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RESEARCH ARTICLE

Comparative Assessment of Forage Legume and Grass-legume Mixture Quality Ensiled with Biological and Chemical Preservatives

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Abstract The paper presents research and production studies evaluating the forage legume ensiling quality treated with biological and chemical additives. Experiments were conducted when ensiling alfalfa in tight-head barrels (30L capacity), treated with bioconservants. The conducted investigation revealed preservatives that significantly improve the acid composition and nutritional value of silages and haylages. Compared to the control sample, silos treated with Bioamid-3 and SilactPro starter cultures showed higher lactic acid content by 46.3 and 59.2%, respectively, and better intake. The most effective chemical preservatives were organic mixtures with propionic acid (PRES No. 3) and low formic acid content (PRES No. 2). Their application increased the lactic acid level by 17.8 and 22.6%, adjusted the silage acidity (pH) at the scale of 3.8–3.9, and improved the feed energy value (NE) by 1.7–2.5%. Despite the proven efficiency of preservatives, compliance with the ensiling technology remains a strict requirement for the guaranteed fodder conservation and quality.

Keywords Ensiling · Preservatives · Bioadditives · Microorganisms · Chemicals · Organic acids

Significance Statement The given research evaluates the efficiency of the most common bioadditives and chemical acids in haylage and silage preparation. Producers of ensiled fodder are giving a greater focus on nutrient quality preservation, better palatability, and more efficient conversion of feed nutrients into meat and milk. Safe chemical and biological preservatives contribute to improving the quality of milk products. Secondly, proven preservatives ensure proper maintenance of animal health, which prolongs their productivity.

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Introduction

Large livestock enterprises rely much on preservatives to store animal feed. In this regard, the search for high-quality additives becomes more relevant for many agricultural enterprises.

The selection of preservatives is mostly based on their typical use provided by the continually growing advertising and the products' relatively low price. As a result, farmers do not get the desired silage conservation and quality.

Preservatives retain nutrients and increase the intake of conserved succulent feed. As a rule, preservatives enlarge the lactic acid content during fermentation, reduce the pH value owing to the higher content of other organic acids, inhibit the undesirable microflora in the silage by more lactic acid bacteria. There are chemical preservatives based on organic acids and their salts and biological ones obtained mainly from lactic acid bacteria. New bioconservants contain one more effective bacteria and enzymes that provide better conservation under an insufficient level of readily available carbohydrates in plants [1, 2].

Preservatives do not make the initial crop better, but they can store their nutritional value. The market provides a wide range of additives, especially biological ones. The efficiency of most preservatives has not been studied and proved. Thus, the goal of the given research was to evaluate the efficiency of the most common bioadditives and chemical acids in haylage and silage preparation.

Material and Methods

Experiments were conducted when ensiling alfalfa in tight-head barrels (30L capacity). Trial samples were treated with the following bioconservants: Biotrof-111 (produced by "Biotrof," Saint Petersburg), Biosib ("Sibbiopharm," Berdsk), Bioamid-3 ("Bioamid," Saratov), SilactPro (private company "BTU Center," Ukraine). An untreated control sample of haylage undertook an unbiased assessment of the studied preservatives. Table 1 presents the qualitative composition of bioadditives, their concentration in 1 g (ml), and application rates.

All biopreparations were made based on lactic acid bacteria. Bioamid-3, Biosib, and SilactPro preservatives were prepared with propionic acid bacteria. SilactPro had the highest CFU level per 1 ml (g) with 3.3*10 billion cells per 1 g of dry preparation. Bioamid-3 consisted of 5×10^8 CFU/g of Lactococcus lactis subsp. lactis PNCIM B3123, not less than 3×10^8 CFU/g of Lactobacillus plantarum PNCIM B-10965 and Propionibacterium sp.PNCIM B-6085.

Bioadditives were inoculated according to the manufacturer's recommendations. They were mixed in well water at the rate of 4–5 L of water per 1 ton of plant raw materials and added into the ensiled crop with a manual dispenser.

The second set of experiments to assess the effect of chemical acids (formic, propionic, and sodium formate) in different combinations was carried out in ensiling the green mass of a pea-barley mixture. The trial feed was put in 50 L polymer barrels. The samples of chemical preservatives were developed by Izagri, LLC (Krasnodar region) based on previously recommended analog preparations produced by Kemira OYJ, Finland.

Preservative characteristics and chemical composition are shown in Table 2. The application rate was 3.0 L per ton for the haylage of 65% moisture or more and 4–5 L per ton for the silage of 45–65% moisture.

Chemical preparations were manually dispensed at the rate of 80–100 ml per barrel (25–28 kg of green mass, depending on the silage moisture). Then, the green vegetation treated with preservatives was tightly packed into plastic barrels. The ensiling process lasted 40 days until fermentation was completed, and the acidity was fully stabilized.

Average samples for laboratory analysis were taken from all layers of the ensiled forage in 1.5–2 kg. The volume of organic acids was measured by the Lepper-Flieg method (the Russian national standard GOST R 55,986–2014); the pH level was determined using an Anion 7000 potentiometer. Nutrients were found using the following methods: crude protein by an arbitration method according to GOST 13,496.4–93; crude fat by the Soxhlet extraction technique according to GOST 13,496.15–2016; crude fiber by the Weende method; neutral and acid-detergent fiber by Van Soest fiber analysis with Velp Fiwe 6 extractor, crude ash by drying in a muffle furnace at 550 °C.

The content of nitrogen-free extracts (NFE), net energy (NE), and net energy of lactation (NEL) for raw nutrients were calculated using the formulas:

$$\text{NFE}\ (\%) = \text{DM} - \text{CP} - \text{CF} - \text{FIB} - \text{ASH} \quad (1)$$

where NFE stands for nitrogen-free extract content, DM is dry matter, CP is crude protein, CF—crude fat, FIB—crude fiber, ASH—ash (given in %);

$$\text{NE} = 10.678 + 0.088\ \text{CP} - 0.332\ \text{CF} - 0.075\ \text{FIB} + 0.006\ \text{NFE} \quad (2)$$

where NE is the net energy (MJ/kg DM) for legume silages;

Table 1 Bioconservant characteristics and application rates

Indicator	Biotrof-111	Biosib	Bioamid-3	SilactPro
Preservative formulation	Liquid	Liquid	Dry	Dry
Bacteria composition	Pediococcus pentosaceus, Lactobacillus plantarum, Lactobacillus buchneri	Lactobacillus plantarum, Lactobacillus lactis P. freudenreichii	Lactococcus lactis, Lactobacillus plantarum, Propionibacterium sp.	Enterococcus faecium, Pediococcus pentosaceus, Lactobacillus plantarum, Propionibacterium subsp.
The CFU level per 1 ml (g)	$1.0*10^9$	$1.0*10^9$	$3.0–5,.0*10^8$	3.3×10^{10}
Application rate per 1 ton of green mass	65 ml	70–80 ml	1.5 g	6–8 g

Table 2 Characteristics and component composition of chemical preservatives

Indicator	PRES No. 1	PRES No. 2	PRES No. 3
Components	1) Formic acid; 2) sodium formate 3) water	1) Formic acid; 2) glycine; 3) sodium formate; 4) water	1) Formic acid; 2) propionic acid; 3) sodium formate; 4) water
Ratio of acids and salts, %	65:25:10	40:25:20:15	40:20:22:18

* PRES stands for a chemical preservative

$$NE = 10.365 + 0.026\,CP + 0.275\,CF - 0.176\,FIB + 0.0476\,NFE \quad (3)$$

where NE is the net energy MJ in 1 kg of dry matter for grain silages, legume-cereal mixtures with moisture of more than 68–70%.

$$NEL = 0.6 * (1 + 0.004 * (NE/GE * 100 - 57)) * NE \quad (4)$$

where GE is the feed gross energy (MJ/kg DM) calculated using the following formula:

$$GE = 0.0239 * CP + 0.0398 * CF + 0.0201 * FIB + 0.0175 * NFE \quad (5)$$

The feed intake was evaluated by means of visual observations. The amount of uneaten residue was determined by weighing.

The cost of biological and chemical preservatives was calculated at average market prices, based on suppliers' commercial offers. The data reliability was determined by the Student's t test for small samples.

Results and Discussion

The content of organic acids and their ratio is a direct indicator that characterizes microbiological processes and grass ensiling quality. The higher the concentration of lactic acid (more than 60%), the greater is the silage energy value.

The laboratory tests proved the absence of butyric acid in all silage samples, including the control one. Good sealing provided proper conservation of ensiled grasses without using preservatives for a short time. Rainy weather causes difficulties in maintaining the necessary moisture of ensiled crops making it above 68–72%. Excessive moisture results in the intense growth of saprophytic, butyric acid bacteria, and other undesirable microorganisms that spoil the feed.

Table 3 indicates that the bioferment application decreased the silage's pH and enlarged the organic acid concentration. However, the experimental preservatives did not have a significant difference in the pH. The silage treated with Biosib preservative was the only variant to have the level of lactic acid and total acidity equal to that of the control sample. The lactic acid content increased when applying dry preservatives. It was higher than the control by 1.46 times in the silage treated with Bioamid-3, by 1.34 times in the sample inoculated with Biotrof-111. Overall, the lactic acid ratio to total acids in all the silage samples exceeded the first quality class requirements with the ratio not less than 65% for perennial and annual plants stipulated by the Russian national standard GOST R 55,986–2014.

Higher synthesis of lactic acid in experimental silages directly affected the microbiological processes of acetic acid formation. The lowest acetate level was in the samples with Bioamid-3 and SilactPro preservatives. The amount of acetic acid in these feeds was 1.88 and 1.52 times lower than the control. It could result from the intense growth of homofermentative lactic acid bacteria introduced in high concentrations in these starter cultures. Besides, propionic acid bacteria inhibited undesirable microflora in the first stages of the silage fermentation. Many large research and production companies, such as the global concern Lallemand, have reasons to include propionic acid bacteria in preparations to ensile hard-to-preserve raw materials [3, 4].

Evaluating the nutritional value of the studied samples revealed that bioconservants had a versatile effect on nutrient retention. The application of dry Biomid-3 and SilactPro preservatives had a positive effect on the feed energy value. The silage treated with Biotrof-111 additive had the highest crude protein content, amounting to 14.04% in the dry matter against 13.63% in control.

The sample with Biomid-3 had moderately higher net energy than other experimental silages. However, the reliable difference in the content of nutrients and net energy between experimental and control silages is not found in Tables 4, 5.

There was a little distinction in the level of organic nutrients in the studied samples, while their raw ash index was lower than control. Thus, the ash content reduction ranged from 4.2 to 4.7% in the samples with Biotrof-111, Bioamid-3, and SilactPro bioadditives.

Table 3 The organic acid content in alfalfa ensiling after wilting with biological preservatives

Ensiling variants	pH	The silage acid content, % of dry matter			Total silage acids, %	Lactic acid ratio to total acids, %
		Lactic	Acetic	Butyric		
Control	4.64	6.48	3.17	Absent	9.6	67.2
Biotrof-111	4.53	8.70	2.69	Absent	11.39	68.9
Biosib	4.60	6.14	2.78	Absent	8.92	76.3
Bioamid-3	4.48	9.48	1.68	Absent	11.16	84.9
SilactPro	4.50	10.32	2.06	Absent	12.38	83.4

Table 4 Nutritional value of alfalfa silage after wilting with different biological preservatives

Treatment sample	Total moisture,%	Dry matter, %	Crude protein, %	Crude fiber, %	Crude fat, %	NFE, %	Raw ash, %	Energy value, MJ	
								NE	NEL
Control	64.5	35.5	4,84* 13.63	9,48* 26.7	1.21	16.90	3.02	*3,19** 8.98	5.21
BIOTROF-111	65.8	34.2	4.80 14.04	9.45 27.6	1.20	15.96	2.79	*3.06* 8.96	5.19
BIOSIB	66.6	33.4	4.59 13.74	9.22 27.3	1.18	15.62	2.79	*2.99* 8.95	5.19
Bioamid-3	64.7	35.3	4.87 13.80	9.46 26.8	1.23	16.87	2.87	*3.18* 9.01	5.23
SILACTPRO	64.3	35.7	4.94 13.84	9.57 26.8	1.27	16.95	2.90	*3.21* 8.99	5.22

Henceforward *- the nutrient value in the numerator is expressed in terms of natural moisture and dry matter in the denominator

Table 5 Content of neutral and acid-detergent fiber in alfalfa silage treated with biological additives

Research target	The fiber level in dry matter	
	Neutral detergent	Acid-detergent
Control	41.33	29.94
BIOTROF-111	42.09	31.15
BIOSIB	41.84	30.44
Bioamid-3	40.66	30.31
SILACTPRO	40.94	30.33

Table 6 Organic acid content when using chemical acids in the pea-barley mixture silage

Ensiling variants	pH	The silage acid content, % of dry matter			Total silage acids, %	Lactic acid ratio to total acids, %
		Lactic	Acetic	Butyric		
Control	3.70	9.03	12.96	Absent	21.99	41.1
PRES No. 1	3.82	10.09	9.74	Absent	19.83	50.9
PRES No. 2	3.89	12.01	6.84	Absent	18.85	63.7
PRES No. 3	3.86	11.06	7.73	Absent	18.79	58.9

Table 7 Nutritional value of pea-barley silage treated with chemical preservatives

Treatment variant	Total moisture,%	Dry matter, %	Crude protein, %	Crude fiber, %	Crude fat, %	NFE, %	Raw ash, %	Energy value, MJ	
								NE	NEL
Control	75.1	24.9	3.68 14.78	7.38 29.63	0.98	11.14	1.73	2.18 8.74	5.02
PRES No. 1	73.9	26.1	3.92 15.02	7.42 28.44	1.05	11.97	1.74	2.36 9.04	5.22
PRES No. 2	74.3	25.7	3.88 15.10	7.48 29.11	0.99	11.59	1.76	2.27 8.84	5.09
PRES No. 3	73.9	26.1	3.9 14.94	7.51 28.77	1.03	11.98	1.68	2.34 8.96	5.17

Indicators of neutral and acid-detergent fiber hydrolysis under the influence of bacterial enzymes did not differ significantly compared to the control silage.

The fiber fractions analysis showed that Bioamid-3 and SilactPro preservatives slightly decreased the neutral detergent fiber content by 0.67 and 0.39%, respectively. There were no significant differences in the amount of acid-detergent fiber, which indicated a low fermentation activity of microorganisms in the bioadditive.

A more objective indicator that characterizes the nutritional value and palatability of the studied silages is their intake. Observations of the nutritional activity of cows showed that preservative-treated silages were eaten first. Fodders prepared with Bioamid-3 and SilactPro were of the highest preference and consumed almost two times faster than the control silage. However, by the end of the observation, all the silage samples were almost entirely eaten.

The next experiment was carried out with the use of chemical organic acids. The best ensiling quality for the barley-pea plant mixture was obtained using chemical preservative (PRES) No. 2 and PRES No. 3 (Table 6).

Adding these acids led to higher lactic acid accumulation, even compared to chemical preservative No. 1, which contains 65% formic acid + 25% sodium formate. At the same time, the use of propionic acid in the composition of PRES No. 3 did not have a direct effect on the synthesis of lactic acid, as evidenced by its ratio to total acids equal to 58.9%, compared to 63.7% when using PRES No. 2 without propionic acid. There was a tendency to reduce the total acid content per unit of dry matter using chemical preservatives with a low content of formic acid and sodium formate. In this regard, due to the low content of acetic acid in silage samples with PRES No. 2 and PRES NO. 3, the silage pH was 0.15–0.2 units higher than control.

The low pH of the control silage being 3.7 is probably due to the high moisture content (73–75.5%) of the plant raw material that resulted in intensive fermentation and epiphytic microflora growth.

The better preservation effect of the organic acids provided a higher protein safety by 1.1–2.1% than in the control silage (Table 7). Although a reliable difference in crude protein content and fiber was not observed, there was a trend of higher crude protein and lower crude fiber.

The use of chemical acids resulted in a slight increase in nitrogen-free extracts (NFE) and dry matter by 0.4–0.84 and 0.8–1.2%, respectively, compared to the control sample. These changes affected the feed energy value, which contributed to higher net energy by 1.1–3.4% and net energy of lactation (NEL) by 1.4–4.0%.

Calculating the economic efficiency of the studied preservatives found that biological additives had a lower cost, in contrast to chemical organic acids. The costs of biological preservatives per 1 ton of silage ranged from 16.6 to 22 rubles. The costs of chemical additives were between 340 and 500 rubles. The lowest costs for preservatives were established when ensiling with liquid Biotrof-111 and Biosib bioadditives being on average 16.6 rubles per 1 ton of the silage mass. The most expensive was chemical preservative PRES No. 3 containing 20% propionic acid (produced by BASF).

More than 30 biological preservatives based on lactic acid bacteria are being used in Russia. Introducing LAB strains is considered one of the most effective ways to better fermentation processes in the feed. Numerous studies have proved that preservatives significantly change feed's chemical composition [2, 5–8].

Thus, Tao et al. [9, 10] treated wilted and chopped alfalfa (45% dry matter) with Lactobacillus buchneri 40,788 (4×10^5 CFU/g), L. buchneri 40,788 (4×10^5 CFU/g), and Pediococcus pentosaceus (1×10^5 CFU/g). The pH level of the silage treated with the third bacterium was the lowest. Samples of groups 1 and 3 had a higher acetic acid content in their composition than the control silage. The scholars found that inoculation of L. buchneri 40,788 and Pediococcus pentosaceus resulted in silage with the highest concentration of 1,2 propanediol after 180 days of ensiling. In other studies [11], the effects of Bacillus subtilis (BS) and Lactobacillus buchneri (LB) and their combinations on fermentation, aerobic stability, and microbial community of alfalfa silage were analyzed. The results showed that the BS-treated silage had a higher concentration of lactic acid and less proteolysis than the control sample.

Mu [8] conducted experiments treating grass mixtures with L. plantarum (L), cellulase (F), and their combinations (LF). The given additives increased the content of Lactobacillus and reduced the level of Weissella, Pediococcus, Lactococcus, lower pH while cutting the amount of acetic acid and ammonia nitrogen. Treated silages had a higher concentration of lactic acid compared to the control sample. LF silage had the highest lactic acid level and the lowest number of enterobacteria during 30 days of ensiling.

Another study identified the effects of the chemical vanillin (V), homofermentative Lactobacillus plantarum (LP), and heterofermentative Lactobacillus brevis (LB) on the distribution of the metabolome, microbial communities, viruses, and antibiotic resistance genes in silage with high moisture content in the maize core. LP and LB were found to improve lactic acid production, while V and LB inhibited protein degradation. There was a significant difference in the metabolic profiles of silage treated with additives and the control one [4, 12].

In the experiments of Ali et al. [5], starter cultures rapidly reduced the fodder's pH at the beginning of the ensiling process. Compared to corn and sorghum silages, clover silage had a higher concentration of lactic acid and lactic acid ratio to acetic acid during the entire study. At the end of ensiling, sorghum silage had a higher pH (> 4.50) and microbial cultures as Sphingomonas, Imageobacter, and Novosphingobium.

Silage with different amounts of soy and corn was treated with lactic acid, Lactobacillus plantarumand, and Lactobacillus buchneri. It was found to have a high lactic acid content and a low pH, no propionic and butyric acids. The nutritional value of silage increased, as evidenced by lower neutral and acid-detergent fibers and higher crude protein [6].

The studies [10] indicated that the use of Lactobacillus hilgardii (LH) and Lactobacillus plantarum (LP) increased the aerobic stability of silage. The longer LH and LP treatment did not significantly affect the pH level, the content of lactic, acetic, and propionic acids of silage. Simultaneously, populations of undesirable microorganisms such as Acetobacter pasteurianus, Paenibacillus amylolyticus, and yeast like Kazachstania humilis were inhibited. This improved fermentation quality, aerobic stability and reduced aerobic spoilage of the product. The control silage was compared with a sample treated with Lactobacillus parafarraginis DSM 32,962 to get the expected concentration. The results showed that the additive significantly improved the aerobic stability of the tested silage [13].

Leandro et al. [14] studied 19 strains of lactic acid bacteria. The research results showed that all strains had different antimicrobial activity, pH tolerance, and antibiotic susceptibility.

Silage was treated with Lactobacillus plantarum (SKP), Lactobacillus plantarum, and Lactobacillus paraplantarum. All silage was ensiled in plastic bottles and incubated at room temperature. Lactic acid (LA), ethanol, and propionic acid (PA) were significantly ($P < 0.05$) higher compared to the control. The amount of dry matter, crude protein, neutral detergent fiber, propionic acid, and acid-detergent fiber did not vary greatly among different treatments at the end of sensitization [15].

Researchers Ranjit et al. [16] ensiled whole-grain corn with the L. plantarum, L. buchneri, propionic acid at the rate of 0.1% of the fresh weight and untreated in 20-L barrels and found that silage treated with Lactobacillus buchneri (1×10^6 CFU/g) had a lower concentration of lactic acid compared to untreated silage. This silage had a higher content of acetic acid (3.60%) and a lower amount of yeast (2.01 log CFU/g) than other samples.

The lactate level was significantly higher in feeds treated with the enzyme or molasses compared to others ($P < 0.05$). The propionate-treated silage had higher acetic acid content ($P < 0.05$) [10].

Experiment results on the effect of biological starter cultures based on *Propionibacterium acidopropioni* F-5 on the ensiling quality conducted in the Kazakhstan scientific and analytical center "Biomedpreparat" agree with those received by the authors of the given paper. Thus, according to scientists Balapanov et al. [17], a characteristic feature of propionic acid bacteria is their ability to include sugar and lactic acid in their metabolism, abundant in acidified silage. The assimilation of lactic acid by propionic acid bacteria inhibits the acidification of the ensiled fodder caused by lactic acid bacteria.

The given research revealed that feed ensiled with propionate had the highest intake level compared to silage treated with formic acid. The control silage had a large amount of acetic acid and was least preferred by cows.

It should be noted that the pea-barley mixture silage ensiled with propionic acid resulted in the highest intake. Propionate is a substrate for carbohydrate metabolism and can inhibit pathogenic microflora during ensiling, increasing feed palatability.

Conclusion

Summarizing scientific and industrial research results, it is necessary to conclude that both biological and chemical preservatives provide better preservation of nutrients and higher energy value of silages due to more uniform and purposeful fermentation.

Applying Bioamid-3 and SilactPro additives resulted in the maximum preservation of nutrients due to a higher concentration of lactic acid by 46.3 and 59.2%, respectively, than in the control silage. Compared with Biotrof-111 and Biosib, the given preservatives had greater lactate concentration by 7.1–16.0%.

Ensiling with a chemical preservative based on propionic acid (PRES No. 3) provided better feed conservation proven by a relative increase in active acidity. There was an 8.0–17.8% increase in lactic acid than the control silage and the sample treated with chemical acids (PRES No. 1). Compared to other silages treated with chemical and biological preservatives, the conserved fodder ensiled with PRES No. 3 was better eaten by animals.

Though chemical acids outcompete biological preservatives, their application is limited at a high cost. While the latter cost 16.6–22 rubles, the cost of chemical organic preservatives reaches 500 rubles per 1 ton of green mass. In this regard, using biological additives with propionic acid bacteria is one of the rational solutions for making high-quality haylage and silage.

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Data availability Data will be available on request.

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