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THE EFFECT OF BIOLOGICAL ADDITIVES ON AEROBIC STABILITY OF SILAGES FROM MEADOW SWARD INTENDED FOR FEEDING AND ENERGY PRODUCTION

Summary

The paper is aimed at summarising long-term studies on the effectiveness of biological ensilage stimulators containing homo- and hetero-fermentative cultures of lactic acid bacteria used in the conservation of meadow sward intended for fodder or energy production. Study material consisted of silages from wilted meadow sward ensiled in big cylinder bales wrapped in a plastic film with the addition of four bacterial preparations made in the Institute of Agricultural and Food Biotechnology: Lactosil, Lactacel-L, Lactosil Plus and Lactosil Biogaz. Control silages were made of the same plant material without additives. Studies included the assessment of aerobic stability of silages and changes in chemical composition and microbiological profile during silage samples storage in aerobic conditions. Dry mass, pH of fresh silage and the amount of lactic, acetic and butyric acids were determined in fresh silage samples and in samples after 12 days of aerobic incubation. The number of yeasts, moulds and lactic acid bacteria was determined as well. The efficiency of applied biological additives varied during the process of ensiling meadow sward, mainly due to their different composition. Lactacel-L appeared most efficient in improving fodder quality from among all applied additives. Lactosil was the best in prolonging aerobic stability. The addition of Lactosil Biogaz helped improve aerobic stability and decreased the content of lactic acid in raw material intended for energy production.
Key words*: aerobic stability, yeasts, moulds, lactic acid bacteria*

WPŁYW DODATKÓW BIOLOGICZNYCH NA STABILNOŚĆ TLENOWĄ KISZONEK Z RUNI ŁĄKOWEJ Z PRZEZNACZENIEM NA CELE PASZOWE I ENERGETYCZNE

Streszczenie

Celem pracy było syntetyczne podsumowanie wieloletnich badań dotyczących efektywności biologicznych stymulatorów kiszonkarskich, zawierających homo- i heterofermentatywne kultury bakterii fermentacji mlekowej, stosowanych w procesie konserwacji runi łąkowej z przeznaczeniem na cele paszowe i energetyczne. Materiał badawczy stanowiły kiszonki z podsuszonej runi łąkowej zakiszanej w dużych belach cylindrycznych owijanych folią z dodatkiem czterech preparatów bakteryjnych opracowanych w IBPRS: Lactosil, Lactacel-L, Lactosil Plus i Lactosil Biogaz. Materiał porównawczy stanowiły sianokiszonki sporządzone z tego samego materiału roślinnego bez dodatku (kiszonki kontrolne). Badania obejmowały ocenę stabilności tlenowej kiszonek oraz rozmiar zmiany w składzie chemicznym i profilu mikrobiologicznym w trakcie przechowywania kiszonek w warunkach tlenowych. W świeżych próbkach kiszonek oraz w próbkach po 12 dniach inkubacji tlenowej oceniano zawartość suchej masy, pH świeżej masy kiszonki oraz ilość kwasu mlekowego, octowego i masłowego. Oceniano również liczebność drożdży, pleśni i bakterii fermentacji mlekowej. Efektywność zastosowanych dodatków biologicznych w procesie zakiszania runi łąkowej była zróżnicowana, co wynikało przede wszystkim z ich składu. Spośród preparatów przeznaczonych do zakiszania runi łąkowej na cele paszowe największą skutecznością pod względem poprawy jakości okazał się dodatek Lactacel-L. W aspekcie wydłużenia stabilności tlenowej najbardziej efektywny był dodatek Lactosil. W przypadku preparatu Lactosil Biogaz, przeznaczonego do konserwacji surowców na cele energetyczne, uzyskano poprawę stabilności tlenowej i zmniejszenie ilości kwasu mlekowego.
Słowa kluczowe*: stabilność tlenowa, drożdże, pleśnie, bakterie fermentacji mlekowej*

1. Introduction

Aerobic stability i.e. the resistance to aerobic decomposition is an important feature of silage [1, 2, 3]. Direct reason of aerobic instability of silage entails the development of yeasts and moulds [4, 3]. The activity of the latter in ensiled plant material is an effect of its excessive drying and improper taking it out of a silo. Intensive growth of yeasts and moulds may change chemical composition of silage, which results in the loss of dry mass and nutritive components like sugars, lactic acid, acetic acid and ethanol. Aerobic instability is a harmful phenomenon unfavourably affecting hygienic state of silages and their palatability, hence their usefulness for feeding [5].

Resistance to aerobic decomposition is an important feature of silages intended for cattle feeding but also for conservation of raw materials being substrate in methane fermentation used in agricultural biogas fermenters [6].
The intensity of aerobic decomposition in silages may be measured by the amount of produced CO_2 during incubation [7] or by temperature increase compared with that in the surrounding [8, 9, 10]. Changes in pH, chemical composition and the number of fungal microflora are also determined during aerobic incubation [9, 10].

An important element of modern technologies of silage production from wilted meadow sward is the addition of biological components stimulating fermentation. However, one of the consequences of increasing lactic acid content in silage inoculated by bacteria is their negative effect on aerobic stability [11, 12]. To improve the resistance of silages to aerobic decomposition, microbial additives containing selected strains of hetero-fermentative bacteria (apart from homo-fermentative lactic acid bacteria) are applied to limit populations of yeasts and moulds in silage. The additives containing strains of *Lactobacillus buchneri* and *Lactobacillus brevis* are particularly effective in limiting growth of microorganisms responsible for aerobic decomposition of silage [11, 13, 14]. The effect of *Lactobacillus buchneri* on aerobic stability is, however, ambiguous [15].

The aim of this paper is to comprehensively summarise long-term studies on the effectiveness of biological silage additives containing homo- and hetero-fermentative cultures of lactic acid bacteria used in the conservation of meadow sward intended for feeding and energy production.

2. Material and methods

2.1. Test material

Studies were performed in Experimental Farm of the Institute of Technology and Life Sciences in Falenty during productive experiments in years 2000-2016. Study material was silages from wilted meadow sward ensiled in big bales wrapped in plastic film with the addition of four bacterial preparation elaborated at the Institute of Agricultural and Food Biotechnology: Lactosil, Lactacel-L, Lactosil Plus and Lactosil Biogaz. Silages produced from the same plant material without additives were the control.

During the whole study, 134 samples of silage were analysed, half of which (67) were the control samples. Characteristics of particular additives are presented in table 1. The first three preparations contained starting cultures of homo-fermentative lactic acid bacteria supplemented with one or two strains of hetero-fermentative *Lactobacillus buchneri* and *Lactobacillus brevis.* Apart from lactic acid bacteria, Lactacel L and Lactosil Plus contained complexes of fodder enzymes. Lactosil Biogaz contained synergistic species and strains of lactic acid bacteria *Lactobacillus* sp. in order to increase the content of acetic and propionic acids in conserved raw materials intended for biogas production.

2.2. Aerobic stability test

Aerobic stability of silages was determined with the temperature test according to the method described by Honig [16]. To do this, about 20 kg of fresh silage were placed in perforated 60 l baskets and kept at constant room temperature for 12 days. Temperature test was performed in a closed chamber at a temperature of 20+/-1^{o}C. Temperature inside silage samples was measured twice a day during 12-day exposition with mercuric thermometer. Stability was expressed as the time (in days) needed to increase temperature in silage samples by 1^{o}C above ambient temperature.

2.3. Chemical and microbiological analyses

Dry mass (with the drying oven method), pH of fresh silage mass (potentiometrically) and the content of lactic, acetic and butyric acids (enzymatically) were determined in fresh silage samples. Silage quality was assessed based on the percent of particular acids in their total content and expressed in scores according to 100-score scale of Flieg-Zimmer. Before aerobic exposition and after its end the number of yeasts, moulds and lactic acid bacteria were determined with the method of cultures on selective media.

2.4. Statistical analysis

Obtained data on the quality and aerobic stability were statistically processed using one-way ANOVA. Comparisons of means and division into uniform groups were performed with the HSD Tukey T test at $p \leq 0.05$. Discrimination analysis was used to assess the effect of selected chemical and microbiological parameters on the intensity of aerobic decomposition. Initial set of variables included: dry mass content, pH, concentrations of lactic, acetic and butyric acids in dry mass, and the number of yeasts, moulds and acid bacteria. These variables were primarily standardised. Analysis was made with the use of Statistica for Windows software, StatSoft Inc. – analytical module: multiple exploration techniques/discrimination analysis.

Table 1. Characteristics of used additives
Tab. 1. Charakterystyka zastosowanych dodatków

Additive	Characteristics	Composition
Lactosil	Bacterial preparation intended for ensiling meadow sward	*Lactobacillus plantarum* C KKP/788/p, *Lactobacillus plantarum* K KKP/593/p, *Lactobacillus brevis* KKP 839, *Lactobacillus buchneri* KKP/907/p
Lactacel L	Two-component bacterial and enzymatic preparation composed of granulated bacterial concentrate and liquid concentrate containing selected complex of fodder enzymes, mostly glucoamylase intended for ensiling meadow sward and leguminous plants	*Lactobacillus plantarum* C KKP/788/p, *Lactobacillus plantarum* K KKP/593/p, *Lactobacillus brevis* KKP 839, *Lactobacillus buchnerii* KKP/907/p, complex of fodder enzymes mostly glucoamylase
Lactosil Plus	Bacterial and enzymatic preparation containing mixed cultures of lactic acid bacteria and selected complex of fodder enzymes intended for ensiling meadow sward and leguminous plants	*Lactobacillus plantarum* C KKP/788/p, *Lactobacillus plantarum K* KKP/593/p, *Lactobacillus buchneri* KKP/907/p, complex of fodder enzymes
Lactosil Biogaz	Bacterial preparation for ensiling raw materials intended for biogas production	*Lactobacillus buchneri* A KKP 2047p, *Lactobacillus reuteri* M KKP 2048p, *Lactobacillus diolivorans* K KKP 2057p

Source: own study / Źródło: opracowanie własne

3. Results

3.1. Chemical and microbiological quality of ensilages

Mean dry mass content of analysed ensilages ranged from 412.1 to 566.8 g·kg^{-1} FM, which was typical of silages made of wilted material. Application of studied preparations significantly affected most qualitative parameters of silages (table 2). Silages with the addition of microbiological preparations had significantly lower pH than the control ones. They also contained more lactic acid but significant differences were noted only for the addition of Lactosil. The exception was silage with Lactosil Biogaz, in which concentration of lactic acid was lower than that in the control silage.

No significant effect was also found of additives on the content of acetic and butyric acids, which was probably the result of very large variability of these parameters in analysed silages. All silages with added preparations had significantly more scores on the Flieg-Zimmer scale than control silages.

Application of preparations exerted significant effect on the number of analysed microorganisms. In all treated silages the number of acid bacteria favourably increased from 2.55 log cfu/g fresh mass in the control silage to the mean of 4.96 log cfu/g fresh mass in silages with additives. The latter is considered sufficient for the optimum proceeding of lactic fermentation [17]. Simultaneously, a significant reduction in the number of yeasts and moulds was noted in all silages. The exception was silage with Lactosil Biogaz, where the number of moulds exceeded 5.0 log cfu/g fresh mass, which probably resulted from too intensive drying of ensiled raw material before harvesting.

Addition of Lactacel-L appeared on average more efficient in improving silage quality. Very good quality was found in as many as 100% of evaluated silages with this additive. The same note was attributed to 74% of silages with Lactosil and to 78% of silages with Lactosil Plus. For comparison, most samples from among control silages obtained good or satisfactory note. Only 24% of control silages were of very good quality.

3.2. Changes of temperature in the course of stability test

Changes of temperature in silage samples subject to aerobic incubation at 20^{o}C are presented in fig. 1. Temperature increased fastest and was highest in control silages. The lowest rate of temperature increase was noted in silages with Lactosil.

Aerobic stability of control silages was 7.4$\pm$3.63 days. The longest stability (9.8$\pm$4.18 days) was attained by silages with Lactosil. Addition of this preparation prolonged aerobic stability by 2.4 days compared to the control but the difference was not statistically significant. Prolongation of aerobic stability by other additives was smaller and compared with the control varied from 0.6 days (Lactosil plus) to 1.2 days (Lactacel-L) (fig. 2).

3.3. Changes of chemical and microbiological parameters of ensilages

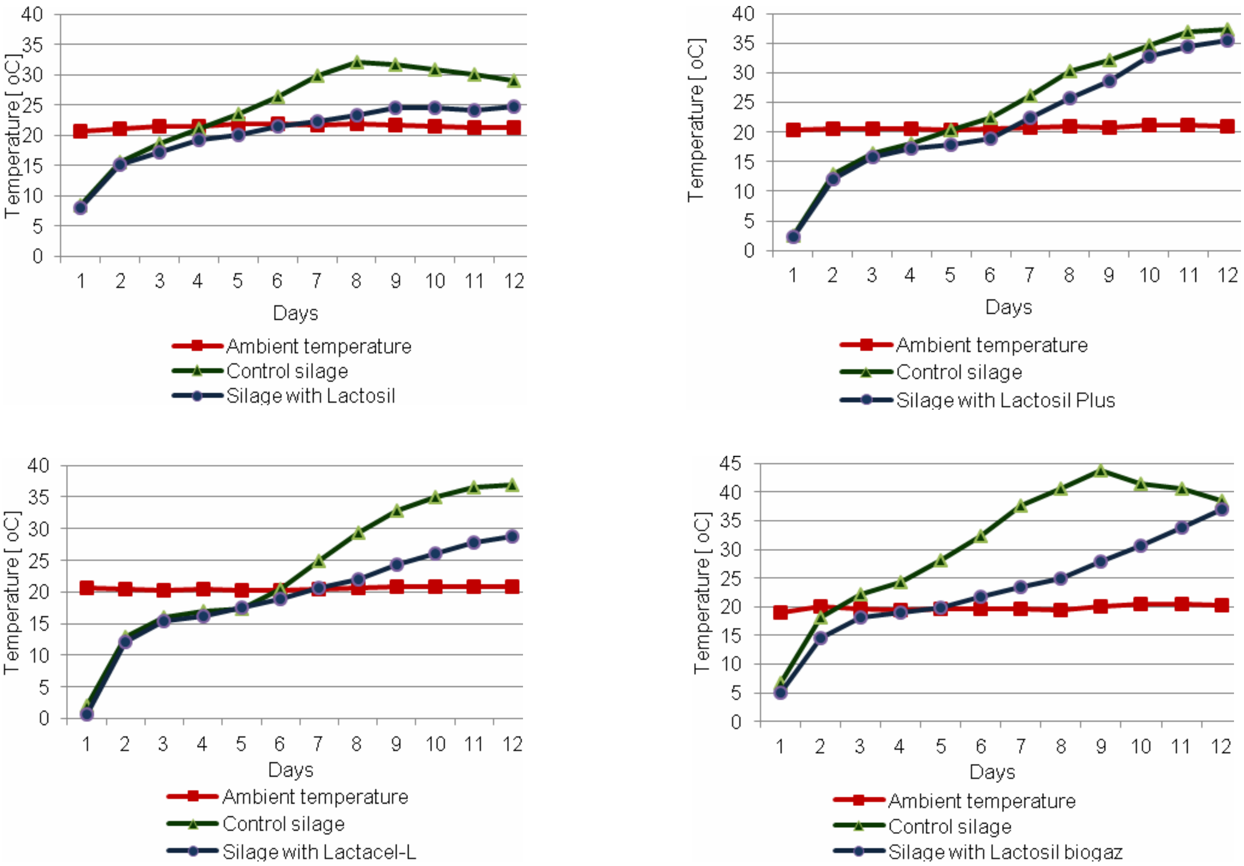
After 12-day aerobic exposition, all silages (except for that with Lactosil Biogaz) showed the increase in dry mass content by 25.2 g kg^{-1} (Lactacel L) or 106.1 g kg^{-1} (Lactosil). Aerobic decomposition was accompanied also by the increase in pH found in all analysed silages (table 2). The content of lactic acid (with the exception of silage with Lactosil Biogaz) and acetic acid decreased while that of butyric acid increased (with the exception of silage with Lactosil) during decomposition. This was an effect of decomposition of lactic acid by aerobic microorganisms, mainly yeasts [4]. The quality of silages expressed in a 100-score Flied-Zimmer scale dropped most in the control (by 16 scores) and less in silages with Lactacel-L and Lactosil Plus. The quality of silages with Lactosil or Lactosil Biogaz did not change.

The number of yeasts increased after aerobic exposition of control silage from 2.70 to 3.14 log cfu g^{-1} fresh mass. In silages with additives the increase was smaller and in those with Latacel-L and Lactosil Biogaz a slight decrease in the number of yeasts was noted. The number of moulds increased in all silages with the exception of that with Lactosil Biogaz. The greatest changes in mould numbers were found in silage with Lactacel-L (increase by 0.90 log cfu g^{-1}). In none of the silages (with the exception of that with Lactosil-Biogaz) the number of moulds exceeded 3-4 cfu g^{-1} fresh mass of silage, the value considered allowable in good silages [18]. The decrease in the number of lactic acid bacteria was also noted in all silages (table 3).

Table 2. Changes in the chemical parameters of silage incubated under aerobic conditions for 12 days at 20^{o}C
Tab. 2. Zmiany parametrów chemicznych kiszonek po 12 dniach tlenowej inkubacji w 20^{o}C

Examined parameters	Day	Silages				
		Control	Lactosil	Lactacel L	Lactosil Plus	Lactosil biogaz
Dry matter [g kg^{-1} FM]	1	425.3	413.5	418.5	435.1	565.8
	12	500.9	519.6	443.7	480.3	544.6
	Increase / Decrease	**75.6**	**106.1**	**25.2**	**45.2**	**-21.2**
pH	1	5.01c	4.72b	4.50b	4.83b	4.24a
	12	5.72	5.37	5.11	5.37	4.53
	Increase / Decrease	**0.71**	**0.65**	**0.61**	**0.54**	**0.29**
Lactic acid [g kg^{-1} DM]	1	30.7b	53.8c	44.3bc	38.7bc	22.8a
	12	21.4a	40.3b	33.7ab	28.9ab	23.9a
	Increase / Decrease	**-9.3**	**-13.5**	**-10.6**	**-9.8**	**1.1**
Acetic acid [g kg^{-1} DM]	1	9.2	9.1	7.9	10.0	3.2
	12	8.3	7.8	6.6	8.7	1.8
	Increase / Decrease	**-0.9**	**-1.3**	**-1.3**	**-1.3**	**-1.4**
Butyric acid [g kg^{-1} DM]	1	1.78	1.07	0.04	0.33	0.09
	12	2.30	0.90	0.30	0.60	0.10
	Increase / Decrease	**0.52**	**-0.17**	**0.26**	**0.27**	**0.01**
Scores acc. to Flieg-Zimmer scale	1	68a	94b	99b	89b	100b
	12	52a	94b	96b	82b	100b
	Increase / Decrease	**-16**	**0**	**-3**	**-7**	**0**

a,b – values with different superscripts differ significantly (P≤0,05)/ wartości oznaczone różnymi literami różnią się istotnie statystycznie (P≤0,05)
Source: own study / Źródło: opracowanie własne



Source: own study / Źródło: opracowanie własne

Fig. 1. Changes in temperature during aerobic exposure of control silage and silage inoculated with tested inoculants
Rys. 1. Zmiany temperatury w kiszonce kontrolnej i w kiszonkach z dodatkami mikrobiologicznymi w trakcie testu stabilności tlenowej

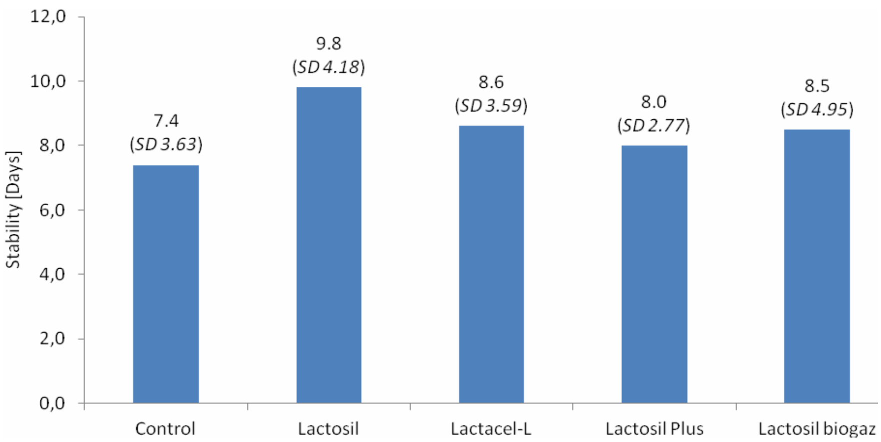


Fig. 2. Aerobic stability of control silage and inoculated silages
Rys. 2. Stabilność tlenowa kiszonki kontrolnej i kiszonek z dodatkami

Source: own study / Źródło: opracowanie własne

Table 3. Changes in microbiological parameters of silage incubated under aerobic conditions for 12 days at 20^{o}C
Tab. 3. Zmiany parametrów mikrobiologicznych kiszonek po 12 dniach tlenowej inkubacji w 20^{o}C

Examined parameters	Day	Silages				
		Control	Lactosil	Lactacel L	Lactosil Plus	Lactosil Biogaz
Yeasts [log cfu g^{-1} FM]	1	2.70b	2.23ab	0.79a	1.52ab	1.58ab
	12	3.14c	2.26bc	0.76a	1.72ab	1.56ab
	Increase/Decrease	**0.44**	**0.03**	**-0.03**	**0.2**	**-0.02**
Moulds [log cfu g^{-1} FM]	1	3.19b	1.15a	0.87a	1.92a	5.52c
	12	3.52b	1.53a	1.77a	2.14a	5.43b
	Increase/Decrease	**0.33**	**0.38**	**0.90**	**0.22**	**-0.09**
Lactic acid bacteria [log cfu g^{-1} FM]	1	2.55a	4.80b	4.94b	5.13b	-
	12	2.27a	4.10b	4.78b	4.73b	-
	Increase/Decrease	**-0.28**	**-0.70**	**-0.16**	**-0.40**	-

Source: own study / Źródło: opracowanie własne

3.4. Discrimination analysis of selected variables characterising silages

Performed stepwise statistical analysis of elimination of insignificant variables showed that the greatest input to discrimination of aerobic stability of silages (the highest F statistics and the lowest Wilks partial lambda values) had consecutively: the number of moulds, concentration of acetic acid, the number of lactic acid bacteria, the number of yeasts, and concentration of lactic acid (table 4). Dry mass content was also an input variable.

Obtained results partly confirmed the results of studies by Ohyama et al. [19] on the effect of selected biochemical and microbiological factors on aerobic stability of silages. Their results demonstrated positive correlation between aerobic stability of silages and the content of: acetic acid (R = 0.44) and butyric acid (R =0.36) and negative correlation for the number of yeasts (R = -0.58) and the content of dry mass in silage (R = -0.47). Wyss [20] found correlation between aerobic stability of silages and the content of acetic acid high and equal to R = 0.51.

As seen from own results and literature data, aerobic stability of silages depends on many factors including the composition and number of microflora and the concentration of fermentation products.

4. Discussion

Performed studies revealed that the efficiency of applied biological additives varied, which was a result of their different composition. The presence of strains of lactic acid bacteria ascribed to the group of relatively homo-fermentative bacteria (able to mainly produce lactic acid [12]) in Lactosil, Lactacel-L and Lactosil Plus directed lactic fermentation in ensiled plant material. This was expressed in decreased pH, increased content in lactic acid at parallel smaller amount of butyric acid, which resulted in the improvement of silage quality compared with the control. Microbiological and enzymatic preparation Lactacel-L showed more positive effect on the process of ensiling and on the quality of silage. Higher efficiency of such preparations can be explained by the presence of appropriate enzymes, which support the activity of lactic acid bacteria through the increase of soluble sugars as substrates in the production of lactic acid. Synergistic action of selected strains of bacteria and enzymes during ensilage disrupts cell walls of plants, partially decomposes starch and non-starch carbohydrates, which also improves digestibility of silages [21].

Worsening of aerobic stability is often a consequence of improved silage quality, which consists in the increase of lactic acid content due to stimulated fermentation [22]. Lactic acid produced by lactic acid bacteria is used by yeasts and moulds responsible for aerobic decomposition of silages [4]. To prevent from decomposition, preparations were supplemented with strains of absolutely hetero-fermentative strains of *Lactobacillus buchneri* and *Lactobacillus brevis.* The former – a component of all studied preparations – has an ability for anaerobic decomposition of lactic acid to acetic acid and 1,2-propanodiole [23]. Undissociated acetic acid is particularly important in preserving aerobic stability of silage. This acid is able to limit the growth of yeasts and moulds, which are responsible for aerobic decomposition [24, 18, 4].

The efficiency of described strains was partly confirmed in performed studies. Despite the fact, that significantly higher concentration of acetic acid was not found in silages with additives, the number of yeasts was lower there. This was also reflected in the improvement of aerobic stability of silages and in restriction of the intensity of aerobic decomposition. Control silages, in which yeasts concentration was significantly higher, warmed up faster than those containing additives and greater changes in the growth of aerobic microorganisms were observed there.

Apart from the number of yeasts, the number of moulds is an important element in the assessment of microbiological quality of silages intended for fodder [25]. Almost all additives, except Lactosil Biogaz, decreased the number of moulds in silages, which was also found in earlier studies of other authors [26, 27].

Another effect was obtained in silages with Lactosil Biogaz, whose composition provided synthesis of volatile fatty acids during ensilage through synergistically acting species and strains of the genus *Lactobacillus* sp. [28]. Increased content of acetic and propionic acids had to ensure aerobic stability and the improvement in profitability of biogas production from silage.

The content of lactic acid in silages with Lactosil Biogaz was smaller by 25% than in the control and smaller by 50% than that in silages with other preparations. Decreased concentrations of lactic acid are favourable in silages intended for biogas production. High content of lactic acid negatively affects the activity of methane fermentation bacteria. Desirable is, however, a high content of acetic acid, which is particularly important in proper course of methane fermentation since about 70% of methane is produced from acetic acid.

Table 4. Results of discriminant function analysis
Tab. 4. Wyniki analizy funkcji dyskryminacyjnej

Estimated factors	Wilks's lambda	Partial Wilks's lambda	*F* discrimination (11,94)	Level *p*	Latitude	Coefficient of canonic correlation R^2
Moulds count [log cfu g^{-1} FM]	0.438	0.768	2.575	0.007	0.812	0.188
Acetic acid [g kg^{-1} DM]	0.422	0.797	2.171	0.022	0.684	0.316
Lactic acid bacteria [log cfu g^{-1} FM]	0.417	0.807	2.041	0.033	0.499	0.501
Yeasts count [log cfu g^{-1} FM]	0.412	0.816	1.927	0.045	0.524	0.476
Lactic acid concentration [g kg^{-1} DM]	0.414	0.813	1.967	0.040	0.461	0.539
Dry matter [g kg^{-1} FM]	0.411	0.819	1.890	0.050	0.498	0.502

Results of the total discrimination statistics: number of variables in model: 6; Wilks's lambda = 0.336; approximation *F* (66.51) = 1.739; p < 0.001

Source: own study / Źródło: opracowanie własne

5. Conclusions

Application of biological additives containing homo- and hetero-fermentative strains of lactic acid bacteria intended for ensiling meadow sward for fodder improved the quality of silages, prolonged the period of aerobic stability and limited changes in chemical and microbiological parameters during aerobic incubation.

The effectiveness of applied biological additives was different. From among preparations intended for ensiling meadow sward for fodder, the greatest efficiency in improving quality was noted for Lactacel-L. Lactosil was most efficient in prolonging aerobic stability.

Lactosil Biogaz composed of three hetero-fermentative strains of lactic acid bacteria and intended for conserving raw material for energetic purposes gave the improvement of aerobic stability and decreased the content of lactic acid.

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