Master's Thesis of Science in Agriculture

Effect of Wilting and Lactic Acid Bacteria Treatment on Fermentation Pattern of Italian Ryegrass (*Lolium multiflorum* Lam.) Silage

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Abstract

The most cultivated forage crop in South Korea is Italian ryegrass (*Lolium multiflorum* Lam.), and its cultivation area is continuously increasing. The purpose of this study was to investigate the effects of wilting and lactic acid bacteria（LAB) treatment on silage fermentation pattern and final quality of Italian ryegrass silage. Italian ryegrass was harvested at heading stage and ensiled four treatments (NWNA: no-wilting no-additive; NWA, no-wilting with additive, WNA, wilting no-additive; WA, wilting with additive). The silage samples were collected at 1, 2, 3, 5, 10, 20, 30 and 45 days after ensiling and the final quality was confirmed at 60 days after storage. Wilting treatment resulted in higher ADF (acid detergent fiber) and NDF (neutral detergent fiber) content of Italian ryegrass silage ($p<0.05$), and the WNA (wilting no-additive) treatment showed the lowest total digestible nutrients (TDN) and *in vitro* dry matter digestibility (IVDMD). However, wilting treatment resulted in lower crude protein and *in vitro* dry matter digestibility ($p<0.05$) and there was no significant differences in ADF, TDN, WSC (water soluble carbohydrate), ammonia nitrogen content and pH ($p>0.05$). The pH of the silage was higher in the wilting group and lower in the additive treatment group. Meanwhile, the decrease in pH occurred sharply on the 3-5th day of storage. The ammonia nitrogen content was significantly lower in the additive treatment ($p<0.05$), and wilting was not affected. As fermentation progressed, the lactic and acetic acid contents were increased and showed the highest content at 30 days of storage. In conclusion, the wilting treatment did not significantly improve the silage fermentation, but the additive treatment improves the quality of the silage. So, inoculant treatment is recommended to prepare the high quality Italian ryegrass silage.

Keywords : Italian ryegrass, Silage, Fermentation, Wilting, Inoculant

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Contents

List of Tables

List of Figures

List of Abbreviations

AA: Acetic acid

ADF: Acid detergent fiber

BA: Butyric acid

CP: Crude protein

DDM: Digestible dry matter

DM: Dry matter

DMI: Dry matter intake

DW: Distilled water

FM: Fresh matter

HPLC: High performance liquid chromatography

IVDMD: *In vitro* dry matter digestibility

LA: Lactic acid

LAB: Lactic acid bacteria

LSD: Least significant difference

NDF: Neutral detergent fiber

NH_3-N: Ammonia nitrogen

RFV: Relative feed value

SAS: Statistical analysis software

TDN: Total digestible nutrients

VFA: Volatile fatty acid

WSC: Water soluble carbohydrate

1.Introduction

1.1 Research background

Two types of ryegrass, perennial and annual, are being cultivated in worldwide. Italian ryegrass (*Lolium multiflorum* Lam.) is a herbaceous annual or biennial grass that is grown for silage, and as a cover crop (Jung et al., 1996). In the United States, *Lolium multiflorum* is sometimes used as a winter cover crop to prevent erosion, build soil structure and suppress weeds. As a palatable forage crop, it can be grazed by livestock and provide feed in years when alfalfa suffers from winter kill (USDA, 2007). It grows on about 1 million ha in the humid, southern United States, being used primarily for winter pasture in clear seeding and in dormant bermudagrass sods (Evers, 1995).

South Korea has four distinct seasons, so crop cultivation during winter are extremely limited. Also, cultivation using paddy fields is dominant because the forage production bases are weak (Kim et al., 2001). Italian ryegrass is one of the wintering crops that is grown after rice cultivation. It was cultivated mainly in some southern coastal areas due to weak winter hardness. However, the cultivated area has been gradually increased due to the development of resistance varieties for cold-winter, and now about 135 thousand hectares of IRG were cultivated in 2015 and it estimated 52% of total forage cultivation areas in South Korea (MAFRA, 2018). Italian ryegrass is one of the fastest-growing forage crops available to farmer. It is widely distributed throughout temperature and tropical or subtropical regions of the world and is one of China's major forage crop used either fresh green-chop or as hay or silage (Shao et al, 2005). It offers rapid establishment and starts growing early in the following spring and has fast regrowth after defoliation.

It thrives on all kinds of soil and is mainly used for conservation in short duration leys, often in mixtures with red clover. New varieties have better persistence and are suitable for 2-3 year leys.

Unfortunately, rains come often at the proper harvest time of the Italian ryegrass in South Korea (early May - mid May), which restrict on the storage methods. Most farmers stored Italian ryegrass as the form of silage, and some farmers are trying to store it as hay. Produced silage (round bale) is wrapped in plastic vinyl and distributed throughout the country (Kim et al, 2001). However, due to the lack of silage preparation technology, the quality of the sold silage is uneven and there is a distrust between producers and consumers.

Ensiling forage crop is well known method of conservation for shortage season. Lactic acid bacteria (LAB) converts water soluble carbohydrates (WSC) under anaerobic condition into lactic acid. As a result of the pH decline, the silage is well preserved (Shao et al*.,* 2002). Acidification of well fermented silage inhibits undesirable microorganism. During the fermentation process, competition takes place between LAB and undesirable microorganism, and fermentation quality always depend on the result of the competition (Krawutschke et al., 2012).

Dry matter content of raw materials has a great influence on silage fermentation, which affects all fermentation characteristics, pH level and quality parameter of silage. Ensiling with low DM content around 25% could cause inferior fermentation and high pH level deducing serious DM loss, compared to higher DM content. The DM at less than 300 g kg^{-1} may also generate an increase in seepage loss and expedite *clostridial* fermentation reduces voluntary intake (McDonald et al., 1991). But, wilting reduces the amount of fermentable carbohydrates required to properly preserve the silage and restricts the growth of undesirable microorganisms (Barnes et al, 2003).

The application of silage additives is normally recommended to ensure and improve silage fermentation (Krawutschke et al., 2012). At present, lactic acid bacteria (LAB) inoculant are the main additives in many parts of the world (Weinberg and Muck, 1996). Although numerous inoculant studies have been conducted, there are often variations in the fermentation results obtained and in their interpretation. LAB additives usually increase the rate of lactic acid production, thereby accelerating the pH decline and reducing post-harvest proteolysis. In addition, rapid acidification results in the inhibition of detrimental microorganisms (Robowsky et al, 2001).

1.2 Aim of research

Generally, wilting result in lower water soluble carbohydrate contents, extensive protein breakdown and sometimes higher total volatile fatty acid (VFA) during ensiling (Uchida *et al*., 1989). Wilting also affect the chemical composition, DM losses, silage fermentation and animal performance (Steen, 1984; Patterson et al., 1996).

So, this study was accomplished to evaluate the effect of wilting and inoculant on fermentation dynamics and qualities of Italian ryegrass silage.

2. Literature review

2.1 Characteristics of Italian ryegrass

Italian ryegrass is an annual or biennial grass that can grow up to 100cm, it is one of the fastest growing grasses available to farmers. It offers rapid establishment and starts growing early in the following Spring and has fast regrowth after defoliation. For many years, Italian ryegrass has been the topic of plant breeding, which means there are a broader range of varieties available commercially. It has high yielding and provides about 18t DM per hectare on suitable soils. Italian ryegrass is native to central and southern Europe, north-west Africa and south-west Asia (Spedding and Diekmahns, 1972). Italian ryegrass is an important forage grass that is widely distributed in Asia, Europe, and North and South America (Lopes et al., 2009). It has now spread, largely as a result of its cultivation as a pasture grass, to temperate regions of all continents. It is normally restricted to lowland habitats, but may grow at higher altitudes where drainage and nutrient status permit (Beddows, 1973), although it has not been recorded above 900 m.

It was concluded in Shao's experiment that the silage made from Italian ryegrass with high moisture content had a good fermentation quality owing to the dominance of lactic acid bacteria and active lactic acid fermentation during the initial stage of ensiling (Shao et al., 2005). And the physical characteristics of Italian ryegrass can delay the onset of lactic acid bacteria fermentation phase by extending the phases of respiration and aerobic microorganisms activity, causing the higher loss of water soluble carbohydrates and the shortage of lactic acid bacteria fermentation substrates (Shao et al., 2005). The stems and leaves of Italian ryegrass are rich in crude protein, crude fat and crude ash. The grass is tender and juicy and has good palatability, which is suitable for high producing dairy cows. Recently years, it has been seeded in late fall, and harvest in the following year. This can provide an excellent double-crop option (Joel, 2014). Italian ryegrass has the potential of high yield, it establishes quickly and suitability for reduced-tillage renovation, so it can use on heavy and waterlogged soils (Hannaway et al., 1999).

2.2 Cultivation of Italian ryegrass

2.2.1 Italian ryegrass in South Korea

In South Korea, Italian ryegrass accounts for about 52% of the total forage cultivation area (MAFRA, 2018), so it is an important variety of forage cultivation. Since 2007, Italian ryegrass has been a very important major winter forage crop in South Korea, and developed 15 Italian ryegrass varieties including early, medium and late maturity variety. But the cold tolerance of Italian ryegrass is significantly affected by weather and climate conditions (Choi et al., 2011). The Ko-variety of Italian ryegrass developed in South Korea have good cold tolerance and adaptability compared with other varieties. The cultivation area of Italian ryegrass has extended from 21,700 ha in 2007 to the 123,600 ha in 2017. The seed market of Italian ryegrass has also increased to 100 million won, and South Korea has been developing on new varieties of resistant and high-yielding products, trying to open the market for seed exports (Ji et al., 2017).

2.2.2 Italian ryegrass in China

In recent years, China has introduced and successfully tested Italian ryegrass in a multi-point trial and large-scale cultivation in the country. Italian ryegrass is widely grown in southern China from December to May of the next year. It is also one type of palatable digestible and leafy annual forage as hay and silage. However, due to the high annual precipitation in southern China and the humid climate, Italian ryegrass supplied unbalancedly as hay. Silage is found to be an ideal way of utilization forage in southern China. It is very positive to explore Italian ryegrass silage for developing grassland animal husbandry in this area. Italian ryegrass is used as a high-quality forage grass species in many countries. In southern China, since the 1990s it has been introduced into winter-fallow paddy fields as part of the Italian ryegrass–rice rotation (IRR) system (Ye et al., 2015). Studies have shown that the effects of different mowing heights of Italian ryegrass on forage yield. When the mowing height is 75 cm, the annual average dry matter yield is 11800 kg / hm^2, which is 58.9% higher than the 30 cm mowing The accumulation speed is the highest (Zhang et al., 2008).

Italian ryegrass was imported to China and its main role was to provide green lawns and pastures, which have been widely planted in recreation areas and used for livestock grazing (Zhang et al., 2009). However, there have been some problems in recent years. Italian ryegrass has invaded wheat fields in many provinces of China and has become an aggressive weed (Hao and Zhang, 2015; Wang et al., 2008). And China is also solving this issue actively.

2.2.3 Italian ryegrass in the world

Italian ryegrass is originally produced in southern Europe and southwestern Africa, mainly distributed in Italy, the United States, the United Kingdom, New Zealand, Australia, temperate Asia, North Africa and other countries. However, in many areas of the United Kingdom, there is a problem because of the increasing of Italian ryegrass as a weed.

Italian ryegrass is a serious and widespread weed problem in grain fields in western Oregon and Washington. Field studies were performed to measure the effects of Italian ryegrass disturbance on winter wheat yield. The results showed that with the increase of ryegrass density, wheat grain yield decreased. In one experiment, when the ryegrass density increased from 0.7 plants / square meter to 93 plants / square meter, wheat grain yield decreased by 4,100 kg / ha. In the case of high ryegrass density, the interference resulted in a decline in wheat yield of 168 kg N / ha greater than 56 kg N / ha. The results obtained in these studies illustrate the importance of good ryegrass control in winter wheat management systems (Appleby et al., 1976).

2.3 Utilization of Italian ryegrass

The grass-legume association has been used in many countries all around the world because a greater total herbage yield may be obtained by growing grass and a legume in an association, rather than in individual swards, where no fertilizer N is applied. Interferences and competition between legumes and associated grasses are of great importance in world pasture production. Such interspecific interferences are likely to be a dominating factor in the nonequilibrium, man-made and

-managed pasture vegetation. It is generally accepted that grasses normally have a competitive advantage over legumes, and they tend to dominate pastures but to maintain high pasture productivity, a balance between grasses and legumes is desirable (Haynes 1980). Except for legume, Italian ryegrass-alfalfa, Italian ryegrass-tall fescue, Italian ryegrass-rice, these collocations are also used far and wide. Italian ryegrass has similar nutritional value to perennial ryegrass and can be made as fresh feed, hay, and silage, or grazing. In the early stage of heading to be cut is better, 3 to 5 times cutting per year, and the production is about 3,000 to 5,000 kg per ha. Usually, the farmers cut Italian ryegrass at 8 weeks after seeding. A cutting height of 4 inches could improve for faster regrowth by leaving energy stored in crowns and leaf areas, thereby improve for photosynthesis. In the United States, it is used for forage predominately and irrigated intermountain valleys (Hannaway et al., 1999).

2.3.1 Grazing

Grazing intensity significantly affected forage quality, the inability to adjust grazing rates to provide equal grazing pressure on all treatments and reproduction of grazing trials may bias production performance per animal and per acre (Mott, 1960). Italian ryegrass grows fast, has many tillers and is resistant to grazing. It is high-quality grazing pasture and one of the highest yields of digestible materials in grasses. Usually unicast or mixed with a variety of pasture crops such as white clover, red clover, and so on. Cattle, sheep, and horses like the mixed-grass, which not only increases the quantity of the meat but also produces more milk. Cattle, horses, and sheep are usually grazing once in two months after sowing and can be grazing once every other month. When grazing, it should be divided into sections to prevent heavy grazing. After each grazing, it is necessary to topdress and irrigate once.Under the same environmental conditions, field measurements of reproductive growth of ryegrass dominant forages have shown that they are 23 to 41% higher than vegetative growth (Noble, 1972) (Anslow and Green, 1967). Multiplier function is to explain the efficiency of above-ground growth in the vegetative stage of the pasture life cycle each year (McCall et al., 2003).

2.3.2 Chopped as feed

Italian ryegrass has high nutritional value and is rich in protein, minerals, and vitamins. It has the characteristics of high-yield cultivation, an efficient agricultural grassland system, high crude protein synthesis, good palatability, and high digestibility. As feed for cattle, sheep, hares, geese, and pigs, it can obviously save grain and reduce breeding costs (Zhang et al., 2009). Castration is preferred, feeding with a chopped or beaten mix. The barley feed should be fed now and not cast too much to avoid waste.

2.3.3 Silage

Silage is a feed that preserves green leaf crops by fermentation and acidification, and ensiling is a popular method of storing forage now. Silage is divided into two phases. The first is the aerobic phase, where oxygen is consumed; then the anaerobic fermentation phase, which converts the sugar into acid, during this period the aerobic respiration stops, the pH decreases, the dry matter loses more, and the hemicellulose is decomposed. Most yeasts that have deteriorated from silage show a high tolerance to lactic acid, but have a low tolerance to butyric acid. They are able to grow and absorb lactic acid at low pH (Cai et al., 1999).

After about a few weeks, the fermentation is over. It is worth noted that if the fermentation process is not properly managed, acid silage will produce an unpleasant smell because of excessive production of ammonia or butyric acid. Italian ryegrass has a high sugar content and is superior to other grass species in response to nitrogen fertilizer, so it has become a popular grass for silage making in recent years.

2.3.4 Hay

Due to Italian ryegrass has so leafy with a unique texture, it is more difficult to make into dry hay than other grasses. Italian ryegrass is easier to make as dry hay in July and August. It can be made into hay anytime and makes excellent baling (Joel, 2014). Italian ryegrass belongs to the stalk grass, which is dry and dehydrated quickly and can be made into fine green hay and hay powder. Generally, in the flowering period, it can be harvested on a sunny day for more than 3 consecutive days during flowering, cut off and spread into a gauzy layer to dry, and when the water content is below 14%, it will be piled up. It can also be made into grass powder, grass blocks, grass cakes, etc., for winter and spring feeding, or as a commercial feed, or mixed with grain feed.

2.4 Effect of wilting in silage

2.4.1 Effect of wilting on animal performance

Formic acid alone or in combination with formalin to non-wilted silage is the best way to enhance subsequent preservation, as silage is achieved by adding an acid-formalin additive to non-wilted or wild silage grass can maximize weight gain (Haigh et al., 1985). The dry matter intake of steer cattle fed with formic acid treatment and inoculated silage was significantly higher than that of non-fed silage. The weight gain of the additive-treated silage was significantly higher. The inoculated silage and formic acid silage increased by 35% and 42%, respectively, compared with the untreated silage. Feed conversion efficiency (FCE) values were significantly higher for animals fed additive-treated silage (Winters et al., 2001). Wilting can increase dry matter (DM) intake (DMI: Dawson et al., 1999; Wright et al., 2000) and reduce the quantity of effluent produced, which is an important part of making silage. There are several experiments' results showed that wilting have impact on animal performance, however, it does not always translate into improved animal performance. A large amount of researches comparing wilted and unwilted silage, the impact of wilting on animal performance is still a mystery (Edmuds et al., 2014).

In general, responses in terms of dry matter intake (DMI) and performance of both dairy and cattle to wilting are mainly a reflexion of the fermentation quality of unwilted silage (Wilkins, 1984), especially ammonia N concentration, relative to its wilted counterpart (Wright et al., 2000). Considering the benefits of wilting in terms of increased DMI and difficulty in predicting production responses, as well as the need for improvement in efficiency of feed utilization and reduction in environmental emissions, it is important to get a learn more about the effects of wilting on the nutritional components and chemical composition of grass silage (Edmunds et al., 2014). According to the review of literature, Wright et al., (2000) established that responses in DMI and animal performance are related to the extent and rate of moisture loss in the field.

2.4.2 Effect of wilting on milk and protein yield

In the study of Edmunds et al., (2014) suggested that rapid wilting has the potential to increase milk protein yield, highly wilted silages may have a similar effect, despite this will depend largely on factors previously described. Some experiments have confirmed that wilting can increase protein yield. However, Purwin *e*t al.*,* (2009) did not observe any difference in milk and protein yield until after 200 d in milk, which they explained was probably due to reduced concentrate supply. Gordon et al*.,* (2000) observed a decrease in milk yield and no change in milk protein yield due to wilting. Conflicting results in production responses due to wilting, despite increased intake, are an indication of the complexity of the interacting effects between metabolism processes in the ruminant, silage quality and whole-ration composition. Therefore, people have raised many possibilities, but the factors that cause the results are very complicated. So, although a general increase in DMI due to wilting, production responses in terms of milk and protein yield are unpredictable.

2.4.3 Wilting period

The weather plays an important role in the wilting (Uslu et al.*,*2017), but the long time of wilting will result in the loss of sugar in the grass. Wilted for 24-36 hours, it will reduce about 6% of sugar. Farmers should be better preferably make a quick pre-wilting treatment before ensiling to achieve the best dry matter content. In favorable weather, 4-6 hours of wilting are sufficient. In the study of Uslu et al., (2017) showed that the dry matter recovery (DMR) of silages increased 3.27% and 6.62% for wilted 1.5 hours and 33 hours compared with unwilted ryegrass silage. And DMR is an important silage feature in economic sense.

There are many researches emphasized that wilting has negative influence on silage acidity due to increasing DM content and restricted fermentation, the same results can be found in Gordon et al., (2000) literature. In the study of Yan et al., (1998) showed that a negative interaction between DM and pH value were made at regions in which wilting took much time because of unfavourable weather conditions causing contamination and decreasing water soluble nutrients, and indicated that wilting grass prior to ensiling was the reason that DMI increased.

The DM content increasing depending on the wilting degree and it was also significantly increased relative feed value (RFV) of silages. Wilting ryegrass till achieving around 40 % could improve pH value and DMR that is important for economical income of farm management as well as RFV which is known related to animal performance (Uslu et al., 2017).

It has the potential to increase the content of sugars, but the precondition is the weather conditions allow for rapid wilt if not, farmers could choose to wilt Italian ryegrass for 24 hours, it should be cut in the afternoon because the sugar content of the Italian ryegrass is at its highest. But, there is a problem with the crop becoming too dry because too long wilting time, which could have a negative effect on the aerobic stability of the silage at feed-out.

2.4.4 Wilting methods

One of wilting methods is tedding. Since the top of the grass is exposed to the air and receives more sunlight, the bottom of the crop has higher moisture than the top part. So, it's an effective way to speed up the drying rate by tedding. Tedding not only increased the drying rate of grass but also bruising the herbage (Murdoch and Bare, 1963). There is a problem that tedding will cause DM losses about 1-3%, and if not managed properly, it will cause greater losses (Murdoch and Bare,1963; Savoie et al., 1982). Because of chemical nutritive of leaves is higher than the stem, tedding losses showed more proportion of leaves (Savoie,1987). At the same time, tedding time should be considered due to the air temperature is different in a day, the tedding time will affect the drying rate. And tedding frequency is also an essential factor because in some reports showed that tedding frequency speed up the drying rate (Dirk and John. 2015). Tedding too frequently has caused the loss of nutrients. The CP content will decrease as increasing of tedding times. NDF after 3 tedding times are higher than before (Kim et al., 2004). In Park's experiment, the drying rate of Italian ryegrass was affected by the tedding frequency after 3 days it was cut. And the result showed tedding frequency had no effect on ADF and NDF, but influenced CP and RFV (Park et al., 2013).

Another way to achieve the target is conditioning. It was reported that 3-4 days is necessary to wilt Italian ryegrass by conditioning and tedding in South Korea (Park et al., 2013). There are many common methods to conditioning, such as chemical, mechanical and other techniques. Mechanical conditioning is widely used by forage producers, and it is through crushing, breaking and abrading to achieve the goal. Mechanical conditioning reduces 1-2 days of field drying than mechanical conditioning and reduces 1-1.5 days than chemical conditioning, and showed higher ADF, NDF, IVDMD, RFV and visual score (Seo et al., 2000). It had provided a small improvement in the digestibility of grass hay (Klinner, 1976).

Chemical conditioning is by using of drying agents to hasten the moisture loss from crop. The agents affect the waxy surface of the crop, allowing moisture to leave the crop more easily. Spraying a 2.8% aqueous solution of potassium carbonate to the crop when it is mowed provides, it is an effective chemical conditioning treatment (Rotz and Thomas, 1985). In a series of field trials, the hay treated by chemical conditioning was of similar quality with untreated hay (Johnson et al., 1983). In another experiment, the hay treated by chemical conditioning had a higher crude protein content than untreated hay when both were dried under relatively good drying conditions (Ehle et al., 1985). *In vitro* neutral detergent fiber and cellulose disappearance were not influenced by the treatment; however, sheep dry matter intake, as well as digestibilities of NDF (neutral detergent fiber) and CP (crude protein), were higher than chemically conditioned hay. When hay received rain during the field curing process, there was no difference in quality between chemically conditioned and control hay (Rotz et al., 1987).

2.5 Effect of inoculant in silage

2.5.1 Inoculant effect

Inoculants used as silage additives can improve storage efficiency and enhance animal performance. Generally, people choose to use lactic acid bacteria, which can quickly produce lactic acid and increase the fermentation rate of natural silage; even if it does not affect the fermentation, specific lactic acid bacteria may affect animal performance (Weinberg et al., 1996). During ensiling, bacteria convert plant sugar into acid, thereby reducing the pH value of silage, which is benefit to the preservation of silage.

Baytok et al., (2003) reported that molasses treatment significantly affected the concentrations of DM, NDF, ADF, and CP, however while the addition of formic acid into silage increased, the addition of molasses decreased the acetic acid concentration of silage. Both concentrations of NDF and ADF were significantly lower in silage treated with molasses compared with control or silage treated with formic acid (Baytok et al., 2003). These decreases in NDF and ADF concentrations may have resulted from increased cell wall digestion because of increased silage fermentation caused by addition of molasses (Bingöl, Baytok, 2003).

High quality silage inoculants can ferment faster and more efficiently than untreated controls, reducing in less energy and dry matter losses and improving animal performance. However, Weinberg and Muck (1996) can not make sure the reason of improved animal performance. Additives have a positive impact on animal production, often indirectly. However, producers expect to increase milk production or milk production efficiency as the main return on investment in silage additives. Unfortunately, little is known about how additives affect intake and milk production (Muck et al., 2008). All crops contain a variety of bacteria. Some are more effective at converting sugars into the correct acid type than others. The most desirable fermentation produces high levels of lactic acid. It has been proved that epiphytic lactic acid bacteria (LAB) play an important role in silage quality during the various stages of fermentation (McDonald et al. 1991).

2.5.2 Inoculants variety

In the past, the fermentation was conducted by indigenous microorganisms, but in recent years, the farmers are used to inoculated with specific microorganisms to speed up fermentation or improve the resulting silage. Lactic acid bacteria (LAB) are widely used as a inoculant in silage, LAB can be divided into two categories:

the homofermentative LAB, which ferment glucose with lactic acid as the primary by-product, the heterofermentative LAB, which not only ferment glucose and lactic acid, but also ethanol/acetic and carbon dioxide (CO_2) as by-product (McDonald et al., 1987). Silage inoculants contain one or more strains of lactic acid bacteria, and the most common is *Lactobacillus plantarum.* Other bacteria used include *Lactobacillus buchneri, Enterococcus faecium* and *Pediococcus species*. The utilization of homofermentative LAB did not result in silage with a more homolactic fermentation profile in baled silages (Keles and Demirci, 2011). In addition to lactic acid bacteria, molasses, hemicellulose, formic acid, etc. are commonly used inoculants.

3.Materials and methods

3.1.General information

The experiment was a split-plot design with three replications. The main plot was four treatments (NWNA; no-wilting no-additive, NWA; no-wilting with additive, WNA; wilting no-additive and WA; wiling with additive) and sub-plot was silage opening dates (1, 2, 3, 5, 10, 20, 30, 45 and 60 days).

These experiments were conducted at the Pyeongchang Campus of Seoul National University, South Korea. The experimental field is located in the mountainous area with an average elevation about 650 meters.

3.2 Silage preparation

Italian ryegrass (IRG) was cultivated in experimental field of Pyeongchang campus, Seoul National University (37.32°N, 128.26°E, 550 m ASL). "Kogreen" variety, developed by the National Institute of Animal Science (Choi *et al*., 2006), was seeded on September 27 at a seeding rate of 40 kg ha^{-1}. At seeding date, 40 kg ha^{-1} of nitrogen, 150 kg ha^{-1} of phosphate, and 75 kg ha^{-1} of potassium were applied as fertilizer. And also 100 kg ha^{-1} of nitrogen and 75 kg ha^{-1} of potassium fertilizer were additional applied in next early March.

IRG was harvested at 16 May and chopped into about 2-3 cm pieces using a forage cutter (Richi Machinery Co., Ltd, Henan, China). Wilting treatment was for five hours. After manual mixing, chopped IRG was treated with silage inoculant ("Chungmi-Lacto" Chung-mi Co., *Lactobacillus plantarum*). Recommended level (over 10^6 cfu g^{-1}) of inoculant was dissolved in tap water and sprayed into mixed samples. Thereafter, approximately 300 g treated material was packed into vinyl bag (20 cm x 30 cm), air was taken out, sealed and stored at the ambient temperature (22-28°C) in the shade.

3.3 Chemical analysis

Three bags per treatment were opened on 1, 2, 3, 5, 10, 20, 30, and 45 days after ensiling, respectively. Collected sample was dried for 72 h in an air-forced drying oven at 65°C and estimated DM (dry matter) content. Dried samples were ground to pass through a 1-mm screen and kept in double-plug type plastic bottles for analysis.

3.3.1 Crude protein analysis

Crude protein (CP) was measured by the Italy machine "Automatic Elemental Analyzer Euro Vector EA3000" utilized from 1997. And Dumas method (Jean-Baptiste Dumas, 1884) was used for CP analysis.

3.3.2 Fiber analysis

These experiments used the method of Van Soest (1991) to determined acid detergent fiber (ADF) and neutral detergent fiber (NDF) . In the NDF procedure used heat-stable amylase and sodium sulfite. Machine "ANKOM 2000 Automated Fiber Analyzer" (Ankom Technologies, Inc., Fairport, NY, USA) were used for ADF and NDF analysis.

3.3.3 Calculation of TDN and RFV

Total digestible nutrient (TDN) and relative feed value (RFV) were calculated by the known formula (Holland et al., 1990). TDN was calculated from ADF value, and RFV was estimated through digestible dry matter (DM) and dry matter intake (DMI) as RFV.

$$TDN(\%)=88.9-(0.79\times ADF)$$

$$RFV=(DMI\times DDM)/1.29$$

$$DDM=88.9-(0.779\times ADF)$$

$$DMI=120/NDF$$

3.3.4 *In vitro* dry matter digestibility analysis

In vitro dry matter digestibility (IVDMD) used the two-stage technique (Tilley and Terry, 1963) to analysis. Weigh 0.5-0.6 g of the samples in filter bags (Ankom Technology, Macedon, NY, USA) which were previously soaked in acetone and completely dried, sealed by heat sealer. After sealed, put these samples into the Daisy Incubator digestion jars (Ankom Technology, Inc., Fairport, NY, USA), added 266ml buffer solution B and 1330ml buffer solution A (1:5 ratio,v/v). Table 1 shows reagents contained of buffer A and B. Rumen fluid was collected from healthy Holstein steers before morning feed, and filtered into 39℃ preheated thermos bottles through 4 layers of cheesecloth, repeated this step in the laboratory. Then added 400ml fluid into Daisy Incubator digestion jars and flushed with CO_2 gas for thirty seconds before securing the screwing cap, then incubated at 39℃ for 48 hours, performed the NDF procedure to get the *in vitro* dry matter digestibility.

Table 1. Reagents of buffer solution A and B

Buffer Solution A	g / 5 liter
KH_2PO_4	50g
$MgSO_4\cdot 7H_2O$	2.5g
NaCl	2.5g
$CaCl_2\cdot 2H_2O$	0.5g
Urea (reagent grade)	2.5g
Buffer Solution B	g / 5 liter

Na_2CO_3	30g
$Na_2S{\cdot}9H_2O$	2g

3.4 Fermentation characteristics

3.4.1 Acidity (pH)

The silage samples were stored in a -80℃ deep freezer. Take 10 grams of the sample into a conical flask after it was thawed with 100ml distilled water and stored in a refrigerator 2 hours. The next step was to filter the mixture through filter paper (Whatman No. 6, AVANTEC), a pH meter (AB 150, Fisher Scientific International, Inc. Pittsbursh, US) was used for measuring the filtrates silage pH measurement.

3.4.2 Organic acid

Weigh 10 g of the silage sample and mix it with 100 ml of distilled water in a 250 ml conical flask, seal it and put it in the refrigerator for 24 hours. Take out the conical flask and shaken by hand every two hours. It was taken out and filtered with a filter paper (Whatman No.6, AVANTEC) and the filtrate was stored in a refrigerator at minus 20℃ for use. Before using HPLC (Agilent Technologies, CA, USA) analysis, 1.5 ml of the filtrate was centrifuged in a Centrifuge Smart 15 (Hanil Science Industrial, South Korea) of 3000 rpm for 15 minutes. After that, the supernatant can be used by the high performance liquid chromatography (HPLC) to analyze the contents of lactic acid (IA), acetic acid (AA), and butyric acid (BA).

Table 2. Instrument conditions of HPLC for determination of organic acid in Italian ryegrass silage

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

3.4.3 Ammonia nitrogen (NH_3-N)

In order to analyze Ammonia nitrogen (NH_3-N), according to the modified phenol-hypochlorite reaction method described by Broderick and Kang (1980), put 12 ml sample filtrate into a 15 ml centrifuge tube, centrifuged at 3000 rpm for 15 minutes. Transfer the 0.2 ml supernatant sample to the 25 ml capacity test tube, add 1 ml phenol reagent and 1 ml alkali-hypochlorite regent. After vortex mixed, it was placed in a water bath at 37 °C for a water bath reaction for 15 minutes, and then vortex mixed again with 8 ml of distilled water and detected at a wavelength of 630 nm.

3.4.4 Water soluble carbohydrate (WSC)

Weigh 0.2 g of the ground sample and 200 ml of distilled water into a labelled Schott bottle, seal the bottle and shake it on the shaker for one hour. The filtrate was filtered through a filter paper (Whatman No.1, AVANTEC). 2 ml of the filtrate was pipetted into a test tube and rapidly mixed with 10 ml of anthrone reagent. The bath was boiled in water for 20 minutes and then cooled with running water for 10 minutes. Finally, it was detected at a wavelength of 620 nm. The WSC content was calculated by the formula:

$$WSC(\%) = G \times D \times E \times 100 \times 0.1 / (W \times \text{Sample lab dry matter } \%)$$

Where: W = sample weight (mg),

G = mg glucose read from graph,

E = Extract volume (200ml),

D = Dilution factor.

3.5 Statistical analysis

Data on fermentation dynamics and chemical composition were subjected to two-way analysis of variance (ANOVA) with the fixed effects of treatments, ensiling days and interaction (treatment × ensiling days) using the general linear model (GLM) procedure of SAS ver. 9.1 (SAS Institute, Cary, NC, USA). Least significant difference tests were used to determine specific differences among means. The level of statistical significance was $P < 0.05$.

4.Results and discussions

4.1 Forage quality of raw material

4.1.1 Dry matter of raw material

The raw materials reflect the nature characteristics of grass species. Dry matter is what remains after the water evaporated from the feed. The fact that grass is usually harvested at 18-20% low dry matter (DM), the fact that storage with> 90% dry matter is troublesome. Instead, grasses can be stored at a lower DM (20-50%) through ensiling (Morten et al., 2013).Livestock need to intake a certain amount of dry matter from feed every day to maintain production. As the table 3 shown that, the dry matter of Italian ryegrass with wilting are higher 47 g kg^{-1} than without wilting, it means that wilting is an effective way of increasing dry matter.

4.1.2 Crude Protein of raw material

Table 3 shows that crude protein has decreased 10.3 g kg^{-1} with wilting (89.2 g kg^{-1}) than without wilting (99.5 g kg^{-1}). Besides dry matter content and availability of water soluble carbohydrates, wilting intensity may exert a strong impact on the crude protein fractions (Thomas et al., 1977). So, the reason why crude protein decrease with wilting maybe is wilting effect on dry matter, and dry matter impact on crude protein. In Edmunds's (2012) research also have the thought that wilting has impact on nitrogen components. Fitzgerals (1996) insisted that wilting affected decrement of crude protein, but Cottyn et al. (1985) reported that there was no significant difference.

4.1.3 Fibers and IVDMD of raw material

As Table 3 presented, the ADF with wilted is 286.00 g kg^{-1}, and ADF of no wilted sample is 275.85 g kg^{-1}, there are no significant change. But NDF with wilting is 516.85 g kg^{-1}, and the NDF without wilted is 492.85 g kg^{-1}. Fresh Italian ryegrass with wilted is higher 24 g kg^{-1} than no wilted ($p<0.05$). The neutral detergent fiber (NDF) content of no-wilting with additive (NWA) treatment was the lowest among treatments ($p<0.05$).

The IVDMD with wilting is 741.5 g kg^{-1}, and the IVDMD without wilted is 795.25 g kg^{-1}. Fresh Italian ryegrass with wilted is lower 44.25 g kg^{-1} than no wilted ($p<0.05$). Wilting has a big impact on the fermentation pattern of silage. In this experiment, wilting decreased the CP, IVDMD content and increased NDF content of raw materials. Kim et al. (2001) reported that IVDMD decreased with increasing wilting period in rye silage, but increased fiber content. The CP and IVDMD contents of wilted materials were lower than those of no wilted ($p<0.05$).

Table 3. Effects of wilting and inoculant treatment, prior to ensiling, on chemical composition of Italian ryegrass.

Item	NWNA	NWA	WNA	WA	Mean	LSD(0.05)
DM (g kg^{-1})	258.8 ^{b}	245.3 ^{b}	291.4 ^{a}	306.7 ^{a}	275.5	16.3
CP (g kg^{-1})	99.8 ^{a}	99.2 ^{a}	87.8 ^{b}	90.6 ^{ab}	94.4	9.66

ADF ($g\ kg^{-1}$)	276.3	275.4	286.6	285.4	280.9	NS
NDF ($g\ kg^{-1}$)	493.5 ^{ab}	491.2 ^{b}	518.5 ^{a}	515.2 ^{ab}	504.6	26.69
IVDMD($g\ kg^{-1}$)	784.6 ^{a}	805.9 ^{a}	742.0 ^{b}	741.0 ^{b}	768.4	32.28
TDN (%)	67.07	67.14	66.26	66.35	66.7	NS

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive

NS:not significant

4.1.4 TDN of raw material

As shown in Table 3, there was no significant change in total digestible nutrients (TDN). The reason why there is no significant difference between the treatments is that the fermentation has not yet started. For expressing nutritional values are crude protein content, crude protein digestibility, nitrogen balance, dry matter intake, and dry matter digestibility are the most suitable criteria, in addition, the nutritional value of the raw material has a lot of relatives with the harvest season of the grass (Milford, 1960). The biggest increase was improved forage, supplemented with TDN> 60% and supplemented with CP intake> 0.05% of BW. When supplemented TDN intake> 0.7% of body weight, feed TDN: CP ratio <7 (sufficient N), or VFI taken alone> 1.75% of body weight, supplements reduce voluntary feed intake (VFI). When supplements increase VFI, forage TDN: CP ratio> 7 (N deficiency), VFI is often very low when fed alone (Moore et al., 1999).

4.1.5 pH ,WSC and NH_3-N of raw material

The chemical composition pH, water soluble carbohydrate (WSC) and ammonia nitrogen contents of fresh Italian ryegrass are exhibited in Table 4.

There was no significant difference in WSC, ammonia nitrogen content and pH among treatments ($p>0.05$).

On the other hand, the water soluble carbohydrate (WSC) content of the material was not significantly different among treatments, and the average level was 153.4 g kg^{-1}. Parker and Batisman (1982) stated that the minimum water soluble carbohydrate content required for silage fermentation should be 25-30 g kg^{-1} and the wilted silage should be higher than 38 g kg^{-1} (Haigh and Parker, 1985). In this experiment, content of WSC was high level. McDonald et al. (1991) insisted that the water soluble carbohydrate content varies depending on the species, growth, daily time, light intensity, temperature, and fertilization level and major five grasses was showed 5-315 g kg^{-1}.

Table 4. Effects of wilting and inoculant treatment, prior to ensiling, on pH, WSC and ammonia nitrogen content of Italian ryegrass.

Item	NWNA	NWA	WNA	WA	Mean	LSD(0.05)
pH	6.22	6.29	6.32	6.29	6.28	NS
WSC (g kg^{-1})	164.1	183.7	153.3	113.0	153.5	NS
NH_3-N/TN(g kg^{-1})	4.3	6.3	10.8	11.8	8.27	NS

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive

NS:not significant

4.2 Forage quality of silage

4.2.1 Dry matter of silage

The DM content of wilted silages were significantly higher than those of no-wilted ($p < 0.05$), but there was not significant difference between wilting treatment silage.

4.2.2 Forage quality

As presented in Table 5, inoculant increased the CP content of Italian ryegrass silage and wilting increased the NDF content and decreased CP content. This result seems to be due to proteolytic degradation of raw materials with wilting. TDN and IVDMD of wilting no-additive (WNA) treated silage was the lowest among treatments ($p<0.05$).

Lactic acid bacteria treatment did not show any difference in crude protein content after fermentation, but it showed a tendency to increase slightly in no wilting silage (NWNA).

The contents of IVDMD, ADF and NDF in Italian ryegrass silage showed a significant difference as wilting, LAB treatment. *In vitro* dry matter digestibility of silage showed a tendency to decreased in WNA treatment silage, but there was no significant difference between no wilting (NWNA and NWA groups). The contents of ADF and NDF increased with the wilting treatment, but there was no significant difference between no wilting group. Keady and Murphy (1996) reported that LAB treatment reduced ADF and NDF content of silage, but was not significant. But, Gordon (1989) and Patterson et al. (1996) showed a tendency to increase in ADF and NDF content.

There was not significant difference between inoculant treatments in TDN content. The results show that a reasonable estimate of the average total digestible nutrients and total digestible energy of ruminant feed can be derived from knowledge of only the crude protein and crude fiber content of the material being fed (Glover et al., 2009).

Table 5. Effects of wilting and Inoculant treatment, after ensiling, on dry matter (DM) content and chemical composition of Italian ryegrass silage.

Item	NWNA	NWA	WNA	WA	Mean	LSD(0.05)
DM ($g\ kg^{-1}$)	225.6 ^{b}	211.7 ^{b}	312.5 ^{a}	329.8 ^{a}	269.9	3.7
CP ($g\ kg^{-1}$)	97.9 ^{ab}	101.6 ^{a}	88.8 ^{c}	93.3 ^{bc}	95.4	5.71
ADF ($g\ kg^{-1}$)	298.6 ^{b}	289.8 ^{b}	310.6 ^{a}	297.0 ^{b}	299.0	8.81
NDF ($g\ kg^{-1}$)	508.2 ^{b}	499.9 ^{b}	540.2 ^{a}	526.4 ^{a}	518.7	15.56
IVDMD($g\ kg^{-1}$)	767.1 ^{a}	739.7 ^{a}	698.7 ^{b}	734.6 ^{a}	735.0	34.28
TDN (%)	65.3 ^{a}	66.0 ^{a}	64.4 ^{b}	65.4 ^{a}	65.3	0.69

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive

Values with different small letter show significant difference among treatments ($p<0.05$).

4.3 Fermentation quality of silage

4.3.1 pH of silage

The chemical composition, pH, water soluble carbohydrate (WSC) and ammonia nitrogen contents of Italian ryegrass silages are exhibited in Table 6.

The pH of silage was lowered by inoculation and the pH of wilted silage was higher (4.12 and 4.66). The pH(3.65) of silage treated with lactic acid bacteria is obviously better than that(4.39) of silage without lactic acid bacteria. The effect of the two treatments on the pH was that the inoculant was greater than the wilting. Malate and citrate are the main buffers in Italian ryegrass, the anion fraction of the plant materials accounted for 68 – 80% of the total buffering capacity, and for 73 – 88% in the silages. Buffering caused by plant proteins was estimated to be 10 – 20% of the total buffering capacity, the organic acids were responsible for most of the buffering effect in herbages and silages (Playne and McDonald, 1966).

4.3.2 WSC and ammonia nitrogen content of silage

There was significant difference in WSC and ammonia nitrogen content among treatments ($p>0.05$). Wilting has no significant effect on the water-soluble carbohydrates and ammonia nitrogen of silage. However, Derbyshire et al. (1976) and Haigh and Parker (1985) also reported that ammonia nitrogen of silage increased by wilting.

The residual contents of WSC after the silage fermentation were significantly higher (60.4 and 57.8 g kg^{-1}) in the additive treatment ($p <0.05$). The ammonia nitrogen content was significantly higher in the non-inoculated silage ($p <0.05$).

Table 6. Acidity (pH), water soluble carbohydrate (WSC) and ammonia nitrogen (NH3-N/TN) content of Italian ryegrass silage.

Item	NWNA	NWA	WNA	WA	Mean	LSD(0.05)
pH	4.12 ^{b}	3.60 ^{c}	4.66 ^{a}	3.69 ^{c}	4.02	0.12
WSC (g kg^{-1})	13.0 ^{b}	60.4 ^{a}	15.6 ^{b}	57.8 ^{a}	36.7	14.01
NH_3-N/TN (g kg^{-1})	224.9 ^{a}	64.4 ^{b}	224.9 ^{a}	62.2 ^{b}	144.1	39.05

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive

Values with different small letter show significant difference among treatments ($p<0.05$). NS:not significant

4.3.3 Organic acid of silage

Table 7 presents the results for silage fermentation characteristics. Lactic acid content of inoculated silage were significantly higher but wilting treatment showed lower. Acetic and butyric acid contents were lower in the additive treated silages and the lowest in NWA silage.

LAB treatment promoted the production of lactic acid in silage and reduced the production of acetic acid and butyric acid. On the other hand, lactic acid / acetic acid ratio showed a tendency to increase by the treatment of lactic acid bacteria additive, which shows that Homo-type fermentation was predominant.

Table 7. Lactic acid, acetic acid, butyric acid content and lactic acid / acetic acid (LA/AA) ratio of Italian ryegrass silage.

Item	NWNA	NWA	WNA	WA	Mean	LSD(0.05)
Lactic acid(g kg^{-1})	23.5 ^{c}	108.1^{a}	18.6 ^{c}	74.3 ^{b}	56.1	20.8
Acetic acid(g kg^{-1})	18.8 ^{a}	8.1 ^{c}	14.3 ^{ab}	11.7 ^{bc}	13.2	4.6
Butyric acid(g kg^{-1})	12.8 ^{a}	0	10.5 ^{ab}	0.8 ^{b}	6.0	2.8
LA/AA	1.25	13.3	1.3	6.4	4.25	

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive

Values with different small letter show significant difference among treatments ($p<0.05$).

4.4 Forage quality on fermentation pattern

4.4.1 Crude protein and *in vitro* dry matter digestibility on fermentation pattern

Table 8 shows the change in forage quality from 1 to 45 days after silage preservation. Crude protein content was increased in NWNA and NWA treated silages during fermentation periods and there was not found the significant difference between wilted silages (WNA and WA silage). But CP content was slightly increased in inoculated silage during fermentation. There was significant difference among treatment and number of fermentation days. As a whole, NWA silage were significantly higher in each elapsed days and lower in WNA and WA silage.In addition, IVDMD decreased with progressed fermentation. It can be seen from the table that after 45 days of fermentation, the IVDMD of the NWNA group without any treatment increased by 5.4 g kg^{-1} compared with one day of fermentation. The other three groups are the longer the fermentation days, the smaller the IVDMD value. In particular, the NWA group reduced the most, with a decrease of 72.6 g kg^{-1}.

Table 8. Effect of wilting and inoculant on content of crude protein (CP), *in vitro* dry matter digestibility (IVDMD) of Italian ryegrass silage.

Item		CP (g kg^{-1})				IVDMD (g kg^{-1})			
Treatment*		NWNA	NWA	WNA	WA	NWNA	NWA	WNA	WA
Ensiling days	1	95	97.3^{b}	85.1	84.0^{c}	773.3^{AB}	802.4^{aA}	734.9^{aC}	746.8^{abBC}
	2	95.8^{B}	103.2^{abA}	85.4^{C}	84.3^{bcC}	769.2^{B}	811.7^{aA}	748.5^{aB}	753.6^{abB}
	3	95.7	96.2^{b}	85.2	90.8^{ab}	762.8^{B}	816.4^{aA}	739.9^{aB}	758.3^{aB}
	5	93.4^{B}	101.4^{abA}	89.4^{B}	88.1^{abcB}	773.0^{B}	809.9^{aA}	742.8^{aC}	756.3^{aBC}
	10	99.7^{A}	101.6^{abA}	84.3^{B}	89.2^{abcB}	753.8^{B}	810.2^{aA}	749.6^{aB}	745.9^{abB}
	20	97.8^{A}	101.2^{abA}	87.8^{B}	89.6^{abcB}	766.2^{B}	808.9^{aA}	728.4^{abC}	753.7^{abBC}
	30	101.6^{A}	108.6^{aA}	88.6^{B}	89.9^{abcB}	781.3^{A}	728.4^{bB}	706.0^{bcB}	726.4^{cB}
	45	97.6^{AB}	102.6^{aA}	89.8^{C}	94.8^{aBC}	778.5^{A}	729.8^{bB}	697.2^{cC}	736.1^{bcB}
Significance**	T	***				***			
	D	***				***			
	T x D	*				***			

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive

**T, treatment; D, ensiling days; T x D, interaction between treatments and ensiling days. * $p<0.05$, *** $p<0.001$

Values with different small letter show significant difference among ensiling days in the same treatment ($p<0.05$).

Values with different capital letter show significant difference among treatments in the same ensiling days ($p<0.05$)

4.4.2 Fiber on fermentation pattern

Table 9 shows the change of fiber content in forage quality from 1 to 45 days after silage preservation. The ADF and NDF contents tended to increase with fermentation and wilted silages were higher. However, there was no significant difference in the ADF and NDF content among fermentation periods of NWNA silage ($p > 0.05$).

In the experiment by Weinberg, their purpose of the experiment was to study the effects of variety, maturity at harvest, lactic acid bacteria (LAB) inoculants and their interactions on aerobic stability of wheat silage, and the same It was concluded that wilting and lactic acid bacteria additives did not significantly affect fiber (Weinberg et al., 2010).

As the fiber part is more difficult to digest, it can be seen from the table that the ADF treated by WA has the lowest growth among the four treatments, and even the NDF has decreased slightly. It shows that the dual treatment of wilting and inoculant is more conducive to the digestion of feed.

Table 9. Effect of wilting and inoculant on acid detergent fiber (ADF), neutral detergent fiber (NDF) content of Italian ryegrass silage.

Item		ADF ($g\ kg^{-1}$)				NDF ($g\ kg^{-1}$)			
Treatment*		NWNA	NWA	WNA	WA	NWNA	NWA	WNA	WA
Ensiling days	1	280.5^{B}	271.4^{cC}	293.3^{aA}	288.5^{bAB}	500.0^{B}	487.3^{abC}	523.9^{bcA}	521.8^{aA}
	2	283.7^{A}	270.2^{cB}	287.8^{cA}	287.8^{bA}	508.5^{A}	483.7^{abB}	514.0^{cdA}	520.5^{bA}
	3	282.5	274.7^{bc}	290.1^{c}	281.2^{c}	508.6^{A}	479.6^{abB}	514.3^{cdA}	504.4^{cdAB}
	5	281.4	281.3^{abc}	288.0^{c}	288.4^{b}	492.6^{B}	485.3^{abB}	508.2^{cdA}	506.1^{cdA}
	10	281.7	283.9^{ab}	297.2^{bc}	290.6^{ab}	489.3	492.1^{ab}	514.6^{cd}	507.9^{cd}
	20	285.7^{AB}	274.4^{bcB}	296.7^{bcA}	289.6^{abA}	485.7^{BC}	472.7^{bC}	516.1^{cdA}	507.9^{cdAB}
	30	291.2	290.6^{a}	306.1^{ab}	292.9^{ab}	499.1	502.1^{a}	532.4^{ab}	513.2^{bc}
	45	293.2^{B}	286.8^{aB}	310.8^{aA}	295.9^{aB}	503.4^{C}	496.7^{aC}	544.3^{aA}	518.6^{abB}
Significance**	T	***				***			
	D	***				***			
	T x D	NS				*			

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive

**T, treatment; D, ensiling days; T x D, interaction between treatments and ensiling days. * $p<0.05$, *** $p<0.001$. NS: not significant

Values with different small letter show significant difference among ensiling days in the same treatment ($p<0.05$).

Values with different capital letter show significant difference among treatments in the same ensiling days ($p<0.05$).

4.4.3 TDN and RFV on fermentation pattern

Table 10 shows the TDN content, estimated as ADF content, of NWNA silage showed no significant difference according to the fermentation period. A model was constructed using neutral detergent fiber (NDF), lignin, crude protein (CP), ash, fatty acid or ether extracts, and insoluble crude protein concentrations of acids and neutral detergents (Weiss et al., 1992). There was a research that studied the effect of dry matter of silage on total digestible nutrients and found no significant effect (Buck et al., 1969). Therefore, it can be inferred that wilting did not affect total digestible nutrients. And according to Okine's experimental results, the additives did not significantly affect total digestible nutrients (Okine et al., 2005).

Relative feed value (RFV) has been used to compare the quality of beans, legumes / grass hay and silage. For livestock producers and hay farmers, it is useful to have an index that prices hay and predicts animal performance, the relative feed value index estimates digestible dry matter (DDM) of the alfalfa from ADF, and calculates the DM intake potential (as a percent of body weight, BW) from NDF. The index is then calculated as DDM multiplied by dry matter intake and divided by 1.29 (Jeranyama et al., 2004). Because there was no significant difference in fibers, there was no significant change in RFV between the four treatments.

Table 10. Effect of wilting and inoculant on TDN, RFV content of Italian ryegrass silage.

Item		TDN %				RFV			
Treatment*		NWNA	NWA	WNA	WA	NWNA	NWA	WNA	WA
Ensiling days	1	66.74^{B}	67.46^{aA}	65.73^{aC}	66.10^{bBC}	124.7	129.2	117.3	118.4
	2	66.49^{B}	67.55^{aA}	66.16^{aB}	66.16^{bB}	122.2	130.4	120.1	119
	3	66.58	67.20^{ab}	65.98^{a}	66.69^{a}	122.4	130.8	119.8	123.6
	5	66.67	66.68^{abc}	66.15^{a}	66.11^{b}	126.7	128.3	121.6	122
	10	66.65	66.47^{abc}	65.42^{ab}	65.94^{bc}	127.2	126.3	118.8	121.2
	20	66.33^{AB}	67.22^{abA}	65.46^{abB}	66.02^{bcB}	127.6	132.9	118.8	121.4
	30	65.9	65.95^{ac}	64.72^{bc}	65.76^{bc}	123.2	122.8	113.5	119.9
	45	65.74^{A}	66.25^{acA}	64.35^{cB}	65.53^{cA}	121.9	124.9	110.3	117.9
Significance**	T		***						
	D		***						
	T x D		NS						

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive

**T, treatment; D, ensiling days; T x D, interaction between treatments and ensiling days. * p<0.05, *** p<0.001. NS: not significant

Values with different small letter show significant difference among ensiling days in the same treatment (p<0.05).

Values with different capital letter show significant difference among treatments in the same ensiling days (p<0.05)

4.5 Chemical analysis on fermentation pattern

4.5.1 Dry matter content on fermentation pattern

Figure 1 shows the dry matter content of the silage. Dry matter content increased continuously as fermentation proceeded, but decreased slightly after 20 days. Wilted silages showed the higher DM content among treatments. Wilting has negative effect on silage acidity due to increasing DM content and restricted fermentation (Uslu *et al*., 2017).

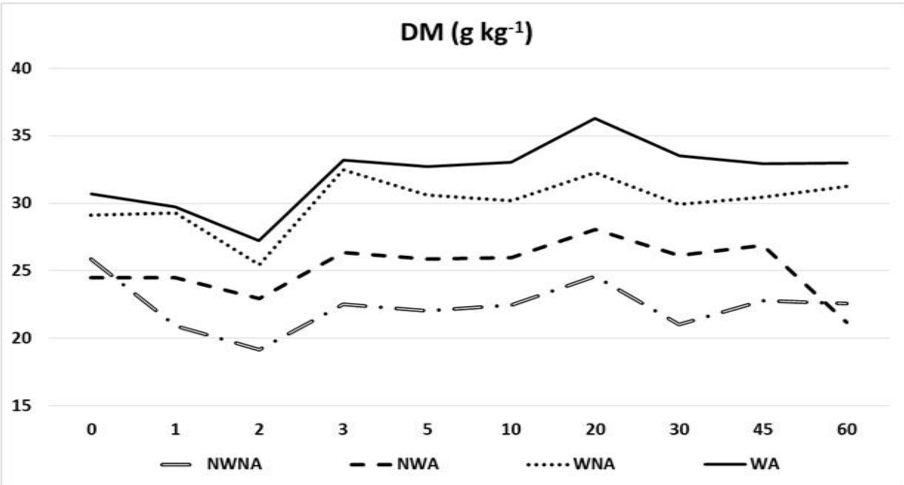

Figure 1. Effect of wilting and inoculant on DM (dry matter) content of Italian ryegrass silage.

4.5.2 pH on fermentation pattern

Figure 2 shows the pH of the silage. The most important changes in treated silage quality was in pH. Wilting resulted in higher silage pH and Inoculant treated silage was lower. Many studies have reported that LAB treatment lowers the final pH of silage (Mayne *et al*, 1990; Harrison *et al*., 1989; Anderson *et al*., 1989). Wilting increased final pH of silage by increasing the DM content of the raw material (Gordon, 1981; William *et al*., 1995; Marsh, 1979). The pH of silages were significantly decreased from 2 to 5 days after fermentation, and the inoculated silage were significantly lower ($p < 0.05$). During silage fermentation, the pH change gradually decreased over time and significantly decreased from 2rd day. However, in the WNA treatment, the pH decrease sharply occurred from slightly late 5th day. However, Zhao *et al*. (2019) reported that the pH change was stabilized after the sharpest decrease by day 3 in analysis of rice straw silage fermentation pattern. The pH of silage was significantly lowered by inoculation and the pH of wilted silage was higher (average 3.86 and 4.18). In addition, wilting treatment was generally delayed in pH decrement.

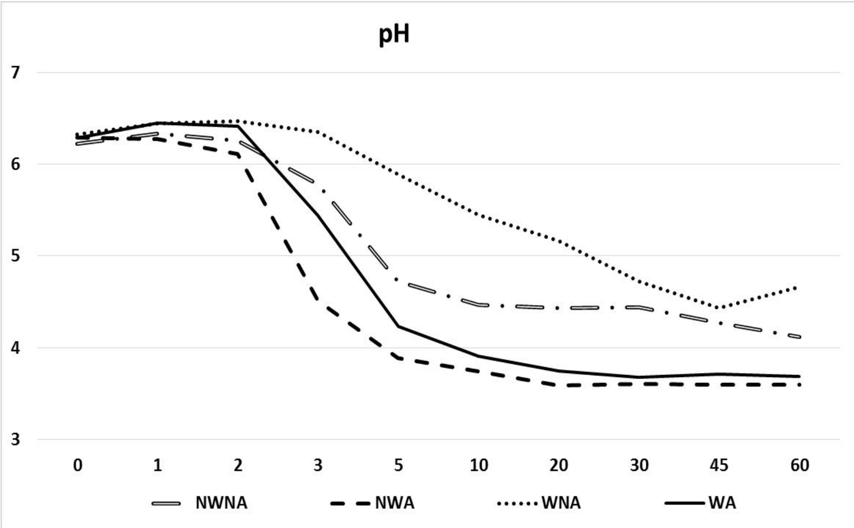

Figure 2. Effect of wilting and inoculant on pH changes of Italian ryegrass silage during fermentation

4.5.3 WSC content on fermentation pattern

Figure 3 shows the WSC content of the silage. WSC content increased until the 2nd day of storage and then decreased sharply again, while WNA silage was highest at 3 days and decreased. Overall, the WSC content of 45th days' silage was significantly lower in no inoculated silage ($p < 0.05$).

The residual contents of WSC after the silage fermentation were significantly higher (60.4 and 57.8 g kg^{-1}) in the additive treatment ($p<0.05$).

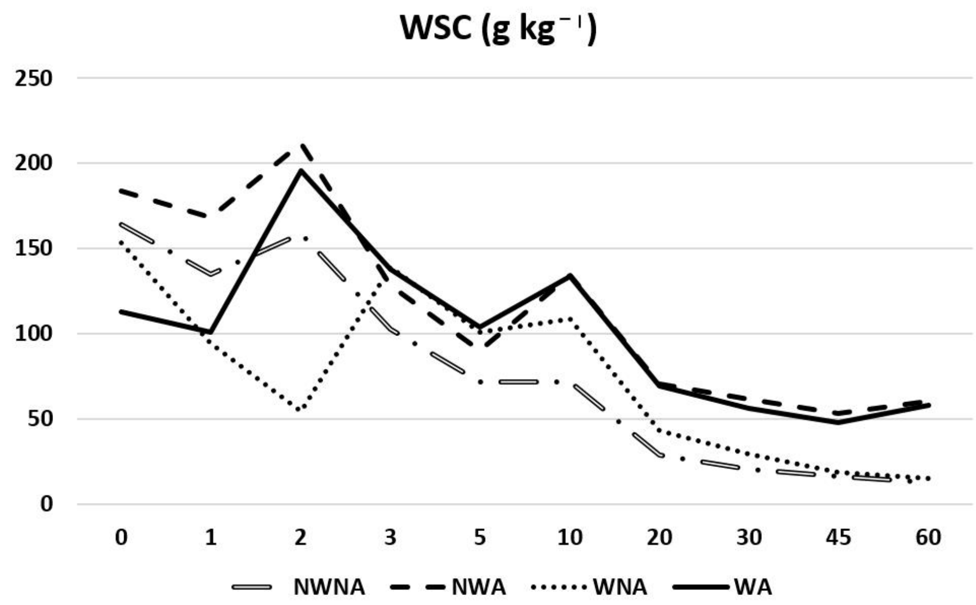

Figure 3. Effect of wilting and inoculant on WSC (water soluble carbohydrate) content of Italian ryegrass silage.

4.5.4 Ammonia nitrogen content on fermentation pattern

Figure 4 shows the ammonia nitrogen content of the silage. Change in ammonia nitrogen content was significantly lower in the additive treated silage ($p<0.05$) and increased steadily in general, and the NWNA silage increased rapidly after 45 days. The ammonia nitrogen content was significantly higher in the WNA and NWNA silage ($p <0.05$).

The NH_3-N/TN ratio, which indicates the degree of proteolytic degradation, was reduced by LAB treatment, and according to Haigh (1996), when the NH_3-N content was less than 10% of the total nitrogen, it was classified as high quality silage. So, the silage of this experiment can be classified as good quality. Sharp *et al.* (1994) also found that the NH_3-N content produced by proteolysis is reduced by the treatment of LAB additives. According to the report of Dawson *et al*. (1999), wilting grass before ensiling increased silage pH and ammonia nitrogen concentration, results that are in agreement with many previous studies (Gordon, 1981; Wilkins, 1984). The concentration of many of the fermentation products in the silage were also reduced as a result of wilting, indicating a more restricted fermentation in the wilted silage.

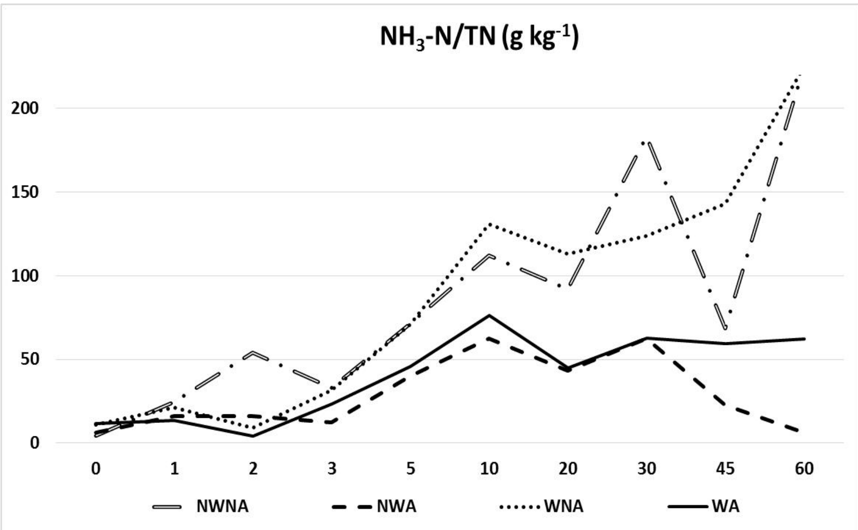

Figure 4. Effect of wilting and inoculant on NH_3-N/TN content of Italian ryegrass silage.

4.5.5 Organic acid on fermentation pattern

Table 11 shows the changes of lactic acid content during silage fermentation. Lactic acid content increased with fermentation and decreased at 30 days after conservation at the highest level in all treatments. Lactic acid content of inoculated silage were significantly higher but wilting treatment showed lower. In addition, lactic acid content was significantly increased by inoculant treatment and highest in NWA silage ($p<0.05$).

The Inoculant treatment increased the lactic acid content of the silage, but the wilting treatment resulted in a decrease. This is considered to be the result of limited fermentation due to the decrease in moisture content. Kim *et al*. (2001) also found that lactic acid content decreased with prolonged wilting period in rye silage.

Table 11. Effect of wilting and inoculant on lactic acid content of Italian ryegrass silage.

Item		Lactic acid ($g\ kg^{-1}$)			
Treatment*		NWNA	NWA	WNA	WA
Ensiling days	1	ND	ND	ND	ND
	2	ND	9.7^{cA}	ND	4.5^{cB}
	3	ND	32.9^{cA}	ND	20.7^{bB}
	5	8.4^{cC}	80.4^{bA}	4.7^{bC}	54.7^{bB}
	10	12.1^{cC}	96.2^{bA}	6.9^{bC}	72.6^{aB}
	20	23.2^{bC}	104.8^{bA}	12.3^{bC}	83.8^{aB}
	30	60.3^{aC}	136.8^{aA}	42.8^{aC}	103.7^{aB}
	45	50.8^{aC}	127.4^{aA}	38.4^{aC}	80.6^{aB}

Significance**	T	***
	D	***
	T x D	**

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive; ND, not-detceted.

**T, treatment; D, ensiling days; T x D, interaction between treatments and ensiling days. * $p<0.05$, *** $p<0.001$

Values with different small letter show significant difference among ensiling days in the same treatment ($p<0.05$).

Values with different capital letter show significant difference among treatments in the same ensiling days ($p<0.05$).

Table 12, 13 shows the changes of Acetic and butyric acid content during silage fermentation. Acetic and butyric acid contents were lower in the additive treated silages and the lowest in NWA silage.

Acetic acid content was also increased up to 30 days, then decreased, and WA silage increased steadily. Butyric acid content was not detected in the inoculant-treated silage or slightly differentiated by 45 days. In NWNA silage, the butyric acid content was detected after 10 days and the content was continuously increased.

Table 12. Effect of wilting and inoculant on acetic acid content of Italian ryegrass silage.

Item		Acetic acid ($g\ kg^{-1}$)			
Treatment*		NWNA	NWA	WNA	WA
Ensiling days	1	ND	ND	ND	ND
	2	ND	ND	ND	ND
	3	ND	ND	ND	ND
	5	22.3^{A}	10.7^{B}	8.4^{bBC}	7.5^{C}
	10	19.4^{A}	11.4^{AB}	9.5^{bB}	8.5^{CB}
	20	18.1^{B}	12.8^{B}	26.0^{bA}	8.4^{C}
	30	23.7^{A}	13.2^{B}	24.8^{aA}	9.7^{C}
	45	17.2^{B}	11.9^{C}	21.7^{aA}	11.0^{C}
Significance**	T			**	
	D			**	
	TxD			**	

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive; ND, not-detceted.

**T, treatment; D, ensiling days; T x D, interaction between treatments and ensiling days. * p<0.05, *** p<0.001

Values with different small letter show significant difference among ensiling days in the same treatment (p<0.05).

Values with different capital letter show significant difference among treatments in the same ensiling days (p<0.05)

Table 13. Effect of wilting and inoculant on butyric acid content of Italian ryegrass silage.

Item		Butyric acid (g kg^{-1})			
Treatment*		NWNA	NWA	WNA	WA
Ensiling days	1	ND	ND	ND	ND
	2	ND	ND	ND	ND
	3	ND	ND	ND	ND
	5	ND	ND	ND	ND
	10	7.4	ND	ND	ND
	20	8.4	ND	ND	ND
	30	10.8^{A}	ND	1.4^{B}	ND
	45	11.3^{A}	ND	2.3^{B}	0.5^{B}
Significance**	T	***			
	D	**			
	T x D	**			

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive; ND, not-detceted.

**T, treatment; D, ensiling days; T x D, interaction between treatments and ensiling days. * p<0.05, *** p<0.001

Values with different small letter show significant difference among ensiling days in the same treatment (p<0.05).

Values with different capital letter show significant difference among treatments in the same ensiling days (p<0.05)

Table 14 shows the changes of Lactic/Acetic acid content during silage fermentation. The lactic / acetic acid content ratio was significantly higher in the additive treated silage, indicating that homo type fermentation was dominant.

On the other hand, acetic acid and butyric acid contents were decreased by treatment with inoculant. In general, it was reported that lactic acid bacteria additives increased lactic acid and decreased butyric acid contents (Haigh *et al*., 1996).

Table 14. Effect of wilting and inoculant on Lactic/Acetic acid content of Italian ryegrass silage.

Item		Lactic/Acetic acid (g kg^{-1})			
Treatment*		NWNA	NWA	WNA	WA
Ensiling days	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	5	0.38^{cB}	7.51^{bA}	0.56^{bB}	7.29^{bA}
	10	0.62^{cB}	8.44^{bA}	0.73^{bB}	8.54^{bA}
	20	1.28^{bB}	8.19^{bA}	0.47^{bB}	9.98^{aA}
	30	2.54^{bB}	10.36^{aA}	1.73^{aB}	10.69^{aA}
	45	2.95^{bB}	10.71^{aA}	1.77^{aB}	7.33^{bA}
Significance**	T	***			
	D	***			
	TxD	***			

* NWNA, no-wilting no-additive; NWA, no-wilting with additive; WNA, wilting no-additive; WA, wilting with additive

**T, treatment; D, ensiling days; T x D, interaction between treatments and ensiling days. * $p<0.05$, *** $p<0.001$

Values with different small letter show significant difference among ensiling days in the same treatment ($p<0.05$).

Values with different capital letter show significant difference among treatments in the same ensiling days ($p<0.05$).

5. Conclusions

Wilting resulted in lower crude protein and *in vitro* dry matter digestibility, but no significant differences in ADF, TDN, WSC, and ammonia nitrogen content ($p>0.05$) in forage, NDF of wilted samples is higher than without wilting. However, wilting treatment resulted in higher ADF and NDF content of Italian ryegrass silage ($p<0.05$). The pH of the silage was higher in the wilting group (WNA and WA) and lower in the additive treatment group. The decrement in pH occurred sharply on the 3-5th day of storage. The ammonia nitrogen content was lower in the additive treatment ($p<0.05$).

Add lactic acid bacteria groups (NWA, WA) can significantly increase the lactic acid content and decrease the acetic acid，butyric acid and ammonia nitrogen content but increased the WSC content than without lactic acid bacteria groups (WNA, NWNA) in Italian ryegrass silage.

As fermentation progressed, the lactic acid and acetic acid contents were increased and showed the highest content at 30 days of storage.

In conclusion, the wilting did not significantly improve the silage fermentation, but the lactic acid bacteria treatment improves the quality of the silage.

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7.요약

국내에서 가장 많이 재배되는 사료작물은 이탈리아 라이그라스 (*Lolium multiflorum* Lam.)이며 재배면적은 지속적으로 증가하고 있다. 본 연구의 목적은 예건(wilting) 및 첨가제(Inoculant) 처리가 이탈리안 라이그라스 사일리지의 발효 양상과 최종 품질에 미치는 영향을 조사하는 것이다. 이탈리안 라이그라스는 출수기에 수확하였고 전체 4개의 서로 다른 처리 (NWNA: 비예건-첨가제 무처리; NWA: 비예건-첨가제 처리; WNA: 예건-첨가제 무처리; WA: 예건-첨가제처리)를 하여 사일리지를 제조하였다. 사일리지의 시료는 조제 후 1, 2, 3, 5, 10, 20, 30 및 45 일에 개봉하여 채취하였고 최종 품질은 저장 후 60 일에 확인 하였다. 예건 처리는 이탈리안 라이그라스 사일리지의 ADF 및 NDF 함량을 높였으며 ($p<0.05$), WNA 처리는 사일리지는 TDN 및 IVDMD 함량을 높였다. 그러나 예건 처리는 CP 및 IVDMD 함량을 낮추었으나 ADF, TDN, WSC, 암모니아태 질소 및 pH에 있어는 통계적인 유의성이 없었다 ($p>0.05$). 예건 처리를 한 사일리지에서 pH가 높게 나타났으며 첨가제를 처리한 사일리지에서는 낮게 나타났다. 반면 사일리지 조제 후 3-5일째까지 pH가 급격하게 감소하는 것으로 나타났다. 암모니아태 질소 함량은 첨가제를 처리한 사일리지에서 유의적으로 낮았으며 ($p<0.05$), 예건은 영향을 주지 않았다. 발효가 진행됨에 따라 젖산 및 초산 함량이 증가되었으며 저장 30일째 가장 높은 함량을 보여주었다. 결론적으로 예건처리는 이탈리안 라이그라스 사일리지의 품질을 유의적으로 개선시키지 않았으나 첨가제 처리는 사일리지의 품질을 개선시켰다. 따라서 젖산균 첨가제 처리는 고품질의 이탈리안 라이그라스 사일리지를 원하는 곳에서는 적극 추천된다.

주요어: 이탈리아 라이그라스, 사일리지, 발효, 예건, 첨가제

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