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EFFECTS OF MICROBIAL INOCULATION ON ALFALFA SILAGE QUALITY

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Mikrobiyel inokulant ile hazırlanan yonca silajının silaj kalitesi üzerine etkisi

ÖZET

Bu çalışma mikrobiyel inokulant ile hazırlanan yonca silajının değişik katkı maddeleri ile birlikte inokulasyon uygulamalarının silaj besin maddeleri ve bazı silaj kalitesi özellikleri üzerine etkilerinin incelenmesi amacıyla planlanmıştır. Deneme'de 4'ü inokulantsız olmak üzere toplam 8 silaj grubu 2x4 faktöriyel deneme düzenine göre oluşturulmuştur. Laboratuvar silosu olarak, yaklaşık 4 kg taze silaj materyali alabilen pet kavanozlardan yararlanılmıştır. Yem katkısı olarak %5 melas, %5 arpa, %1.5 tuz katılmış, inokulantlı gruplarda ise bu katkılara ilaveten her bir silaja Lactobacillus plantarum ve Streptococcus faecium 'dan oluşan bakteri kültürü (Pioneer 1174) ilave edilmiştir. Silolar aynı ortamda 240 gün kapalı tutulduktan sonra açılmıştır. Katkı maddeleri incelenen tüm parametreler üzerinde etkili olmuştur. Buna karşılık inokulant ilavesi KM, HS, NDF, pH, KM kaybı değerleri üzerine etkili olurken HP, ADF ve HK değerlerinde etkisi görülmemiştir. Araştırmada elde edilen sonuçlara göre inokulantların, yem maddelerinin tekniğine uygun olarak silolanması halinde silaj kalitesini artırıcı etkiler sağlayabileceği kanaatine varılmıştır.

ANAHTAR KELİMELER: Bakteriyel inokulant, silaj, silaj özellikleri

SUMMARY

This study was designed for investigating the effects of inoculated alfalfa silage with various additives on silage nutrients and silage quality. A 2x4 factorial design was used including 8 silage groups. Of these groups 4 of them are inoculated but rest were left non-inoculated. As laboratory silos, plastic jars that have 4 kg fresh silage material capacity were used. Bacteria cultures (Pioneer 1174; Lactobacillus plantarum and Streptococcus faecium) and additives (5% molasse, 5% barley and 1.5% salt) were added into these silage groups. The silos were stored in the same conditions and were opened after 240 days. We found that inoculation affected dry matter, crude fibre, neutral detergent fibre, pH and dry matter losses, but have no effect on crude protein, acid detergent fibre and crude ash. As a result, it may be concluded that supplementation of inoculant may have beneficial effects on silage quality only if feeds are ensiled with in proper technical conditions.

KEY WORDS: Bacterial inoculant, silage characteristics

INTRODUCTION

Silage is the feedstuff produced by anaerobic fermentation of crop, forage or agricultural by-products generally contains adequate moisture content. Objective of making silage is to store of feedstuff with minimum nutrients losses (Mc Donald 1981). There are many factors affected the silage quality such as; DM (Dry Matter) level of crops, content of water soluble carbohydrates (WSC), microorganism (naturally present on the plant), oxygen (air), temperature and DM loses (Bolsen et al. 1990, 1996a, 1996b, 1996c, Atwal 1985, Haigh and Parker 1985, Honig 1986, Pahlow and Zimmer 1985, Woolford et al. 1978, Woolford 1984).

Several silage additives may be supplied for increasing silage quality, nutrient contents and digestibility. These additives are mainly classified; as fermentation inhibitors (formic acid, propionic acid, acetic acid, urea, ammonia, i.e), fermentation stimulants (amylase, cellulase, hemicellulase, pectinase, protease, molasses, glucose, sucrose, dextrose, grains and bacterial inoculants containing lactic acid bacteria (LAB) culture (Lindgren et al.

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1983, Bolsen et al. 1996a, 1996c**,** Nadeau et al. 2000).

Microbial inoculants (or bacterial inoculants) containing LAB (lactic acid bacteria) have a major concern in ensiling of feedstuffs, crops and other by-products (Pahlow 1984, Seale 1986, Kung et al. 2003). Additives containing live LAB are called for biotechnologic silage additives (Pahlow 1989). LAB is a mixture of several microorganism including Lactobacillus plantarum, Pediococcus acidilactici, Streptococcus faecium provide homolactic fermentation in silages (Bolsen and Heidker 1985, Lindgren and Dobrogosz 1990, Pahlow and Honig 1986, Wilkinson 1985). It was reported that bacterial inoculant improve silage quality by decreasing pH, NH_3-N and dry matter (DM) loses or increasing ratio of lactic acid/acetic acid and aerobic stability (Hinds et al. 1985, Haigh et al. 1987, Kung et al. 1991, Kung and Ranjit 2001, Whiter and Kung 2001). Also, forming the fermentation gases, protein degradability and sensitivity to air (O_2) are decreased by inoculation of bacterial cultures (Pahlow 1984, Pahlow and Honig 1986, Taylor et al. 2002).

The objective of this study was to evaluate the effects of microbial inoculant on alfalfa silage quality for laboratory silos.

MATERIALS and METHODS

One field of second cutting alfalfa (25.73% DM) were obtained from the Konya Animal Research Institute. As silage additives; grounded barley, salt (NaCl) and molasses were used. Mixtures of Lac.plantarum and Str. faecium were used as a bacterial inoculant (Pioneer 1174; Pioneer HI-Bred International, Inc., Des Moines, IA, USA). Second cutting alfalfa was harvested at flower line stage with a silage chopper (5-10 cm theoretical length of cut) and transferred to feed analysis laboratory at the Faculty of Veterinary Medicine University of Selçuk. These material were minced and homogenised for filling into laboratory silos.

A total of 8 silage groups, of which 4 groups were not inoculated, were designed for 2x4 factorial design and five laboratory silos were used in each group. Plastic jars with 4 kg fresh silage material capacity were used as laboratory silos. Silage groups and additives were shown in table 1; crude nutrient analyses of silage material used in treatment were presented in table 2. An amount of 80 mg inoculant was suspansed in 180 ml chlorine free water, and 9 ml of the suspantion was used for each 20 laboratory silos. Weighed the fresh alfalfa for each plastic jar (approximately 4 kg) were spread thinly on the nylon wrap and the additives were homogenously supplied on this material. Then they were filled into laboratory silo. Then, the inoculant was sprayed into fresh silage material. Silage materials filled into plastic jars were pressed by heavy shot and these silos were covered and stored at laboratory for 8 months. After the opening top 10-15 cm of the silos were removed, and analysis of crude protein (CP) and pH were immediately made in silage material obtained from several areas of the silo. Rest of samples were dried at 60 ^{0}C for 48-72 h and grounded for nutrient analysis. The silos were weighed prior to the opening, and the DM levels of rested material in silos were measured. These values were subtracted from previous DM levels and DM loses levels were determined. Crude nutrient values in the feedstuffs used in the experiment were determined by AOAC methods (1984), acid detergent fibre (ADF) and neutral detergent fibre (NDF) levels were measured by methods of Van Soest and Robertson (1985).

SPSS programme (SPSS, 1998) was used for statistical analysis. Two factors analysis of variance was also used for any correlation (Table 3). The data were analysed by using Student's paired t-test and analysis of variance. Duncan statistical methods were used when the results were statistically different after analysis of variance.

RESULTS and DISCUSSION

In this study, the effects of inoculation and several additives on silage nutrients and quality were examined. We found that inoculation affected DM, crude fibre (CF), NDF, pH, DM losses, but have no effect on crude protein (CP), ADF and crude ash (CA). Table 3 represents the data analysed by analysis of variance according to 2x4 factorial design.

Means of DM levels between the inoculated and non inoculated groups were found different ($P<0.001$), and these values in inoculated group were higher (24.70%) than the control group (23.50%; Table 4). Results from the studies with microbial inoculants in several silage materials could be different related to silage DM level. Heron et al.(1988) reported that the DM levels of inoculant and inoculant plus glucose added laboratory ryegrass (15.33%DM) silage were 15.7% and 16.8% respectively. These results were similar with present study. Molasses supplementation to inoculated silage increased the DM level (26.54%). This increase may be related to LAB and some additives such as molasse and glucose activating the LAB. Also, increases in DM levels may be explained by wilting time of the fresh material after the cropping and removing the fresh material water in the ensiling period. In a study prepared in laboratory silos with grass plus legume silage, Lindgren et al. (1983) reported that the DM levels of control, inoculant, inoculant plus grain (10%) and only grain (10%) group were 16.6%, 17%, 23.2% and 23.4%, respectively. As expected, inoculation and inoculation plus grain supplementation to silage caused the increases of the silage DM levels. This result may be related to supplementation of grains containing high DM level. In the present study, DM levels of groups including barley (5%) and inoculated plus barley (5%) silage were found as 23.09% and 25.03%, respectively.

Table 1. Silage groups and feed supplements used in the study

Groups	Supplements and used ratios
1 (Control)	--
2	Molasses (%5)
3	Barley (%5)
4	Salt (%1.5)
5	Microbial Inoculant (1 g/ton)
6	Molasses (%5) + Microbial inoculant (1g/ton)
7	Barley (%5) + Microbial inoculant (1g/ton)
8	Salt (%1.5) + Microbial inoculant (1g/ton)

Table 2. Amounts of crude nutrients in the fresh alfalfa used in the study (based on DM)

Nutrients	%
Dry matter (DM)	25.73
Crude protein (CP)	16.18
Ether extract (EE)	3.01
Crude ash (CA)	9.04
Crude fibre (CF)	27.06
Acid detergent fibre (ADF)	39.72
Neutral detergent fibre (NDF)	51.06

Table 3. Tables of two-way ANOVA

		Dry matter (DM)		Crude fibre (CF)		Neutral detergent fiber (DM loss)		PH		Dry matter loss (NDF)	
Source	DF	MS	P	MS	P	MS	P	MS	P	MS	P
Total	39	5.92		7.57		20.86		0.02		61.24	
Between inoculant	1	14.54	0.000	3.36	0.042	4.55	0.013	0.36	0.000	242.12	0.000
Between additives	3	67.92	0.000	86.69	0.000	253.91	0.000	0.11	0.000	664.99	0.000
Interaction	3	0.84	0.067	2.56	0.029	0.295	0.721	0.03	0.000	6.99	0.180
Error	32	0.32		0.75		0.67		0.00		4.08	

DF= Degree of Freedom, MS= Mean of Squares, P= Probability

Table 4. The dry matter values of silages prepared by inoculant and different feed additives (in dry matter,%)

Additive Groups	Inoculant groups				Significance	General	
	Without inoculant		Inoculated				
	N	X ± Sx	N	X ± Sx		N	X ± Sx
Control	5	19.85 ± 0.21 c	5	21.00 ± 0.41 c	-	10	20.42 ± 0.29 c
Molasses	5	25.34 ± 0.23 a	5	26.54 ± 0.13 a	-	10	25.94 ± 0.23 a
Barley	5	23.09 ± 0.12 b	5	25.03 ± 0.15 b	-	10	24.06 ± 0.34 b
Salt	5	25.70 ± 0.29 a	5	26.23 ± 0.33 a	-	10	25.97 ± 0.23 a
Significance		***		***			***
General	20	23.50 ± 0.54	20	24.70 ± 0.52	***		

***: P< 0.001; - : P> 0.05; a-c: Means with no common superscripts within a column are significantly different

Table 5. The crude fibre values of silages prepared by inoculant and different feed additives (in dry matter, %)

Additive groups	Inoculant groups				Significance	General	
	Without inoculant		Inoculated				
	N	X ± Sx	N	X ± Sx		N	X ± Sx
Control	5	34.04 ± 0.22 a	5	33.77 ± 0.09 a	-	10	33.91 ± 0.12 a
Molasses	5	26.69 ± 0.51 d	5	26.94 ± 0.39 c	-	10	26.82 ± 0.31 d
Barley	5	31.48 ± 0.39 b	5	29.43 ± 0.22 b	**	10	30.46 ± 0.40 b
Salt	5	29.44 ± 0.28 c	5	29.20 ± 0.67 b	-	10	29.32 ± 0.34 c
Significance		***		***			***
General	20	30.42 ± 0.64	20	29.84 ± 0.60	*		

***: P< 0.001, **: P< 0.01, *: P< 0.05, -: P> 0.05; a-d: Means with no common superscripts within a column are significantly different

Table 6. The NDF values of silages prepared by inoculant and different feed additives (in dry matter, %)

Additive groups	Inoculant groups				Significance	General	
	Without inoculant		Inoculated				
	N	X ± Sx	N	X ± Sx		N	X ± Sx
Control	5	53.80 ± 0.42 a	5	53.34 ± 0.29 a	-	10	53.57 ± 0.25 a
Molasses	5	43.77 ± 0.45 d	5	42.64 ± 0.34 d	-	10	43.21 ± 0.33 d
Barley	5	51.13 ± 0.30 b	5	50.38 ± 0.15 b	-	10	50.76 ± 0.20 b
Salt	5	44.36 ± 0.28 c	5	43.99 ± 0.54 c	-	10	44.18 ± 0.29 c
Significance		***		***			***
General	20	48.26 ± 1.00	20	47.59 ± 1.03	*		

***: P< 0.001, *: P< 0.05 -: P> 0.05; a-d: Means with no common superscripts within a column are significantly different

Table 7. The pH values of silages prepared by inoculant and different feed additives (in fresh samples)

Additive groups	Inoculant groups				Significance	General	
	Without inoculant		Inoculated				
	N	X ± Sx	N	X ± Sx		N	X ± Sx
Control	5	5.02 ± 0.03 a	5	4.69 ± 0.01 a	***	10	4.86 ± 0.06 a
Molasses	5	4.94 ± 0.04 a	5	4.84 ± 0.02 a	-	10	4.89 ± 0.03 a
Barley	5	4.78 ± 0.01 b	5	4.56 ± 0.03 b	***	10	4.67 ± 0.04 b
Salt	5	4.79 ± 0.03 b	5	4.68 ± 0.02 b	*	10	4.73 ± 0. 02b
Significance		***		***			***
General	20	4.88 ± 0.03	20	4.69 ± 0.03	***		

***: P< 0.001, *: P< 0.05, -: P> 0.05; a-b: Means with no common superscripts within a column are significantly different

Table 8. The dry matter losses of silage prepared by inoculant and different feed additives (in dry matter, %)

Additive groups	Inoculant groups				Significance	General	
	Without inoculant		Inoculated				
	N	X ± Sx	N	X ± Sx		N	X ± Sx
Control	5	25.72 ± 0.86 a	5	20.56 ± 1.62 a	-	10	23.14 ± 1.22 a
Molasses	5	12.85 ± 0.87 c	5	8.29 ± 0.52 c	-	10	10.57 ± 0.90 c
Barley	5	22.92 ± 0.42 b	5	15.92 ± 0.62 b	-	10	19.42 ± 1.22 b
Salt	5	6.74 ± 0.72 d	5	3.79 ± 1.03 d	-	10	5.27 ± 0.77 d
Significance		***		***			***
General	20	17.06 ± 1.79	20	12.14 ±1.57	***		

***: P< 0.001, -: P> 0.05; a-d: Means with no common superscripts within a column are significantly different

These results can be explained that microorganisms which found the adequate levels of WSC (water soluble carbonhydrates) in silage medium may grow up and give a good quality silage with the lower DM loses. The results from Cleale et al. (1990) and Gordon (1989) agree with the present study, and it was found that the DM levels were higher in the inoculated silage groups. It was reported that the DM levels were high (P<0.05) in inoculated corn and alfalfa silage (Hunt et al. 1993, Kung et al. 1991, 1993, Whiter and Kung 2001).

There was a significant interaction between the groups of inoculant and additives (P<0.05) in terms of crude fibre values. (Table 3). However, crude fibre values were decreased in inoculation plus barley silage only (P<0.01; Table 5). The difference of level of crude fibre between the inoculant and non-inoculant groups could be result of the additives used. In a laboratory silo with corn silage (32.4% DM), there was no difference in terms of hemicellulose and cellulose between the inoculated and non-inoculated groups (Hunt et al. 1993). But, these values have been significantly decreased in both of these groups relative to fresh silage material (P<0.05). The dramatic decrease in hemicellulose level could be concluded that hemicellulose has been more hydrolysed than other components of cell wall. In present study, the mean NDF values obtained from inoculated and non-inoculated groups were found 48.26% and 47.59%, respectively; therefore, it was clear that inoculation was also found to be effective on decreasing of NDF values (P<0.05; Table 6). Harrison et al.**(**1989) tested grass (28% DM) in laboratory condition and the NDF values in DM as 54.2% and 53.1% in control and inoculated groups respectively (P<0.05). It was also reported that inoculation significantly decreased (P<0.001) the NDF values in grass silage (Anderson et al. 1989) and in corn silage (Hunt et al. 1993). These significant decreases in NDF values can be related to the increase of LAB numbers in silage condition and effective homofermentation, by means of increases in the cell wall digestibility. However, no knowledge is available how LAB destruct the cell wall components. However, it is reported that the low pH values provided by the inoculant supplementation increases the acid hydrolysis and breakdown the cell wall fractions (Bolsen et al. 1996a).

The inoculant addition decreased the pH values in control (P<0.001), barley (P<0.001) and salt (P<0.05) groups, while no significant difference was found in molasse added group (Table 7). Zimmer (1986) reported the mean pH values were as 4.30 in 436 feed silage, in which microbial inoculants used. Because of hard fermentation of alfalfa, inoculants are effective on pH values in alfalfa silage (Seale et al. 1986, Kung et al. 2003). However, it was reported that inoculants were less effective in corn and grass silage (Bolsen et al. 1995, Henderson et al. 1986). Lindgren et al. (1983) found the pH values 4.4, 4.4, 4.3 and 4.3 in control, inoculant, inoculant plus 10% grain and 10% grain groups respectively in a experiment performed by the grass silage (17% DM) in laboratory conditions. In the present study, barley added non inoculated group showed a pH value of 4.8 while the pH value (4.56) in barley plus inoculant added groups was decreased (P<0.001; Table 7).

However, others (Anderson et al. 1989, Haigh et al. 1987) found higher pH values in inoculant added groups, and they also stated that there was no significant effect of the inoculation on the pH values. The common properties of silage matters in these studies were having the low DM ratio. These results were different from the our findings. It is difficult to explain why the high pH values obtained from inoculant supplementation groups having low DM values. Amount of DM loss (17.06%) in non-inoculated groups was found higher (P<0.001) than those of inoculated groups (12.14%; Table 8). Honig (1986) reported that the loses of DM decreased by the addition of inoculant. In this study, silages were made by material including 30-40% DM in laboratory and field conditions, and the levels of DM losses were found to be close together (5-10%). Lindgren et al. (1983) found the amount of DM loses lower (5.6%) in inoculated group, and higher (7.6%) in inoculant plus grain (10%) added group comparing to control (6.3%) in the grass silage (17%DM) performed in laboratory conditions. In the present study the loss of DM values in barley plus inoculant groups were found higher than the other groups, except non-inoculated control group (Tablo 8). But, it is interesting to obtain this kind of result in presence of nutrients which can be utilised by LAB. Haigh et al. (1987) found the DM loses lower (27.4%) in inoculated group (P>0.05) than those of control (28.2%) in the farm- scale silage performed by grass and legume mixture (16% DM). This values of DM loses are higher than those of determined values in inoculated and non-inoculated groups of the present study. The determination of higher DM loses and poor results of the inoculant supplementation may be related to the silage material in which low DM levels. For instance, previous research performed in silages with sufficient DM levels (>25%) suggested lower DM losses (Honig 1986). Moreover, microbial inoculants were found to be effective in decreasing the DM loses in such silage (Zimmer 1986).

As a result, it may be concluded that supplementation of inoculant may have beneficial effects on silage quality only if feeds are ensiled with in proper technical conditions.

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