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THE EFFECT OF SILAGE ADDITIVES ON QUALITY OF THE MIXTURE SILAGES OF MAIZE AND DENDROMASS

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ABSTRACT

The aim of this work was to evaluate the influence of biological and chemical ensilaging additives on the quality of the fermentation process and nutrient content in silages produces from a mixture of maize plants (70 % in fresh matter) and oak and spruce twigs (dendromass) as a potential feed for wild ruminants. Mixture 1 was composed of 30 % fresh twigs of forest trees (50 % oak twigs, 50 % spruce twigs). The content of dry matter in Mixture 1 was 32.9 % of fresh matter. The nutrient content reached the crude protein concentration 7.8 % of dry matter, crude fibre 22.4 %, total sugar 9.4 % and starch 19.2 % of dry matter. Mixture 2 was composed of 30 % fresh twigs of forest trees (75 % oak twigs, 25 % spruce twigs). The content of dry matter in Mixture 2 was 32.1 % of fresh matter. The nutrient content reached the crude protein concentration 7.5 % of dry matter, crude fibre 23.1 %, total sugar 9.0 % and starch 16.7 % of dry matter. The fermentation process during ensilaging was observed in one control variant without treatment and two experimental variants, in which the ensilaged matter was treated with additives: T1 (*Lactobacillus buchneri* DSM 13573) and T2 (22.9 % sodium benzoate, 8.3 % sodium propionate). The influence of the treatment of the silage mixture on the nutrient content of the silage was low. Chemical additive applied to the ensilaged matter of both mixtures improved the quality of the fermentation process of the produced mixture silages for wild ruminants. This was confirmed by the highest lactic acid content, lowest content of volatile fatty acids and alcohol, but also the lowest losses of dry matter in the fermentation process. Positive effect of applying the biological inoculant based on heterofermentative bacteria of lactic fermentation was determined only in Mixture 1 silage produced from dendromass compound of 50 % oak twigs and 50 % spruce twigs. Our results indicate that application of ensilaging additives is beneficial in production of maize-dendromass mixture silages for wild ruminants and it improves the fermentation process as well as the quality of the produced silages.

Key words: mixture of maize and dendromass; silage additives; fermentation process; quality of silages, wild ruminants

INTRODUCTION

The basis of the majority of wild ruminants' feeding systems is pasture, however, supplemental feeding is a very frequent form of management, especially during the winter season, and not only in Slovakia, but also in the northern parts of Europe and America. It has its significance especially in the areas where it is important to improve the conditions for survival of the animals. No less important is the prevention of damage from forest tree browsing by the animals (Smith, 2001; Peek *et al.*, 2002).

The botanical composition of red and roe deer and mouflon diet was studied by many authors. Kamler and Homolka (2016) focused on the proportion and quality of agricultural crops and natural forest plants and estimated quality of the herbivore diet. Red deer, roe deer and mouflon ingested all cultivated plants growing close to forest. The average proportion of corn for red deer was 40 %. Cultivated plants were well

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accessible for herbivores in the study area and during vegetation period formed an important part of their diet, but the importance of cultivated plants for herbivores was lower compared to natural food resources present in the forests during vegetation period. Although the main natural food sources had lower nutritional value, they formed the main part of herbivore diet.

In wild ruminant nutrition, new methods and systems for supplemental feeding are being implemented. One of the relatively new systems, which is spreading in practice, is the utilization of mixture feed rations. Mixture of feeds is preserved most often by ensilaging and after fermentation process it is offered to the animals. In livestock, the individual feeds are usually preserved first and only then combined to produce feed mixtures. For wild ruminants, this method is complicated from the management aspect and therefore there is emphasis on systems that will allow to first combine the feeds and then ensilage them.

The quality of the resulting silage mixture can then be evaluated in the same way as the quality of other silages. Supplementing silage mixtures to wild ruminants is a risk, because when silages are subjected to anaerobic conditions for an extended period of time, yeasts and fungi can be activated, which can then lead to undesirable fermentation processes, causing loss of nutrients and damage to the hygienic quality of the produced feed (Hrbek *et al.*, 2013). Vodňanský *et al.* (2007) pointed to the effect of the different silage quality feeding on the bark browsing intensity by red deer. According to Kung and Shaver (2001), production of silage is a complex process involving physiological, chemical and microbiological changes, which have to take place in the silage matter in order to achieve a stable product of high quality. Because the fermentation process is spontaneous and the conditions to ensure high quality silage are not always optimal, Bíro *et al.* (2014) recommend to apply ensilaging additives to improve the fermentation process in forage. Kaiser and Piltz (2004) consider quality silage to be that, which shows low losses of dry matter and nutrients with the concentration of crude protein the same or not significantly lower than in fresh unsilaged feed. Nijboer *et al.* (2003) determined that well-produced silages, when ensilaging dendromass for winter supplemental feeding of the wild ruminants, must have stable pH values. The pH value should be between 3.7 and 5.0 depending on the character and dry matter content of the ensilaged feed.

In our previous experiments (Rajský *et al.*, 2018), we explored the option of producing mixture silages of maize and dendromass composed of oak and spruce twigs in order to produce palatable feed rich in energy, which would also provide sufficient crude fiber content for wild ruminants. We found out that thanks to the high portion of easily ensilaged maize (70 %), the maize-dendromass mixture silages were of good quality.

The 30 % portion of dendromass in our experimental silages was created in two variants of different oak and spruce ratios. The first ratio variant was 50 : 50 %, which we considered to the default, resp. neutral. The second ratio variant was 75 % oak and 25 % spruce. Lower ratio of spruce was chosen because coniferous trees contain certain antinutritional and irritating components, therefore the higher ratio of broadleaf trees is preferable for the digestive system and metabolism of wild ruminants.

We investigated the use of coniferous and broadleaf trees as components in feed mixtures for game because trees represent a natural food source of wild ruminants. In Slovakia, mixed forests are common and the wild ruminants, depending on the tree species, vegetation phase and the percentage of the tree species, consumes broadleaf and coniferous trees with high intensity, often favouring them over pastures and meadows of lesser quality.

In this study, we focused on improving of mixture silages by applying ensilaging additives to the silage matter. Our objective was to evaluate the influence of biological and chemical ensilaging additives on the quality of the fermentation process and on the nutritional value of silages produced by combining twigs of oak and spruce with maize, with maize constituting the majority 70 % of the resulting mixture.

MATERIAL AND METHODS

In the experiment, two different mixtures were produced by combining whole maize plants and twigs of conifer and broadleaf trees. Maize was harvested at the stage of wax ripeness and it represented 70 % of both mixtures (fresh matter content). In Mixture 1, the remaining 30 % were fresh twigs of forest trees compound of 50 % oak twigs and 50 % spruce twigs (Dendro 1). In Mixture 2, the remaining 30 % were fresh twigs of forest trees compound of 75 % oak twigs and 25 % spruce twigs (Dendro 2). The mixtures were without wilting chopped to 3 cm (maximally) and after homogenization ensilaged in 1.7 l glass laboratory silos. Measure for the equal intensity of compression of ensilaging mass was the equal weight of the laboratory silos. Anaerobic conditions were achieved by sealing the silos. Each variant was repeated 5 times.

The fermentation process during ensilaging was observed in one control variant without treatment of the mixture and two experimental variants, in which the ensilaged matter was treated with additives: T1 (biological silage additive, *Lactobacillus buchneri* DSM 13573; the application rate was 2 $l.t^{-1}$ feed) and T2 (chemical additive, 22.9 % sodium benzoate, 8.3 % sodium propionate; the application rate was 3.5 $l.t^{-1}$ feed). The filled experimental silos were stored in a dark room with stable temperature at 22 ± 1 °C. During the fermentation process, changes in weight were monitored and based on those, loses of dry matter weight were determined in %. The experiment finished 180 days after ensilaging.

Samples of feed components as well as silage samples were chemically analysed. The following parameters of organic analysis were determined: dry matter content (gravimetric analysis), crude protein content according to Kjeldahl, content of crude fibre, saccharides, ash, ether extract and starch according to Decree MP SR no. 2145 /2004-100 (2004), acid detergent and neutral detergent fibre according to Van Soest *et al.* (1991).

In addition to the basic parameters of organic analysis, parameters of fermentation process were also determined for the silage samples: pH in the aqueous extract was determined using electrometric method, lactic acid and volatile fatty acids (acetic, propionic and butyric acid) content was determined by gas chromatography and alcohol content by micro diffusion method. These chemical analyses were performed according to Decree MP SR no. 2145/2004-100 (2004), too.

Results were statistically processed by one-way analysis of variance, by the ANOVA multifactorial procedure and by the subsequent POST-HOC Tukey test.

Table 1. The nutrient content of ensilaged mixtures and their compounds (in $g.kg^{-1}$ dry matter)

Item	Mixture 1	Mixture 2	Maize	Dendro 1	Dendro 2
Dry matter in $g.kg^{-1}$ FW	329.01	321.48	287.32	457.69	485.98
Organic matter	946.49	951.93	952.51	940.39	947.81
Crude protein	78.28	75.02	78.37	76.29	63.50
Crude fibre	223.72	230.67	190.41	349.56	367.25
ADF	265.61	278,.04	213.47	429.86	447.63
NDF	564.02	573.41	512.64	528.99	602.00
Hemicelluloses	298.41	295.37	299.17	99.13	154.37
Nitrogen-free extract	613.65	619.83	659.14	474.28	489.37
Total sugars	94.44	90.45	110.25	39.82	39.78
Reduced sugars	83.95	88.36	106.32	38.84	31.78
Starch	192.31	167.43	245.50	0.00	0.00
Ether extract	30.84	26.41	24.59	40.25	27.68
Ash	53.51	48.07	47.49	59.61	52.19

FW – fresh weight, ADF – acid detergent fibre, NDF – neutral detergent fibre
Mixture 1: 70 % maize + 30 % Dendro 1, Mixture 2: 70 % maize + 30 % Dendro 2
Dendro 1 – dendromass mixture in rations of 50 % oak twigs and 50 % spruce twigs
Dendro 2 – dendromass mixture in rations of 75 % oak twigs and 25 % spruce twigs

RESULTS AND DISCUSSION

Table 1 presents the nutrient content in mixtures of whole maize plants with twigs of conifer and broadleaf trees. In addition to the nutritional value of the experimental mixtures it shows also the nutrient content of each feed components from which the mixtures were produced. The results indicate, that concentrations of nutrients in Mixture 1 and Mixture 2 reflect the composition of the individual feed components. Both mixtures can be characterised as feed with lower level of crude protein (78 and 75 g.kg^{-1} dry matter), ether extract (31 and 26 g.kg^{-1} dry matter) and ash (54 and 48 g.kg^{-1} dry matter), with average crude fibre content (224 and 231 g.kg^{-1} dry matter) and high content of neutral detergent fibre (564 and 573 g.kg^{-1} dry matter) as well as the reduced sugars (84 and 88 g.kg^{-1} dry matter). Starch concentration in the mixtures (192 and 167 g.kg^{-1} dry matter) corresponded to the ration of maize and dendromass. According to Weissbach (2003), concentrations of crude protein and saccharides soluble in water directly affect buffer capacity and acidification potential of feed, which determine its ensilagibility. Low crude protein content and high saccharide content in our experimental mixtures indicated their good ensilagibility.

Regarding the production of high quality silage and high production of usable nutrients, Juráček *et al.* (2012) recommend ensilaging maize of 30 – 35 % dry matter content. Considering that in our silages, maize represented 70 % of the silage, we aimed to produce silage mixture of dry matter content in this margin. The content of dry matter in the experimental silage mixtures at ensilaging was 32.9 and 32.1 %.

Table 2. The nutrient content and parameters of fermentation process in silages from Mixture 1 (in g.kg^{-1} dry matter)

Item	Control		T1		T2	
	x̄	SD	x̄	SD	x̄	SD
Dry matter v g.kg^{-1} FW	299.75^{a}	5.09	301.54^{a}	4.21	320.35^{b}	6.35
Dry mater losses in %	9.98^{a}	1.58	9.45^{a}	1.29	3.00^{b}	1.69
Organic matter	946.71	2.34	947.96	3.18	949.75	2.23
Crude protein	80.30	0.89	80.94	3.48	78.75	5.36
Crude fibre	272.19^{a}	5.84	250.87^{b}	6.67	258.43^{a,b}	10.79
ADF	330.71	6.12	325.68	10.30	322.87	13.27
NDF	558.65	8.75	560.05	15.98	545.74	18.09
Hemicelluloses	227.94	3.27	234.37	13.87	222.87	15.97
Nitrogen-free extract	561.78^{a}	4.56	582.89^{b}	11.86	582.35^{b}	9.70
Starch	203.53	23.16	223.86	9.77	205.81	22.25
Total sugars	3.82^{a}	0.55	7.14^{a}	2.37	14.33^{b}	1.23
Reduced sugars	2.43^{a}	0.95	5.46^{a}	2.09	12.20^{b}	1.55
Ether extract	32.44	0.70	33.26	1.17	30.22	2.67
Ash	53.29	2.34	52.04	3.18	50.25	2.23
pH	4.27^{a}	0.06	4.12^{b}	0.01	3.92^{c}	0.02
Acids						
– lactic	36.26^{a}	3.21	52.34^{b}	1.20	60.15^{c}	1.32
– acetic	19.69^{a}	1.76	26.18^{b}	3.06	13.74^{c}	1.61
– propionic	1.18^{a}	0.24	0.78^{a,b}	0.29	0.62^{b}	0.20
– butyric	1.69^{a}	0.20	1.12^{a,b}	0.35	0.77^{b}	0.20
Alcohol	7.89^{a}	0.64	8.16^{a}	0.52	2.34^{b}	0.28

n = 5, FW – fresh weight, ADF – acid detergent fibre, NDF – neutral detergent fibre, T1 – biological additive (*Lactobacillus buchneri* DSM 13573), T2 – chemical additive (22.9 % sodium benzoate, 8.3 % sodium propionate), Different superscripts within a row mean statistical difference (P ≤ 0.01); ^{ab} vs ^{a,b} is not different

In order to improve the fermentation process and the silage quality, the mixtures were treated with one biological ensilaging additive based on heterofermentative bacteria of lactic fermentation (T1) and one chemical additive based on salts of organic acids (T2). Control was a silage without treatment by ensilaging additives. The results of nutritional content and fermentation process of the produced mixtures Mixture 1 and Mixture 2 are presented in Tables 2 and 3.

In silages of Mixture 1, which contained dendromass in rations of 50 % oak twigs and 50 % spruce twigs, only low influence of ensilaging additives on nutrition value was determined. Highly significant differences were determined between T2 silage and the other silages in the content of dry matter (320 compared to 300 and 302 $g.kg^{-1}$ dry matter) and sugars, but also in the crude fibre content between the control and T1 silage. The dry matter losses during the fermentation process were from 3.00 % to 9.98 %. The conclusively lowest losses were determined in the silage treated with the chemical ensilaging additive. Low losses of dry matter prove the lowest degree of nutrient degradation and the highest effect of conservation during the fermentation process. In the silage treated with biological ensilaging additive fermentation losses were determined to be only 0.53 % lower compared to the untreated control silage, which indicates low effect of this additive. The differences in pH values and lactic acids and acetic acid concentrations between the experimental Mixture 1 silages were highly statistically significant. In the untreated control silage we determined the highest pH (4.27), highest butyric acid (1.69 $g.kg^{-1}$ dry matter) and propionic acid (1.18 $g.kg^{-1}$ dry matter) concentrations and the lowest concentration of lactic acid (36.26 $g.kg^{-1}$ dry matter). This confirms that the lowest quality of fermentation process of all Mixture 1 silages was in the untreated silage control. The highest content of acetic acid was determined in the silage treated with the biological additive (26.18 $g.kg^{-1}$ dry matter) and the highest lactic acid content was determined the silage treated with the chemical additive (60.15 $g.kg^{-1}$ dry matter).

In silages produced using Mixture 2, which contained dendromass in rations of 75 % oak twigs and 25 % spruce twigs, the lowest dry matter losses during the fermentation process were determined in the silage treated by chemical additive T2 (6.07 %), which were highly statistically significant differences ($P \leq 0.01$) compared to other silages (9.23 and 10.54 %). The influence of ensilaging additives on the majority of nutrient content was insignificant. Highly significant differences were determined only between T2 silage and control in crude fibre content (242 compared to 260 $g.kg^{-1}$ dry matter) and between all silages in the content of total and reduced sugars. Fermentation process was adequate. The pH values were determined to be between 3.96 and 4.13, with the lowest values determined in the silage treated using chemical additive and the highest in the untreated control silage. Highly statistical significant differences in acid content were determined between T2 silage and the other silages in lactic acid (55.51 compared to 45.31 and 45.75 $g.kg^{-1}$ dry matter, acetic acid (12.76 compared to 18.10 and 19.08 $g.kg^{-1}$ dry matter) and alcohol (2.87 compared to 6.26 and 7.20 $g.kg^{-1}$ dry matter). T1 silage showed the lowest butyric acid content (0.55 $g.kg^{-1}$ dry matter), in which there was a highly statistical significant difference only in comparison to the untreated silage (1.34 $g.kg^{-1}$ dry matter).

Silage production is process resulting in physiological, chemical and microbiological changes, which need to take place in the feed being ensilaged in order to produce a quality product. In order to achieve the expected quality of the produced silage, a number of measures is necessary to control the fermentation process and to minimize the losses during fermentation. In addition to following the technological process, it is possible to also apply ensilaging additives of biological and chemical character (Spörndly and Pauly, 2008). Silage additives encompass a wide range of products including bacterial inoculants, fermentable substrates and enzymes, all of which are designed to promote growth of desirable organisms and appropriate fermentation products, as well as to inhibit growth of undesirable organisms and prevent poor quality silage (Schroeder, 2004).

Ward (2000) as well as Kung and Shaver (2001) consider the most helpful analyses for the determination of good silage composition to be the dry matter, acidity, crude protein, acid detergent fibre, neutral detergent fibre, lignin, calcium and phosphorus. The use of a fermentation profile including organic acids such as lactic, acetic, propionic and butyric acids is also a helpful tool. The monitored parameters of the nutrient composition and fermentation process correspond to their recommendations. Though lignin, calcium and phosphor were not monitored in ours experimental silages, the focus was on sugars, starch, ether extract, ash and alcohol, which are also important parameters determining the quality of the produced silages.

Based on the results, we have determined that the influence of treatment by biological and chemical additives on nutrient content of Mixture 1 and Mixture 2 silages was low. Notable is the decrease of crude fibre content compared to untreated silage. Fermentation process had the most significant influence on utilizability of total and reduced sugars. The highest content of reduced sugars was determined in the silage treated by chemical additive and the lowest in the untreated control silage, in both mixture silages.

One of the parameters to evaluate the quality of fermentation process is the losses of dry matter during fermentation. The lower the losses, the lower are also the losses of nutrients and therefore the fermentation process was better. The lowest losses were determined in silages treated with chemical additive, in both types of mixtures (Mixture 1 and Mixture 2).

As known, the preserving effect in silage is obtained by suppressing the aerobic microbes by exclusion of air and eliminating the remaining harmful anaerobic microbes, which require no oxygen, through reducing the pH value by means of enrichment of lactic acid. Whether the harmful acid – sensitive microbes can be eliminated, particularly the *Enterobacteria* and

Table 3. The nutrient content and parameters of fermentation process in silages from Mixture 2 (in g.kg^{-1} dry matter)

Item	Control		T1		T2	
	x̄	SD	x̄	SD	x̄	SD
Dry matter v g.kg^{-1} FW	295.71	4.38	291.52	6.96	304.24	6.16
Dry mater losses in %	9.23^{a}	1.36	10.54^{a}	2.15	6.07^{b}	1.27
Organic matter	948.66	1.61	946.41	2.04	949.59	3.56
Crude protein	78.05	2.09	80.38	3.04	76.77	2.21
Crude fibre	260.16^{a}	3.55	247.51^{a,b}	10.84	241.95^{b}	8.26
ADF	304.08	8.77	296.57	13.61	291.45	8.68
NDF	534.16	10.52	537.65	10.75	550.87	18.45
Hemicelluloses	230.08	9.94	241.08	11.24	259.42	15.85
Nitrogen-free extract	580.65	17.82	584.53	15.12	601.66	9.21
Starch	231.63	11.33	227.76	14.35	217.51	7.77
Total sugars	1.46^{a}	0.01	5.47^{b}	1.66	11.52^{c}	0.73
Reduced sugars	1.01^{a}	0.01	3.53^{b}	0.60	8.60^{c}	1.39
Ether extract	29.80	0.91	33.99	2.28	29.21	1.49
Ash	51.34	1.61	53.59	2.04	50.41	3.56
pH	4.13^{a}	0.08	4.04^{a,b}	0.04	3.96^{b}	0.02
Acids						
– lactic	45.31^{a}	3.12	45.75^{a}	1.71	55.51^{b}	3.68
– acetic	18.10^{a}	1.23	19.08^{a}	1.59	12.76^{b}	1.47
– propionic	0.52^{a}	0.14	1.33^{b}	0.41	0.59^{a}	0.11
– butyric	1.34^{a}	0.38	0.55^{b}	0.19	0.83^{a,b}	0.23
Alcohol	6.26^{a}	0.56	7.20^{a}	0.71	2.87^{b}	0.13

n = 5, FW – fresh weight, ADF – acid detergent fibre, NDF – neutral detergent fibre, T1 – biological additive (*Lactobacillus buchneri* DSM 13573), T2 – chemical additive (22.9 % sodium benzoate, 8.3 % sodium propionate), Different superscripts within a row mean statistical difference (P ≤ 0.01); ^{ab} vs ^{a,b} is not different

Clostridia, depends on the green fodder's potential for acidification (Weissbach, 2003). Bíro *et al.* (2014) considers the decrease of the pH values during the fermentation process to be one of the main preservation factors, which inhibits the multiplication of undesirable microorganisms in silages. For easily ensilaged forage, decrease of pH < 4.3 is recommended. Mitrík (2010) also highlights the importance of silage acidification and suggests that for evaluation of the quality of silages of maize and other easily ensilaged forages, the following formula should be used to determine the maximum pH value of the silage:

$$pH \leq 0.0026 \times \text{Dry matter in } \% + 3.694$$

Mixture 1 and Mixture 2 silages reached in our experiment pH values corresponding to the recommendations of the aforementioned authors. In both mixture types, the highest pH value was determined in the untreated control silage and the lowest pH value in the silage treated by chemical additive.

Few authors engage in the research on silages containing dendromass. Jeon *et al.* (2003) determined in detailed research of such silages, that forest by-product silage had a fermentative quality of 4.1 pH and 8.9 % lactic acid (DM basis). In the silages in our experiment, the same pH values but significantly lower lactic acid concentrations (3.6 – 6.0 %) were determined.

It is essential to have a good microbial fermentation process to produce high quality silage. A good fermentation process is not only dependent on the type and quality of the forage crop, but also on the harvesting and ensiling technique. Many additives have been used to improve fermentation quality of made silages (Oude Elferink *et al.*, 2011).

Quality of fermentation process in silage is characterised by the content of fermentation products. Favourable is a high lactic acid content and low volatile fatty acids and alcohol content. The results of the fermentation process in our silages show that the effect of biological additive application to Mixture 2 silage was very low. The only demonstrably positive influence was from the chemical additive, which was manifested in the highest concentration of lactic acid and the lowest concentrations of acetic acid, propionic acid and alcohol. In Mixture 1 silages, more significant differences were determined between the silages treated with additives and the untreated control, which confirm the positive influence of treatment of the silage mixture. Fermentation process was the most successful in silages treated with the chemical additive, which is confirmed by the highest lactic acid content and the lowest content of volatile fatty acids and alcohol.

From this it can be determined that the chemical additive applied to the silage mixture composed of 70 % whole maize and 30 % fresh twigs shoots of forest trees conclusively improved the quality of the produced silage mixtures for wild ruminants. Positive influence of applying the biological inoculant based on heterofermentative bacteria of lactic fermentation had only partial effect, determined only in Mixture 1 silage, which was produced using the dendromass compound of 50 % oak twigs, 50 % spruce twigs.

The gathered data indicate that application of ensilaging additives has its purpose in production of maize-dendromass mixture silages for wild ruminants and it improves the fermentation process as well as the quality of the produced silages.

CONCLUSION

In this study, we evaluated the influence of selected biological and chemical ensilaging additives on the quality of fermentation process and nutritional value of silage produced from mixture of dendromass and maize, with 70 % maize. Two mixtures were created, which differed in the ratios of oak and spruce twigs in the dendromass. The ensilaging additives were more effective in case of Mixture 1 silage, which was produced using dendromass compound of 50 % oak and 50 % spruce twigs. Of the applied silage additives, the chemical additive, based on salts of organic acids, was determined to be more effective in improving the quality of the produced silages.

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