

Impact of Silo Twice biopreservative on haylage quality

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Abstract. The complex biological starter (biopreservative) “Silo Twice” is related to microbiology in feed production, in particular to the technology for obtaining complex biopreservatives - preparations used for silage of feed. In the finished biopreservative fermented Silo Twice product, all nutrients of the starting material are preserved and the relative value of the feed has increased 17 units. Compared to the classic self-preservation of haylage, the introduction of Silo Twice biopreservative provides a decrease in protein losses of 2.0–2.2 times. Feeding milk cows with haylage obtained when applying Silo Twice biopreservative provides an increase in the milk yield by 8.0%, milk fat content by 0.4%. The use in the diet of dairy cows of haylage obtained with the introduction of the biopreservative Silo Twice contributed to a reduction in the cost of exchange energy and concentrates by 2.43% and 2.95%, respectively. The use in the diet of dairy cows of haylage obtained with the introduction of biopreservative Silo Twice led to the optimization of processes in the scar, an increase in the level of volatile fatty acids and bacteria, with a decrease in the amount of ammonia by 16.3%. This fact indicates an improvement in the digestibility and absorption of nitrogen in the scar of cows. Feeding milk cows for 60 days with haylage obtained when the Silo Twice biopreservative was introduced provided a savings of 2250 rubles per cow.

1 Introduction

One of the main most priority and continuously developing areas of animal husbandry is dairy cattle breeding. The most important factor in realizing the biological potential of cows is balanced feeding, taking into account the physiological need of the animal body.

Currently, the solution of issues in the field of animal feeding is impossible without taking into account the preservation of plant nutrients in feed production. At the same time, the aspect of processing green mass into canned food is of particular relevance.

Haylage is a feed made from herbs harvested in the early phases of the growing season, boiled to a humidity of 45...55% and preserved in anaerobic conditions. Haylage canning is achieved as a result of the insufficient water content in boiled plants, when most bacteria, due to physiological dryness of the environment, cannot extract the water necessary for intensive development. As a result, acid formation in haylage is limited, putrid and oily - acidic bacteria develop weakly, which contributes to the better preservation of nutrients and, above all, sugar. Therefore, it is important for the humidity of the feed to be within 45...55%. Then the water of the feed is inaccessible to bacteria. The water-retaining force of plant cells is at a humidity of 50... 55% - 52 atm and the maximum sucking force of most bacteria is about 50 atm. But if putrid and other bacteria cannot develop under such humidity, molds that have a sucking force of more than 200 atm are intensively developed. Their development can only be prevented by careful isolation of the mass from the air. Without air access, the

breathing of plant cells is also stopped and the development of thermophilic bacteria, which cause strong heating of the mass, is eliminated. The nutritional value of feed is determined by the content of exchange energy in 1 kg of natural feed or dry matter. In the norms of feeding animals, along with their need for exchange energy, energy rationing is also indicated in feed units. Sources of metabolic energy are carbohydrates, fats and proteins. To obtain 4000... 4500 kg of milk from a cow, 800... 1000 g of the average daily growth when fattening young cattle and 450... 500 g when fattening pigs, it is necessary to harvest at least 40... 45 c of food for each conditional unit per year. Units are with content of 1 feed. Units have 110... 115 g of digestible protein. At the same time, in the annual structure of feed, haylage is 7%, silage is 23%, or at least 1.5... 2 tons of haylage and 5... 6 tons of silage should be stored per 1 head of cattle. Starting with grass fermentation, traditional and new technologies of silage and haylage preparation from whole plants and partially combed stems have the same technological operations: mowing of plant raw materials with grinding, transportation of mass, its laying in storage, leveling, compaction and sealing. To compress the mass, the degree of grinding for both silage and haylage plays a crucial role. Beveled herbs on silage and boiled on haylage to optimal humidity are ground by fodder harvesting machines into particles 20... 50 mm long. The production of feed with a high nutrient content is largely determined by the harvest time of the plants. Silos should be prepared from plants that are freshly milled or tilted to a humidity of 60... 70% of the ground

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mass. Quality silage is obtained from plants mowed in the following phases of growing. Corn and sorghum are from wax, milk-wax ripeness of grain, sunflower from the beginning of flowering. Sudanese grass is from sweeping panicles, perennial legumes - budding, perennial legume grasses – budding. But it should not be later than the flowering phase, perennial cereal grasses is at the end of the exit phase into the tube - the beginning of scaling. Grass mixture is from the above-mentioned growing phases of the predominant component. Annual legume grasses and legume-cereal grass mixtures are mowed in the phase of wax ripeness of legume seeds in two or three lower tiers. To obtain haylage, legume perennial herbs are mowed in the budding phase, but not later than the beginning of flowering. Legume annual plants, legume-cereal and their mixtures are mowed no later than the phase of bean formation in two or three lower tiers, perennial cereal - at the end of the pipe-forming phase until the beginning of spitting. Legumes should be boiled to a humidity of 45... 55%, cereals - 40... 55% [7; 8; 11].

Due to climatic conditions and imperfection of storage facilities, harvesting of feed in Russia often occurs in conditions of high humidity, which negatively affects the quality of feed, including silage and haylage. After depressurization of canned food, deterioration of its quality begins; putrefactive bacteria and other pathogenic microflora lead to significant losses of feed nutrients, cause a number of diseases and a decrease in animal productivity [1; 2]. This can be avoided by the use of chemical and biological preservatives containing microorganisms capable of producing high levels of lactic acid and fermenting a number of hard-to-reach plant compounds. The use of chemical preservatives has significant disadvantages: the resulting feed may contain toxic substances, which is unsafe for health [3]. Therefore, it was decided to create a preservative of biological origin that does not harm animal health and has a beneficial effect on the quality of haylage and milk productivity.

The purpose of the work is to create a new comprehensive third-generation biopreservative for silting the green mass of all plant species, flattened grain and preserving hay.

2 Material and methods

The research material was as follows.

Lactic acid-producing bacilli *Enterococcus faecium* had the content of enterococci in plant and animal raw materials that does not allow staphylococci, listeria and *E. coli* to multiply in the finished product. The main factor of antagonistic activity of enterococci is their ability to produce antimicrobial peptides - enterocins [4].

Lactic acid bacteria are *Lactobacillus plantarum*, *Lactobacillus buchneri*, *Lactobacillus fermentum*, producing lactic acid, which contributes to a rapid decrease in the pH of the silage mass, prevents the development of anaerobic microbes that cause food damage [5].

Propionibacterium freudenreichii subsp. *Shermanii* produce propionic acid and its salts (propionates), acetic and lactic acids having a pronounced bactericidal effect.

Enzyme complex contained:

- cellulase catalyzing hydrolysis of glycoside bonds in cellulose to form glucose;
- amylase catalyzing the cleavage of starch, glycogen and saccharides to simple monosaccharides which are readily digestible in the intestine.
- glucanase, which destroys beta-glucans and other non-starch polysaccharides of grain raw materials to form simple sugars;
- xylanase, which promotes the release of protein, starch, pectin from plant cells and the formation of simple sugars by decomposing arabinoxylans to a xylose molecule [6].

The method for preparation of the Silo Twice biopreservative is microbiological synthesis followed by freeze-drying and mixing of preparation components with each other and dry milk whey to normalize titer. As a result, a new bacterial substance is obtained, containing highly active strains of bacteria and a complex of enzymes for the fermentation of haylage, silage and flattened grain.

3 Results and discussion

Microbiological and physicochemical indicators of the quality of microorganisms and enzymes included in the Silo Twice biopreservative comply with the developed TS 10.89.19-012-09967133-2021 and contain the following strains and colony-forming units (CFU/g):

- *Enterococcus faecium* VOA-1 ECM V-28720 – $2,3 \times 10^{10}$ CFU/g;
- *Lactobacillus plantarum* VKPM V-11264 - $1,2 \times 10^{11}$ CFU/g;
- *Propionibacterium freudenreichii* subsp. *Shermanii* VKPM B-5592 - $2,2 \times 10^9$ CFU/g;
- *Lactobacillus fermentum* VKPM B-7573 - $1,2 \times 10^{10}$ CFU/g;
- *Lactobacillus buchneri* VKPM V-7641 - $1,2 \times 10^{11}$ CFU/g;
- amylases - 300 U/g;
- glucanases - 300 U/g;
- xylanases - 300 U/g;
- cellulases - 100 U/g.

Starter microorganisms provide fast preservation and enhanced effect of aerobic stability directed against putrefactive bacteria, yeast, molds and other fungi. Live *Lactobacillus plantarum* bacteria provide a high level of lactic acid formation in the preserved food, minimize the appearance of undesirable acids and other compounds during fermentation of the green mass, provide a high level of carotenoid preservation in the product, increasing its biological value, and have antagonistic properties with respect to undesirable microflora leading to its deterioration. It has been found that efficient processing of feed butyric acid by *L. fermentum* strain into hydrogen peroxide (H_2O_2) allows one to additionally

fight emerging molds, fungi and pathogenic bacteria, protect the digestive tract of the animal from foodborne infections, and helps to prevent oxidative damage of feed products. The Silo Twice biopreservative enzyme complex (cellulase, amylase, gluconase, xylanase) contributes to the cleavage of non-starchy polysaccharides and the release of additional nutrients, in particular protein, thereby improving the preservation quality of difficult-to-silage plants.

The use of the Silo Twice biopreservative consists in its introduction into the embedded green mass at the rate of 1.0 g of the product (with an activity of at least 1×10^{11} CFU) per 1 ton of the silted raw material, diluting it in the required amount of water. The ready solution of the preparation is introduced into the silusable mass by means of spraying devices on the combine or in trench. In the absence of such devices, the drug can be introduced into the trench with a watering bottle with small holes. The silo mass must be carefully sealed after its laying and compaction for 1-2 days, since lactic acid bacteria are able to multiply strictly without air oxygen access.

There were no adverse events and complications with the use of Silo Twice biopreservative at the recommended doses. There are no contraindications to the use of the Silo Twice biopreservative. The biopreservative Silo Twice is compatible with all feed ingredients, other feed supplements and drugs. Products from animals (including birds and fish) after the use of the Silo Twice biopreservative can be used for food purposes without restrictions, as confirmed by the results of laboratory tests.

When injecting the Silo Twice biopreservative at a dose of 1.0 g/t during haylage of the green mass of Sudanese grass harvested in the panicle sweeping phase, haylage was obtained, the chemical composition of which is presented in parentheses, after the indicators of Sudanese grass.

So, the moisture content of the grass was 71.43%, including hay (76.64%), crude 3.51% protein (2.80%), 0.06% starch (1.06%), simple 0.66% sugars (0.32%), lactic acid 1.34% (1.95%), acetic acid 0.07% (0.30%), butyric acid 0.00% (0.00%), ammonia 0.03% (0.13%), the relative value of the 84 feed (101).

Therefore, in haylage, part of the protein passed into acid, which is proved by an increase in ammonia, simple sugars passed into alcohol-soluble carbohydrates and organic acids. The relative value of feed increased by 17 units compared to raw materials. Compared to the classic self-preservation of haylage, the introduction of the Silo Twice biopreservative provides a decrease in protein losses of 2.0-2.2 times.

The closest in functional properties to the Silo Twice biopreservative is a second-generation biopreservative (prototype), represented by a drug consisting of hetero-enzyme lactic acid and propionic acid bacteria with possible addition of enzymes. It contains a combination of strains of lactic acid bacteria: *Lactobacillus diolivorans*, *Lactobacillus buchneri* and *Lactobacillus rhamnosus* contribute to the rapid and intensive fermentation processes in the first weeks of silage of starch-rich cultures. The dosage is 1 g per ton of the silted mass [2].

Protopit has a significant drawback: a rather high cost due to foreign production.

Feeding dairy cows with haylage obtained by applying the Silo Twice biopreservative led to optimization of scar processes, an increase in the level of volatile fatty acids and bacteria with a decrease in the amount of ammonia by 16.3%, which indicates an improvement in the digestibility and absorption of nitrogen in the scar of cows. As a result, the milk yield increased by 8.0% compared to that of the control group, milk fat content increased by 0.4% (Table 1).

Table 1. Comparative analysis of the preserved food by the example of haylage from Sudanese grass (in dry substance (CB) feed).

Indicators	Control (prototype)	Experience I self-conservation	Experience II preservative-biopreservative-volume Silo Twice
Crude protein (CP), %	11.85	11.20	11.99
Rhodium-rhyme protein, % CP	56.3	61.4	57.0
Split in the scar protein, % CP	67.3	59.0	66.0
Transit protein, % CP	32.7	41.0	34.0
Starch, %	4.51	3.86	4.56
Lactic acid, %	8.28	5.30	8.33
Vinegar acid, %	1.40	0.89	1.30
Energy exchange, MJ/kg	9.45	8.68	9.93
Permafrost energy, MJ/kg	11.32	10.12	11.70

Relative fodder value	100	82	101
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The amount of raw protein in all three haylage samples is normal. The optimal value of the crude protein should be < 190 g/kg CB or, translated into percentages, < 19% [9].

Soluble protein is the percentage of crude protein soluble in the buffer that is responsible for the pH of the scar. The indicator is given in % of the total raw protein. The high content of soluble raw protein suggests that a large amount of protein will be available in the scar. As a result, a high content is cleaved in the scar and a low content of protein is absorbed in the intestine. Thus, soluble crude protein is an indicator of protein quality. This parameter gives an idea of the amount of protein absorption in the scar. A high rate suggests that more protein is available to bacteria than they can absorb, so the protein is lost (not used) in the environment. Where the scar cleavage rate is low, the amounts of energy and protein are in equilibrium, whereas the negative scar cleavage rate indicates a lack of protein in the scar, which means insufficient bacterial growth. This indicator in all test groups of samples is within the limit.

Transit protein means a non-cleavable protein, this is the amount of protein that does not break down in the scar, but passes through the scar when transiting for subsequent absorption in the small intestine. The normal indicator is more than 14.0%; therefore, all test samples for this indicator correspond to the norm.

Starch levels correlate with lactic acid levels. The highest starch index (preferably) was noted in the second test group, where the Silo Twice biopreservative was used, a slightly lower amount of starch was noted in the prototype, and in the group where self-preservation was used, the lowest value was noted.

Lactic acid is one of the acids formed during the preservation process. Sugar is a fuel for lactic acid bacteria that produce lactic acid. The standard is 3-15%. The largest amount of lactic acid is contained in the second experimental group, where the biopreservative Silo Twice was used (8.33%), a slightly smaller amount in the prototype (8.28%), and the lowest value was noted (5.30%) in the group where self-preservation was used.

Acetic acid is very important, as it helps to prevent the feed from overheating. Grass silage/haylage with a low proportion of acetic acid is very sensitive to overheating. The optimal acetic acid content is 1.0-2.0% or 10-20 g/kg CB. The largest amount of acetic acid is contained in the second experimental group, where the biopreservative Silo Twice was used (1.30%), a slightly larger amount in the prototype (1.40%), and the lowest value was noted (0.89%) in the group where self-preservation was used.

The content of exchange energy in a feeding product is the amount of energy available for metabolism. Digestible (digestible) energy is gross energy minus the energy released with manure. Digestible (digestible) energy adjusted for the amount of energy lost with the

release of urine and gas (mainly methane) is considered as the amount of exchange energy. This energy is available for use in the metabolic process. In the process of metabolism, most of the energy is spent on heat production. Heat-adjusted exchange energy is considered to be ultimately clean energy. Clean energy is used for growth, milk production and reproduction. The optimal level of exchange energy is > 10.5 MJ/kg CB [9]. The largest amount of exchanged and digested energy was noted in the second experimental group. The highest fodder value was noted in the second experimental group.

Calculating the cost of the diet with the introduction of haylage to each of the experimental groups, the following result was obtained: feeding milk cows for 60 days of haylage obtained when the biopreservative Silo Twice was introduced, provided a savings of 2250 rubles per cow.

4 Conclusion

After conducting a comparative assessment of the effect of preservatives on haylage quality, the following conclusions were made.

1. The extended preservation and bactericidal effect of the Silo Twice biopreservative complex of live bacteria and enzymes on the greasy plant mass led to the preservation of the raw material nutrients in the product and an increase in their digestibility compared to the self-preserving product.
2. The cost of the diet using the biopreservative Silo Twice turned out to be lower than the cost of the diet with the imported biopreservative.

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