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Research article

Synergy of lactic acid and yogurt blends optimizes napier (*Pennisetum purpureum*) silage by enhancing fermentation quality, nutritional value and field stability

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A B S T R A C T

Ensiling fodder is an effective way of preserving forage quality to alleviate persistent feed shortages. We hypothesized that combining an inoculant of lactic acid bacteria and lactic acid in the Napier *(Pennisetum purpureum)* silage would result in better fermentation quality, efficient dry matter recovery, and increased silage stability under field conditions. Therefore, our objective was to determine the efficacy of yogurt, lactic acid, and their combinations on the yield and nutritional quality of Napier silage. The treatments were designated as T_0 (Control) = Silage without Lactic acid + Yogurt; T_1 = Silage containing Lactic acid @ 1.00 g/kg; T_2 = Silage containing Yogurt @ 10.0 g/kg; T_3 = Silage containing Lactic acid @ 1.00 g/kg + Yogurt @ 10.0 g/kg. After 42 days of ensiling, the pH and chemical composition of the silage were analyzed. The lowest ($P<0.01$) pH and the highest ($P<0.01$) crude protein content were observed in the Yogurt + Lactic acid inoculated silage. Silages inoculated with either yogurt or lactic acid had higher ($P<0.05$) dry matter content than the control. However, the crude fiber, ether extract, and ash contents were lowest ($P<0.01$) in the Yogurt + Lactic acid inoculated silage. It was concluded that combination of lactic acid and yogurt substantially improved the fermentation quality and nutritional value of the prepared silage. It was therefore, recommended that lactic acid and yogurt may help improve silage quality under field conditions.

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1. INTRODUCTION

On-farm feed preservation is crucial for conserving the nutritional quality of feed to prevent the loss of nutrients by microorganisms from unwanted microbial contaminations (Jaipolsaen et al., 2022). The ensiling technique was first developed as a management tool mostly for ruminant production to preserve surplus feed resources from times of overproduction to use at a later time during times of shortage. But lately, its significance has grown, particularly in high-input "zero-grazing" systems that raise animal output per unit of land (Muck et al., 2018). Presently, silage stands as the most extensive fermentation process globally, with an approximate production of 287 million tons inside the European Union. Lactic acid bacteria (LAB) use the process of ensiling to break down carbohydrates in anaerobic conditions primarily into lactic acid and other byproducts like acetic or butyric acid which lowers the pH level, maintains feed value and stops the growth of unwanted microorganisms to prolong the shelf life of forages up to one or two years under typical circumstances (Ferraretto et al., 2015).

The constant supply of high-quality nutrients during periods of underproduction is a universal problem. Silage furnishes high-quality succulent green fodder to livestock round the year throughout the globe. The main components of the inoculants used for ensiling function by reorienting the fermentation pattern of silage in a way that enhances crop preservation. It occurs when the lactic acid bacteria in the inoculant outnumber the lactic acid bacteria of the preserved crop (Fabiszewska et al., 2019). These days, there are two primary kinds of silage inoculants, i. e., *Lactobacillus buchneri*, a hetero-fermentative type, and the classic homofermentative type, which includes *Lactobacillus plantarum*, *Pediococcus* spp., and *Enterococcus faecium*. The homo-fermenters derive their name from the fact that they convert six-carbon sugar molecules into lactic acid as their sole product. Conversely, the hetero-fermenters produce a diverse group of products (Oliveira et al., 2017).

In hetero-fermentation, a 6-carbon sugar can be transformed into one of the following components: one ethanol + one lactic acid + CO_2 or one lactic acid + one acetic acid + CO_2. Lactic acid is a strong organic acid produced by the rumen microbes that efficiently prevents the decomposition of silage (Pang et al., 2011; Addah et al., 2014; Chen et al., 2017; Muck et al., 2018; Carvalho et al., 2021; Kim et al., 2021; Jaipolsaen et al., 2022). On the other hand, acetic acid is a mild acid that functions effectively as a spoiling inhibitor. Ethanol is a somewhat neutral spoilage inhibitor that is partly fermented in the rumen (Jaipolsaen et al., 2022). The lactic acid bacteria further increase the fermentation rate, causing a more rapid decline in pH, with a slightly lower final pH. It can thus be hypothesized that the use of cost-effective hetero-fermentative inoculum may produce better quality silage. To the best of our knowledge, very limited work has so far been done in Bangladesh regarding silage production inoculated with yogurt and lactic acid. The current study, therefore, aims to compare the efficacy of Yogurt, Lactic acid, and their combinations on yield and chemical parameters (p^H, dry matter, crude protein, crude fiber, ash, and ether extract) of Napier (*Pennisetum purpureum*) silage under existing farm conditions in Bangladesh.

2. MATERIALS AND METHODS

Statement of the experiment

The study was carried out in a commercial dairy farm in Chattogram district, Bangladesh from March to June 2023. The financial support was provided by Young Power in Social Action (YPSA).

Study design

Three different types of additives, i.e., yogurt (LAB inoculants), and lactic acid, and their combinations were used to prepare three types of Napier silage. After 42 days, representative samples were taken immediately following harvest. Samples were sent in airtight polythene bags for analysis in the postgraduate laboratory of Chattogram Veterinary and Animal Sciences University. The pH of the silage was measured using a digital microprocessor pH meter (Hanna Instruments, USA). The silage samples were tested for proximate analysis to determine moisture, crude protein, crude fiber, ash, and ether extract (AOAC, 2019).

Statistical analysis

Data were compiled into Microsoft Excel Professional 2020 (Microsoft Corporation, USA). Outliers in the data set were tested by Mahalanobis and Jackknife distances and multicollinearity was checked by the variance inflation factors. The equality of variances in the response variable was tested by the Shapiro-Wilk test. The data were finally used for fitting a generalized linear model (GLM) considering the effect of lactic acid, yogurt, and lactic acid + yogurt as the fixed effect. When the effects were deemed significant ($P<0.05$), the Duncan's New Multiple Range Test (DMRT) was used to compare the means. All statistical analyses were performed using Stata 14.1 SE (Stata Corp LP, College Station, Texas, USA) and SAS JMP Pro 16.2 (SAS Inc., USA). The following statistical model was used:

$$Y_{ijkl} = \mu_i + A_{ij} + B_{ik} + C_{il} \ldots e_{ijkl}$$

Where,

Y_{ijkl} = Observed effects of the trait i for j^{th} lactic acid, k^{th} yogurt, l^{th} lactic acid + yogurt.

μ_i = Overall population mean for the trait i;

A_{ij} = Fixed effects of j^{th} lactic acid for the trait i (0=1,2,…n);

B_{ik} = Fixed effects of k^{th} yogurt for the trait i (0=1,2,…n);

C_{il} = Fixed effects of l^{th} lactic acid + yogurt for the trait i (0=1,2,…n);

e_{ijkl} = Random error which is normally and independently distributed with mean '0' and variance 'σ^2', i.e., $\varepsilon i \sim NID(0,\sigma^2)$.

3. RESULTS

Supplementation of yogurt plus lactic acid substantially reduced (P<0.01) 12.0% silage p^H compared with the control group (Table 1). Similarly, ether extract and ash content decreased (P<0.01) by 14.3% and 22.5%, respectively because of yogurt plus lactic acid supplementation without affecting (P>0.05) crude fiber content of the silage biomass. Unlike, ether extract, ash, and crude fiber, the crude protein content significantly (P<0.01) increased by 33.3% in the yogurt plus lactic acid supplemented group at the expense of dry matter (DM%) compared with control because of vigorous desirable hetero-fermentation (Table 1).

Table 1. Effect of lactic acid, yogurt, and their combinations on p^H and chemical composition of Napier (*Pennisetum purpureum*) silage

Parameter (%)	Treatment[1]				Estimate[2]			
	T_0	T_1	T_2	T_3	SEM	R^2	RMSE	P-value
pH	5.0^a	4.7^b	4.8^b	4.4^c	0.072	0.99	0.02	<0.001
Dry matter	20.6^b	22.2^a	22.8^a	20.4^b	0.403	0.95	0.34	0.004
Crude protein	6.6^b	7.0^b	8.1^a	8.6^a	0.308	0.97	0.19	0.001
Crude fiber	40.0	38.9	38.0	37.2	0.434	0.82	0.69	0.059
Ether extract	2.1^b	$1.9b^c$	2.4^a	1.8^c	0.086	0.98	0.04	0.007
Ash	16.0^a	13.3^b	12.5^c	12.4^c	0.560	0.99	0.15	<0.001

[1]T_0 = Silage without Lactic acid + Yogurt; T_1= Silage containing Lactic acid @ 1.00 g/kg; T_2= Silage containing Yogurt @ 10.0 g/kg; T_3= Silage containing Lactic acid @ 1.00 + Yogurt @ 10.0 g/kg.
[2]SEM = Standard error of the means; R^2 = Regression co-efficient; RMSE = Root mean square error.

4. DISCUSSION

Ensiling, one of the most vibrant and robust globally accepted standard anaerobic preservation technique was introduced mainly in the ruminant production systems to satisfy the scarcity period feed demand by preserving excess feed resources produced in a particular peak season of the year. The lactic acid bacteria (LAB) serve as the most crucial component in achieving successful silage fermentation while silage is widely employed as cattle feed in several countries (Pang et al., 2011; McEniry et al., 2014). The effects of lactic acid, yogurt, and their combinations on the yield and nutritional quality of napier (*Pennisetum purpureum*) silage under existing farm conditions in Bangladesh were investigated. After a field trial, the inoculated napier silage exhibited a considerably lower pH, increased dry matter and crude protein content, and attained the appropriate fermentation quality faster than the non-inoculated silage in the current study.

Recently, several types of additives have been developed to improve the ensiling process of the desired forage biomass. Among them, the biological supplements are useful as they are safe, non-corrosive to machinery, environment friendly, and natural. Currently, bacterial inoculants are being added to the silage to stimulate lactic acid fermentation accelerating the decrease in pH which eventually contributes to the preservation quality of the silage. They generate large amounts of lactic acid in the silage promptly ensuring minimal wastage and consistent stability. The LAB possesses the ability to enhance homo-lactic fermentation resulting in a vigorous production of lactic acid (Chen et al., 2017). Consequently, the pH drops dramatically which contributes to the conservation of nutrients and enhancement of silage fermentation (Muck et al., 2018).

To ensure the production of superior silage and prevent the proliferation of harmful micro-organisms, LABs are frequently used for controlled fermentation (Carvalho et al., 2021). The inoculation of LAB cultures in our study resulted in a substantially lower pH in the napier silage due to increased lactic acid content. These findings appear aligned with previous studies on pasture fermentation which reported that the lactic acid bacteria can efficiently metabolize sugars into lactic acid and exhibit resilience to increased acidity during storage (Alhaag et al., 2019; Bai et al., 2021). Thus, the incorporation of LAB starter during silage production has been proven to be advantageous as it hinders the development of aerobic bacteria and enhances the overall quality of the silage (Nadeau et al., 2000).

In our study, the use of yogurt (LAB culture) improved the characteristics of napier silage. Similar findings from earlier studies using starter culture reported improved fermentation quality of grasses and legumes, although, the scenario differed on corn, sorghum, and sugar cane (Oliveira et al., 2017). In the ensiling process, starter cultures may prevent undesirable microbes and enhance the quality of silage fermentation (Kim et al., 2021). One possible explanation for this may be due to the faster pH drop and lactic acid production. Therefore, growth of Enterobacter in acidic conditions may be hindered. At the same time, *Enterobacter* and numerous LAB might compete for their nutrients throughout the fermentation (Muck et al., 2018) resulting in the population of inoculated silage *Enterobacter* being reduced than that of the non-inoculated.

Ensiling involves the fermentation of organic acids by bacteria to lower the pH, which is a complex procedure (Ding et al., 2020). Compared to other organic acids, lactic acid is a little stronger. hence, in our study, as an aerobic bacteria inhibitor, lactic acid addition decreased the silage pH and increased acidity as opposed to the control group. It indicated that lactic acid was effective in the inhibition of aerobic bacterial activity and stimulation of the hetero-fermentative LAB growth. Therefore, it may be claimed that lactic acid might also be utilized as an additional inoculant in forage biomass to restrain the growth of aerobic bacteria effectively.

In our study, the pH of all the silages decreased substantially during the ensiling process, and the pH levels of the yogurt and LA-inoculated silages were lower than those of the control group. Among the silages, the lowest pH was observed in the silage inoculated with yogurt plus LA ($P<0.01$). The starter cultures were important in napier silage fermentation. In our study, the LAB dominated the fermentation process which was evident from the decreased pH of the inoculated silage (Zhang et al., 2022). This is likely because the sufficient water-soluble carbohydrate content of napier grass accelerated the rapid LA fermentation process (Yang et al., 2020).

The mystery of yogurt culture as a good silage inoculant is the microbes that dominate all the other bacteria (Shao et al., 2007). The added culture incorporated in this study increased the crude protein and dry matter contents of the silage. Hence, silage inoculated with yogurt and LA independently had higher ($P<0.05$) dry matter content than the control. Accordingly, among the treatments the highest protein content ($P<0.01$) was observed in Yogurt plus LA-inoculated silage. The noticeably elevated protein contents in these silages might be due to the added and produced lactic acid from LAB which decreased the pH sharply and eventually prevented the growth of *Clostridium spp*. (Nadeau et al., 2000; Tian et al., 2014). Ammonia nitrogen is typically generated from protein decomposition in silage materials by Clostridium bacteria (Xing et al., 2009). The LAB starter in our study effectively improved the characteristics of silage which was in agreement with a previous study (Shao et al., 2007). A similar result was also reported in another study which demonstrated that adding more fermented juice resulted in more loss of silage crude protein and dry matter (Jin-ling et al., 2013).

Ether extract and ash content decreased ($P<0.01$) in yogurt and LA-inoculated silages, and the crude fiber content of the silage was lowest in yogurt plus lactic acid inoculated silage ($P>0.05$). One of the key objectives of this study was to increase the number of LAB bacteria and boost up their fermentation process so that the abundance and diversity of a specific type of desirable microorganisms were ensured for high-quality silage production (Shao et al., 2007). The utilization of LAB starter cultures played a crucial role in producing high-quality silage from plant material such as napier grass which lacks a significant abundance of LAB bacteria.

Current and future developments

Some studies have recommended that mixed inoculants for silage production may have a more pronounced impact on silage fermentation than the use of a single culture. A series of previous studies have been conducted on the development and evaluation of alternatives to improve the nutritive value of whole-plant silage. These attempts have aimed to maximize both the physical and chemical characteristics of whole-plant silage to optimize dairy profitability. Future investigations ought to focus on more evaluation of the efficacy of probiotic LAB to produce superior silage with low pH and high lactic acid. The establishment of assessment techniques for optimizing harvest efficiency, and nutritional modeling is therefore, recommended.

5. CONCLUSION

The study aimed to elucidate the effects of yogurt, lactic acid, and their combination on p^H, yield, and chemical composition of napier (*Pennisetum purpureum*) silage. It was evident that p^H substantially reduced and the crude protein content significantly increased in the yogurt plus lactic acid supplemented group at the expense of dry matter compared with control because of vigorous desirable hetero-fermentation. It was concluded that the incorporation of locally available, cheap, heterofermentative inoculum, i.e., sour yogurt substantially increased crude protein content and improved other physical attributes of Napier silage. Hence, the idea could be practiced and implemented under field conditions for dairy farmers under the initiatives of the NGOs. More comprehensive intensive studies with wide sample sizes could be carried out in the future to investigate the temporal and spatial pattern of the problem.

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REFERENCES

Addah, W., Baah, J., Okine, E.K., Owens, F.N. and McAllister, T.A. 2014. Effects of chop-length and a ferulic acid esterase-producing inoculant on fermentation and aerobic stability of barley silage, and growth performance of finishing feedlot steers. Animal Feed Science and Technology, 197: 34–46.

Alhaag, H., Yuan, X., Mala, A., Bai, J. and Shao, T. 2019. Fermentation characteristics of Lactobacillus plantarum and Pediococcus species isolated from sweet sorghum silage and their application as silage inoculants. Applied Sciences, 9: 1247.

AOAC, 2019. Official methods of analysis of the AOAC (Association of the Official Agricultural Chemists) International, 21st edn., Rockville, Maryland, USA.

Bai, J., Ding, Z., Ke, W., Xu, D., Wang, M., Huang, W., Zhang, Y., Liu, F. and Guo, X. 2021. Different lactic acid bacteria and their combinations regulated the fermentation process of ensiled alfalfa: ensiling characteristics, dynamics of bacterial community and their functional shifts. Microbial Biotechnology, 14: 1171–1182.

Carvalho, B.F., Sales, G.F.C., Schwan, R.F. and Ávila, C.L.S. 2021. Criteria for lactic acid bacteria screening to enhance silage quality. Journal of Applied Microbiology, 130: 341–355.

Chen, L., Yuan, X. jun, Li, J. feng, Wang, S. ran, Dong, Z. hao and Shao, T. 2017. Effect of lactic acid bacteria and propionic acid on conservation characteristics, aerobic stability and in vitro gas production kinetics and digestibility of whole-crop corn based total mixed ration silage. Journal of Integrative

Agriculture, 16: 1592–1600.

Ding, Z., Bai, J., Xu, D., Li, F., Zhang, Y. and Guo, X. 2020. Microbial Community Dynamics and Natural Fermentation Profiles of Ensiled Alpine Grass Elymus nutans Prepared From Different Regions of the Qinghai-Tibetan Plateau. Frontiers in Microbiology, 11: 1–15.

Fabiszewska, A.U., Zielińska, K.J. and Wróbel, B. 2019. Trends in designing microbial silage quality by biotechnological methods using lactic acid bacteria inoculants: a minireview. World Journal of Microbiology and Biotechnology, 35: 1–8.

Ferraretto, L.F., Crump, P.M. and Shaver, R.D. 2015. Effect of ensiling time and exogenous protease addition to whole-plant corn silage of various hybrids, maturities, and chop lengths on nitrogen fractions and ruminal in vitro starch digestibility. Journal of Dairy Science, 98: 8869–8881.

Jaipolsaen, N., Sangsritavong, S., Uengwetwanit, T., Angthong, P., Plengvidhya, V., Rungrassamee, W. and Yammuenart, S. 2022. Comparison of the Effects of Microbial Inoculants on Fermentation Quality and Microbiota in Napier Grass (Pennisetum purpureum) and Corn (Zea mays L.) Silage. Frontiers in Microbiology, 12: 1–12.

Jin-ling, H., Li-ke, W. and Si-fa, D. 2013. Effects of Previously Fermented Juice on Nutritive Value and Fermentative Quality of Rice Straw Silage. Journal of Northeast Agricultural University, 20: 48–52.

Kim, D., Lee, K.D. and Choi, C. 2021. Role of LAB in silage fermentation: Effect on nutritional quality and organic acid production—An overview. AIMS Agriculture and Food, 6: 216–234.

McEniry, J., Forristal, P. D. and O'Kiely, P. 2014. Factors influencing the conservation characteristics of baled and precision-chop grass silages. Irish Journal of Agricultural and Food Research, 50: 175–188.

Muck, R. E., Nadeau, E. M. G., McAllister, T. A., Contreras-Govea, F. E., Santos, M. C. and Kung, L. 2018. Silage review: Recent advances and future uses of silage additives. Journal of Dairy Science, 101: 3980–4000.

Nadeau, E. M. G., Russellt, J. R. and Buxton, D. R. 2000. Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. Journal of Animal Science, 78: 2980–2989.

Oliveira, A. S., Weinberg, Z. G., Ogunade, I. M., Cervantes, A. A. P., Arriola, K. G., Jiang, Y., Kim, D., Li, X., Gonçalves, M. C. M., Vyas, D. and Adesogan, A. T. 2017. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. Journal of Dairy Science, 100: 4587–4603.

Pang, H., Qin, G., Tan, Z., Li, Z., Wang, Y. and Cai, Y. 2011. Natural populations of lactic acid bacteria associated with silage fermentation as determined by phenotype, 16S ribosomal RNA and recA gene analysis. Systematic and Applied Microbiology, 34: 235–241.

Shao, T., Zhang, L., Shimojo, M. and Masuda, Y. 2007. Fermentation quality of Italian ryegrass (*Lolium multiflorum Lam.*) silages treated with encapsulated-glucose, glucose, sorbic acid and pre-fermented juices. Asian-Australasian Journal of Animal Sciences, 20: 1699–1704.

Tian, J., Yu, Y., Yu, Z., Shao, T., Na, R. and Zhao, M. 2014. Effects of lactic acid bacteria inoculants and cellulase on fermentation quality and in vitro digestibility of Leymus chinensis silage. Grassland Science, 60: 199–205.

Xing, L., Chen, L. J. and Han, L. J. 2009. The effect of an inoculant and enzymes on fermentation and nutritive value of sorghum straw silages. Bioresource Technology, 100: 488–491.

Yang, F., Wang, Y., Zhao, S. and Wang, Y. 2020. Lactobacillus plantarum Inoculants Delay Spoilage of High Moisture Alfalfa Silages by Regulating Bacterial Community Composition.

Zhang, L., Li, X., Wang, S., Zhao, J., Dong, Z., Zhao, Q., Xu, Y., Pan, X. and Shao, T. 2022. Effect of Sorbic Acid, Ethanol, Molasses, Previously Fermented Juice and Combined Additives on Ensiling Characteristics and Nutritive Value of Napiergrass (*Pennisetum purpureum*) Silage. Fermentation, 8: 528.