercept;
ED = effective degradability, calculated at an outflow rate of 0.05 = k
$ED = a + (b.c) \div c + k$ where k is assumed to be the rate of particulate outflow from the rumen (Ørskov & McDonald, 1979).

Statistical Analysis: Data obtained on silage fermentation and on the same incubation time were analyzed using ANOVA in a randomized block design, while means differences between treatments determined using multiple range test procedures (Duncan, 1955 and Snedecor and Cochran, 1980).

Results and Discussion

Characteristics of cell wall contents, buffering capacity and epiphytic lactic acid bacteria of fresh material Elephant grass and silages: Chemical composition and number of epiphytic lactic acid bacteria (LAB) prior to ensiling of the tropical Elephant grass and silages are presented in (Table 1). The crop had higher neutral detergent fiber (60.8%), acid detergent fiber (38.0%), acid detergent lignin (8.8%) and 29.2% cellulose contents but lower water-soluble carbohydrate (3.2%), crude protein (CP = 9.1%) and 22.8% hemicellulose contents. Most tropical forages especially grasses contain less nitrogen but more cell wall constituents (De-Visser, 1998 and Yahaya *et al.*, 1999).

Table 1:Characteristics of fresh material Tropical Elephant grass and silages

	Fresh Elephant grass prior to ensiling	Elephant grass silages			
		Control	FJLB	ACE	SE
Dry matter	24.59	22.9[b]	23.2[a]	23.8[a]	0.1
			% DM		
Crude protein (CP)	9.1	9.6[c]	10.4[a]	8.9[b]	0.1
Neutral detergent fiber (NDF)	60.8	59.6[a]	57.7[c]	57.9[b]	0.1
Acid detergent fiber (ADF)	38.0	39.1[b]	37.1[c]	39.7[a]	0.1
Acid detergent lignin (ADL)	8.8	9.5[b]	8.8[c]	11.1[a]	0.3
Hemicellulose	22.8	20.5[a]	20.7[a]	18.2[b]	0.1
Cellulose	29.2	29.6[a]	28.3[b]	28.6[b]	0.3
Water soluble carbohydrates (WSC)	3.2	ND	ND	ND	
pH	6.22	5.45[a]	4.33[b]	5.45[a]	0.23
Buffering capacity (M.eq.DM)	266.75	ND	ND	ND	ND
Epiphytic lactic acid bacteria (cfu/gFW)	3.12×10^5	ND	ND	ND	ND
FJLB after 48 h incubation					
FJLB + 0 additive					
pH	4.77	ND	ND	ND	ND
LAB (cfu/ml)	2.1×10^9	ND	ND	ND	ND
FJLB + 5% glucose		ND	ND	ND	ND
pH	3.98	ND	ND	ND	ND
LAB (cfu/ml)	5.4×10^9	ND	ND	ND	ND

Means followed by different superscripts within the same row differ ($P<0.05$)
Each value represent a mean of three silos except for fresh Elephant grass
FJLB = Fermented juice of epiphytic lactic bacteria
Control = Without additive
FJLB = 5% additive of fermented juice of epiphytic lactic bacteria (w/v)
ACE = 5% additive of Acetic acid powder (w/v)
SE = Standard error, nd = not determined

Table 2: Effects of treatments on losses and fermentation quality (%) of Elephant grass silages

	Elephant grass			
	Control	FJLB	ACE	SE
Disappearence (%)				
Dry matter	5.7[b]	2.6[c]	8.8[a]	0.3
Hemiecellulose	13.1[b]	11.7[b]	20.3[a]	1.1
Cellulose	2.4b	5.7[a]	1.5[b]	0.4
Fermentation quality				
pH	5.45[a]	4.33[b]	5.45[a]	0.23
Oragnic fatty acids (%DM)				
Lactic acid	0.94[b]	3.47[a]	3.28[a]	0.23
Acetic acid	0.67	0.74	0.22	0.18
Propionic acid	0.25	-	0.06	-
Iso-butyric acid	0.09	-	0.36	-
N-butyric acid	0.07	-	-	-
Total VFA	2.12[b]	4.21[a]	4.02[a]	0.31
VBN (% Total N)	12.70[a]	6.00[b]	9.42[b]	0.27
Ethanol (%DM)	2.51[a]	1.61[b]	2.07[a]	0.07
V score	68.2[b]	94.4[a]	75.6[b]	3.95

Means followed by different superscripts letters in the same row differ ($P<0.05$)
Each value represent a mean of three silos
Control, FJLB, ACE and SE = refered to Table 1

Czerkawski (1986) found out that tropical forages accumulated more starch instead of fructans during growth and contained less soluble sugars, hemicellulose and CP contents compared to temperate grasses. Apart from species differences these variations may be caused by high temperature intensity, which is a typical reflection of tropical weather condition (Stephen and Michael 1960 and Van Soest 1987). This is because high temperature which is a typical characteristic of tropical weather condition increase metabolic rate to expense more sugar in crops.
Similarly, due to the high temperatures of tropical weather condition, Elephant grass showed lower number of epiphytic lactic acid bacteria (LAB) of 3.6 X 10^5 compared to 4.2 X 10^5 cfu/g FW obtained in temperate Italian ryegrass (Yahaya *et al.*, 2003b). This is because lactic acid bacteria prefer moderate warm weather, mainly overcast with relatively high humidity, calm with low evaporative tendency (Gibson *et al.*, 1958; Weise and Wermke, 1973). Whereas clostridia which are obligate anaerobes associated with bad fermentation process are little affected by climatic factors, as they mostly occur on the growing plant in the endospore form only (Micheal, 1984).
The amount of buffering capacity which is a milli-equivalent kg^{-1} DM of alkaline requires to change the pH from 4 to 6 is an important factor in lowering the pH value of ensiled crops. Tropical Elephant grass showed a higher buffering capacity probably due to appreciable contents of anions (organic acid salts, orthophosphates, sulphates, nitrates, and chlorides), low proteins (McDonald *et al.*, 1991) and rapid lignifications of cell walls constituents (Yahaya *et al.*, 1999). These negatives characteristics have been observed to exert some resistant in lowering the pH values of the crop during fermentation (Yahaya *et al.*, 2003a).
The chemical composition of the tropical Elephant grass silages showed that cell walls ADF and ADL were decreased ($P<0.05$) with the addition of FJLB (LAB) at ensiling, indicating an improvement in the silage feeding values (Yahaya *et al.*, 2002b). In the contrast, the Control treatment had higher ($P<0.05$) NDF and cellulose contents compared to the other treatments due to higher disappearance of readily soluble carbohydrate hemicellulose fraction leaving the less-degeradable fraction in the silages (Yahaya *et al.*, 2003b). One other reason for the higher disappearance of these components during ensilage is because tropical elephant grass contains less soluble sugars and hemicellulose and both carbohydrates are less resistance to acid hydrolysis (Yahaya *et al.*, 2002c). However even though cell walls NDF and ADF contents were decreased in Control treatments compared to ensiled original fresh materials, the amount of these components decreased in the FJLB treated silages was significant indicating the beneficial effects of the treatment in improvement of silage nutritive value resulting to an increased in *in-situ* silage DM and NDF degradability (Fig. 1 and 2).

Loses and Fermentation Quality of Elephant Grass During Ensiling: The disappearance and fermentation characteristic of the tropical Elephant grass silages are presented in Table 2. The DM losses in silage treated with FJLB additive was small 2.6% and within the acceptable range of < 5% reported by Yahaya *et al.* (2001a) with hemicellulose and cellulose disappearance accounting to 11.7% and 5.7% respectively. Higher values of 8.8% DM and 20.3% hemicellulose were obtained in ACE treated silage. The decreasing degradation observed in DM and hemicellulose contents in FJLB treated silages could be partially associated with the better fermentation of original

Table 3: Ruminal degradability of Elephant grass and subsiquent silages

		In situ remen degradability			
	Grass	Control	FJLB	ACE	SE
DM disappearance (%)					
a	12.15	14.25^{b}	15.05^{a}	14.35^{b}	0.62
b	42.25	35.72^{b}	50.86^{a}	38.43^{b}	3.30
c	0.08	0.06^{b}	0.09^{a}	0.08^{b}	0.01
Total degradability a+b	54.40	49.97^{b}	65.91^{a}	52.78^{ab}	3.50
Undegradable fraction (%)	45.60	50.04^{a}	34.09^{b}	47.23^{ab}	3.50
Effective degradability (%)	54.45	50.02^{b}	65.96^{a}	52.83^{ab}	3.50
NDF disappearance (%)					
a	0.02	0.02^{b}	0.04^{a}	0.01^{b}	0.01
b	44.30	48.96^{ab}	53.74^{a}	50.68^{ab}	1.97
c	0.30	0.28^{b}	0.34^{ab}	0.31^{ab}	0.01
Total degradability a+b	44.32	48.97^{ab}	53.78^{a}	50.69^{ab}	1.97
Undegradable fraction (%)	55.68	51.03^{ab}	46.22^{b}	49.31^{ab}	1.97
Effective degradability (%)	44.37	49.02^{ab}	53.83^{a}	50.74^{ab}	1.97

Means followed by different superscripts within the same row differ ($P<0.05$)
Effective degradability in the rumen (assuming rate of passage of $0.05/h^{-1}$)
Control, FJLB, ACE and SE = refered to Table 1

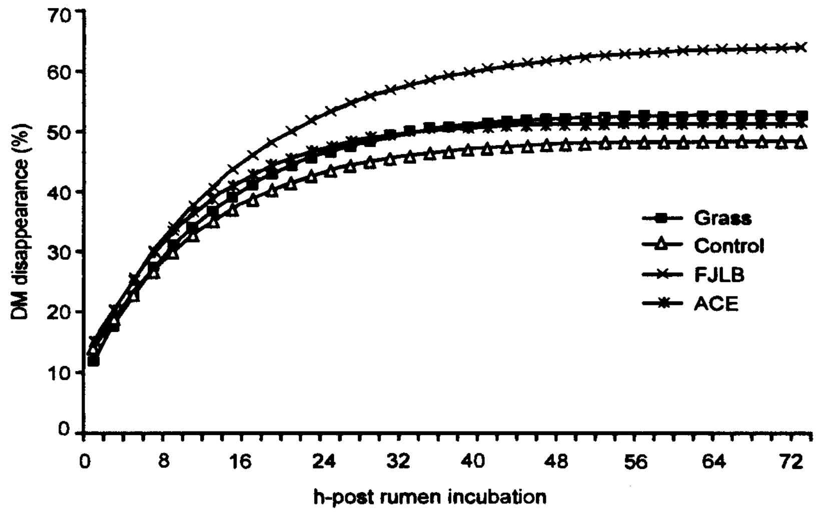

Fig. 1: *In situ* DM disappearances of Tropical Elephant grass and silages at various h-post rumen incubation

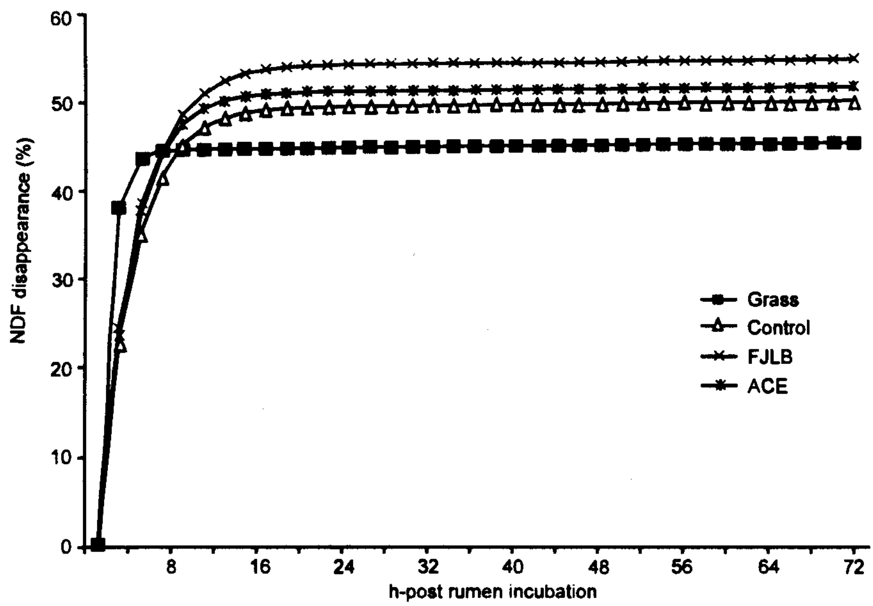

Fig. 2: *In situ* NDF disappearances of Tropical Elephant grass and silages at various h-post rumen incubation

ensiled material fresh matter resulting from the increased number of LAB in the FJLB additives bringing the pH value of the silages beyond the critical pH level which is not conducive for the development of clostridia which are associated with bad fermentation.

The silages from treatment of epiphytic lactic acid bacteria had lowered ($P<0.01$) pH values and increased ($P<0.01$) lactic acid compared to the Control. The treatment also improved the nutritive values of the silages by decreasing the levels of ammonia and ethanol contents, accounting a V-score value of 94 % compared to 76% and 68 % respectively, in Control and ACE treatments (Butler and Bailey, 1973 and Yahaya *et al.*, 2003a). The assessment of the overall fermentation quality of the three treatments showed a clostridial type of fermentation with present of propionic, iso-butyric and n-butyric acids in silages, even at small concentration in the Control and ACE compared to FJLB silage. This is strongly assumed to be associated with lack of FJLB additive which resulted in the lower numbers of epiphytic LAB in the Control and ACE silages, a part from higher fiber and low sugar contents in the original fresh ensiled tropical Elephant grass (McDonald *et al.*, 1991 and Yahaya *et al.*, 2001b).

The Rate and Extent of in Situ Rumen Degradation of Elephant Grass and Silages: The estimate of the *in situ* DM and NDF degradability and their soluble fractions of (a), (b) and (c) are shown in Figures 1 and 2 and Table 3 respectively.

The digestibility of DM and NDF were higher ($P<0.05$) in the FJLB silage than in the Control or ACE silages. This is because for all incubation times the rate at which (b) was degraded was considerably higher for the FJLB silage [(c) = 0.091 for DM and (c) = 0.34 for NDF] than for the Control [(c) = 0.06 for DM and (c) = 0.28 for NDF] and the ACE silage [(c) = 0.08 for DM and (c) = 0.31 for NDF]. Similarly, the total or cumulative rate (a+b) of DM (65.91%) and NDF (53.78%) digestibility was higher in the FJLB silage, but, that between ACE and Control treatments were not significantly different. The high values of the potential digestible and degradable fractions (a+b) for DM and NDF in the FJLB silage could be attributed to its differences in the chemical composition such as lower contents of ethanol, lower DM and hemicellulose losses, absence of propionic, butyric acids but appreciable amount of lactic acid and CP contents. This silage provides more nutrients to rumen microbes due to it high potential degradable and digestible fractions (a+b) than the other treatments (Table 3). Piero *et al.*, (1999) reported similar differences of immediately soluble fractions (a) of DM, starch and total degradable fractions of DM, N and starch obtained from two models equations.

The results from ACE silage indicated a higher potential digestible fraction during early stage of incubation compared to fresh material Elephant grass but, quickly decreased after 24h of incubation. Since tropical forages have appreciable acetate content, it may be assumed that the additive of ACE at ensiling did not improve the fermentation of Elephant grass, *in situ* rumen degradability as well as amount of ADF content of silage. Therefore it is paramount to ensure a proper selection of additives as well as the ensiling method is correct to avoid excessive loss of nutrients in the silo during making of Elephant grass silage.

Conclusion

The results in this study revealed that FJLB treatment increased lactate concentration and decreased losses of nutrients in silages as well as gave highest potential digestible and degradable fractions and hence had high nutritive value. In contrast Control treatment gave higher DM, hemicellulose losses and higher ethanol and VBN levels in silages.

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