Contents available at: Sri Lanka Journals Online

Tropical Agricultural Research

Journal Home Page: https://tar.sljol.info

RESEARCH

Potential of the Locally Produced *Lactobacillus* Inoculants in Ensiling Guinea Grass (*Megathyrsus maximus*) Forage

K.M. Devindi[1], U.L. Karunadhipathi[2], R. Priyantha[3], N. Liyanagunawardhana[3], W.M.P.B. Weerasinghe[3], R.G.S.C. Rajapaksha[4] and M.B.P. Mahipala Kumara[5]*

[1] *Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka.*
[2] *Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.*
[3] *Veterinary Research Institute, Peradeniya, Sri Lanka.*
[4] *Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka.*
[5] *Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.*

ARTICLE INFO

Article history:
Received: 01 July 2024
Revised version received: 23 December 2024
Accepted: 25 December 2024
Available online: 01 January 2025

Keywords:
Lactobacillus plantarum
Lactobacillus rhamnosus
Lactobacillus oris
Nutritive Value
Silage Quality

Citation:
Devindi, K.M., Karunadhipathi, U.L., Priyantha, R., Liyanagunawardhana, N., Weerasinghe, W.M.P.B., Rajapaksha, R.G.S.C. and Kumara Mahipala, M.B.P. (2024). Potential of the Locally Produced *Lactobacillus* Inoculants in Ensiling Guinea Grass (*Megathyrsus maximus*) Forage. Tropical Agricultural Research, 36(1): 99-105.

DOI:
https://doi.org/10.4038/tar.v36i1.8886

Kumara Mahipala M.B.P.
https://orcid.org/0000-0002-1756-1301

*Corresponding author- pmahi@agri.pdn.ac.lk

ABSTRACT

Lactic acid bacteria inoculants are employed to accelerate forage ensiling ensuring silage quality. Inoculants for ensiling are not produced locally and are restricted for importation in Sri Lanka. This study evaluated the potential of locally produced, freeze-dried *Lactobacillus* inoculants containing *Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus oris,* on ensiling of guinea grass *(Megathyrsus maximus)* forage. The experiment was conducted as a complete randomized design (CRD). Guinea grass forage harvested at the 10 % flowering stage were chopped and inoculated with each *Lactobacillus* (i.e. treatments) species (i.e. 10^6 CFU/g fresh forage) or sprayed with deionized water (i.e. control). Six laboratory silos were prepared with each inoculant and one silo was the control. Silage samples were taken from 3 silos opened at 2 weeks and from the remaining 3 silos at 52 weeks post-ensiling. All samples were assessed for pH value and lactic acid content. In addition, the samples taken at 52 weeks were analyzed for organic matter digestibility (OMD) and metabolizable energy (ME) content. Guinea grass ensiled with *Lactobacillus* exhibited significantly ($P<0.05$) high lactic acid content and low pH value compared to the control as early as 2 weeks ensiling. At 52 weeks, guinea grass inoculated with *L. rhamnosus* recorded, significantly ($P<0.05$) higher lactic acid content compared to that inoculated with *L. plantarum*. Nevertheless, at 52 weeks guinea grass ensiled with, both *L. rhamnosus* and *L. oris* recorded significantly lower ($P<0.05$) pH values than those inoculated with *L. plantarum*. Inoculation of guinea grass with *Lactobacillus* significantly ($P<0.05$) increased the OMD and ME content of silage. This study concluded that locally produced, freeze-dried *L. rhamnosus*, and *L. oris* inoculants have the potential to enhance the ensiling guinea grass.

INTRODUCTION

Guinea grass (*Megathyrsus maximus)* Type A is a naturalized, and most abundant forage for feeding dairy cattle in Sri Lanka (Weerasinghe, 2019). The species is a medium-quality forage which reported 20.21 % dry matter (DM), 9.14 % crude protein (CP), and 40.45 % *in-vitro* organic matter digestibility (OMD) (de Moura Zanine *et al.*, 2018). Growth and yield of the grass depend on the bimodal monsoon rainfall pattern of the country. Therefore, conserving of excess guinea grass for utilization during the dry seasons would be one of the potential strategies to overcome forage scarcity in feeding dairy cows in Sri Lanka.

Forage ensiling is primarily driven by lactic acid bacteria (LAB), which anaerobically ferment water-soluble carbohydrates (WSC) in forage resulting in organic acids. Strong lactic acid reduces the pH value of forage thereby preserve the quality of resultant silage. The species of bacteria in microbial community play a crucial role in silage fermentation (Driehuis *et al.*, 2018). Epiphytic LAB naturally present on forage crops are responsible for silage fermentation and also influences silage quality (Cai *et al.*, 1998). The production of high-quality silage in tropical environment is challenging due to the low density of naturally occurring epiphytic LAB and low level of WSC present in forage (Khota *et al.*, 2016). In addition, contamination of forage during ensiling process, potentially introduce pathogenic or undesirable microbes to silage (Queiroz *et al.*, 2018). Lactic acid bacteria inoculants are commonly used to enhance silage quality by optimizing the fermentation process and enhancing the aerobic stability of the silage.

Pasebani *et al*. (2011) have shown that guinea grass forage inoculated with epiphytic LAB isolated from guinea grass at 10^5 colony forming units (CFU)/g reduced the pH value within 14 days post-ensiling. Importation of forage ensiling microbial inoculants to Sri Lanka is strictly restricted by the biodiversity concerns. In this context, attempts have been made to develop a potential inoculant locally, for ensiling process. Dissanayake *et al.* (2022) isolated *Lactobacillus plantarum, Lactobacillus rhamnosus* and *Lactobacillus oris* from maize, sorghum and guinea grass silage, respectively. Devindi *et al.* (2023) have freeze-dried them, successfully. The present experiment was design to investigate the influence of those freeze-dried LAB as inoculants on ensiling guinea grass forage.

METHODOLOGY

The ethical clearance for the research was obtained from the Ethical Clearance Committee of the Faculty of Agriculture, University of Peradeniya (ECC/2022/E/049). The experiment was conducted at the laboratories of the Department of Animal Science, Faculty of Agriculture, University of Peradeniya and Veterinary Research Institute (VRI), Peradeniya.

Forage preparation

Guinea grass forage which was re-grown 4 weeks after harvesting to the height of approximately 60-70 cm with around 10 % flowering was harvested. The fresh forage was chopped into pieces of about 10 mm using a commercial forage chopper and homogenized. The DM content of the chopped forage was 19 %.

Inoculation and ensiling procedure

Freeze-dried *L. plantarum*, *L. rhamnosus,* and *L. oris* inoculants produced by Devindi *et al,* (2023) by isolating the species from maize, sorghum, and guinea grass silage, respectively, were used as inoculants. The viable colony forming units (CFU) of each freeze-dried *Lactobacillus* inoculant was tested by inoculating them on Man, Rogosa and Sharpe (MRS) agar followed by incubation for 48 hours at 39° C. The *L. plantarum, L. rhamnosus,* and *L. oris* inoculants had 3 x 10^9 CFU/mL, 1.6 x 10^9 CFU/mL and 3.4 x 10^9 CFU/mL, respectively. The three treatments were prepared by using three freeze-dried *L. plantarum, L. rhamnosus,* and *L. oris* inoculants. Each inoculant was separately dissolved in 15 mL deionized water and sprayed on 1 kg of freshly, chopped, homogenized forage at 10^6 CFU/g of fresh forage concentration. Forage for the control was sprayed with deionized water (15 mL/ kg of fresh forage) without inoculant. The forage was then pressed into airtight 1 L Weck glass jars (i.e. Laboratory silo). Six replicate silos were prepared with each *Lactobacillus* inoculant (i.e. treatment) and control. They were ensiled together at room temperature (25 - 30° C). The experiment was conducted as a complete randomized design (CRD).

Determination of silage quality and data analysis

Silage samples from 3 of the silos representing each inoculant and control at 2 weeks post-ensiling and at 52 weeks post-ensiling were taken. The 2 sampling stages were determined based on McDonald *et al.* (1991) who described the critical stages in ensiling process. According to them, the primary ensiling occurs during the initial 1-3 weeks period, during which lactic acid bacteria lower the pH value to preserve the forage. Secondary fermentation may occur depending on conditions such as oxygen exposure or excessive moisture, leading to undesirable microbial activity. When properly sealed and stored under the right conditions, forage silage can last for 1 to 2 years, or even longer, without significant loss of nutritional value.

The pH value (AFIA, 2011), DM, lactic acid (Barnett, 1951), and soluble carbohydrate (AFIA, 2003) of the ensiled forage were determined. The determination of *in-vitro* OMD and *in-vitro* ME content were carried out for forage ensiled for 52 weeks by performing *in-vitro* gas fermentation assay (Menke and Steingas, 1988). The data were subjected to analysis of variance (ANOVA) procedures ($P<0.05$). Means were compared by Duncan's multiple range test ($P<0.05$).

RESULTS AND DISCUSSION

The low DM, WSC, and energy content and high buffering capacity are the major inferior qualities of the guinea grass for silage production (Santos *et al.,* 2013). Pre-ensile guinea grass forage used in the present study had 19 % DM. However, Ashbell and Weinberg, (2003) suggested that ensiling of forage containing DM percentage exceeding 30 % ensures favorable fermentation and prevents undesirable fermentation by *Clostridia*. Inoculants often accelerate forage ensiling by enhancing acidification, thereby lowering the pH value in silage (Weinberg and Muck, 1996). Microbial inoculants are used to influence the quality parameters of silage including lactic acid content and pH value. The effect of inoculation of guinea grass forage with LAB inoculants (10^6 CFU/g fresh matter) on lactic acid content and pH value at 2 weeks and 52 weeks ensiling has been presented in Table 1. At week 2, guinea grass inoculated with *Lactobacillus* species recorded significantly ($P<0.05$) higher lactic acid content than the guinea grass ensiled without inoculation (i.e. control). High lactic acid formation has resulted in significantly ($P<0.05$) low pH value in inoculated forage as early as 2 weeks ensiling. However, at 52 weeks ensiling, the lowest ($P<0.05$) lactic acid content and the highest ($P<0.05$) pH value were reported in guinea grass forage inoculated with *L. plantarum*. The results of the present study are comparable to those reported by Lin *et al.* (1992), who stated that *L. plantarum* was not effective in improving the quality of ensiling guinea grass. They also reported that inoculants were not always successful in improving silage quality. Muck (2010) reported the fermentation of forage silage in detail. Accordingly, lactic acid which is highly efficient at lowering the pH value of silage is the primary organic acid produced by LAB, such as *Lactobacillus.* Nevertheless, other organic acids, which may produce during the fermentation process, also contribute to the acidification. For example, acetic acid or butyric acid can be formed during silage fermentation, depending on the bacterial strains such as heterofermentative *Lactobacillus buchneri*. These acids, though less efficient than lactic acid can still lower the pH value of the silage. The observation of low lactic acid (3.22 %) with a low pH value (4.57) in guinea grass inoculated with *L. oris* could indicate a shift in the fermentation pathway, where heterofermentative *L. oris* produced a mixture of acetic acid, propionic acid, etc., leading to acidification despite the lower lactic acid content. *Lactobacillus oris* isolated from naturally ensiled guinea grass (Dissanayake *et al.,* 2022) was used to produce *L. oris* inoculant for the present experiment. The fermentation pathways of tropical forage silage are different from those of temperature silage and the factors responsible for forage conservation are not fully understood (Miller *et al.,* 1966). Further, acetic acid rather than lactic acid may be the main preservative in tropical forage ensiling process (Panditharathna, 1984). However, organic acid contents other than lactic acid were not measured in the present experiment. The pH values recorded in the present experiment are comparable with that reported by Santos *et al.* (2014) for guinea grass silage (5.04) inoculated with *L. plantarum* and ensiled for 35 days. The pH value reflects the ultimate acidity of the ensiled product and serves as one of the key indicators of both ensiling efficiency and quality of silage. Whiter and Kung (2001) reported that a low silage pH value often associated with poor silage intake and reduced stability in rumen digestion while high silage pH value often linked to elevated ammonia nitrogen content, resulting in low silage intake and reduced the ability to conserve ensiled forage. Therefore, *in-vivo* feeding experiments are to be conducted in the determination of the effect of *Lactobacillus* inoculants on guinea grass ensiling. During the forage ensiling process, rapid acidification is a key characteristic of microbial inoculants, as it conserves forage by inactivating plant proteases and inhibiting the growth of pathogenic and spoilage microorganisms (Dunière *et al.,* 2013).

Guinea grass inoculated with *Lactobacillus* significantly (P<0.05) increased the *in-vitro* OMD and ME contents of silage at 52 weeks (Table 2). In commercial silage production, *Lactobacillus* inoculants are used to increase the ratio of lactic acid to acetic acid, to reduce proteolysis, and increase dry matter recovery. Aydin and Denek (2023) reported that rapid lowering of pH value by lactic acid reduces proteolytic enzyme activity and thereby prevents break down of forage proteins during ensiling. Nevertheless, *in-vitro* response of microbial inoculants on OMD greatly depends on the species or strain of microorganism, and the substrate. The key determinant of successful microbial inoculant is the compatibility of ensiling forage and microorganisms (Muck, 1988). Compared to the present experiment, greater *in-vitro* OMD (60.1 %) reported for guinea grass silage (Panditharathna,1984) may be due to the difference in the stage of maturity of the ensiling forage which greatly influence both fermentation characteristics and the nutritive value of the post-ensiled material.

As described earlier the *L. plantarum, L. rhamnosus,* and *L. oris* inoculants used in this study were isolated from maize, sorghum, and guinea grass silage, respectively (Dissanayake *et al.,* 2022). Microbial inoculants sourced from strains in environments different from the environment of harvested forage, and from different forage types, can still perform effectively in the ensiling process (Puntillo *et al.,* 2020). Therefore, the *L. rhamnosus,* and *L. oris* inoculants, which produced silage with significantly low pH value (P<0.05) as early as 2 weeks and significantly increased *in-vitro* OMD and ME, at 52 weeks are promising options to enhance the ensiling guinea grass.

Table 1: Effect of inoculation of guinea grass with *Lactobacillus* (10^6 CFU/g Fresh Forage) on lactic acid content and pH value of silage

Inoculated *Lactobacillus* species	2 weeks[1]		52 weeks[1]	
	Lactic acid content (%)	pH value	Lactic acid content (%)	pH value
Lactobacillus plantarum	14.705 ± 0.145a	4.900 ± 0.030b	0.595 ± 0.075c	5.670 ± 0.001a
Lactobacillus rhamnosus	15.044 ± 0.603a	4.845 ± 0.275b	17.015 ± 0.465a	4.960 ± 0.001c
Lactobacillus oris	14.443 ± 0.289a	5.225 ± 0.115b	3.220 ± 0.150b	4.570 ± 0.001d
Without inoculum (Control)	0.9020 ± 0.187b	5.600 ± 0.230a	2.675 ± 0.485b	5.520 ± 0.001b

[1], Mean ± SE.
Means within a column followed by different lower-case letters are significantly different (P<0.05).
The dry matter and crude protein percentage of silage produced from Guinea grass harvested at 4 weeks re-growth ranged from 12.32% to 18.10% and 6.89% to 8.88%, respectively.

Table 2: Effect of inoculation of guinea grass with *Lactobacillus* inoculation (10^6 CFU/g fresh forage) on *in-vitro* organic matter digestibility and metabolizable energy content of silage at 52 weeks

Inoculated *Lactobacillus* species	Organic Matter Digestibility (%)	Metabolizable Energy content (MJ/kg DM)
Lactobacillus plantarum	33.540 ± 0.643a	4.833 ± 0.097a
Lactobacillus rhamnosus	32.406 ± 0.255a	4.653 ± 0.037a
Lactobacillus oris	32.793 ± 0.860a	4.703 ± 0.132a
Without inoculum (Control)	29.926 ± 0.673b	4.246 ± 0.130b

[1], Mean ± SE.
Means within a column followed by different lower-case letters are significantly different (P<0.05).
Dry matter and crude protein percentage of silage produced from Guinea grass harvested at 4 weeks re-growth ranged between 12.32 to 18.10 and 6.89 to 8.88, respectively.

CONCLUSION

The present study concludes that inoculating guinea grass forage with freeze-dried *L. rhamnosus* and *L. oris* at 10^6 CFU/g fresh forage concentration can ensure a rapid drop of pH value during ensiling as early as 2 weeks and show a potential to improve the quality of guinea grass silage.

ACKNOWLEDGMENT

This study was supported by the University Research Council (URC) of the University of Peradeniya through the Multidisciplinary Research Grant (MR 381). The technical support by the staff of the Department of Animal Science, University of Peradeniya and the Veterinary Research Institute are gratefully acknowledged.

REFERENCES

AFIA – Laboratory Methods Manual, A reference manual of standard methods for the analysis of fodder. (2003). Australian Fodder Industry Association Limited, 70-72.

AFIA – Laboratory Methods Manual, A reference manual of standard methods for the analysis of fodder. (2011). Version 7, Australian Fodder Industry Association Limited, 80-81.

Ashbell, G., and Weinberg, Z.G. (2003). Silage from tropical cereals and forage crops.

Aydin, S.S. and Denek, N. (2023). Effect of Adding Lactic Acid Bacteria to Maize Silage on Nutritive Guality, Fermentation Properties and in Vitro Digestibility. *Tarim Bilimleri Dergisi*, 29(4), 1050–1058.

Barnett, A.J.G. (1951). The colorimetric determination of lactic acid in silage. *Biochem Journal* , 49 (4), 527–529.

Cai, Y., Benno, Y., Ogawa, M., Ohmomo, S., Kumai, S., Nakase, T. (1998). Influence of Lactobacillus spp, 64(8), 2982–2987.

de Moura Zanine, A., Bonelli, E.A., Ferreira, D., Souza, A.L., Santos, E.M., Pinhlo, R.M.A., Parente, H.N., Parente, M.O.M. (2018). Fermentation and chemical composition of guinea grass silage added with wheat meal *and Streptococcus bovis*, *New Zealand Journal of Agricultural Research*, 61(4), 487–494.

Devindi, K.M., Priyantha, R., Liyanagunawardena, N, Weerasinghe, W.M.P.B., Kumara Mahipala, M.B.P. (2023). Assessment of the effect of freeze-dried Lactobacillus inoculants on ensiling fodder Sorghum and Guinea grass. In, Proceedings of the International Symposium on Agriculture and Environment 2023 (pp. 120). Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, 81100, Sri Lanka.

Dissanayake, L.M.J.N.K., Kumara Mahipala, M.B.P., Apeksha Uthpali, N., Senanayaka, S.D., Rajapakse, R.G.S.C., Jinadasa, H.R.N., Weerasinghe, W.M.P.B., Esham, M. (2022). Proceedings of 4th International Conference of Agricultural Sciences, Sabaragamuwa University of Sri Lanka, 252-255

Driehuis, F., Wilkinson, J.M., Jiang, Y., Ogunade., Adesogan. (2018). Silage review: Animal and human health risks from silage, *Journal of Dairy Science*, 101(5), 4093–4110.

Dunière, L., Sindou, J., Chaucheyras-Durand, F., Chevallier, I., Thevenot-Sergentet, D. (2013). Silage processing and strategies to prevent persistence of undesirable microorganisms, *Animal Feed Science and Technology*, 182(1–4), 1–15.

Khota, W., Pholsen S., Higgs, D., Cai, Y. (2016). Natural lactic acid bacteria population of tropical grasses and their fermentation factor analysis of silage prepared with cellulase and inoculant, *Journal of Dairy Science*, 99(12), 9768–9781.

Lin, C., Brent, B.E., Bolsen, K.K. (1992). Epiphytic lactic acid bacteria succession during the pre-ensiling and ensiling periods of alfalfa and corn, *Kansas Agricultural Experiment Station Research Reports*, 0(1), 113–119. .

McDonald, P., Henderson, A. R., & Heron, S. J. E. (1991). The Biochemistry of Silage. 2nd Edition, Chalcombe Publications.

Menke, K.H., Steingas, H. (1988). Estimation of the energetic feed value obtained from chemical analyses and in vitro production using rumen fluid. *Animal Research and Development*, 28, 7–55.

Miller, W.J., Clifton, C.M., Fowler, P.R., Cameron, N. W. (1966). Ensiling Characteristics of Tift Sudangrass and Coastal Bermudagrass, *Journal of Dairy Science*, 49(5), 477–485. Available at: https://doi.org/10.3168/jds.S0022-0302(66)87900-6.

Muck, R.E., (2010). Silage additives and management issues. Proceedings of Idaho Alfalfa Forage Conference, Best Western Burley Inn, Burley, Idaho, USA. 16 – 17 February 2010. pp. 49-55.

Muck, R. E. (1988). Factors Influencing Silage Quality and Their Implicationsfor Management. *Journal of Dairy Science*,71, 2992–3002. doi: 10.3168/jds.S0022-0302(88)79897-5

Panditharathne, s. (1984). Ensiling characteristics, digestibility and palatability of tropical grasses as affected by growth stage, chopping length and additives. Ph. D. Dissertation, VPI and SU, USA.

Pasebani, M., Yaakub, H., Alimon, A.R. and Sijam, K. (2011). Effect of epiphytic lactic acid bacteria isolated from guinea grass on nutritional value of the silages. *African Journal of Agricultural Research*, 6(19), 4447-4450.

Puntillo, M., Gaggiotti, M., Oteiza, J.M., Binetti, A., Massera, A., Vinderola, G. (2020). Potential of Lactic Acid Bacteria Isolated From Different Forages as Silage Inoculants for Improving Fermentation Quality and Aerobic Stability, *Frontiers in Microbiology*, 11(December).

Queiroz, O.C.M., Ogunade, I. M., Weinberg, Z., Adesogans, T. (2018). Silage review: Foodborne pathogens in silage and their mitigation by silage additives, *Journal of Dairy Science*, 101(5), pp. 4132–4142.

Santos, E.M., Silva, T. C., Macedo, C. H. O., Campos, F. S. (2013). Lactic acid bacteria in tropical grass silages. Pages 335–362 in Lactic Acid Bacteria: R&D for Food, Health and Livestock Purposes.M. Kongo ed. InTech, Rijeka, Croatia.

Santos, E.M., Pereira, O.G., Garcia, R., Ferreira, C.L.L.F., Oliveira, J.S., Silva, T.C. (2014). Effect of regrowth interval and a microbial inoculant on the fermentation profile and dry matter recovery of guinea grass silages, *Journal of Dairy Science*, 97(7), 4423–4432.

Whiter, A. G., & Kung, L. (2001). The effect of a dry or liquid application of Lactobacillus plantarum MTD1 on the fermentation of Alfalfa Silage. *Journal of Dairy Science*, *84*(10), 2195–2202. https://doi.org/10.3168/jds.S0022-0302(01)74666-8

Weerasinghe, W.M.P.B. (2019). Livestock feeds and feeding practices in Sri Lanka. 181-206. In: Samantha, K., Bokhtiar., S.M & Ali, M.Y. (Ed). Livestock feeds and feeding practices in South Asia. SAARC Agriculture Center, Dhaka, Bangladesh.

Weinberg, Z.G., Muck R.E. (1996). New trends and opportunities in the development and use of inoculants for silage *FEMS Microbiology Reviews,* (19), 53–68. doi: org/10.1016/0168-6445(96)00025-3.