Evaluation of Fermentation Quality of a Tropical and Temperate Forage Crops Ensiled with Additives of Fermented Juice of Epiphytic Lactic Acid Bacteria (FJLB)

M. S. Yahaya*, M. Goto, W. Yimiti, B. Smerjai[1] and Y. Kawamoto[1]

Faculty of Bioresources, Mie University, 1515 Kamihama-cho, Tsu city 514-8507, Japan

ABSTRACT : This study aimed to examine the fermentation quality of a tropical Elephant grass (*Pennisetum purpuereum*) and temperate Italian ryegrass (*Lolium multiflorum*) forages ensiled additive of fermented juice of epiphytic lactic acid bacteria (LAB) and to determine what factor affects the fermentation characteristics of the crops. In both species cell walls neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents in silages were consistently decreased ($p<0.05$) with the addition of FJLB at ensiling more then Control treatment. The FJLB additive increased number of LAB (cfu) and lactate concentration in the silages in both species. The Control treatment without additive underwent a clostridial type of fermentation with traces of propionic, iso-butyric, n-butyric acids contents with higher ($p<0.01$) levels of volatile basic nitrogen (VBN %TN) and had appreciable decreased of nutrient in silages. FJLB treatment improved silage nutritive value with little contents of VBN %TN, ethanol and very small amount of dry matter (DM) and hemicellulose losses ($p<0.05$) between 2 to 5% and 7 to 3% respectively, in Elephant grass and Italian ryegrass species. The results in this study indicates that while among the factors affecting silage fermentation butyric type of fermentation was more pronounced in tropical elephant grass compared to the temperate Italian ryegrass, FJLB additive revealed a better silage fermentation products in both species. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 7 : 942-946)***

Key Words : Tropical and Temperate Forage Crops, Fjlb Additive, Silage Fermentation

INTRODUCTION

There are many conflicting reports on factors that lead to development of lactic acid bacteria during ensiling process. While previous studies indicate that higher population of epiphytic lactic acid bacteria on aerial parts of forages influences its good fermentation quality than amount of its sugar content, leading to the wide use of commercial LAB additives (Micheal, 1984). However, recent reports indicate that sugar and dry matter contents of crops are principally important factors for the development of lactic acid bacteria (LAB) during ensilage (Jones et al., 1992; Yahaya et al., 2002a). Although most of the tropical forage crops have high dry matter yields potential, they are characterized with rapid deterioration of protein, lower sugar contents and high buffering capacity (Yahaya et al., 1999). Furthermore the population of the indigenous epiphytic microorganism particularly LAB essential for good fermentation is affected by higher tropical weather condition (Gibson et al., 1958; 1961).

Although temperate species have high contents of water-soluble sugars (Yahaya et al., 2002b) and lower buffering capacity compared to tropical grasses, studies with temperate lucerne crop indicate that the use of epiphytic LAB improved fermentation quality of silage more than that of commercial additives of LAB inocula (Ohshima et al., 1997a,b; Li-man et al., 2002; Yahaya et al., 2003a). Information on the utilization of epiphytic lactic acid bacteria (LAB) as additives during fermentation of tropical forages is scarce relative to that available for temperate forage crops.

This study aimed to evaluate the fermentation quality of a tropical Elephant grass and a temperate Italian ryegrass ensiled with additives of fermented juice of epiphytic lactic acid bacteria (FJLB) and to determine the factors that affect the fermentation characteristics of the crops.

MATERIAL AND METHODS

Preparation of fermented juice of epiphytic lactic acid bacteria (FJLB)

The tropical forage of Elephant grass (*Penisetum purpureum*) and a temperate crop of Italian ryegrass (*Lolium multiflorum*) was cut at fifth growth stage from Ryukyus University Experimental Research Farm, Okinawa and at first growth stage from Mie Science and Technology Promotion Centre Farm, Mie prefecture, Japan, respectively. To prepare the FJLB, the fresh matter of each species harvested was immediately cut into 2 to 3 cm lengths using a mechanical forage cutter. The entire lots from each species were thoroughly mixed and about 200 g fresh matter was immediately macerated with a liter (1,000 ml) of sterilized distilled water. This was collected through sterilized double cheesecloth and juice transferred into a

* Corresponding Author: M. S. Yahaya. Tel: +81-059-231-9494, Fax: +81-059-231-9494, E-mail: saniyahya@hotmail.com

[1] Ryukyu University, Nishihara-cho 2903-0213, Okinawa, Japan.

Received July 31, 2003; Accepted April 1, 2004

Table 1. Chemical composition (%) of fresh material tropical and temperate forage crops

	Fresh material tropical elephant grass	Fresh material temperate Italian ryegrass
pH	6.22	6.10
Dry matter	24.6	22.1
	%DM	
Crude protein	7.1	9.3
Neutral detergent fiber	60.8	54.8
Acid detergent fiber	43.6	35.1
Acid detergent lignin	11.8	6.6
Water soluble carbohydrates	2.7	3.1
Hemicellulose	16.2	19.7
Cellulose	31.8	28.5
Buffering capacity M.eq.DM	266.75	252.2
Epiphytic lactic acid bacteria (LAB. cfu/gFW)	3.12×10^5	4.2×10^5

bottle of one liter capacity. About 5 and 2% (w/v) sugar (glucose) was added into the extract solutions and incubated for 2 days at 30°C. respectively. for Elephant grass and Italian ryegrass. 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 during ensilage. 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-$CaCo_3$ agarplate (Masuko et al., 1992).

Silage preparation

The fresh matter from Elephant grass and Italian ryegrass representing 24.6 and 22.1% DM. respectively. was cut into 2-3 cm length. The entire lot from each species was thoroughly mixed and a representative samples obtained. About 500 g fresh matter of Elephant grass and Italian ryegrass were ensiled in three silos of 1.000 ml capacity with the two treatments of T1 (Control i.e., without any additive) and T2 with the additives of 5 and 3% (w/v) of FJLB, respectively. Six silos from the two species (in total. 12 silos) were opened after 40 days and weighed to determine the extent of DM and structural carbohydrate loss. A representative samples from each silo was mixed. and frozen at -15°C for chemical analysis. Silage extracts was prepared immediately by macerating a 50 g silage samples with a 300ml of distilled water. These was collected through double cheesecloth and used to determine pH value and concentrations of volatile fatty acids and volatile basic nitrogen and ethanol.

Chemical analyses

Dry matter (DM) contents of fresh materials and resultant silage from the two species were determined by freeze drying. Crude protein (CP) content was determined as described previously (Yahaya et al., 2002a). 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 volatile fatty acids (i.e., formate, lactate, acetate, propionate, n- and iso-butyrate. n- and iso-valerate) in the silage extracts were determined by HPLC equipment (Jasco co. Tokyo Japan) using an ion exchange column (Shimadzu SCR-102 (H). 12 mm ID×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 as described previously (Conway and O' Malley, 1942). The buffering capacity of the two species was determined according to the methods described by Plane and McDonald (1966).

Statistical analysis

Silage fermentation data were analyzed using ANOVA in a randomized block design. while means differences between treatments determined using multiple range test procedures (Duncan. 1955; Snedecor and Cochran. 1980).

RESULTS AND DISCUSSION

Characteristics of cell wall contents, buffering capacity and epiphytic lactic acid bacteria of fresh material forage crops

Chemical composition and number of epiphytic lactic acid bacteria (LAB) prior to ensiling of the tropical Elephant grass and temperate Italian ryegrass are presented in (Table 1). The higher dry matter (DM). neutral detergent fiber (NDF), acid detergent fiber (ADF). acid detergent lignin (ADL) and cellulose contents observed in fresh Elephant grass compared to Italian ryegrass could be due to differences in species and partly due to ecological factor. In contrast water-soluble carbohydrate (WSC). crude protein (CP) and hemicellulose contents of elephant grass appeared lower compared to Italian ryegrass. Most tropical forages

Table 2. Fermentation quality of a tropical and temperate forage crops silages

	Elephant grass		SE	Italian ryegrass		SE
	Control	FJLB		Control	FJLB	
pH	5.45^a	4.33^b	0.1	3.86^a	3.77^b	0.01
Oragnic fatty acids (%DM)						
Lactic acid	0.9^b	3.5^a	0.5	1.9^b	2.4^a	0.1
Acetic acid	0.7	0.7	0.2	0.2	0.3	0.1
Propionic acid	0.3	-	-	-	-	-
Iso-Butyric	0.1	-	-	-	-	-
N-Butyric acid	0.1	-		<0.1	-	-
Total VFA	2.1^b	4.2^a	0.3	2.2^b	2.8^a	0.2
VBN % TN	12.7^a	6.0^b	0.3	12.1^a	4.1^b	0.2
Ethanol (%DM)	2.5^a	1.6^b	0.1	nd	nd	nd
V score	68.2^b	94.4^a	5.4	nd	nd	nd

Means followed by different letters ($^{a, b, c}$ and ^{d}) differ ($p<0.05$).
Each values indicates mean of three silos. FJLB: Fermented juince of lactic acid bacteria. SE: Standard error. nd: not determined.

Table 3. Chemical composition (%) of tropical and temperate forage crops silages

	Elephant grass silages		SEM	Italian ryegrass silages		SEM
	Control	FJLB		Control	FJLB	
Dry matter (DM)	22.9^b	23.2^a	0.1	22.1^a	20.9^b	0.1
	----------------%DM-----------			------------------%DM-------------		
Crude protein	9.6^b	10.4^a	0.1	10.7	11.0	0.2
Neutral detergent fiber	53.8^a	51.7^b	0.1	55.1	55.0	0.2
Acid detergent fiber	39.7^a	37.1^b	0.1	35.9^a	34.8^b	0.3
Acid detergent lignin	10.1^a	8.8^b	0.3	6.7^a	5.8^b	0.2
Hemicellulose	13.1^b	14.6^a	0.1	19.3^b	20.1^a	0.3
Cellulose	29.6^a	28.3^b	0.3	29.2	29.0	0.3

Means followed by different letters (^{a} and ^{b}) differ ($p<0.05$). Each Values indicates mean of three silos except for fresh italian ryegrass.
Elephant grass: tropical forage crop. Italian ryegrass: temperate forage crop. SEM: Standard erorr of mean.

especially grasses contain less nitrogen but more cell wall constituents (De-Visser. 1998; Yahaya et al., 1999). 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; 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 Elephant grass showed lower number of epiphytic lactic acid bacteria (LAB) of 3.6×10^5 compared to 4.2×10^5 cfu/g FW in temperate Italian ryegrass. This is because lactic acid bacteria prefer moderate warm weather, mainly overcast with relatively high humidity, calm with low evaporative tendency (Weise and Wernke, 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) and proteins (McDonald et al., 1991) and rapid lignifications of cell walls constituents. These negatives characteristics have been observed to exert some resistant in lowering the pH values of the crop during fermentation (Yahaya et al., 2003a).

Fermentation quality of the two forages during ensiling

The fermentation characteristic of the tropical Elephant grass and temperate Italian ryegrass silages are presented in Table 2. In both species the treatment of epiphytic lactic acid bacteria lowered ($p<0.01$) the 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 marks in Elephant grass silage (Butler and Bailey, 1973; Yahaya et al., 2003a). Fermentation of the Control treatments in both forages showed a clostridial type with detection of n-butyric acids in silages, even at small concentration. But from the assessment of the overall fermentation quality of the two forages the Italian ryegrass silage revealed a better fermentation without traces of propionic, iso-butyric and n-butyric acids in silage compared to tropical elephant grass

Table 4. Dry matter and structural carbohydrates degradation of Elephant grass (*Pennisetum purpereum*) and Italian ryegrass (*Lolium multiflorum*) during ensiling

	Elephant grass		SE	Italian ryegrass		SE
	Control	FJLB		Control	FJLB	
Dry matter	3.8[a]	2.6[b]	0.3	7.3[a]	5.3[b]	0.6
Hemicellulose	13.1[a]	7.1[b]	1.1	12.4[a]	2.9[b]	1.2
Cellulose	5.1[a]	2.4[a]	0.3	6.6[a]	3.3[b]	1.0

Each value represent a mean of three silos. FJLB: 5% (w/v) for elephant grass and 3% (w/v) for Italian ryegrass.

silage. This is strongly assumed to be partly associated with differences in species, lower numbers of epiphytic LAB and nitrogen with higher fiber contents in the original fresh material ensiled tropical elephant grass (Table 1). One other reason for the better fermentation quality of the Control treatment in temperate Italian ryegrass silage could be due to its higher value of sugar contents. Among the temperate grasses the Italian ryegrass have been reported to have the higher soluble sugars contents one of the principal substrates for microorganism during ensilage (McDonald et al., 1991; Yahaya et al., 2001b).

Effects of treatments on dry matter and cell walls contents of the two forage crops silages

The chemical composition of the tropical Elephant grass and temperate Italian ryegrass silages are presented in Table 3. In all the silages evaluated the cell walls ADF and ADL were consistently 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 ($p<0.05$) cells walls but lower ($p<0.05$) hemicellulose in both species compared to the original ensiled fresh material 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 and probably leading to an increase in silage digestibility in the rumen (Table 3).

Dry matter loss and disappearance of structural carbohydrates hemicellulose and cellulose during ensiling

The Losses of dry matter and structural carbohydrate disappearance in the two forage crops are presented in Table 4. The DM losses in silages treated with FJLB additive were small and within the acceptable range of 2 to 5% reported by Yahaya et al. (2001a) with hemicellulose disappearance accounting to 7% in FJLB additive compared to higher values of 13% in Control treatment in Elephant grass. The same trend of lower ($p<0.05$) hemicellulose disappearance of 3% in FJLB treated silage was obtained compared to higher values of 12% in Control treatment in Italian grass. Control treatments consistently showed higher DM losses in both in species evaluated, the same was observed for cellulose losses appearing higher in the Control treatment due to lack of additives. The decreasing degradation observed in DM and hemicellulose contents in FJLB treated silages could be partially associated with the better fermentation of original 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 conducive for the development of clostridia which are associated with bad fermentation (Table 4).

CONCLUSION

The results in this study revealed that ensiling of Elephant grass and Italian grass with additive of FJLB treatment increased lactate concentration and decreased losses of nutrients in both silages. In contrast Control treatment gave higher DM, hemicellulose losses and higher ethanol and VBN levels in silages. More research is needed to determine the type of microroganism in the FJLB and mathematically quantify their effectiveness during fermentation and *in vivo* digestibility of tropical and temperate forage crops silages.

ACKNOWLEDGEMENTS

The authors are sincerely grateful to Japan Society for the Promotion of Science for providing the funds through the award of JSPS Postdoctoral Fellowship for Foreign Researchers Program and making it possible to undertake this research in Japan.

REFERENCES

Butler, G. W. and R. W. Bailey. 1973. Criteria for assessing the efficiency of fermentation process. In: (Ed. G. W. Butler and R. W. Bailey) Chemistry and Biochemistry of Herbage, 3:33-80.

Conway, E. J. and E. O' Malley. 1942. Microdiffusion method: ammonia and urea using buffer absorbents (revised methods for

ranges greater than 10 (g N) Biochemistry Journal, 36:655-661.
Czerkawski, J. W. 1986. Structural carbohydrates in forages, In: (Ed. J. W. Czerkawszki) An introduction to rumen studies, 1st edition, Pergamon press pp. 1-170.
Deriaz, R. E. 1961. Routine analysis of carbohydrates and lignin in herbage. J. Sci. food Agric. 12:153-160.
De-Visser, H., C. Klop, C. J. Van der Kolen and A. M. Vuuren. 1998. Starch Supplementation of grass harvested at two stages of maturity prior to ensiling. Intake, digestion and digestibility by dairy cows. J. Dairy Sci. 81:2221-2227.
Ducan, D. B. 1955. Multiple range test and multiple F test Biometrics 11:1-42.
Gibson, T., A. C. Sterling, R. M. Keddie and R. F. Rosenberger. 1958. Bacteriological changes in silages made at control temperatures, Journal of general microbiollogy, 19:112-129.
Gibson, T., A. C. Sterling, R. M. Keddie and R. F. Rosenberger. 1961. Bacteriological changes in silages as affected by laceration of the fresh grass, Journal of general applied bacteriology, 34:60-70.
Goering, H. K. and P. J. Van Soest. 1970. Forage Fiber Analysis Apparatus, Reagents, Procedures and some Applications, Handbook, Vol. 379 ARS-USD, Washington, DC.
Jones, B. A., L. D. Satter and R. E. Muck. 1992. Influences of bacterial inoculant and substrate addition to lucerne ensiled at different dry matter contents. Grass and Forage Science, 47:19-27.
Li-man, C., M. Goto and M. Ohshima. 2002. Variation in the fermentation characteristics of alfalfa silage of different harvest time as treated with fermented juice of epiphytic lactic acid bacteria, J. Grassland Sci. (Japan) 47:583-587.
McDonald, P., A. R. Henderson and S. J. E. Heron. 1991. Principles of ensiling. In: (P. McDonald, A. R. Henderson and S. J. E. Heron) Biochemistry of silage, 2rd edition Chalcombe Publication, Lincol, UK, pp. 9-40.
Matsuko, T., S. Okada, T. Uchimura and K. Awaya. 1992. Effects of inoculation with lactic acid bacterial calture at ensiling on the fermentative quality and flora of lactic acid bacteria of grass silage, J. Grassland Sci. (Japan) 63:1182-1187.
Matsuko, T., H. Yoji, T. Yuki, C. Li-man, M. Goto and M. Ohshima. 2002. Effect of addition of fermented juice of epiphytic lactic acid bacteria prepared from timothy and orchardgrass on fermentation quality of silages, J. Grassland Sci. (Japan), 48:120-125.
Micheal, K. W. 1984. Ensiling process. In: (Ed. I. L. Allen, I. M. Rechard) Silage fermentation Marcel Dekker, New York, pp. 1-22.
Ohshima, M., E. Kimura and H. Yokota. 1997a. A methods of making good quality silage from direct cut alfalfa by spraying previously fermented juices, Anim. Feed Sci. Technolo. 66:129-137.
Ohshima, M., Cao L. M. Yokota, E. Kimura, Y. Ohshima and H. Yokota. 1997b. Influence of addition of fermented green juice to alfalfa ensiled at different moisture contents, J. Grassland Sci. (Japan), 43:56-68.
Plane, M. J. and P. McDonald. 1966. The buffering constituents of herbage and of silage, J. Sci. Food and Agric. 17:264-268.
Snedecor, G. W. and M. G. Cochran. 1980. Data classification and analysis of variance. In: (G. W. Snedecor and M. G. Cochran), statistical methods 7th edition Iowa State University press pp. 255-333.
Stephen, J. W. and J. N. Micheal. 1960. The ensiling process and action of microorganism In: (J. W. Stephen and J. N. Micheal) The conservation of grass and forage crop Oliver and Boyd Edinburgh London pp. 215-225.
Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition, J. Dairy Sci. 74:3583-3597.
Weise, F. and M. Wermke. 1973. The effect of weather on epiphytic micro-organism of smooth stalked meadow fescue (Poa proteanese L.). Doe. Wirtshafseigene Futter, 19:22.
Yahaya, M. S, J. Takahashi, S. Matsuoka and A. Kibon. 1999. Effect of supplementary feeding of cotton seed cake on feed intake, water consumption and work output of work bulls in Borno state Nigeria Anim. Feed Sci. Technol. 79:137-143.
Yahaya, M. S., A. Kimura, J. Harai, H. V. Nguyen, M. Kawai, J. Takahashi and S. Matsuoka. 2001a. Effect of length of ensiling on silo degradation and digestibility of structural carbohydrates of lucerne and orchardgrass. Anim. Feed Sci. Technol. 92:141-148.
Yahaya, M. S., A. Kiimura, J. Harai, M. Kawai, J. Takahashi and S. Matsuoka. 2001b. Evaluation of structural carbohydrates losses and digestibility in alfalfa and orchardgrass during ensiling Asian-Aust. J. Anim. Sci. 14(12):1701-1704.
Yahaya, M. S., M. Kawai, J. Takahashi and S. Matsuoka. 2002a. The effects of different moisture content at ensiling on silo degradation and digestibility of structural carbohydrates of orchardgrass, Anim. Feed Sci. Technol. 101:127-133.
Yahaya, M. S., M. Kawai, J. Takahashi and S. Matsuoka. 2002b. The effects of different moisture and ensiling time on silo degradation of structural carbohydrates of orchardgrass, Asian-Aust. J. Anim. Sci. 15(2):213-217.
Yahaya, M. S., H. V. Nguyen, M. Kawai, J. Takahashi and S. Matsuoka. 2002c. The effects of growth stage at ensiling on degradation of structural carbohydrates of lucerne, Malaysian J. Anim. Sci. (MJAS) 7(2):49-54.
Yahaya, M. S., Goto M., W. Yimiti, S. Karita, B. Smerjai and Y. Kawamoto. 2003a. Fermentation characteristics of two tropical forage crops ensiled with the additives of fermented juice of epiphytic lactic acid bacteria and commercial acetic acid powder (submitted to J. Grass and Forage Science British Grassland Society and European Grassland Federation).
Yahaya, M. S., M. Kawai, J. Takahashi and S. Matsuoka. 2003b Effect of prolonging the time of filling into silo on silo degradation and digestibility of structural carbohydrate of orchardgrass (JAVA) J. Anim. Vet. Adv. 2:23-29.