Use of Biological Additives with Grass Containing Medium and High Levels of WSC for Effective Conservation and Aerobic Stability

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Abstract
The objective of the trials was to determine the effect of two silage inoculant strains *Lactobacillus buchneri* and *Propionibacteria acidipropionici* on grass containing medium and high levels of WSC for fermentation characteristics and aerobic stability of silages. The basic raw materials originated from second growth cut grasses from 2 different plots of the farm: 1st.grass components were a mixture of grass- and leguminous species, contained medium 1.5-3.0% WSC/FM. The 2nd grass components was mainly grass species; with high>3% WSC/FM content.
We stored the filled micro (4.2 litre) containers on ambient temperature. It was proven that both inoculant strains significantly decreased lactic acid content ($P<0.01$) and increased acetic acid content ($P<0.001$) of silages and significantly increased the aerobic stability as well. The best aerobic stability>240 hours was the *Lactobacillus buchneri* treated silages originated from medium WSC/FM grass. The main advantage of treatment of *L. buchneri* on grass compared to *P. acidipropionici* is the longer aerobic stability of silage ($P<0.001$).
There was no significant differences among the microbiological profile neither of treated nor of control silages.

Keywords: aerobic stability, grass silage, *Lactobacillus buchneri*, *Propionibacterium acidipropionici WSC*

1. Introduction

The concentration of WSC in forage for ensilage is important to ensure that the crop is acidified rapidly by lactic acid producing bacteria thereby conserving nutrients, reducing losses and providing effective preservation of the ensiled herbage on long-term storage of the ensiled herbage. Lack of availability of WSC or an inappropriate epiphytic microbial population on the crop often results in the production of silages of poor nutritional and hygienic quality and reduced aerobic stability [1].
Silages when exposed to air sooner or later deteriorate as a result of aerobic microbial activity. The aerobic deterioration usually results the loss of important nutritional components by the oxidation of lactic acid and water soluble carbohydrates (WSC) [2, 3, 4]. The concentrations of lactic acid and WSC usually decrease very rapidly as they are used as substrate by the aerobic microorganisms [5, 6].
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. Recently the heterofermentative *L. buchneri* is regarded to be the most promising lactic acid bacteria for increasing aerobe stability. Applied by itself it may show a negative effect by reducing the speed of fermentation. According to Ruser et al. [7] 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 et colab. [8] 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 unidentified metabolites with

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antifungal activity. Some studies showed that the primary reasons for the ineffectiveness sometimes for the fermentation of propionic acid bacteria such as *Propionibacterium acidipropionici* include the facts that they are strict anaerobes, slow growing, relatively acid intolerant and they have proteolytic activity. They are able to convert lactic acid and glucose to acetic and propionic acids that are more antifungal than lactic acid, therefore can improve the aerobic stability of silage [9].

2. Materials and methods

Grasses with two different WSC content were harvested for making silage.

The basic raw materials originated from second growth cut grasses from 2 different plots of the farm: 1st.grass components were a mixture of grass- and leguminous species, contained medium 1.5-3.0% WSC/FM. The 2nd. grass components were mainly grass species; with high>3% WSC/FM content.

The a dry matter (DM) content of raw materials: 24% and 26% respectively, and the determined WSC content was 2.9% and 5.2% on FM basis.

The grass was mechanically harvested at a chop lenght of 2.4 cm

The applied treatments were: *L. buchneri* NCIMB 40788 at 2 dosages: 1.0 x 10^5 cfu/g FM (T2) or 3.0 x 10^5 cfu/g FM (T3), and *P. acidipropionici* MA 26/4U at 1.0 x 10^5 cfu/g FM (T4).

Each bacterial inoculant was diluted in distillated water and then sprayed onto the fresh forage. The negative control (T1) received the same amount of water than the other 3 treatments. For each treatment, 6 small sized containers of 4.2 l cubic capacity each were used, closed by screwed cap (altogether 24-24 pieces). The mini-silos were stored at 20-22ºC ambient temperature and opened after 135 and 185 days ensiling (3 replicates each). The laboratory examinations focused primarily on the fermentation products and the microbiological analysis for lactic acid bacteria (LAB), yeasts and moulds count. The aerobic stability of silages was determined by the system of Völkenrode, Honig (1990) and set for the time to reach a 3°C increase above ambient temperature. Data were compared using the student-t-test of Microsoft Excel program. Significance was declared for P<0.05.

3. Results and discussion

3.1. Silages originated from medium WSC content of grass

The density of silos was 470-485 kg/m^3 which correspond to a density of 113-116 kg DM/m^3. Lactic acid content was lower respectively in the treated T2, T3 and T4 silages than that of T1 control silage (P<0.001). Acetic acid content was essentially higher in all treated silages compared to the control. The difference was very strong (P<0.001) in case of T2 and T3 treatments and still pronounced (P<0.01) in case of T4 silages. Ethanol production was almost twice as much higher (P<0.05) in treated silages than in control (Table 1).

Control silage was stable for 146 hours, while it lasted 234 hours for T4 treated silages (Figure 1). The stability of T2 and T3 silages surpassed the control and T4 silages, they remained unspoiled longer than the experimental period (>240 hours) (P<0.001). Less DM losses were detected in aerobic condition for all treated silages (P<0.001). The fresh material and the treated silages were healthy, containing very small number of yeasts and moulds.

The microbial profile of silages showed that the LAB treated forages did not increase the number of mesophyl LAB at the end of fermentation and storage (Ns).

3.2. Silages originated from high WSC content of grass

The density of silos was 496 kg/m^3 which correspond to a density of 127 kg DM/m^3.

The statistical analysis confirmed the similar density of the microsilos. There was no significant difference (P>0.1) among the density of different treated microsilos.

Table 2 shows that the pH of the silages was between 4.3-4.8. Control silages have the lowest pH while treated silages have significantly higher pH.

The statistical analysis showed significant differences in pH between T1 control and T2 (P<0.001). T1-T3 (P<0.01) and T1-T4 (P<0.001) level. The pH of T3-T4 was also different (P<0.01).

Table 1. Fermentation products and aerobic stability of *Lactobacillus buchneri* and *Propionibacterium acidipropionici* treated grass silages on DM basis (Silages originated from medium WSC content of grass)

Parameters		Treatments							
		T1		T2		T3		T4	
		Untreated control		LB $1x10^5$ cfu/g FM		LB $3x10^5$ cfu/g FM		PA $1x10^5$ cfu/g FM	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd
Dry matter	%	24.91	1.97	22.43	0.18	23.95	0.51	24.19	0.29
pH		4.53^{a}	0.30	4.96^{b}	0.06	5.02^{b}	0.04	4.93^{b}	0.07
Lactic acid	%	7.21^{a}	0.89	3.03^{b}	0.23	2.94^{b}	0.72	2.61^{b}	0.36
Acetic acid	%	1.92^{a}	0.70	3.31^{b}	0.31	3.28^{b}	0.22	2.77^{c}	0.13
Butyric acid	%	0.000	0.00	0.000	0.00	0.000	0.00	0.007	0.02
Propionic acid	%	0.02^{a}	0.04	0.13^{b}	0.05	0.09^{b}	0.04	0.14^{b}	0.05
Ethanol	%	1.05^{a}	0.14	1.83^{b}	0.49	2.03^{b}	0.30	1.67^{b}	0.31
Ammonia	% of total N	17.36	3.43	18.56	1.09	19.61	0.85	17.56	1.38
DM losses	%	6.09^{a}	1.95	0.00^{b}	0.00	0.00^{b}	0.00	0.75^{b}	1.83
Aerobic stability	hours	146^{a}	27.7	$>240^{b}$	-	$>240^{b}$	-	234^{b}	14.3

a,b,c: mean values within a row with no common superscripts differ significantly (p<0.05)

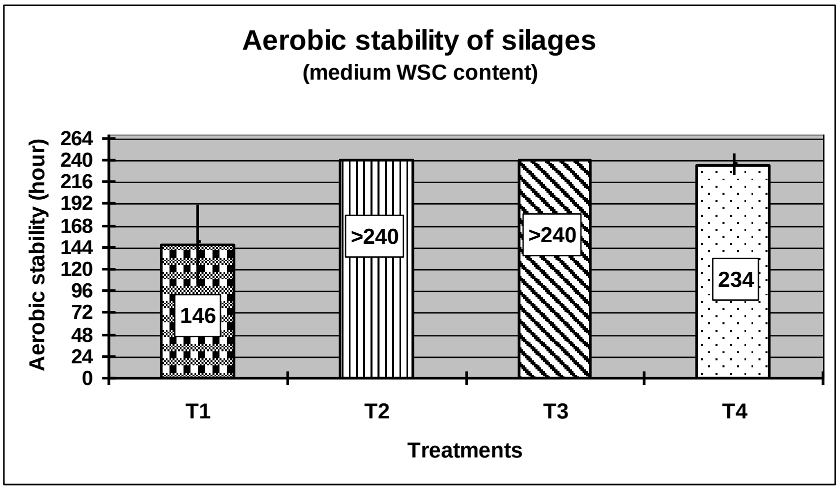

Figure 1. Aerobic stability of silages (medium WSC content)

Lactic acid content was lower respectively in the treated silages than that of control. Compare to the control silage: there was significantly less lactic acid content in T1 silage (p<0.01) and T3 silage (P<0.05) and there was a trend for the difference (P<0.1) between T1 and T4 silages.

Acetic acid content was essentially higher in all treated silages compare to the control. The difference was very strong (P<0.001) in all bacterial treatments.

Propionic acid could be found only T2, T3 and T4 silages, which differed significantly from control in case of T2 and T4 silages.

Ethanol production was similar in control, T2 and T3 silages, and twice more ethanol produced in T4 silages, which differed (P<0.05) level) of significance (T1-T4).

Less ammonia production was detected in control silages than that of treated ones. The level of significance was the following: (P<0.01) in T1-T2 and T1-T4, and there was no significant differences (ns) in T1-T3 treated silages.

DM losses in bacterial treated silages were lower compare to the control during the aerobic stability experiments. Essentially less -only a quarter of DM loss was detected in T2 and T3 treated silages compared to control (see Table 2).

Table 2. Fermentation products and aerobic stability of Lactobacillus buchneri and Propionibacterium acidipropionici treated grass silages on DM basis (Silages originated from high WSC content of grass)

Parameters		Treatments							
		T1		T2		T3		T4	
		Untreated control		LB 1x10^5 cfu/g FM		LB 3x10^5 cfu/g FM		PA 1x10^5 cfu/g FM	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd
Dry matter	%	27.61	0.34	25.54	0.24	27.99	0.51	24.77	0.08
pH		4.26^a	0.15	4.60^{bc}	0.13	4.51^b	0.15	4.76^c	0.16
Lactic acid	%	3.26^a	0.81	2.11^b	0.55	2.36^b	0.54	2.58^{ab}	0.67
Acetic acid	%	1.59^a	0.18	4.03^b	0.41	3.43^b	0.69	3.92^b	0.25
Butyric acid	%	0.00	-	0.00	-	0.00	-	0.00	-
Propionic acid	%	0.00^a	-	0.031^b	0.02	0.011^a	0.02	0.032^b	0.01
Ethanol	%	1.34^a	0.68	1.57^a	0.78	1.46^a	1.11	2.79^b	1.29
Ammonia	% of total N	9.07^a	0.77	11.04^b	1.43	10.19^{ab}	0.71	11.14^b	0.94
DM losses	%	3.62	2.29	0.92	1.42	0.97	1.85	2.59	3.08
Aerobic stability	hours	128^a	22	$>205^b$	55	$>211^b$	45	146^a	58

a,b,c: mean values within a row with no common superscripts differ significantly ($p<0.05$)

Figure 2 shows the aerobic stability of silages. It can be stated, that all bacterial treated silages remained stabile longer than the control.

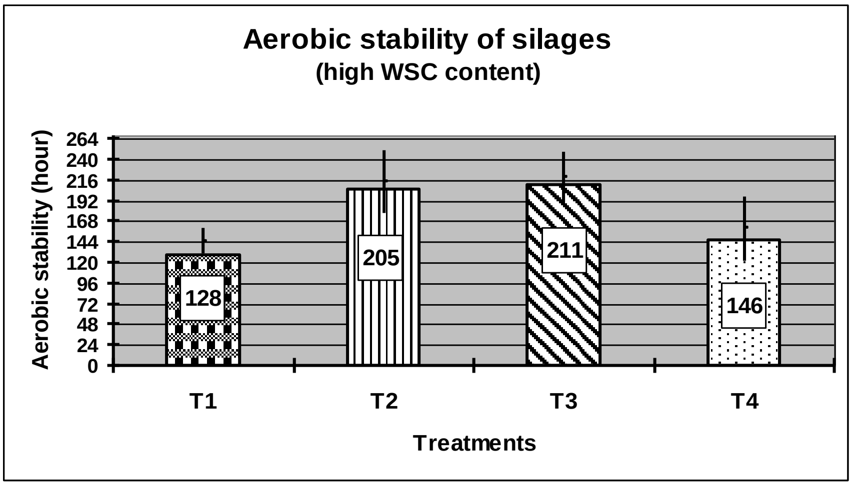

Figure 2. Aerobic stability of silages (high WSC content)

The control silages were stabile until 128 hours, while T4 treated silage until 146 hours. The T2 silages were stabile till 205 hours and T3 silages till 211 hours average.

The statistical analysis proved significantly increased aerobic stability of T2 and T3 silages ($P<0.01$) compare to control (Table 2.)

There was no significant difference between T1-T4 samples.

The grass raw material for ensiling contained very small number of mesophyl lactic acid producing bacteria. The raw material was healthy, containing small number of yeasts and moulds.

The microbial profile of silages shows that the treatments with lactic acid bacteria increased the number of mesophyl lactic acid bacteria. The remaining number of lactic acid bacteria was 10^5 respectively in T2, T3 and T4 silages at opening, while it was 10^3 in the control.

The silages remained healthy, there were no yeasts in considerable amount (<10). The number of moulds decreased compared to the raw material (9.8x 10^2 cfu/g) down to 7.5 – 2.5 x 10^1.

It can be proven, that the deterioration was the largest in control silages.

Among bacterial treated silages, the T4 treated samples spoiled earlier than T2 and T3 ones.

4. Conclusions

Treatments with biological inoculants improved fermentation and aerobic stability both of grass silages originated either medium- or high WSC containing row materials.

Lactobacillus buchneri (NCIMB 40788) on grass compared to *Propionibacterium. acidipropionici* (MA 24/4U) resulted in longer aerobic stability of silage.

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