The Effects of Lactic Acid Bacteria+Enzyme Mixture Silage Inoculant on Wheat Silage[1]

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This study was carried out to determine the effects of a commercial lactic acid bacteria+enzyme inoculants used as silage additive on the fermentation, crude nutritient contents, cell wall fractions and *in vitro* dry and organic matter digestibilities wheat (*Triticum aestivum L.*) harvested and ensiled at milk and dough stages of maturity. Sil-All (Altech, UK) containing water soluble *Pediococcus acidilactici, Lactobacillus plantarum* and *Streptococcus faecium bacteria* with cellulase, hemicellulase, pentosonase and amylase was used as bacterial inoculants. The inoculant was applied to the silages at 6.0 $\log_{10}$ cfu/g levels. Wheats were ensiled in 2 liter glass jars and stored at 25 ±2 °C in the laboratory. Three jars from each group were sampled for pH, ammonia nitrogen, water soluble carbohydrates, organic acids (acetic, butyric and lactic), crude nutritients, cell wall fractions and microbiological analyses following the 75-day ensiling period. In additions *in vitro* dry and organic matters digestibility of the silages were determined with enzymatic methods. The inoculant improved fermentation characteristics, decreased neutral and acid detergent fiber contents of wheat silages. However, the *in vitro* dry and organic matter digestibilities of the silages were not affected by the treatments.

Keywords: Silage, inoculants, fermentation, maturity stage, wheat

Laktik Asit Bakteri+Enzim Karışımı Silaj İnokulantının Buğday Silajı Üzerine Etkileri

Bu çalışma silaj katkı maddesi olarak kullanılan ticari bir laktik asit bakteri+enzim karışımı inokulantın, süt ve hamur olum dönemlerinde hasat edilip, silolanan buğday silajlarının fermantasyon özellikleri ile ham besin maddeleri içerikleri, hücre duvarı kapsamı, *in vitro* kuru ve organik madde sindirilebilirlikleri üzerindeki etkilerinin saptanması amacıyla düzenlenmiştir. İnokulant olarak *Pediococcus acidilactici, Lactobacillus plantarum* ve *Streptococcus faecium* bakterileri ile birlikte sellülaz, hemisellülaz, pentozanaz ve amilaz içeren Sil-All (Altech, UK) kullanılmıştır. İnokulant silajlara 6.0 $\log_{10}$ cfu/g düzeyinde katılmıştır. Silolama 2 litrelik cam kavanozlarda 25±2 °C sıcaklıktaki laboratuar koşullarında gerçekleştirilmiştir. Silolanmadan sonraki 75. günde kavanozlar açılarak, silajlarda pH ölçümleri, amonyak azotu, suda çözünebilir karbonhidrat, organik asit (asetik, bütrik ve laktik), ham besin madde, hücre duvarı fraksiyonlarının belilenmesi ve mikrobiyolojik analizler yapılmıştır. Ayrıca, enzimatik yöntemle silajların *in vitro* kuru ve organik madde sindirilebilirlikleri saptanmıştır. Laktik asit bakteri+enzim inokulantları buğday silajlarının fermantasyon özelliklerini olumlu yönde etkilemiş, nötr ve asit deterjanda çözünmeyen karbonhidrat kapsamlarını düşürmüş, bunlara karşın *in vitro* kuru ve organik madde sindirilebilirliklerini etkilememiştir.

Anahtar kelimeler: Silaj, inokulant, fermantasyon, olgunluk dönemi, buğday

[1] This study is based on Seda BAŞKAVAK's MSc thesis.

Introduction

Wheat is one of the major crops for silage in Turkey. Wheat for silage is part of the double cropping system. It is harvested at the milk or dough stage of maturity in the spring, and the fields are cleared for summer crops such as maize or sorghum. Filya (2003) reported that growing wheat resulted in substantial increases in dry matter (DM) content and DM yield. Stage of maturity at harvest also effects forage yield, nutritive value and animal performance.

In order to improve the ensiling process, various chemical and biological additives have been developed. Biological additives are believed to be convenient to use, safe, non-corrosive to machinery, do not present environmental hazards, and regarded as natural products (Muck, 1993; Filya et al., 2000). Bacterial inoculants are added to silage in order to stimulate lactic acid fermentation by accelerating the decrease in pH, and thus improving silage preservation. If sufficient lactic acid bacteria are not present on the crop at ensiling, a slow rate of pH decrease will be resulted. The pH should drop rapidly to below 5 in order to prevent growth of anaerobic clostridia tyrobutyricum, bacteria degrade protein and producing butyric acid, lowering palatability and lower voluntary feed intake (Meeske et al., 2002). Bacterial inoculants generally increase lactic acid levels, reduce silage pH, acetic acid, butyric acid and ammonia-nitrogen levels in silage (Sheperd et al., 1995; Aksu et al., 2004). Inoculation with lactic acid bacteria (LAB) can increase fermentation efficiency but it is less efficient if fermentable substrate is insufficient. When LAB is combined with enzymes a stronger effect should be expected by releasing fermentable sugars to produce more lactic acid in proportion to other products (Kung et al., 1991; Chen et al., 1994; Nadeau et al., 2000a; Nadeau et al., 2000b).

This study was carried out to determine the effect of commercial LAB+enzymes inoculants used as silage additive on fermentation, crude nutritient contents, cell wall fractions, *in vitro* DM and organic matter (OM) digestibilities of wheat (*Triticum aestivum L.*) harvested and ensiled at milk and dough stages of maturity.

Material and Methods

Whole crop wheat (*Triticum aestivum L.*) was harvested at milk and dough stage of maturity. After harvest the wheat was chopped to about 1.5 cm and ensiled in 2 liter special anaerobic jars. Three jars from each group were sampled for chemical and microbiological analysis. Half of the crop was treated with inoculants + enzymes, a mixture consisting of *Pediococcus acidilactici, Lactobacillus plantarum, Streptococcus faecium* bacteria and cellulase, amylase, hemicellulase and pentosanase enzymes applied at a rate of 6.0 $\log_{10}$ cfu LAB/g of fresh forage (Sil All, Altech, UK). Jars were kept in a dark room at 25±2 °C. After 75 days of fermentation the silages were examined for nutritional composition, fermentation parameters and *in vitro* DM and OM digestibility.

DM contents of the fresh material and silage samples were determined by oven drying for 72 h at 60 °C, followed by milling through a 1-mm screen and drying for another 3 h at 103 °C. Ash content was obtained after 3 h at 550 °C. Crude protein content were determined following the procedure of Association of Official Analytical Chemists (AOAC, 1990). The pH values of both fresh material and silage samples were measured as reported by Chen et al. (1994). Buffering capacity (Bc) of fresh material was estimated as described by Playne and McDonald (1966). The ammonia-nitrogen (NH_3-N) and water soluble carbohydrate (WSC) contents of silages were determined, according to Anonymous (1986). For the analysis of silo acids (lactic, acetic and butyric) the shortened version of Lepper's method (Karabulut and Canbolat, 2005) was employed. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was performed according to Goering and Van Soest (1983). Hemicellulose was calculated as the difference between NDF and ADF and cellulose as the difference between ADF and ADL. DM and OM digestibilities were estimated according to Aufrère and Michalet-Doreau (1988), with a three-stage technique: Pre-treatment with pepsin in hydrochloric acid (0.2% pepsin in 0.1 N HCl), starch hydrolysis, attack by cellulase (Onozuka R 10 from *Trichoderma viride*, Merck). LAB, yeast and mould counts were obtained according to the methods reported by Seale et al (1990). Accordingly, as the incubation medium; MRS agar was used for LAB and malt extract was

used for mould and yeast. LAB, mould and yeast counts of the samples were obtained at 30 °C degrees following 3 days incubation period and converted into logarithmic coli-form unit (cfu/g).

One-way analysis of variance with Duncan's multiple range test (SAS, 1988) were utilized in the statistical analysis.

Results and Discussion

The chemical composition of the whole crop wheat harvested at milk and dough stage of maturity are given in Table 1.

DM content and pH of the crop harvested at dough stage were higher than that harvested at milk stage whereas Bc value was lower. In general, an increase in dry matter contents is expected with maturity. Bergen et al. (1991) reported 175 and 166 meq/kg DM for Bc, 5.90 and 5.90 for pH value in whole crop wheat harvested at milk and dough stages, respectively. Macgregor and Edwards (1968) observed a negative relationship between crop maturity and Bc. The WSC content of whole-crop cereal forage and showed a considerable decrease at dough stage when compared to milk stage. The drop in WSC concentration at dough stage seems to be a limiting factor for the ensiling process. The insufficiency of WSC seems to be pronounced in the initial phases of fermentation, which depressed the activity of LAB. This is reflected in poor lactic acid production and a slow pH decrease giving little protection in the silage against the spoilage of micro flora.

A number of main and interceptive effects of maturity stage and LAB+enzyme inoculant application on the chemical and microbiological composition of wheat silages were presented in Table 2.

The mean pH value of milk stage silage was significantly lower than that of dough stage silages ($P<0.001$). It was reported that as the harvesting time was delayed silage pH level could increased parallel to the increased in DM content (Gonçalwes et al.,1999; Demirel et al., 2006). Dry matter contents of dough stage silage were significantly higher than that of milk stage silages ($P<0.001$), whereas, CP, ADF, NDF and cellulose contents were significantly lower ($P<0.001$). The dry matter content of whole crop wheat was decreased by about 4.5% during ensiling for both stages. The decreases in ADF and NDF contents with maturation arise from the fact that seed ration increases and the fact seed has less cell wall components (Demirel et al., 2006). Various reports indicating that cell wall components decrease as harvesting time is delayed (Crovetto et al., 1998; Filya, 2003; Demirel et al. 2006). Crude protein content tended to decrease as maturity proceeded. This could be due to the relatively higher leaf content at the earlier maturity stages (Tolera et al., 1998). *In vitro* DM and OM digestibilities were not affected by harvesting time ($P>0.05$). This is in agreement with the results of Crovetto et al. (1998).

There are various publications related to the effect of LAB+enzyme inoculation on silage fermentation. It is generally reported that LAB+enzyme inoculation has positive effects on the silage fermentation by decreasing pH, acetic acid and NH_3-N; but increased lactic acid and LAB (Kung et al., 1991, Chen et al., 1994, Nadeau et al., 2000a; Nadeau et al., 2000b; Meeske et al., 1999; Filya, 2002).

Table 1. Chemical composition of whole crop wheat harvested at two stages of maturity

Chemical Composition	Stage of Maturity	
	Milk Stage	Dough Stage
DM, %	34.01	37.49
pH	6.15	6.24
Bc, meq NaOH/kg DM	139.85	111.99
CP, % DM	12.28	10.34
WSC, g/kg DM	87.40	56.50

DM: Dry Matter; Bc: Buffering Capacity; CP: Crude Protein; WSC: Water Soluble Carbohydrates

Table 2.Chemical and microbiological composition of wheat silages harvested at two stages of maturity

Treatment	Milk Stage		Dough Stage		Contrast (P<)			
	Control	LAB+E	Control	LAB+E	SEM	M	LAB+E	MxLAB+E
pH	4.27 c	4.09 d	4.64 a	4.49 b	0.065	<0.001	<0.001	0.628
DM, %	32.19 c	33.65 b	35.74 a	36.69 a	0.558	<0.001	0.017	0.537
WSC, g/kg DM	12.30 b	20.17 a	5.60 c	12.50 b	1.625	<0.001	<0.001	0.676
NH_3-N, g/kg TN	78.85 b	68.19 b	102.41 a	74.17 b	4.914	0.007	0.024	0.243
AA, % DM	1.04 a	0.83 b	0.70 b	0.76 b	0.047	0.011	0.256	0.066
BA, % DM	0.00 b	0.00 b	0.09 a	0.00 b	0.016	0.111	0.111	0.111
LA, % DM	3.78 b	4.37 a	3.08 c	3.73 b	0.150	<0.001	0.003	0.832
LA/AA	3.69 c	5.27 a	4.49 bc	4.94 ab	0.238	0.549	0.024	0.161
LAB, $\log_{10}$ cfu/g FM	3.31 b	4.60 a	3.26 b	4.48 a	0.195	0.486	<0.001	0.746
Yeast, $\log_{10}$ cfu/g FM	0.77 b	1.43 b	2.96 a	3.24 a	0.386	0.006	0.401	0.729
Mould, $\log_{10}$ cfu/g FM	2.58 ab	2.63 ab	3.30 a	1.56 b	0.261	0.692	0.084	0.069
CP, % DM	13.39 a	13.09 a	9.99 b	10.37 b	0.504	<0.001	0.932	0.478
NDF, g/kg DM	571.3 a	553.7 b	539.7 b	514.0c	0.672	0.000	0.004	0.469
ADF, g/kg DM	350.6 a	330.8 a	321.8 b	309.5 b	0.539	0.007	0.048	0.599
ADL, g/kg DM	66.3	56.6	62.5	57.1	0.177	0.601	0.037	0.504
Hemicellulose, g/kg DM	220.6	223.0	217.9	204.5	0.448	0.282	0.563	0.416
Cellulose, g/kg DM	284.4 a	274.2 ab	259.3 bc	252.5 c	0.452	0.004	0.185	0.784
in vitro DMD, %	50.74	52.12	52.37	54.14	0.548	0.094	0.139	0.847
invitro OMD, %	52.03	54.26	55.29	55.42	0.556	0.117	0.123	0.590

M: Maturity; LAB+E: Lactic acid bacteria+enzyme; DM: Dry Matter; CP: Crude Protein; NH_3-N:Ammonia nitrogen; WSC: Water Soluble Carbohydrate; LA: Lactic acid , AA: Acetic acid, BA: Butyric acid; LAB: Lactic acid bacteria; cfu: Colony forming unit ; FM: Fresh material; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; Hemicelluloses: NDF-ADF; Cellulose: ADF-ADL; DMD: Dry matter digestibility; OMD: Organic matter digestibility.

a-d Means, within a column with no common superscript differ significantly, P<0.05.

The result also indicates that silage treated with LAB+enzyme inoculant had lower ($P<0.001$) pH and lower ($P<0.05$) NH_3-N concentrations than that of control silage of both maturity stage and a higher ($P<0.01$) lactic acid concentration and lactic acid/acetic acid ration than that of control silages. The lactic acid/acetic acid ratio indicates the extent of homolactic fermentation in relation to heterolactic fermentation of sugar to lactic acid during ensiling where also acetic acid is produced (Jones et al., 1992). Extensive proteolysis occurred during ensiling indicated by the nearly 13% and 28% greater NH_3-N concentrations in control compared to LAB+enzymes inoculants silages at milk and dough stages, respectively.

In order to obtain the necessary level of fermentable WSC for the lactic acid fermentation in crops low in WSC the use of cell-wall degrading enzymes have been suggested (Stokes, 1992; Muck, 1993). In some studies, LAB+enzyme mixture inoculants decreased cell wall contents of silages (Nadeau et al., 2000 a;b). In contrast some reports showed that LAB+enzyme inoculants did not decrease significantly cell wall contents of silages (Meeske et al., 1993; Filya et al., 2001). In the present study, silages treated with LAB+enzyme inoculant had lower NDF ($P<0.004$) and ADF ($P<0.048$) contents than control silage at both maturity stages.

LAB +enzymes inoculants did not affected *in vitro* DM and OM digestibilities of wheat silages ($P>0.05$). Addition of LAB+ enzyme inoculants to forage tended to increase *in vitro* DM and OM digestibilities. There are various reports indicating that inoculants did not effect ruminal DM and OM degrabilities or digestibility of silages (Filya et al, 2000; Nadeau et al., 2000b; Hristov and McAlliester, 2002); however in some studies, inoculants improved, degradability or digestibility (Nadeau et al., 2000a).

Conclusions

The use of a LAB inoculants with enzymes during ensiling whole crop wheat resulted in an improvement preservation as indicated by lower pH and NH_3-N, higher lactic acid and increased numbers of LAB.

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