ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 12, Issue, 03, pp.10680-10683, March, 2020

DOI: https://doi.org/10.24941/ijcr.38271.03.2020

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

THE EFFECTS OF LACTIC ACID BACTERIA AND INOCULANTS MIXTURE ENZYME ON FERMENTATION AND FEED VALUE OF VETCH (*VICIA NARBONENSIS L.*) SILAGE

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ARTICLE INFO

Article History:
Received 04th December, 2019
Received in revised form
20th January, 2020
Accepted 18th February, 2020
Published online 30th March, 2020

Key Words:
Vetch, Silage, Fermentation, Silage Additive, Silage Quality.

ABSTRACT

Narbon vetch (*Vicia narbonensis* L.) requires lower rainfall than chickpeas, faba beans and lentils and may be proven a better legume alternative in ratio systems. Vetch was used in this trial as the silage material. This study carried out to determine the effects of lactic acid bacteria and inoculants as silage additives, on the fermentation and aerobic stability of vetch silage. *Vicia narbonensis* was harvested pot setting period and ensiled in silos type of glass containers. Each application consists of 3 parallel. 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, LAB and LAB + enzyme groups of dry matter 23.03, 21.83, 22.52, pH 4.11, 4.00, 4.15, ammonia-nitrogen; 70.19, 129.44, 106.00 found. In conclusion, it was evaluated that chemical, pyhsical, and microbiological qualities increease with the addition of LAB and LAB + enzyme to the narbon vetch silage. Also use to narbon vetch silage material useful for storage conditions.

Citation: ***Coskuntuna, L. and GÜL, S.*** **2020.** "The effects of lactic acid bacteria and inoculants mixture enzyme on fermentation and feed value of Vetch (*Vicia narbonensis L.*) silage", *International Journal of Current Research*, 12, (03), 10680-10683.

INTRODUCTION

Narbon vetch (*Vicia narbonensis* L.) has greater temperature and esser humidity requirements, which make it possible to grow advantageously in warm dry areas. It tolerates cold and is not damaged by frost. It can be used as forage crop (Altınok, 2002; Altınok and Hakyemez, 2002; Kendir *et al*., 2009). Fresh, dried or preserved forage legumes are highly suitable for use as roughage in animal diet. Because of their richness in protein, vitamins and mineral matter (Vasijeviç *et al*., 2009). The objective of the current work was to determine the ability of vetch to be ensiled and effects of LAB and LAB+Enzyme on the quality of narbon vetch (*Vicia narbonensis* L.) silage.

MATERIALS AND METHODS

Forage production: The study was conducted 2016 in Tekirdağ (41.0 ºN, 27.5 ºE), western Turkey located at about 5 m altitude above sea level and with a total precipitation of 482 mm on average and an annual mean temperature of 10.5 ºC. *Vicia narbonensis* was harvested pot setting period. Forage was chopped (1.0-1.5 cm theoretical length of cut). Silage materials were divided into three trial groups for the control, LAB and LAB+Enzyme treatments. (1) The chopped forage was mixed and divided into distilled water, denoted as treatment control; (2) inoculant, a mixture of lactic acid bacteria (LAB) consisting of *Lactobacillus plantarum* and *Enterococcus faecium* applied at a rate of 6.00 log10 cfu LAB·g^{-1} 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^{-1} 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-Offlingen, Germany) equipped with lids that enabled gas release only. The jars were stored under constant room temperature (20 ± 1 °C). Three jars per treatment were sampled on days 45 for pH, DM, WSC, LA content measurement, and LAB; mould 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 terephtahlate). 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

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during aerobic exposure is absorbed in the base and determined by titration with 1 N HCl. In addition, change in pH, yeast and mould 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 mould 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 moulding and five being completely moulded samples (Filya et al., 2000).

Analytical procedure: Chemical analyses were performed on triplicate samples. DM was determined by oven drying for 48 hat 60 °C. The pH in fresh material and silage samples was measured according to the British standard method (Anonymous, 1986). The ammonia nitrogen (NH3-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). Lactic acid (LA) were determined by the spectrophotometric method (Koc and Coskuntuna, 2003). 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 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 moulds 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 °C for 48-72 h.

Statistical analysis: Statistical analyses were performed with the general linear model (GLM) procedure of Duncan's multiple range test performed with the Statistical Analysis System (2005) Software (SAS, Cary, N.C.).

$Y_{ij} = \mu + a_i + e_{ij}$
Y_{ij}= studied traits
μ= overall mean
$a_{i=}$ = fixed effect of the treatment
e_{ij}= random effect

For all statistical comparisons, a probability level of $P<0.05$ was accepted as statistically significant. When significant associations were identified, the mean values for each effect were contrasted using Duncan test.

RESULTS

We investigated the effect of LAB inoculants as silage additives for the fermentation and aerobic stability of vetch silage. The results showed that the pH of all silage groups decreased faster. The pH content was significantly different in the control, LAB, and LAB+Enzyme silages in the current study ($P<0.05$) (Table 1). The concentration of ammonia-N after 21 days of ensiling was significantly higher ($P<0.05$) (Table 1). The DM contents of silages after 21 days of ensiling was found to be significantly different ($P<0.05$) (Table 1). The dry matter content of the 21 day ranged from 22.29 to 25 (Table 3). The same situation was identified for the DM content after five days in the control group ($P<0.01$) (Table 1). The ash content of the 45 day ensiling ranged from 9.36 to 9.84, and it was significantly different ($P<0.05$) (Table 2). In the study, no significant difference in the lactobacilli yeast

Table 1. Chemical analysis of Vetch (*Vicia narbonensis*) (pot setting) silages

Days of ensiling	Treatment	DM (% in FM)	pH	CP (%)	WSC g/kg	NH_3N/TN g/kg TN	LA (%)	Weight Loss (%)
0		23.14	5.75	17.66	70.16	68.16		
2	Control	22.40±0,54	4.68±0,06	17.75±0,03	50.40±5,84	55.02±2,81	0.83±0,05	6.72±1,16
2	LAB	23.42±0,30	4.73±0,02	17.26±0,36	60.25±2,39	55.51±1,64	0.78±0,06	7.57±0,61
2	I	23.94±0,66	4.65±0,03	17.54±0,07	45.48±3,96	56.52±0,42	0.75±0,06	7.88±0,76
5	Control	24.03±0,54 a **	4.73±0,01 a	17.47±0,01 a	43.19±1,96	63.83±1,52	0.95±0,03	7.96±0,82
5	LAB	22.66±0,24 b	4.33±0,01 b	17.24±0,03 b	42.15±4,01	62.89±2,96	0.97±0,04	6.87±0,66
5	I	23.45±0,12 ab	4.30±0,01 b	16.56±0,05 c	44.11±2,75	70.60±2,22	1.02±0,07	6.38±0,40
21	Control	23.37±0,62 b *	4.63±0,01 a**	16.63±0,06	19.65±2,50	86.70±3,15 b*	1.11±0,09	7.24±0,39
21	LAB	22.29±0,04 b *	4.25±0,01 b**	16.36±0,26	20.06±1,41	115.69±5,54 a *	1.22±0,06	7.73±0,41
21	I	25.00±0,35 a *	4.24±0,01 b**	16.10±0,02	20.35±2,90	124.85±2,46 a*	1.23±0,09	7.59±0,57
45	Control	23.03±0,20 a	4.11±0,01 a *	17.18±0,04	9.32±2,18	70.19±8,86 b *	1.80±0,06	6.78±0,46
45	LAB	21.83±0,30 b	4.00±0,01 b*	17.23±0,08	7.77±1,92	129.44±4,06 a *	1.72±0,07	7.33±0,35
45	I	22.52±0,17 ab	4.15±0,03 a*	16.95±0,22	8.49±1,53	106.00±4,32 a*	1.81±0,07	8.17±0,52

*Values with different superscripts within in the same column are different ($P<0.05$).
**Values with different superscripts within in the same column are different ($P<0.01$).
DM: dry matter; CP: crude protein; WSC: water soluble carbohydrate; NH_3-N: ammonia nitrogen.

Table 2. Chemical composition of Vetch (*Vicia narbonensis*) (pot setting) silage after 45 days of ensiling (%)

Days of ensiling	Treatmen	DM	CF	Ash	NDF	ADF	ADL
45	Control	23,03±0,20 a	23,03±0,50	9,36±0,03 b	36,43±0,34	30,30±0,49	13,18±0,06
45	LAB	21,83±0,30 b	21,83±0,20	9,84±0,15 a *	36,56±0,23	27,24±0,36	11,99±0,28
45	I	22,52±0,17 ab	22,52±0,43	9,43±0,03 b *	36,29±0,36	29,00±0,31	12,98±0,24

*Values with different superscripts within in the same column are different ($P < 0.05$).
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*) (pot setting) silages

Days	Treatment	Lactobacilli (log_{10} cfu)	Yeast (log_{10} cfu)	Mould (log_{10} cfu)
2	Control	0,71±0,06	0,56±0,08	0,18±0,04
2	LAB	0,61±0,07	0,71±0,13	0,23±0,06
2	I	0,61±0,07	0,65±0,08	0,22±0,07
5	Control	0,80±0,07	0,66±0,07	0,29±0,05
5	LAB	0,85±0,06	0,76±0,13	0,34±0,08
5	I	0,80±0,04	0,73±0,09	0,33±0,04
21	Control	1,02±0,07	0,95±0,07	0,48±0,06
21	LAB	1,03±0,11	0,91±0,07	0,57±0,06
21	I	1,02±0,14	0,98±0,09	0,61±0,08
45	Control	1,22±0,10	1,10±0,09	0,78±0,10
45	LAB	1,41±0,06	1,18±0,117	0,81±0,10
45	I	1,44±0,12	1,27±0,09	0,94±0,08

Table 4. Aerobic stability Vetch (*Vicia narbonensis*) (pot setting) silages.

Days	Treatment	pH	Yeast (log_{10} cfu)	Mould (log_{10} cfu)	CO_2 (log_{10} cfu)
	Control	8,81±0,02 a*	6,09±0,55	5,70±0,18	22,01±3,16
	LAB	8,67±0,01 b*	5,94±0,43	5,79±0,14	20,49±3,63
	I	8,47±0,07 b*	6,03±0,31	5,60±0,24	22,51±3,66

mold content detected among the control, LAB, and LAB+Enzyme treatments groups. In the silages evaluated, the CO_2 level of the LAB+Enzyme group was higher than the control and LAB groups. But was not significantly different (Table 4). Aerobic stability experiment identified that pH content of all silage groups were significiant difference ($P<0.05$). It's ranged from 8.47 to 8.81 (Table 4). An aerobic stability experiment established that the pH content of all silage groups was significantly different ($P<0.05$), ranging from 8.47 to 8.81 (Table 4).

DISCUSSION

For good quality silage, the fermentation 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). All silages were well preserved. In the study, LAB and LAB+Enzyme improved the fermentation parameters of narbon vetch silage. The pH of all the silages decreased faster and to a greater extent. In this study, it was established that the pH value was higher in the control and LAB+Enzyme groups. The pH value was found to be 4.11, 4.00, 4.15 for the control, LAB and LAB+Enzyme groups, respectively. The findings of Baytok & Muruz (2003), Koç et al.(2008), Basole (2010), and Elmalı & Duru (2012). support this study. In the experiment, the LA content of all vetch silage groups were not significantly different. Both inoculants produced more LA in the silages in agreement with pH values. The addition of LAB inoculants at ensiling was intended to ensure rapid and vigorous fermentation that results in faster accumulation of LA with a lower pH 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. The weight loss findings of the all silage groups were not significantly different. The findings of this study differ from the findings of Koç et al. (2009). This may have been the result of different temperatures and the formic acid that was used. The CP values of all of the groups of silage were not significantly different. Baytok et al. (2005) reported that the DM, CA, OM, and CP values were not significantly impacted by the organic acid addition to the silage. Additionally, Rowghani and Zamiri (2009) reported that DM and CP increase with the addition of organic acid to the corn silage. The ash content of vetch silage after was significantly different after 45 days ($P<0.05$). The findings of Elmalı & Duru (2012) support the findings of this study. Silage fermentation is a complex process which depends on many factors: The silage materials that contribute to a good fermentation are the dry matter content's physiological properties of epiphytic bacteria and most importantly, the quantity of soluble carbohydrates (Zanine et al., 2019). A decline in pH values inhibit the proliferation of spoilage microorganisms, which allow the nutritive values of the silage to be preserved.

Conclusion

The result of this experiment showed that addition of LAB and LAB+Enzyme addition to the vetch silage has possitive effect on the chemical and microbiological characteristics and ability of the vetch to be ensiled as well as the decrease in pH and thus improving silage preservation. Also addition LAB and LAB+Enzyme increased concentration of ammonia-N of vetch silage.

Acknowledgment

This work was supported by Namık Kemal University NKUBAP.024.YL.09.09 faundation Turkey. The authors would like to thanks fort his financial support. Namık Kemal University, Scientific research Project Commission

Conflict of interest

There is not any conflict of interest in this study all authors.

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