THE EFFECT OF WILTING AND SODIUM BENZOATE BASED ADDITIVE ON SILAGE QUALITY OF GRASS AND LEGUME-GRASS MIXTURE CROP

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SUMMARY: *The aim of the present investigation was to improve silage quality by using silage additive, based on sodium benzoate and hexamine or by wilting of the herbage.*

Six forage stands were used in this study. Four stands consisted of pure grasses and two stands were legume-grass mixtures composed as follows: cocksfoot, bromegrass, reed canarygrass, timothy, lucerne-grass mixture and white clover-grass mixture. For each forage stand three cutting frequencies were applied, i.e. 4-, 3- and 2-cuts. The first three consecutive cuts of 4-cuts and from the first two cuts of 3-cut treatments were ensiled in 3 litre glass silos for 100-120 days. The harvest times and herbage development stages varied but most of the grasses were at the leaf stage.

The following silage treatments were applied: (a) untreated, (b) benzoate/hexamine (Superben) treated at an active matter of 1600/700 g ton^{-1} fresh matter (FM) and (c) crop wilted in the field for 22- 24 hours, without September.

The quality of the untreated silage was satisfactory but a poor fermentation was obtained, reflected in high pH values, in low levels of lactic acid and in relatively high concentrations of butyric acid. Application of Superben to direct cut herbage halved the concentration of butyric acid when compared with untreated herbage ($P<0.0001$). Wilting doubled the silage DM concentration. The microbial activity was restricted due to osmosis and less butyric acid, other organic acids and ammonia were produced in the wilted silage. The fermentation pattern of the legume-grass mixture silage was somewhat superior to that of the grasses. The mixed silage had a higher nutritive value and slightly lower butyric acid, acetic acid and ammonia concentrations at a significance level of $P<0.05$. The conclusion is that addition of Superben to various silage crops, or crop wilting improves silage fermentation.

Key words: *butyric acid, dry matter, fermentation, hexamine, lactic acid, organic acid.*

Introduction

Silage making is easy to adopt to early harvesting and in the optimal time when the nutrient concentration of the herbage is expected to be sufficiently high. A high nutritive value of the forage has a great impact on feeding cattle and dairy cows at high productivity levels. Young, fresh forage is usually rich in crude protein (CP) and low in DM but the water soluble carbohydrate (WSC) concentration varies to a great extent (Pettersson, 1988). Thus, adequate fermentation is not always guaranteed. In addition, legumes are generally low in WSC and have a high buffering capacity, features which can cause difficulties on ensiling (McDonald *et al.*, 1991). A common measure for overcoming the principal problems with a freshly cut crop is rapid water reduction in the herbage by intensive wilting. Thereby the microbial activity is restricted due to osmosis and lower amounts of undissociated organic acids, mainly lactic acid are needed to suppress undesired microbes (Lindgren, 1991). Wilting increases the WSC concentration in the water phase, thereby increasing the concentration of available substrate for the lactic acid bacteria. Thus, more favourable fermentation conditions are created by wilting to obtain a high quality silage without serious effluent losses.

On the other hand, under Estonian climatic conditions it is often impossible to wilt grass when necessary. Therefore, chemical or biological additives have to be applied. On large cooperative farms in Estonia the liquid silage additive *Siloben*, based on the industrial byproduct benzoic acid, has been used in making bunker silage. *Siloben* is an aqueous solution of 30-35 % *sodium benzoate* which is added to herbage at an application rate of 8-15 litre ton^{-1} FM depending on the silage crop. In 1987, the Estonian Research Institute of Agriculture developed an improved variant, namely Superben. It is based on Siloben, but contains 11-17 % of hexamethylenetetraamine (hexamine). An appreciable amount of hexamine would effectively inhibit the growth of silage microorganisms and is effective against yeasts and moulds (Woolford, 1975). Benzoic acid is a relatively weak acid, but it and its salts possess disdinct antimicrobial properties and therefore benefits preservation. Sodium benzoate has been shown to inhibit heterofermentative lactic acid bacteria and encourage homolactic fermentation in silage (McDonald *et al.*, 1991). When *Siloben*, *Superben*, *Ester-3* (containing acetic acid and formaldehyde) and *AIV-2* (containing mainly formic acid) were used in large bunker silos at the Laboratory of Feeds during 1984-1988, these additives had variable effects on the quality of grass silage. Sodium benzoate-treated silages had a higher stability when exposed to air: for 9-10 days, *vs*. 5-7 days when *Ester-3* and *AIV-2* were used. In some respect the silages treated with *AIV-2* and *Ester-3* were of better quality. However, the sodium benzoate treatment also tended to have a preservative effect at higher pH and prevented it declining (Older *et al.*, 1991).

Objective

The aim of this experiment was to study the effectiveness of an additive based on sodium benzoate and hexamine and the effect of wilting on silage quality of various types of crops. The crops comprised pure grass species and legume-grass mixtures harvested at different frequencies and stages of emergence.

Materials and Methods

Six forage stands were ensiled in this study. Four stands consisted of grasses only and two stands were legume-grass mixtures as follows: cocksfoot ”Y 220” (*Dactylis glomerata*), bromegrass ”Lehis” (*Bromus inermis*), reed canarygrass ”Pedja” (*Phalaris arundinacea*), timothy ”Y 54” (*Phleum pratense*), lucerne-grass mixture (*Medicago sativa, Festuca rubra*, about 40 % lucerne and 60 % grass) and a white clover-grass mixture (*Trifolium repens, Festuca pratensis, Phleum pratense, Lolium perenne, Festuca rubra*, about 70 % white clover and 30 % grasses). Stands were established on a 0.8 ha paddock at the Juuliku Experimental Farm in order to study the forage grass at different cutting regimes (see *Materials and Methods* of the paper entitled *Relations between yield and nutritive value of grass or grass-legume mixtures at different cutting regimes*). The forage for silage was taken from the first three consecutive cuts of 4-cuts and from the first two cuts of 3-cut treatments of six stands. The herbages were at the leaf development stage, except for the first cut of the 3-cut treatments at the heading stage. The results of the chemical analyses of the silage crops are presented in Table 1. Treatments were as follows: (a) untreated, (b) *Superben*-treated and (c) material wilted in the field for 22-24 hours. An untreated variant served as a negative control. *Superben* was applied to a direct cut herbage at an application rate of 5 litre ton^{-1} FM (1600 g sodium benzoate and 700 g hexamine). When the crop was wilted, no additive was applied. Treatments were randomly assigned. The herbage was chopped to 5 cm length, mixed thoroughly with additive where applicable and ensiled in 3 litre glass jars with 3 replicates each. A total of 90 treatments × 3 replicates = 270 silos were prepared. The jars were closed immediately after filling with rubberglove. Silos were opened 100-120 days after filling and samples were taken for chemical analyses. Samples of 3 replicates of each herbage treatment were pooled and one sample was submitted for analysis, thus 90 samples in all. The following determinations were made: toluene DM (dried and corrected for volatiles according to Lingvall & Ericson, 1981), pH, crude protein (a Kjeldahl method), crude fibre (according to Kürschner & Hanak, modification of Lenkeit & Becker), crude fat (Niine, 1982), WSC (Potshinok, 1958), ammonia nitrogen (volatile N fraction in silage juice distilled on a Kjeltec Autosystem, then N determination of the distillate by a Kjeldahl method), lactic acid and VFA (used gas cromathograph and according to Hacker, Block, Weissbach, 1983) and metabolisable energy (Oll, 1994).

Table 1. The chemical composition of silage crop. The results are averaged over herbage species and cuts

Crop	n	DM[a]	CP[b]	CF[c]	CFT[d]	Crude ash	WSC[e]	ME[f]
		$g\ kg^{-1}$	$g\ kg^{-1}$ DM					$MJ\ kg^{-1}$ DM
Grasses	20	215±41	139±34	274±31	26±6	77±12	78±22	10.5±0.6
Legume-grass mixtures	10	229±50	164±45	235±25	23±4	92±16	70±14	10.8±0.6

[a] Dry matter
[b] Crude protein
[c] Crude fibre
[d] Crude fat
[e] Water soluble carbohydrates
[f] Metabolizable energy

Statistical methods. Results were examined statistically by analysis of variance according to the following model:

Model: $Y_{ijk} = \mu + \text{treatment}_i + \text{crop}_j + (\text{crop} \times \text{treatment})_{ij} + \text{error}_{ijk}$

(i=3 treatments, j=2 crop type, grasses and legume-grass mixtures)

μ – overall mean

n=90

All statistical analyses were carried out by the GLM procedure of the computer package SAS (SAS Institute Inc., 1989).

Results

Results will focus on the general effects of a) an additive based on sodium benzoate and hexamine and b) herbage wilting compared to negative control on chemical composition and fermentation of silage. The results of the chemical analyses on the silages are presented in Tables 2 and 3. The average DM concentration of silage was similar to that of the ensiled crop. When the crop was wilted, the silage DM was approximately doubled. The differences in chemical composition between treatments were only marginal and not significant (Table 2). The average CP concentration of the grass silages was at a moderate level, while the CP concentration was 18 % higher in legume-grass mixture silage (Table 3). Because the crude fibre (CF) concentration of the mixed crop was 11 % lower than that of the grasses, the mixed silage also had higher ME concentration.

The untreated silages had the lowest quality. In this silages the concentration of butyric acid was higher than acceptable for good quality. The rapid fermentation is followed by a corresponding decrease in pH, but the pH value of the untreated silage was higher than acceptable for a low DM concentration. Butyric acid production was related to ammonia production (Table 2). The application of *Superben* reduced the butyric acid concentration to about half of that in the untreated silage. The pH value of the *Superben* treatment was slightly higher than that of the untreated silage, but due to the special qualities of this additive it improved silage quality. The total ammonia value (83 g NH_3-N kg^{-1} tot-N) was close to the limit for good quality silage (below 80 g kg^{-1} of tot-N), but after subtraction of NH_3 from the *Superben* the value was 34 g NH_3-N kg^{-1} tot-N which is a low level.

The wilting of herbage restricted microbial activity and less fermentation products were produced. The concentrations of lactic acid and acetic acid were considerable lower in wilted than in untreated silage, but the proportion of acetic acid was slightly increased. This is evidence of increased activity of heterofermentative lactic acid bacteria as opposed to homofermentative bacteria. The pH value was highest in wilted silage, but pH is not a good indicator at high dry matter levels. The concentrations of butyric acid and ammonia, as parameters of silage quality, were lowest (Table 2).

Table 2. The treatment effect on chemical composition and fermentation products of silage. The results are averaged over treatments, (n=30) each treatment

Treatment	pH	DM[a]	CP[b]	CF[c]	CFT[d]	Crude ash	NH_3-N[e]	Lactic acid	Acetic acid	Butyric acid	ME[f]
		gkg^{-1}	$gkg^{-1}DM$					$gkg^{-1}FM$			$MEkg^{-1}DM$
Untraeted	4.82	206	144	290	33	102	90	7.5	2.6	1.7	9.9
Superben ($5\ l\ ton^{-1}$ FM)	4.95	218	148	285	35	100	83 (34)[i]	6.6	2.5	0.8	10.0
Wilted	5.19	398	141	296	36	96	65	2.6	1.1	0.7	9.8
Average	4.99	272	145	290	35	100	79	5.6	2.1	1.1	9.9
LSD[g]	0.18	21.3	11.6	14.4	3.5	4.8	10.8	2.2	0.5	0.4	0.2
P>F[h]	0.001	0.0001	0.43	0.35	0.22	0.06	0.0001	0.0001	0.0001	0.0001	0.24

[a] Dry matter
[b] Crude protein
[c] Crude fibre
[d] Crude fat
e Ammonium content, g NH_3 kg^{-1} total-N
[f] Metabolizable energy
[g] Least significant difference of a treatment mean; n=30
[h] Probability of a larger F-value
i Corrected for NH_3-N in the silage additive

Table 3. The effect of crop type on chemical composition and fermentation products of silage. The results are averaged over grasses n=60, and legume-grass mixtures, n=30

Silage group	pH	DM[a]	CP[b]	CF[c]	CFT[d]	Crude ash	NH_3-N[e]	Lactic acid	Acetic acid	Butyric acid	ME[f]
		gkg^{-1}	$gkg^{-1}DM$					$gkg^{-1}FM$			$MEkg^{-1}DM$
Grasses	4.97	278	136	301	36	95	83	6.4	2.3	1.2	9.8
Legume-grass mixtures	5.02	260	161	268	34	109	73	4.0	1.6	0.8	10.1
Average	4.99	272	145	290	35	100	79	5.6	2.1	1.0	10.0
LSD[g]	0.16	21.2	13.4	13.7	3.4	5.0	9.4	2.0	0.56	0.38	0.19
P>F[h]	0.51	0.11	0.001	0.0001	0.23	0.0001	0.001	0.02	0.03	0.04	0.01

[a] Dry matter
[b] Crude protein
[c] Crude fibre
[d] Crude fat
e Ammonium content, g NH_3 kg^{-1} total-N
[f] Metabolizable energy
[g] Least significant difference of a treatment mean; n=30
[h] Probability of a larger F-value

In Table 3 the results corresponding to the silage crop are presented. The average butyric acid and ammonia concentrations were rather lower in the legume-grass mixture than in the grass silage. Despite the fact that forage legumes are considered difficult to ensile, the silages containing legumes appeared to be of superior quality.

Discussion

The success of fermentation and consequently silage quality is dependent on several factors. Weissbach (1974) concluded that silage fermentation is determined by the sugar concentration of the crop, its buffering capacity and dry matter concentration. Weissbach (1974) summarized the results of earlier studies and expressed the fermentability coefficient of herbage as follows: FC=DM+80 X, (where DM, g kg^{-1} and X=the ratio of available sugars to buffering capacity) which is applicable in ideal circumstances and which is actually based on the critical pH-theory of Virtanen. According to this theory a butyric acid-free silage is expected below the critical pH level which depends on the buffering capacity and on the amount of organic acids, mainly lactic acid, produced during fermentation. In silage with a DM concentration below 250 g kg^{-1}, pH 4.2 is regarded as satisfactory (McDonald *et al.*, 1991). With an increase in DM bacterial multiplication is delayed and fermentation restricted, resulting in higher pH-values. At DM concentrations above 350 g kg^{-1}, the pH-value is no longer a good parameter for fermentation (Pettersson, 1988). By the formula of Weissbach (1974) butyric acid fermentation is normally not expected when FC>450. This value can easily be obtained for high DM crops. When the DM is low, sufficient sugars are needed to reach this value or to obtain a critical pH.

In the present study the concentrations of WSC and DM of the silage crops varied to a large extent, but were similar for grasses and legume-grass mixtures. In general, the early cut crop had lower DM and higher CP concentrations. The WSC concentration tended to be lower in early harvests. In general, the results indicated that untreated silages had the highest average butyric acid and ammonia concentrations, but they fell within satisfactory limits. Concentations of butyric acid below 1, from 1 to 3 and higher than 3 g kg^{-1} FM are accepted as indicating good, moderate, and bad silage, respectively (Spördnly, 1993). Butyric acid is mainly produced by vegetative saccharolytic clostridial cells. Despite the fact that other microorganisms are able to produce butyric acid (e.g. yeasts, bacillus), their contribution is usually small (McDonald *et al.*, 1991).

The results showed the importance of using additives or herbage wilting to improve the quality of silage made from freshly cut herbage. The intensive wilting of herbage for 22-24 hours increased silage DM concentration up to 398 g kg^{-1}. Under anaerobic conditions this DM level generally is high enough to restrict the growth of undesirable miroorganisms, particularly certain clostridia. Woolford (1984) claimed that the activity of clostridia is already restricted at a DM level above 300 g kg^{-1}. However, at higher DM concentrations inhibition may be even greater. It has been estimated that there is no necessity for additives beyond 400 g DM kg^{-1} and butyric acid free silage may be expected at DM concentrations above 450 g kg^{-1} (Lingvall, 1995). These results are in accordance with those generalized by Weissbach (1974). The results of the present study confirm these earlier findings. Wilting considerably restricted microbial activity and less fermentation products, i.e. volatile fatty acids, lactic acid and ammonia, were produced. Ammonia is released mainly when the proteolytic clostridia deaminate amino acids. Because the activity of clostridia was restricted, proteolytic processes were also supressed in the wilted herbage. The effects of wilting on silage quality were comparable to those of adding Superben to direct cut herbage. An important aspect of wilting was to eliminate silage effluent. Küntzel (1991) claimed that only ensiled forage grass of less than 300 g DM kg^{-1} produces effluent.

The main effect of using *Superben* was to lower the butyric acid concentration to about half of that untreated silage. Weissbach *et al.* (1991) reported that silage treated with the salts of acids undergo a restricted fermentation. A higher pH-value of the silage treated with *Superben* may thus be expected. The inhibitory effect of *Superben* arose from the interaction of sodium benzoate and hexamine. Woolford (1975) suggested that the minimum inhibitory concentrations of sodium benzoate and hexamine against clostridia at pH 5 were 7 and 1 mM, respectively. At lower pH both components are more effective against various groups of microorganisms, especially yeasts and moulds. Hexamine liberates formaldehyde and ammonia under acid conditions and in the presence of available water. Formaldehyde, in addition to sodium benzoate has an even greater inhibitory effect on the silage microorganisms. However, despite the fact that silage quality was improved considerably when using 1600 g of sodium benzoate and 700 g kg^{-1} FM of hexamine in active matter, butyric acid production was not avoided. This was obviously due to the high pH-value. The activity of lactic acid bacteria was restricted, probably due to excessive doses of hexamine, resulting in high pH value. However, this gave the clostridia some competitive advantage. Gross and Beck (1972) suggested the effective mixture of hexamine and sodium nitrite to be at least 400 g and 800 g ton^{-1} FM, respectively.

Conclusions

The quality of silage made from various direct cut crops was satisfactory without treatment. Either, application of an additive based on sodium benzoate and hexamine or crop wilting favoured the fermentation and resulted in good quality silage. Silages made from legume-grass mixtures were slightly better fermented and had a higher nutritive value.

KOKKUVÕTE: Närvutamise ja naatriumbensoaati sisaldava konserveerimislisandi mõju kõrrelistest ja ristikust-kõrrelistest koosnevast rohust valmistatud silo kvaliteedile.

Käesoleva töö eesmärgiks oli silo kvaliteedi parandamine, kasutades konserveerimislisanditena naatriumbensoaati ja heksamiini või valmistades silo närbutatud rohust.

Uuriti 6 rohukooslust, neli neist olid puhasliigilised, kaks ristiku-kõrreliste segud. Puhasliikidest olid katses harilik kerahein, ohtetu püsikluste, paelrohi ja põldtimut. Iga rohukoosluse rohu saamisel oli rakendatud kolme niitmissagedust: kahe-, kolme- ja neljakordset niitmist vegetatsiooniperioodil. Neljakordsel niitmisel sileeriti esimesed kolm, kolmekordse niitmise puhul aga esimesed kaks niidet. Sileeriti 3 l klaasnõudesse ning lasti silol valmida 100…120 päeva. Niitmisaeg ja sellele vastav taimede kasvustaadium varieerus mõneti, kuid enamasti oli rohi kõrsumise staadiumis.

Katsevariantideks olid: a) kontroll (lisandeid ei kasutatud), b) lisati bensoaadi ja heksamiini segu (Superbeni) 1600/700 g tegevainet ühe tonni rohu kohta, c) rohtu närvutati põllul 22…24 tundi, konserveerimislisandit ei kasutatud.

Kontrollvariandi silo oli rahuldava kvaliteediga, kuid fermentatsioon oli tagasihoidlik, mida iseloomustas suhteliselt kõrge pH-väärtus, vähene piimhappe- ja rohke võihappesisaldus. Konserveerimislisandid ja rohu närvutamine parandasid silo kvaliteeti. Superbeni *kasutamine vähendas poole võrra võihappesisaldust võrreldes lisandita valmistatud siloga (P<0,0001). Närvutamise tulemusena silo kuivainesisaldus kahekordistus. Närvutatud rohu käärimine oli vähem intensiivne osmoosi tõttu, võihapet, teisi orgaanilisi happeid ning ammoniaaki tekkis vähem. Ristiku-kõrreliste silos olid fermentatsiooniprotsessid mõnevõrra intensiivsemad kui kõrreliste silos, selle toiteväärtus oli kõrgem, ka oli või- ja äädikhapet ning ammoniaaki vähem (P<0,05).*

Järeldus: Superbeni *lisamine ja rohu närvutamine aitavad silo kvaliteeti parandada.*

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