Effects of Inoculating *Lactobacillus plantarum*, Molasses and Urea on the Fermentation of Whole Crop Rice Silage

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Abstract

A study was conducted to examine the effects of inoculating *Lactobacillus plantarum* and adding molasses and urea on the fermentation of whole crop rice silage (WCRS). Three treatments were included in the study: Treatment 1 - a total amount of lactic acid bacteria (LAB) $1x10^5$ CFU/g *L. plantarum* isolated from WCRS was sprayed onto whole crop rice chips, Treatment 2 - *L. plantarum*, 5% molasses and 0.5% urea were added to whole crop rice chips, Treatment 3 - Chikuso (containing *L. plantarum*) was added to the whole crop rice chips and Treatment 4 (Control) - no *L. plantarum* and additives were added to the whole crop rice chips. All samples were ensiled for 60 days and analyses of water soluble carbohydrate, buffer capacity, pH, lactic acid and acetic acid were conducted at 10-day intervals. LAB Treatment 1 showed the lowest pH value throughout the ensiling period with pH values of 3.50 and 3.80 at days10 and 60, respectively. The water soluble carbohydrate of Chikuso of Treatment 3 recorded the lowest value (0.38% DM, $P < 0.05$) followed by Treatment 1 (0.66% DM). Buffer capacity of Treatment 2 of LAB with urea and molasses increased significantly from day 0 (1.98 meq/L) to day 60 (10.33 meq/L) of the ensiling period. Lactic acid content in Treatment 1 was higher ($P>0.05$) than those of Treatment 2 and the Control treatments. Acetic acid content of the Control treatment recorded the highest value at day 60 (1.38% DM) compared to the treatments using LAB (1.32% DM) and LAB with urea and molasses (1.24% DM).

Keywords: ***Lactobacillus plantarum*, urea, molasses, additives, whole-crop rice silage**

Introduction

In Malaysia, rice straw is one of the most abundant agricultural by-products. Traditionally the farmers fed the fresh rice straw to the ruminants. Many studies have been conducted on this material for ruminant feed especially through ensilaging process. Whole crop rice silage (WCRS) is currently being used as animal feed in Japan, Taiwan, China and Korea, especially for dairy cattle. WCRS is different from rice straw because it also contains grain, thus WCRS can provide better nutrition compared to rice straw. Many researchers have reported the use of lactic acid bacteria for the production of WCRS (Cai, 2005; Cai, 2006 and Kim *et al*., 2005). Silage feeding is a way of enhancing livestock production in the tropics during periods of inadequate supply of fresh forage. As good quality silage requires production of lactic acid to rapidly reduce pH, the plant material should contain sufficient fermentable carbohydrate for fermentation purposes (McDonald *et al*., 1995). Several studies have shown the beneficial effects of bacterial inoculation on corn silage preservation (Higginbotham *et al*., 1998; Ranjit and Kung, 2000). Bacterial inoculants work by increasing the rate and extent of lactic acid fermentation which lower the pH of silage, thus reducing the likelihood of clostridia activity that produces butyric acid**.** Additives are used to improve silage preservation by ensuring lactic acid bacteria dominating the fermentation phase in the ensiling process (Titterton and Bareeba, 1999). Molasses is often added to silage as a sugar additive and is well known to increase fermentation and feeding quality (Bolsen *et al*., 1996; Humphreys, 1991; Yokota *et al*., 1992). The addition of four percent molasses to the ensiled material generally improve silage quality by increasing lactic acid content (Aminah *et al*., 1999). Addition of urea is a common and cheap method of increasing nitrogen supply to ruminants fed with silage. Although urea addition raised total nitrogen content, it decreased the fermentation quality of silage by increasing pH with the release of ammonia (Pancholy *et al*., 1994). The aim of this study was to evaluate the effect of *Lactobacillus plantarum* isolated from WCRS, molasses and urea on the fermentation of WCRS.

Materials and Methods

Fresh whole crop rice plants (paddy straw with grain) of MR 219 variety at matured stage were chopped into small pieces (2-3 cm in length) using an OTOSIL shredding machine. Moisture content was fixed at approximately 60% by air drying. The rice straw chips were divided into 4 parts. In Treatment 1, $1x10^5$ CFU/g (fresh weight of silage *L. plantarum*) lactic acid bacteria (LAB) was sprayed to the rice straw chips. Other than *L. plantarum*, 5% molasses and 0.5% urea were added to the rice straw chips in Treatment 2 (LAB+UM). Chikuso, a commercial silage innoculant containing *L. plantarum* was used as Treatment 3 (Chikuso). No additive was added to Treatment 4 of the rice straw chips and it served as a control treatment (Control). The experiment was conducted using a complete randomised design with 3 replicates. *L. plantarum* used in this experiment was formerly isolated from WCRS of MR 219 variety. The rice straw chips and additives were mixed thoroughly and sealed in plastic pouch bags using a vacuum pack sealer. Each bag contained 600g of the mixture. All samples were ensiled for 60 days. Determination of water soluble carbohydrate (Dubois *et al*.,1956), buffer capacity (Moharrery, 2007), pH, lactic acid and acetic acid (Anon, 2001) were carried out on the samples at 0, 10, 20, 40 and 60 days of fermentation.

Data on pH, buffer capacity, lactic acid and volatile fatty acid were analysed using one-way ANOVA with Duncan Multiple Range Test for mean comparison upon significant F test at $P<0.05$ using SAS programe version 9.1 (SAS Institute, Inc., Cary, NC, USA).

Results and Discussion

The value of pH decreased in all treatments as the duration of ensiling increased (Figure 1). LAB treatment showed the lowest pH value throughout the ensiling period with pH values of 3.50 and 3.80 at day 10 and 60, respectively. Chikuso treatment showed the second lowest pH value after LAB treatment with pH value of 3.82 and 4.02 at days 10 and 60, respectively. Similar results were reported previously where the pH of silage reduced significantly as compared to the control treatment, which was attributed to *L. plantarum* inoculation (Adesogan, 2008; Gao *et al*., 2008; Huisden, *et al*., 2009). Lactic acid bacteria used in this experiment was the *L. plantarum* that was formerly isolated from WCRS. This lactic acid bacteria was the best isolate selected after screening in the whole crop rice extract medium.

Water soluble carbohydrate content (WSC) decreased in all treatments throughout the ensiling period (Figure 2). WSC content in LAB, LAB+UM and Chikuso were not significantly different at day 10, (Table 1) nevertheless control treatment recorded the highest value of WSC (3.94% DM). The WSC of Chikuso treatment recorded the lowest value (0.38% DM) and significantly different compared with control treatment (1.43% DM). LAB treatment showed the second lowest value of WSC (0.66% DM) followed by LAB+UM treatment (0.81% DM) at day 60. The WSC content in silage is positively correlated to pH reduction in silage (Jones, 1970). During fermentation, water soluble carbohydrate was converted to lactic acid resulting in reduced pH. In this experiment, *L. plantarum* in Chikuso and LAB treatments converted WSC to lactic acid effectively, as shown by WSC (0.66%) and pH value (3.8) on LAB treatment and the WSC (0.38% DM) and pH value (3.82) of Chikuso treatment at day 60. This result was similar to those reported by McDonald (1991).

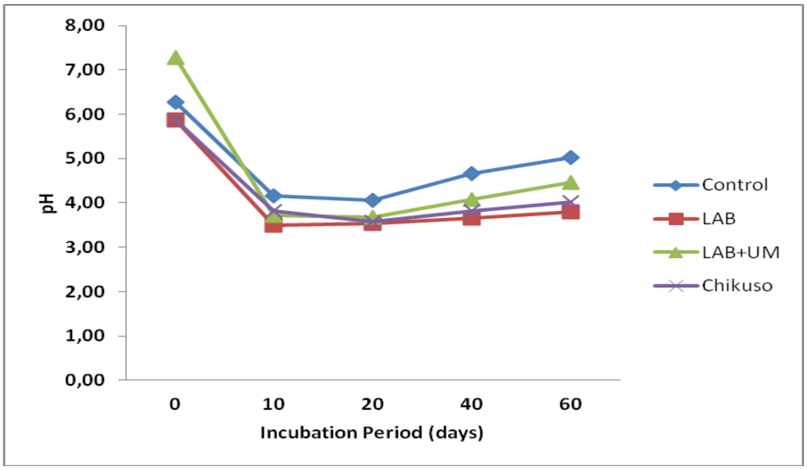

Figure 1: pH profile of WCRS silage

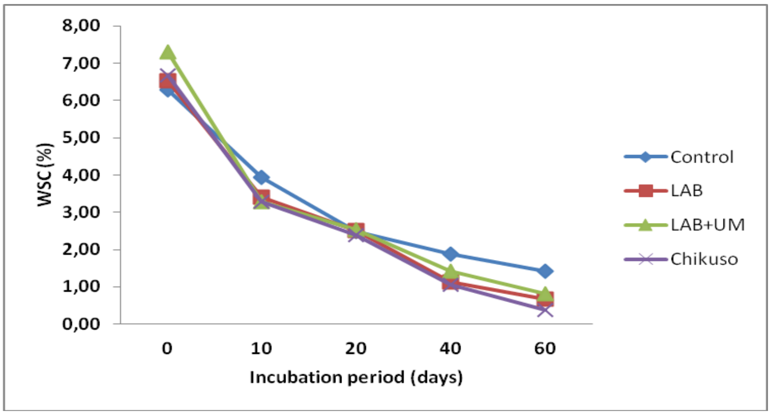

Figure 2: Water soluble carbohydrate profile of WCRS silage

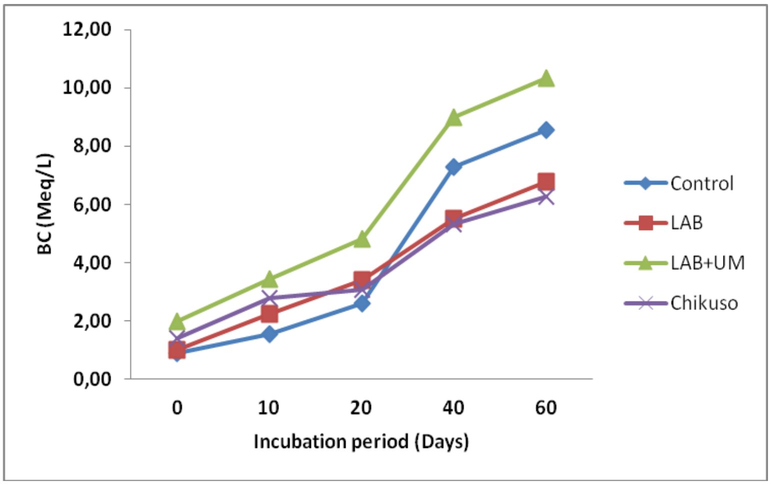

Figure 3: Buffer capacity profile of WCRS silage

Table 1: Profile of fermentation products by treatments in whole crop rice silage

Fermentation end products	Control	LAB	LAB+ UM[1]	Chikuso
Day 0				
WSC (%)	6.29[b]	6.52[b]	7.29[a]	6.67[b]
pH (%)	6.28[a]	5.88[b]	7.27[a]	5.87[b]
LA (%)	1.43[a]	1.41[a]	1.52[a]	1.06[a]
AA(%)	0.65[a]	0.69[a]	0.45[a]	0.52[a]
BC (%)	0.88[b]	1.02[b]	1.98[a]	1.42[ab]
Day 10				
WSC (%)	3.94[a]	3.41[b]	3.29[b]	3.28[b]
pH (%)	4.17[a]	3.50[c]	3.71[c]	3.82[b]
LA (%)	3.89[b]	4.37[a]	3.72[b]	4.50[a]
AA(%)	1.11[a]	0.99[ab]	0.74[c]	0.81[bc]
BC (%)	1.56[c]	2.23[bc]	3.43[a]	2.78[ab]
Day 20				
WSC (%)	2.48[a]	2.50[a]	2.54[a]	2.38[a]
pH (%)	4.06[a]	3.53[c]	3.67[b]	3.57c
LA (%)	3.99[c]	4.93[a]	4.33[bc]	4.79[ab]
AA(%)	1.28[a]	1.17[a]	1.14[a]	1.18[a]
BC (%)	2.60[c]	3.41[b]	4.82[a]	3.09[bc]
Day 40				
WSC (%)	1.89[a]	1.12[b]	1.41[a]	1.06[b]
pH (%)	4.67[a]	3.65[d]	4.08[b]	4.02[a]
LA (%)	3.57[b]	4.52[a]	3.96[ab]	4.20[a]
AA(%)	1.38[a]	1.3[1ab]	1.21[b]	1.18[b]
BC (%)	7.28[b]	5.50[c]	8.98[a]	5.31[c]
Day 60				
WSC (%)	1.43[a]	0.66[b]	0.81[b]	0.38[c]
pH (%)	5.02[a]	3.80[c]	4.46[a]	3.82[b]
LA (%)	3.30[b]	4.45[a]	3.65[b]	4.10[a]
AA(%)	1.38[a]	1.32[b]	1.24[b]	1.12[c]
BC (%)	8.56[b]	6.78[c]	10.33[a]	6.25[c]

[abc]Means with different superscripts within rows are significantly different ($p<0.05$)
[1]LAB+UM = Lactic acid bacteria + urea-molasses

Buffering capacity (BC) in silage can be defined as the degree to which forage material resists change in pH (Playne 1963: McDonald 1991). Silage with higher BC value could inhibit pH reduction. BC (meq/L) was increased in all treatments (Figure 3). BC value of LAB+UM increased significantly from day 0 (1.98 meq/L) to day 60 (10.33 meq/L) of the ensiling period. Chikuso treatment recorded the lowest value of BC at day 60 compared with the other treatments. pH value is related to BC in the silage. In this experiment, it could likely that the elevation of BC value be attributed to the urea in LAB+UM treatment. During ensilation process, plant cell urease converts urea into ammonia. The alkaline property of ammonia can delay the drop of pH (Andre *et al*., 2011).

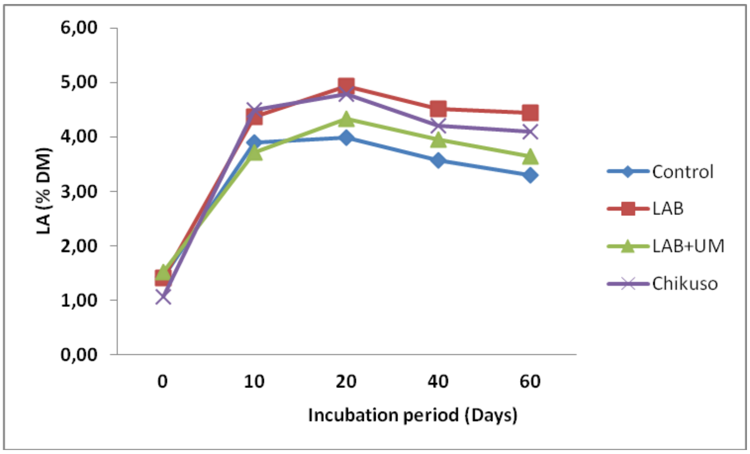

Figure 4: Lactic acid profile in WCPS

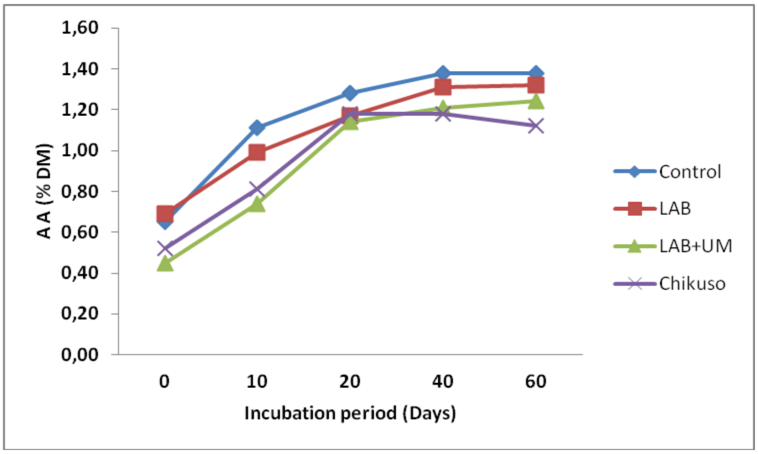

Figure 5: Acetic acid profile in WCPS

Lactic acid content increased from days 0 to 10 but leveled thereafter and all treatment groups showed similar trend (Figure 4). Lactic acid content in LAB treatment was insignificantly higher than those of LAB+UM and the control treatments. As shown in Figure 5, acetic acid content of control treatment recorded the highest DM value at day 60 (1.38% DM) compared to LAB (1.32% DM) and LAB+UM (1.24% DM). Acetic acid begins to be produced in the second phase of silage fermentation by acetic acid bacteria. The increasing acid inhibits acetic acid bacteria, causing termination of phase II. Lower pH enhances the growth and development of another anaerobic group of bacteria, those producing lactic acid (Danner *et al*., 2003). Under the condition of the experiment, the increment of lactic acid in silage treated with bacterial inoculants clearly indicated that the *L. plantarum* which was isolated from WCRS could enhance lactic acid production. This result is in agreement to that reported by McDonald (1991).

Conclusion

Inoculation of *L. plantarum* isolated from WCRS enhanced the fermentation of whole crop rice silage. Water soluble carbohydrate content was the main causal factor for reduced pH in the silage. Addition of urea increased the buffer capacity in whole crop rice silage. The performance of *L. plantarum* used in this experiment was comparable with Chikuso, a commercial silage inoculant containing *L. plantarum*.

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References

Adesogan, A.T. 2008. Recent advances in bacterial silage inoculants technology. Proc. Florida Ruminant Nutrition Symposium. January 29-30 2008. Gainesville, FL. p 53-56.

Aminah, A., Abu Bakar, C. and Izham, A. 1999. Silages from tropical forages: nutritional quality and milk production. In: L.'t Mannetje (Ed.) Proc. FAO e-Conf. on Trop.Silage. FAO Plant Prod. and Protect. Paper 161. 1 Sept. - 15 Dec. 1999.

Andre, A.F. P., Armando, A. R., Waldomiro, B. and Gilberto, B.S. 2011. Fermentation parameters, quality and losses in sugarcane silages treated with chemical additives and bacterial inoculants. R. Bras. Zootec. 40 (11): 2318-2322.

Anon, 2001. Guide Book for Forage, Association of Forage Evaluation. Japan Grassland Agriculture Forage and Seed Association, Tokyo, pp 36-38.

Bolsen, K.K., Ashbell, G. and Weinberg, Z. G. 1996. Silage fermentation and silage additive: Review. Asian-Aus. J. Anim Sci. 9: 483-493.

Cai, Y. 2005. Quality improving technique of whole crop rice silage. Inte. Symp.Production and Utilization Techniques of Whole Crop Forage Rice. pp 103-135.

Cai, Y. 2006. Development of lactic acid bacteria inoculants for whole crop rice silage in Japan. Proc. X11th AAAP Animal Science Congress 2006, Busan, Korea.

Danner, H, Holzer, M., Mayrhuber, E. and Braun, R. 2003. Acetic acid increases stability of silage under aerobic conditions. Appl. Environ. Microbiol. 69(1): 562–567.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A and Smith, F. 1956. Calorimetric methods for determination of sugars and related substances. Anal. Chem. 28(3): 350-356.

Gao, L., Yang, H., Wang, X., Huang, Z., Ishii, M., Igarashi, Y. and Cui, Z. 2008. Rice straw fermentation using lactic acid bacteria. Bioresource Technol. 99: 2742-2748.

Higginbotham, G.E., Mueller, S.C, Bolsen, K.K. and Depeters, E.J. 1998. Effect of inoculants containing propionic acid bacteria on fermentation and aerobic stability of corn silage. J. Dairy Sci. 81: 2185-2191.

Huisden, C.M., Adesogan, A.T., Kim, S.C. and Ososanya, T. 2009. Effect of applying molasses or inoculants containing homofermentative or heterofermentative bacteria at two rates on the fermentation and stability of corn silage. J. Dairy Sci. 92: 690-697.

Humphreys, L. R. 1991. Tropical pasture utilization. Cambridge University Press New York, pp. 143-148.

Jones, D.I.H. 1970. The ensiling characteristics of different herbage species and varieties. J. Agric. Sci. (Camb) 75: 293-300.

Jong, G. K., Eui, S.C., Jun, S.H., Sei, H.Y., Young, C.L.and Sung, S. 2006. Development of lactic acid bacteria inoculant for whole crop rice silage in Korea. Proc.X11th AAAP Animal Science Congress 2006, Busan, Korea.

McDonald, P. 1991. The Biochemistry of Silage. Chichester. publ, A Willey Interscience.

McDonald, P., Edwards, R.A., Greenhagh, J.F.D. and Morgan, C.A. 1995. Animal Nutrition 5th Ed. Longman Scientific and Technical. New York. pp. 451-464.

Moharrery, A. 2007. The determination of buffering capacity of some ruminant's feedstuff and their cumulative effects on TMR ration. American J. Anim.Vet.Sci. 2 (4): 72-78.

Pancholy, R., Mali, P.C. and Marthur, D. 1994. Effect of urea-molasses and lactic culture on silage fermentation of *Cenchrus ciliaris*. Annal. Arid Zone. 33:147-150.

Playne, M.J. 1963. Buffering capacity of sweet sorghum: the effect of nitrogen content, growth stage and ensilage. J. Sci. Food Agric. 14(7): 495-498.

Ranjit, N.K. and Kung, K. 2000. The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum* or a chemical preservative on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 83: 525-535.

Yokota, H., Kim, J. H., Okajima, T. and Ohshima, M. 1992. Nutritional quality of napier grass (*Pennisetum purpureum* Schum) ensiled with or without molasses. Asian-Aus. J. Anim. Sci. 5: 673-679.

Titterton, M. and Bareeba, F. B. 1999. Grass and legume silages in the tropics. In: L.'t Mannetje (Ed.) Proc. FAO e-Conf. on Trop. Silage. FAO Plant Prod. and Protect. Paper 161. 1 Sept. - 15 Dec. 1999.