UDC 636.085.52: 631.563.6

## INOCULANT EFFICIENCY IN CORN SILAGE PREPARATION

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**Abstract**. *Specialists of the Institute of Food Resources of the National Academy of Sciences (Kyiv, Ukraine) have developed a new inoculant, which includes highly active strains of lactobacilli, namely: Lactobacillus plantarum, L. brevis, L. rhamnosus, L. buchneri. All strains were isolated from natural sources. Studies have shown that the use of an inoculant based on lactic acid bacteria in corn silage allows obtaining first-class silage according to the following indicators: dry matter content, pH, lactic acid content, lignin and specific proportion of ammonia nitrogen in total nitrogen. In experimental samples of silage 120 days after the start of the process, the content of dry matter is higher by 8%, crude protein by 0.5%. Silage analysis showed a lactic to acetic acid ratio of 2.62 for the control and 1.23 for the experimental samples, and no butyric acid was detected. Silage from corn, prepared without microbial additives, was not acidified during ensiling to standard pH values, instead, the introduction of lactic acid inoculant made it possible to obtain silage with an optimal pH value of up to 3.9. The addition of inoculant resulted in an increase in ammonia nitrogen to 5.17% of total nitrogen compared to the control of 0.93%.*
**Keywords**: *cattle, silage, corn silage, inoculant, lactic acid bacteria, feed efficiency.*

## ЖУГУГУ СИЛООСУН ДАЯРДОО ҮЧҮН ИННОКУЛЯНТТЫ ПАЙДАЛАНУУНУН НАТЫЙЖАЛУУЛУГУ

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**Аннотация**. *Украинанын Улуттук илимдер академиясынын тамак-аш ресурстары институтунун адистери (Киев, Украина) жаңы эмдөөчү каражатты иштеп чыгышты, анын курамына лактобациллалардын жогорку активдүү штаммдары, атап айтканда: Lactobacillus plantarum, L. brevis, L. rhamnosus, L. buchneri кирет. Бардык штаммдар табигый булактардан бөлүнүп алынган. Изилдөөлөр жүгөрүнү силостоодо сүт кислотасынын бактерияларынын негизиндеги сепкичти колдонуу төмөнкү көрсөткүчтөр боюнча биринчи класстагы силос алууга мүмкүнчүлүк түзөрүн көрсөттү: катуу заттардын курамы, pH, сүт кислотасынын курамы, лигнин жана аммиак азотунун жалпы азоттогу салыштырма үлүшү. Силостун эксперименталдык үлгүлөрүндө 120 күн өткөндөн кийин кургак зат 8%га, чийки белок 0,5%ке көп болот. Силостун анализи сүт жана уксус кислотасынын катышы контролдук үчүн 2,62, эксперимент үчүн 1,23 болгон, май кислотасы табылган эмес. Микробдук кошумчаларсыз даярдалган жугеру силосу силостоодо стандарттуу pH маанисине чейин кычкылдаштырылган эмес, ал эми суттук ферментти киргизуу pH 3,9га чейинки оптималдуу силосту алууга мумкундук*

*берди. Эмдөөчү каражатты кошуу аммиак азотунун 0,93% контролго салыштырмалуу жалпы азоттун 5,17% га көбөйүшүнө алып келди.*
**Өзөктүү сөздөр**: *мал, силос, жүгөрү силосу, инокулянт, сүт кислотасы бактериялары, тоюттун эффективдүүлүгү.*

## ЭФФЕКТИВНОСТЬ ИСПОЛЬЗОВАНИЯ ИННОКУЛЯНТА ДЛЯ ПРИГОТОВЛЕНИЯ КУКУРУЗНОГО СИЛОСА

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**Аннотация**. *Специалистами Института продовольственных ресурсов НААН (г. Киев, Украина) разработан новый инокулянт, в состав которого входят высокоактивные штаммы лактобактерий, а именно: Lactobacillus plantarum, L. brevis, L. rhamnosus, L. buchneri. Все штаммы были выделены из природных источников. Исследования показали, что применение инокулянта на основе молочнокислых бактерий в силосовании кукурузы позволяет получить силос, соответствующий первому классу по следующим показателям: содержание сухих веществ, pH, содержание молочной кислоты, лигнина и удельная доля аммиачного азота в общем азоте. В опытных образцах силоса через 120 дней после закладки содержание сухого вещества больше на 8%, сырого протеина на 0,5%. Анализ силоса показал, что соотношение молочной и уксусной кислоты – 2,62 для контроля и 1,23 для опыта, масляной кислоты не обнаружено. Силос из кукурузы, приготовленный без микробных добавок, не подкислялся в процессе силосования до стандартных значений pH, а внесение молочнокислой закваски позволило получить силос с оптимальным значением pH до 3,9. Добавление инокулянта привело к увеличению аммиачного азота до 5,17% от общего азота по сравнению с контролем 0,93%.*
**Ключевые слова**: *крупный рогатый скот, силос, кукурузный силос, инокулянт, молочнокислые бактерии, эффективность корма.*

### 1. Introduction

Different biological technologies are among the critical innovative technologies that determine the progress of the economy of to-day, they are becoming quite an actual phenomenon. In Ukraine, the share of products produced using biotechnological methods, in particular microbiological technologies, is unreasonably small, which contradicts current trends, since modern biotechnologies most fully correspond to the basics of an innovative model for the development of the agro-food sector of the economy, the growth of its profitability, and a gradual increase in the level of economic intensity of the whole processing industry [1,2].

Developments in the field of biotechnology, in particular microbiology, belong to the core areas of activity of the Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine (IFR NAAS) and are the main area of competence of the Biotechnology Department, which develops and successfully introduces into production innovative direct introduction starter cultures for the production of fermented milk products of increased biological activity based on the use of probiotic strains of bifidobacteria and lactic acid bacteria, effective starter preparations for the production of hard rennet cheeses etc. Taking into account the extensive experience of biotechnologists of the IFR NAAS in the targeted selection of microorganisms, as well as the need for domestic animal husbandry in modern effective microbiological preparations, a number of products were developed for the effective preparation of silage and feeding calves [3].

For the production of nutritious and animal-safe silage, modern technologies widely use biological products that allow regulation of microbiological processes and guarantee the functional activity and high quality of this feed, which is enriched with biologically active metabolites of microflora, is easily digestible and environmentally friendly. Various chemical and microbiological preservatives are used to ensure the efficiency of ensiling and the proper biological and zootechnological properties of the resulting feed and to minimize the loss of nutrients during the technological process. The most common biological preservatives are based on lactic acid bacteria [4,5]. They dominate spontaneous microflora, and lactic acid is a valuable precursor of nutrients in metabolic processes in the animal body and a preservative agent, which inhibits undesirable biochemical processes in the silage mass, in particular, the breakdown of protein compounds. Only due to lactic acid fermentation, a rapid decrease in pH occurs, which suppresses the vital activity of other microorganisms, while long-chain carbohydrates (fiber, starch), proteins and vitamins are not degraded. Another important function of lactic acid bacteria in the composition of the silage is the inhibition or termination of the vital activity of undesirable microflora – putrefactive and butyric bacteria, yeast, mold, etc.

The above considerations are also confirmed in the works of numerous scholars. Thus, an American specialist in the field of fodder production, J. Begg, states [6] the positive effect of lactic acid in the preparation of silage, which primarily consists in increasing the yield of dry mass of fodder. In the case when silage fermentation proceeds uncontrollably, a significant amount of carbon dioxide is released into the environment, causing loss of solids and leading to compaction of the silage mass. Part of the silage mass is inevitably lost during the fermentation therefore the visible compaction of the silage can be caused not only by its shrinkage, but also by fermentation actions. A rationally selected microbiological preparation can reduce the loss of dry matter from 15% to 12-13%. Positive, in this sense, is the use of lactic acid bacteria, which convert vegetable sugars into lactic acid very quickly and with the least energy loss, which does not exceed 3-5%. According to F. Gross [7], any bacterial agent of this purpose should contain viable lactic acid bacteria in the amount of 105 - 106 bacteria per gram of plant material, because in this case they can dominate over spontaneous microflora.

The use of lactic acid fermentation in the preservation of silage has a number of significant advantages. Lactic acid is a valuable nutrient precursor in metabolic processes in animals and, as a preservative, inhibits other decomposition processes in the silage mass, in particular the breakdown of protein compounds. It is lactic fermentation that provides a rapid decrease in pH, which neutralizes the activity of all other microorganisms (with the exception of yeast), while long-chain carbohydrates (fiber, starch), proteins and vitamins are not decomposed [3,5].

Based on the inhibition of microflora, the process of spoilage of silage by the products of natural fermentation of some representatives of the epiphytic microflora of plant materials causes a sharp decrease in the pH of silage. Such representatives are lactobacilli. Their enzyme systems decompose water-soluble carbohydrates mainly to such organic acids as lactic, acetic, propionic, as a result of which an environment with a pH of 4.0-4.2 is formed in the ensiled mass, which is lower than the level of spoilage microbiota survival [8, 9].

The process is usually divided into four phases, each of which is characterized by certain features. The first phase is aerobic. Oxygen still present between the plant particles and the pH value is 6.0-6.5.

Accordingly, under such conditions, plant respiration occurs, combined with a high activity of aerobic and facultative aerobic microorganisms. This phase is characterized by a mixed composition of microflora. The second phase is fermentation lasting from a few days to a few weeks after the silsilage becomes anaerobic after being sealed. After all the oxygen in the silage has been consumed, the aerobic microorganisms stop growing. In addition, during this phase, the predominant microflora is represented by lactic acid bacteria, which, by producing lactic and other acids, lower the pH level to 3.8-5.0. The third phase is stable. Without air intake, all parameters change little. At a low pH value, the activity of lactic acid bacteria also decreases. The fourth phase is the opening of the silage. During this phase, aerobic microorganisms are reactivated by exposure to air. Therefore, a number of studies are devoted to methods for ensuring the aerobic stability of silage [10].

It has been established that silage spoilage is caused by epiphytic microorganisms competing with lactic acid bacteria for carbohydrates. Putrefactive and thermophilic bacteria (Erwinia herbicola, Escherichia coli, Hafnia alvei and Serratia fonticola) have the greatest influence on the decrease in the nutritional value of silage. They carry out decarboxylation and deamination of amino acids in silage, NO3 reduction, with the accumulation of ammonia and biogenic amines [11].

Mold in silage is represented by some species of the genera Fusarium and Alternaria; Aspergillus flavus and Aspergillus parasiticus; forms that are endophytic symbionts in grasses or cereals, such as Claviceps and Neotyphodium species; and forms that develop in silage without controlling its biochemical parameters, such as Penicillium roqueforti and Penicillium paneum, Aspergillus fumigatus, Monascus ruber, Byssochlamys nivea, Rhizopus nigricans and Chrysonilia sitophila [12,13]. The latter group is most often encountered during storage of silage or usually occurs as a result of its aerobic spoilage [13].

A widespread problem with silage on many dairy farms is their low aerobic stability, which is the result of slow filling rates or insufficient pack compaction during ensiling. In addition, any poor control on the surface of the silage exposes the silage mass to prolonged exposure to air. Based on this, yeasts (for example, Candida, Endomycopsis, Hansenula, Pichia degrade lactic acid into carbon dioxide and water, producing an excess amount of heat (silage warming process), which leads to loss of nutrients [14]). Lactic acid degradation also raises the pH of the silage to a level that allows opportunistic bacteria (eg Bacillus) and molds (eg Aspergillus, Fusarium and Penicillium) to grow and further reduce silage quality.

The use of lactobacilli in ensiling has 3 strategies for use for corn silage, which are manifested in the use of the following groups of silage starters [15]: based on obligate-homofermentative obligate-heterofermentative lactic acid bacteria, combined inoculum-homofermentative or facultative-heterofermentative lactic acid bacteria.

Facultative heterofermentative lactic acid bacteria differ from obligate homofermentative ones in that they have phosphoketolase. This enzyme allows them to decompose pentoses, producing primarily lactic and acetic acids. General facultative-heteroenzymatic strains include mainly representatives of lactobacilli Lactobacillus plantarum, Lactobacillus casei. Silage treated with one or more of these bacteria often have low pH, acetic, butyric and ammonia levels, and a higher lactic acid content, which in general reveals a better recovery of solids compared to untreated silage [16]. Inoculation also increases the amount of yeast in the feed and reduces their aerobic stability [17]. Since yeasts are usually the initiators of aerobic spoilage, the reduced concentration of acetic acid from inoculation with homofermentative lactic acid bacteria contributes to a faster growth rate of the yeast and thus to a decrease in the aerobic stability of the silage.

Specialists used heterofermentative inoculants somewhat later than homofermentative ones. Hetero-fermented corn silages violate traditional quality measures, for example, they have a markedly reduced ratio of lactic acid to acetic acid. However, the available data for grain silage do not show a negative effect of heteroenzymatic silage on the productivity of cows [18]. The most promising representatives of heterofermentative lactic acid bacteria are Lactobacillus buchneri, Lactobacillus diolivorans, Lactobacillus brevis, Lactobacillus kefiri, Lactobacillus hilgardii.

The development of starter cultures that combine facultative and obligate heterofermentative lactic acid bacteria aims to achieve the advantages of both types of inoculants in one product. Facultative-heterofermentative will control the period of early active fermentation, thereby reducing the populations of enterobacteria, clostridia and other microorganisms, therefore, reducing proteolysis and fermentation losses of dry matter. During this period of active fermentation, the pH is expected to drop faster and to a lower value than in untreated silage. It is the duty of heterofermentative lactic acid bacteria (in most cases L. buchneri) to then slowly convert lactic acid into acetic acid (and also propionic acid) during the period of active silage fermentation, lower the pH value and improve the aerobic stability of the silage [19].

Researchers D. Koms and P. Hoffman [20] draw attention to the fact that the use of homofermentative bacterial cultures sometimes causes a decrease in the stability of silage upon contact with oxygen compared to silage obtained without the use of these cultures, since in the presence of oxygen homofermentative acid is produced and actively used by some types of yeast and mold. In this sense, the addition of 5x105 CFU of L. buchneri preparation per 1 g of fresh green mass to the silage can help. In this case, aerobic stability becomes more pronounced for materials such as wet corn and silage from it, as well as silage from alfalfa and fine grained cereals. It is believed that the positive effect of L. buchneri is associated with the formation of acetic acid, as well as with its inhibitory effect on the growth of some types of yeast, which, upon contact with oxygen, leads to heating of the mass. The use of L. buchneri results in lower levels of yeast and mold, in particular after the silage has been exposed to oxygen.

Silage made with the use of microbial starter cultures-inoculants is not inferior to the quality of products obtained with the use of chemical preservatives, it properly provides the feed needs of animals. The ensiling process meets the requirements of labor protection and environmental protection, and at the same time is the most cost-effective. According to the approximate calculations of J. Begg [6], the use of a microbiological preparation costs 1.5-2.5 USD per 1 ton of silage. Even if dry matter losses are reduced by only 3%, reducing the compaction of the silage will already have an economic effect. In addition, the shelf life of silage will increase and its nutritional properties will improve.

**2. Materials and methods**

The biochemical indicators of the quality of corn silage were determined using a new inoculant developed by specialists from the Institute of Food Resources of the National Academy of Sciences of Ukraine. The sais inoculant includes highly active strains of lactobacilli, namely: Lactobacillus plantarum, L. brevis, L. rhamnosus, L. buchneri. All strains were isolated from natural sources.

The number of bacteria in 1 g is not less than 1.0x1011 CFU of bacteria, the need for application per 1 ton of silage is 1 g of the preparation.

Technological studies on ensiling were carried out in accordance with the requirements of the "Methodology for conducting experiments on fodder production and animal feeding" [21].

The process began with laying the silage in a concrete trench. At the same time, experimental (using inoculant) and control (according to traditional technology) silage lots were prepared. The raw material was corn with a moisture content of 70-75%. After preparing, the silage was compacted for proper density and covered with foil. After three months of storage, the quality indicators of silage, its chemical composition and aerobic stability were determined. The finished silage was analyzed for the content of organic acids, acidity, solids matter (SM) retention, crude protein (CP) content, ammonia, lignin, neutral- and acid-detergent fiber (NDF and ADF, respectively) were determined. These indicators characterize the quality of fermentation. The smell of silage was determined by rubbing a small portion of it between the fingers. Silage analysis was carried out according to generally accepted methods [22].

For the study, two samples of silage were selected, one of which was experimental, that is, with the addition of an inoculant, and the second was a control, without inoculation.

**3. Research results**

Among other crops, corn is often ensiled because it has a high sugar content with a relatively low content of buffer substances (crude protein and ash) [23,24]. The nutritional value of corn lies in the fact that a significant part of its starch in the rumen is not split. If starch is well absorbed by ruminants (90-100%), then fiber is absorbed by no more than 50%. The composition of the cell fiber includes lignin (practically not digestible in the animal body), cellulose and hemicellulose (well absorbed, digestibility ranges from 20 to 90%). In the process of plant maturation, the amount of lignin in its cells increases, which means that the digestibility and nutritional value of the feed decrease. Taking into account the above considerations, scientists and specialists of the IFR NAAS created an inoculant for ensiling corn, legumes and other typical types of plant raw materials. The preparation contains highly active strains of lactic acid bacteria Lactobacillus plantarum, L. brevis, L. rhamnosus, L. buchneri. That is, the composition of the preparation includes effective producers of lactic acid, such as L. plantarum and L. rhamnosus, and the obligate heteroformative species L. brevis L. buchneri, which together provide a targeted synergistic effect of the preparation, guarantee compliance with optimal fermentation parameters with simultaneous effective suppression of the development of putrefactive microorganisms and mold due to the active development of highly active microflora in the mass. The use of this bacterial composition guarantees a reduction in the loss of dry matter and nitrogen, the silage is rich in vitamins and organic acids, and its high biological value is ensured by the active development of lactic acid bacteria. These microorganisms contribute to normal digestion and improve feed conversion. The industrial use of the developed preparation for corn ensiling has shown that it contributes to the ensiling process, providing a higher quality feed with a pleasant smell and acceptable color. Analysis of the sensorial characteristics of the silage is presented in table 1.

According to the results presented in Table 1, the silage fermented on the basis of a biological product is characterized by a very good quality of sensorial parameters, due to

**Table 1.** Sensorial characteristics of silage

| Parameter | Experiment | Control |
|---|---|---|
| Color | Mostly green, sometimes of green-yellow hew | Brown-light-green color |
| Odor | Pleasant fruit odor of high quality silage | Unpleasant smell |
| Taste | Pleasant sour taste | Sour taste |
| Structure | The crushed pieces have a clear structure and are easily separated from each other, without signs of mucus. | The structure is preserved, the first signs of mold have appeared |

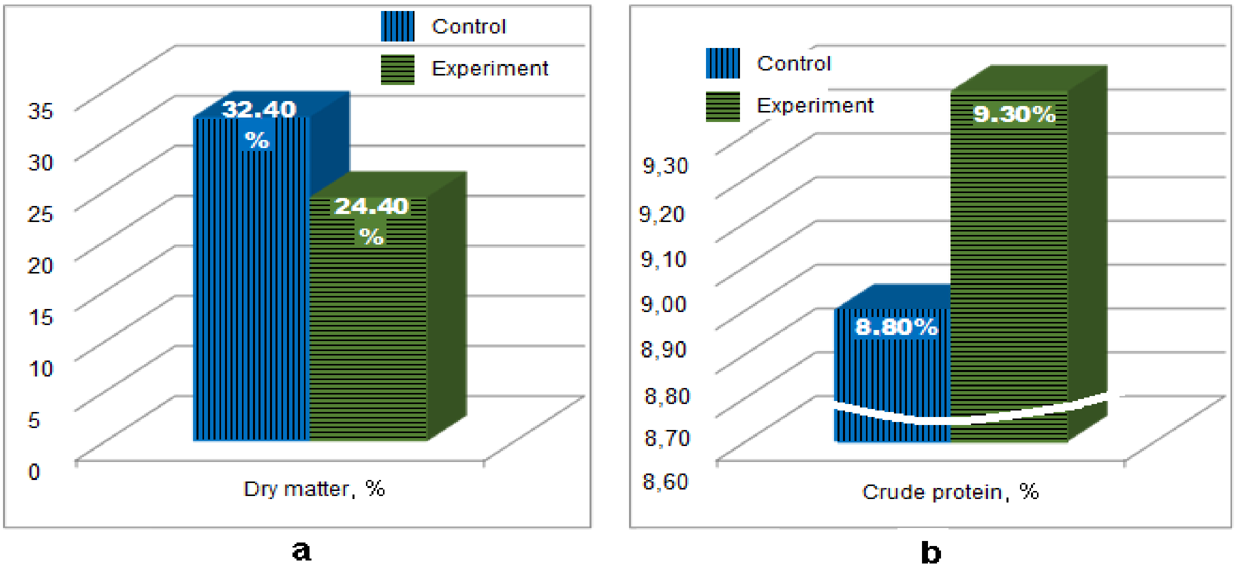


**Figure 1.** Content, % (by mass) of: a) dry matter; b) crude protein.

which a high nutritional attractiveness for animals is ensured.

Therefore, the test sample had a green-yellow color, while the dark brown color predominated in the control sample. The benign test sample had a fruity odor and the control sample had an acetic acid odor. The samples almost did not differ in consistency, but the control sample had the first signs of mold.

The results of the analysis of samples by chemical composition are shown in Fig. 1

As shown in Fig. 1, the silage obtained with the use of the preparation has a lower dry matter content after 120 days of fermentation - 24.4% compared to the control silage, for which this value was 32.4%. Such losses are explained by the conversion of water-soluble carbohydrates in silage by cultures of the drug into lactic and acetic acids. According to DSTU 4782:2007 [25], in terms of dry matter content up to 25%, silage belongs to the first class of silage and 20% to the second class.

According to the content of crude protein, the experimental sample was characterized by the preservation of this indicator at the level of 9.3%, compared with the value of the control - 8.8%. Accordingly, the value lies within the normal range for the first class of silage - 10% and the second - 9%. This protein retention is especially important for the nutritional value of protein-poor feeds such as corn.

In accordance with Figure 2 it can be seen that the silage treated with the preparation has a higher content of lactic acid

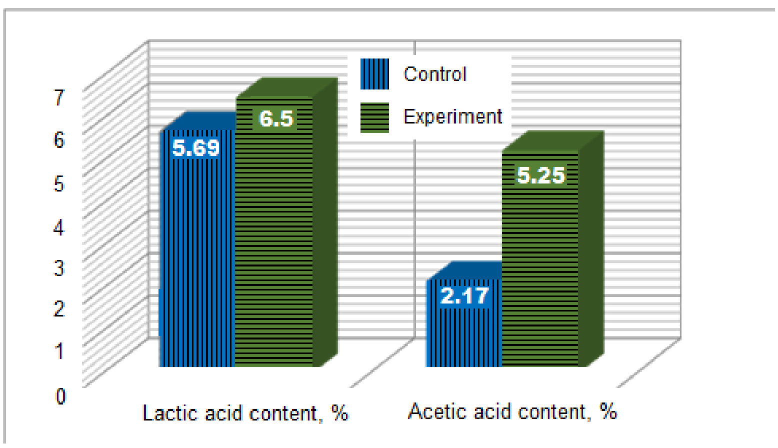


**Figure 2.** Content, % by weight, of lactic and acetic acid in the control and experimental silage.

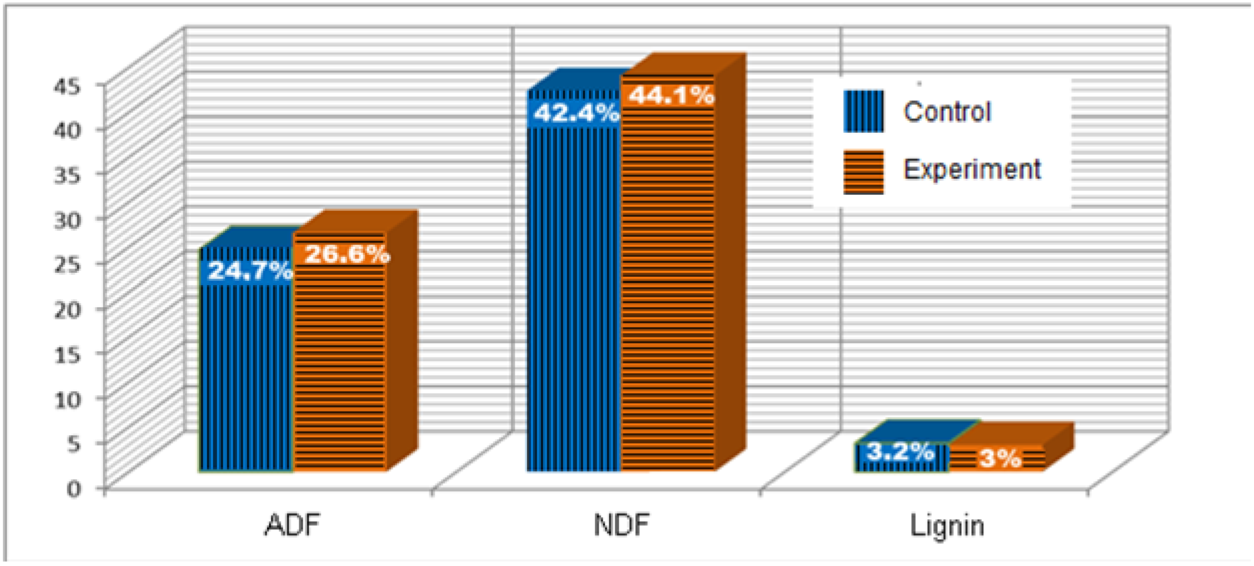


**Figure 3.** Parameters of silage digestibility, %, by weight, where ADF and NDF are acid and neutral detergent fiber

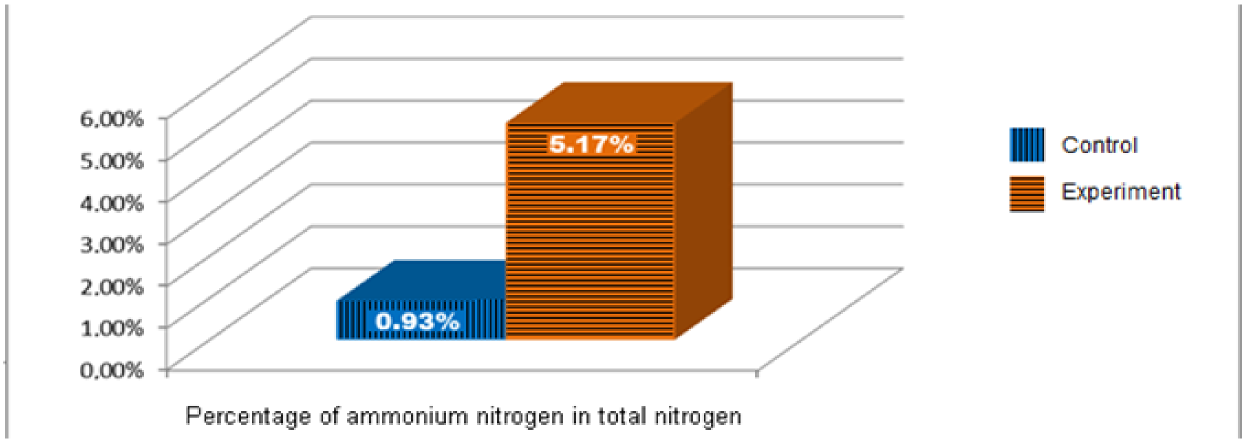


**Figure 4.** Silage digestibility parameter: share, % by mass, of ammonia nitrogen in total nitrogen

(6.5% compared to 5.69% of the control) and, accordingly, a lower pH value (3.8 to 3.84 in the control), although only by 0.04 units. This value fully complies with the requirements for pH in silage with a dry matter content of 15-25%, which is in the range of 3.8-4.3 units.

Acetic acid plays a special role in the preservation of such silage (5.25% to 2.17 in the control), because it provides a reduction in the content of yeast and mold during the last phase of ensiling. However, in accordance with DSTU 4782:2007 [25], the content of acetic acid should be less than 3.5%, which indicates an increased acetogenic activity of the microbiota during the fermentation process. Thus, despite the best preservation of silage, this content of acetic acid has a negative impact on the nutritional value for animals.

Further analysis was aimed at studying the indicators of feed digestibility (Figure 3). The value of these parameters showed that the treatment with the drug in terms of the content of acidic and neutral detergent fiber in relation to the total dry matter content increased by 1.9% for ADF and 1.7% for NDF. However, with respect to the lignin content, this value improved compared to the control, namely, it decreased by 0.2%.

Figure 4 shows the content of ammonium nitrogen in relation to total nitrogen. As can be seen from the diagram, the addition of the drug resulted in an increase in ammonium nitrogen to 5.17% of total nitrogen, compared to the control of 0.93%. In accordance with established state standards, this figure in the silage of the first class should not exceed 10%.

**4. Discussion**

Feed fiber is not only cellulose, but also the lignin associated with it. There are different forms of fiber - NDF, soluble in neutral detergent, and ADF, soluble in acidic detergent. The digestibility of the first is about 50%. It should be noted that the higher the proportion of ADF, the lower the digestibility of the feed and the energy concentration. Lignin is the heaviest fraction of NDF for digestion. A higher lignin content corresponds to a lower fiber quality. In the process of maturation, the content of lignin in plant cells increases, the digestibility and nutritional value of the feed decrease. NDF is an indicator of the quality (digestibility

and nutritional value) of plant foods. According to the content of NDF, the potential effectiveness of the diet (rumen fullness) of animals is also assessed, taking into account the capacity of the rumen. It is recommended to ensure the content of NDF in the diet is not less than 28%. The optimal value of NDF, at which the best digestibility of fiber in the rumen is observed, is 37% of the dry matter mass. In the experimental sample, the value of NDF was 35.2%.

Therefore, the essence of ensiling with the use of biological products lies in the intensive enrichment of the silage mass with lactic acid bacteria. This contributes to the rapid accumulation of lactic acid and the suppression of putrefactive microflora in the first days of ensiling, prevents the development of butyric fermentation, which is observed during ensiling of protein-rich and insufficiently compacted raw materials. In general, the use of bacterial preservatives for ensiling makes it possible to obtain a better and safer product compared to the use of chemical preservatives, as evidenced by the moderate intensity of fermentation processes, the favorable ratio of organic acids, and less bacterial contamination by foreign microorganisms.

**5. Conclusion**

The results showed that the use of an inoculant based on lactic acid bacteria in corn ensiling makes it possible to obtain first-class silage in accordance with DSTU 4782:2007 [25] in terms of the following indicators: solids content, pH, content of lactic acid, lignin, and the specific fraction of ammonia nitrogen in total nitrogen.

So, in the experimental samples of silage 120 days after laying, the content of dry matter is more by 8%, crude protein by 0.5%. The analysis of the silage showed that the ratio of lactic and acetic acid was 2.62 for the control and 1.23 for the experiment; butyric acid was not detected.

Tests using the inoculant showed that silage prepared without corn microbial additives did not acidify during ensiling to standard pH values. The introduction of lactic acid starter made it possible to obtain silage with an optimal pH value of up to 3.9. It was shown that the addition of the inoculant led to an increase in ammonium nitrogen to 5.17% of total nitrogen compared to the control of 0.93%. It was revealed that the overall digestibility is less compared to the control.

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