



## RESEARCH ARTICLE

### INVESTIGATIONS INTO THE FEED VALUE OF VETCH (*VICIA NARBONENSIS*) SILAGE

\*<sup>1</sup>Levent COŞKUNTUNA and <sup>2</sup>Sevilay GÜL

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, Namık Kemal University, Tekirdağ, Turkey

<sup>2</sup>Vocational School of Technical Sciences, Plant and Animal Production Department, Namık Kemal University, Tekirdağ, Turkey

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#### ABSTRACT

Narbon vetch (*Vicia narbonensis* L.) is an important forage species among vetches of central Europe, the Mediterranean, the Near East, Ethiopia, central Asia and India. Vetch was used in this trial as the silage material. The aim of this study was to determine the effects of lactic acid bacteria and inoculants as silage additives on the fermentation and aerobic stability of grass silage. *Vicia narbonensis* was harvested early in the flowering period (flowering 1/10) and ensiled in silo-type glass containers. Each application consisted of three parallels. Chemical and microbiological analyses were conducted on the silage, which was opened on the 45th day after it was ensiled. According to the analysis, control, lactic acid bacteria (LAB) and inoculants (LAB+enzyme) groups of KM 13,16, 12,28, 13,47, pH 4.57, 4.51, 4.41, NH<sub>3</sub>-N: 87.29, 90.19, 92.86 were found. In conclusion, it was evaluated that chemical, physical and microbiological qualities increase with the addition of LAB and inoculant to the narbon vetch silage. Also, the use of narbon vetch silage material is useful for storage conditions.

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## INTRODUCTION

Narbon vetch (*Vicia narbonensis* L.) is one of the important self-pollinated forage species and has a natural distribution ranging throughout central Europe, the Mediterranean, the Near East, Ethiopia, central Asia and India. It can be found in all regions of Turkey, except for Northern Anatolia (Davis and Plitman, 1970). Narbon vetch has greater temperature and lower humidity requirements, which makes it possible for it to grow advantageously in warm dry areas. It tolerates cold, and is not damaged by frost. It can be used as forage crop green manure and has great importance in crop rotation (Altinok, 2002; Altinok and Hakyemez, 2002). It has the potential to replace traditional barley-fallow rotation in the Eastern Mediterranean (Oram and Belaid, 1990) and is recommended as a forage crop in fallow years in dry farming areas of Turkey (Bakir, 1981). The importance of evaluating the nutritional worth of ruminant animal feedstuffs is taken into consideration. (ARC, 1980). The forage can either be grazed in early spring or cut for hay and silage (Allden and Geytenbeek, 1980). The purpose of the current work was to extend the study of the effect of LAB and inoculant on the silage microbiology, chemical composition and aerobic stability of Narbon vetch (*Vicia narbonensis* L.) silages.

## MATERIALS AND METHODS

### Forage production

*Vicia narbonensis* was harvested early in the flowering period (flowering 1/10). Forage was chopped (10-15 cm theoretical length of cut). Silage materials were divided into three trial groups for the control, lactic acid bacteria and inoculant treatments. (1) The chopped forage was mixed and divided into distilled water, denoted as treatment control; (2) inoculants (LAB+enzyme), a mixture of lactic acid bacteria (LAB) consisting of *Lactobacillus plantarum* and *Enterococcus faecium* applied at a rate of 6.00 log<sub>10</sub> cfu LAB/g of fresh forage (Pioneer 1188, USA), treatment LAB; (3) enzymes, a mixture of enzymes consisting of cellulase, amylase, hemicellulase and pentosanase enzymes applied at a rate of 0.01 mg/g of fresh forage (Enzyme, Global Nutritech 41600 Kandira, Kocaeli-Turkey), treatment Enzyme. The application rate determined by the manufacturers stated the level of LAB and enzyme in the products. On the day of the experiment, inoculants and enzymes were suspended in 10 ml of tap water and the whole suspension was sprayed over 5 kg (wet weight) of the chopped forage spread over a 1 x 4 m area. All inoculants and enzymes were applied to the forages in a uniform manner with constant mixing (Özdüven *et al.*, 2009; Özdüven *et al.*, 2010). The material mixed with additive was pressed in 36 1.0-l glass jars (Weck, Wher-Ofllingen,

\*Corresponding author: Levent COŞKUNTUNA,

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, Namık Kemal University, Tekirdağ, Turkey.

Germany) equipped with lids that enabled gas release only. The jars were stored under constant room temperature ( $20 \pm 1^\circ\text{C}$ ). Three jars per treatment were sampled on day 45 for pH, DM, WSC, LA content measurement, and LAB; mold and yeast enumeration. At the end of the experiment, the silages were also subjected to an aerobic stability test, lasting five days, in a system developed by Ashbell *et al.*, (1991). The system is constructed from two parts of recycled soft drink bottles (polyethylene terephthalate). The upper part (1 l) is filled with about 250 g (wet weight) loosely packed silage and the lower part with 100 ml 20% KOH. Gas is exchanged through 1 cm holes in the upper part; carbon dioxide produced during aerobic exposure is absorbed in the base and determined by titration with 1 N HCl. In addition, change in pH, yeast and mold counts and visual appraisal also serve as indicators for aerobic spoilage. Visual appraisal of samples exposed to air was performed by a panel of three according to the extent of mold cover, texture, and their odor. The panel evaluation was converted into a numeric scale from 1 to 5, with one being good quality silage with no apparent molding and five being completely molded samples (Fila *et al.*, 2000).

### Analytical procedure

Chemical analyses were performed on triplicate samples. DM was determined by oven drying for 48 h at  $60^\circ\text{C}$ . The pH in fresh material and silage samples was measured according to the British standard method (Anonymous, 1986). The ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) content of silages was determined according to Anonymous (1986). The WSC content of silages was determined by spectrophotometer (Shimadzu UV-1201, Kyoto, Japan) after reaction with antron reagent (Thomas, 1977). LA and acetic acid (AA) were determined by the spectrophotometric method (Koc and Coskuntuna, 2003).

### Statistical analysis

Statistical analysis of the silage chemical analysis results included a one-way analysis of variance and Duncan's multiple range test performed with the Statistical Analysis System (2005) Software (SAS, Cary, NC).

## RESULT AND DISCUSSION

The chemical composition of the fresh and ensiled narbon vetch (*vicia narbonensis*) silages is given in Table 1. In the experiment, LAB and inoculant improved the fermentation parameters of narbon vetch silage. The pH of all silages decreased faster and to a greater extent. During fermentation, significant differences were shown between the pH values of control, LAB and inoculant inoculated silages ( $P < 0.01$ ). For good quality silage fermentation aerobic requirements and reduced pH should be ensured. The pH value usually drops through the fermentation of lactic acid bacteria sugar with lactic acid (Van Soest 1994). In this study it was evaluated that the pH value was higher in the control group. PH value was identified to be 4.51 and 4.41 for LAB and inoculant (LAB+Enzyme), respectively. Other findings support this study (Baytok and Muruz 2003; Koç *et al.*, 2008; Basole 2010; Elmalı and Duru 2012). It was identified that the addition of LAB and inoculant to the vetch silages led to an increase in DM. CP:DM ratios 2.1 days after ensiling were significantly higher ( $P < 0.01$ ). The same situation was found at five days after ensiling ( $P < 0.05$ ). CP values two and five days after ensiling were significantly higher ( $P < 0.01$ ). Baytok *et al.*, (2005) reported that DM, CA, OM and CP values are not significantly impacted by the organic acid addition to the silage. Rowghani and Zamiri (2009) reported that DM and CP increase with the addition of organic acid to the corn silage.

Table 1. Chemical analysis of Vetch (*Vicia Narbonensis*) silages

Days of ensiling	Treatment	DM	pH	CP	WSC	NH3N/TN	Lactic Acid	Weight Loss
0		11,83	5,82	24,28	48,14	73,14		
2	Control	13,91 $\pm$ 0,05 a**	4,50 $\pm$ 0,01a**	22,22 $\pm$ 0,06b**	35,89 $\pm$ 0,55	72,33 $\pm$ 1,70a*	1,34 $\pm$ 0,02c**	4,84 $\pm$ 0,72
2	LAB	13,27 $\pm$ 0,09 b**	4,42 $\pm$ 0,01b**	23,88 $\pm$ 0,02a**	30,36 $\pm$ 2,00	57,47 $\pm$ 1,95b*	1,84 $\pm$ 0,04a**	5,73 $\pm$ 1,04
2	I	13,52 $\pm$ 0,17ab**	4,42 $\pm$ 0,01b**	23,97 $\pm$ 0,15a**	33,21 $\pm$ 1,16	61,54 $\pm$ 0,56b*	1,62 $\pm$ 0,02b**	6,30 $\pm$ 0,85
5	Control	13,29 $\pm$ 0,11b*	4,99 $\pm$ 0,01a**	22,32 $\pm$ 0,05b**	28,25 $\pm$ 1,10	84,55 $\pm$ 2,96a*	2,56 $\pm$ 0,02c**	5,99 $\pm$ 0,86
5	LAB	13,35 $\pm$ 0,20b*	4,80 $\pm$ 0,01c**	24,30 $\pm$ 0,02a**	27,10 $\pm$ 0,91	73,25 $\pm$ 5,71b*	2,00 $\pm$ 0,02a**	5,30 $\pm$ 0,82
5	I	14,26 $\pm$ 0,16a*	4,90 $\pm$ 0,01b**	24,35 $\pm$ 0,11a**	28,15 $\pm$ 1,00	66,23 $\pm$ 0,27b*	1,70 $\pm$ 0,04b**	5,89 $\pm$ 0,88
21	Control	13,21 $\pm$ 0,07b**	4,58 $\pm$ 0,01a**	20,69 $\pm$ 0,16	20,21 $\pm$ 0,95	104,76 $\pm$ 6,11	2,10 $\pm$ 0,04	6,83 $\pm$ 0,72
21	LAB	12,93 $\pm$ 0,10b**	4,52 $\pm$ 0,01b**	20,77 $\pm$ 0,11	21,14 $\pm$ 0,99	111,12 $\pm$ 2,31	2,19 $\pm$ 0,07	5,63 $\pm$ 0,61
21	I	13,57 $\pm$ 0,11a**	4,35 $\pm$ 0,01c**	20,35 $\pm$ 0,01	20,15 $\pm$ 1,01	94,49 $\pm$ 1,31	2,02 $\pm$ 1,12	6,10 $\pm$ 0,68
45	Control	13,16 $\pm$ 0,05a*	4,57 $\pm$ 0,01a**	19,22 $\pm$ 0,01c**	11,16 $\pm$ 1,05	87,29 $\pm$ 3,14	2,27 $\pm$ 1,05b*	6,41 $\pm$ 0,78
45	LAB	12,28 $\pm$ 0,22b*	4,51 $\pm$ 0,01b**	20,86 $\pm$ 0,01b**	9,64 $\pm$ 1,49	90,19 $\pm$ 1,43	2,78 $\pm$ 1,07a*	5,99 $\pm$ 0,90
45	I	13,47 $\pm$ 0,32a*	4,41 $\pm$ 0,01c**	21,73 $\pm$ 0,02a**	9,35 $\pm$ 1,79	92,86 $\pm$ 1,22	2,70 $\pm$ 1,04a*	7,62 $\pm$ 0,60

\*Values with different superscripts within the same column are different ( $P < 0.05$ ).

\*Values with different superscripts within the same column are different ( $P < 0.01$ ).

DM: dry matter; CP: crude protein; WSC: water soluble carbohydrate; NH3-N: ammonia nitrogen.

Fermentation losses during storage were estimated by weight loss calculated separately for each jar by the difference in the weight at the beginning and end of the ensiling period. Crude protein (CP) and crude fiber (CF) were determined following the procedure of the Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the method of Van Soest (1982). Microbiological evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK). Yeast and molds were determined by pour-plating in malt extract agar (Oxoid CM59) that had been acidified after autoclaving by the addition of 85% LA at a concentration of 0.5% vol/vol. Plates were incubated aerobically at  $32^\circ\text{C}$  for 48-72 h.

The findings of Aksu and Duru (2012) support this study. LAB and inoculant treatments had an effect on concentration of ammonia-N 2., 5. and days ensiling found significantly higher ( $P < 0.05$ ). Treatments did not affect the concentration of ammonia-N of days ensiling. In the experiment, LA level was 2., 5. Days ( $P < 0.01$ ) and days ensiling ( $P < 0.05$ ) found significantly higher. Both inoculants produced more LA in the silages than was found in the control silages, in agreement with pH values. The addition of LAB inoculants at ensiling is intended to ensure rapid and vigorous fermentation that result in faster accumulation of LA and lower pH values at earlier stages of ensiling and improved forage preservation. The findings of Filya *et al.*, (2000), Koç *et al.*, (2008) and Özdüven *et al.*, (2009) support this study.

**Table 2. Chemical composition of Vetch (*Vicia Narbonensis*) silage after 60 days of ensiling**

Days of ensiling	Treatment	DM	CF	Ash	NDF	ADF	ADL
0		11,83	18,34	14,35	37,83	28,41	10,43
45	Control	13,16±0,05 a*	18,59±0,09 a*	13,76±0,01 a**	37,71±0,39	31,50±0,50a*	10,50±0,50
45	LAB	12,28±0,22 b*	18,22±0,21 ab*	12,32±0,01 c**	37,47±0,27	27,50±0,50b*	11,50±0,50
45	I	13,47±0,32 a*	17,66±0,22 b*	13,04±0,02 b**	37,29±0,41	27,00±0,01b*	11,00±0,25

\*Values with different superscripts within the same column are different ( $P < 0.05$ ).

\*\*Values with different superscripts within the same column are different ( $P < 0.01$ )

DM: dry matter; CF: crude fiber; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin.

**Table 3. Microbiological analysis of Vetch (*Vicia Narbonensis*) silages**

Days	Treatment	Lactobacilli	Yeast	Mold
0		0,70	0,50	0,10
2	Control	0,83±0,06	0,65±0,08	0,12±0,02
2	LAB	0,74±0,08	0,75±0,10	0,16±0,03
2	I	0,83±0,05	0,70±0,08	0,15±0,03
5	Control	1,07±0,05	0,77±0,08	0,26±0,05
5	LAB	1,00±0,06	0,86±0,08	0,21±0,04
5	I	1,08±0,05	0,90±0,06	0,30±0,06
21	Control	1,30±0,06	1,07±0,11	0,36±0,06
21	LAB	1,22±0,03	1,09±0,04	0,37±0,08
21	I	1,26±0,06	1,21±0,5	0,43±0,07
45	Control	1,55±0,10	1,19±0,07	0,48±0,04
45	LAB	1,61±0,04	1,16±0,09	0,40±0,08
45	I	1,56±0,07	1,20±0,03	0,51±0,11

**Table 4. Aerobic stability Vetch (*Vicia Narbonensis*) silages**

Days	Treatment	pH	Yeast	Mold	CO <sub>2</sub>
0					
	Control	8,08±0,15	5,50±0,28	4,48±0,26	31,95±4,27
	LAB	8,50±0,01	5,15±0,20	4,49±0,16	35,28±2,93
	I	8,29±0,06	5,27±0,16	4,43±0,25	31,84±5,31

The weight loss of the silages is given in Table 1. No significant difference was detected among the control, LAB and inoculant treatment groups. The findings of this study are different from those of Koç *et al.*, (2009). This may have been the result of the different temperature and formic acid that was used. In the experiment, the WSC in all silages decreased with the decrease in pH. Control, LAB and inoculant inoculated vetch silages showed no significant difference. Table 2. shows fiber composition CF, Ash, NDF, ADF, and ADL content of the vetch after 45 days. CF and ADF contents were found to be significant in all groups (control, LAB and inoculant ( $P < 0.05$ )). The findings of Baytok *et al.*, (2005) and Elmalı and Duru (2012) support the CF content of this study. ADF contents were not found to be similar in the findings of Koç *et al.*, (2009) and Elmalı and Duru (2012). This may have been the result of the different additive and different temperature that were used. Baytok *et al.*, (2005) reported that DM, CA, OM and CP values are not significantly impacted by the organic acid addition to the silage. Compared with the LAB and inoculant groups, ash contents were lower than ( $P < 0.01$ ) the control. This finding is different from those of Elmalı and Duru (2012). This may have been the result of the malic acid that was used.

NDF and ADF levels of the study are given in Table 2. No significant differences were found among all the groups (control, LAB and inoculant). The results of the aerobic stability test are given in Table 4. In the experiment, no significant differences could be detected in the lactobacilli, yeast, and mold contents detected between the control, LAB and inoculant treatment groups. Also, the CO<sub>2</sub> level of the silage found that LAB group was higher than the control. Inoculant groups had no significant difference.

## Conclusion

The result of this experiment showed that LAB and inoculant addition to the vetch silage has positive effects on the physical and chemical characteristics and the ability of the vetch to be ensiled as well.

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