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Full Length Research Paper

Effect of epiphytic lactic acid bacteria isolated from guinea grass on nutritional value of the silages

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An investigation was carried out to evaluate the effects of epiphytic lactic acid bacteria on the nutritional values of guinea grass silages. The four epiphytic lactic acid bacteria, namely, *Weissella confusa*, *Weissella paramesenteroides*, *Leuconostoc mesenteroides* ssp. *dextranicum*, and *Lactococcus lactis* ssp. *hordniae* were individually applied in inoculation of guinea grass silages at the rate of 1 × 10^5 CFU/g. Treatments were opened in three replications on days 14, 21 and 28. Value of pH, Proximate and Van Soest analysis and ammonia nitrogen were measured for each silage sample. Epiphytic lactic acid bacteria effectively increased crude protein (P<0.01), while acid detergent fiber (P<0.05), neutral detergent fiber, ammonia nitrogen and pH value (P<0.01) decreased significantly. It seems that increasing duration of silage making improved nutritional value of the silages (P<0.01).

Key words: Lactic acid bacteria, inoculation, nutritional value, guinea grass silage.

INTRODUCTION

Guinea grass is native to Africa, but now is widely distributed and grown in many countries of warm climate regions. Guinea grass is in category of perennial C_4 warm season grasses which provide the C_4 carbon fixation pathway and hence, high growth rates, high water and N efficiencies, and relatively low nutritive value (Barnes and Nelson, 2007). Silage is a fermented fodder with high moisture content that can be fed to animals as roughage source (McDonald, 2002). The production of high quality silage requires grasses or fodders that contain high water soluble carbohydrate, buffering capacity and high number of lactic acid bacteria, which is difficult to achieve in the tropical pastures (Bureenok et al., 2005). Lactic acid bacteria can be used as additives or starter cultures in the process of silage making to improve efficiency of the preservation procedure (Weinberg and Muck, 1996).

MATERIALS AND METHODS

Four epiphytic lactic acid bacteria isolated from guinea grass (*Panicum maximum*) comprised of *Weissella confusa, Weissella paramesenteroides, Leuconostoc mesenteroides* ssp. *dextranicum* and *Lactococcus lactis* ssp. *hordniae* were considered as the inoculants (Pasebani et al., 2010). Overnight pure culture of each isolates was prepared individually at the rate of 1 × 10^5 CFU/g fresh grass (McAllister, 1998; Rizk, 2005). MRS broth in amount of 120 ml was considered as a carrier for the inoculations thereby, controls without broth were proposed to evaluate the effect of carrier separately.

The guinea grass plot at Field 2, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia was harvested in August 2009 at the age of 42 days of re – growth and with height approximately 60 to 70 cm. The fresh guinea grass contained 7.6% crude protein (CP), 44.7% acid detergent fiber (ADF), 79.9% neutral detergent fiber (NDF) and 38.3% dry matter (DM). The cut grasses were chopped at 2 to 3 cm of length and ensiled in the PVC container with a capacity approximately 12 kg. Treatment groups for silage making namely, two controls (grass without any additive: Grass A, and grass with MRS broth but without any bacterial inoculants; Grass B, grass with both MRS broth and one of the bacterial inoculants (grass with *W. confusa*: Grass-WC; with *W. paramesenteroides*: Grass-WP, *L. mesenteroides* ssp. *dextranicum*: Grass- LM; *L.lactis* ssp. *Hordniae*: Grass-LL). Containers were opened in three replications on day 14, 21 and 28 of fermentation periods across the treatment groups. Silage samples were taken for determination of pH value, nutritional analysis, such as proximate analysis (AOAC, 2007), Van Soest analysis (Soet, 1994), and ammonia nitrogen (Byrne and McCormack, 1978). The general linear model procedure of the SAS (SAS® Institute, 2004) program

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Table 1. The pH of different type of guinea grass silages either with or without inoculants opened at day 14, 21 and 28 of ensiling.

Type of silages	Day 14	Day 21	Day 28
Grass-A	4.9^{ax}	4.6^{abx}	4.3^{bx}
Grass-B	4.8^{axy}	4.4^{bxyz}	4.2^{bxy}
Grass-WC	4.5^{ay}	4.3^{axyz}	4.2^{axy}
Grass-WP	4.1^{ay}	4.1^{az}	4.1^{ay}
Grass-LM	4.2^{ay}	4.2^{ayz}	4.2^{xy}
Grass-LL	4.6^{axy}	4.5^{axy}	4.2^{axy}

a, b, and c: Means in a row with no common superscripts is significantly different at p <0.01. x, y, and z: means in a column with no common superscripts is significantly different at p <0.01.

was used for statistical analyses, with means separated by the Duncan Multiple range test.

RESULTS

The standard plate counts of bacteria for the fresh guinea grass was represented of the amount of bacteria equal to 2.65×10^5 CFU /g while the epiphytic lactic acid bacteria were equal to 8.3×10^3 CFU/g by modification of 3 h activation time in MRS broth.

Table 1 shows the pH changes of different type of guinea grass silages prepared in this study either with or without inoculants at different period of ensiling process. Statistical analyses by using Duncan grouping shows by lowercase letters on the right side of the data(s).

Results shows that pH values in treatments inoculated with the epiphytic LAB were significantly decreased in comparison with the control ones (p<0.01). Increasing duration of silage making also were effective factor to decrease or maintenance of low pH value (p<0.01). A significant interaction between treatment and time was shown in the parameter, pH value (p<0.05). Table 2 shows the percentage of DM, CP, ADF, NDF and Ammonia (NH_3 – N) (mean ± se) of guinea grass silages in different treatments and times.

Results show that DM was not changed significantly by using the LAB inoculants as additives. Increasing the duration of ensiling in different treatments was changed the DM content significantly (P<0.05). The interaction between different treatments and different time of opening containers is also significant (P<0.05). CP was increased significantly (P<0.01) by using both bacterial inoculants and increasing duration of silage making. Significant interactions was between different treatments and times (P<0.01). ADF of the silages were decreased significantly (P<0.05) by using the bacterial inoculants and reduced significantly by increasing the duration of silage making (P<0.01). There was an interaction between treatment and time for this parameter (P<0.01). NDF was declined by the delay in opening of containers as well as using the bacterial inoculants (P<0.01). There was no interaction between treatment and time for this parameter. Ammonia was deducted by the effect of time and bacterial inoculants and the interaction between these two was also significant (P<0.01).

DISCUSSION

Acceptable pH value range for silage is depended DM content of the grass. Values of pH and DM content have direct influence on the quality of silage and hence, pH range of 4.6 to 4.7 can be considered as good silage for dry matter range of 35 to 40% (Henderson, 1993; Weinberg and Muck, 1996).

DM content, the presence of oxygen, pH and temperature play important role in degradation of proteins by plant enzymes. Rapid decline in pH and increase in DM content cause amino acid degradation (Mandel et al., 1989).

In ruminants, ammonia is derived either from deamination of amino acids or from absorption from the digestive tract. This ammonia is highly toxic for the animal cells and has to be eliminated. It is generally accepted that well preserved silage should have ammonia-N content less than 80 g/kg total nitrogen (Wright, 1995).

In agreement with previous studies, pH value, NH_3-N was decreased and CP increased (Zahiroddini et al., 2004; Wright, 1995). It is clear that more decline in pH of inoculated silages, resulted to a stop in proteolysis and hence, deamination of forage proteins (Zahiroddini et al., 2004; Wright, 1995; Henderson, 1993).

Due to the decrease in ADF and NDF, it would be described that lignin remains unchanged during silage making and only a small fraction (less than 5%) which occur in the cellulose fraction decrease (Morrison, 1979).

Conclusion

The inoculation of the epiphytic lactic acid bacteria at a

Table 2. The percentage of DM, CP, ADF and NDF (mean ± se) of guinea grass silages in different treatments and times.

Composition	Type of silage					
	Grass-A	**Grass-B**	**Grass-WC**	**Grass-WP**	**Grass-LM**	**Grass-LL**
DM (%)						
Day 14	38.1±0.3^{au}	38.0±0.3^{au}	38.2±0.3^{au}	38.1±0.3^{au}	37.7±0.3^{au}	38.0±0.3^{au}
Day 21	38.3±0.3^{au}	38.3±0.3^{au}	38.1±0.3^{au}	38.0±0.3^{au}	37.5±0.3^{au}	37.8±0.3^{au}
Day 28	38.2±0.3^{au}	38.2±0.3^{au}	37.5±0.3^{auw}	36.5±0.3^{bw}	37.5±0.3^{auw}	37.4±0.3^{auw}
CP (%)						
Day 14	7.3±0.14^{cz}	7.5±0.14^{cz}	8.0±0.14^{cy}	8.5±0.14^{cx}	10.4±0.14^{au}	9.4±0.14^{bw}
Day 21	8.5±0.14^{by}	8.6±0.14^{bxy}	9.0±0.14^{bx}	9.6±0.14^{bw}	10.5±0.14^{au}	10.3±0.14^{au}
Day 28	9.4±0.14^{aw}	9.6±0.14^{aw}	9.5±0.14^{aw}	10.7±0.14^{au}	10.6±0.14^{au}	10.3±0.14^{au}
ADF (%)						
Day 14	43.8±0.58^{au}	43.4±0.58^{au}	43.1±0.58^{au}	42.5±0.58^{bu}	42.2±0.58^{au}	42.9±0.58^{au}
Day 21	42.7±0.58^{aw}	42.7±0.58^{aw}	42.1±0.58^{bw}	44.9±0.58^{au}	42.4±0.58^{aw}	43.1±0.58^{aw}
Day 28	40.8±0.58^{bu}	42.4±0.58^{au}	40.1±0.58^{cuw}	41.5±0.58^{bu}	41.5±0.58^{au}	38.3±0.58^{bw}
NDF (%)						
Day 14	74.4±0.36^{au}	74.5±0.36^{au}	73.4±0.36^{auw}	73.0±0.36^{aw}	72.6±0.36^{aw}	73.1±0.36^{aw}
Day 21	72.3±0.36^{buw}	72.5±0.36^{bu}	72.1±0.36^{buw}	72.0±0.36^{buw}	71.1±0.36^{bw}	72.1±0.36^{buw}
Day 28	72.1±0.36^{bu}	72.3±0.36^{bu}	72.0±0.36^{bu}	71.2±0.36^{bu}	70.1±0.36^{bw}	71.9±0.36^{bu}
NH_3 – N (%)						
Day 14	5.5±0.13^{au}	5.4±0.13^{au}	3.3±0.13^{ax}	4.3±0.13^{aw}	4.0±0.13^{aw}	4.4±0.13^{aw}
Day 21	3.1±0.13^{bu}	2.7±0.13^{buw}	2.6±0.13^{bw}	3.0±0.13^{buw}	2.8±0.13^{buw}	2.9±0.13^{buw}
Day 28	2.4±0.13^{cu}	2.2±0.13^{cuw}	2.0±0.13^{cw}	2.5±0.13^{cu}	2.1±0.13^{cw}	2.4±0.13^{bu}

u, w, x, y, and z: Means in a row with no common superscripts is significantly different; a, b, and c: means in a column with no common superscripts is significantly different.

rate of 1 × 10^5 CFU/g was effective to decrease pH value, ADF, NDF, and NH3-N and also increase CP. In overall, inoculated silages opened at day 28 were gave better result compared to day 14 or 21. Lactic acid bacteria inoculants whether they are homo-fermentative or hetro-fermentative would be suggested to use as additive in silage making, so as to improve its nutritional value.

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