Influence of lactic acid and cellulolytic bacteria on the physicochemical parameters of alfalfa silage

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Abstract. The study is aimed at studying the effect of a microbiological preparation consisting of lactic acid bacteria, thermophilic streptococci and cellulolytic bacteria on the quality of alfalfa silage with different dry matter content. Determined pH, content of organic acids, dry matter and nutrients, including carbohydrates. Inoculant application increased crude protein, crude fat and crude fiber and reduced water-soluble carbohydrates in silage. The preservation of nutrients in the test samples was higher than that of the control. pH corresponded to optimal values; however, lactic acid fermentation proceeded more intensively in the sample with an increased dry matter content.

Keywords: silage, alfalfa, silage additive, acidity, silage quality

1. Introduction

As a fodder crop, alfalfa is widely distributed in the world grass growing, occupying the largest areas [1]. The crop is characterized by high yields and hay yields, resistance to drought and pests, young plants successfully resist the spread of weeds. Its ability to fix atmospheric nitrogen makes it superior to other crops in quality and quantity of protein and essential amino acids. Alfalfa protein is well digested and assimilated by all kinds of animals, including highly productive dairy cows [2, 3]. Therefore, methods of preserving alfalfa fodder for year-round use are of great importance, one of which is ensilaging. It is an anaerobic preservation method that preserves nutrients and improves the palatability of green fodder. Silage is one of the traditional bulk feeds used for animals in the world [4-6].

During silage, lactic acid bacteria break down water-soluble carbohydrates and produce lactic acid, which lowers the pH of the feed, which helps to suppress putrefactive microflora. They also inhibit the growth of undesirable epiphytic microorganisms by competing for nutrients and synthesizing antimicrobial and antifungal metabolites [7]. However, the high buffer capacity of alfalfa, the low number of epiphytic lactic acid bacteria populations and insufficient sugars for the development of beneficial microflora can complicate the fermentation process [8].

Bacterial preparations are used to obtain good quality silage from alfalfa, to minimize nutrient losses and to enhance fermentative processes [2, 9]. It is also believed that the fermentation of silage is influenced by the amount of dry matter in the raw material [10]. Objective: to study the quality of finished alfalfa silage with different dry matter content and the use of microbiological preparation.
2. Materials and methods.
Lucerne variegated (Medicago x varia T. Martyn) of the Blagodat variety, collected in the phase of budding, with a dry matter content of 25.76% and 37.14% were ground and treated with a microbiological preparation consisting of lactic acid bacteria (Lactobacillus rhamnosus, Lactobacillus paracasei, Lactococcus helveticus), bifidobacteria (Bifidobacterium animalis), thermophilic streptococci (Streptococcus thermophilus) and cellulolytic bacteria. The total number of microorganisms was 15*10^8 CFU/cm³. Alfalfa was placed in 0.5 l laboratory containers for silage. After 40 days of fermentation, physico-chemical parameters of the silage were determined according to standard methods.

3. Results and discussion
The pH values in alfalfa silages with different dry matter content prepared with the microbiological preparation corresponded to the optimal values (pH 3.9-4.3) (Table 1). This may be due to the acidifying effect resulting from the use of lactic acid and cellulolytic bacteria [11]. Application of the studied additive had the effect of statistically significant increase in the total amount of acids in alfalfa silage samples with a dry matter (DM) content of 37.14% by 0.43% compared with the index in the control (P ≤ 0.05). The increase in this parameter was due to an increase in lactic acid by 0.45% (P ≤ 0.05), which is important in the process of silage acidification and contributes to a decrease in pH. Our results are consistent with previously published data [12, 13]. The decrease in pH can be attributed to the use of cellulolytic bacteria. They improve the decomposition of plant fibers and contribute to an increase in water-soluble carbohydrates, the nutrient medium of lactic acid bacteria. This stimulates the fermentation process, resulting in lower pH and improved preservation of silage [13, 14].

### Table 1. Content of organic acids in silage (n = 3, mean ± SE)

| Silage variant | pH             | Ratio of acids in the natural substance, % |
|----------------|----------------|-------------------------------------------|
|                |                | total | lactic | vinegar | butyric |
| Alfalfa (DM 25.76%) | Control 4.17±0.01 | 2.80±0.19 | 2.33±0.25 | 0.37±0.05 | 0.11±0.01 |
|                | Experiment 4.08±0.02 | 2.51±0.36 | 2.10±0.35 | 0.33±0.01 | 0.08±0.02 |
| Alfalfa (DM 37.14%) | Control 4.86±0.12 | 2.27±0.07 | 1.89±0.07 | 0.27±0.05 | 0.12±0.06 |
|                | Experiment 4.10±0.04 | 2.70±0.08 | 2.34±0.08* | 0.22±0.02 | 0.15±0.02 |

Control - silage prepared without microbiological preparation, experiment - silage prepared with microbiological preparation; DM - dry matter; * - reliability of difference in comparison with control P ≤ 0.05

The content of butyric acid, which is an indicator of clostridia activity, in silage with the addition of microbial inoculum was quite low and did not differ significantly from the same indicator in the control samples. The data we obtained may indicate the effective suppression of butter-acid bacteria as a result of rapid accumulation of lactic acid and low silage pH [15, 16].

It was found that the DM content in the forages prepared with the microbiological preparation was lower compared to the control values (Table 2). In the experimental sample of alfalfa silage with a CB content of 37.14%, this index had a statistically significant difference and was lower by 11.55% compared with the value in the control (P ≤ 0.05). Previously published studies have shown that a significant increase in DM in the silage green matter leads to a reduction in total acid formation, which affects the quality of silage harvested [17, 18]. This is consistent with the data obtained in our study (Table 1). Nevertheless, in the feeds preserved with the microbial inoculum, there was no loss of DM relative to its content in the original raw material. This indicates that lactic acid bacteria mainly produce only lactic acid without by-products and inhibit the development of putrefactive microorganisms [19].
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Table 2. Biochemical composition of green mass and obtained silage (n = 3, mean ± SE)

| Silage variant | DM, %     | Contained in DM, % |
|---------------|-----------|--------------------|
|               | Crude Protein | Crude Cellulose | Crude Fat | Sugar | Starch |
| Alfalfa (DM 25.76%) |           |                   |           |       |        |
| Green mass    | 25.76±4.81  | 22.02±0.65        | 23.29±1.09| 2.91±0.08| 8.48±0.26 | 2.20±0.35 |
| Control       | 31.63±0.53  | 22.71±0.10        | 25.07±0.18| 3.59±0.21| 3.97±0.08 | 1.13±0.01 |
| Preservation of nutrients, % | 122.79 | 103.13 | 107.64 | 123.37 | 46.82 | 51.37 |
| Experiment    | 31.07±1.04  | 22.74±0.62        | 25.24±0.23| 3.83±0.04| 0.90±0.07 | 0.99±0.01 |
| Preservation of nutrients, % | 120.61 | 103.27 | 108.37 | 131.62 | 10.61 | 45.00 |
| Alfalfa (DM 37.14%) |           |                   |           |       |        |
| Green mass    | 37.14±2.21  | 22.03±1.12        | 22.42±2.25| 3.14±0.22| 8.71±0.89 | 2.29±0.37 |
| Control       | 50.70±0.87  | 19.03±0.34        | 24.29±2.03| 1.72±0.14| 3.74±0.01 | 0.88±0.13 |
| Preservation of nutrients, % | 136.51 | 86.38 | 108.34 | 54.78 | 42.79 | 38.43 |
| Experiment    | 39.15±0.24* | 21.56±0.14*       | 24.85±0.24| 3.08±0.71*| 0.90±0.07*| 0.76±0.01* |
| Preservation of nutrients, % | 105.41 | 97.87 | 111.02 | 98.09 | 10.33 | 33.19 |

Control - silage prepared without microbiological preparation; experiment - silage prepared with microbiological preparation; * - reliability of difference in comparison with control P ≤ 0.05.

It should be noted the better preservation of crude protein (CP) in silage with the use of microbiological preparation compared with similar data in the control. In the experimental sample of dried alfalfa silage CP content was statistically significantly higher by 2.53% compared with the control sample (P ≤ 0.05) (Table 2). The decrease in SP indicator during fermentation may be due to its decomposition, which leads to an increase in ammonia nitrogen production, which leads to a deterioration in silage quality [20, 21].

An increase in crude fat content (CF) in the experimental silages was found, with a significant excess of 1.26% in the alfalfa sample with a DM of 37.14% (P ≤ 0.05) over that of the control. This may indicate better preservation of this component in the experimental samples, which agrees with the works of other authors [21, 22].

As noted earlier, the addition of cellulolytic bacteria potentially increases the availability of nutrient components for lactic acid bacteria [23]. Therefore, the significant reduction in sugar in silage samples harvested with the microbial preparation appears to be due to the active reproduction of lactic acid bacteria, which used sugar in the process of life activity [13, 24].

The study revealed that silage prepared using microbiological preparation from alfalfa with different moisture content differed slightly in nutrient content, but the lactic acid fermentation process was more intense in the sample with higher DM content. This is indicated by the higher amount of lactic acid in alfalfa silage with a DM content of 37.14%. Scientists have noted an increase in epiphytic lactic acid bacteria on alfalfa during wilting, which enhances lactic acid fermentation [25].

It is worth noting that quality silage was obtained from alfalfa with a DM content of 25.76% without the use of inoculant. This may be due to optimal laboratory conditions and the possible presence of beneficial epiphytic microflora in the green matter before silage.

4. Conclusion

Thus, a microbiological preparation consisting of lactic acid bacteria (Lactobacillus rhamnosus, Lactobacillus paracasei Lactococcus helveticus), bifidobacteria (Bifidobacterium animalis), thermophilic streptococci (Streptococcus thermophilus) and cellulolytic bacteria showed potential for
use as a preservative supplement for green alfalfa. Improvement of fermentation processes was found when using the inoculum, as indicated by a lower pH value and increased amount of lactic acid in the experimental silage samples. In the forages prepared with the use of microbiological preparation, the preservation and content of nutrients were higher than in the control samples. These indicators indicate that better quality silage was obtained. Lactic acid fermentation was more intense in the experimental silage made from dried alfalfa. Nevertheless, further more detailed studies are needed to confirm the effectiveness of the used microbiological preparation for silage harvesting.

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