

Effects of Bacterial Inoculants on Fermentation and Aerobic Stability of Baled Triticale-Hungarian Vetch Silage and Lamb Performance

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Summary

The effects of bacterial inoculants containing homofermentative lactic acid bacteria (HM LAB) alone or in combination with *Lactobacillus buchneri* on conservation characteristics of baled triticale-Hungarian vetch silage and performance of Konya merino female lambs were investigated. The herbage was mowed at milk stage of maturity of triticale. Field wilted herbage was treated with LAB additives at 1.0×10^6 cfu/g and baled. Bales were wrapped with six layers of plastic white stretch-film. At the end of 3 m ensiling, silages made with or without LAB preserved well. Inoculation with HM LAB had little effect on fermentation characteristics of silages. However, silage treated with the HM LAB in combination with *Lactobacillus buchneri* resulted in silage with a higher ($P < 0.05$) concentration of acetic and propionic acid. These silages did not heat throughout 500 h of monitoring. There were no ($P > 0.05$) treatment effects on any variables measured on the lamb performance. Overall effects of additives on rumen fluids were relatively small. It is concluded that the use of HM LAB in combination with *Lactobacillus buchneri* is preferable because the combination of these bacteria could improve aerobic stability of silages via accumulation of acetic and propionic acid without reduced concentration of lactic acid. It is also concluded that elevated acetic and propionic acid in well fermented silages do not depress the dry matter intake of ruminants.

Keywords: Bacterial inoculants, Baled silage, *Lactobacillus buchneri*, Lamb, Triticale

Bakteri İnokulantlarının Balyalanmış Tritikale-Macar Fiği Silajının Fermantasyon ve Aerobik Stabilitesi ile Kuzularda Performansa Etkileri

Özet

Bu çalışmada homofermantatif laktik asit bakterilerini (HM LAB) yalnız ya da *Lactobacillus buchneri* ile kombinasyon halinde içeren bakteri inokulantlarının balyalanmış tritikale-Macar fiği silajının fermantasyon özellikleri ile Konya Merinosu dişi kuzularda performans üzerine olan etkileri araştırılmıştır. Silajlık materyal tritikalenin süt olum döneminde hasat edilmiştir. Tarlada soldurulmuş materyal LAB ile 1.0×10^6 kob/g düzeyinde muamele edilerek balyalanmıştır. Balyalar 6 kat beyaz streç film ile sarılmışlardır. Üç aylık silolama sonucunda LAB ilave edilerek ya da edilmeden silolanmış silajlar iyi fermente olmuşlardır. Silajların fermantasyon özellikleri üzerine HM LAB ilavesinin belirgin etkisi tespit edilmemiştir. Bununla beraber, HM LAB'ın *Lactobacillus buchneri* ile kombinasyon halinde ilavesi ile silajların asetik ve propiyonik asit içerikleri artmış ($P < 0.05$), bu silajlar 500 saatlik ölçüm sürecince ısınmamışlardır. Kuzuların performansı üzerine muamelelerin etkisi önemsiz ($P > 0.05$) olurken, katkı maddeleri ilavesinin rumen sıvısı üzerine olan genel etkileri de düşük bulunmuştur. Araştırma sonucunda HM LAB'ın *Lactobacillus buchneri* ile kombinasyon halinde kullanılması ile aerobik stabilitenin, silajın laktik asit içeriği azalmadan, artan asetik ve propiyonik asit içeriğine bağlı olarak arttığı ve bu nedenle de bu bakterilerin kombinasyon halinde kullanılmasının tercih edilebileceği değerlendirilmiştir. Ayrıca iyi fermente olmuş silajların içerdiği yüksek asetik ve propiyonik asidin ruminantların kuru madde tüketimini etkilemediği sonucuna varılmıştır.

Anahtar sözcükler: Bakteri inokulantları, Balyalanmış silaj, *Lactobacillus buchneri*, Kuzu, Tritikale



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INTRODUCTION

Silage fermentation is not a totally controlled process and conditions are not always optimal to ensure satisfactory fermentation. Therefore, efficient ensiling of forage requires stimulation of ensiling process.

The application of silage additives during ensiling is sometimes used to encourage beneficial microbial activity and/or inhibit detrimental microbial activity. It is possible to use both chemical and biological additives in silage making, in order to promote adequate fermentation patterns, especially under sub-optimal condition.

Bacterial inoculants are more advantageous than chemical additives because they are safer, easy to use, non-corrosive to machinery, do not pollute the environment, and are regarded as natural products. Homofermentative lactic acid bacteria (HM LAB) have been widely used in silage inoculants to improve or stimulate the silage fermentation due to their ability to produce lactic acid faster and more efficiently¹. Therefore, increasing HM LAB prior to ensiling could assist to take well preserved baled silages where the onset of fermentation is slower and the pH and overall concentration of fermentation acids are lower compared with silages made from precision-chopped herbage² and they could positively affect ruminant performance¹. In addition, *Lactobacillus buchneri*, a heterofermentative lactic acid bacterium, alone or in combination with HM LAB has been showed to increase the aerobic stability of silages through the accumulation of acetic acid³. Thus, application of *Lactobacillus buchneri* in combination with HM LAB could also make bale environment more inhibitory for the activities of undesirable microorganism. However, compared with the homolactic fermentation, heterolactic fermentation could be considered disadvantageous because of water and gas formation during the fermentation of sugar to fermentation acids⁴.

Another challenge in using *Lactobacillus buchneri* alone or in combination with the HM LAB as silage additive is that high level of acetic acid may suppress the dry matter intake (DMI) in ruminants^{5,6}.

Few studies have been conducted simultaneously comparing the responses to HM LAB, alone or in combination with the *Lactobacillus buchneri* for baled silages rather than conventional silage which made from precision chopped and for baled silage made from high dry matter (DM) triticale-hungarian vetch that was fed to lambs. The objective of this study was to compare the effects of HM LAB, alone or in combination with the *Lactobacillus buchneri*, on conservation characteristics and aerobic stability of baled triticale-Hungarian vetch silages. Their effects on DMI and live weight gain (LWG) of lambs fed with these silages were also investigated.

MATERIAL and METHODS

Silage Making

The experiment was conducted at Bahri Dagdas International Agricultural Research Institute, Konya (37°51'N, 32°33'E), Turkey. The mixture of triticale-Hungarian vetch herbage (30:70 seed ratio, respectively) from one field divided into three 0.5 ha plot. Plots were mown in every 20 min to a stubble height of approximately 5 cm with a conventional mower conditioner (Pottinger, Catnova 3100T) set to place wide windrows on the stubble at milk stage of maturity of triticale. Herbage was wilted in windrows to target DM of about 450 g/kg, for approximately 5 h. In order to provide the information necessary to achieve the target application rate for additives, a series of bales (1.25 m diameter and 1.20 m wide) were made throughout the field, weighed and the amount of water used for each bale was measured. Bales were then made and wrapped with a round baler (New Holland, BR560A, combi) equipped with an applicator for liquid additives. The additive treatments were; (1) Control - no additive (C), (2) HM LAB inoculant (HM LAB; *L. plantarum* and *E. faecium*, 1132, Pioneer® Hi-Bred, Int., Inc., USA) applied at 1.0x10⁶ cfu/g of herbage, (3) homofermentative + heterofermentative LAB inoculant (HM+HT LAB; *L. buchneri*, *L. plantarum*, *E. faecium*, 11G22, Pioneer® Hi-Bred, Int., Inc., USA) applied at 1.0x10⁶ cfu/g of herbage. All bacterial inoculants, in powder form, were dissolved in 9 L of de-ionised water and spread evenly over herbage on the sward from the applicator at the time of baling. The same amount of deionised water was also spread on the control herbage from the applicator. Bales were wrapped with six layers of white plastic stretch-film (25 µm film thickness) providing quadruplicate for each treatment in about 20 min per treatment. In order to prevent microbial contamination 2 bales from each treatment were also made. Wrapped bales were then weighed and stored outdoors for approximately 3 m on their curved side, on a soil base.

Feeding Trial

The feeding trial consisted of a 12 d acclimatization period followed by 63 d of an experimental period. A total of 27 Konya merino female lambs were housed in pens (1.7 m x 1.5 m) with individual water troughs and feeders. Lambs were acclimatized to silage by gradually changing their diets from pasture to silages. There were 9 lambs in each of the C, HM and HM + HT LAB treatments. Silages were offered *ad libitum* in experimental period with a concentrate (2.550 kcal/kg ME, 16.1% crude protein) equivalent to 1% of individual live weights of lambs. Silages were offered 1.2 of the silage intake. Dry matter intake was measured daily. Lambs were weighed on two consecutive days every three weeks from start to end of the experiment.

Rumen fluids were taken from 6 lambs per treatment on two consecutive days by using a hand operated suction

probe at the end of the feeding trial before fresh silage given to lambs in the morning. Rumen fluid taken two consecutive days analyzed separately and the mean values of two days used in statistical analysis.

Analytical Procedures

In order to sample bales, a 10 cm silage layer from one bale end was removed. From the freshly exposed silage face a 10 cm wide, 5 cm deep, the silage layer was taken across the vertical diameter of the bale. This silage layer was then mixed and sampled for subsequent chemical and microbiologic analysis and aerobic stability.

Silage samples were assayed for DM by oven drying at 60°C for 48 h. Crude protein was determined by Kjeldahl method according to AOAC ⁷. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) level of silages were determined according to Van Soest ⁸. Twenty g of sampled silage was blended (Waring Blender, 8010ES, US) with 180 ml of distilled water for 1 min at high speed. The resulting homogenate was then filtered through Whatman 1 filter paper. The pH of the filtrate was measured with a pH meter (WTW, Inolab 720, Germany). A proportion of the filtrate (50 ml) was acidified with 100 µm of 50% H₂SO₄, centrifuged at 6.000x g for 15 min and then frozen, before being used for determination of concentration of lactic acid ⁹, water soluble carbohydrates (WSC) ¹⁰ and ammonia-N. Ammonia-N was determined in the Kjeldahl unit without a digestion step but with the addition of base. A further proportion of the filtrate (5 ml) was acidified with 1 mL meta-phosphoric acid (vol/vol, 25%), centrifuged at 4.000x g for 10 min and then frozen prior to analysis for volatile fatty acids (VFAs; acetic, propionic and butyric acids) concentration. The VFAs were measured by gas chromatography (GC-15A, Shimadzu, Kyoto, Japan) according to Supelco ¹¹.

The pH of rumen liquid was measured immediately after taken with a pH meter (WTW, Inolab 720, Germany) on a sub-sample. For rumen liquid VFAs, samples were filtered through two layers of cheesecloth. Five ml of filtrate was mixed with 1 mL meta-phosphoric acid (vol/vol, 25%) and centrifuged at 2.000x g for 10 min and frozen at -20°C before being used for determination of rumen VFAs and ammonia-N. Rumen VFAs were analyzed as mentioned in silage VFAs. Rumen ammonia-N was determined by the procedure described by Weatherburg ¹².

Enumeration of LAB performed on MRS agar (CM 0361 Oxoid, oxoid LTD., England). Plates were incubated for 48 h at 35°C. Yeasts and moulds were enumerated on DRBC agar (Dichloran-Bengalrot-Chloramphenicol-Agar, Merck KGaA, Germany). Plates were incubated for 5 d at 25°C.

Aerobic stability of the baled silages was also assessed. Approximately 1 kg silage sample from each bale was placed loosely in a 5-L jar. A layer of cheesecloth was placed over the jar and silage was exposed to air at ambient

temperature (22±2°C). The temperature of the silage mass and ambient temperature were recorded by a data logger (CR1000, Campbell Scientific, Inc., Logan, UT), adjusted to record every 10 min and averaged over a period of every 2 h. The index of aerobic stability was expressed as the interval in hours until the temperature of silage mass rose more than 2°C above ambient temperature. Aerobic stability of silages was measured for 500 h.

Statistical Analysis

All data from silage composition, feeding trial and rumen fluid study were analyzed by one-way analysis of variance (ANOVA) in a completely randomized design in SPSS (1993). Comparisons between treatments were made using the Duncan's multiple range test. Statistical significance was declared at P<0.05.

RESULT

Baled Silages Composition

The chemical and microbial compositions of baled silages are presented in [Table 1](#). There were no (P>0.05) treatment effects on DM, crude protein, ADF, concentration of butyric acid and ammonia-N and, microbial composition of silages at the end of 3 months ensiling period.

The application of HM + HT LAB resulted in an increase (P<0.05) in concentration of acetic acid, propionic acid and NDF compared with the control and HM LAB silages, while application of HM LAB resulted in a decrease (P<0.05) in silage pH, compared with the control silages. Concentrations of lactic acid and WSC were the highest (P<0.05) in silages treated with HM LAB, but they were the lowest (P<0.05) in silages treated with HM + HT LAB. The highest (P<0.05) aerobic stability value was also in silages treated with HM + HT LAB, while it was the lowest (P<0.05) in silages treated with HM LAB.

Feeding Trial

The pH of rumen fluid, concentration of total VFAs and molar proportions of acetic, propionic, isobutyric, isovaleric, valeric and lactic acid were not different (P>0.05) among the treatments ([Table 2](#)). Rumen fluid from lambs fed with the silages treated with HM + HT LAB had lower (P<0.05) molar proportion of butyric acid, while rumen fluid from lambs fed with the control silages had lower (P<0.05) molar proportion of valeric acid. Ammonia concentration of rumen fluid ranged from 110 to 116 mmol/L and it was not affected (P>0.05) by the treatments.

Performance of lambs fed with control and inoculated silages for 63 d are presented in [Table 3](#). There were no treatment effects on any variables measured in feeding trial. All lambs from each treatment consumed all their concentrate given equivalent to 1% of individual live weights of lambs.

Table 1. Conservation characteristics of baled triticale-Hungarian vetch silages
Tablo 1. Balyalanmış triticale-Macar fiği silajlarının özellikleri

Variable	Treatments ¹ (X±Sx)		
	C	HM LAB	HM + HT LAB
Dry matter, %	44.0±1.8	41.1±2.0	44.2±1.3
Crude protein, % DM	13.6±0.43	13.3±0.39	13.6±0.30
NDF, % DM	55.5±0.65 ^b	52.7±0.56 ^b	62.9±3.66 ^a
ADF, % DM	39.2±3.0	34.8±3.51	38.0±1.76
pH	4.6±0.07 ^a	4.3±0.09 ^b	4.6±0.02 ^{ab}
Lactic acid, % DM	5.3±0.42 ^{ab}	6.3±0.64 ^a	4.5±0.16 ^b
Acetic acid, % DM	2.3±0.32 ^b	1.8±0.08 ^b	4.1±0.66 ^a
Propionic acid, % DM	0.08±0.05 ^b	0.03±0.01 ^b	0.45±0.16 ^a
Butyric acid, % DM	0.03±0.01	0.17±0.09	0.05±0.02
WSC ² , % DM	4.9±0.59 ^{ab}	6.0±0.28 ^a	3.4±0.79 ^b
Ammonia-N, % DM	0.10±0.01	0.08±0.01	0.09±0.01
LAB ³ , log10 cfu/g	5.78±0.29	6.15±0.02	5.92±0.22
Yeasts, log10 cfu/g	0.00±0.00	0.00±0.00	1.64±0.95
Moulds, log10 cfu/g	0.62±0.62	0.50±0.50	0.83±0.83
Aerobic stability, h	418±49.8 ^{ab}	303±51.8 ^b	500±0.00 ^a

¹ C: Control; HM LAB: Homofermentative LAB; HM + HT LAB: Homofermentative + heterofermentative LAB
² WSC: Water soluble carbohydrates, ³ LAB: Lactic acid bacteria
Within a column, means followed by different letters differ significantly (P<0.05)

Table 2. Rumen fluid characteristics of lambs fed with baled triticale-Hungarian vetch silages
Tablo 2. Balyalanmış triticale-Macar fiği ile beslenen kuzuların rumen sıvısı özellikleri

Variable	Treatments ¹ (X±Sx)		
	C	HM LAB	HM + HT LAB
pH	7.00±0.07	7.01±0.08	6.97±0.04
Total VFA, mmol/L	131.9±19.0	128.7±10.0	164.7±13.4
Acetic acid, %	49.2±0.79	48.7±0.61	50.4±0.54
Propionic acid, %	23.9±0.53	24.0±0.57	25.0±0.31
Butyric acid, %	20.8±0.50 ^a	20.3±0.87 ^a	18.2±0.60 ^b
Izobutyric acid, %	3.0±0.23	2.9±0.15	2.5±0.10
Izovaleric acid, %	1.4±0.37	1.8±0.34	2.1±0.06
Valeric acid, %	0.2±0.17 ^b	1.1±0.15 ^a	0.9±0.06 ^a
Acetic acid :Propionic acid	2.1±0.07	2.0±0.06	2.0±0.03
Lactic acid, %	1.4±0.34	1.2±0.11	0.8±0.05
Ammonia-N (mmol/L)	116±8.1	111±6.3	110±9.8

¹ C: control; HM LAB: Homofermentative LAB; HM + HT LAB: Homofermentative + heterofermentative LAB
Within a column, means followed by different letters differ significantly (P<0.05)

DISCUSSION

Baled Silages Composition

Satisfactory fermentation depends on maintaining anaerobic conditions during ensilage and on inhibiting the activity of undesirable anaerobic microorganisms⁴. In this study, the pH of the silages ranged from 4.3 to 4.6 and these values were satisfactory to ensure effective conservation with a DM content of silages between 400-450 g/kg according to Weissbach¹⁴. Low concentration of butyric acid and ammonia-N, and low count of yeast and

mould also indicated that silages with no additives or with additives preserved well. This reflected that anaerobic conditions prevailed within the bales and evidence for undesirable microbial activity was quite small.

When homofermentative LAB is effective in silage fermentation, the resultant silage usually has more lactic acid and less acetic and butyric acid together with a low final pH. These are an indication of a more homolactic fermentation that prevailed during the silage fermentation¹⁵. In the current study, silage inoculated with the HM LAB had only a pH value that was lower than control silage had, while

Table 3. Performance of lambs fed with baled triticale-Hungarian vetch silages
Tablo 3. Balyalanmış triticale-Macar fiği silajları ile beslenen kuzuların performansları

Variable	Treatments ¹ (X±Sx)		
	C	HM LAB	HM + HT LAB
Weight of lambs at day 0, kg	31.4±0.30	31.2±0.38	31.3±0.38
Weight of lambs at day 63, kg	35.8±0.43	36.8±0.34	36.4±0.54
Live weight gain, g/d	70.6±5.09	88.8±5.6	80.7±6.56
Silage intake, g DM/d	592±18.5	600±20.2	643±19.3
Total intake, g DM/d	882±20.9	892±20.6	933±21.1
FCR ²	20.6± 3.9	17.0±2.4	17.6±3.3

¹ C: control; HM LAB: Homofermentative LAB; HM + HT LAB: Homofermentative + heterofermentative LAB
² Feed conversation ratio (total intake/live weight gain)

other parameters that would show silage treated with the HM LAB underwent more homolactic fermentation had not been affected by the HM LAB treatment. This is in line with the findings of Sucu and Filya ¹⁶ and Ranjit and Kung ¹⁷ who also reported that inoculation of maize with HM LAB had lack of effectiveness on concentration of lactic acid which is one of the most important indicators of a more homolactic fermentation. Under condition where the control silage had a good conservation characteristic with a lactic acid dominant fermentation already prevailing, the application of HM LAB had little impact on final fermentation products of baled triticale-Hungarian vetch silage. In the prevailing condition of this experiment, high herbage DM and a harsh stalk of triticale reduced the efficiency of chopping units of round baler which has stationary cutting blades. As a result, the silages were nearly unchopped. Therefore, long particle length and high DM of herbage may have limited the onset of the fermentation and thus the opportunity for HM LAB to fulfil their potential may have been restricted. This is suggested due to lack of mechanical treatment of herbage in this experiment such as chopping and mincing, prior to ensilage, coupled with high DM of herbage may have prevented a rapid release of fermentable nutrients for LAB and occurrence of intimate mixing between forage and additives ¹⁸. These factors could make the number of lactobacilli present initially less important, and thus, could contribute to explain the smaller than expected effects of HM LAB.

Silages treated with the HM + HT LAB showed the evidence of well preserved silages that were dominated by heterolactic fermentation as indicated by having higher acetic and propionic acid concentration relative to control silages. Silages treated with HM + HT LAB had nearly equal concentration of acetic acid and lactic acid without increased concentration of butyric acid and ammonia-N. An explanation for these outcomes is that *L. buchneri* could shift the fermentation course during 3 m ensilage period with anaerobic conversion of lactic acid to acetic acid and propionic acid ¹⁹. When *L. buchneri* applied without HM LAB at barley ²⁰, and sorghum and maize ²¹ the resultant silages had higher concentration of acetic acid and lower concentration of lactic acid than control

silages. In the current study, silages treated with the HM + HT LAB had a lactic acid level that was not different from control silages. This was in line with the findings of Filya ²¹ that when *L. buchneri* used in combination with the HM LAB, the resultant silages had not reduced lactic acid concentration.

Kleinschmit and Kung ², in their meta-analysis, reported that the mean aerobic stability value of small-grain silages inoculated *L. buchneri* at $>1.0 \times 10^5$ cfu/g was 39 h longer than control silages (206 and 245 h, for control and *L. buchneri* treatment, respectively) with an average DM of 318 g/kg, which is similar to the result presented here. The higher aerobic stability value of control silage is due to the higher DM content of silages. The heating of silages treated with HT + HM LAB would take longer if assessment of aerobic stability of silages were longer than 500 h. The lowest aerobic stability of silages treated with HM LAB suggested that HM LAB could decrease aerobic stability of silages and result in increased aerobic deterioration of silages at feedout in line with the findings of Cai *et al.* ²², Ozduven *et al.* ²³ and McEniry *et al.* ²⁴.

The ADF and crude protein of silages were not altered by the LAB treatment. This indicates that LAB either homofermentative or heterofermentative have no direct effects on these nutrients. The higher concentration of NDF in silages treated with HM + HT LAB suggests less acid hydrolysis of the hemicellulose.

Feeding Trial

Rumen fluids were taken in this experiment to help to explain the differences that would occur between the additive treatments. However, there were only treatment effects on butyric and valeric acid concentration in rumen fluid. Rumen fluid pH was unaffected with feeding silages containing 4.1% DM acetic acid and this in line with the Anil *et al.* ⁵. But, they also reported that the concentration of acetate in rumen fluid was increased by acetate infusions during the 0-3 h and remain high 2 h after infusion. In present experiment, concentration of acetic acid was not different among the treatments.

Under condition where HM LAB had little effects on the conservation characteristics of high DM baled triticale-Hungarian vetch silage, total or silage intake and LWG of lambs fed with these silages did not differ from the lambs fed with untreated silage.

It could be speculated that elevated acetic acid in silage could be associated with reduced DMI from the finding of Anil *et al.*⁵ who reported that different degree of sodium acetate infusions caused a dose-related depression in hay intake. However, Mbanya *et al.*²⁵ reported that sodium acetate itself does not depress the DMI and revised data⁶ showed that reduced energy corrected milk yield associated with the elevated total VFA in the silage. It is more likely that if acetic acid associates with the reduced silage intake, this is probably in poorly fermented silages where ammonia-N and butyric acid concentration are also high as indication of clostridial fermentation. These silages often contain high acetic acid as well. In the present experiment, HM+HT LAB treated silages contained nearly twice as much acetic acid as untreated silage did (4.1 and 2.3% DM, respectively) without elevated concentration of ammonia-N and butyric acid. As a result, lambs fed with the HM + HT LAB inoculated silages had no lower silage intake than all other lambs fed with the untreated or HM LAB treated silages. This is in agreement with other works done with *L. buchneri* in dairy cows^{26,27} and in sheep²⁸ that was also showed that elevated acetic acid after successful inoculation with *L. buchneri* did not suppress the DMI. This outcome suggests that feeding silages elevated acetic acid alone is not responsible for depressing DMI of ruminants.

In conclusion, the effect of HM LAB on silage fermentation patterns of high DM baled triticale-Hungarian vetch forage was little. Using HM LAB in combination with *L. buchneri* is preferable because the combination of HM LAB and *L. buchneri* could improve aerobic stability of silages via accumulation of acetic and propionic acid. It is also concluded that elevated acetic and propionic acid in well fermented silages do not depress the DMI of ruminants.

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