Effects of Amino Acids Fermentation By-product on Fermentation Quality and *In situ* Rumen Degradability of Italian Ryegrass (*Lolium multiflorum*) Silage

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ABSTRACT : The experiment of silage for preservation of fresh Italian ryegrass (*Lolium multiflorum*) was carried out to examine whether the fermentation quality and microbial degradation in the rumen can be altered by the treatment of amino acids fermentation byproduct (AFB). The plant was ensiled for 40 days with 4 treatments of different ratios of AFB and sugarcane molasses (SCM) mixture. The treatment 2 (T2, AFB:SCM=100:0) and treatment 3 (T3, AFB:SCM=40:60) silages showed higher ($p<0.05$) concentrations of lactic acids, lower ($p<0.05$) pH and dry matter (DM) losses than the Control (T1, none additive) and treatment (T4, AFB:SCM=0:100) silages. The treatments 2 and 3 contained higher ($p<0.05$) DM and crude protein contents in silages compared to treatments 1 and 4 silages. The NDF, ADF and cellulose contents were also lower ($p<0.05$) in T2, T3 and T4 silages than T1 silage and fresh material before ensiled. The *in situ* rumen DM, NDF, ADF, hemicellulose and cellulose degradability was also higher ($p<0.05$) in T2, T3 and T4 silages than T1 silage, while the highest improvement was achieved with addition of AFB:SCM at level of 40:60 at ensiling. The result in this study indicates that the addition of AFB and SCM additives improved the silage fermentation and cell wall degradability of Italian ryegrass silage. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 5 : 633-637)***

Key Words : Amino Acids Fermentation Byproducts, Cell Wall Composition, Italian Ryegrass, Rumen Degradability, Silage Fermentation

INTRODUCTION

Amino acids fermentation byproducts (AFB) is liquid type of nitrogenous compound produced from the purification and processing of amino acids substances in the food and feed industries. Unlike sugarcane molasses (SCM), the AFB are low energy material but effectively used for agricultural and aquaculture fertilizers for upgrading soil fertility and growing plant planktons of shrimp feeds. It also could serve as nitrogen sources for rumen microbes by livestock industry, as shown by the evaluation of TDN 24.7% and total nitrogen 7.3% on the fresh matter basis (Agriculture, Forestry and Fisheries Research Council Secretariat, MAFF, 1999).

Since the AFB has off-flavor and higher concentrations of ammonium sulfate and ammonium chloride, ensiling AFB as an additive might reduce its flavor and improve the cell wall digestibility of forages, without losing high palatability and balanced supply of energy and nitrogenous sources, like a ammonia treatment. Also treatment of AFB could be effective on silage fermentation because it contains certain amounts of soluble carbohydrate residues for microbial fermentation, although ammonia and other chemical treatments are generally targeted to dried feedstuffs such as rice, oat and barley straw (Ørskov et al., 1983). Very limited information is, however, available on effects of treatments of AFB on fermentation quality and chemical composition and rumen degradability of silages.

In this study, laboratory scale experiment of ensiling Italian ryegrass (*Lolium multiflorum*) was carried out to examine whether the fermentation quality and microbial degradation in the rumen can be altered by the treatment of AFB liquid.

MATERIALS AND METHODS

Silage preparation

The first growth of Italian ryegrass was harvested at heading stage in June in Mie Science and Technology Promotion Center (Ureshino-cho, Japan). The crop was cut into 2 to 3 cm lengths using a hand cutter and the entire lots thoroughly mixed and ensiled with different levels of AFB and SCM mixtures, while the AFB liquid was diluted to a concentration of 70% by distilled water. A 500 g fresh matter of Italian ryegrass was ensiled into bottle silos of 1 litre capacity with four treatments of T1=Control (none additive), T2=AFB:SCM (100:0), T3=AFB:SCM (40:60) and T4=AFB:SCM (0:100). Treatments were run in triplicate.

At 40 days after ensiling, 3 silos from each treatment (total 12 silos) were opened and weighed. Three representative samples of each treatment were thoroughly mixed, freeze dried and finely ground to pass through a 2.5 mm sieve and kept in a plastic bottle at room temperature for chemical analysis and determination of *in situ* rumen

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Table 1. Chemical composition and physicochmical characteristic of Italian ryegrass, amino acids fermentation byproducts and sugarcane molasses

	Italian ryegrass	Amino acids fermentation byproducts	Sugarcane molasses
Moisture (%)	78.0	39.5	27.3
Chemical composition (%DM)			
Crude protein (CP)	9.3	40.4	3.2
Neutral detergent fiber (NDF)	54.8	ND	ND
Acid detergent fiber (ADF)	35.1	ND	ND
Acid detergent lignin (ADL)	6.6	ND	ND
Hemicellulose	19.7	ND	ND
Cellulose	28.5	ND	ND
Reducing sugars	3.3	1.9	9.2
Physicochemical characteristics			
pH	6.1	4.4	4.5
Buffering capacity (meq·kg^{-1} DM)	252.2	308.4	98.8
Specific gravity (g/ml)	ND	1.3	1.5
Viscosity (pa s)	ND	0.1	0.4

ND: not determined.

Table 2. Effects of amino acids fermentation byproducts on dry matter and fermentation quality of Italian ryegrass silages

	pH	VBN	Organic acids (% DM)						DM	Ethanol %DM
		(% TN)	LAC	ACE	FOR	PRO	N-BU	Total	loss (%)	
T1=Control	3.86^{a}	2.1^{c}	16.9^{c}	1.2^{d}	0.2	-	0.4	18.7^{c}	7.3^{a}	3.26^{a}
T2=AFB:SCM (100:0)	3.80^{b}	8.8^{a}	23.1^{a}	1.5^{b}	-	0.2	-	24.8^{a}	5.3^{b}	2.23^{bc}
T3=AFB:SCM (40:60)	3.72^{c}	4.3^{b}	18.4^{b}	1.3^{c}	-	-	-	19.7^{b}	4.8^{b}	1.57^{c}
T4=AFB:SCM (0:100)	3.68^{c}	1.2^{d}	16.9^{c}	1.6^{a}	-	-	-	18.5^{c}	7.6^{a}	2.83^{b}
SEM	0.02	0.02	0.1	0.01	-	-	-	0.1	0.4	0.04

Means (n=3) followed by different letters ($^{a, b, c, d}$) significantly differ ($p<0.05$).
AFB: amino acids fermentation byproducts. SCM: sugarcane molasses. SEM: standard error of mean.
VBN: volatile basic nitrogen. LAC: lactic acid. ADE: acetic acid. FOR: formic acid. PRO: propionic acid. N-BU: n-butyric acid.

degradability. Silage extracts were prepared immediately by macerating 50 g silage sample with 300 ml of distilled water. This was collected through a double layer of cheesecloth and, used to determine pH and concentrations of volatile fatty acids and volatile basic nitrogen (VBN).

Chemical compositions and fermentation characteristics

Dry matter contents of fresh Italian ryegrass and resultant silages from 4 treatments was determined by freeze drying for a minimum of 24 h. Contents of NDF and ADF were determined according to the methods of Van Soest et al. (1991) without using sodium sulfite and amylase. Acid detergent lignin (ADL) was determined using 72% H_2SO_4 solution as modified by Van Soest et al. (1991). Crude protein (CP) content was determined as described by procedures 954.01 and 954.02 respectively. (AOAC, 1990). Concentrations of volatile fatty acids (i.e., formate, lactate, acetate, propionate, iso-butyrate, n-butyrate and iso-valerate) in silage extracts were determined by using HPLC equipment (Jasco Co. Tokyo, Japan) attached with an ion exchange column (Shimadzu SCR-102 (H), 12 mm ID×30 cm, Shimadzu Co., Japan). The pH of silage extracts was determined with a glass electrode while ammonia as a % total N was analyzed as described previously by the method of Conway and O'Malley (1942) and Goto et al. (2003a). Buffering capacity and the soluble sugar were determined according to the method described by Plane and McDonald (1966) and Deriaz (1961), respectively. Viscosity of AFB and SCM was measured using a standard viscosity cup.

Determination of chemical composition and rumen degradability

In situ rumen degradability of DM and NDF, ADF, hemicellulose and cellulose was assessed by the nylon bag (42 μm pore size) technique (Ørskov et al., 1980). A triplicate of 5 g from each treatment was weighed into a nylon bag (7×12 cm). The weight of the bags plus the sample was recorded, fixed into four plastics tubes and finally incubated into the rumen of a fistulated dry cow for 24 h. The cow was maintained on 4 kg of chopped Italian ryegrass hay and 300 g of concentrate meal (60% maize grain, 36% soybean meal and 4% minerals and vitamins on DM basis). After 24 h incubation, nylon bags were taken and immediately hand-washed under tap running water until water running out of the bags was clear. The bags containing the indigestible residues were dried in the oven at 55°C for 48 h and re-weighed. The DM and cell walls degradabilities were determined from the DM loss and after the chemical analysis, respectively.

Statistical analyses

Data of silage fermentation, chemical compositions and,

in situ degradability were analyzed using ANOVA in a randomized block design, with mean differences determined using a multiple range test (Snedecor and Cochran. 1980; Goto et al., 2003b).

RESULTS AND DISCUSSION

Chemical composition

The first growth of Italian ryegrass used in this study has a moisture content (78.0%) of suitable range for ensiling as well as high soluble sugars (3.3%) that could lead to its good fermentation quality (Table 1). The crop was less fibrous (NDF; 54.8%) as compared to the general observations of cell walls constituents arising from many studies (Dunny, 1993). While both AFB and SCM additives were characterized of low moisture contents (respectively, 39.5 and 27.3%) and highly soluble substances. AFB had higher nitrogenous compounds (40.4%) such as ammonium sulfate and ammonium chloride but SCM had more reducing sugars (9.2%) than nitrogenous compounds (3.2%).

Effects of treatments on fermentation characteristic

In general. Italian ryegrass silages with better fermentation quality were observed to be predominantly colonized with superior silage lactic acid bacteria such as *Lactococcus plantarum* (*L. plantarum*) and *Lactococcus fermentum (L. fermentum*), showing lower DM loss and VBN value and higher concentration of lactic acid (Micheal. 1984). In this study, silages with additives of AFB and SCM had lower ($p<0.05$) pH value compared to the T1 (Control) silage. which showed a low pH value of 3.86 but some traces of formic and n-butyric acids with lower total concentrations of lactic acid and VFA (Table 2). Lactic acid was the major product throughout all silages. but was higher ($p<0.05$) in the AFB-treated (T2 and T3) silages. Acetic acid was less variable among treatments although it was significant ($p<0.05$). Total concentrations of lactic acid and VFA were also higher ($p<0.05$) in the T2 silage and followed by the T3 silage, while T1 and T4 were the lowest ($p<0.05$). Therefore. the T2 and T3 silages appeared to be more acceptable for the feeding guidelines as reported by Chamberlain and Wilkinson (1996) and Yahaya et al. (2002) as compared to T1 and T4 silages.

The concentration of VBN was also the highest ($p<0.05$) with the T2 silage and followed by T3 silage, being resulted from higher concentrations of nitrogenous compounds. mainly ammonium nitrogen in AFB liquid (Table 1). Also DM loss after the ensiling was higher ($p<0.05$) with the T1 and T4 silages than T2 and T3 for silages. being 7.3-7.6% with T1 and T4 silages and 4.8-5.3% with T2 and T3 silages. The latter two silages were thus within acceptable ranges of 4-5%. DM loss previously reported by McDonald et al. (1991) and Yahaya et al. (2001). Therefore. the AFB additive was more effective for further improvement of fermentation quality of Italian ryegrass silage. which showed good quality even without any additive and wilting. This was consistent with previous result which showed that the AFB additive enhanced the pure culture growths of *Pediococcus acidilactici*. *Leuconostoc mesenteroides*, *L. plantarum* and *L. fermentum* and also the fermentation qualities of barley whole crop silage, although it was not known of which nutrients in the AFB liquid can be utilized by those lactic acids bacteria (Yimiti et al.. 2003).

Effects of treatments on chemical composition and in

Table 3. Effects of amino acids fermentation byproducts on the chemical composition of Italian ryegrass silages

	DM (%)	Chemical composition (%)					
		CP	NDF	ADF	ADL	Hemicellulose	Cellulose
T1=Control	21.0^{c}	10.7^{c}	55.1^{a}	35.9^{a}	6.7^{a}	19.2	29.2^{a}
T2=AFB:SCM (100:0)	22.6^{a}	16.4^{a}	52.2^{b}	33.1^{b}	6.2^{b}	19.1	26.9^{b}
T3=AFB:SCM (40:60)	22.5^{a}	11.9^{b}	51.0^{b}	32.1^{b}	6.3^{b}	18.9	25.8^{b}
T4=AFB:SCM (0:100)	22.0^{b}	9.5^{d}	51.4^{b}	32.7^{b}	6.3^{b}	18.7	26.4^{b}
SEM	0.1	0.2	0.3	0.4	0.2	0.5	0.5

Means (n=3) followed by different letters (a, b, c, d) significantly differ ($p<0.05$).
AFB, SCM, CP, NDF, ADF, ADL and SEM were referred to Tables 1 and 2.

Table 4. Effects of amino acids fermentation byproducts on the *in situ* rumen degradability of Italian ryegrass silages

	In situ rumen degradability (%)				
	DM	NDF	ADF	Hemicellulose	Cellulose
Original grass	37.8^{d}	17.7^{d}	26.4^{c}	21.3^{c}	12.2^{d}
Silages					
T1=Control	43.1^{c}	23.4^{c}	30.3^{b}	25.4^{b}	12.9^{d}
T2=AFB:SCM (100:0)	45.6^{b}	28.7^{b}	33.3^{a}	30.1^{a}	18.7^{c}
T3=AFB:SCM (40:60)	48.5^{a}	32.4^{a}	33.0^{a}	31.8^{a}	29.3^{a}
T4=AFB:SCM (0:100)	44.9^{b}	25.9^{bc}	27.8^{c}	22.8^{c}	22.4^{b}
SEM	0.5	1.0	0.7	1.7	1.2

Means (n=3) followed by different letters (^{a,b,c,d}) significantly differ ($p<0.05$).
AFB, SCM, NDF, ADF, and SEM were referred to Tables 1 and 2.

situ degradability

Treatments of T2 and T3 at ensiling increased ($p<0.05$) DM and CP contents in Italian ryegrass silages by 22.6-22.5% and 16.4-11.9%, respectively, compared to those of treatments of T1 (21.0 and 10.7%) and T4 (22.0 and 9.5%) silages (Table 3). Similarly, NDF, ADF and cellulose contents were lower ($p<0.05$) with the T2, T3 and T4 silages compared to T1 silage and fresh material before ensiled.

Different levels of AFB to SCM liquid at ensiling increased ($p<0.05$) *in situ* rumen degradability of DM, NDF, ADF, hemicellulose and cellulose of the silages compared to the T1 silage without additive and fresh material of Italian ryegrass (Table 4). Especially among all silages treated with additives, the T3 silage showed the highest ($p<0.05$) degradability of DM and cell wall constituents. The fact might be explained as a phenomenon of a slow rate of the breakdown at *in situ* rumen incubation of highly fibrous materials such as T1 silage, since T1 silage had higher cell wall constituents than the others. In contrast, lower NDF, ADF and ADL contents and their higher degradabilities observed in the treated silages can be explained by acidic hydrolysis of cell walls by organic acids produced during the ensiling. That would be supported by previous result, which showed that the *in vivo* DM and cell wall digestibility of alfalfa (*Medicago sativa* L.) was increased with the improvement of lactic acid fermentation using the inoculum of epiphytic lactic acid bacteria (Masuko et al., 1992; Cao et al., 2002). It was also probable that inorganic acids in the AFB additive, such as sulfuric acid and hydrogen chloride might directly affect acidic hydrolysis of the cell walls. However, ammonia that can be released from the AFB additive was not known of whether it worked on the breakdown of cell wall structures of Italian ryegrass under acidic conditions (Goto et al., 1993). Thus, the nutritive values of silages can be improved with addition of the AFB. Further research is needed to elucidate mechanisms of the improvement of the fermentation quality and rumen degradability of the silages as treated with AFB additive. It is also interesting to clarify responses of the improvement of those nutritive values with levels of AFB additive.

CONCLUSION

The results in this study showed that addition of AFB additive enhanced more lactic acid fermentation as well as improved CP contents in Italian ryegrass silage. It was also clear that AFB additive improved *in situ* rumen DM, NDF, ADF, hemicellulose and cellulose degradabilities.

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