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EFFECTS OF Lactobacillus spp. ON MOULD AND YEAST GROWTH IN THE ENSILING PROCESS

ABSTRACT: The paper includes the results of effect of preparations Biomax SI (Lactobacillus plantarum) and Biomax WS (L. plantarum and Pediococcus pentosaceus) on mould and yeast growth in the ensiling process. The following has been analyzed: total number of mould and yeast, specific rate of mould and yeast growth, pH, content acid (lactic, acetic and butyric acid), moisture and temperature in bales during 30 days of lucerne ensiling.

KEY WORDS: bacterial inoculates, Biomax SI, Biomax WS, ensilage of lucerne, lactic acid bacteria, Lactobacillus plantarum, lucerne

INTRODUCTION

In the case of intensive animal production concept of feed production should be based on the maximum use of natural resources. The aim of this concept is the produce enough quantity of ponderous feed during the whole year. Ponderous feed is mostly made from hay, silage and ensilage of lucerne, grass leguminoze mixture, silage of corn etc. There is a need for higher quality of lucerne ensilage during the whole year. In order to provide the best quality ensilage, bacterial inoculates, Biomax SI and Biomax WS, were used.

Using bacterial inoculates as additives, which main metabolic production is lactic acid, is a natural way to intensify the process of fermentation in silage, and decrease the pH value in order to provide efficient conserving of nutritive components. The importance of using bacteria as additives for ensiling is in numerous advantages they have over chemical additives: easy use, safe action, they are not corrosive for machines, and they do not pollute the environment (Antov et al., 2004).

According to Bolsen (1999) and Bolsen et al. (2000), in USA 150 additives are used, of which 80 are bacterial additives. There are 203 additives available on the market of the European Union, of which 87 are bacterial additives.
The main aim of this research was to investigate the effects of Biomax SI and Biomax WS preparations on moulds and yeasts during the ensilage process of lucerne.

**MATERIAL AND METHODS**

In these experiments, ensilage of lucerne, harvested in 2005 in Vojvodina, on the parcels of company “PIK-Bečej” A.D. “Poljoprivreda” was used. In the first experiment, harvested lucerne was treated with Biomax SI. In 100g of Biomax SI 100L water without chlorine was added, and well homogenized. Two litres of this solution were sprayed on one tone of lucerne sheeted to 20—30 cm layer. Then, lucerne baling, using six layered of polyethylene foil was performed by Krone press machine and bales of 500kg weight were formed. In the second experiment, 60g of Biomax WS was diluted with 200L of water without chlorine and well homogenized. Two litres of this solution were sprayed on one tone of lucerne, and bales of 500kg weight were formed in the same way as in the first experiment. The samples were taken from the bales treated with Biomax SI and Biomax WS, during the first six days and then on the 12th, 18th, 24th and 30th day of ensiling.

Biomax SI is an additive for silage of grass, clover, fresh lucerne, corn and silage of whole plant. It is produced by CHR Hansen Denmark and it contains *Lactobacillus plantarum* (50 billions CFU/g).

Biomax WS is an additive for silage of corn and whole plant. It is produced by CHR Hansen Denmark and it contains *Lactobacillus plantarum* and *Pediococcus pentosaceus* (50 billions CFU/g).

All used materials were sampled according to the feed sampling methods regulated by the “Pravilnik o metodama uzimanja uzoraka i metodama fizičkih, hemijskih i mikrobioloških analiza — Sl. list SFRJ br. 15/1987”.

Investigation of the effects of Biomax SI and Biomax WS preparations on moulds and yeasts, during the ensilage process of lucerne, was performed in two experiments. In the first experiment, the samples were taken from bales treated with Biomax SI, during the first six days, and then on the 12th, 18th, 24th and 30th day of ensiling for performing microbiological and chemical analyses. Microbiological investigation included the determination of total number of moulds and yeasts, and specific rate of mould and yeast growth. The following analyses were done: measurements of pH value, acid content (lactic, acetic and butyric acid), moisture and temperature in bales of lucerne during 30 days of ensiling. In the second experiment, the samples were taken from bales treated with Biomax WS, in the same manner as described above.

The evaluation of total number of moulds and yeasts was performed according to the indirect Koch’s method (Škriñjar, 2001).

The evaluation of specific rate of mould and yeast growth was achieved using the equation (Pejin, 2003): \( \mu = \frac{\ln N (t) - \ln N (o)}{t} \) (\( \mu \) — specific rate of microorganism growth, \( N (t) \)-number of microorganisms in time \( t \), \( N (o) \)-number of microorganisms in time zero, i.e. at the beginning, \( t \)-time).

Chemical analyses (measurements of pH value, acid content (lactic, acetic and butyric acid content by distilation), moisture and temperature in bales of
lucerne) were performed according to the methods of Kolarški and Pavličevič (1970).

RESULTS

The effects of Biomax SI and Biomax WS preparations on the total number of moulds are presented in Table 1 and Figure 1.

Tab. 1 — Effects of preparations Biomax SI and Biomax WS preparations on total number of moulds during 30 days of lucerne ensiling.

<table>
<thead>
<tr>
<th>NM</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
<th>12th day</th>
<th>18th day</th>
<th>24th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>1.280</td>
<td>3.450</td>
<td>3.650</td>
<td>3.200</td>
<td>2.580</td>
<td>1.580</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WS</td>
<td>1.950</td>
<td>4.550</td>
<td>5.500</td>
<td>5.450</td>
<td>4.200</td>
<td>2.900</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NM — number of moulds; SI — Biomax SI; WS — Biomax WS

Fig. 1 — Effects of Biomax SI and Miomax WS preparations on total number of moulds during 30 days of lucerne ensilage

The effects of preparations Biomax SI and Biomax WS preparations on the specific rate of mould growth are presented in Figure 2.
The effects of Biomax SI and Biomax WS preparations on total number of yeasts are presented in Table 2. and Figure 3.

<table>
<thead>
<tr>
<th>NY</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
<th>12th day</th>
<th>18th day</th>
<th>24th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>180</td>
<td>650</td>
<td>1.000</td>
<td>1.110</td>
<td>1.130</td>
<td>1.120</td>
<td>120</td>
<td>110</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WS</td>
<td>260</td>
<td>710</td>
<td>1.250</td>
<td>1.350</td>
<td>1.400</td>
<td>1.390</td>
<td>150</td>
<td>120</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NY — number of yeasts (x 10^6); SI — Biomax SI; WS — Biomax WS
The effects of Biomax SI and Biomax WS preparations on the specific rate of yeast growth are presented in Figure 4.

![Figure 4](image)

**Fig. 4.** — Specific speed of yeast growth during 30 days of ensiling lucerne treated with Biomax SI and Biomax WS

Figure 5. represents the changes in pH value of lucerne ensilage treated with Biomax SI and Biomax WS during 30 days of ensiling.

![Figure 5](image)

**Fig. 5** — pH value in lucerne ensilage treated with Biomax SI and Biomax WS during 30 days of ensiling

Figure 6. represents the percentage of lactic acid in lucerne ensilage treated with Biomax SI and Biomax WS during 30 days of ensiling, and Figure 7.
represents the percentage of free and total acetic acid in lucerne ensilage treated with Biomax SI and Biomax WS during 30 days of ensiling.

Fig. 6 — The percentage of lactic acid in lucerne ensilage treated with Biomax SI and Biomax WS during 30 days of ensiling

Fig. 7 — The percentage of free and total acetic acid in lucerne ensilage treated with Biomax SI and Biomax WS during 30 days of ensiling

In Table 3. and Table 4. contents of free and total butyric acid in lucerne ensilage treated with Biomax SI and Biomax WS during 30 days of ensiling are displayed.
Tab. 3 — Effects of Biomax SI and Biomax WS preparations on free butyric acid during 30 days of lucerne ensiling

<table>
<thead>
<tr>
<th></th>
<th>FBA</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
<th>12th day</th>
<th>18th day</th>
<th>24th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WS</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

FBA — free butyric acid; SI — Biomax SI; WS — Biomax WS

Tab. 4 — Effects of preparations Biomax SI and Biomax WS preparations on total butyric acid during 30 days of lucerne ensiling

<table>
<thead>
<tr>
<th></th>
<th>TBA</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
<th>12th day</th>
<th>18th day</th>
<th>24th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0,01</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WS</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0,01</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

TBA — total butyric acid; SI — Biomax SI; WS — Biomax WS

In Figure 8. and Fig. 9. moisture in lucerne, and temperature in bales of lucerne ensilage treated with Biomax SI and Biomax WS during 30 days of ensiling are shown.

![Graph showing moisture and temperature changes](image)

Fig. 8 — Moisture in lucerne during 30 days of ensiling treated with Biomax SI and Biomax WS
DISCUSSION

From the obtained results, it can be seen that both preparations, Biomax SI and Biomax WS, completely suppressed the growth of moulds on the 12th day of ensiling (Table 1, Fig. 1. and Fig. 2).

The presence of moulds in silage can change its savor, and nutritive value and decrease the content of carbohydrates and proteins in silage. Yet, the most important is that moulds in silage can disintegrate proteins and produce mycotoxins. The presence of moulds does not always mean the production of mycotoxins, but under certain conditions, the production of mycotoxins can be very fast and toxins can be very stable for a long period of time after the stunt of moulds. *Aspergillus fumigatus* is capable of producing toxic alkaloid klavin (Edwards and McDonald, 1989).

The conditions that favour the growth of moulds are (Antov et al., 2004): moisture above 13%, relative moisture above 70%, temperature over 13°C, easily acceptable nutritive ingredients, pH value above 5, presence of oxygen.

The growth of moulds in silage will be inhibited by low pH value, however it can be supported by the presence of non fermentable carbohydrates.

Toxigenic strains of moulds can provoke intensive intoxications in humans and animals. Toxigenic strains belong to genera *Aspergillus, Fusarium, Penicillium, Stachybotris, Claviceps, Ustilago* and *Puccinia*. The strains grow and produce mycotoxins in food and feed only under appropriate conditions of moisture and temperature. One should bear this mind during storing and preserving feed, particularly grains (Radanov-Pelagić, 2000).

The number and types of filospherical microorganisms depend on: plant type, composition and amount of exudates, pH value, temperature and mois-
ture. According to Govedarica and Jarak (2001) there are $10^6$ and $10^8$ CFU/g of bacteria and yeasts on plants which are used for feed preparation. These findings are in accordance with the results gained in this paper. The initial number of yeasts was $180 \times 10^6$ in the ensilage of lucerne treated with Biomax SI (Table 1), and in the ensilage of lucerne treated with Biomax WS was $260 \times 10^6$ (Table 2).

From the results shown in Table 2. and Figure 3. it can be seen that both preparations (Biomax SI and Biomax WS) reduced the number of yeasts totally on the 24th day of ensiling.

According to Antov et al. (2004), the majority of yeasts grow on temperature between 0 and 37°C, but several of them are adapted to temperatures above 43°C. The number of yeasts increases if the temperature is higher than 40°C during the aerobic spoilage. They are more sensitive on higher temperatures than clostridiums. It has been noticed that the number of yeasts usually grow during wilting of sow, that is obvious for fermentative yeasts and yeasts that are using lactate. That explains why aerobic spoilage is more frequent when ensiling drier mass with low compactness. Since soil is a reservoir of yeasts, the possibilities of contamination during wilting are higher, especially after the mechanical turning over of wilting mass. Silages with yeast population that counts $10^6$CFU/g are considered especially susceptible to aerobic spoilage. After two or three days of aerobic exposure the total number of yeasts can increase to $10^{12}$CFU/g. Diffusion of oxygen is very important for population of yeasts that assimilate lactate. Yeasts usually grow in the range of pH values from 3 to 8, while the optimal level for the most of them ranges from 3.5 to 6.5. Under aerobic conditions, they tolerate the presence organic acids (lactic, acetic, citric, malatic, propionic, fumaric acid) better than other microorganisms.

It can be concluded that both preparations have the same effects on the specific rate of yeast growth during 30 days of lucerne ensiling (Figure 4).

Lucerne, grass, small grain corn with high moisture content, usually react well to microbiological inoculation with homofermentative lactic acid bacteria. Recapitulating the results of the researches that were published in the period 1985—1990, Muck and Bolsen (1990) concluded that in more than 2/3 in inoculated silages was a significant decrease in pH value and increase in lactic acid content, which is similar to the results obtained in this paper (Figure 5). In the experiment of lucerne ensiling with Lactobacillus plantarum (Antov and Čobič, 1991), the obtained lactic acid content varied from 8,94 to 10,95% in the silage with 30% of dry matter (d.m.) and in the silage with 50% d.m., the lactic acid content ranged from 4,59 to 6,91%, which is not in agreement with the results achieved in this paper, where the lactic acid content ranged from 0,80 to 2,50% for lucerne ensilage (44,20% d.m.), treated with Biomax SI and from 0,66 to 1,96% for lucerne ensilage (44,30% d.m.), treated with Biomax WS (Figure 6).

Regarding the effects of Biomax SI and Biomax WS preparations on acetic acid content (Figure 7) in lucerne ensilage (44,20% d.m.) treated with Biomax SI, the acetic acid content ranged from 0,40 to 1,13%, and in lucerne ensilage (44,30% d.m.) treated with Biomax WS, the acetic acid content ran-
Percentage of free butyric acid was 0 in both treatments, while very low content of total butyric acid (0.01%) was found in lucerne ensilage treated with Biomax SI on the 24th day of ensiling, and on the 5th day of ensiling in lucerne ensilage treated with Biomax WS. These results prove the good technological procedure during the ensiling as well as positive effects of Biomax SI and Biomax WS on the ensiling process (Table 3. and Table 4). At the end, it should be emphasized that bacterial additives as well as enzymes, are not so omnipotent to produce qualitative ensilage from low quality material, using inappropriate technological procedure (Antov et al., 2004).

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УТИЦАЈ LACTOBACILLUS SPP. НА ПЛЕСНИ И КВАСЦЕ У ПРОЦЕСУ СЕНАЖИРАЊА ЛУЦЕРКЕ

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Резиме

Рад садржи резултате испитивања утицаја два препарата Biomax SI (Lactobacillus plantarum) и Biomax WS (Lactobacillus plantarum и Pediococcus pentosaceus) на раст плесни и квасца у процесу сенажирања луцерке. Рађене су следеће анализе: укупан број плесни и квасца, специфична брзина раста плесни и квасца, pH, садржај киселина (млечна, сирћетна и бутерна киселина), затим влaga и температура под утицајем оба препарата у процесу сенажирања током 30 дана.