Improvement of technological methods of raw fodder grain preservation

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Abstract. Increasing the efficiency of the livestock industry is largely determined by the quality of the most valuable component of diets - forage grain. The properties of reliable preservation of its quality today possess the technology of preservation of raw fodder grain with chemical and biological preparations. Biopreparations with strains of lactic acid bacteria are the cheapest and most environmentally safe. Among chemical preparations powdered sulphur is cheap and environmentally safe one. The two-component mixture of \textit{L. casei} + \textit{L. lactis} had a certain advantage for preserving raw barley grain from three strains of homofermentative lactic acid bacteria: \textit{Lactobacillus species} (\textit{L. species}), \textit{Lactobacillus casei} (\textit{L. casei}) and \textit{Lactococcus lactis} (\textit{L. Lactis}). The mixture of \textit{L. casei} + \textit{L. lactis} in a ratio of 1:1 and a dose of 1 l/40 t was called Biosil NN. When processing grain with a moisture content of about 35 \% using Biosil NN, it was optimally acidified with lactic acid, which is dominant in the composition of fermentation products, and had a high energy value. Careful grain ramming enhanced the preservation effect of Biosil NN. A similar effect on the preserved grain was provided by powdered sulphur at a dose of 1 kg/t. The most preserving effect of preparation was at an average degree of compaction of the flattened grain with a moisture content of about 25 \%. According to the quality of fermentation of preserved grain the chemical preservative Promir had worse results than those stimulated with Biosil NN and powdered sulfur.

1. Introduction

The genetic potential of highly productive animals cannot be realized without feeding significant amounts of concentrated feed. The main type of concentrated fodder is fodder grain which is the most valuable; when feeding pigs and poultry, fodder grain is the predominant type of feed that provides growth of productivity and improvement of quality of livestock products. Under these conditions, the species and qualitative composition of grain concentrates is particularly important as market relations dictate lower production costs, and the cheapest feed is feed of own production [1]. In this regard, the most important problem of modern livestock breeding is the most complete preservation of feed quality before feeding in particular farm.

When choosing the technology of fodder preservation, the main attention is paid to the simplicity of its implementation and economic efficiency. In the recent past, xeroanabiosis was the main method of preserving the fodder grain – ensuring product safety as a result of partial or complete dehydration [2]. In this method of preservation the fodder grain was stored in a dry state at a moisture content below the critical values. For the main grain crops (oats, barley) they were in the range of 14.5-15.5\%
[3]. In favorable conditions for harvesting grain, the dry state, which guarantees reliable preservation of fodder grain for a long time, was achieved when it was threshed in the phase of full ripeness. In unfavourable weather conditions, grain moisture content (15%) was brought to standard value for dry matter by artificial drying, which is related to energy consumption. The higher the moisture content of the grain, the more energy was required to dry it. The sharp increase in energy prices in the market conditions made this method of bringing the fodder grain moisture content to standard (especially at its values of 25% and higher) economically impractical [4].

At the same time it is impossible to preserve raw fodder grain without taking urgent measures, especially in rainy weather during the harvest period. It begins to self-heat in 2-3 days, and then to germinate, mold and deteriorate with the loss of fodder and technical qualities. Therefore, since the mid-1960s in the countries of Western Europe and the mid-1970s in the former USSR, active work has been carried out to find opportunities to replace drying by other methods of effective fodder grain preservation [5, 6].

As a result, in recent years the use of grain harvesting technology for forage in dough stage with following preservation and storage under anaerobic conditions became the solution to this problem [7]. A number of advantages over traditional methods of preserving grain contributed to its rapid and wide spread. Such advantages are: 1) earlier (for 2-3 weeks) harvesting and less dependence on weather conditions; 2) increasing (by 10-15%) nutrient yield from the unit of sowing area; 3) reduction of losses from grain shedding and from being eaten by birds; 4) saving significant amounts of energy and facilities; 5) possibility of growing later and more productive varieties of grain; 6) improving conditions for sub-cover crops development; 7) elimination of processing and use of green, small and destroyed grain for food; 8) replacement of grain crushing as a method of preparation for feeding with less energy-intensive flattening; 9) increasing the efficiency of feeding own grain to farm animals; 10) reducing the need for purchased concentrated feed; 11) using straw as a better quality feed, etc. This was also facilitated by spreading of roller flatteners in farms, which simplify the preparation of wet grain for feeding, and plastic sleeves, which eliminated the shortage of sealed storage facilities [8-12]. At the same time the grain moisture content could be increased to 40%, which only simplified and reduced the cost of technology [13, 14]. It should be noted that all advantages inherent in harvesting grain with the high moisture content are preserved. In general, due to the reduction of material expenses the prime cost of grain harvested using this technology was 10-50% lower than the dried one [15].

Use of anaerobic storage technology of raw fodder grain is inextricably connected with its treatment with chemical preparations or additives improving preservation conditions [4, 16]. Preservation of grain during chemical preservation is ensured by bactericidal and fungicidal properties of preparations used for these purposes. Due to specifics of feed product fungicidal properties are especially important, so the reliable preservation of the nutritional value of feed grain (according to a number of authors [17, 18]) is provided by processing it with organic acids, among which propionic acid should occupy a significant place. Technological and engineering solution of chemical preservation is not difficult [19]. However, interest in it is gradually decreasing due to the high cost of organic acids and environmental problems [20]. Therefore, scientists are constantly searching for new chemicals that would be able to successfully cope with the task, but would be characterized by lower cost and safety of use. In our opinion powdered sulphur can be attributed to such preparations. Thus sulphur is an inert substance in this form and its transition to chemically active form without influence of any external factors is impossible, sulphur is environmentally safe [21, 22]. Its conversion to reactive forms (sulphur-containing gases, sulphuric and sulphurous acids, sulphuric anhydride) takes place either under the influence of thiobacteria (serobacteria) or when it is dissolved in methyl and ethyl esters of lactic and acetic acids, as well as in aldehydes formed during lactic acid fermentation [23]. Moreover, the second way is most plausible when preserving feed using lactic acid fermentation.

The choice in favor of biological means to increase the reliability of fodder grain preservation is justified by the fact that the use of biopreparations is ecologically safer and economically more profitable than the use of the most common chemical preservatives for this purpose, although the latter
often show a more reliable result of its preservation. For this reason, the use of such preparations (microbiological inoculants) is becoming more widespread in the world, and scientists are conducting research to improve existing and create new effective biological preservatives and additives [24, 25].

Since the preservation of raw grain in anaerobic conditions, as well as during silage, is based on lactic acid fermentation, research on the development of preparations based on homofermentative lactic acid bacteria has become quite natural. The low concentration of these bacteria in epiphytic microflora of grain was an additional argument for the creation of such preparations. It was assumed that the introduction of such bacteria into raw grain would contribute to the rational transfer of simple sugars mainly in lactic acid, which preserves the feed by quickly acidifying it (reducing the pH). Positive results of preservation of fodder grain with high moisture content by such preparations consisted in reduction of food nutritional value losses during storage, improvement of its quality, increase of eatability and digestibility [26-32].

Despite the similarity of airtight storage technology of high moisture content grain with silage due to specificity of the preserved material, it undoubtedly has its own features, knowledge of which will allow more rational use of preservative additives, increasing their efficiency. In this regard, the purpose of our research was to determine the effectiveness of processing raw fodder grain with lactic acid ferments and powdered sulphur as well as to assess the impact of a compaction degree and use of chemical and biological preparations on the results of preservation.

2. Materials and methods

The strains of Lactobacillus species (L. species), Lactobacillus casei (L. casei) and Lactococcus lactis (L. lactis) were used in laboratory experiments while conducting research to determine the effectiveness of lactic acid bacteria ferments for preserving raw fodder grains. Bacterial cultures were used both separately and in various combinations with each other. Raw flattened barley grain was treated with biological preparations. The dose of biopreparations was 1l/40t. The grain was stored in airtight glass containers with volume of 1 dm³. The grain was carefully compacted to a condition excluding the presence of voids in grain mass.

In experiment to determine the optimal composition of mixture of Lactobacillus casei (L. casei) and Lactococcus lactis (L. lactis), raw flattened barley grains were treated with binary biopreparation composition of 1:3, 1:1 and 3:1. The application dose was 1l/40 t.

Studying the powdered sulphur effect on fermentation quality in the preservation of raw flattened barley grains, the preparation was used at a dose of 1.0; 1.5 and 3.0 kg/t.

In experiment conducted using generally accepted methods in the laboratory [33], we studied the effect of chemical and biological preparations in comparison with control storage without additives and positive control (chemical preservative Promir) on acidification of a raw barley grain. The grain was stored in a whole (moisture content about 35%) and flattened (moisture content about 25%) form without compaction, with medium and strong ramming. Biological preparation Biosil NN, powdered sulphur and chemical preservative Promir were used to treat the grain. Biopreparation Biosil NN consists of homofermentative lactic acid bacteria Lactobacillus caseii and Lactococcus lactic. Application dose is 1l/40t. Powdered sulphur is an amorphous yellow powder containing at least 99.5% sulphur, no more than 0.2% moisture content and 0.05% ash (SU 1099937). Application dose is 1 kg/t. The preparation Promir is manufactured by the Swedish concern PerstorpGroup. The preparation contains 43-48% formic acid, 18-23% propionic acid and 4-8% ammonium formate. It is added to fodder grain with high moisture content at dose of 3 l/t.

The results of research were processed by the method of variation statistics on PC Pentium IV using a standard set of statistical programs.

3. Results

To study the effect of lactic acid bacteria on fermentation quality, experiment on preservation of raw barley grains using L. species, L. casei and L. lactis separately and their possible combinations was made. The application dose recommended by the manufacturer was 1l/40t of silage mass. The effect of
biopreparations on organoleptic characteristics of preserved grain was in many ways the same. Grain colour and its structure are examples of such characteristics. The smell of fermentation products was felt in grain with preparations consisting of separate cultures of lactic acid bacteria, as well as with triple mixture and mixture of *L. casei* + *L. lactis*. A mixture of lactic acid bacteria of species *Lactobacillus* gave the silage a smell of bread dough.

Analyzing the composition of fermentation acids, it was found that most organic acids, including lactic acid, were in grain preserved with mixtures of *L. casei* with *L. lactis* and *L. species* with *L. casei*, less in *L. casei*.

The preserved raw flattened grain contained approximately the same amount of dry matter. The grain with *L. lactis* and with *L. casei* + *L. lactis* was the only exception, because it contained a little more dry matter than grain without additives.

The base of fermentation acids in the preserved grain was lactic acid. However, used lactic acid fermentations did not increase its proportions compared to the control (grain without additives). The equal level of this characteristic was only in grain with *L. casei* + *L. lactis*. Optimum degree of grain acidification was observed almost in all versions of its preservation. The accumulation of ammonia nitrogen in grain was insignificant (table 1).

**Table 1.** Degree of acidification, a part of lactic acid and a level of ammonia nitrogen in the grain.

| Variant of preservation | Dry matter, % | Ammonia nitrogen, g/kg RV | pH | Part of lactic acid, % from acids sum |
|------------------------|---------------|---------------------------|----|--------------------------------------|
| without additives      | 65,08 ± 0,61  | 0,051 ± 0,015             | 4,13 ± 0,02 | 86,9                                |
| with *L. species*      | 65,59 ± 0,34  | 0,122 ± 0,044             | 4,12 ± 0,02 | 72,0                                |
| with *L. casei*        | 65,17 ± 0,64  | 0,184 ± 0,009a            | 4,23 ± 0,03 | 85,9                                |
| with *L. lactis*       | 66,77 ± 0,52c | 0,070 ± 0,005             | 4,12 ± 0,02 | 75,3                                |
| with *L. species* + *L. casei* | 65,42 ± 0,38 | 0,148 ± 0,013a            | 4,05 ± 0,00 | 79,0                                |
| with *L. species* + *L. lactis* | 66,15 ± 0,15 | 0,135 ± 0,023b            | 4,20 ± 0,03 | 83,4                                |
| with *L. casei* + *L. lactis* | 66,76 ± 0,56 | 0,104 ± 0,017c            | 4,30 ± 0,00 | 86,2                                |
| with *L. species* + *L. casei* + *L. lactis* | 65,72 ± 0,16 | 0,127 ± 0,005c            | 4,10 ± 0,10 | 84,9                                |

\[ a - P<0,01 \]
\[ b - P<0,05 \]
\[ c - P<0,10 \]

A comprehensive evaluation of a bacterial preparations effect on the preservation process revealed the superiority of a two-component mixture based on lactic acid bacteria strains – *Lactococcus lactis* and *Lactobacillus casei*.

Determination of the optimal ratio of bacterial components in a mixture for raw flattened grain preservation in comparative analysis of the use of biological and chemical preparations showed that the quality of fermentation was high in most variants. The grain preserved with biopreparations, as well as treated with classical chemical preservative, had an optimal pH that was significantly (P < 0.05) lower than in grain without additives (table 2).

Grain with different ratio of bacterial preparations had optimal acidity (pH 4.1), contained 7.6 to 13.5 g/kg of RV organic acids, from which 90% or more was lactic acid. At the same time lactic acid ferment with equal ratio of components *L. lactis* and *L. casei* provided maximum acid formation, highest output and mass part of lactic acid (more than 90 %) in a total sum of fermentation acids during grain preservation. In general, chemical treated grain did not differ from grain preserved by biological additives. The proportion of butyric acid in fermentation products was insignificant and did not exceed 2.2% from organic acids sum. The equal content of components in lactic acid-based
biopreparation maintained the high nutritional value of the treated grain. It shows the highest concentration of exchange energy in comparison with other preservation options (table 2).

Table 2. Results of raw flattened grain preservation with different compositions of biopreparation L. casei + L. lactis

| Characteristics   | without additives | with L. casei + L. lactis in ratio: | Promir |
|-------------------|-------------------|------------------------------------|--------|
|                   |                   | 1:3                                | 1:1    | 3:1    |        |
| Dry matter, %     | 68,8±0,4          | 67,4±0,4                           | 68,2±0,4| 68,0±0,4| 68,3±0,5|
| pH                | 4,30±0,05         | 4,07±0,02b                         | 4,07±0,02b| 4,07±0,02b| 4,08±0,02b|
| Organic acids, g/kg RV: |                     |                                    |        |        |        |
| total             | 13,6±2,8          | 7,6±0,95                           | 13,5±1,8| 10,6±1,4| 11,7±2,0|
| including: lactic | 11,6±2,1          | 6,8±0,9f                           | 12,4±1,8| 9,6±1,2 | 10,3±1,9|
| acetic            | 2,0±0,6           | 0,7±0,2f                           | 0,8±0,04| 0,8±0,4 | 1,2±0,3 |
| butyric           | 0,03±0,03         | 0,07±0,03                          | 0,3±0,04| 0,23±0,18| 0,2±0,1 |
| Part of lactic acid, % from acids sum | 85,1               | 89,9                               | 91,9   | 90,3   | 88,0   |
| Exchange energy, mJ/kg RV |              | 12,56±0,01                        | 12,50±0,02| 12,57±0,01| 12,49±0,00| 12,51±0,06|

b - P≤0,05  
c - P≤0,10

According to comprehensive evaluation of single-strain and mixed biological preparations brought in preserved grain, the best results in content of dry matter, accumulation of organic acids (including lactic, as well as its dominance among fermentation acids) were provided by preparation from mixture of lactic acid bacteria L. lactis + L. casei at dose of 1 liter per 40 tones of preserved grain. This preparation, called Biosil NN, proved to be the most effective at preserving raw fodder grain in anaerobic storage conditions.

In the following experiment we defined the influence of powdered sulphur use on results of preservation of raw flattened grain. Grain processing with lactic acid ferment Biosil NN was a positive control (table 3).

Table 3. Fermentation quality and nutritiveness of the raw barley grain in powdered sulphur processing

| Characteristics                  | without additives | Biosil NN | powdered sulphur, kg/t |
|----------------------------------|-------------------|-----------|------------------------|
|                                  |                   |           | 1                      | 1,5               | 3                   |
| Dry matter, %                    | 66,6±0,3          | 66,7±0,2  | 66,6±0,2               | 66,6±0,2          | 67,3±0,1            |
| pH                               | 4,50±0,05         | 4,22±0,02 | 4,30±0,00              | 4,28±0,02         | 4,23±0,02           |
| Organic acids, g/kg RV:          |                   |           |                        |                    |                     |
| lactic                           | 19,3±0,3          | 7,4±0,3   | 17,8±0,8               | 10,1±0,2          | 13,7±0,6            |
| butyric                          | 0                 | 0,1±0,06  | 0                      | 0                 | 0                   |
| Part of lactic acid from fermentation acids sum, % | 82,8               | 80,4      | 86,8                   | 84,9              | 83,5                |
| Contained in dry matter:         |                   |           |                        |                    |                     |
| raw protein, %                   | 13,45±0,20        | 13,54±0,42| 13,12±0,17             | 13,15±0,20        | 13,43±0,37          |
| exchange energy, mJ/kg           | 12,42±0,08        | 12,43±0,10| 12,62±0,07             | 12,63±0,02        | 12,52±0,10          |

The fermentation quality in most variants of grain preservation was high. Application of biological and chemical preparations normalized the conditions of grain acidification. It formed a sufficient
amount of organic acids to acidify the feed to standard pH, and in all experimental groups of grain, acidification was significantly lower than in a control variant and comparable to its processing with biopreparation Biosil NN.

The best result in all characteristics of fermentation quality was grain, in which 3 kg of sulphur was added per 1 ton. However, the values of grain preserved with the same preparation, but added at dose of 1 kg/t, were not very different from it. Moreover, in a composition of fermentation acids in the grain of this preservation variant, the mass part of lactic acid and the concentration of exchange energy in dry matter (i.e., its energy nutritiousness) were higher. It should also be noted that there is no butyric acid in sulphur powdered grain. There were no significant differences in protein content between variants for preserving raw barley grains. In terms of concentration of exchange energy in dry matter, the sulphur powdered grain exceeded the grain of control variant (without additives) and with Biosil NN (table 3).

The effectiveness of preparations used to improve the results of preservation is influenced by the conditions of their use. Such conditions include the physical form of preserved material, its moisture content, degree of compaction, reliability of sealing, temperature regime of storage, etc. In the following experiment biological and chemical preparations were used to treat whole (moisture content about 35%) and flattened (moisture content about 25%) raw grain stored without forced compaction (self- compaction or self-sealing), with medium and strong ramming.

Preservation of raw fodder grain in airtight conditions is related to degree of its acidification by organic acids formed during spontaneous or organized fermentation. The dependence of acidity of the whole grain on the degree of compaction without additives, with biological and chemical preparations is shown in table 4.

On average for all compaction variants, better acidification of the whole grain was observed when it was preserved with biopreparations Biosil NN. Slightly worse results were obtained from the use of powdered sulphur and the chemical preservative Promir for this purpose. However the acidity of grain of these test variants was within 4.38-4.35 units pH, i.e. the difference with Biosil NN grain was not significant (didn’t exceed 0.1 units pH or 2.3%). In relation to grain stored without additives, the difference between grain with preparations was 0.19-0.26 units of pH or was 4.2-5.7 % higher, indicating improvement in preservation results using specified biological additive and chemical preservatives (table 4).

| Degree of compaction | Grain without additives | with Biosil NN | with sulphur | with Promir | Average |
|----------------------|-------------------------|---------------|-------------|------------|---------|
| Self-sealing         | 4.55±0.05               | 4.35±0.05     | 4.42±0.04   | 4.50±0.05  | 4.46    |
| Medium               | 4.70±0.10               | 4.27±0.01 b   | 4.38±0.03   | 4.28±0.04 b| 4.41    |
| Strong               | 4.37±0.02               | 4.22±0.01 a   | 4.33±0.02   | 4.26±0.06  | 4.30    |
| **Average**          | **4.54**                | **4.28**      | **4.38**    | **4.35**   | **4.39** |

a - P≤0.01
b - P≤0.05

Increasing the ramming degree on acidification of whole raw barley grain had the most beneficial effect. Stored with high compaction grain was significantly better acidified in all preservation variants. This situation is clearly illustrated in figure 1, which shows that the trends in acidity of raw grain were exactly the same depending on compaction of its storage treated with different preparations.
From minimum values in strongly compacted grain (taken as 100%), at average intensity of ramming the pH values increased slightly and reached maximum at self-sealing. This trend was most contrasted with grain treated by chemical preservative Promir, the deviation of pH values at self-sealing significantly exceeded the average values of the experiment. Changes of this characteristic looked slightly different (depending on the degree of compaction) only in grain without additives. In this variant the lowest value was also obtained with strong compaction, but its maximum value was noted in medium rammed grain, and the discrepancy with minimum characteristic was the highest (7.6%) (figure 1).

In general, for all variants of grain preservation the effect of compaction degree on its acidification looked like: strong → medium → self-sealing.

As it has already been noted, grain with Biosil NN was better acidified at any storage compaction (table 4). In relation to grain in control variant (without additives) it had significantly (P≤0.05-0.01) lower pH value at the end of storage period. It was followed by the results of grain acidification with powdered sulphur without ramming and with Promir at medium and strong compaction. At any degree of compaction, grain without additives was worse acidified than in other preserving variants. This is clearly confirmed by figure 2, which shows the absence of pH deviations during preservation of whole raw grain with Biosil NN. This means that all minimum values of this characteristic (taken as 100%) are in this variant of experiment or that at any degree of compaction grain with this preparation had the lowest pH, i.e. was better acidified.

Maximum deviations from minimum pH values (from 3.6 to 10.1%) are noted in grain without additives, which mean its weakest acidification at any storage compaction. The intermediate value was the deviations of grain acidity during its preservation with powdered sulphur and Promir: 1.6-2.6 and 0.2-3.4%. At the same time deviations were less than average characteristic in experiment at medium and strongly rammed grain with chemical preservative Promir and at self-sealed grain with powdered sulphur. In terms of acidification degree grain with chemical preparation Promir was little inferior to grain with Biosil NN, especially at medium compaction of its storage (figure 2).
Figure 2. Dependence of whole grain acidity (pH) from type of additives and storage compaction.

At all values of compaction of whole raw grain for storage, according to the level of influence on the degree of acidification, the preparations used in the experiment were arranged in the following decreasing sequence: biopreparations Biosil NN → chemical preservative Promir → powdered sulphur. Grain without additives in equal storage conditions was acidified much worse than grain with additives.

According to the same scheme flattened grain was put into storage. Its only difference was the lower moisture content (about 25%). The results of the research showed that the less moist flattened grain was significantly worse acidified than rawer whole in all variants of preservation (tables 4-5).

The highest degree of acidification of such grain was provided by powdered sulphur. The value pH grain in this variant decreased by 0.9 units pH or for 14.4% on average and was significantly lower (P≤0.01) when stored without additives. The degree of compaction played a role. Thus, if the difference in pH between grain without additives and powdered sulphur was 0.38 units (6.1%) in self-sealing, 0.8 units (12.7%) in strong compaction, then it would be 1.5 units (24.2%) in medium compaction. Thus the highest acidifying capacity of powdered sulphur was at medium storage compaction of grain (table 5).

| Degree of compaction | without additives | with Biosil NN | with sulphur | with Promir | Average |
|----------------------|-------------------|----------------|--------------|-------------|---------|
| Self-sealing         | 6.23±0.02         | 6.22±0.06      | 5.85±0.08    | 6.23±0.02   | 6.13    |
| Medium               | 6.28±0.07         | 6.20±0.05      | 4.78±0.12    | 6.93±0.06   | 6.05    |
| Strong               | 6.20±0.00         | 6.25±0.03      | 5.40±0.17    | 5.97±0.03   | 5.96    |
| Average              | 6.24              | 6.22           | 5.34         | 6.38        | 6.04    |

* - P≤0.01

The preservative Promir had less significant impact on this characteristic. When grain treated with this preparation was self-compacted, it was acidified in the same way as the grain without additives. At medium storage compaction of grain, the reaction of its environment was close to neutral and pH was higher (0.65 units or 9.4%; P < 0.01) than in grain without additives. More dense grain storage with Promir helped to improve the grain acidification in comparison with storing it without additives (pH value decreased by 0.23 units or 3.7 %; P<0.01), but powdered sulphur effect on this
characteristic was stronger. Biopreparation Biosil NN did not improve acidification of preserving flattened grain in comparison with the results of spontaneous fermentation (grain without additives) (table 5).

Increasing the ramming degree in experiment variants had a weak effect on acidification of flattened raw barley grain. This situation is illustrated in figure 3. It shows that the trends in acidity changes of raw flattened grain depending on the compaction of its storage during processing with Biosil NN and spontaneous fermentation (without additives) were weakly pointed and did not differ much from the average values in experiment.

![Figure 3](image-url)  
**Figure 3.** Acidifying activity of preparations depending on the compaction of flattened raw grain for storage.

The compaction effect of flattened grain for storage had the opposite effect on acidifying activity of chemical preparations Promir and powdered sulphur. Acidifying activity of sulphur was maximum at medium degree of compaction, of Promir – at strong compaction; minimum acidifying activity was in sulphur at self-sealing and in Promir at medium compaction degree of grain (figure 3).

As it had already been noted, the best acidification of flattened grain was provided by processing it with powdered sulphur, which confirms the absence of deviations in acidity indicators of grain at this variant in figure 4.

The most insignificant deviations of acidity indicators from minimum pH values in grain with sulphur in other variants of grain preservation (barely exceeding 6% limit) were noted when it was stored without forced compaction. Grain with Promir had the smallest deviation from the minimum with strong compaction. In general, these deviations were close to average characteristics for experiment variants. Finally, the highest level of deviation (up to 45%) was at medium compaction of grain storage. This was the result of the most powerful acidifying action of powdered sulphur at this degree of compaction. It was particularly contrasting for grain with Promir (figure 4).
Figure 4. Relative acidifying capacity of preservative additives at different degrees of compaction of raw flattened grain.

4. Discussion
Studying of effective using of lactic acid bacteria in the form of monocultures or mixtures to improve fermentation quality in preservation of raw fodder grain allowed us to establish that the best results were achieved from the use of two-component mixture L. lactis+ L. casei. The superiority of polycOMPONENT Lactic acid ferments compared to monocomponent ones is marked out by other researchers [34]. According to their opinion multi-bacterial preparations combining lactic acid bacteria of initial stage of silage (starting fermentation at high values of pH) and bacteria of final stage of silage fodder ripening (powerful producers of lactic acid, completing preservation) realize the maximum rate of acidification. They explain less effectiveness of mono-bacteria by their low activity at initial stage of silage, which makes it possible for undesirable microflora to ferment sugars and destroy protein.

In our experiment, grain preserved with biological products had an optimal pH value for silage fodder (4.0–4.2), which provides stability of its composition during long-term storage. This result was obtained by stimulating of acid formation with bicomponent mixture, including synthesis of lactic acid, which was dominant among fermentation acids. The relationship of these characteristics is confirmed by highly reliable degree of direct dependence (r = 0.90; P < 0.01) of total organic acids sum (formed in preserved grain) from lactic acid content. At the same time there is limited accumulation of ammonia nitrogen in grain, which indirectly indicates that the protein is well preserved during preservation and storage.

When determining the optimal composition of biological preparation, it turned out that acidification conditions were normalized in grain with each composition of preparation, as well as with chemical preservative Promir. However, the composition of biopreparation with equal ratio of components in grain preservation was characterized by more active acid formation, mainly due to increase in formation of lactic acid (which dominated in composition of fermentation acids) and fermentation itself was close to homofermentative lactic acid. Such composition of fermentation products provides fodder stability during anaerobic storage. Similar result of using L. lactis + L. casei mixture with equal ratio of components was obtained due to the fact that bacterium L. lactis was a producer of antibiotic nizin. This antibiotic actively counteracts the functioning of a range of pathogen bacteria, such as clostridium. The antimicrobial activity of nizin is also expressed in suppression of many gram-positive bacteria (staphylococcus, streptococcus, etc.), a number of acid-resistant bacteria, including spore-forming ones. It is due to the high reactivity of unsaturated amino acid residues that interact with groups of SH ferments [35]. Limiting of wrong types of fermentation and stimulating
homofermentative type of lactic acid fermentation contributed to the most economical consumption of nutrients for fermentation, which increased the energy saturation of grain treated with such biopreparation. As a result, this grain contained the maximum amount of exchange energy in dry matter. Therefore, in future, such composition of biological preparation (called Biosil NN) was used for grain preserving.

The bactericidal properties of sulphur derivatives acquired during activating of powdered preparation oppress the development of undesirable microflora without suppressing the vital activity of lactic acid bacteria [22, 36]. Different doses of application during preservation of raw fodder grain, as well as application of biopreparation Biosil NN (unlike storage of grain without additives), normalized fodder acidification, where lactic acid formed the basis of fermentation acids. Since lactic acid was the main fermentation product in raw barley grain preserved by powdered sulphur, a direct dependence on the amount of total acid formation was quite expected, which was confirmed in the course of correlation analysis (r=0.997; P<0.01).

The dominance of lactic acid among the organic acids formed during fermentation provided the level of acidification necessary for successful preservation of raw fodder grain, which is indicated by presence of pH value feedback with mass part of lactic acid in fermentation acids sum (r = -0.74; P<0.10). In other words, increase of this part was accompanied by decrease of pH value of grain, i.e. an improvement of its acidification.

Rapid acidification of grain to the optimal level inhibits butyric acid fermentation, as it was confirmed by results of powdered sulphur use for raw grain preservation, in which butyric acid could not be detected in biochemical analysis.

With proper fermentation of feed, the predominant amount of acetic acid is a by-product of lactic acid fermentation. In this direction of fermentation, the volume of its formation, even in heterofermentative lactic acid fermentation, is very limited (usually not exceeding 30% of total amount of fermentation acids). The amount of acetic acid outside these ranges can be associated with both aerobic acetic acid fermentation (with delays in sealing feed) and with other types of fermentation for which acetic acid is one of the main products. In this case mass acidification may be slowed down, which leads to development of rotten processes in it with release of substances with alkaline properties that increase the buffering of preserved raw materials. Therefore, a direct dependence of pH value of grain from acetic acid level (found in course of correlation analysis) was quite natural. The more acetic acid is produced in feed, the weaker it is acidified (r = 0.85; P <0.10).

A significant (P < 0.10-0.01) reduction of raw fiber content in raw fodder grain preserved with powdered sulphur (probably due to its hydrolysis with sulphur derivatives) has a positive value; thus the nutritional value of feed is increased as a result. As a result of such changes in dry matter of grain, there was an increase of concentration of exchange energy in comparison with other variants of its preservation.

It should be noted that of all tested doses of powdered sulphur, application of the lowest dose (1 kg/t) allowed to regulate the course of fermentation much better. So we used this exact dose for further research.

One of the most important characteristics that should be considered as the main preserving factor is the actual acidity (pH value) of grain stored in airtight conditions. The better grain is acidified, the more reliable it is protected from the influence of harmful and wrong microflora, active reproduction of which leads to its decomposition and spoilage. For this reason, comparative tests of preserving capacity of biological and chemical preparations at different compaction of raw fodder grain for anaerobic storage were carried out using this characteristic.

The results of research showed that application of each preservative additive had improved the acidification of raw grain stored in anaerobic conditions, i.e. it caused the manifestation of acidifying abilities of grain microflora or inoculated lactic acid microflora.

Since the moisture content of whole grain was about 35%, the rules of its preservation largely coincided with the technology of silage. For this reason, the use of lactic acid ferment Biosil NN has proved to be the most effective in preserving such grain among all test variants at any degree of
compaction. Grain treated with this preparation was better acidified. Chemical preservative Promir comparable to Biosil NN had an effect on acidification at medium and strong compaction of stored grain. It should be noted that in this case acidification was achieved, perhaps not only due to the formed fermentation acids, but also due to the organic acids included in preparation. Although powdered sulfur also stimulated improvement in grain acidification in comparison to storing it without additives, it was worse than other preparations.

Increasing grain storage compaction also contributed to improvement its acidification in all test variants. However, if the grain with powdered sulphur at each stage of transition from self-sealing to medium and from medium to strong storage compaction, the value of pH reduction was approximately the same, then the main improvement in acidification occurred at the first stage of increasing the compaction degree (i.e. at medium compaction) using Biosil NN and especially Promir.

Preservation with the same preparations of flattened grain with moisture content about 25% gave slightly different results of its acidification. The use of powdered sulphur showed the best acidifying capacity both on average for all compaction variants and separately in each of them. Its most acidifying effect was observed at average grain compaction for storage, at which optimal conditions were created for its manifestation. The acidifying effect of biopreparation was not different from the results of spontaneous fermentation, i.e. self-preservation of grain. In other words, lactic acid bacteria included in preparation showed no activity at such moisture content of preserved grain. Chemical preservative Promir affected differently on acidification at various grain compaction for storage. The pH value of grain treated with Promir did not differ from grain characteristics in control variant (without additives) in self-sealing, i.e. such conditions had a weak effect on acidification. In medium compaction grain with chemical preservative was acidified worse than control variant, probably due to suppression of microbiological processes by organic acids included in this preparation. Improvement of grain acidification with this preparation was observed only with strong compaction, conceding only to grain with powdered sulphur.

Average trends of influence of compaction degree of flattened grain on its acidification were reduced to its improvement as ramming increased, but the difference in pH values at various compactions was insignificant. Grain acidity increased during transition from self-sealing to medium compaction of storage with Biosil NN and powdered sulphur, in grain without additives and with chemical preservative it decreased. The result was contrary during transition from medium to strong degree of compaction; moreover the range of deviations was significant using sulphur and Promir. In other words, the most favorable conditions for enhancing the acidifying capacities of Biosil NN and powdered sulphur were formed at medium compaction of grain for storage, the acidifying capacities of Promir and preservation without additives – at strong compaction.

5. Conclusion

Lactic acid bacteria used to treat raw fodder grain have a positive effect on fermentation quality. Among the single-strain lactic acid bacteria and theirs mixtures, two-component composition consisting of bacteria from initial (L. lactis) and final (L. casei) fermentation stages in preservation of the raw barley grain had the best effect on this characteristic in flattened barley grain. It provided optimization of feed acidification by enhancing acid formation, primarily through synthesis of lactic acid, which dominates in composition of fermentation acids. The optimal composition of lactic acid ferment based on these bacteria was preparation with equal ratio of components, which ensured the best fermentation quality during preservation of raw fodder grain. The use of powdered sulphur in doses from 1 to 3 kg/t for preservation of raw grain had also a positive effect on fermentation quality. The most useful dose was 1 kg/t, which (equally with other doses of preparation) normalized the feed acidification, excluded the formation of butyric acid and increased its energy value. The maximum formation of lactic acid (which was the base of fermentation acids) was observed at this dose of application. According to the acidification degree of raw fodder grain, the preservative effect of Biosil NN (L. lactis + L. casei), powdered sulphur and chemical preservative Promir was clearly shown in comparison with spontaneous fermentation at its storing without additives. The whole grain (with
moisture content about 35%) was changed most contrasting by preservation with Biosil NN, flattened grain (with moisture content about 25%) was changed most contrasting by preservation with powdered sulphur. The highest degree of acidification of whole grain was observed at strong compaction using biopreparation, the highest degree of acidification of flattened grain was observed at medium compaction using powdered sulphur. On average of experiment strengthening of grain compaction increased the degree of its acidification: greater at preserving of whole grain, less at preserving flattened grain.

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