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Effect of Lactic Acid Bacteria Inoculant on Fermentation Quality of Sweet Sorghum Silage

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***Abstract*—**Sweet sorghum is considered one of the best plants for silage production and is now a more important feed crop in many countries worldwide. It is simple to ensile because of its high water-soluble carbohydrates (WSC) concentration and low buffer capacity. This study investigated the effect of adding *Pediococcus acidilactici* AZZ5 and *Lactobacillus plantarum* AZZ4 isolated from elephant grass on the fermentation quality of sweet sorghum silage. One commercial bacteria *Lactobacillus Plantarum*, Ecosyl MTD/1(CB), and two strains were used as additives *Pediococcus acidilactici* (AZZ5), *Lactobacillus plantarum* subsp. *Plantarum* (AZZ4) at 6 log colony forming units (cfu)/g of fresh sweet sorghum grass in laboratory silos (1000 g). After 15, 30, and 60 days, the silos for each treatment were opened. All of the isolated strains enhanced the silage quality of sweet sorghum silage compared to the control, as evidenced by significantly ($P < 0.05$) lower ammonia nitrogen (NH_3-N) content and undesirable microbial counts, as well as greater lactic acid (LA) contents and lactic acid/acetic acid (LA/AA) ratios. In addition, AZZ4 performed better than all other inoculants during ensiling, as evidenced by a significant ($P < 0.05$) reduction in pH and ammonia-N contents and a significant increase in LA contents.

***Keywords*—**Fermentation, *Lactobacillus plantarum*, lactic acid bacteria, *Pediococcus acidilactic*, sweet sorghum

I. Introduction

IN the past few years, there has been a growing demand for dairy products in numerous developing countries, particularly in the tropical and subtropical areas of Asia and Africa. Nevertheless, the production of silage for dairy farming faces obstacles in these regions due to the ensiling process, which heavily relies on local environmental conditions [1]. The identification and adoption of acid-tolerant, thermophilic lactic acid bacteria (LAB) or homolactic acid fermented LAB as starter strains is required for the stable production of high-quality silage in these environments[2].

Ensiling is a fermented fodder technique for economically feeding dairy cows [3]. Silage is a key source of roughage produced by anaerobic fermentation of fresh grasses. Fresh fodder crops can be stored for an extended period of time without degradation [4]. Sorghum is one of the best plants for ensilage and it is becoming increasingly significant in many parts of the world [5]. Sweet sorghum has a high amount of WSC and a low buffer capacity, making it simple to ensile [6]. Due to its high fodder yield and drought tolerance, sorghum may be a good option for silage production in marginal areas [7]. The addition of inoculant increased silage quality by lowering pH and increasing the population of LAB [8].

On the other hand, sorghum is one of the most appropriate plants for silage production and becoming an increasingly significant forage crop in many regions of the world [5]. Due to its high WSC content and low buffer capacity, it is easy to ensile [6]. In regions with marginal conditions, it could serve as a viable choice for silage production, given its capacity for high fodder yield and resilience to drought [7]. Inoculants have been shown to increase silage quality by lowering pH and increasing the population of LAB [8]

The purpose of this study is to look at how microbial inoculants affect the fermentation quality of sweet sorghum silage.

II. Material and Methods

A. Forage Harvesting

Sweet sorghum (Sorghum bicolor) was grown at the experimental field of Nanjing Agricultural University, Jiangsu, China (Latitude 32°01_19" N, Longitude 118°51_08"E, at altitude 17 m above sea level). The sweet sorghum at dough stage was harvested for the first cutting on 13 October 2017, and chopped manually to an approximate length of 2-3 cm.

B. Silage Preparation

The chopped grasses were inoculated with three isolated strains of LAB, *Pediococcus acidilactici* (AZZ5), *Lactobacillus plantarum* subsp. *plantarum* (AZZ4) and a commercial LAB *Lactobacillus plantarum*, Ecosyl MTD/1 (CB) Ecosyl Product Inc. USA. All strains were isolated from previously fermented juice of elephant grass silage in our laboratory, identified by phenotype, 16S rRNA, and RecA gene analysis, then suspended in 20% glycerol and stored at -20 °C. There were four treatments: (i) no additives as a control, (ii) AZZ5 inoculant, (iii) AZZ4 inoculant, and (iv) CB. The grass was subsequently mixed homogeneously, packed, and compressed manually into approximately 1 L (9.5 cm diameter × 18.7 cm height), and then sealed airtight with a screw top. Lactic acid bacteria inoculant was applied as additives at 1.0×106 CFU/g of fresh material to

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sweet sorghum silage, control treatment was sprayed with equal distilled water. Additives were applied using a hand sprayer by spraying uniformly onto the ensilage material. After treating and thorough mixing, each treated batch was used to fill a silo, which was sealed with a screw top and plastic tape. A total of 36 laboratory silos were made (3 days × 4 treatments × 3 replicates) for each treatment and kept at 25 °C in ambient temperature. Triplicate jars for each treatment were opened on days 15, 30 and 60 of ensiling.

C. Chemical Analyses

The phenol-hypochlorite reaction technique was used to determine the concentration of ammonia-N (NH_3-N) [9]. The pH of fresh grasses and silage was measured using a pH meter. Organic acids such as butyric acid (BA), LA, propionic acid (PA), and AA were investigated using high-performance liquid chromatography according to Liu et al. [10].

D. Microbial Population

The chopped grass was immediately collected for the determination of DM loses, buffer capacity and the population of epiphytic micro-organism. Grass sample (10 g) of wet silage of each sample was added to 90 mL of sterilized saline solution (8.50 g L^{-1} NaCl), completely immersed, and shaken well for 10 min; serial dilutions (10^1 through 10^6) were also prepared with this solution. Decimal dilutions of 10^{-1} to 10^{-6} were prepared from these extracts for microbiological counting. The enumeration of LAB, aerobic bacteria and yeast was carried out by using de Man, Rogosa, and Sharpe agar, nutrient agar, and potato dextrose agar, respectively. The Petri dishes were incubated at 37 ºC, and the bacteria enumeration was done manually and determined for the growth of the microorganism (48 to 72 hours). Finally, the overall microbial data were transformed to log10 and presented based on fresh weight basis.

E. Statistical Analysis

The data of silage fermentation quality, chemical composition and microbial counts were analysed using the General Linear Model (GLM) procedure according to the model for a factorial treatment design as follows: Yij = l + Ii + Tj + I + T) ij + eij, where Yij is the dependent variable; l represents the overall mean; Ii represents the influence of LAB inoculation; Tj represents the effect of fermentation days; (I+T)ij represents the effect of the interaction between LAB inoculation and days; and eij indicates the residual error. The effects were deemed significant at P 0.05, and Turkey's tests were performed to separate the means [11]. All statistical procedures were performed according to the GLM procedure of Statistical Analysis System [10].

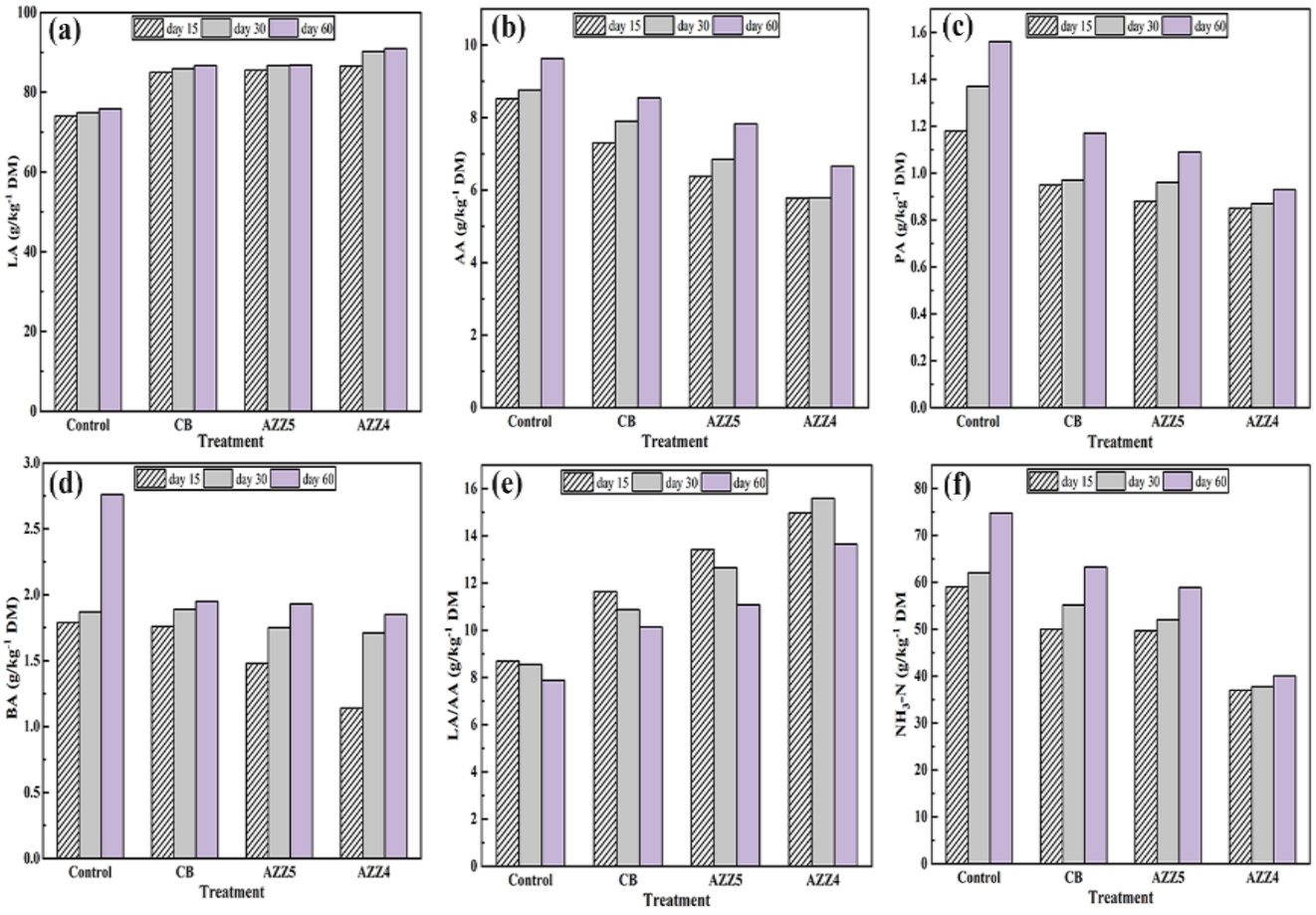

Fig. 1 Effect of LAB on organic acids and ammonia nitrogen of sweet sorghum: (a) LA, (b) AA, (c) PA, (d) BA. (e) LA/AA, (f) NH_3-N, AZZ4: *Lactobacillus plantarum* subsp. *Plantarum*, AZZ5: *Pediococcus acidilactici*, CB: Commercial bacteria

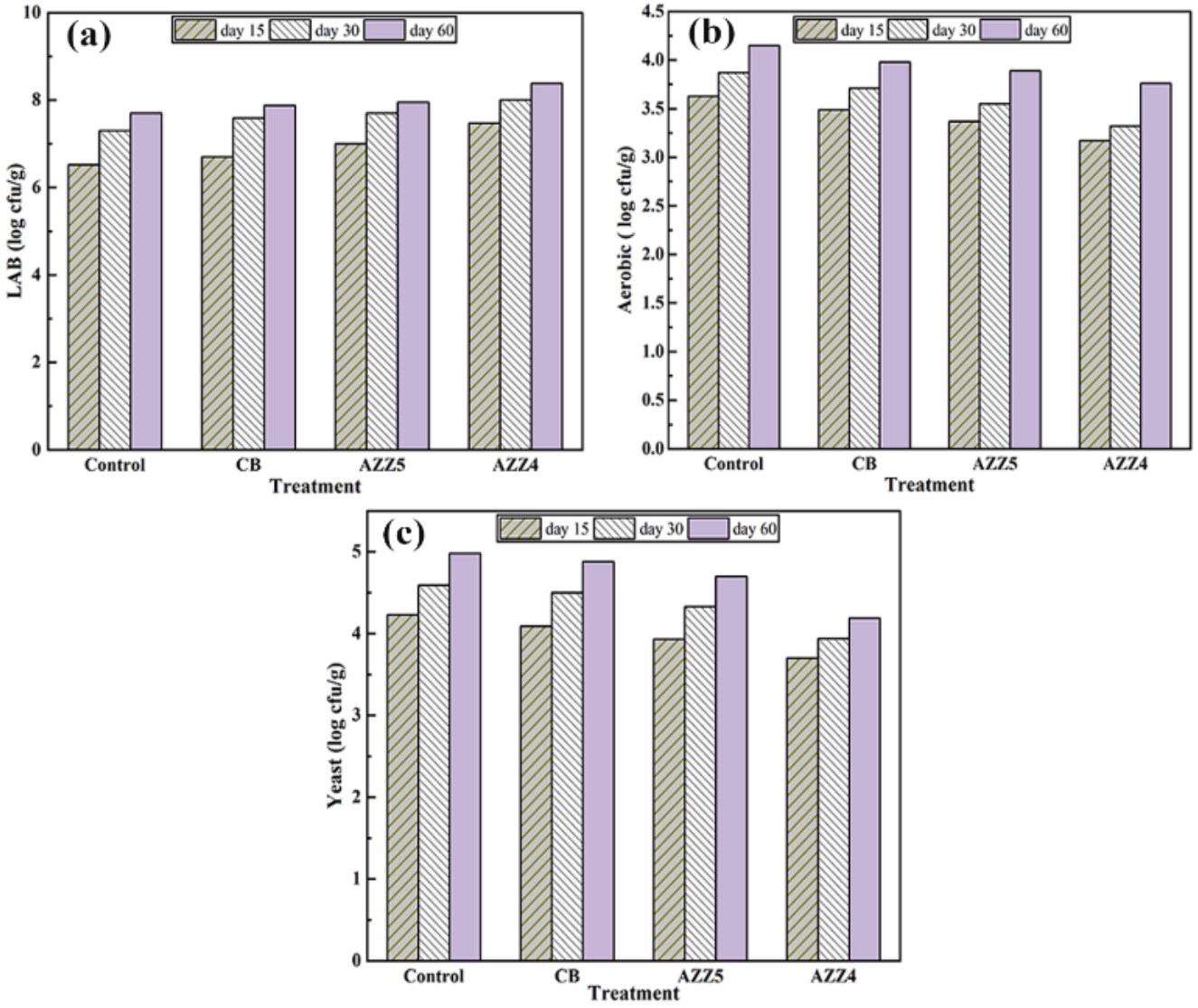

Fig. 2 Effect of LAB on microbial composition of sweet sorghum silage during fermentation period, (a) LAB counts, (b) Aerobic bacteria counts, (c) Yeast counts of sweet sorghum silage. AZZ4: *Lactobacillus plantarum subsp. Plantarum*, AZZ5: *Pediococcus acidilactici*, CB: Commercial bacteria

III. Results and Discussion

A. Effect of LAB on Organic Acids and Ammonia Nitrogen of Sweet Sorghum

Effect of LAB on organic acids and ammonia nitrogen of sweet sorghum silage is shown in Fig. 1. When LAB isolates were added, the level of L.A. increased, resulting in a greater decrease in pH and ammonia content than the control. The AA content of all silages increased from 15 to 60 days of ensiling, while the AA content of inoculated silages was relatively low ($P < 0.05$) than the control. The LA/AA ratios in control silage gradually decreased during ensiling. PA and BA content is increased during ensiling, and inoculated silage contained less PA and BA than the control.

Silage organic acids were affected ($P < 0.05$) by LAB, ensiling day and interaction between them (Fig. 1). The LA content changes of all sweet sorghum silages present the opposite pattern with the pH, and the LA concentration of all silages increased rapidly at the beginning of ensiling. Inoculated silages had higher ($P < 0.05$) LA content compared to the control during ensiling. After 15 days of ensiling silages inoculated with AZZ4 and AZZ5 had higher LA than the CB ($P < 0.05$), the same trend was shown on 60 days of ensiling. The contents of AA of all silages increased from 15 d to 60 days of ensiling, whereas the AA in the inoculated silages were lower ($P < 0.05$) than the control. The ratios of LA/AA in control silage decreased gradually during ensiling. PA and BA contents increased during ensiling and inoculated silage had lower PA and BA content than the control.

B. Effect of LAB on Microbiological Compositions of Sweet Sorghum Silage

The effects of AZZ4 and AZZ5 on the microbiological composition of the sweet sorghum silage after 15, 30 and 60 days of ensiling are shown in Fig. 1. Microbial population was

significantly ($P < 0.05$) affected by LAB isolates, time of fermentation and interaction between them. After 15 days of ensiling, the microbial population of LAB in inoculated silage increased rapidly which was significantly higher than those of the control silage ($P < 0.05$). The count of the total LAB in silage inoculated with AZZ4 and AZZ5 was significantly ($P < 0.05$) higher than those in control, AZZ5 and CB inoculated silage ($P < 0.05$). All of the inoculated silage showed a decreasing trend of the yeast and aerobic population after 15 days of ensiling. Compared with the control and CB inoculated silage, AZZ4 inoculated silage had a significantly ($P < 0.05$) lower yeast and aerobic bacteria count during ensiling.

LA inoculants improved the fermentation quality of sweet sorghum silage by increasing LA production and decreasing pH more quickly. However, in the experiment, all inoculants enhanced fermentation quality by reducing pH rapidly than the control, which is similar to previous research [12], [13] that revealed the pH reduce.in inoculate d maize silage as compared with the control.

During ensiling days, strain AZZ4 generally performed better than other inoculants indicated by higher LA, ratio of LA/AA and LAB count, and lower pH, NH_3-N content, aerobic bacteria and yeasts counts. Hence, a potential explanation for this phenomenon is that strain AZZ4 exhibited noticeably faster rates of growth and acid production compared to other inoculants. Ammonia-nitrogen level reflected the CP degradation in silage, which represents an important parameter for evaluating silage. In this study, all the inoculants decreased the NH_3-N contents compared with the control [14]. It could be related to the rapid reduction in pH caused by the addition of the inoculants, which inhibited the growth and proteolytic activity of micro-organisms such as clostridia [14].

Bacterial inoculants are added to forage during ensiling to promote LA fermentation, hastening the pH reduction and thereby enhancing the preservation of silage [15]. The ensiling fermentation was enhanced by all inoculants, as evidenced by the accelerated pH decrease in sweet sorghum silage. This observation aligns with findings from previous studies [12], [13]. Nevertheless, our current study contradicts the findings of Xing et al. [16], who documented no impact on pH when adding bacterial inoculation during the ensiling of sweet sorghum straw. This may be attributed to the ample content of WSC in sweet sorghum, leading to a swift decline in silage pH even without the use of additives. All LAB isolates exhibited a substantial decrease in pH compared to the control. This phenomenon aligns with the findings of Filya et al. [17], where it was observed that silage inoculants elevated LA production, leading to a significant reduction in pH and minimized dry matter losses. Throughout the ensiling period, strain AZZ4 consistently demonstrated superior performance compared to other inoculants, as evidenced by higher levels of LA, a higher ratio of LA to AA (LA/AA), and LAB count, along with lower pH, NH_3-N content, aerobic bacteria, and yeast counts. Two plausible explanations for this phenomenon exist. One possibility is that strain AZZ4 exhibited noticeably faster rates of growth and acid production than other inoculants. According to McDonald et al. [18], the competitiveness of LAB could be heightened through a combination of faster growth rates and an extended pH range, potentially leading to variations in their competitiveness within the silage. The second potential explanation lies in the broader spectrum of carbohydrate sources available to strain AZZ4 compared to other inoculants. Saarisalo et al. [19] identified that the capacity of LAB to utilize various substrates found in forage crops could confer an advantage in their competition with other microorganisms. Ammonia-nitrogen level reflected the CP degradation in silage, which represents an important parameter for evaluating silage. In this study, all the inoculants decreased the NH_3-N contents compared with the control. It could be related to the rapid reduction in pH caused by the addition of the inoculants, which inhibited the growth and proteolytic activity of micro-organisms such as clostridia [14].

After 15 days of ensiling, the concentration of AA increased and the ratio of LA/AA tended to decrease in the treated silages. This could be due to a shift in fermentation pattern from homofermentation to heterofermentation, which is consistent with other studies such as [20], which discovered a significant shift in LAB activity from homofermentative to heterofermentative after 15 days of ensiling. BA and PA contents were dramatically reduced in all strains. BA levels may have dropped due to the pH drop generated by the addition of the isolates, which may have reduced the growth and proteolytic activity of microorganisms such as clostridia [21]. Lower pH is thought to reduce proteolytic activity in ensiled forage. Kleinschmit et al. [22] discovered similar results for aerobic bacteria populations in silages treated with LAB additions.

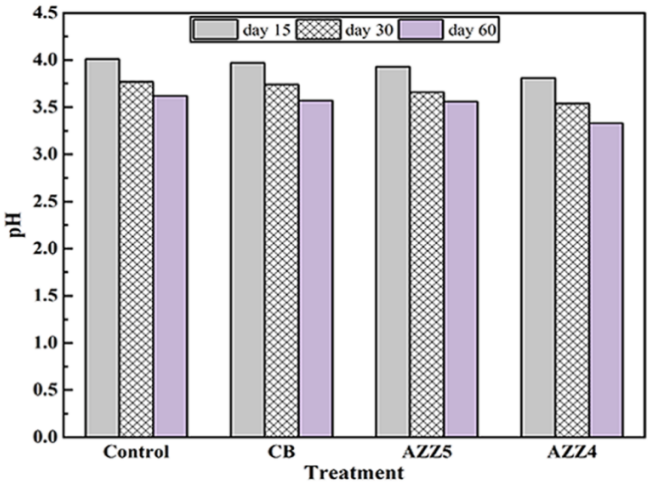

Fig. 3 Effect of LAB on pH of sweet sorghum during fermentation period, C.B.: Commercial bacteria, AZZ5: *Pediococcus acidilactici*, AZZ4: *Lactobacillus plantarum subsp. Plantarum*.

IV. Conclusions

The addition of AZZ1, AZZ4, and AZZ7 as inoculants reduced the pH of the sweet sorghum silages and continued to improve silage quality in this experiment. Inoculants improved fermentation quality by lowering NH_3-N and dry matter losses in sweet sorghum silage.

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