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BIOLOGICAL PRESERVATIVE IN WHOLE CROP WHEAT ENSILAGE

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ABSTRACT – Biological preservative in whole crop wheat ensilage
Ensilage of whole crop wheat is popular in Europe and America for feeding of ruminant animals, but it is quite rare in Hungary. It can be introduced for replacement of shortage of silomaize silages in drought season. The quality of wheat silage could improve by biological inoculants.
Silage additives are expected to ensure a more efficient fermentation phase as well as reduce the risk of aerobic deterioration when silages are exposed to air. Many additives have been developed to improve the ensiling process and nutritive value of silage.
Nowadays the 3rd generation biological inoculants –containing lactic acid bacteria and enzymes – are used in order to coordinate the fermentation in such a way that they increase lactic acid production at the beginning of the fermentation and improve the quality and stability of silage during the fermentation and feeding. The quality of raw material (maturity of plant, chop length, spreading of inoculant uniformly) and the proper filling, compacting, covering and wrapping have a great influence on the effectiveness of the inoculant. The mycotoxin content of malfermented silages is an undesirable risk factor.
The objective of our research was to determine the effect of two silage inoculant strains *Lactobacillus buchneri* and *Pediococcus acidilactici* mixture combined with *amilase-, glucanase-, xylanase* and *galactomannase* enzymes on whole crop wheat silage fermentation characteristics, nutritive value and aerobic stability compare to untreated control.
Experimental ensilage procedure started with the basic whole crop raw material originated from waxen ripeness of wheat (hard cheddar stage of maturity of seeds) at the time of harvesting. The DM content of chopped raw material was 44%.
The LAB inoculants were applied to raw material at $2.5x10^5$ CFU/g fresh material (FM).
Because of quite good quality of untreated silages also, the priority of LAB treatment could not proven in the aerobic stability test. The biological preservative (LAB+enzymes) promoted better fermentation and forced back the undesirable butyric acid production in the silages.

Keywords: whole crop wheat silage, lactic acid bacteria, fermentation, aerobic stability

INTRODUCTION

Wheat is used for bread-stuffs first of all, or for animal feed. Ensilage of whole crop wheat is popular in Europe and America (WOOLFORD ET AL., 1982), but very rare in Hungary until now. The reason of making whole crop wheat silage for ruminant animals is the following: The uncertainty of selling of over production could be avoided in the high yield years. The wheat harvesting is occurred 2-2,5 months earlier than for maize means: whole crop wheat silage is available the same time earlier, so it could be replace the shortage of other forages at that time. Early entry of reseeds is possible. There is no environmental pollutant effluent. Lower clamp cost, eg. straw ball walls are sufficient.

Additives are expected to ensure a more efficient fermentation phase as well as reduce the risk of aerobic deterioration when silages are exposed to air (KEADY AND MURPHY, 1998, SZUCS AND SINDOU, 2005). Many additives have been developed to improve the ensiling process and nutritive value of silages (NIA AND WITTEMBER, 1999, KUNG ET AL., 2003).

Acids: under difficult ensiling conditions (rainy weather) acids are best choice as an aid to preservation. *Enzymes:* additives containing fibriolytic enzymes provide additional sugar through the breakdown of plant fibre. *Bacterial inoculants:* they can improve fermentation characteristics by speeding up the fall in pH and lowering ammonia levels into the silo. At present biological additives are preferred because they are non-toxic, non-corrosive to machinery, do not present environmental hazards and are regarded as natural products.

Mixtures of different types of additives: they can improve the effect of usage them selves. Nowadays the *bacterial inoculants* with cell-wall degrading *enzymes* -so called 3rd generation biological inoculants- one of the most popular additives are used in order to coordinate the fermentation in such a way that they increase lactic acid production by leaps and bounds at the beginning of the fermentation and improve the quality and stability of silage during the fermentation and feeding (KUNG ET AL., 1991).

There is an unsettled discussion around this issue: the circumstances provided in the silo do not always fit the optimal conditions which are necessary for the functioning of the enzymes. For example cellulase originating from *Trichoderma reseei* fungi, has an optimal activity between pH 4.8 and 5.2 while from *Trichoderma viride* is between 4 and 5. The optimal temperature for these cellulase enzymes: namely 55-65 °C and 40-50 °C respectively (KNABE, 1987). We need to make a compromise regarding the maturity and dry matter content of plant for ensiling as well. The suggested domain of dry matter content for ensiling of grass and lucerne is 28-33% (SCHMIDT ET AL., 2001) as the hard cheddar/ wax maturity of cereals (SZUCS AND AVASI, 2005), in which domain both lactic acid bacteria and cell wall degrading enzymes can work in a sufficient manner.

Silage additives produce variable results on aerobic deterioration of silages. A high concentration of lactic acid cannot provide aerobic stability for sure SUCU AND FILYA (2006). Recently the heterofermentative *L. buchneri* is regarded to be the most promising lactic acid bacteria for increasing aerob stability (DREIHUIS ET AL., 1996, 1999, WEINBERG ET AL., 2002). Applied by itself it may show a negative effect by reducing the speed of fermentation, but its combination with homofermentative lactic acid bacteria does compensate this disadvantage (FILYA, 2003).

According to RUSER AND KLEIMAN (2005) it takes effect on stability in the 2nd phase: during the 1st phase lactic acid originates from sugar and in the 2nd phase acetic acid and 1,2 propandiol are generated from lactic acid. OUDE ELFERINK ET AL. (1999) emphasize the role of propionic acid originating from 1,2 propandiol and 1 propanol in stability (1,2 propandiol and 1 propanol are not found in untreated silage). *L.buchneri* may produce other yet unindentified metabolites with antifungal activity BAX AND SINDOU (2005). Applying suitable biological preservatives may be an effective method for the promotion of lactic acid fermentation and preserving forage nutritive value during ensiling and on exposure to air at feed-out (WEDDEL, 2001).

The quality of raw material for ensilage (maturity of plant, chop length, spreading of inoculant uniformly) and the proper filling, compacting, covering and wrapping have a great influence on the effectiveness of the inoculant. The mycotoxin content of malfermented silages is undesirable risk factor (NADEU, 2007, SZUCS –AVASI, 2005).

MATERIAL AND METHODS

Table 1. Composition of applied biological (LAB+ enzymes) inoculant

Lactobacillus buchneri NCIMB 40788	> 3.00 x 10^{10} CFU* /g
Pediococcus acidilactici MA 18/5M	> 2.00 x 10^{10} CFU /g
Beta-glucanase	> 6000 IU/**g
Alfa-amilase	> 2400 IU/g
Xylanase	> 2400 IU/g
Galactomannase	> 1200 IU/g

**Colony Forming Unit* ***Activity Unit*

Treatments

- T1: Control (untreated) for 4 weeks storage
- T2: LAB inoculants 2.5x10^5 CFU/g fresh material (FM) for 4 weeks storage
- T3: Control (untreated) for 6 weeks storage
- T4: LAB inoculants 2.5x10^5 CFU/g fresh material (FM) for 6 weeks

The certain amounts of inoculants (*Table 1.*) were diluted in 100ml of distilled water, and spread on 25 kg of raw materials as follows:
We evenly spread 25 kg/treatment of chopped whole crop wheat on micro-silo ensiling on plastic foil, then we vaporized the silage additive on the weighed portions, finally it was mixed.

Micro-size ensiling process
Small size containers (4.2 l cubic capacity / each), which we used were closed by screwed cap. We filled 5 containers for each treatment, altogether 20 pieces. Storage took place on 20-22C° interior temperature. We stored the filled micro containers 4 or 6 weeks. We performed laboratory examinations on them. The examination focused primarily on the products of fermentation and the aerobic stability.

Chemical analysis
Dry matter (MSZ ISO 6496; 2001)
Crude protein with Kjehl-Foss technique on Gerhard Vapodest 40 types of equipment (MSZ 6830-4; 1981)
Crude fat: Soxhlet system (MSZ 6830-6; 1981
Crude fiber: Henneberg Stohmann system (MSZ EN ISO 6865; 2001)
NDF, ADF, ADL: Van Soest system (1967)
Crude ash: sample burning on 600 Celsius° (MSZ ISO 5984; 1992)
Examination of pH: used electric Digital pH Meter OP-211/1
Examination of NH_3: We measured NH_3 from watery extract, with OP264/1 NH_3 measuring device
Organic acids: lactic acid and volatile fatty acids (acetic-, butyric-, propionic acid) with the type of Young Lin 6100 Acme 6100 gaschromatograph device, applied FID detection. The type of directing softwere Autochro 3000.
Examination of ethanol: We determined it from watery extract with $K_2Cr_2O_7$ solution and through titration with Mohr-salt-solution.

Water soluble carbohydrates(WSC)system:D.Hillegis-G.Pahlow, FAL Braunscweig, Germany

Determination of aerobic stability – System Völkenrode (Honig, 1990)
Principle: To determine aerobic stability of the silages a method by Honig (1990) was modified and implemented. It is based on monitoring temperature wich rises due to increased microbial activity of samples exposed to air. The measurement was continued for 7 days. The registration of the temperature of the samples was realized in every hour by computer.
Evaluation: The time till that the silage is supposed to be stable is the registration unit shows a temperature rise of 3°C above ambient temperature (last at least 48 h).

Statistical analyses:
Full statistical analyse was using an internationally recognised statistical procedure.
We processed data by means of IBM PC computer with the aid of Microsoft Excel program. As method of mathematical statistics, we used the method of comparison of calculated mean values and significance. Significance was declared for $P<0.05$.

RESULTS

The most important characteristics of whole crop wheat and silages are shown in *table 2 and 3.*

Table 2. Chemical composition and nutritive value of whole crop wheat and silages

Parameters		Fresh raw material	Silages			
			4 weeks storage		6 weeks storage	
			Control T1	Treated T2	Control T3	Treated T4
		Mean n=5	Mean n=5	Mean n=5	Mean n=5	Mean n=5
Dry matter	**%**	43.7	45.7	45.9	45.6	45.2
Crude protein	**g/kg DM**	89	81	80	79	78
MPE	**g/kg DM**	71	66	66	65	65
MPN	**g/kg DM**	53	49	48	47	47
Crude fat	**g/kg DM**	26	18	15	18	20
Crude fibre	**g/kg DM**	392	314	321	324	327
Crude ash	**g/kg DM**	69	61	59	67	65
NE(m)	**MJ/kg DM**	5.56	5.31	5.27	5.23	5.27
NE(g)	**MJ/kg DM**	3.14	2.92	2.89	2.85	2.89
NE(l)	**MJ/kg DM**	5.22	5.19	5.17	5.13	5.15
WSC	**g/kg DM**	72	26	23	26	29
NDF	**g/kg DM**	743	615	611	633	620
ADF	**g/kg DM**	408	319	334	336	338
ADL	**g/kg DM**	68	68	73	66	61
Cellulose	**g/kg DM**	340	251	261	270	277
Hemicellulose	**g/kg DM**	335	296	276	297	282

Table 3. Fermentation products in whole crop wheat silages

Parameters		Fresh raw material	Silages			
			4 weeks storage		6 weeks storage	
			Control T1	Treated T2	Control T3	Treated T4
		Mean n=5	Mean n=5	Mean n=5	Mean n=5	Mean n=5
Dry matter	**%**	43.7	45.7	45.9	45.6	45.2
Buffer capacity		15	--	--	--	--
Lactic acid	**% of DM**	--	12.5	20.5	12.3	13.7
Acetic acid	**% of DM**	--	3.3	9.5	3.0	9.0
Butyric acid	**% of DM**	--	4.8	2.5	4.3	1.5
Propionic acid	**% of DM**	--	0.0	0.3	0.0	0.6
Ethanol	**% of DM**	--	1.2	1.1	0.9	1.5
Ammonia	**% of total N**	--	8.3	7.2	8.1	7.4
pH		--	4.1	4.1	4.1	4.2

CONCLUSIONS

The conclusion of whole crop wheat ensiling experiments-are the followings:
-The basic whole crop raw material originated from waxen ripeness of wheat (hard cheddar stage of maturity of seeds) at the time of harvesting. The DM content of chopped raw material was 44%.
-The average net weight of filled micro silo-containers was 1878-1882 g, the density of silos was 0,442-0,443 t /m^3 which corresponds to a density of 194 kg DM/m^3 .
The statistical analyse confirmed the similar density of the microsilos. There was no significant difference (P>10%) among the density of different treated microsilos.
-The average pH at silo opening was 4,1-4,2 suggesting that the treated and also untreated silages were well fermented.
-There was no significant effect of treatments on water soluble carbohydrate, protein- and netto energy content of silages .
-Lactic acid and acetic acid were the main fermentation products. High concentrations of acetic acid were found in the treated silages, indicating a hetero fermentative pathway thanks to the activity of *Lactobacillus buchneri.*
-There were significant differences between higher lactic acid content of treated silages and the the control after 4 weeks of fermentation (P< 5%) but there were no significant differences on 6 weeks samples.
-Lactic acid and acetic acid ratio was better in treated silages, which results better palatability and consumption for ruminant animals.
-The inoculant-treated silages contained less undesirable butyric acid than that of control silages. The difference is significant (P< 5) after 6 weeks of storage. The butyric acid is an undesirable substance of silages, because it is dangerous for the health of ruminant animals.
-The protein degradation was higher in control silages which was showed by their higher NH_3 content.
-On aerobic condition neither treated nor control silages did not heat up more than 3 C° during 7 days of exposure to the air.

-DM losses in bacterial treated silages were lower compare to the control -both after 4 and 6 weeks storage silages- during aerobic stability experiment, but the difference was not significant.
-Because of the same good stability of untreated silages, the advantages of LAB treatment could not proven by the aerobic stability test.
-The applied dosage of biological preservative (LAB+enzymes) promoted better fermentation and forced back the undesirable butyric acid production in the silages.

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