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RESEARCH ARTICLE

Optimization of Fermentation Time and Stability of King Grass at storage by application of *Lactobacillus plantarum*

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ABSTRACT

This experiment compared the consequences of fermentation by *Lactobacillus plantarum* inoculation on fermentation properties, microbial interactions and shelf-life of king grass silage. There was a control (CK) and *L. plantarum* inoculated (LP) group of silage treatments. The parameters of fermentation were observed at 3 days, 7 days, 14 days, and 30 days and storage stability was observed using aerobic exposure tests up to 28 days. Silage treated with LP had a better pH drop, greater concentration of lactic acid, reduced ammonia nitrogen and better lactic acid bacteria (LAB) dominance, and inhibited yeasts and molds. Aerobic stability of LP silage has been greatly enhanced when compared to the control. These findings prove that *L. plantarum* is a good method of maximizing fermentation duration and increasing the storage stability of king grass silage, which is a viable approach to better forage storage.

1. Introduction

Pennisetum purpureum × Pennisetum typhoides, also known as king grass, is a high-yield tropical forage that is easily cultivated to feed ruminants because of its rapid growth, great biomass, and capacity to be grown in various climatic conditions [1]. It has good nutritive value to livestock, but has high moisture level and the deterioration after harvesting poses a great challenge to its preservation. The main technique used to conserve forage is ensilage, which is an anaerobic fermentation of lactic acid bacteria (LAB) to produce organic acids to reduce the pH and prevent the growth of unwanted microorganisms like molds and clostridia [2].

Although it has advantages, ensiling king grass is likely to be complicated by a high buffering capacity and a rather low amount of water-soluble carbohydrates (WSC) a necessary substrate of LAB metabolism [3]. Reduced acidification rates during ensiling may lead to prolonged fermentation, more proteolysis, and low silage quality, with higher concentrations of ammonia nitrogen and lower nutrient retention [4]. Further, fermentation can lead to aerobic stability when the ferment is prolonged and when the acidification is not optimum, and in such case, the silage is vulnerable to spoilage when exposed to air during feed-out.

In order to overcome these shortcomings, LAB inoculation has been a popular area of research to enhance the effectiveness of fermentation and silage quality. The *Lactobacillus plantarum* is the most commonly used homofermentative species of LAB strains because of its capacity to rapidly convert WSC to lactic acid and generate the conditions that prevent the unwanted microorganisms [5]. Past research has proved that the inoculation of grass silages with *L. plantarum* does not only improve the fermentation properties of the feed but also the aerobic stability, ammonia nitrogen concentration, and the preservation of crude protein [5,6].

A number of researches have reported the usefulness of *L. plantarum* in tropical forages. As an example, studies on Napier grass and other tropical grasses found that the inoculated silages had higher levels of lactic acid, low PH, and less yeast and mould growth as compared to the control groups [7,8]. Besides, the inoculated silages were more aerobically stable during feed-out, and they also lasted longer in storage and minimized nutrient losses. These results imply that LAB inoculation is specifically significant in high moisture forages such as king grass where natural fermentation can be inadequate aiding spoilage.

Although the advantages of *L. plantarum* are known, there is limited knowledge of research on king grass silage. The fermentation time optimization and the analysis of the storage stability under the conditions of real practice is important to optimize the nutritional benefits and feed efficiency of this valuable forage. Thus, the proposed research will explore the impact of *L. plantarum* inoculation on the fermentation dynamics, chemical composition, microbes, and aerobic stability of king grass silage. The findings should yield some insightful information on how to enhance preservation of forage when used as a means of livestock production in tropical areas.

2. Materials and Methods

2.1. Experimental Materials

New king grass at vegetative stage was cut and cut into 2-3 cm. *Lactobacillus plantarum* was cultivated in MRS broth by using a culture collection. All analyses were done using analytical grade chemicals.

2.2 Inoculum Preparation

Activation of the strain was done in MRS broth at temperature 37 C with 18-24 h with subculture twice. To obtain the desired cell concentration, the concentration of the cells was adjusted to 1 × 10 8 CFU/mL.

2.3 Silage Preparation

King grass chopped was split into two treatments: 1. CK: No inoculant of Control 2. LP: L. plantarum 1 × 106 CFU/g fresh matter was inoculated. About 500 g of treated forage were packed and compacted in anaerobic silage bags which are then sealed, and stored at temperatures of 2530 o C.

2.4 Fermentation Evaluation

Sample was taken at 3, 7, 14 and 30 days and the pH, lactic acid, acetic acid, butyric acid, ammonia nitrogen and microbial counts (LAB, yeasts, molds) were taken.

2.5 Storage Stability and Aerobic Exposure.

Silage was anaerobically stored within 28 days after 30 days. Stability of aerobic samples was estimated by keeping samples in room temperature and exposing them to air where temperature increase, pH, and visible spoilage were recorded over 5 days.

2.4 Statistical Analysis

Each treatment was done thrice. ANOVA was used to analyze the data and the significance was $p < 0.05$.

Results

During fermentation, Fermentation Characteristics LP silage experienced a quicker pH reduction than CK (Figure 1). The level of lactic acid increased at a high rate in the silage treated with LP and acetic acid and butyric acid were low (Table 1). The ammonia nitrogen was found to be very low in LP silage, which means that it was more preserved in the form of proteins. The count of LAB counts was more and dominated in LP silage, and yeast counts and mould counts were repressed (Figure 2, Table 1). Silage - in storage Silage that was treated with LP- had low pH, high lactic acid and LAB superiority (Table 2). Aerobic exposure tests revealed that there was delayed temperature increase and less spoilage in LP silage than CK, indicating the improved aerobic stability (Figure 3).

Table 1. Fermentation Characteristics of King Grass Silage after 3, 7, 14, and 30 Days

Parameter	**Day 3**	**Day 7**	**Day 14**	**Day 30**
pH	CK: 5.6 ±0.05 LP: 5.2 ±0.04*	CK: 5.3 ±0.04 LP: 4.7 ±0.03*	CK: 5.0 ±0.03 LP: 4.4 ±0.02*	CK: 4.8 ±0.03 LP: 4.2 ±0.03*
Lactic acid (% DM)	CK: 0.8 ±0.05 LP: 1.5 ±0.07*	CK: 1.2 ±0.08 LP: 2.0 ±0.10*	CK: 1.5 ±0.09 LP: 2.5 ±0.12*	CK: 1.5 ±0.10 LP: 2.8 ±0.12*
Acetic acid (% DM)	CK: 0.5 ±0.03 LP: 0.4 ±0.02*	CK: 0.6 ±0.04 LP: 0.4 ±0.03*	CK: 0.6 ±0.04 LP: 0.4 ±0.03*	CK: 0.6 ±0.04 LP: 0.4 ±0.03*
Butyric acid (% DM)	CK: 0.05 ±0.01 LP: ND	CK: 0.06 ±0.01 LP: ND	CK: 0.07 ±0.01 LP: ND	CK: 0.08 ±0.01 LP: ND

NH_3–N (% total N)	CK: 8.5 ±0.6 LP: 5.2 ±0.4*	CK: 9.2 ±0.7 LP: 5.8 ±0.4*	CK: 10.1 ±0.8 LP: 6.2 ±0.5*	CK: 12.5 ±0.7 LP: 6.8 ±0.5*
LAB (log CFU/g)	CK: 7.0 ±0.2 LP: 8.0 ±0.2*	CK: 7.2 ±0.2 LP: 8.6 ±0.2*	CK: 7.3 ±0.2 LP: 9.0 ±0.1*	CK: 7.2 ±0.2 LP: 9.1 ±0.1*
Yeasts & Molds (log CFU/g)	CK: 5.5 ±0.3 LP: 4.2 ±0.2*	CK: 5.6 ±0.3 LP: 3.8 ±0.2*	CK: 5.7 ±0.3 LP: 3.5 ±0.2*	CK: 5.3 ±0.3 LP: 3.2 ±0.2*

CK = Control silage (no inoculant); LP = Silage inoculated with *Lactobacillus plantarum*. ND = Not detected. ** Indicates significant difference from control at $p < 0.05$

4. Discussion

The current research shows that fermentation performance and storage stability were greatly enhanced through inoculation of king grass silage by *Lactobacillus plantarum*, thus indicating that it can be used as an inoculant of silage. The major result was the swift decrease in the pH of the LP-treated silage as opposed to the control. The quick acidification is crucial to the maintenance of the forage nutrients and retard the growth of microorganisms that lead to spoilage, including clostridia and enterobacteria that cause proteolytic growth and the production of butyric acid [2]. The increased rate of pH decrease in LP silage is agreeable to earlier accounts, with *L. plantarum* increasing the production of lactic acid and decreasing the fermentation duration in tropical grass silage (Xie et al., 2023).

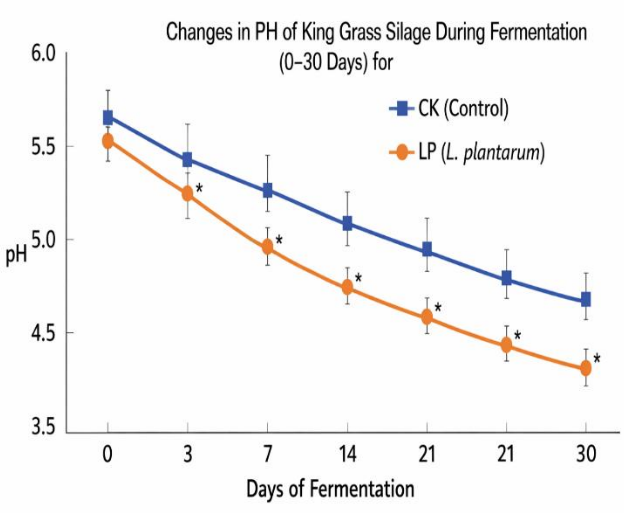

Figure 1. *Changes in pH of king grass silage during fermentation (0–30 days) for CK and LP treatments. (*p < 0.05).*

The high levels of accumulated lactic acid in the LP-treated silage also explain the high quality of fermentation. The main organic acid that caused the reduction of pH during ensiling is lactic acid, and its increased level in inoculated silage presents the idea that *L. plantarum* used available water-soluble carbohydrates (WSC) as substrates efficiently [5]. As a contrast, control silage had lower lactic acid accumulation, indicating the lack of active LABs and slowing down the fermentation kinetics. This observation is similar to the work of other scholars regarding tropical forages, where homofermentative LAB inoculation highly improved the lactic acid level and lowered spoilage possibilities (Wu et al., 2022).

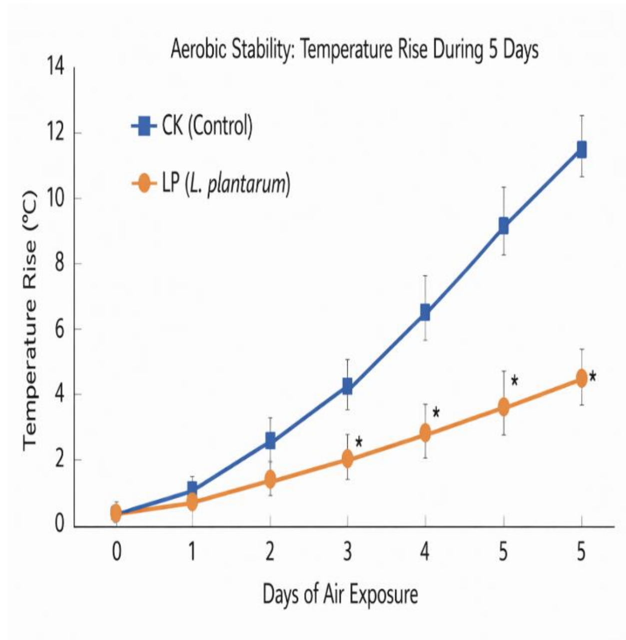

Figure 3. *Aerobic stability: temperature rise during 5 days of air exposure for CK and LP. (*p < 0.05).*

Another significant result was protein preservation as shown by the reduction in ammonia nitrogen (NH 3-N) levels in LP silage. The build-up of NH 3-N is a consequence of the activity of undesirable microbes and high concentrations are linked to nutrient loss and low feed value [9]. Depression of NH 3 -N in LP-treated silage indicates that the quick acidification and LAB supremacy had been able to prevent proteolytic microorganisms. This observation is not isolated because Gao et al. [5], report that inoculation with LAB enhances the retention of nitrogen in silages.

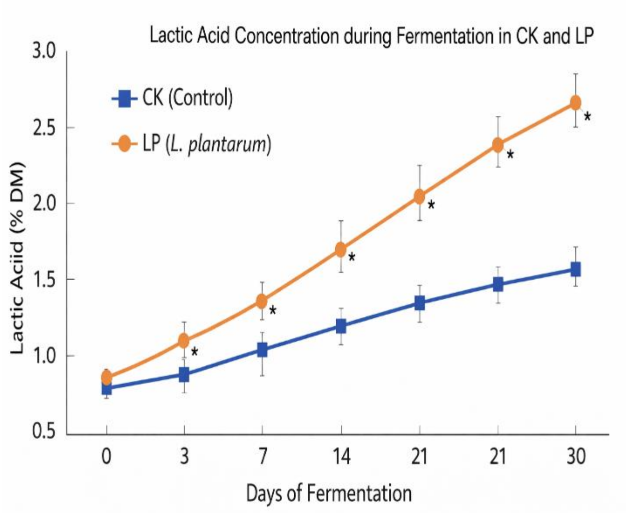

Figure 2. *Lactic acid concentration during fermentation in CK and LP silages. (*$p < 0.05$).*

Microbial dynamics also justify the positive effects of L. plantarum inoculation. The LAB populations were found to be more abundant in the LP silage over the fermentation times and the count of yeast and moulds were always lower than in the control. It is a critical change in microbial ecology that allows the stability of silage during storage and during feed-out. Aerobic spoilage is mostly caused by yeasts and molds which cause heating, loss of nutrients, and visual degradation. LP-treated silage showed an extended ability to resist temperature increase in our aerobic stability tests which showed an improved aerobic stability. The findings have been consistent with past literature, in which *L. plantarum* inoculation enhanced the aerobic stability of tropical grass silages by inhibiting the growth of microorganisms that cause spoilage [5].

The gains that are being experienced with the use of LP in silage also underscore the need to ensure that the inoculant strains used with tropical forage such as king grass adhere to high moisture content and low WSC and large buffering capacity characteristics. These features have the potential to restrain natural fermentation and predispose it to spoilage. *L. plantarum* targeted inoculation guarantees the adequate production of lactic acid, fast pH reduction, and microbial control, which in turn enhances the fermentation and storage results [8,9]

Overall, supplementation with *L. plantarum* seriously contributed to the promotion of fermentation, preservation of nutrients, and aerobic stability of king grass silage. The effect of the rapid acidification, an increased level of Lactic acid, lower level of NH3-N and a manageable microbial populations were all beneficial in improving the quality of silage and increase the storage potential. These findings clearly indicate that *L. plantarum* inoculation is an applicable and effective approach to the enhancement of the preservation and utilization of tropical forage resources [10].

Table 2. Storage Stability and Aerobic Exposure of King Grass Silage

Treatment	**Storage Period (Days)**	**pH**	**Lactic Acid (% DM)**	**LAB (log CFU/g)**	**Yeasts & Molds (log CFU/g)**	**Aerobic Stability (Time to Temp Rise >2°C, h)**	**Visible Spoilage**
CK	0 (end of fermentation)	4.8 ±0.03	1.5 ±0.10	7.2 ±0.2	5.3 ±0.3	–	–
CK	7	4.9 ±0.04	1.4 ±0.08	7.0 ±0.2	5.5 ±0.3	24	Yes
CK	14	5.0 ±0.05	1.3 ±0.08	6.8 ±0.2	5.7 ±0.3	22	Yes
CK	28	5.1 ±0.05	1.2 ±0.07	6.5 ±0.2	5.8 ±0.3	20	Yes
LP	0 (end of fermentation)	4.2 ±0.03	2.8 ±0.12	9.1 ±0.1	3.2 ±0.2	–	–
LP	7	4.3 ±0.03	2.7 ±0.11	9.0 ±0.1	3.3 ±0.2	72	No
LP	14	4.3 ±0.03	2.6 ±0.10	8.9 ±0.1	3.4 ±0.2	70	No
LP	28	4.4 ±0.03	2.5 ±0.10	8.8 ±0.1	3.5 ±0.2	68	No

CK = Control silage (no inoculant); LP = Silage inoculated with *Lactobacillus plantarum*. Aerobic stability = hours until silage temperature rises >2°C above ambient during air exposure. Visible spoilage = presence of mold, off-odor, or texture deterioration.

5. Conclusion

The inoculation of *lactobacillus plantarum* has shown great occurrence of fermentation quality and storage stability in king grass silage by accelerating acidification and enhancing lactic acid, ammonia nitrogen and microbial dynamics. The aerobic stability of silage in LP was better and spoilage was lower which validated the potential usefulness of LAB inoculation of silage to preserve tropical forage. These results justify its application in sustainable livestock feeding systems and indicate the significance of microbial inoculants in enhancing the quality of silage.

References

[1]. dos Santos Nascimento, G., de Souza, T.A.F., da Silva, L.J.R. and Santos, D., 2021. Soil physico-chemical properties, biomass production, and root density in a green manure farming system from tropical ecosystem, North-eastern Brazil. *Journal of Soils and Sediments*, *21*(6), pp.2203-2211.

[2]. Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C. and Kung Jr, L., 2018. Silage review: Recent advances and future uses of silage additives. *Journal of dairy science*, *101*(5), pp.3980-4000.

[3]. Liu, W., Si, Q., Sun, L., Wang, Z., Liu, M., Du, S., Ge, G. and Jia, Y., 2023. Effects of cellulase and xylanase addition on fermentation quality, aerobic stability, and bacteria composition of low water-soluble carbohydrates oat silage. *Fermentation*, *9*(7), p.638.

[4]. Guo, W., Liu, Y., Na, M., Zhang, Y. and Na, R., 2025. Effects of rumen-degradable starch levels on in vitro rumen fermentation and microbial protein synthesis in alfalfa silage. *Fermentation*, *11*(2), p.106.

[5]. Zhao, X., Sun, Y., Chang, Z., Yao, B., Han, Z., Wang, T., Shang, N. and Wang, R., 2024. Innovative lactic acid production techniques driving advances in silage fermentation. *Fermentation*, *10*(10), p.533.

[6]. Wang, X., Song, J., Liu, Z., Zhang, G. and Zhang, Y., 2022. Fermentation quality and microbial community of corn stover or rice straw silage mixed with soybean curd residue. *Animals*, *12*(7), p.919.

[7]. Chen, P., 2021. Lactic acid bacteria in fermented food. In *Advances in probiotics* (pp. 397-416). Academic Press.

[8]. Wu, B., Hu, Z., Wei, M., Yong, M. and Niu, H., 2022. Effects of inoculation of Lactiplantibacillus plantarum and Lentilactobacillus buchneri on fermentation quality, aerobic stability, and microbial community dynamics of wilted Leymus chinensis silage. *Frontiers in Microbiology*, *13*, p.928731.

[9]. Xie, H., Xie, F., Guo, Y., Liang, X., Peng, L., Li, M., Tang, Z., Peng, K. and Yang, C., 2023. Fermentation quality, nutritive value and in vitro ruminal digestion of Napier grass, sugarcane top and their mixed silages prepared using lactic acid bacteria and formic acid. *Grassland Science*, *69*(1), pp.23-32.

[10]. Ni, Q., Ranawana, V., Hayes, H.E., Hayward, N.J., Stead, D. and Raikos, V., 2020. Addition of broad bean hull to wheat flour for the development of high-fiber bread: Effects on physical and nutritional properties. *Foods*, *9*(9), p.1192.