**Master's Thesis of Science in Agriculture**

# Forage Potential of Proso Millet (*Panicum miliaceum* L.) and Effects of Additives on Silage Fermentation

기장 (***Panicum miliaceum*** **L.**)의 조사료 이용 가능성 및 첨가제 처리가 사일리지 발효에 미치는 영향

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**Shengnan Wei**

**Department of International Agricultural Technology**

**Graduate School of International Agricultural Technology**

**Seoul National University**

# Forage Potential of Proso Millet (*Panicum miliaceum* L.) and Effects of Additives on Silage Fermentation

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By

Shengnan Wei

Supervised by
Prof. Jong Geun Kim

Major of International Agricultural Technology
Department of International Agricultural Technology
Graduate School of International Agricultural Technology
Seoul National University

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Approved as a qualified thesis
for the Degree of Master of Science in Agriculture
by the committee members

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| **Chairman** | **Sang Kee Kang, Ph.D.** | ______________ |
| **Member** | **Jong Geun Kim, Ph.D.** | ______________ |
| **Member** | **Kyoung Hoon Kim, Ph.D.** | ______________ |

# Abstract

Silage corn, sorghum-sudangrass hybrid and proso millet are versatile summer forage crops that can be fed as soilage or conversed as silage. However, in South Korea, proso millet is rarely used as silage to feed ruminants. This experiment was carried out at Pyeongchang, Korea, in order to compare the productivity, the fermentation dynamic and the effects of different additives on silage fermentation of the three forage crops. The studies were conducted from May to December, 2019. Proso millet ("Golden") was sown on June 8th and harvested on September 5th. Silage corn ("Gwangpyeongok") and sorghum-sudangrass hybrid ("Turbo-gold") were planted on May 10th and harvested on September 10th. Yield was significantly affected by crop species. The fresh yield of sorghum-sudangrass hybrid (121,733 kg/ha) was significantly higher than those of proso millet (25,350 kg/ha) and corn (67,557 kg/ha) ($p$<0.05). The highest yield of total digestible nutrients (TDN) was corn (14,378 kg/ha), while the lowest was proso millet (4711 kg/ha).

The fermentation dynamics of proso millet, corn and sorghum-sudangrass hybrid silages were evaluated at 1, 2, 3, 5, 10, 15, 20, 30, and 45 days after ensiling. The results showed that during the ensiling period, the dry matter (DM), crude protein (CP) and water soluble carbohydrate (WSC) content of all crops decreased significantly ($p$<0.05). As the fermentation proceeds, the content of in vitro dry matter digestibility (IVDMD) decreased slightly, and corn was always higher than proso millet and sorghum-sudangrass hybrid. The pH of all crops dropped rapidly in the early stage of fermentation and stabilized in the later stage.

The lactic acid bacteria (LAB) counts of the three crops silage reached the maximum on the 10th day were proso millet 6.90 log10 cfu/g FW, corn 7.77 log10 cfu/g FW and sorghum-sudangrass hybrid 6.95 log10 cfu/g FW. As the ensiling progressed, the lactic acid (LA) and acetic acid (AA) content of the three crop silages increased significantly ($p<0.05$).

For the effect of additives on crop fermentation, treatments included control (without additive), with *Lactobacillus plantarum* (LP, $1.0 \times 10^6$ CFU/g fresh matter), and formic acid (FA, 98%, 5ml/kg). All silages were prepared and stored for 60 days. The results showed that additives had significant effects on improving the fermentation quality of crops, and different additives had different effects on different crops. All additives significantly increased the CP content and IVDMD of silages, and reduced the content of ammonia nitrogen ($NH_3$-N). Compared with the control, whether FA or LP was added, the WSC of the three crops were largely preserved. The WSC in the proso millet treated with FA was the highest. The use of LP significantly increased the LA content of silage, while the use of FA significantly increased the content of AA ($p<0.05$). The highest count of LAB was detected in the treatment of LP in corn.

Based on the results of this study, proso millet is also a good choice for silage. In addition, when preparing silage, formic acid and lactic acid bacteria inoculant improved the quality and fermentation pattern of silage.

**Keywords:** Silage corn, sorghum-sudangrass hybrid, proso millet, productivity, fermentation dynamic, additives, fermentation quality.

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# List of Abbreviations

AA: Acetic acid

ADF: Acid detergent fiber

ADS: Acid detergent solution

BA: Butyric acid

BC: Buffering capacity

BMR: Brown mid-ribs

C: Control

CEC: Cation exchange capacity

CFU: Colony forming unit

CP: Crude protein

DDM: Digestible dry matter

DM: Dry matter

DMI: Dry matter intake

DW: Distilled water

FA: Formic acid

FM: Fresh matter

FW: Fresh weight

GLM: General linear model

HPLC: High performance liquid chromatography

IVDMD: *In vitro* dry matter digestibility

LA: Lactic acid

LAB: Lactic acid bacteria

LP: *Lactobacillus plantarum*

MRS: De Man, Rogosa and Sharpe agar

NDF: Neutral detergent fiber

$NH_3$-N: Ammonia nitrogen

NPN: Non-protein nitrogen

PCA: Plate count agar

PDA: Potato dextrose agar

RFV: Relative feed value

TDN: Total digestible nutrients

TM: Total microorganisms

TN: Total nitrogen

WSC: Water soluble carbohydrate

# 1. Introduction

## 1.1 Research background

South Korea is a country with scarce agricultural resources. Two thirds of its land area is mountains and hills. The cultivated land area only accounts for 22% of the land area. It is one of the countries with the lowest per capital cultivated land area in the world. The livestock industry accounts for almost 40% of total agricultural production in South Korea (Korean Statistical Information Service, 2017). In addition, beef production ranks second (30% of meat produced) among sources of meat production in South Korea. Feed is the most important cost in livestock industry, which often accounts for more than half of the production cost. In fact, the feed costs of Hanwoo (Korean native cattle) and dairy cattle account for 38% and 58% of the total cost of beef production, respectively (Statistics Korea, 2017). Thus, with the development of livestock industry, the forage industry has attracted more and more attention. Forage industry is the basis for the survival and development of livestock industry. However, South Korea's current self-sufficient feed resources are relatively weak, and some feed still needs to be imported from the United States and other countries. As the most basic production source of animal products, feed will affect the sustainable development of the whole livestock industry once there are problems. In order to stabilize livestock industry and production, the production of high-quality forage can reduce feed cost and produce import substitution effect.

Forage has always been an important source of nutrition for livestock. In addition, the content of crude fiber in forage is above 18% in dry matter (Solaiman, 2010). They provide fiber in the diet to improve the proper digestion of feed consuming animals. Forage promotes rumen operation by stimulating saliva secretion. It also maintains normal pH in the rumen, which helps with fiber digestion. Different forages differ in their composition and nutritional value, and their contributions to the productivity of the livestock industry are also different. Decide whether to feed the forage to animal or what kind of forage to feed based on the quality of forage. And the most effective way to judge the quality of forage is animal performance. There are many factors affecting the quality of forage, among which the most important and basic is the forage species and cultivar. Species differences include the difference between grasses and legumes and warm season and cool season grasses. There are other factors, such as any forage quality decline with the increase of maturity. And plants grown at high temperatures produce lower quality forage due to lignification (Buxton et al., 1994).

It is very important for farmers to choose a suitable crop variety. It is necessary to consider not only the high quality of forage crops, but also the economic cost and accessibility of crops. In recent years, corn and sorghum-sudangrass hybrids are the two most common forage crops used to feed animals. They have low production costs, high yields and relatively high nutritional value. Corn and sorghum-sudangrass hybrids are both warm season crops. Warm season crops contain less protein than cool season crops, but the protein may be more efficiently used by animals since a portion of the protein may bypass degradation in the rumen where microbes would utilize some of the protein (Haag, 2019). For South Korea, which is heavily dependent on imported feed at present, it is of great significance to find and develop a new feed. As one of the most valuable grain, proso millet is an important summer crop in Asia and Africa. It is also heavily planted in Jeju, South Korea. According to its characteristics, it is easy to grow and manage, and the requirements for climatic conditions are not strict. Proso millet is a potential development possibility.

The primary methods of harvesting and preserving forage crops include silage, hay making, green chopping and grazing. Silage is a type of animal feed produced by the fermentation of crops or by-products under anaerobic conditions. Hay is grass, legumes, or other herbs that are cut and dried and used as fodder for animals. Compared with hay, the silage making is less affected by weather conditions. More importantly, silage preserves more of the nutrients in the raw material. Silage can reduce nutrition loss (10-15%) (Moran ,2005). More and more attention has been paid to how to produce high quality silage to improve animal production performance. Ensiling time is one of the factors that affect the quality of fermented silage. In the fermentation process, with the increase of ensiling time, the nutritional value of silage will change. Silage with different ensiling time has different quality. Hoffman et al. (2011) reported that the starch-protein matrix was degraded by proteolytic activity over an extended ensiling time. At the same time, the fermentation process of silage is also affected by different factors. In recent years, in order to improve the fermentation quality of silage, various additives are used to promote or inhibit fermentation, reduce fermentation losses, and improve animal performance.

## 1.2 Aim of research

The aim of this thesis consists of two parts. The one is to compare proso millet as a new crop with the main commonly used forage crops. To study the fermentation dynamic of these crops and compare their changes of feed nutritional value with the increase of ensiling time. Second, how to improve the fermentation quality is also very important. Different types of additives have different effects on the fermentation process of silage. Compare the effects of different additives on the fermentation of these crops. To obtain better quality silage and improve animal performance.

# 2. Literature review

## 2.1 Forage crop

In the diet of ruminants, forage provides important nutrients and is a key factor affecting ruminant productivity. The most direct and effective method of judging forage quality is the performance of ruminants. The species of forage crops in the world is highly diverse and widely distributed, and the quality of forage crops varies with the species of forage crop, climate, soil, etc. Species differences include the difference between grasses and legumes and warm season and cool season grasses. The nutrient content of grasses and legumes varies depending on many factors, such as species, maturity, fertilization and soil fertility, growth environment and harvest conditions. Grasses contain higher concentrations of acid detergent fiber and neutral detergent fiber than legumes (Amiri, 2012). The crude protein (CP) concentration of legumes is higher than grasses. Compared with warm season grasses, cool season grasses contain higher water soluble carbohydrates.

### 2.1.1 Silage corn

Corn (*Zea mays* L.) is a world famous cereal used mainly in food, livestock feed and industrial raw materials. Corn is also known as the king of the grass with global annual production exceeds wheat and rice. Also due to its high productivity under various climate, corn is the world's most widely grown crop (USDA, 2018). Fresh matter yields of corn green fodder range from 10 to 50 t/ha (FAO, 2016). Corn is warm season grass, compared with cool season grass, corn has some advantages in capturing solar energy in warm weather. Silage corn is a high-yield crop that makes good use of nutrients in the soil. Among the forage crops, the crop with the highest yield is corn, which has excellent productivity and feed value, especially when silage is prepared, corn has high sugar content, good fermentation quality and high palatability of livestock (Kim, 1991). And it is the most common silage crop in Korea even in the world because it has a high and easily digestible carbohydrate content and a suitable buffering capacity. This crop can be used as an alternative source of silage in cold and dry areas (Crovetto et al., 1998). Compared with other feed grains, corn is lower in protein and slightly higher in energy. Corn contains approximately 72 percent starch on a dry-matter basis (Lardy, 2002). Due to the high energy content, with the increase of silage corn planting area, milk production increased significantly (Fitzgerald et al., 1999).

### 2.1.2 Sorghum - sudangrass hybrids

Sorghum-sudangrass hybrids (*Sorghum bicolor x S. bicolor* L.) are abundant among various grasses. Sorghum-sudangrass hybrids are also warm season plants with high drought and disease resistance. This crop is a hybrid of forage-type sorghum and sudangrass. Under water and temperature stress, sorghum-sudangrass hybrids show high production potential to accumulate green and dry matter (Kikindonov et al., 2015). They can be used by livestock for harvesting or harvested as hay or silage. Compared to corn, they have a smaller leaf area, more secondary roots and a more waxy leaf surface, and these features help them better withstand drought (Sarrantonio, 1994). Sorghum-sudangrass hybrids can efficiently use sunlight and moisture in the soil, thereby rapidly accumulating large amounts of biomass. They also can effectively increase soil organic matter content in sunny areas (Valenzuela et al., 2002). They are also highly and stable productive, and their products are good and nutritious. Sorghum-sudangrass hybrids generally have total digestible nutrient values in excess of 53%–60% and crude protein concentrations of 9%–15% (Dennis Hancock, 2020). Due to its high unit yield, it is one of the most cultivated species in the world. Research shows that sorghum-sudangrass hybrids with brown midribs (BMR) have higher fiber digestibility (Dann et al., 2008). Sorghum-sudangrass hybrids are relatively easy to obtain and stable returns are possible. They have good palatability and digestibility, increases feed intake and improves animal performance.

### 2.1.3 Proso Millet

Proso millet is of the genus *Panicum miliaceium*. The proso plant is considered a short-day plant and usually an erect annual, 30- to 100-cm tall, with few tillers and an adventitious root system (Baltensperger, 2002). Compared with sorghum-sudangrass hybrids, the nutritional value and yield of proso millet are slightly inferior. However, it may be more suitable for cool, poorly drained soils, and they also can tolerate lower pH soils. In addition to adapting to poorly drained soils, proso millet is also hardy under drought conditions. Like sudangrass, some millet varieties will regrow after harvest. It has no risk of livestock poisoning due to prussic acid toxicity. Proso millet has high drought tolerance and strong resistance to many diseases affecting corn (Kumar et al., 1993). Proso millet is high in nutrition and dietary fiber. They are good sources of protein, micronutrients and phytochemicals. Studies have shown millet contains 7-12% protein, 2-5% fat, 65-75% carbohydrates, and 15-20% dietary fiber (Dayakar et al., 2017). The essential amino acids of proso millet protein are better than those of corn and other grains.

Corn, sorghum-sudangrass hybrids and proso millet produce less lignin and are therefore usually more digestible. In addition to the nutrients, these crops are cheap and easy for farmers to obtain, which can lead to greater profits.

## 2.2 Preservation method

With the development of livestock industry, the demand of feed for ruminant is increasing. Grazing is one of the ways for livestock to get forage. However, the following problems are the shortage of livestock feed in non-forage growing season and the low forage production efficiency that cannot meet the demand of livestock. In this case, the preservation of feed is the key to solve this problem (Muck et al., 2001).

Forage preservation has been defined as " the preservation of forage plant material to provide feed for livestock at a time after the primary period of growth of these plant" (Gallaher et al., 2000). Providing feed for deficit seasons has been an important component of increasing agricultural production. Also in agriculture, the main goal of forage preservation is to produce an idealized product that is very close to the original herbage in forage value, with low nutrient loss and good palatability. The preservation methods of forage include ensiling and haymaking. Ensiling is a process in which the green fodder is fermented to produce acid under anaerobic conditions to achieve the preservation effect. Haymaking is the process of reducing the moisture in green fodder without spoilage during storage. Deciding which method to use varies by region, by climate. More needs to be considered in combination with forage crop species, feed value, cost, yield and technology. Haymaking is more dependent on weather conditions than ensiling. For example, in areas with high humidity or bad weather conditions, hay production will cause higher harvesting losses due to the forage can't get to the right level of dryness. Ensiling avoids most of the harvesting losses encountered in haymaking because ensiling is treated at a higher moisture level, where it is less susceptible to mechanical losses. At the same time, because of the lower moisture content of hay, it avoids a series of losses and spoilage caused by respiration and fermentation during storage. Due to frequent and heavy rainfall in the eastern mountain area of

South Korea, silage has been a more common forage preservation practice (Li et al., 2017).

Ensiling is a technique for acidifying and preserving forage crop under anaerobic conditions (Ramos et al., 2016). It is a widely used method for preserving forages (Wilkinson and Toivonen, 2003). The most important thing that must occur during ensiling is to ensure an oxygen free environment. In this anaerobic environment, bacteria control the fermentation process. Because it prevents the growth of unwanted aerobic bacteria, yeasts, and molds that compete with beneficial bacteria for substrate. The basic principle of ensiling is to convert the sugar in forage crop into lactic acid and reduce the pH of crop to around 4.0 or lower. This effectively reduces the possibility of forage crop spoilage. In order to obtain the best fermentation effect, forage crops should have high content of soluble carbohydrate to provide fermentation substrate.

In the fermentation process, microorganisms are the most critical, especially lactic acid bacteria (LAB). Lactic acid is produced by LAB through fermentation, thus reducing the pH. The low pH limits plant enzymatic activity. At the same time, the clostridia bacteria are an equally important bacterium because it causes higher dry matter loss.

High quality silage can be better maintaining the nutritional composition of raw materials. During ensiling, the fermentation of forage is affected by many factors, just like the harvest time, the harvest stage, the extent of chopping, the moisture content, the temperature and so on. For the harvest time, the composition of the forage changes daily (Greenfield et al., 1974; Lechtenberg et al., 1971). For the same crop, the water soluble carbohydrate content in the late afternoon is higher than in the morning (Cheeke et al., 2010).

As a preservation method, the nutritional value of ensiling depends on the quality of the forage used and the efficiency of the preservation process, especially in terms of preventing nutrient loss.

## 2.3 Silage fermentation

Silage is produced by fermenting fresh grass containing sufficient water soluble carbohydrates under anaerobic conditions. It is produced by sugar fermentation bacteria naturally present on the surface of the forage to produce acid to prevent the forage from rotting.

### 2.3.1 Ensiling process

Efficient fermentation is designed to create a more palatable and digestible feed which encourages dry matter intake and improves performance. Five phases occur during the silage fermentation process:

A. Aerobic state: This phase begins at the time of harvest. At this stage, microorganisms such as molds and yeasts continue to consume oxygen and water-soluble carbohydrates and release carbon dioxide, water and heat. This phase usually lasts several hours until oxygen is depleted.

Plant enzymes are also very active at this stage, causing proteins to be first reduced to amino acids, then to amines, and finally to ammonia. During this process, up to half of the plant protein may be broken down (Shabtai Bittman, 1999). Make the pH value of fresh crops within an appropriate range (pH 6.0-6.5) (Moran, 2005).

B. Anaerobic state: It starts when the trapped oxygen is exhausted and lasts differently due to different crops and different fermentation environments. At this stage, anaerobic fermentation occurs. The main bacteria at this stage is Enterobacter. They produce both acetic and lactic acid. Although they can reduce pH, they take longer and cause nutrients loss. When the pH drops below 5, homo-fermenters predominate and silage fermentation begins.

C. Fermentation state: The process at this stage is performed under anaerobic conditions. This stage is mainly dominated by lactic acid bacteria. Lactic acid bacteria convert WSC into lactic acid, which lowers the pH of the silage and helps to preserve the silage. This is the longest stage in the ensiling process because it continues until the pH (around 4.0) of the silage is sufficient to inhibit the growth of all bacteria (Rocky Lemus, 2010).

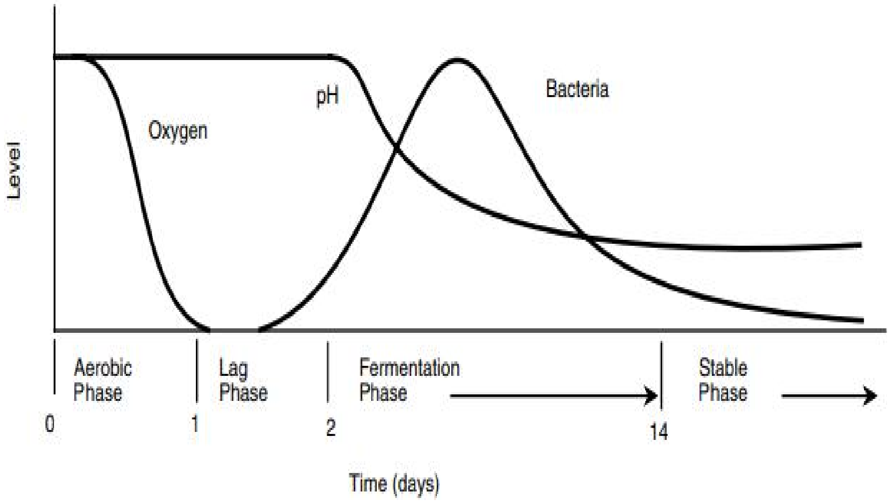


Figure 1. The phase of silage fermentation (Pitt, 1990)

D. Stable state: When the pH decreases and as long as oxygen and water do not penetrate the silage, the fermentation process is stable. Stable in pH 3.5~4.2.

E. Feeding state: After 45~72days, the fermentation is stable, and then open the silage to feed out. The silage is exposed to oxygen, which causes the secondary aerobic degradation of the forage by microorganisms, and also promotes the growth of yeast and fungi (Fransen, 2013). To minimize losses, silage should be used as quickly as possible once it has been removed from the silo.

### 2.3.2 Chemical changes of nutrients

Chemical changes are inevitable during ensiling due to the conversion of soluble carbohydrates into organic acids and the degradation of fresh crop fibers and proteins. With the progress of ensiling, the content of water-soluble carbohydrates is decreasing. Corresponding to this is the increase in organic acid content which can effectively reduce the pH and inhibit the growth of bad microorganisms. During ensiling, proteolytic plant enzymes degrade proteins into non-protein nitrogen (NPN) compounds under aerobic conditions (Gasior et al., 2002).

When the ensiling time is long, the loss and change of nutritional value are greater. Overall, the fermentation phase is considered to last from 7 to 45 days. However, fermentation will continue as ensiling continues. In general, prolonged storage resulted in considerable dry matter losses. After the main fermentation phase, the stable phase is entered, during which the silage undergoes a further slight but continuous fermentation (Pahlow et al., 2003). Therefore, with the prolongation of ensiling time, not only the fermentation products are increased, but storage loss is also increased. In the experiment of Saricicek et al. (2016), they found the silage was stable for the first 90 and 104 days of ensiling, whereas, after the 118th days of ensiling, a decline in the stability was observed with the prolonged ensiling time.

## 2.4 Silage microbiology

Silage is a complex microbial symbiotic system. Microorganisms play a leading role in the fermentation of silage and determine the quality of the silage.

### 2.4.1 Lactic acid bacteria

Lactic acid bacteria play an important role in the preservation of green fodder crops. There are many types of lactic acid bacteria, the most common are *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Streptoccus* (Pahlow et al., 2003) and *Weissella* (Cai et al., 1998). The lactic acid bacteria can be divided into homofermentative lactic bacteria and heterofermentative lactic bacteria.

The homofermentative lactic bacteria convert glucose to lactic acid.

$$C_6H_{12}O_6 \text{ (Sugar)} \rightarrow 2C_3H_6O_3 \text{ (Lactic acid )}$$

The heterofermentative lactic bacteria convert sugars to a range of products like lactic acid, ethanol, and acetic acid.

$$C_5H_{10}O_5 \rightarrow C_3H_6O_3 \text{ (Lactic acid )} + C_2H_4O_2 \text{ (acetic acid)}$$

$$C_6H_{12}O_6 \text{ (Sugar)} \rightarrow C_3H_6O_3 \text{ (Lactic acid )} + C_2H_5OH \text{ (ethanol)} + CO_2$$

$$C_6H_{12}O_6 + H_2O \rightarrow C_3H_6O_3 \text{ (Lactic acid )} + C_2H_5OH \text{ (ethanol)}$$

$$+2C_6H_{14}O_6 \text{ (Mannitol)}$$

Fermentation of homofermentative bacteria is preferred because lactic acid reduces pH more effectively than does acetic acid and because it avoids dry matter loss caused by gas production via heterofermentative bacteria. The production of lactic acid effectively reduces the pH of the silage in the initial of fermentation and helps to inhibit the growth of harmful microorganisms. When the pH drops to a certain level, it also inhibits its own growth.

### 2.4.2 Enterobacteria

Enterobacteria is a facultative anaerobic microorganism that competes with lactic acid bacteria for available carbohydrates. Enterobacter is preferred when the pH is neutral. So in the early stage of fermentation, enterobacter is more active. As the pH drops below 5, enterobacteria will decrease sharply. The main product of enterobacter is acetic acid. Compared with lactic acid, acetic acid is a weak acid, and it takes longer to lower the pH. During this period, it took a long time to suppress the growth of undesirable microorganisms in time, causing unnecessary losses. Although acetic acid can improve aerobic stability, it is less palatable to livestock than silage dominated by lactic acid (Bolsen, 1955).

### 2.4.3 Yeasts and Molds

Yeasts in silage are undesirable microorganisms. The growth of yeast can cause heating, loss of dry matter (DM), nutrients and energy. Under aerobic conditions, yeasts decompose lactic acid into carbon dioxide and water, which leads to an increase of pH in silage and the growth of microorganisms such as mold. Under anaerobic conditions, yeasts compete with lactic acid bacteria to use sugar to ferment sugar to ethanol and carbon dioxide. Ethanol production also negatively affects the taste of milk (Randby et al., 1999).

Mold is an aerobic microorganism. In the presence of oxygen and increased acidity, molds grow significantly. Molds can utilize the glucose in respiration and degrade the fiber and protein. Mycotoxins are products of the secondary metabolism of molds. Mycotoxins-containing silages can cause serious damage if consumed by animals, such as fatal poisoning and subclinical symptoms, including suppressed immune systems and hormonal imbalance (Bennett et al., 2003; Vila-Donat et al., 2018).

### 2.4.4 Clostridia

Clostridia are anaerobic bacteria which ferment carbohydrates, organic acids, proteins and amino acids, producing ammonia, reducing the availability of silage for livestock. Clostridia can be divided into two groups: one mainly consumes glucose and organic acid, such as C. *tryobutyricum and* C. *sphenoides;* the other consumes protein like C. *bifermentants*. Clostridia is particularly sensitive to the availability of water, and in very wet crops, even a pH as low as 4.0 does not inhibit its growth (Driehuis et al., 2000). The main product of clostridia is butyric acid, which not only has a pungent odor, reduces the palatability of silage, but also increases the pH of the silage. Silage with butyric acid content exceeding 5g / kg dry matter content and high ammonia and amine content are typical clostridium silage (McPherson et al., 1966).

$$2C_3H_6O_3 \text{ ( Lactic acid ) } \rightarrow CH_3CH_2CH_2\text{-}COOH \text{ ( Butyric acid ) } + 2H_2 + 2CO_2 + \text{ Heat}$$

### 2.4.5 Aerobic bacteria

Aerobic bacteria are present on plants and can damage the quality of silage due to respiratory metabolism. They degrade the glucose to water and $CO_2$ which result in dry matter loss. Aerobic bacteria are more active in the early stages of silage until oxygen is depleted.

## 2.5 Silage additive

At present, in order to improve the fermentation quality of silage and reduce the nutrients loss of raw materials during ensiling process, silage additives are widely used. The ideal silage additive is safe, can reduce dry matter loss, improve silage quality, limit secondary fermentation, etc (Merensalmi et al., 1991). It's worth noting that silage additive can only improve the quality of silage to a certain extent, but cannot turn the poor quality silage into good quality silage. Silage additive can be categorized as fermentation stimulants, fermentation inhibitors, aerobic deterioration inhibitors, nutrients, and absorbents (McDonald et al., 1991).

### 2.5.1 Fermentation stimulants

Fermentation stimulants include inoculants, carbohydrates sugar sources and enzymes. The aim of them is to improving fermentation or increasing the production of lactic acid.

Microbial inoculant is now the predominant technology employed to influence the silage fermentation. A small amount of lactic acid bacteria exists naturally on raw materials. The addition of lactic acid bacteria during silage fermentation process can promote the rapid reproduction of lactic acid bacteria, produce a large amount of lactic acid, lower the pH, thereby resist the activities of harmful microorganisms, reduce the loss of dry matter, and obtain ideal silage.

Homo-fermentative inoculants such as *Lactobacillus plantarum* can rapidly produce a large amount of lactic acid, reducing the pH value below 4.0, thus effectively inhibiting the growth of other microorganisms, to ensure the safety and quality of silage. In about half of the experiments, Kung and Muck (1997) found that homo-fermentative inoculants can improve animal performance by 3 to 5%. Tao (2005) inoculated with lactic acid bacteria for silage, compared with the non-inoculated silage, the pH decreased significantly, and the quality of the obtained silage were improved.

Hetero-fermentative inoculants like *Lactobacillus buchneri* can convert lactic acid to acetic acid and other products. And high concentration of acetic acid can inhibit the growth of mold. Also have research (Kung et al., 1999) observed that the aerobic stability in high moisture corn had been improved when it was treated with *Lactobacillus buchneri*. Microbial inoculants should be kept refrigerated for maximum survival when used.

Enzymes include amylases, cellulases and hemicellulases. The purpose of adding the enzyme is to reduce the fiber content in the silage, so as to improve the digestibility of the ruminants. In addition, enzymes can degrade more sugar for fermentation by lactic acid bacteria. Enzymes also contribute to the degradation of acid detergent fibers and neutral detergent fibers, thus improving lactic acid fermentation, dry matter recovery and animal performance. Sanchez et al.(1996) reported that cellulase enzymes addition was improving fiber degradation during silage fermentation.

Molasses and sugars are added to the silage as carbohydrate to additional fermentation substrate to support the growth of lactic acid bacteria. Research by scholars has shown that silage with added molasses has better appearance quality, and molasses significantly improves the quality of silage (Arbabi et al., 2008).

### 2.5.2 Fermentation inhibitors

Fermentation inhibitors are mainly acids, organic salts and other chemical inhibitors.

Among them, formic acid and propionic acid are widely used as acids. When acid is added to the silage, the pH of the silage decreases rapidly, which can inhibit the activity of microorganisms and plant enzymes, and reduce the fermentation loss of protein and carbohydrates. Adding formic acid for silage can effectively inhibit the fungal and plant enzyme activities in the silage and reduce protein loss (Wilson et al., 1973). At the same time, studies have shown that the silage color after adding formic acid is more green and had a fragrant odor. Considering the corrosiveness of the acid and the effect on animal performance, a reasonable amount of addition is also necessary consideration. There are reports (Silveira et al., 1973) that elephant grass treated with 0.5% formic acid not only has improved fermentation, but also has higher intake and digestibility compared to the untreated control.

Formaldehyde can inhibit harmful bacteria and prevent spoilage. In the rumen, the protein in the silage is directly decomposed into ammonia and consumed. Formaldehyde can be combined with the protein in the silage to form a difficult-to-dissolve complex, which can prevent the decomposing protein from rumen microorganisms, thereby increasing livestock absorption and utilization of protein.

### 2.5.3 Aerobic deterioration inhibitors

We know that in the storage of silage, it is impossible to completely remove air, so we need to minimize the aerobic degradation of silage. Obviously, in order to suppress aerobic spoilage, the activities and growth of spoilage organisms, especially microorganisms such as yeasts that cause spoilage, must be suppressed. Some additives include chemical additives based on volatile fatty acids such as propionic acid and acetic acid, and biological additives based on bacteriocin-producing microorganisms such as *Lactobacillus* and *Bacillus* have been shown to help improve aerobic stability (Peter McDonald et al., 1991; Woolford, 1975). Studies have shown that compared with lactic acid, propionic acid and acetic acid have better yeast inhibition, and a mixture of lactic acid and propionic acid or acetic acid has a synergistic inhibitory effect (Moon, 1983).

### 2.5.4 Nutrients

Common nutrients include urea, ammonia, and minerals. Their use is mainly due to the lack of certain nutrients in the silage, which can improve the fermentation quality of the silage. For example, the addition of ammonia can increase the content of crude protein in silage. If phosphoric acid and calcium powder are added to the silage, the calcium and phosphorus needs of livestock growth and development can be well satisfied.

### 2.5.5 Absorbents

When crops with high moisture content are used for silage, there is a lot of effluent from the silage, which will not only cause loss of nutrients, but also have certain pollution. So using absorbent to reduce effluent is also one of the methods to ensure better fermentation of silage. Grains, straw, sugar beet pulp, etc. can be used as adsorbents. Sugar beet pulp has a good water adsorption capacity, which can not only reduce the effluent of the silage, but also increase the feed intake of the silage. When the researcher examined the effect of inclusion of sugar beet pulp with grass at ensiling, the effluent of silage is greatly reduced (Ferris et al., 1990).

## 2.6 Evaluation of silage quality

After obtaining the silage, we need to determine its fermentation quality. The evaluation of the fermentation quality of silage includes many indicators, including acidity, dry matter, crude protein, organic acid content and so on.

The Deutche Lan Dwirtschafts Geseutschaft (DLG) has its own evaluation indicators for the quality of silage (Wei and Jingkun, 1994), including the content of butyric acid, ammonia nitrogen, the acidity, acetic acid and propionic acid, the deterioration ratio of bacteria and mold, and the feeding effect is mainly determination of silage digestibility.

The lactic, acetic and butyric acid were used to assess the quality of silages according to the Flieg-Zimmer scale (Podkowka., 1978.). German scientists Flieg proposed this method for scoring silage in 1938, which was modified by Zimmers and is widely used until now.

The quality assessment of silage in Japan includes four parts (Liu, 2009): sensory evaluation, chemical method evaluation (acidity, ammonia nitrogen content, volatile fatty acid as a proportion of total acid, etc.), the content of various bacteria in microorganisms and the livestock evaluation method (feed intake, digestibility, milk yield).

Sensory evaluation mainly includes color, odor, taste, etc. Silage color can indicate potential fermentation problems. Brown to black silage usually indicates heating from fermentation and moisture damage (Greub and Cosgrove, 2006). The content of different indicators represents different levels of fermentation. For the high quality silage, pH should be around 4.0. The high ammonia nitrogen content in the silage indicates a large amount of protein degradation during the fermentation process and the poor fermentation of the silage. In good silage, the butyric acid levels should be low, which has bad effects on the silage and the feed intake of ruminants (Oetzel, 2007).

# 3. Materials and Methods

## 3.1 General information

This experiment was conducted at the experimental field of Seoul National University, Pyeongchang Campus during the 2019 summer season (located at 37° 32’ 40’’ N, 128° 26’ 33’’ E, where, average altitude is about 550m above sea level, more information is registered as annual mean temperature 12.1 ℃, average annual precipitation 69.2 mm, average annual humidity 67.7 %, Sin-ri, Pyeongchang, Republic of Korea). More detail meteorological information involved during the experimental period are shown Figure 2 and 3.

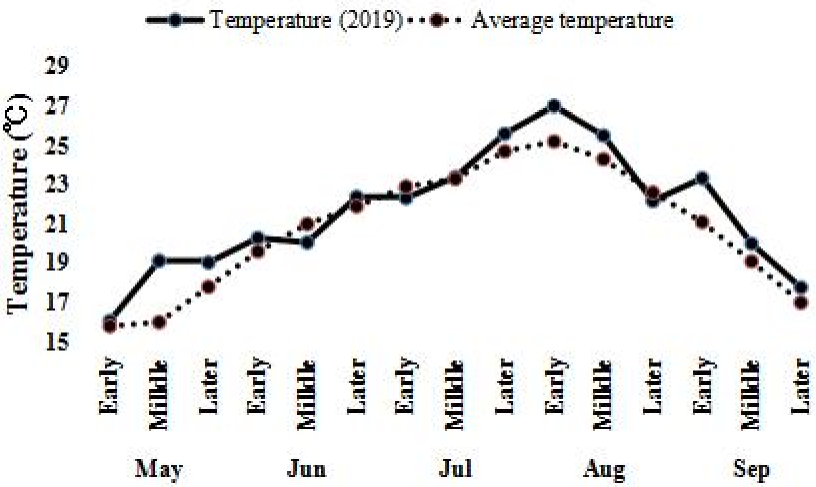


Figure 2. Comparison of temperature during the experiment period and normal year.

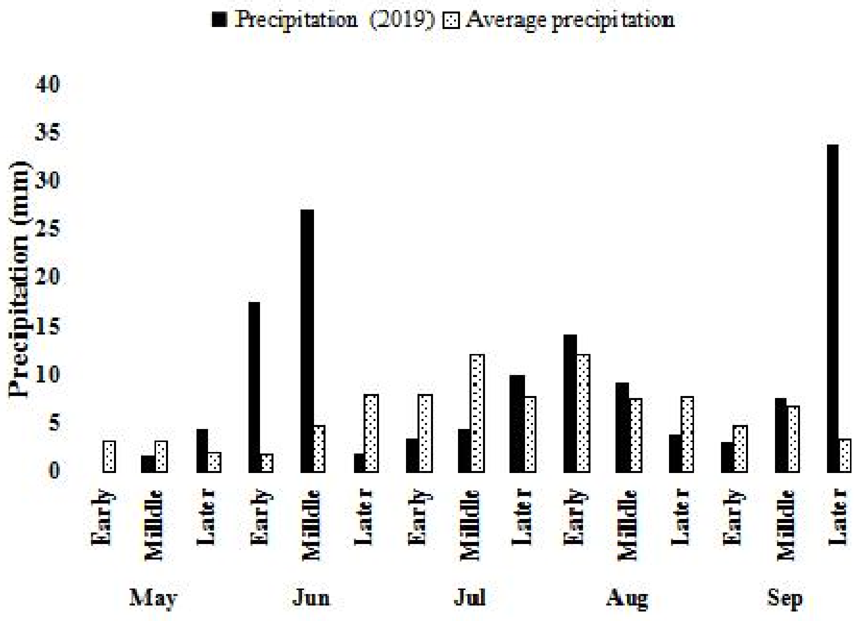


Figure 3. Comparison of precipitation during the experiment period and normal year.

The pH of experimental soil was 6.55 which close to neutral. The contents of organic matter in the soil was in the medium state (14.08 %) while the total nitrogen content was lower, only 0.12 %. The available $P_2O_5$ content was lower (79.12 mg/kg). The cation-exchange capacity of the soil was 16.52 cmol/kg, which was at a low level.

Table 1. Chemical properties of the soil before experiment

| pH (1:5) | OM (%) | TN (%) | Av. $P_2O_5$ (mg/kg) | Exchangeable cation(mg/kg) | | | | CEC (cmol/kg) |
|---|---|---|---|---|---|---|---|---|
| | | | | K | Ca | Mg | Na | |
| 6.55 | 14.08 | 0.12 | 79.12 | 4.01 | 1.75 | 0.92 | 0.10 | 16.52 |

*OM : organic matter, TN : Total nitrogen, CEC : Cation exchange capacity

## 3.2 Materials preparation

### 3.2.1 Raw materials preparation

3.2.1.1. Proso millet

The proso millet ("Golden") was planted on June 8. The test area was 15 $m^2$ (3m×5m). Plants were sown at a row spacing of 50 cm. And seeding rate of millet was 20 kg/ha. Fertilizes were applied at a rate of 200 kg/ha of nitrogen, 150 kg/ha of phosphorus and 150 kg/ha of potassium to raise the fertility of soil. On September 5, before harvesting the millet, agronomic characteristics of millet were measured. After harvesting, weighed the fresh samples and took around 300g fresh samples out to determinate the dry matter content. Dried in 65 °C air-forced drying oven for 72 hours.

3.2.1.2 .Silage corn

The variety of silage corn ("Gwangpyeongok") was used for experiment. The sowing date of corn was May 10. Corn was planted in the test area which acreage was 15 $m^2$ (3m×5m). Corn was sown at a plant-to-plant spacing of 20 cm and an inter-row distance of 75 cm. And the fertilizers of corn were 200 kg/ha of nitrogen, 150 kg/ha of phosphorus and 150 kg/ha of potassium. Four months later, the corn was harvested while it was at the yellow ripen stage. Before harvesting, measured the plant height, leaf number, leaf length, width and other indicators of corn. Measured separately grain yield and corn stoves yield. For determination of dry matter content, one row fresh corn was collected and dried in 65 ℃ air-forced drying oven for 72 hours.

3.2.1.3. Sorghum - sudangrass hybrids

Sorghum-sudangrass hybrid ("Turbo-gold") was planted on May 10. Sorghum-sudangrass hybrid seeds were applied to the plot which area was 15 $m^2$ (3m×5m). Meanwhile, the sowing space was 50 cm b/w row by row and the seeding rate was 40 kg/ha. Plots received 200 kg/ha of nitrogen, 150 kg/ha of phosphorus and 150 kg/ha of potassium when planted. Sorghum-sudangrass hybrid was harvested on September 10 when it was at heading stage. Measured its agronomic characteristics, such as plant height, leaf length, the number of leaf and so on. Around 300g fresh materials were collected and dried in 65 ℃ air-forced drying oven for 72 hours to determinate the dry matter.

### 3.2.2 Silage making

After been harvested, all crops were chopped into 2-3cm length approximately using a fodder chopper (Richi Machinery Co., Ltd, Henan, China). For part 1 experiment: Approximately 600 g samples are packed into plastic film bags (28 cm×36cm, Korea), and the bags were sealed with a vacuum sealer (Zhejiang Hongzhan Packing Machinery Co., Ltd). The film bag silos are stored at dark-dried ambient temperature. Each has three replications. All samples are randomly opened on days 1, 2, 3, 5, 10, 15, 20, 30, and 45 of ensiling to follow fermentation quality. Wet weights of silages were determined to measure DM loss by an electronic scale before and after ensiling.

For part 2 experiment: The chopped crop materials were spread flat on the plastic film and sprayed with an equal amount of different additives. Additives include *Lactobacillus plantarum* ( $1.5\times10^{10}$ CFU/g fresh matter, CMbio, Anseong, Korea), and formic acid (98%). The silage treatments were as follows: (1) control (distilled water), (2) inoculant LAB ($1.0 \times 10^6$ CFU/g fresh matter), (3) formic acid (5ml/kg). Then the chopped crop materials were ensiled into 20 L mini silos maximumly and sealed tightly with lids. Three replications were performed for each treatment. And the 27 mini silos were preserved in dark-dried ambient temperature for 60 days before opening. Wet weight of silages was determined to measure DM loss by an electronic scale before and after ensiling.

### 3.2.3 Sensory evaluation of silage

Sensory evaluation of silage is the most direct way to evaluate the quality of silage. The Deutche Lan Dwirtschafts Geseutschaft (DLG, 2004) evaluates the quality of silage based on its color, odor and structure (Table2). First, the silage was scored according to the parameters, and then the grades were classified according to the scores. High-quality silage maintains the color of the raw materials, has a clear fragrance, and the structure becomes soft without spoilage.

Table 2 . Sensory evaluation of silage

| Index | Scoring criteria | | | Score |
|---|---|---|---|---|
| Odor | Without butyric acid smell, but aromatic fruit taste or obvious bread aroma | | | 14 |
| | With weak butyric acid smell, but strong acidity, weak aromatic flavor | | | 10 |
| | Strong butyric acid smell or have pungent anxious burnt smell or musty taste | | | 4 |
| | Strong butyric acid smell or ammoniacal odour and almost no acid smell | | | 2 |
| Structure | Structure of stem and leaf is complete | | | 4 |
| | Structure of leaf is incomplete | | | 2 |
| | Structure of stem and leaf is incomplete, or is mild pollution | | | 1 |
| | Stem and leaf decay or badly pollute | | | 0 |
| Color | Similar with the raw material, after drying it showed hazel | | | 2 |
| | Color become pale yellow or brown | | | 1 |
| | Color become deep green or yellow, strong musty taste | | | 0 |
| Total | 20-16 | 15-10 | 9-5 | 4-0 |
| point grade | Excellent | Good | Medium | corruption |

## 3.3 Chemical analysis

All fresh samples were collected around 300g and then dried in 65 ℃ air - forced drying oven for 72 hours. After being taken out, they were cooled and weighed to detect the content of dry matter (DM). The dried samples were grounded to pass through a 1 mm screen (Thomas Scientific, Inc., New Jersey, USA) and put into plastic bottles with labeling. Preserved them in 4℃ dark-dried environment prior to analysis.

### 3.3.1 Crude protein analysis

Crude protein (CP) was measured via Dumas method as described by Jean-Baptiste Dumas (1884). The instrument "Automatic Elemental Analyzer Euro Vector EA3000" (EVISA Co., Ltd, Milan, Italy) was used for CP analysis.

### 3.3.2 Fiber analysis

Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured by the method of Van Soest et al. (1991). Most of the cell contents of crop, including fat, sugar, starch and protein, are dissolved in detergent by neutral detergent fiber, and the insoluble residue is neutral detergent fiber (NDF). Acid detergent can further decompose the components in the neutral detergent fiber. The part soluble in acid detergent is acid detergent solution (ADS), and the remaining residue is acid detergent fiber (ADF). The American machine "ANKOM 2000 Automated Fiber Analyzer" (Ankom Technologies, Inc., Fairport, NY, USA) was utilized.

### 3.3.3 Calculation of TDN

Total digestible nutrient (TDN) was calculated by the formula described by Holland et al. (1990). TDN is directly related to digestible energy and is often calculated based on ADF.

Legumes and grasses: TDN% = 88.9 - (0.79 × ADF%)

Corn silage: TDN%=87.84-(0.70 × ADF%)

TDN yield of corn: TDN yield= (DM yield of corn stover × 0.582) + (DM yield of corn ear × 0.85)

Where, numbers 0.582 and 0.85 are constant factors used to calculate TDN.

### 3.3.4 Calculation of RFV

Relative feed value (RFV) was calculated by the formula described by

Holland et al. (1990). RFV was estimated through digestible dry matter (DDM% = 88.9 - 0.779 × ADF%) and dry matter intake (DMI% = 120 / NDF%) as RFV = (DMI% × DDM%) / 1.29.

### 3.3.5 Water soluble carbohydrate (WSC)

Water soluble carbohydrate (WSC) is a measure of the total soluble sugars which are present in a forage. It was analyzed via modifying the anthrone method proposed by Yemm and Willis, (1954). 0.2g of ground sample covered with 200ml distilled water and shaking for one hour on shaker. Then filtered through filter paper (Whatman No. 1, AVANTEC). 2ml of the filtrate was pipetted into glass tubes, rapidly added 10ml of anthrone reagent and mixed by shaking. Loosely screwed cap and placed in the boiling water bath for 20 minutes, then followed by cooled in tap water for 10 minutes. Measured the absorbance at 620 nm wavelength in a 1 cm optical cell. The WSC content was calculated by the formula:

$$WSC\ \% = G \times D \times E \times 100 \times 0.1 / (W \times DM\ \%)$$

Where: W = sample weight (mg)

G = mg glucose read from graph

E = Extract volume (200ml)

D= Dilution factor

A blank determination is carried throughout the sample preparation and colour development stages.

## 3.4 Fermentation characteristics

Fresh silage was taken about 300 g and stored in - 80 ℃ deep freezer (TSE400D, Thermo Fisher Scientific, USA) for sequentially determination of microorganism populations, pH, organic acid and ammonia nitrogen. The lactic, acetic and butyric acid were used to assess the quality of silages according to the Flieg-Zimmer scale (Podkowka, 1978).

Table 3 . Criterion of silage organic acid content

| | Ratio of total acid (%) | Score | Ratio of total acid (%) | Score | Ratio of total acid (%) | Score |
|---|---|---|---|---|---|---|
| LA | 0.0~25.0 | 0 | 44.1~46.0 | 10 | 64.1~66.0 | 20 |
| | 25.1~27.5 | 1 | 46.1~48.0 | 11 | 66.1~67.0 | 21 |
| | 27.6~30.0 | 2 | 48.1~50.0 | 12 | 67.1~68.0 | 22 |
| | 30.1~32.0 | 3 | 50.1~52.0 | 13 | 68.1~69.0 | 23 |
| | 32.1~34.0 | 4 | 52.1~54.0 | 14 | 69.1~70.0 | 24 |
| | 34.1~36.0 | 5 | 54.1~56.0 | 15 | 70.1~71.2 | 25 |
| | 36.1~38.0 | 6 | 56.1~58.0 | 16 | 71.3~72.4 | 26 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 38.1~40.0 | 7 | 58.1~60.0 | 17 | 72.5~73.7 | 27 |
| | 40.1~42.0 | 8 | 60.1~62.0 | 18 | 73.8~75.0 | 28 |
| | 42.1~44.0 | 9 | 62.1~64.0 | 19 | above75. | 30 |
| AA | 0.0~15.0 | 20 | 26.8~28.0 | 13 | 36.1~37.4 | 6 |
| | 15.1~17.5 | 19 | 28.1~29.4 | 12 | 37.5~38.7 | 5 |
| | 17.6~20.0 | 18 | 29.5~30.7 | 11 | 38.8~40.0 | 4 |
| | 20.1~22.0 | 17 | 30.8~32.0 | 10 | 40.1~42.5 | 3 |
| | 22.1~24.0 | 16 | 32.1~33.4 | 9 | 42.6~45.0 | 2 |
| | 24.1~25.4 | 15 | 33.5~34.7 | 8 | above45. | 1 |
| | 25.5~26.7 | 14 | 34.8~36.0 | 7 | | |
| BA | 0.0~1.5 | 50 | 12.1~14.0 | 7 | 30.1~32.0 | -1 |
| | 1.6~3.0 | 30 | 14.1~16.0 | 6 | 32.1~34.0 | -2 |
| | 3.1~4.0 | 20 | 16.1~17.0 | 5 | 34.1~36.0 | -3 |
| | 4.1~6.0 | 15 | 17.1~18.0 | 4 | 36.1~38.0 | -4 |
| | 6.1~8.0 | 10 | 18.1~19.0 | 3 | 38.1~40.0 | -5 |
| | 8.1~10.0 | 9 | 19.1~20.0 | 2 | above40. | -10 |
| | 10.1~12.0 | 8 | 20.1~30.0 | 0 | | |

Note: LA: lactic acid ; AA: acetic acid ; BA: butyric acid

1) The ratio of every organic acids in total acid is meq. 2) The conversion relation of organic acids in fresh sample with meq as follow:

lactic acid(mg equivalent)=lactic acid (%)×11.105

acetic acid(mg equivalent)=acetic acid (%)×16.658

butyric acid(mg equivalent)=butyric acid (%)×11.356

### 3.4.1 Acidity(pH)

Weigh out 10 g fresh chopped silage sample into 250 ml conical flask and cover with 100 ml distilled water. Shaking for one hour on shaker

(Green Sseriker, Vision Scientific, Korea) and stored in refrigerator for 24 hours, during which, the conical flasks were shaken by hand every 2 hours. The mixture was filtered through filter paper (Whatman No. 6, AVANTEC) and the filtrate was used to measure the pH of the silage with a pH meter (AB 150, Fisher Scientific International, Inc., Pittsburgh, US).

### 3.4.2 Organic acid

10g fresh chopped silage sample mixed with 100 ml distilled water into 250 ml conical flask. Shaking for one hour on shaker and then stored at 4℃ for 24 hours. The extracts were filtered through filter paper (Whatman No. 6, AVANTEC) and retained in -20 ℃ refrigerator. Before analyzing, thaw the sample. Take 1.5 ml of filtrate and centrifuged at 3000 rpm, 4℃ for 15 minutes using Centrifuge Smart 15 (Hanil Science Industrial, South Korea). Then take 700ul supernatant of sample solution with syringe (KOVAX-SYRINGE 1 mL) and syringe filter (13mm Syringe Filter, w/0.45 μm PVDF Membrane). Then contents of organic acids were analyzed in high performance liquid chormatography system (HPLC, Agilent Technologies, Santa Clara, CA, US) equipped with a refractive index detector. The condition of instrument was shown in Table 4.

Table 4 . Instrumental conditions of HPLC for determination of organic acid

| | |
|---|---|
| Column | Agilent Hi-Plex H, 7.7 x 300 mm, 8 µm (p / n PL1170-6830) |
| Mobile phase | 0.005 M $H_2SO_4$ |
| Gradient | Isocratic |
| Flow rate | 0.7 ml / min |
| Injection | 20 µL |
| Temperature | 60 ℃ |
| Pressure | 4.6 MPa (46 bar, 670 psi) |
| Detector | UV ( 55 ℃) |

### 3.4.3 Buffering Capacity

The buffering capacity (BC) is defined as resistance against change in pH (Spiekers et al., 2009). 10g fresh silage sample was macerated with 100 ml distilled water. Shaking for one hour on shaker and then stored at 4℃ for 24 hours. The extracts were filtered through filter paper (Whatman No. 6, AVANTEC). The buffering capacity is measured by titrating the filtrate under continuous stirring to pH of 4.0 with 0.1 N hydrochloric acid and by titrating it from pH of 4.0 to pH of 6.0 with 0.1 N sodium hydroxide (Playne et al., 1966). BC was expressed as the amount of acid or base required to produce a unit change in pH of silage sample (Bujňák et al., 2011).

### 3.4.4 Ammonia nitrogen ($NH_3$-N) / Total nitrogen (TN)

Ammonia nitrogen ($NH_3$-N) was analyzed via the method described by Broderick and Kang (1980). An extract is prepared from a 10g chopped silage sample mixed with 100 ml distilled water and stored in 4 ℃ for a period of at least 24 hours, and centrifuged at 3000 rpm for 15 minutes. The 0.02 ml of supernatant sample mixed with 1 ml phenol reagent and 1 ml alkali-hypochlorite reagent. After heating for 15 minutes in a water bath at 37 ℃ and added 8 ml distilled water, the absorbance of sample was detected in 630 nm wavelength of determination ammonia nitrogen of dry matter, and total nitrogen was calculated by CP / 6.25.

## 3.5 Microbial analysis

For microbial analysis, the spread-plate method (Madigan and Michael et al., 2012) was used. The samples (10g) were diluted with 90 ml of sterilized saline solution (8.50 g/L NaCl) and was shaken for one hour. Serial dilutions ($10^{-1}$~$10^{-5}$) were streaked on de Man, Rogosa and Sharpe agar (MRS) medium, plate count agar (PCA) medium and potato dextrose agar (PDA) medium, respectively. The lactic bacteria (LAB), molds and total microorganisms (TM) were counted on MRS, PDA and PCA agar medium, respectively.

Table 5. The incubation temperature and time according to medium

| Agar medium plate | Microorganism | Temperature (℃) | Time (hour) |
|---|---|---|---|
| MRS | LAB | 37 | 24~48 |
| PCA | TM | 37 | 48~72 |
| PDA | Mould | 25 | Above 48 |

All operations must be sterile. A plate with a colony count of 20 to 200 was selected for counting. The colony-forming units per gram (CFU / g) of microorganisms were calculated according to dilution factor. Finally, microbial counts were converted to $\log_{10}$.

## 3.6 *In vitro* digestibility analysis

*In vitro* dry matter digestibility (IVDMD) is an index used to analyze the nutrient digestibility of feed materials in animals (Tilley and Terry, 1963). It consists of two stages of digestion. First stage rumen liquor digestion: Nylon filter bags (ANKOM F57, ANKOM Tech., Fairport, NY) were each 50 mm×55 mm, made from polyester/polyethylene extruded ®laments in a three-dimensional matrix claimed to retain particles >25 mm. They were rinsed in acetone and allowed to air dry before drying at 100 ℃ for 24 h, after which dry bag weight was recorded. Number all bags using a solvent resistant marker. 0.5 - 0.6 g of ground sample were weighed into filter bags and sealed by heat sealer (#HS: 100V ~120V / #HSi: 220V ~240V). Samples should be evenly distributed on both sides of Daisy Incubator digestion jars (Ankom Technologies, Inc., Fairport, NY, USA). Add 1330 ml of buffer solution A and 266 ml of buffer solution B into each jars. Selected two healthy cannulated Holstein steers. Collected their rumen fluid which need through four layers of cheesecloth before morning feed. 400ml of rumen fluid was added to the buffer solution and samples. Purge the digestion jar with $CO_2$ gas for thirty seconds and secure lid. Incubate at 39 ℃ for 48 hours. And then follow the procedure for determining NDF to get the *in vitro* dry matter digestibility.

Table 6. Reagent of buffer solution A and B

| Buffer Solution A | g / liter |
|---|---|
| $KH_2PO_4$ | 10.0 |
| $MgSO_4 \cdot 7 H_2O$ | 0.5 |
| NaCl | 0.5 |
| $CaCl_2 \cdot 2H_2O$ | 0.1 |
| Urea (reagent grade) | 0.5 |
| **Buffer Solution B** | **g / liter** |
| $Na_2CO_3$ | 15.0 |
| $Na_2S \cdot 9H_2O$ | 1.0 |

## 3.7 Statistical analysis

All data were subjected to analysis of variance using General Line Model (GLM) of SPSS (SPSS 20.0 program SPSS Inc., Chicago, Illionis, USA). Mean treatment differences were obtained by Duncan's multiple range tests with a level of statistical significant of 5%.

# 4. Results and Discussions

## 4.1 Productivity of forage crop

The agronomic characteristics and yield of forage crops were shown in Table 7 and Table 8. The plant height of the sorghum-sudangrass hybrid was significantly higher than corn, with the height of 297.60cm ($p<0.05$). The plant height of proso millet was 148.90cm. The leaf length of corn was the shortest which was average of 80.30cm. For leaf width, corn was significantly higher than the sorghum-sudangrass hybrid, almost twice the width of the sorghum-sudangrass hybrid ($p < 0.05$).

Table 7. Agronomic characteristics of forage crops

| Item | Corn | SSH | Proso millet |
|---|---|---|---|
| Plant height (cm) | $274.70^{b}$ | $297.60^{a}$ | $148.90^{c}$ |
| Panicle length (cm) | -- | -- | 11.20 |
| Leaf length (cm) | $80.30^{b}$ | $93.70^{a}$ | -- |
| Leaf width (cm) | $9.30^{a}$ | $4.90^{b}$ | -- |
| Number of leaf (No./plant) | $15.30^{a}$ | $8.70^{b}$ | -- |

SSH: Sorghum-sudangrass hybrid, Different lowercase letters in the same row indicate significant differences （$p<0.05$）

Forage yield was significantly affected by crop species ($p<0.05$) (Table 8). The fresh matter yield of proso millet was especially low (25350 kg/ha), about 1/6 of that of the sorghum-sudangrass hybrid (121,733 kg/ha). The similar yield of proso millet was also found by Shin et al. (2006). The fresh yield of corn stalk of 48,180 kg/ha was significantly higher than the fresh yield of ear (19,378 kg/ha). Same as fresh yield, the highest dry matter yield was detected in sorghum-sudangrass hybrid, followed by corn and proso millet. At the same time, TDN yield of proso millet was much lower than corn and sorghum-sudangrass hybrid, which is about one-third to theirs.

Table 8. Fresh, dry matter and TDN yield of forage crops

| Item | | Corn | SSH | Proso millet |
|---|---|---|---|---|
| FM yield (kg/ha) | Stover+Ear | 48,180+19,378 | | |
| | Total | 67,558$^{b}$ | 121,733$^{a}$ | 25,350$^{c}$ |
| DM yield (kg/ha) | Stover+Ear | 9,799+10,205 | | |
| | Total | 20,004$^{b}$ | 23,510$^{a}$ | 7,467$^{c}$ |
| TDN yield (kg/ha) | | 14,378$^{a}$ | 12,719$^{b}$ | 4,711$^{c}$ |

FM: Fresh matter, DM: Dry matter, TDN: Total digestible nutrient, SSH: Sorghum-sudangrass hybrid, Different lowercase letters in the same row indicate significant differences （$p<0.05$）

## 4.2 Analysis of forage quality of raw material

### 4.2.1 Chemical compositions and feed values

Table 9. Chemical composition and feed values of forage crops

| Species | DM | CP | ADF | NDF | IVDMD | TDN | RFV |
|---|---|---|---|---|---|---|---|
| | g /kg | | | | | % | |
| Proso millet | 303.40[a] | 61.30 | 326.40[b] | 607.20[b] | 649.50[b] | 63.10[b] | 97[b] |
| Corn | 277.30[b] | 59.30 | 287.80[c] | 530.10[c] | 863.20[a] | 67.70[a] | 117[a] |
| SSH | 192.80[c] | 54.69 | 439.90[a] | 662.50[a] | 636.60[b] | 54.10[c] | 77[c] |

DM: dry matter, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber, IVDMD: in vitro dry matter digestibility, TDN: total digestible nutrient, RFV: relative feed value, SSH: Sorghum-sudangrass hybrid. Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

The nutritional composition of the raw materials of each sample was shown in Table 9. Among the three crops, millet had the highest dry matter content, while the dry matter content of sorghum-sudangrass hybrid (192.80 g/kg) was lowest. This may be because sorghum-sudangrass hybrid has no grain compared to the other two crops, and usually the grain has a higher dry matter content.

Crude protein (CP) content is an important quality parameter in dairy diets. Crude protein content in feed is critical for livestock nutrition intake (Chadd et al., 2002). There was no significant difference in crude protein content among the three crops ($p$>0.05). But, the crude protein content of sorghum-sudangrass hybrid was the lowest of the three crops at 54.69 g/kg, which was similar to the results of previous studies (Jeon et al., 2012). The crude protein content of corn was also not high (59.30 g/kg), which is similar to the result of 54.60 g/kg in the previous study (Kim et al., 2013). There are also reports that corn had a lower crude protein content than other crops (Lardy, 2002).

As Table 9 presented, lowest ADF and NDF content detected in corn, and it was significantly lower than proso millet and sorghum-sudangrass hybrid ($p<0.05$). Both NDF and ADF contents were corn < proso millet < sorghum-sudangrass hybrid. In our research, the ADF and NDF contents of corn were 287.80 g / kg and 530.10g / kg, respectively. Similarly, Lee et al. (2019) detected that the ADF and NDF contents of corn were 28.10% and 51.10%, and there was no significant difference between the two. However, among the contents of IVDMD, corn was the highest (863.20 g/kg), proso millet was in the middle (649.50 g/kg), and sorghum-sudangrass hybrid was the lowest (636.60 g/kg). The content of IVDMD was negatively correlated with the content of ADF and NDF, which is consistent with the conclusions of previous studies (Ammar et al., 2004).

The total digestible nutrients (TDN) is related to ADF content among forage crops. TDN in corn was significantly higher than the other two crops ($p<0.05$). And the lowest was found in sorghum-sudangrass hybrid (54.10 %) (Table 9). In general, the results indicated that corn had the highest digestibility among the studied forage crops. This result is consistent with the conclusion of Jahansouz et al (2014).

In our study, the highest value of RFV was obtained from corn (117), whereas the lowest value was found in sorghum-sudangrass hybrid (77).

When RFV is between 103 and 124, the forage is considered as good (Horrocks et al., 1999). This also confirms the high quality of corn.

### 4.2.2 Pre-ensiled characteristics

As shown in the Table 10, the pH of corn (5.80) was significantly lower than that of proso millet and sorghum-sudangrass hybrid ($p$<0.05). Generally , the pH value of forage before ensiling is between 5.50 and 6.00 (Lemus et al., 2017). The pH of sorghum-sudangrass hybrid detected in this experiment is not in this range, but it is also reasonable.

Corn had the highest $NH_3$-N/TN content (34.60 g /kg), significantly higher than the other two crops ($p$<0.05). The $NH_3$-N/TN content of millet and sorghum-sudangrass hybrid were 29.80 g /kg and 14.40 g /kg, respectively.

Table 10. Pre-ensiled characteristics of forage crops

| Species | pH | $NH_3$-N/TN | WSC | BC |
|---|---|---|---|---|
| | | -------------- g /kg | ------------- | mEq kg/DM |
| Proso millet | 6.00$^{a}$ | 29.80$^{b}$ | 170.00$^{a}$ | 32.00$^{b}$ |
| Corn | 5.80$^{b}$ | 34.60$^{a}$ | 144.15$^{b}$ | 24.20$^{c}$ |
| SSH | 6.11$^{a}$ | 14.40$^{c}$ | 136.70$^{b}$ | 55.50$^{a}$ |

SSH: Sorghum-sudangrass hybrid, $NH_3$-N: Ammonia nitrogen, TN: Total nitrogen, WSC: water soluble carbohydrate, BC: Buffering capacity. Different lowercase letters in the same row indicate significant differences （$p$<0.05）

WSC in proso millet was significantly higher than corn and sorghum-sudangrass hybrid ($p$<0.05). The highest WSC content was detected in proso millet (170.00 g/kg) followed by corn (144.15 g/kg), and the lowest was measured in sorghum-sudangrass hybrid (136.70 g/kg). It is reported that the initial WSC content between 60 and 80 g/kg DM is sufficient to produce good quality silage (Amer et al., 2012). So all forage crops in this study contain enough WSC to ferment into good quality silage.

All forage crops have different buffering capacities. Forage crops with high buffering capacity require more acid to reduce pH. The higher the buffering capacity, the more difficult it is to reduce pH. Combining the data, it can be seen that the BC content in corn was the lowest (24.20 mEq kg/DM) and that in sorghum-sudangrass hybrid was the highest (55.50 mEq kg/DM). This indicates that in the early ensiling period, the pH of corn decreased fastest, proso millet was in the middle, and sorghum-sudangrass hybrid was the slowest. Corresponding to the change in pH of different crops from day 0 to day 1, corn declined the fastest. Low CP content in forage crop may reduce the buffering capacity of the silage, thereby lowering the pH value, so as to obtain good silage with minimal loss (Cherney et al., 2003). Proso millet had a high crude protein content, so even though 0-day buffering capacity content of millet was lower than sorghum-sudangrass hybrid, the rate of pH decline was slower than sorghum-sudangrass hybrid.

### 4.2.3 Microorganisms

In each crop, the total number of microorganisms in corn was significantly higher than other two crops, which were 6.95 log10 cfu/g FW in proso millet, 7.60 log10 cfu/g FW in corn, and 6.90 log10 cfu/g FW in sorghum-sudangrass hybrid, respectively ($p<0.05$). That means that when ensiling starts, microbial activity in corn would be active. Similarly, the highest content of lactic acid bacteria was also detected in corn (6.15

sorghum-sudangrass hybrid), followed by 5.91 log10 cfu/g FW in proso millet and 5.88 log10 cfu/g FW in sorghum-sudangrass hybrid. Research of Cai et al. (1999) shows that when the content of epiphytic lactic acid bacteria is less than 5.00 log10 cfu/g FW, it cannot dominate the fermentation. And in our experiment, the contents of lactic acid bacteria in all crops were higher than 5.00 log10 cfu/g FW.

The highest mold content in millet was 4.53 log10 cfu/g FW. In addition, the population of epiphytic microorganisms in silage crops may be affected by forage species, maturity stage, weather and field wilting (Fenton, 1987).

Table 11. Microbial population of forage crops

| Microbes | Proso millet | Corn | SSH |
|---|---|---|---|
| LAB (log10 cfu/g FW) | $5.91^{b}$ | $6.15^{a}$ | $5.88^{c}$ |
| Molds (log10 cfu/g FW) | $4.53^{a}$ | $4.28^{b}$ | $3.30^{c}$ |
| TM (log10 cfu/g FW) | $6.95^{b}$ | $7.60^{a}$ | $6.90^{b}$ |

SSH: Sorghum-sudangrass hybrid, LAB: Lactic acid bacteria, TM: Total microorganism, CFU: Colony forming unit, FW: Fresh weight. Values with different small letters show significant differences among ensiling days with same species. ($p$<0.05)

## 4.3 Analysis of fermentation dynamics of silage

### 4.3.1 Chemical composition during ensiling

Loss of dry matter during the ensiling process is inevitable, which reduces the feed quality of the product (Borreani et al., 2018). The Table 12 showed the change of DM content of forage crops during ensiling. From that, we can see the dry matter content of all forage crops decreased with the progress of ensiling. And the dry matter content on the 0 day and the 45th day was significantly different ($p < 0.05$). Among them, the dry matter of proso millet decreased by 37.00 g/kg, corn decreased by 16.57 g/kg, and the dry matter content of sorghum-sudangrass hybrid decreased from 192.80 g/kg to 174.70 g/kg. The dry matter loss in proso millet was the most serious which was significantly higher than corn and sorghum-sudangrass hybrid ($p<0.05$). This may be caused by too many epiphytic molds growing on the proso millet.

During the ensiling process, the loss of crude protein also occurred, and the content of crude protein showed a downward trend. Crude protein was significantly affected by forage crops ($p<0.05$). Among them, the change of the crude protein content in proso millet was the most significant ($p<0.05$). Proso millet, corn, and sorghum-sudangrass hybrid decreased by

Table 12. DM content, DM loss and chemical compositions during ensiling

| Item | Species | Storage periods (days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 5 | 10 | 15 | 20 | 30 | 45 |
| DM | Proso millet | 284.40$^{aA}$ | 284.10$^{aA}$ | 278.60$^{abA}$ | 278.90$^{abA}$ | 272.20$^{bcA}$ | 270.20$^{cA}$ | 269.60$^{cA}$ | 266.80$^{cA}$ | 266.40$^{cA}$ |
| | Corn | 275.16$^{aB}$ | 274.57$^{aB}$ | 274.67$^{aB}$ | 272.52$^{aA}$ | 272.10$^{aA}$ | 271.02$^{aA}$ | 268.36$^{abA}$ | 264.08$^{bcA}$ | 260.73$^{cA}$ |
| (g/kg) | SSH | 190.30$^{aC}$ | 187.50$^{abC}$ | 183.10$^{abcC}$ | 180.00$^{bcB}$ | 174.30$^{cB}$ | 177.70$^{cB}$ | 176.70$^{cB}$ | 178.40$^{bcB}$ | 174.70$^{cB}$ |
| DM | Proso millet | 19.00$^{cA}$ | 19.30$^{cA}$ | 24.80$^{bcA}$ | 24.50$^{bcA}$ | 31.20$^{abA}$ | 33.20$^{aA}$ | 33.80$^{aA}$ | 36.60$^{aA}$ | 37.00$^{aA}$ |
| loss(g | Corn | 2.14$^{cB}$ | 2.73$^{cB}$ | 2.63$^{cB}$ | 4.78$^{cB}$ | 5.20$^{cC}$ | 6.28$^{cC}$ | 8.94$^{bcC}$ | 13.22$^{abB}$ | 16.57$^{aB}$ |
| /kg) | SSH | 2.50$^{cB}$ | 5.30$^{bcB}$ | 9.70$^{abcC}$ | 12.80$^{abAB}$ | 18.50$^{aB}$ | 15.10$^{aB}$ | 16.10$^{aB}$ | 14.40$^{abB}$ | 18.10$^{aB}$ |
| CP | Proso millet | 62.30$^{abA}$ | 61.00$^{abcA}$ | 59.80$^{aA}$ | 58.30$^{abcdA}$ | 60.30$^{abcdA}$ | 59.90$^{bcd}$ | 57.90$^{cdA}$ | 59.60$^{bcdA}$ | 57.10$^{d}$ |
| | Corn | 57.40$^{aB}$ | 56.50$^{abB}$ | 58.40$^{abA}$ | 54.60$^{abB}$ | 54.50$^{abB}$ | 53.40$^{b}$ | 52.70$^{abB}$ | 53.00$^{abB}$ | 50.80$^{ab}$ |
| (g/kg) | SSH | 53.40$^{aC}$ | 48.90$^{abC}$ | 49.50$^{abB}$ | 49.60$^{abBC}$ | 48.20$^{abC}$ | 46.20$^{ab}$ | 46.60$^{bC}$ | 46.60$^{abC}$ | 47.50$^{b}$ |
| ADF | Proso millet | 324.90$^{abcB}$ | 327.30$^{abcB}$ | 324.30$^{abcB}$ | 342.40$^{bcB}$ | 344.40$^{abcB}$ | 340.10$^{aB}$ | 347.10$^{abB}$ | 330.10$^{cB}$ | 345.80$^{bcB}$ |
| | Corn | 260.50$^{aC}$ | 256.20$^{abC}$ | 251.50$^{abC}$ | 243.40$^{cC}$ | 248.90$^{bC}$ | 249.20$^{bC}$ | 246.80$^{bcC}$ | 241.50$^{cC}$ | 252.40$^{abC}$ |
| (g/kg) | SSH | 419.10$^{A}$ | 419.70$^{A}$ | 420.40$^{A}$ | 414.90$^{A}$ | 420.50$^{A}$ | 437.10$^{A}$ | 427.60$^{A}$ | 403.20$^{A}$ | 415.10$^{A}$ |
| NDF | Proso millet | 608.50$^{abB}$ | 615.80$^{abB}$ | 604.30$^{abcdB}$ | 606.60$^{bcdB}$ | 601.80$^{abcB}$ | 613.50$^{aB}$ | 600.10$^{abcB}$ | 586.00$^{dB}$ | 605.50$^{cdB}$ |
| | Corn | 496.10$^{abC}$ | 491.00$^{aC}$ | 477.60$^{abC}$ | 445.30$^{abC}$ | 459.40$^{abC}$ | 455.90$^{aC}$ | 455.60$^{abC}$ | 445.50$^{bC}$ | 449.20$^{abC}$ |
| (g/kg) | SSH | 678.70$^{aA}$ | 673.60$^{abA}$ | 655.80$^{abcA}$ | 650.50$^{cA}$ | 666.20$^{abcA}$ | 679.90$^{abcA}$ | 667.00$^{abcA}$ | 635.00$^{cA}$ | 640.10$^{bcA}$ |

DM: dry matter, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber, SSH: Sorghum-sudangrass hybrid. Values with different small letters show significant differences among ensiling days with same species. Values with different capital letters show significant differences among species in the same ensiling days ($p$<0.05).

4.20 g/kg, 8.50 g/kg and 7.10 g/kg from day 0 to 45, respectively.

NDF are structural carbohydrates in the forage. These structural carbohydrates are characteristic of chewing activity (Liu, 2009). Moreover, the content of NDF also affect the dry matter intake (DMI) of ruminants (Tjardes et al., 2002). The NDF content of all forage crops showed a significant downward trend ($p$<0.05). From the 0 day to the 45th day, the NDF content of millet dropped from 607.20 g/kg to 605.50 g/kg. Compared to the decrease of 80.90 g/kg in corn and 22.40 g/kg in sorghum-sudangrass hybrid, the change of proso millet was smaller. Chen et al. (2019) mentioned that lower NDF content in silages could also be due to the loss of hemicellulose occurred in the ensiling process. This loss could be due to a combination of enzymatic and acid hydrolysis of the more digestible cell wall fractions during the fermentation. The ADF content of corn and sorghum-sudangrass hybrid also decreased after 45 days of ensiling, which were 35.40 g/kg and 24.80 g/kg respectively. In contrast, the proso millet ADF content increased slightly from 326.40 g / kg on the 0 day to 345.80 g/kg on the 45th day. This may be due to the large amount of soluble components in proso millet being consumed during the ensiling process, resulting in a relatively high fiber content. The decrease in ADF and NDF levels indicates that with the progress of ensiling, the nutrients in the crops are converted into nutrients that animals can more easily absorb, which is also a benefit of fermentation.

### 4.3.2 Fermentation quality during ensiling

The change of pH during forage crops ensiling is shown in the Figure 4. With the prolong of ensiling time, the pH showed a downward trend, and it decreased rapidly in the early period of ensiling. The pH of all crops fell below 5 on the third day, meeting the requirements for good silage (Ruhua et al., 2007). The pH of the late ensiling was stable and the pH of corn was significantly lower than the pH of the other two crops ($p<0.05$). This may be because corn contains more lactic acid bacteria (Table 15). During the 45-day ensiling period, the pH of corn decreased by 1.94, millet by 1.65 and sorghum-sudangrass hybrid by 2.04.

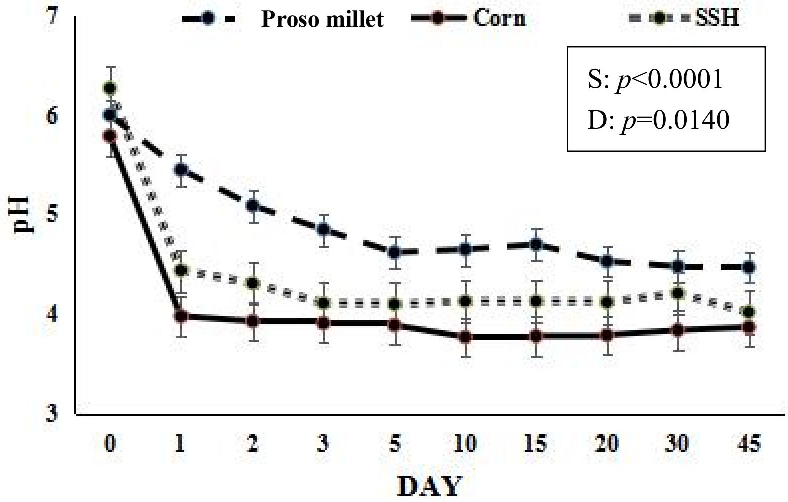


Figure 4. The pH value of forage crops during ensiling. n=9, bars indicate standard error of the means. S: species, D: ensiling time.

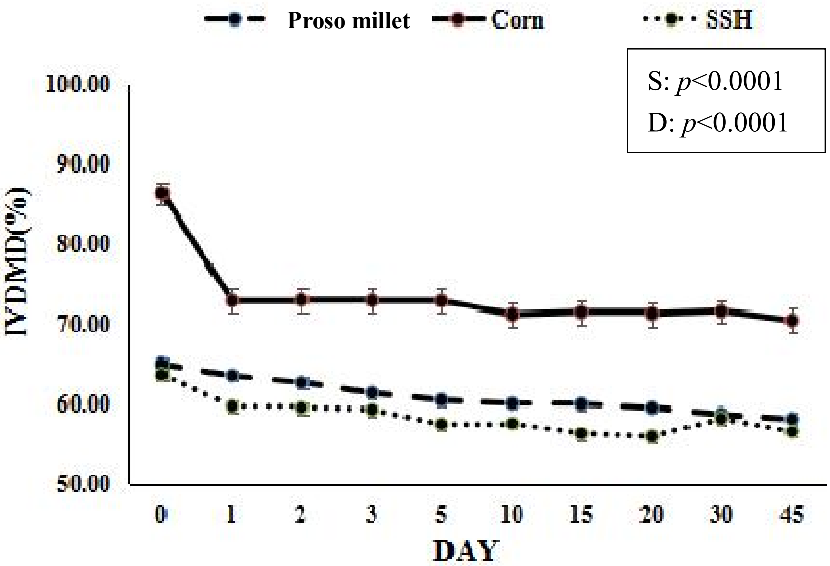


Figure 5. The IVDMD content of forage crops during ensiling. n=9, bars indicate standard error of the means. S: species, D: ensiling time.

Combining the Figure 5, we can see that with the increase of ensiling time, the IVDMD of all forage crops did not change significantly. And the IVDMD of corn was significantly higher than that of proso millet and sorghum-sudangrass hybrid ($p$<0.05), which was negatively correlated with ADF content (Melton et al., 1975).

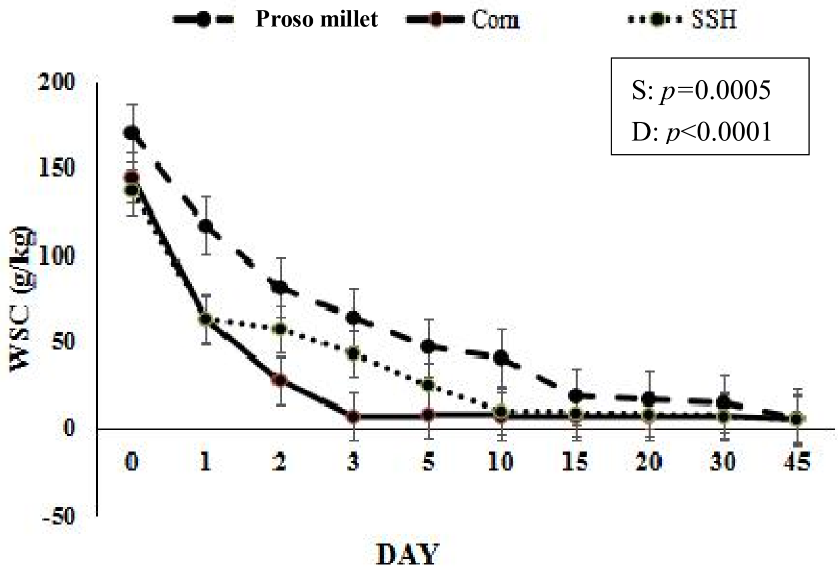


Figure 6. The WSC content of forage crops during ensiling. n=9, bars indicate standard error of the means. S: species, D: ensiling time.

WSC in forage are the raw materials that lactic acid bacteria use to convert to produce lactic acid. Good fermentation requires high WSC content in forage crops, which can be consumed by lactic acid bacteria, produces a large amount of lactic acid, reduces pH, and inhibits the activities of undesirable microorganisms. The WSC content of the three forage crops decreased rapidly from the 0 day to the 3rd day, and slowly decreased to stabilize after 15 days. This may be caused by the activities of microorganisms and plant enzymes in the crops early stage of ensiling. Among them, the WSC content of corn decreased most rapidly, from 62.59 g/kg on the 1st day to 6.67 g/kg on the 3rd day. It showed that the activity of lactic acid bacteria in corn was high at the beginning of ensiling.

The $NH_3$-N/TN in different periods of all forage crops in this experiment are shown in the Figure 7. The $NH_3$-N/TN ratio indicates the decomposition of protein during the ensiling process. The larger the ratio is, the more serious the protein decomposition is, and the worse the quality of the silage is. From the Figure 7, we can see that the $NH_3$-N/TN of all three crops has increased significantly ($p<0.05$), among which millet increased by 115.94 g/kg, corn increased by 42.05 g/kg, and sorghum-sudangrass hybrid increased by 40.80 g/kg. This indicates that protein degradation was most severe in proso millet. When the ratio is less than 12.50%, the quality of the silage is excellent (McDonald et al., 1973). Expect the high $NH_3$-N/TN ratio due to excessive degradation of protein and amino acid in millet, the other two crops were excellent.

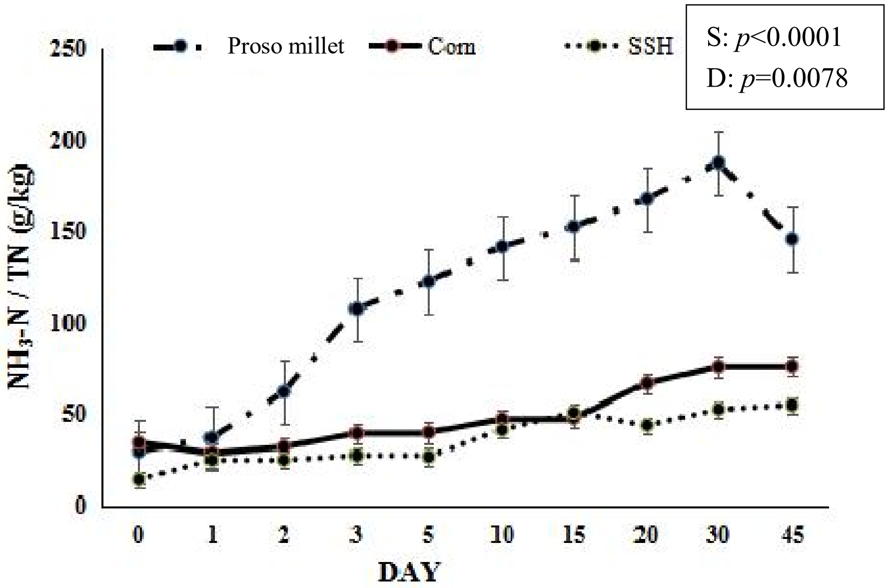


Figure 7. The $NH_3$-N/TN content of forage crops during ensiling. n=9, bars indicate standard error of the means. S: species, D: ensiling time.

### 4.3.3 Organic acids of silage during ensiling

The Table 13 shows the changes in organic acid content of different forage crops during ensiling. The contents of lactic acid and acetic acid increased with the progress of ensiling. Lactic acid is produced by lactic acid bacteria consuming water soluble carbohydrates in forage crops and has a certain influence on the fermentation of forage crops. On the 1st day, the content of lactic acid in sorghum-sudangrass hybrid was 31.32 g/kg, while the content of proso millet and corn was 10.11 g/kg and 17.64 g/kg, respectively. During the 45-day ensiling period, the lactic acid content showed an upward trend, and reached its maximum on the 45th day, which were proso millet 42.51 g/kg, corn 67.67 g/kg, and sorghum-sudangrass hybrid 126.59 g/kg. Moreover, the content of lactic acid was also affected by the type of crop, and the difference is significant ($p<0.05$). During 45 days of ensiling, the lactic acid content of sorghum-sudangrass hybrid had been significantly higher than that of proso millet and corn ($p<0.05$).

The production of acetic acid mainly comes from acetic acid bacteria and heterofermentative lactic acid bacteria. The acetic acid bacteria are relatively active in the presence of aerobic conditions during the initial fermentation to produce a large amount of acetic acid. Although acetic acid can also reduce the pH of the silage, it has poor palatability (Gaiying et al., 2009). The acetic acid is a weaker acid which need more time to reduce the pH than lactic acid. The acetic acid content of all three forage crops were

Table 13. Organic acids of silage during ensiling

| Item | Species | Storage periods (days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 5 | 10 | 15 | 20 | 30 | 45 |
| LA (g/kg) | Proso millet | 10.11$^{eB}$ | 14.08$^{deB}$ | 23.18$^{cB}$ | 21.56$^{cdB}$ | 29.62$^{bcC}$ | 21.53$^{cdC}$ | 40.03$^{aB}$ | 37.47$^{abC}$ | 42.51$^{aC}$ |
| | Corn | 17.64$^{eB}$ | 28.58$^{dA}$ | 33.56$^{dAB}$ | 42.94$^{cA}$ | 44.34$^{cB}$ | 48.00$^{cB}$ | 57.45$^{bA}$ | 62.00$^{abB}$ | 67.67$^{aB}$ |
| | SSH | 31.32$^{eA}$ | 36.14$^{deA}$ | 39.70$^{deA}$ | 45.23$^{dA}$ | 77.86$^{cA}$ | 71.04$^{cA}$ | 69.33$^{cA}$ | 98.39$^{bA}$ | 126.59$^{aA}$ |
| AA (g/kg) | Proso millet | 5.76$^{eC}$ | 11.10$^{deB}$ | 14.67$^{cdeC}$ | 14.07$^{cdeC}$ | 26.34$^{cC}$ | 25.51$^{cdC}$ | 62.67$^{aA}$ | 48.97$^{bB}$ | 41.71$^{bB}$ |
| | Corn | 10.30$^{dB}$ | 14.83$^{dB}$ | 22.62$^{cB}$ | 27.08$^{cAB}$ | 37.61$^{bB}$ | 35.71$^{bB}$ | 25.96$^{cB}$ | 48.37$^{aB}$ | 37.96$^{bB}$ |
| | SSH | 15.86$^{cA}$ | 58.63$^{abA}$ | 49.63$^{bcA}$ | 63.27$^{abA}$ | 47.83$^{bcA}$ | 77.50$^{abA}$ | 62.62$^{abA}$ | 83.21$^{abA}$ | 100.25$^{aA}$ |
| BA (g/kg) | Proso millet | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| | Corn | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| | SSH | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| LA/AA | Proso millet | 1.76$^{a}$ | 1.27$^{abcAB}$ | 1.58$^{abA}$ | 1.53$^{abA}$ | 1.12$^{abcB}$ | 0.84$^{cdB}$ | 0.64$^{dB}$ | 0.77$^{cdB}$ | 1.02$^{bcdB}$ |
| | Corn | 1.71$^{bc}$ | 1.93$^{abA}$ | 1.48$^{cdA}$ | 1.59$^{bcdA}$ | 1.18$^{dB}$ | 1.34$^{cdA}$ | 2.21$^{aA}$ | 1.28$^{cdA}$ | 1.78$^{abcA}$ |
| | SSH | 1.97$^{a}$ | 0.62$^{bB}$ | 0.80$^{bB}$ | 0.71$^{bB}$ | 1.63$^{aA}$ | 0.92$^{bB}$ | 1.11$^{abB}$ | 1.18$^{abA}$ | 1.26$^{abB}$ |

LA: lactic acid, AA: acetic acid, BA: butyric acid, SSH: Sorghum-sudangrass hybrid. ND: No detected. Values with different small letters show significant differences among ensiling days with same species. Values with different capital letters show significant differences among species in the same ensiling days ($p$<0.05) increasing, and the increase rate was larger in the earlier period. The acetic acid content in proso millet (5.76 g/kg) was significantly lower than that in corn and sorghum-sudangrass hybrid ($p<0.05$). The acetic acid content of sorghum-sudangrass hybrid was the highest during the entire fermentation process, and reached 100.25 g/kg at the 45th day. As with lactic acid, the highest levels of acetic acid were still detected in sorghum-sudangrass hybrid. This may be because sorghum-sudangrass hybrid had higher moisture content than the other two forage crops, and the activity of microorganisms was more active and produced more acids. This conjecture is consistent with the previous study's conclusion that reduced moisture content would limit fermentation (Kim et al., 2001).

Butyric acid is produced by clostridia, and its presence is related to the degree of silage spoilage. During the 45-day ensiling period, the butyric acid was not detected.

There was no obvious regularity of LA / AA ratio change trend, but compared with the 1st day LA / AA ratio of each crop, it showed a downward trend. The decrease in the ratio of LA / AA indicates that the fermentation pattern was changed from homofermentation to heterofermentation and is consistent with other studies reported by Shao et al (2002; 2005). The ratio of LA / AA in corn was significantly high ($p$<0.05), indicating that the homofermentation was dominant in corn.

### 4.3.4 Microbial compositions of silage during ensiling

4.3.4.1 Lactic acid bacteria (LAB)

The change of microbial population with fermentation time during ensiling was shown in the Table 14. The number of lactic acid bacteria in the early ensiling period showed an upward trend, reaching the highest peak on the 10th day, with 6.96 log10cfu/g FW for proso millet, 7.77 log10cfu/g FW for corn, and 6.95 log10cfu/g FW for sorghum-sudangrass hybrid. Among them, corn had significantly higher lactic acid bacteria content than proso millet and sorghum-sudangrass hybrid ($p$<0.05). After 10 days the number of lactic acid bacteria began to decline, at day 45, the lactic acid bacteria content of proso millet, corn and sorghum-sudangrass hybrid were 5.34 log10cfu/g FW, 6.08 log10cfu/g FW and 5.89 log10cfu/g FW, respectively. This trend is consistent with the trend of lactic acid bacteria in previous experiments (Ren, 2007). This can be explained by the fact that low pH and lack of fermentable substrates can cause bacterial death, so LAB would decrease over time (Xu et al., 2017). And at all stages of ensiling, the LAB content in corn was almost significantly higher than that of proso millet and sorghum-sudangrass hybrid ($p$<0.05).

4.3.4.2 Mold

The content of molds in forage crops is one of the factors affecting the fermentation quality of crops. Molds are aerobic microorganisms present in silage which can cause spoilage during ensiling (Muck, 2010). From the data in Table 15, it can be seen that mold was always present in each crop during fermentation. And most of the time, mold in corn was significantly lower than proso millet and sorghum-sudangrass hybrid *(p*<0.05). This may be due to the rapid and low pH decline in corn, which can inhibit the growth of undesirable microorganisms. Studies have also shown that in natural ermentation, with the progress of fermentation, when the product of LAB is insufficient, it is not enough to lower the pH value and inhibit bad microorganisms (Zheng et al., 2015). In this case, mold would increase. In the case of low nutrient content in the silage, the number of molds would be reduced due to the lack of substrate. Therefore, there was no regularity in the change of mold, and the phenomenon of increase and decrease occurs repeatedly.

Table 14. Microbial compositions of silage during ensiling

| Item | Species | Storage periods (days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 5 | 10 | 15 | 20 | 30 | 45 |
| LAB (log10 cfu/g FW) | Proso millet | $6.48^{bB}$ | $6.88^{aA}$ | $6.94^{aB}$ | $6.93^{aB}$ | $6.96^{aB}$ | $6.48^{bC}$ | $5.78^{cC}$ | $5.78^{cC}$ | $5.34^{dB}$ |
| | Corn | $6.84^{fA}$ | $6.85^{fA}$ | $7.23^{dA}$ | $7.30^{cA}$ | $7.77^{aA}$ | $7.61^{bA}$ | $7.04^{eA}$ | $6.60^{gB}$ | $6.08^{hA}$ |
| | SSH | $5.08^{fC}$ | $5.68^{eB}$ | $6.60^{bcC}$ | $6.53^{cC}$ | $6.95^{aB}$ | $6.60^{bcB}$ | $6.95^{aB}$ | $6.70^{bA}$ | $5.89^{dA}$ |
| Mold (log10 cfu/g FW) | Proso millet | $3.49^{dA}$ | $4.23^{cA}$ | $4.30^{cA}$ | $4.20^{cA}$ | $5.00^{abA}$ | $5.38^{aA}$ | $4.34^{bcA}$ | $4.04^{bcA}$ | $4.80^{abcA}$ |
| | Corn | $3.18^{eB}$ | $3.00^{fC}$ | $4.00^{cB}$ | $3.00^{fC}$ | $4.08^{cB}$ | $4.00^{cC}$ | $3.85^{dB}$ | $4.67^{aA}$ | $4.18^{bB}$ |
| | SSH | $3.48^{eA}$ | $3.30^{fB}$ | $3.95^{bB}$ | $3.70^{cB}$ | $3.30^{fC}$ | $5.11^{aB}$ | $3.60^{dC}$ | $3.30^{fB}$ | $3.00^{gC}$ |
| TM (log10 cfu/g FW) | Proso millet | $7.43^{dA}$ | $7.56^{cA}$ | $7.44^{dA}$ | $7.78^{bB}$ | $7.86^{aB}$ | $7.26^{eA}$ | $7.04^{fB}$ | $6.30^{hB}$ | $6.60^{gB}$ |
| | Corn | $7.05^{cB}$ | $7.51^{cA}$ | $7.18^{cB}$ | $8.10^{bA}$ | $8.85^{aA}$ | $7.11^{cA}$ | $7.85^{bA}$ | $7.04^{cB}$ | $7.12^{cA}$ |
| | SSH | $6.57^{eC}$ | $6.85^{dB}$ | $6.48^{eC}$ | $7.11^{cC}$ | $7.40^{aC}$ | $6.95^{dB}$ | $7.04^{cB}$ | $7.30^{bA}$ | $6.51^{eC}$ |

LAB: lactic acid bacteria, TM: total microorganism, SSH: Sorghum-sudangrass hybrid, CFU: Colony forming unit, FW: Fresh weight. Values with different small letters show significant differences among ensiling days with same species. Values with different capital letters show significant differences among species in the same ensiling days ($p$<0.05)

4.3.4.3 Total microorganism

Total microorganisms are all bacteria present in the crop. Crop fermentation is dominated by microorganisms, including beneficial bacteria and undesired bacteria. The number of microorganisms in all crops increased first and then decreased significantly ($p$<0.05), and all peaked on the 10th day, with proso millet at 7.86, corn at 8.85, and sorghum-sudangrass hybrid at 7.40 log10cfu/g FW. This change may be related to the reduction of the pH of the forage crops, which would inhibit the growth of microorganisms when the pH of the crops decreases to a certain degree. And for the impact of different crop species on the total microorganisms, the total microorganisms number of corn was significantly higher than that of other crops ($p$<0.05).

## 4.4 Analysis of effects of additives on silage

### 4.4.1 Sensory evaluation of silage

According to the scoring method of the Deutche Lan Dwirtschafts Geseutschaft, on the 60th day after ensiling, sensory evaluations were performed on each of the silage in bottles with additives. The odor, structure, and color were evaluated, and the score was graded. In odor, they all have aromatic smell (Table 2), but compared to proso millet and corn, sorghum-sudangrass hybrid had a weaker smell. The stem and leaf structure of the crops remained well. However, due to the higher moisture content in sorghum-sudangrass hybrid, the structure was relatively loose. The color was basically pale yellow. Each score was above 14 and they were excellent silage.

Table 15. Sensory evaluation of silage

| Species | Treatment | Odor | Structure | Color | Total | Grade |
|---|---|---|---|---|---|---|
| Proso millet | C | 12.33 | 4.00 | 0.97 | 17.30 | Excellent |
| | FA | 12.33 | 4.00 | 1.00 | 17.33 | Excellent |
| | LP | 12.67 | 4.00 | 1.50 | 18.17 | Excellent |
| Corn | C | 10.67 | 4.00 | 1.00 | 15.67 | Good |
| | FA | 13.00 | 4.00 | 1.00 | 18.00 | Excellent |
| | LP | 11.00 | 4.00 | 1.00 | 16.00 | Excellent |
| SSH | C | 11.00 | 3.00 | 1.00 | 15.00 | Good |
| | FA | 10.67 | 3.00 | 1.00 | 14.67 | Good |
| | LP | 10.00 | 3.00 | 1.00 | 14.00 | Good |

C: control, FA: formic acid, LP: *Lactobacillus plantarum*, SSH: Sorghum-sudangrass hybrid. The grade (0-20) were ranked into four grades with Corruption (0-4), Medium (5-9), Good (10-15) and Excellent (16-20).

### 4.4.2 Chemical composition of silage with different additives

The chemical composition of silage with different additives was shown in Table 16. The effects of different additives on silage were different among the same crops. In the treatment of proso millet and sorghum-sudangrass hybrid, the content of DM in both FA and LP treatments was higher than that in the control group. Correspondingly, the DM loss of proso millet and sorghum-sudangrass hybrid with additives were lower than that of the control group. This also confirms the conclusion that silage additives can reduce dry matter loss (Henderson, 1993). The use of formic acid as an additive can effectively restrict the fermentation of silage, thereby reducing the loss of dry matter. This conclusion has been confirmed in other experiments (Roughani et al., 2009). The addition of *Lactobacillus plantarum* in the silage can produce a large amount of lactic acid, which can reduce the pH of the silage in a short time, effectively inhibit the growth of undesired microorganisms, and reduce losses. This is agreement with the conclusion of Weinberg et al. (2002). In the corn treatment group, the DM content of silage with LP was 2.70 g/kg higher than that of the control group, but the DM content of FA group was significantly lower than that of the LP group and the control group ($p$<0.05). In most experiments, the addition of FA should inhibit the growth of undesirable microorganisms and reduce losses. However, some experiments (Rooke et al., 1988) have shown that the silage after adding formic acid cannot completely inhibit the growth of yeast that can consume WSC in the silage and accompany the loss of DM, which is consistent with our experimental data. Compared with the other two species, proso millet had the highest DM content at 60 days, but its DM loss was also significantly higher than that of corn and sorghum-sudangrass hybrid ($p<0.05$). This may be because the pH value of proso millet silage was relatively high, and the content of harmful microorganisms such as mold was high, so that the dry matter of the silage was excessively consumed.

Low CP content observed in the control could be attributed to the microbial activity which facilitated proteolytic during ensiling. Crude protein content was affected by treatments and was higher in LP treated silage ($p<0.05$). Among them, CP content in LP treatment of proso millet was 5.50 g/kg higher than that of the control group, while that of corn was 5.10 g/kg and that of sorghum-sudangrass hybrid was 20.40 g/kg. The highest CP content was detected in LP treated groups can be explained by the LP application induced rapid acidification thereby suppressing protein degradation by undesired microorganisms. It is consistent with the conclusions of Zhao et al. (2019). The FA treatments also enhanced preservation of CP content. That may be due to the restriction of fermentation, deamination and decarboxylation of proteins after the addition of FA (Chamberlain et al., 1990; Rooke et al., 1988). In the three crops, the effect of different additives on the CP content of corn was not significant.

Different additives have different effects on different crops. For proso millet, the content of NDF and ADF treated with additives decreased significantly compared with the control group ($p<0.05$). This conclusion is the same as Baytok et al. (2005). Among proso millet treatments, the ADF contents added with FA and LP decreased by 19.90 g/kg and 39.60 g/kg compared with the control group, while the NDF decreased by 3.90 g/kg and 49.70 g/kg, respectively. Added LP into silage, lactic acid bacteria can effectively reduce the composition of the cell wall, thereby reducing fiber content, which is consistent with experimental results of Tao (2005). The studies of Desta et al. (2016) have shown that the addition of formic acid directly reduces the pH value by quickly acidifying the raw materials,

Table 16. DM content, DM loss and chemical composition of silages with different additives

| Species | Treatment | DM | DM loss | CP | ADF | NDF | IVDMD | TDN | RFV |
|---|---|---|---|---|---|---|---|---|---|
| | | ------ | ------ | ------ | g /kg | ------ | ------ | % | |
| Proso millet | C | 266.00 | 37.40 | 56.60$^{b}$ | 358.90$^{a}$ | 624.30$^{a}$ | 580.30$^{b}$ | 60.55$^{c}$ | 91$^{c}$ |
| | FA | 266.60 | 36.80 | 58.20$^{ab}$ | 339.00$^{ab}$ | 620.40$^{a}$ | 639.20$^{a}$ | 62.12$^{b}$ | 94$^{b}$ |
| | LP | 273.3 | 30.10 | 62.10$^{a}$ | 319.30$^{b}$ | 574.60$^{b}$ | 627.80$^{ab}$ | 63.68$^{a}$ | 104$^{a}$ |
| | Mean | 268.63$^{A}$ | 34.77$^{A}$ | 58.97 | 339.07$^{B}$ | 606.43$^{A}$ | 615.77$^{B}$ | 62.12 | 96$^{B}$ |
| Corn | C | 263.00$^{a}$ | 14.30$^{b}$ | 52.00 | 265.20 | 465.10$^{b}$ | 684.60 | 69.28 | 137$^{a}$ |
| | FA | 257.40$^{b}$ | 19.90$^{a}$ | 52.20 | 275.90 | 510.80$^{a}$ | 688.40 | 68.53 | 123$^{b}$ |
| | LP | 265.70$^{a}$ | 11.60$^{b}$ | 57.10 | 268.50 | 467.60$^{b}$ | 716.10 | 69.05 | 135$^{a}$ |
| | Mean | 262.03$^{A}$ | 15.27$^{B}$ | 53.77 | 269.87$^{C}$ | 481.17$^{B}$ | 696.37$^{A}$ | 68.95 | 132$^{A}$ |
| SSH | C | 175.40 | 17.40 | 46.90$^{b}$ | 428.00 | 654.80 | 547.10 | 55.09 | 79 |
| | FA | 186.70 | 6.10 | 49.80$^{b}$ | 348.10 | 553.60 | 634.00 | 61.40 | 104 |
| | LP | 180.50 | 12.30 | 67.30$^{a}$ | 388.10 | 617.50 | 612.70 | 58.24 | 88 |
| | Mean | 180.87$^{B}$ | 11.93$^{B}$ | 54.67 | 388.07$^{A}$ | 608.63$^{A}$ | 597.93$^{B}$ | 58.24 | 90$^{B}$ |

DM: dry matter, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber, IVDMD: *in vitro* dry matter digestibility, TDN: total digestible nutrient, RFV: relative feed value, C: control, FA: formic acid, LP: *Lactobacillus plantarum*, SSH: Sorghum-sudangrass hybrid. Different lowercase letters in the same column indicate significant differences among additives ($p$< 0.05). Different uppercase letters in the same column indicate significant differences among species ($p$<0.05).

significantly reducing the content of cellulose and hemicellulose in the grass silage. Similarly, the NDF and ADF content of the sorghum-sudangrass hybrid treated with additives decreased, but not significantly ($p>0.05$). Compared with fresh crops, the NDF and ADF of each treatment of corn are significantly reduced. Among them, the NDF in the corn control group was significantly lower than the additive group ($p<0.05$), while the ADF content was not significantly different. This may be because the additive treatment group inhibited the activity of plant enzymes and reduced the degradation of the cell wall.

For IVDMD, whether adding FA or LP increased the content of IVDMD. LP and FA addition significantly ($p<0.05$) improved IVDMD of proso millet silages, while no notable differences were found in IVDMD among the corn and sorghum-sudangrass hybrid silages. This may be because the use of additives significantly improves the quality of silage fermentation, inhibits unfavorable microbial fermentation, especially inhibits protein digestion and hydrolysis, thereby increasing the IVDMD content (Fliya, 2002). Li et al. (2016) also found that treatments with organic acid could increase the IVDMD.

The TDN content is related to the content of ADF. For proso millet and sorghum-sudangrass hybrid, the lowest TDN content was detected in the control group, while the TDN content of the control group corn was higher than that of the additive group without significant difference ($p>0.05$).

The RFV of different crops was also different under the action of different additives. Among the three crops, the corn treatment group had the highest RFV, followed by proso millet and sorghum-sudangrass hybrid. The control group corn had the highest RFV (137), while the control group sorghum-sudangrass hybrid had the lowest RFV (79).

### 4.4.3 Fermentation quality of silage with different additives

In the process of ensiling, microbial fermentation produces organic acids, causing the pH of the silage to drop. Adding FA as an acid to silage would directly reduce the pH of the silage, while adding LP would increase the number of lactic acid bacteria, and then produce more lactic acid to lower the pH. Compared with the pH of fresh crops, the pH of all treatments decreased significantly. And from Figure 8, we can see that the pH of the additive-treated groups were significantly lower than that of the control groups ($p<0.05$). This finding has been verified in other experiments (Zhang et al., 2018). And there was no significant difference in pH between FA treatment group and LP treatment group.

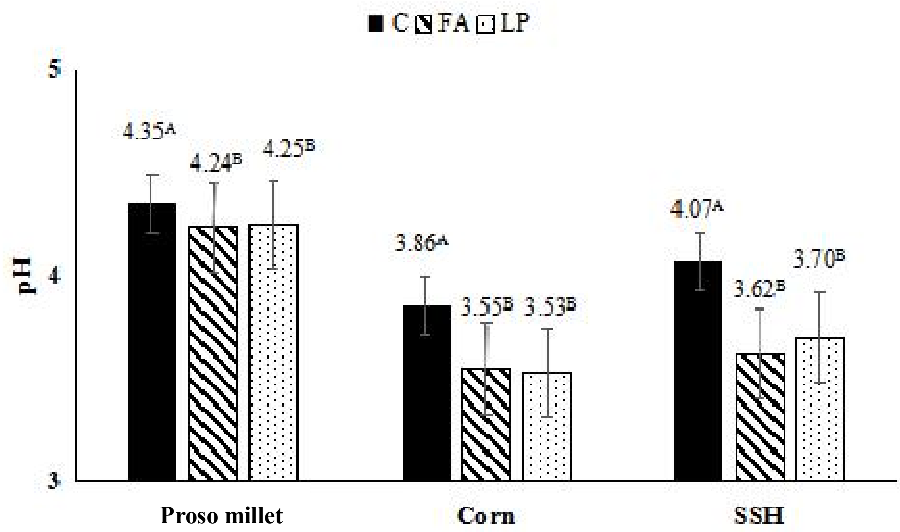


Figure 8. The pH value of silage with control (C), formic acid (FA) and *Lactobacillus plantarum* (LP). The vertical bars represent the standard error of the means (n=9). Means with different letter in the same crops are significant at $p<0.05$.

Water-soluble carbohydrates are considered to be important substrates for LAB growth during proper fermentation, and WSC is continuously consumed as fermentation proceeds. The WSC concentrations of silages were higher ($p<0.05$) in FA compared to C and LP of each forage species.

And among the three crops, the residual amounts of WSC were C < LP < FA. As with our study, treatment of silage with FA additives had shown increased residual WSC concentration, thus indicating that partial inhibition of fermentation results in WSC not being continuously consumed (Silva et al., 2015). Compared with the control group, the WSC content of LP treatment also increased. This may be because after the addition of LP, a large amount of lactic acid bacteria consumes WSC while

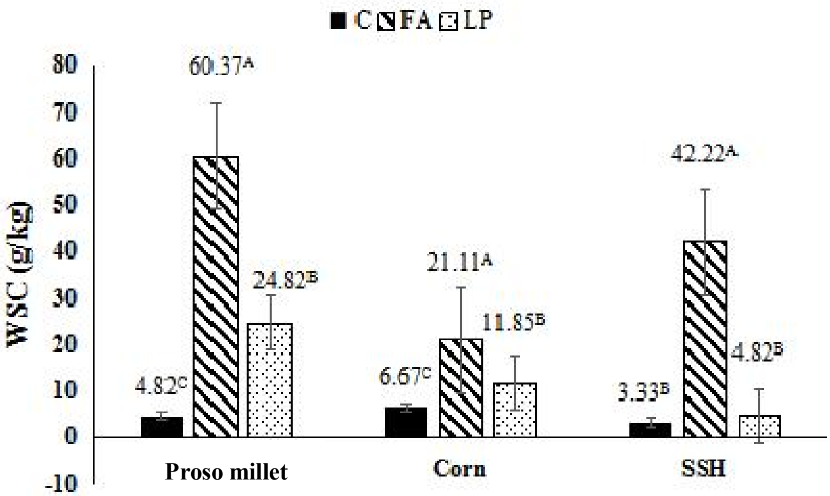


Figure 9. The WSC content of silage with control (C), formic acid (FA) and *Lactobacillus plantarum (LP)*. The vertical bars represent the standard error of the means (n=9). Means with different letter in the same crops are significant at $p$<0.05.

reducing the pH of the silage, inhibiting the growth of undesirable microorganisms, thereby reducing their consumption of WSC. This also indicates that the LP treated group reaches a steady state quickly, so that part of the WSC was retained. Meeske et al. (2002) also reported that bacterial additives have no effect on WSC during fermentation, which indicates that WSC has the same utilization rate in control and LP treated silage. At the same time, we can also see from Figure 9 that the effect of additives on proso millet was significantly higher than that of corn and sorghum-sudangrass hybrid, retaining a large amount of WSC.

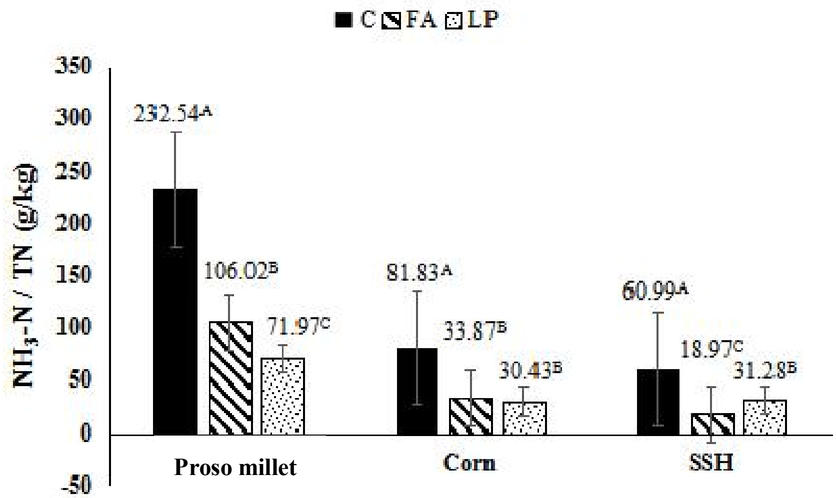


Figure 10. The $NH_3$-N / TN content of silage with control (C), formic acid (FA) and *Lactobacillus plantarum (LP).* The vertical bars represent the standard error of the means (n=9). Means with different letter in the same crops are significant at $p$<0.05.

Protein hydrolysis in silage leads to an increase in soluble N and $NH_3$-N during ensiling (Kung et al., 2018). Well-preserved silages should contain less than 100 g $NH_3$-N / kg TN (McDonald et al., 1991). As can be seen from Figure 10, except for the control group of proso millet, the rest of the treatments meet the requirements of well-preserved silages. For the three crops, the $NH_3$-N/TN content of the additive-treated groups were much lower than that of the control groups. This is consistent with the conclusion of Contreras-Govea et al. (2013). The lower $NH_3$-N/TN contents in silage indicated inhibition of proteolysis during fermentation and therefore the efficiency of nitrogen synthesis by rumen microorganisms is improved (Nsereko et al., 1999).

### 4.4.4 Organic acids of silage with different additives

Table 17 shows the organic acid content of the silage treated with different additives. Additives had significant ($p<0.05$) effects on the lactic acid, acetic acid and ration of lactic acid / acetic acid. Across the three species, the LP treatment had the highest lactic acid concentration, followed by control and FA ($p<0.05$). And among the three crop silages, the lactic acid content of the LP-treated silages were not much different, namely proso millet 76.67 g/kg, corn 70.42 g/kg and sorghum-sudangrass hybrid 78.37 g/kg. In all crops, the use of the LP additive increased the concentration of lactic acid compared to the control group. That may be the lactic acidification in LP silages was clearly enhanced by addition of exogenous *Lactobacillus plantarum*, which favoured homofermentation of silage, thus resulting in a higher production of lactic acid (Adesogan et al., 2002). Similarly, Contreras-Govea et al. (2013) also found that inoculant containing *Lactobacillus plantarum* was successful in increasing the concentration of lactic acid in maize silage. Many reports indicate that the addition of formic acid to silage would limit the fermentation of silage, thereby reducing the content of lactic acid in silage (Kennedy, 1990; Tyrolová et al., 2017). This conclusion is consistent with our experimental data.

As can be seen from Table 17, among the treatments of three crops, the FA treatment group had the highest acetic acid content, which was significantly higher than that of the control group and the LP treatment group ($p<0.05$). This showed that the addition of organic acid can provide a favorable environment for the growth of heterofermentative fermentation bacteria, and thus produce more acetic acid. The same results were observed during ensiling of maize after the addition of formic acid (Tyrolová et al., 2017). Sorghum-sudangrass hybrid had the highest content of acetic acid, followed by corn and proso millet. It may be because the value of the buffering capacity of sorghum-sudangrass was higher, which prolongs the fermentation time, resulting in a large amount of acetic acid production

In all treatments, butyric acid was only detected in the control group of millet and the FA treatment group of sorghum-sudangrass hybrid, 2.27 g/kg and 5.91 g/kg respectively. For proso millet, it may be because the activity of clostridia in untreated silage was not restricted. For sorghum-sudangrass hybrid, it may be due to the high moisture content of sorghum-sudangrass hybrid, which creates an environment suitable for the growth of clostridia, which leads to the production of butyric acid. It is agreement with the result that clostridia fermentation occurs particularly when ensiled crop is low in WSC and DM contents at high temperatures (Oladosu et al., 2016).

As Jones et al. (1992) demonstrated, the lactic acid / acetic acid ratio is a efficiency indicator of homofermentative or heterofermentative fermentation. In our study, the highest value of 4.12 ($p<0.05$) was observed for the LP treated silage of proso millet. This indicates that the LP additive used made the fermentation more homofermentative. Similarly, in corn and sorghum-sudangrass hybrid, the ratio of LP treatment group was also higher. On the contrary, in the treatments of three crops, the LA/AA ratio of FA treatment group was significantly lower than that of control group and LP treatment group ($p<0.05$). This is consistent with the above conclusion, formic acid additives increase acetic acid content but also reduce lactic acid content.

From the Flieg-Zimmer score and grade, we can see that except for the formic acid treatment group of sorghum-sudangrass hybrid, all the silage fermentation quality was better. Among them, the proso millet and corn supplemented with LP were excellent.

Table 17. Effects of additives on organic acids and quality grade of silage

| Species | Treatment | pH | LA (g/kg) | AA (g/kg) | BA (g/kg) | LA/AA | Flieg-Zimmer score | Grade |
|---|---|---|---|---|---|---|---|---|
| Proso millet | C | $4.35^{a}$ | $40.08^{b}$ | $14.14^{b}$ | 2.27 | $2.83^{b}$ | $47^{b}$ | Average |
| | FA | $4.24^{b}$ | $21.02^{c}$ | $35.91^{a}$ | ND | $0.59^{c}$ | $53^{b}$ | Average |
| | LP | $4.25^{b}$ | $76.67^{a}$ | $18.63^{ab}$ | ND | $4.12^{a}$ | $98^{a}$ | Excellent |
| | Mean | $4.28^{A}$ | $45.92^{B}$ | $22.89^{C}$ | $0.76^{B}$ | $2.51^{A}$ | $66^{B}$ | Good |
| Corn | C | $3.86^{a}$ | $61.45^{b}$ | $48.60^{b}$ | ND | $1.26^{b}$ | $61^{b}$ | Good |
| | FA | $3.55^{b}$ | $58.83^{b}$ | $96.20^{a}$ | ND | $0.61^{b}$ | $53^{b}$ | Average |
| | LP | $3.53^{b}$ | $70.42^{a}$ | $24.37^{b}$ | ND | $2.89^{a}$ | $92^{a}$ | Excellent |
| | Mean | $3.65^{B}$ | $63.57^{A}$ | $56.39^{B}$ | ND | $1.59^{B}$ | $69^{A}$ | Good |
| SSH | C | $4.07^{a}$ | $76.70^{a}$ | $45.23^{b}$ | ND | $1.70^{a}$ | $65^{a}$ | Good |
| | FA | $3.62^{b}$ | $32.61^{b}$ | $97.81^{a}$ | 5.91 | $0.33^{c}$ | $21^{b}$ | Bad |
| | LP | $3.70^{b}$ | $78.37^{a}$ | $80.70^{ab}$ | ND | $0.97^{b}$ | $63^{a}$ | Good |
| | Mean | $3.80^{B}$ | $62.56^{A}$ | $74.58^{A}$ | $1.97^{A}$ | $1.00^{B}$ | $50^{C}$ | Average |

LA: lactic acid, AA: acetic acid, BA: butyric acid, C: control, FA: formic acid, LP: *Lactobacillus plantarum*, SSH: Sorghum-sudangrass hybrid, ND: No detected. The Flieg-Zimmer scores (0-100) were ranked into five grades with Poor (0-20), Fair (21-40), Average (41-60), Good (61-80) and Excellent (81-100). Different lowercase letters in the same column indicate significant differences among additives($p$<0.05). Different uppercase letters in the same column indicate significant differences among species ($p$<0.05).

### 4.4.5 Microbial counts of silage with different additives

Combining with Table 18, we can find that additives have a significant effect on the numbers of silage microorganisms ($p$<0.05). All crops were the same, and lactic acid bacteria were the highest in silage treated with LP additives. The FA treated silages had fewer LAB than did either control or LP treated silages. The same result was also found by Da Silva et al. (2015) and Tyrolová et al. (2017). In all FA treatment groups, both the total microorganisms and the molds numbers were significantly lower than those of the control and LP groups ($p$<0.05). This may be because FA treatment can inhibit the growth of undesirable microorganisms. It can also be seen from the data that the LP treatment also effectively reduced the growth of mold compared to the control group. The number of lactic acid bacteria and total microorganisms were not significantly different among species. The mold in corn silage was significantly lower than that in millet and sorghum-sudangrass hybrid ($p$<0.05). This may be because the low pH of corn silage effectively inhibits the growth of undesirable microorganisms.

Table 18. Microbial compositions of silages after 60 days of ensiling

| Species | Treatment | LAB (log10 cfu/g FW) | Mold (log10 cfu/g FW) | TM (log10 cfu/g FW) |
|---|---|---|---|---|
| Proso millet | C | $5.12^{b}$ | $4.15^{a}$ | $6.55^{b}$ |
| | FA | $5.00^{b}$ | $3.70^{b}$ | $6.08^{c}$ |
| | LP | $6.82^{a}$ | $3.98^{ab}$ | $7.26^{a}$ |
| | Mean | $5.65^{C}$ | $3.94^{A}$ | $6.63^{B}$ |
| Corn | C | $6.15^{b}$ | $3.16^{a}$ | $7.10^{b}$ |
| | FA | $5.54^{c}$ | $3.05^{b}$ | $6.89^{b}$ |
| | LP | $7.20^{a}$ | $3.01^{b}$ | $7.70^{a}$ |
| | Mean | $6.30^{A}$ | $3.07^{B}$ | $7.23^{A}$ |
| SSH | C | $5.88^{b}$ | $4.28^{a}$ | $6.35^{c}$ |
| | FA | $5.12^{c}$ | $3.08^{c}$ | $6.75^{b}$ |
| | LP | $7.14^{a}$ | $3.30^{b}$ | $7.35^{a}$ |
| | Mean | $6.05^{B}$ | $3.55^{AB}$ | $6.82^{B}$ |

LAB: lactic acid bacteria, TM: total microorganism, C: control, FA: formic acid, LP: *Lactobacillus plantarum,* SSH: Sorghum-sudangrass hybrid, CFU: Colony forming unit, FW: Fresh weight. Different lowercase letters in the same column indicate significant differences among additives($p$<0.05). Different uppercase letters in the same column indicate significant differences among species ($p$<0.05).

# 5. Conclusion

In terms of productivity, sorghum-sudangrass hybrid had the highest fresh yield, followed by corn and proso millet. The highest TDN yield was detected in corn, while the lowest TDN yield was proso millet. For the nutritional value of corn, the content of ADF and NDF was the lowest, the content of IVDMD, TDN and RFV were also the highest. Proso millet had the highest DM and CP content. At the same time, the IVDMD of proso millet was also slightly higher than sorghum-sudangrass hybrid.

With the progress of ensiling, the chemical composition and nutritional value of various crops have changed. During the ensiling period, the DM, CP and WSC content of all crops decreased significantly ($p$<0.05). Among them, the dry matter content of proso millet was the most serious. The ADF and NDF contents of corn and sorghum-sudangrass hybrid showed a downward trend with the increase of ensiling time. In contrast, proso millet's ADF content increased significantly by 19.40 g / kg during the 45-day ensiling period. The IVDMD of millet decreased significantly during the ensiling period ($p$<0.05), while the changes of corn and sorghum-sudangrass hybrid were not obvious. The pH of all crops dropped rapidly in the early stage of fermentation and stabilized in the later stage. Correspondingly, during the ensiling, buffering capacity continues to increase. In counts of LAB and TM, both rose to the maximum on the 10th day and then decreased. As the ensiling progressed, the lactic acid and acetic acid content of the three crop silages increased significantly ($p<0.05$). And from the Flieg-Zimmer scores, the three crops were above the average fermentation level during the 45-day ensiling period.

For the effect of additives on crop fermentation. Compared with the control group, the additive groups reduced CP and WSC consumption. Using FA can save a lot of WSC than using LP. And the highest content of WSC was detected in the FA additive group of proso millet. At the same time, the $NH_3$-N and pH of the three crop silage additive groups were lower than those of the control group. In addition to the corn additive treatment group, the ADF and NDF contents of the proso millet and sorghum-sudangrass hybrid additive treatment groups were reduced. The addition of LP increased the content of LA in silage, while the use of FA increased the content of AA in silage. BA was only detected in the control group of proso millet and the FA treatment group of sorghum-sudangrass hybrid. Combined with the Flieg-Zimmer score, except for the FA treatment group of sorghum-sudangrass hybrid, the rest of the silages showed above average fermentation levels, and the fermentation grade of proso millet and corn with LP treated were excellent. LP also increased the counts of LAB and TM in silage. FA inhibited the growth of LAB. And the additive groups effectively inhibited the growth of mold.

Forage suitability evaluations revealed valuable information regarding forage establishment, yield, and quality of proso millet as livestock forage.

Overall, the production of proso millet is not very high compared to corn and sorghum-sudangrass hybrid, but it has the advantage of a short season. And proso millet has high drought tolerance and strong growth in saline land. In terms of nutrition, its feeding value is higher than sorghum-sudangrass hybrid. Combining planting conditions and its own nutritional value, millet is a good choice as forage. The use of additives improves the fermentation quality of silage, which can help to improve ruminant performance.

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# 7. 요약

사일리지용 옥수수, 수수-수단그라스 교잡종 그리고 기장은 청예 또는 사일리지 형태로 가축에 급여할 수 있는 다목적 여름철 사료작물이다. 그러나 한국에서는 기장의 경우 가축의 사료로 거의 이용되지 않는다. 본 시험은 사일리지용 옥수수, 수수-수단그라스 교잡종 그리고 기장에 대하여 생산성의 검토와 발효 양상 그리고 서로 다른 첨가제의 처리가 사일리지 발효에 미치는 영향을 비교하기 위해 강원도 평창에서 수행되었다. 이 연구는 2019 년 5 월부터 12 월까지 수행되었다. 기장( “Golden” )은 6 월 8 일에 파종하였고, 9 월 5 일에 수확하였다. 사일리지용 옥수수( “광평옥” )과 수수-수단그라스 교잡종( “Turbo-gold” )은 5 월 10 일에 파종하였으며, 9 월 10 일에 수확하였다. 수량은 작물의 종류에 따라 유의적인 차이를 나타내었다. 생초수량은 수수-수단그라스 교잡종이(121,733 kg/ha) 기장(25,350 kg/ha)과 옥수수(67,557 kg/ha)보다 유의적으로 많았다($p<0.05$). TDN 수량은 옥수수가 가장 많았고(14,378 kg/ha) 기장이 가장 적었다(4,711 kg/ha).

기장, 사일리지용 옥수수 및 수수-수단그라스 교잡종 사일리지의 발효양상을 구명하기 위해 사일리지 저장 1, 2, 3, 5, 10, 15, 20, 30 및 45 일후에 조사를 하였다. 저장기간동안 건물(DM),

조단백질(CP) 및 수용성 탄수화물(WSC) 함량은 모든 작물에서 유의적으로 감소하였다($p<0.05$). 발효가 진행됨에 따라 *in vitro* 건물 소화율(IVDMD)은 약간 감소하였고 옥수수는 기장 및 수수-수단그라스 교잡종 보다 항상 높았다. 공시된 작물들의 pH 는 발효 초기에 급격히 감소하였고 후기로 갈수록 안정되었다. 젖산균수는 발효 10 일째에 모두 최고에 도달하였는데 기장은 6.90 log 10 cfu/g FW 이었고 옥수수는 7.77 그리고 수수-수단그라스 교잡종은 6.95 10 cfu/g FW 이었다. 사일리지 발효가 진행됨에 따라 모든 작물의 사일리지에서 젖산 및 초산 함량이 유의적으로 증가하였다($p<0.05$).

사일리지 발효에 대한 첨가제 효과를 규명하기 위해 무처리(Control), 젖산균(LP, *Lactobacillus plantarum*, $1.0\times10^{6}$ CFU/g fresh matter) 그리고 개미산(FA, formic acid, 98%, 5ml/kg) 처리하였다, 모든 사일리지는 조제 후 60 일동안 보관하였다. 첨가제 처리는 작물들의 발효 품질을 개선시키는 효과가 있었다. 서로 다른 첨가제는 각각의 작물별로 다른 효과를 나타내었다. 모든 첨가제는 사일리지의 조단백질과 *in vitro* 건물 소화율을 증가시켰고 암모니아태 질소 ($NH_3$-N) 함량을 감소시켰다. 대조구와 비교할 때 개미산 및 젖산균 처리구는 모든 작물에서 수용성 탄소화물이 대부분 보존되었다. 특히 개미산을 처리한 기장 사일리지에서 수용성 탄수화물이 가장 높았다. 젖산균첨가제 처리는 사일리지의 젖산함량을 유의적으로 증가시켰으며, 반면 개미산 처리는 초산함량을 유의적으로 증가시켰다($p<0.05$). 젖산균을 처리한 옥수수 사일리지가 젖산균수가 가장 많았다.

본 시험 결과에 의하면 기장은 사일리지로의 이용 가능성이

매우 높았다. 또한 사일리지 조제시 개미산 및 젖산균 첨가제는 사일리지의 품질 및 발효양상을 개선시켜 주었다.

주요어: 사일리지용 옥수수, 수수-수단그라스 교잡종, 기장, 생산성, 발효양상, 첨가제, 발효품질

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