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Research Article

Effect of Moisture Content and Storage Periods on Nutrient Composition and Organic Acids Change in Triticale Round Bale Silage

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ABSTRACT

Livestock production costs are heavily influenced by the cost of feed, The use of domestically grown forages is more desirable for livestock feed production. As part of this study, triticale, which is an extremely palatable and easily cultivable crop in Korea, was used to produce low moisture silage bales with lactic acid bacteria (LAB) and then stored for different periods. We examined the nutrient content of silage, such as crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF), as well as their organic acids, including lactic acid, acetic acid, butyric acid, at different storage periods. The nutrient content of silages, such as crude protein, ADF, and NDF, did not change significantly throughout storage periods. Organic acid data indicated that lactic acid concentrations increased with increasing moisture contents and storage periods up to nine months. However, further extending storage to 12 months resulted in a reduction in the lactic acid content of all silages as well as an increase in their pH. Based on the present results, it suggested that the production of low moisture silage with the LAB may be able to preserve and maintain its quality without altering its nutritional composition. Also, the lactate content of the silage remained significant for at least nine months.

(Key words: Feed, Livestock, Nutrient content, Silage, Storage periods, Triticale)

Ⅰ. INTRODUCTION

The feed that is given to domestic animals plays a major role in ensuring the health of the animals in the domestic livestock. The Korean cattle feeding system relies on both domestically produced rice straw and imported grain. Round baleages made from rice straw account for more than 35% of all round baleages made. Increasing rice straw stockpiles for a long time has put an economic burden on the government (Kim et al., 2020). In recent years, the government has encouraged farmers to cultivate other forage crops in their rice fields. Forages are mainly imported from the USA, particularly alfalfa, timothy and tall fescue (Chang, 2018; Lisa Allen, 2019; Kim et al., 2020). A major contribution to the livestock production is the cost of feeding which accounts for more than half of the total production costs of livestock. It has been found that the grain has recently been used by Korean farmers for the production of animal feed, which has resulted in an increase in the importation of grains. Furthermore, the price of grain has increased due to several reasons, some of which are climate change, changes in market conditions, the need to produce a bioenergy, population raises, and the production and mobilization issues due to the Russia-Ukraine conflict. Therefore, utilizing domestically produced forages is desirable for forage based animal feed production and requires long-term alternative strategies to reduce feed costs for raw materials and import of forages, so we must promote domestic production of high quality baleage and other raw materials.

Triticale is a breed plant derived from crossing wheat and rye, and it is widely used as a feed for domestic and farm animals in various ways. A high content of crude protein and carbohydrates makes it ideal for producing concentrated feed for animals. It can replace oats, barley, and other cereals. Further, triticale is resistant to cold, wet, and other adverse weather conditions. It is highly palatable to livestock and has several leaves, making it well adapted to poor environments, such as barren land. A high crude protein concentration of

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triticale can lead to the production of silage for ruminants (GlamoČLija et al., 2018; Soundharrajan et al., 2020; Jung et al., 2022). There have been few studies examining the production of silage using triticale inoculated with LAB (Pieper et al., 2011; Harper et al., 2017). Compared with other silage crops, triticale has a higher biomass, and fast spring growth (GlamoČLija et al., 2018). It is a key advantage of triticale cultivation that it is cultivated everywhere in Korea. Korea has recently increased its forage production. Lack of silage manufacturing technology and transportation can cause aerobic deterioration, loss of nutrients, and undesirable microbial growth, leading to degraded lactate content and the accumulation of anti-nutrients in silage. Thus, livestock farmers have become more vocal about their complaints.

In general, lactic acid bacteria (LAB) are crucial to the production of silage from grasses, legumes, and other plants. Ensiling is becoming more popular because it provides a reliable, consistent and predictable source of forage for farm animals. LAB, when added to a forage sample during ensiling, promotes silage acidification. This is because they produce lactic acid and, to a lesser extent, acetic acid, butyric acid, succinic acid, among other organic acids. In an acidified environment, yeasts, molds, and Listeria species cannot grow, preventing aerobic deterioration during fermentation (Arasu et al., 2014; Soundharrajan et al., 2020; Jung et al., 2022). In order to expand the use of triticale, it is important to improve its quality and storage. A major objective of this study is to produce low moisture silage bales (42% to 30%) from triticale and examine their nutrient content and organic acid changes during storage (3-12 months).

Ⅱ. MATERIALS AND METHODS

1. Location and cultivation of triticale

This experiment was executed as a randomized design during the May 2021-May 2022. The triticale cultivar “Joseong” was cultivated at Jangsoo, Chunbuk, South Korea (Latitude: 35.6185318 and longitude: 127.5107881). According to the guidelines provided by the Rural Development Administration, Korea, Triticale seed was planted in soil by drill seeding method at the rate of 180 kg/ha on 25 October, 2021. NPK fertilizer applied at the respective rates (120 kg N/ha, 100 kg P/ha, 100 kg K/ha). N Fertilizer was applied to the soil surface in two parts. 60 kg/ha of nitrogen fertilizer was applied as the basal dressing and the rest amount was applied at the start of the growth period in early spring. However, phosphoric acid and potassium were applied as the basal dressing (Jung et al., 2022).

2. Silage production

Triticale was harvested in early heading stage (8th May, 2021) at the experimental field at Jangsoo, Chunbuk, South Korea. Ttriticale was mowed using a disc-mower conditioner and wilted under field condition for 60-72 h to make low moisture silage bales (25-30%), and 48-55h to produce 30-42% moisture silage bales. In this study, tedder (2 times/day) was used to promote the drying rate; because tedder can improve drying conditions by spreading and fluffing the triticale swath. After reaching expected moisture, the silage bales were made with dimensions set for 125 cm height by 120 cm diameter. Lactic acid bacteria used in this study contained L. *plantarum* KCC-10, *Lactobacillus brevis* KCC-44 and *Pediococcus pentosaceus* KCC-45 (Ratio:2 × 10^{10} CFU/g, each). Three kinds of lactic acid bacteria were isolated and characterized (Valan Arasu et al., 2013; Soundharrajan et al., 2019). The cocktails prepared from these strains at Jungnong Bio Inc, South Korea. The additives (100g / 50 tone of Top triticale silage, South Korea) were sprayed automatically during silage bale making and then bales were individually wrapped six times with plastic film (IHL SHIN Chemical Co. Korea) by a silage bale wrapper.

3. Nutrients and organic acids determination

All silage bales were stored in a room temperature (Hanwoo farm) throughout experimental storage periods (3, 6, 9, and 12 months) and opened in respective storage periods. The samples were taken from each bale silages according to a respective storage periods and transferred into a Laboratory (National institute of Animal Science, Seonghwan-eup, Cheonan, Korea) for analysis of contents of dry matter, crude protein, acid detergent fiber (ADF) and neutral detergent fiber (NDF) , pH and fermentative metabolites (lactic acid, acetic acid, butyric acid). Contents of CP, ADF and NDF were analyzed (AOAC, 2000; Goering and Van Soest, 1970).

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For analysis of pH and fermentative metabolites, a sample of 10 g was mixed with 90 mL of distilled water for 1 hr. at high speed shaker and then filtered with Whatman No.6 paper to obtain water extract. pH of silages was measured by pH meter (HI 9024; HANNA Instrument Inc. UK). The acetic acid and butyric acid was determined by Gas-Chromatography (VARIAN 450 with FID detector) and lactic acid content was quantified by HPLC method (Water-e2695 with 2998 PDA detector).

Ⅲ. RESULTS

The present study, silage bales from heading triticale cultivar in the presence of lactic acid bacteria at different moisture levels were prepared and stored them for different periods of time (3, 6, 9, and 12 months). We then determined organic acids such as lactic acid, acetic acid, butyric acid, and nutrient contents such as crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) during respective storage periods. The nutrient content (CP, ADF, and NDF) of the low moisture (28.80% ~ 30.44%) silages did not change during all storage periods as shown in Table 1. Throughout the experimental period, the CP level in silage ranged from 8.2 to 8.9%, the ADF level from 32.3 to 34.3%, and the NDF level from 56.8 to 60.8%. In addition, pH levels of the silage were reduced while storage periods were extended. Also the concentrations of organic acids, such as lactic acid and acetic acid were decreased as storage periods were extended. The acid content of silages at 12 months was drastically reduced compared to other storage periods.

Silage nutrient content (CP, ADF, and NDF) did not significantly change throughout storage periods at different moisture conditions (37.78%-39.86%). The pH values of the silages ranged from 5.29 to 5.87 during different storage periods. The lowest pH value was 5.29 and the highest pH value was 5.87 in the silage bales after six months and twelve months, respectively (Table2). It was found that organic acids in silage increased over the period of 3 to 9 months but there is no significant change in lactate content of silages between 3 , 6 and 9 months. There was a significant increase in acetic acid concentration in silage after storage for 6 months ($p = 0.05$) in comparison to other storage periods. The level of acetic acid was higher at 9 months of storage compared to 3 and 12 months. However, it was lower compared to the level at 6 months of storage. These organic acids, such as lactic and acetic acids were tended to decrease in concentration as the storage period was extended (Table 2).

There was no significant change in the nutrient content of

Table 1. Nutrient composition and organic acids level in very low moisture silage (〈30%) at different storage periods

Storage Periods (Months)	pH	Moisture (%)	LA (DM%)	AA (DM%)	BA (DM%)	ADF (%)	NDF (%)	CP (%)
3	5.79±0.14	30.13±1.5	1.38±0.05^{a}	0.05±0.08^{b}	-	33.7±0.6	56.8±1.4	8.9±0.40
9	5.48±0.02	28.80±0.4	1.44±0.03^{a}	0.24±0.10^{a}	-	34.3±0.3	60.8±0.5	8.8±0.06
12	5.45±0.28	30.44±0.6	0.07±0.01^{b}	-	-	33.2±1.4	59.0±1.5	8.2±0.25

The data are expressed as mean± STD of three replicates (n=3). A different alphabet within a row indicates significance at the 0.05 level. DM: Dry matter content; LA: Lactic acid; AA: Acetic acid; BA: Butyric acid; ADF: Acid detergent fiber; NDF: Neutral detergent fiber; CP: Crude protein.

Table 2 Nutrient composition and organic acids level in low moisture silage (〈40%) at different storage periods.

Storage Periods (Months)	pH	Moisture	LA (DM%)	AA (DM%)	BA (DM%)	ADF (%)	NDF (%)	CP (%)
3	5.62±0.2	37.78±1.3	1.45±0.03^{a}	0.02±0.01^{c}	-	36.4±1.0	60.7±3.1	9.00±0.9
6	5.29±0.1	38.55±1.7	1.62±0.09^{a}	0.25±0.02^{a}	-	36.0±1.6	61.9±1.8	8.60±0.3
9	5.48±0.0	38.13±1.8	1.53±0.20^{a}	0.18±0.001^{b}	-	36.3±0.3	63.4±0.7	8.31±0.15
12	5.87±0.0	39.86±0.2	0.07±0.00^{b}	-	-	34.5±0.1	59.2±0.3	8.54±0.03

The data are expressed as mean± STD of three replicates (n=3). A different alphabet within a row indicates significance at the 0.05 level. DM: Dry matter content; LA: Lactic acid; AA: Acetic acid; BA: Butyric acid; ADF: Acid detergent fiber; NDF: Neutral detergent fiber; CP: Crude protein.

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Table 3. Nutrient composition and organic acids level in low moisture silage (41– 43%) at different storage periods.

Storage Periods (Months)	pH	Moisture	LA (DM%)	AA (DM%)	BA (DM%)	ADF (%)	NDF (%)	CP (%)
3	5.44±0.24	41.64±0.4	1.54±0.13[a]	0.27±0.2[a]	-	35.0±2.5	60.3±1.3	9.3±0.5
6	5.35±0.05	42.80±1.0	1.49±0.25[a]	0.24±0.1[a]	-	35.8±0.8	61.2±0.9	8.2±1.3
9	5.49±0.03	42.11±1.0	1.80±0.001[a]	0.23±0.2[a]	-	36.7±0.4	64.2±0.06	8.2±0.07

The data are expressed as mean± STD of three replicates (n=3). A different alphabet within a row indicates significance at the 0.05 level. DM: Dry matter content; LA: Lactic acid; AA: Acetic acid; BA: Butyric acid; ADF: Acid detergent fiber; NDF: Neutral detergent fiber; CP: Crude protein.

silage (CP, ADF, and NDF) during the storage periods at moisture levels ranging from 41.64% to 42.80% (Table 3). There was almost no difference in the pH of the silage during all of the storage periods (pH 5.35 to 5.49). All of the storage periods (3 to 9 months) of the silage had a significant increase in the amount of lactic acid in the silage. However, the amount of acetic acid in all storage periods was similar (Table 3).

Ⅳ. DISCUSSION

Domestic animals' health is greatly influenced by their diet. Feeding costs account for more than half of livestock production costs. Therefore, domestically grown forages are advantageous for livestock feed production. Thus, triticale, which is a highly palatable and easily cultivable crop in Korea, was used in this study to produce different moisture silage bales with lactic acid bacteria cocktail by an ensiling method. Forage preservation by ensiling method has gained much attention as it can provide reliable, consistent and predictable food supplies for ruminants. Plant oxidation, enterobacterial growth, proteolytic activity, undesired microbial fermentation, deamination and decarboxylation of amino acids severely affect the quality of forages/silage. As a result, energy loss and anti-nutritional compounds are increased in forages (Oliveira et al., 2017). The preservation of ensiled forage samples for prolonged periods is based on the strong acidification by the microbial inoculants. Faster acidification inactivates proteolytic enzymes activities and controls the diversity and richness of enterobacteria which grow until low pH reached (Muck, 2010). The major objectives of the silage and feed industry are to improve the nutritional content, aerobic stability and absorbance efficiency of plant silages. In general, lactic acid bacteria (LAB) play a major role in the preservation of forages at ensiled condition by rapid induction of fermentation of silages. But, in the feed industry, indigenous LAB population in the plant is not sufficient to meet the current industry requirements. Therefore, additional inoculums are required to make good quality silages with preserved nutrients, particularly LAB. In this study, silage bales were prepared from an early heading triticale cultivar at different moisture conditions. Different types of additives are needed to stimulate the ensiling process in order to develop high quality silage. Inoculating silage with LAB is expected to increase lactic acid concentration, lower acetic acid and butyric acid concentration, reduce proteolysis, and increase dry matter recovery (Richard, 2013; Kim et al., 2017). Thus, in the present study, *L. plantarum* KCC-10, *Lactobacillus brevis* KCC-44 and *Pediococcus pentosaceus* KCC-45 were used in the production of silage bales to improve the controlled fermentation process, resulting in a reduction in the pH of the silage as we expected The acidification process of silage produced with LAB at low moisture level (28.0% to 30.44%) was significantly enhanced throughout the storage periods. Another set of silage with moisture between 37.78% and 39.86% was produced with the same LAB strains, leading to a pH reduction maximum at six months storage (pH 5.29). Later, pH levels were slightly increased in a time-dependent manner. The quality of the silage was maintained at a significant level even after 9 months of storage with the LAB, as indicated by the pH value and lactate content of the silage. Production of silage with moisture ranges 41 to 42.8% shown that the pH values were reduced at all the storage periods. Current study, throughout all moisture and storage periods, pH values were greater than 5 in all silage bales. In general, silage quality is usually determined by pH, and a range between 3.8 and 4.2 is desirable for successful ensiling (Ahmadi et al., 2019). However, the current investigation revealed that the pH values were higher than

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those for high quality silage indicators. This may be due to an insufficient supply of water-soluble carbohydrates (WSC) to the LAB at very low moisture levels. Despite slightly higher pH levels in all silages, significant lactate levels were observed in all silages until nine months of storage. It has been demonstrated that adding LAB during the production of low moisture triticale silage significantly improved the quality of the silage and its lactate content. Sevral reports claimed that LAB has the advantage of utilizing water-soluble carbohydrates (WSC) and converting them into lactic acid, an important and major acid present in silages and considered as a marker for preserved high quality silages (Hashemzadeh-Cigari et al., 2014; Ahmadi et al., 2019). As key acids in the ensiled silages, lactic acid, acetic acid, and butyric acid have been identified and represent the highest levels of acids produced by LAB from WSC (Liu et al., 2019; Yang et al., 2019). Particularly, lactic acid was found to be more concentrated in silage than any other acid, which contributed to reducing the pH of the silage and preventing undesirable microbial growth (Kung et al., 2018). The current study found an increase in lactic acid content in silage bales between 3 and 9 months at all moisture levels. It has been suggested that the increasing moisture content of silage and storage periods could influence pH reduction by producing lactic acid and marginal levels of acetic acid. There is not much change in both acidification and lactate content of silages in response to moisture conditions. When silage stored for 12 months, the pH increased slightly because organic acids in all silages were reduced. Based on this study, it was found that the production of low moisture silage (ranges between 28 and 42%) with biological additives such as LAB improved the quality silage by enhancing lactate production. However, the prolonged storage period reduced the lactate or organic acids level and slightly raised the pH of the silages, possibly due to the degradation of lactate or organic acids by undesirable microbial growth. Under very low moisture conditions, plant based silage could have stiff stems, which may damage the film used for silage production, its allowing air to enter bailed silage or silos. In such conditions, aerobic bacteria are more likely to grow in the silage, which enhance the degradation of organic acids and raise the pH level of the silage. As the nutrient composition of silage (CP, ADF, and NDF) did not significantly change throughout its storage period.The use of LAB in storing low moisture silages may be considered to be useful for preserving the native form of nutrients with signifinificant level of lactate for a long period of time, perhaps as long as nine months.

Ⅴ. CONCLUSIONS

This study examined the production of low moisture silage bales from triticale with the addition of lactic acid bacteria (LAB) and their storage for different periods of time. Nutrient content of silages, including crude protein, ADF, and NDF, did not change significantly during the experiment. Further, organic acids such as lactic acid, acetic acid, and butyric acid were quantified at different storage periods. This suggests that organic acids, particularly lactic acid content, increase with increased moisture content and storage periods until 9 months. However, further extending storage to 12 months resulted in a reduction in lactic acid content and an increase in pH. Based on the present results, it seems that the production of low moisture silage with the LAB may be capable of preserving and maintaining its quality without altering its nutritional composition, but the lactate content of the silage may remain significant for at least nine months after storage.

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