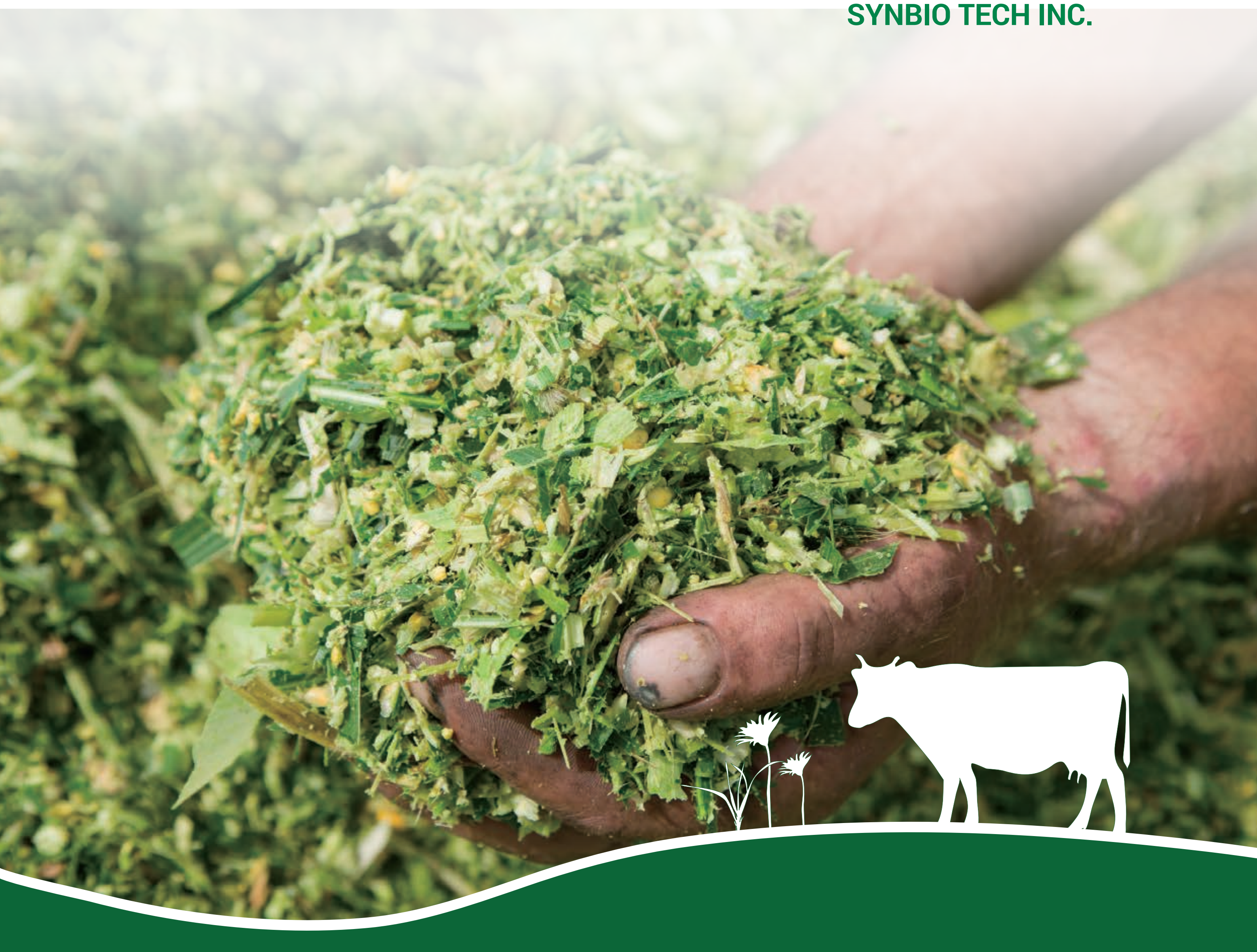
SYNFERM Report

The quality and effects of nature silage and inoculated silage on animal performance

SYNBIO TECH INC.



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Abstract:
The production of silage is very important for preserving fresh forage as a steady and stable feed for animals. The forage, climate, ensiling process, and feed management are all factors that determine the final silage quality. Silage additives, especially the bacterial inoculants that ferment carbohydrates into large amounts of organic acids that reduce the pH, speed up the ensiling process, extend aerobic stability, and improve the nutritional value and palatability of the silage as well as economic performance of the animals.

In order to provide animals with a long-term stable silage of high nutrition content and to avoid fresh fodder decay, the fermentation of fresh grass into silage is very important. However, the silage fermentation process is difficult to control and can result in a significant loss of dry matter. The objective of developing microbial additives is to ensure the quality of fermentation and minimize the loss of dry matter. Additionally, some microbial additives can increase animal feed intake and performance.

1 Effect of Additives on the Quality of Silage

1.1 Factors required for silage fermentation

There are many types of animal feed, of which silage is a type of feed that can achieve long-term preservation and nutrient retention, providing a sustainable and stable supply of feed for animal consumption[1]. Silage production is a dynamic and complex process. The various factors affecting its fermentation quality include water-soluble carbohydrates (WSC), dry matter rate (DM%), buffer capacity (BC), rainfall, temperature, humidity, maturity, and microorganisms. These factors are discussed below:

(1) Water-soluble carbohydrates (WSC):

WSC in plants can be used as a substrate for fermentation and be converted into lactic acid for preservation[2-4]. Different plants contain WSC in varying levels. For example, the WSC content of barley is 10-20% and of corn is 3-10% [2-4]. Additionally, the WSC content decreases with increasing plant maturity.

(2) Buffer capacity (BC):

The crude protein content in plants shows a proportional and positive correlation with BC and the BC affects the pH-lowering ability[5]. Specifically, the higher the BC, the poorer the pH-lowering ability of forage silage, which is disadvantageous for silage preservation. In addition, the coefficient of fermentation (FC) can be used to assess the fermentation ability of grass silage $FC = DM\% + 8WSC/BC$; $FC<35$ is poor, $35<FC<45$ is moderate, and $FC>45$ is excellent. However, the FC is not a complete value; there is a need to also refer to the quantity of lactic acid bacteria attached to the plant. Therefore, the evaluation index for plant silage cannot rely on a single indicator, but must be composed of multiple indicators.

(3) Microorganisms:

a. Lactic acid bacteria:

When the count of lactic acid bacteria is less than 105 colony forming units per gram (CFU/g) fresh grass, the capacity for good fermentation is extremely low even if FC>35[6]. Even though the dry matter and maturity used are the same (i.e., DM 35.8% and 2/3 milk line, respectively), the quantity of lactic acid bacteria attached to the plants are affected by different factors such as temperature, humidity, and rainfall during harvest. Thus, the quantity of lactic acid bacteria can still show significant fold differences[7,8]. In addition to lactic acid bacteria, other microorganisms present on plant surfaces can also affect silage quality. However, lactic acid bacteria can convert soluble carbohydrates into lactic acid, increasing the amount of hydronium ($H+$) ions, lowering the pH, and thereby inhibiting the growth of microbes such as *Clostridium* (Clostridia) and *Enterococcus* (Enterobacteria), which are detrimental to the growth of silage microorganisms and achieving preservation effects [9].

b. *Clostridium* (Clostridia):

These bacteria are present on crops and farmland soil as spores. They can produce butyric acid and degrade proteins in anaerobic conditions, resulting in decreased silage quality and poor palatability.

c. *Enterococcus* (Enterobacteria):

This microorganism produces acetic acid and can also degrade proteins during the silage process. *Enterococcus* carries out heterofermentation, which converts soluble carbohydrates into acetic acid and generates gases such as carbon dioxide, resulting in the decrease of dry matter, nutrient loss, and loss of nutritional value of the fodder.

d. Yeast:

After unsealing silage, which results in it being exposed to air, aerobic microorganisms will start to grow. Yeasts are usually the first microorganisms that are active, resulting in decreased silage stability after unsealing. When the yeast density reaches 10^5 CFU/g DM, it begins to cause silage degradation. The use of yeast species that consume only soluble carbohydrates does not cause changes in quality, but yeasts that can utilize lactic acid as well, such as *Candida albicans* (Candida) and brewer's yeast (*Saccharomyces cerevisiae*) will cause an increase in pH. Spoilage bacteria begin to grow after the pH rises and molds will begin to cause silage decomposition[2,10,11].

Thus, microbial changes in fodder considerably affect silage quality with high variability. Different batches of fodder may have different bacterial, WSC, BC, or DM problems. Therefore, increasing the quantity of lactic acid in the fodder during silage production can improve silage quality and success rate.

1.2 *Lactobacillus* additive usage

Silage additives include enzymes, organic acids, and lactic acid bacteria preparations. Lactic acid bacteria as a biological additive can not only improve the success rate, but can also increase the silage nutritional value. Additionally, it does not burden the environment and is less corrosive, which extends the lifespan of agricultural equipment[12,13]. When the quantity of lactic acid bacteria reaches 10^5 to 10^6 CFU/g, its probability of becoming the dominant species increases[14, 15], and it can quickly produce

large amounts of lactic acid, lower the pH, reduce the loss of dry matter, and retain the nutritional value of plants. Kung recommends the addition of 1×10^5 CFU/g fresh grass[16]. Adding 2-3 times this additive amount can improve the silage quality. However, there were no significant differences in silage quality improvement when the quantity was increased to 1×10^6 CFU/g fresh grass[16].

1.3 Changes in fodder after inoculation

Lactic acid bacteria additives can improve the quality of silage for alfalfa, high moisture corn, forage, and grains. Some examples are listed below:

(1) Changes in pH and organic acid improve silage quality

After alfalfa had withered and turned into semi-dry silage, *Lactobacillus plantarum* strain D41 was added at 4 weeks, 8 weeks, and 12 weeks. The pH in these groups was lower than in the group without the addition of D41. The lactic acid content of the groups with addition of lactic acid bacteria was higher than that of the group without addition. Moreover, groups with the same type of lactic acid bacteria had better performance than the control group[17]. The silage pH values in guinea grass (*Panicum maximum*) after adding *L. plantarum* and *L. rhamnosus* were 4.21 and 4.1, respectively, which was lower than the pH of 5.30 found in natural silage. The addition of bacteria increased the lactic acid content and decreased the acetic acid and butyric acid content[18]. When Lactobacillus was added to barley silage, its lactic acid content was higher than that in the natural group, and had a lower endpoint pH. Addah[7] and McAllister and Hristov[19] obtained similar results in experiments that involved addition of *Lactobacillus* to barley. Corn silage with *Lactobacillus* added may not necessarily increase the organic acid content or decrease the pH compared to natural silage. This is because corn itself typically contains many attached *Lactobacillus*, which could exceed the effects caused by additives[21, 22]. the neutral detergent fiber (NDF) and the acid detergent fiber (ADF) in barley and corn starch are not affected by Lactobacillus[2,7,20], due to the lack of enzymes available to hydrolyze the plant cell wall[2,5,12]. Overall, the use of *Lactobacillus* additives can increase the success rate and consistency of silage quality.

(2) Enhancement of nutritional or economic value

Bolsen[21] pointed out that *Lactobacillus* additives can improve the dry matter recovery rate by 1.3% and also increase overall economic benefits by 1.8%. Furthermore, *Lactobacillus* used in high moisture corn silage can inhibit the growth of spoilage bacteria and improve fermentation quality[23]. Muck and Kung[24] reviewed the literature and reported that the inoculated silage has a lower pH. They also found that 60% of the studies reviewed suggest a decrease in ammonium nitrogen. Additionally, the recovery rate of dry matter increased and the service life of the horizontal silo was extended in 35% of the articles reviewed.

1.4 Stability after unsealing

Silage production is carried out under anaerobic conditions. When silage is opened for feeding, the silage fodder is exposed to air and the anaerobic state is destroyed, especially in the unsealed section. At this time, aerobic microorganisms, particularly yeasts whose fast-growth consumes lactic acid, cause the pH to rise. Molds and other spoilage bacteria become active, leading to silage deterioration[10, 11]. This results in a decline in silage stability and an increase in the proportion of non-nutritional and low-quality forage, thus eliminating the benefits of silage fodder.

Acetic acid is an organic compound with diverse bacteria-inhibiting activities. Heterofermentative lactic acid bacteria such as *L. buchneri* can metabolize carbohydrates and produce lactic acid and acetic acid. This bacterium grows slowly, although it consumes higher amounts of energy during the

saccharides heterofermentation process. The increase in acetic acid has positive effects on aerobic stability and in minimizes losses due to the secondary fermentation of corn silage. Some reports have noted that the homofermentation process shows a negative impact on aerobic stability after unsealing, due to decreased acetic acid content[25]. However, Broberg *et al.*[26] found that silage *Lactobacillus* produces 3-hydroxydecanoic acid that can suppress the growth of *Pichia* (*P. anomala*), *Aspergillus fumigatus* and *Penicillium roqueforti*, thus maintaining the stability of forage after unsealing. Lactic acid bacteria can improve aerobic stability by increasing the amount of lactic acid and metabolism of bacteriostatic substances.

2 Inoculated Silage Has a Positive Effect on Ruminant Animals

Compared to corn silage without any additives, the silage supplemented with *L. plantarum* and *E. faecium* showed improved digestibility of dry and organic matter, feed intake, and production in buffalo, thus significantly increasing production of milk by 0.23 kg/day, total solids by 0.072 kg/day, and fats by 0.035 kg/day. Silage supplemented with bacteria had better fodder feed efficiency and economic benefits[27]. However, if these additives are added directly to the feed as bacterial powder, there is no significant difference in digestibility[28-31]. Therefore, animal performance may have been affected by a metabolite of lactic acid bacteria during silage fermentation.

Muck[32] compared the digestion of natural silage with that of inoculated silage in the rumen and found that inoculated silage reduces gas generation while generating more volatile fatty acids or rumen microorganisms. Beef cattle were observed to gain 3.6 pounds/ton of weight after being fed[33]. Silage treated with *L. plantarum* MTD1 increased 4.8% of feed intake and 4.6% of milk production in beef cattle[34]. Moran and Owen[35] showed that beef cattle can increase dry matter intake by 7.5% and body weight gain by 11.1%. Experiments on sheep also demonstrated that guinea grass silage supplemented with *L. plantarum* and *L. rhamnosus* improved animal performance[18], including increasing dry matter digestibility from 59.4% to 64.1% and 64.4%; organic matter digestibility from 60.2% DM to 64.9% DM and 65.0% DM, independent intake from 638 g DM/day to 885 g DM/day and 928 g DM/day, respectively. Kung and Muck noted that among the published literature, 28% reported a positive response for feed intake, 53% for body weight gain, and 47% for elevated amount of milk[24].

3 Conclusions

The silage fermentation process is difficult to control. The added *Lactobacillus* becomes the dominant species and can increase lactic acid content during fermentation, lowering the pH to achieve preservation and increase dry matter recovery. For raw materials with high moisture, protein content, and buffer capacity, adding *Lactobacillus* may reduce protein degradation and formation of ammonia nitrogen, thus improving silage quality. Feeding on inoculated silage has a positive impact on the animal's weight gain, milk production, dry matter digestibility, raw milk quality, and feed intake. It can also reduce ruminal gases and improve rumen microorganism production of volatile fatty acids, resulting in better animal performance.

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