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Effects of Different Levels of Malic Acid on the Some Quality and Micro Flora of Vetch (*Vicia sativa* L.) Silage

[1]Dilek Aksu Elmali and [2]Metin Duru
[1]Department of Animal Nutrition and Nutritional Disease, Faculty of Veterinary Medicine,
[2]Department of Animal Science, Faculty of Agriculture, University of Mustafa Kemal,
31040 Hatay, Turkey

Abstract: The aim of this study was to explore the usability of different levels of malic acid as a vetch (*Vicia sativa* L.) additive. Vetch was used in this trial as the silage material. Groups with five repetitions which contained different levels of malic acid, i.e., 0-5 g kg^{-1} (M0-M5, respectively) were formed vetches cut in a mean length of 1.5-2 cm were ensiled in jars of approximately 1 L which let merely gas out. Physical, chemical and microbiological analyses were conducted on the silage which was opened on the 120th day after it was ensiled. During physical analyses, only the group which had been added 5 g kg^{-1} of malic acid was evaluated to have obtained the full score in each parameter, namely in smell, outer view and color. With the addition of malic acid, significant increases in dry matter, crude protein and nitrogen free extract values were observed ($p<0.001$) in groups M3-M5 while the same groups showed reduction in crude fiber and crude ash values ($p<0.001$). Lactic acid bacteria count was found to be below the detection limit in the control group while it was found to be above the detection limit in the groups which were added malic acid. Furthermore, the existence of mould was confirmed in the control group. In conclusion, it was evaluated that chemical, physical and microbiological qualities could increase with the addition of malic acid to the vetch silage.

Key words: Malic acid, silage, silage additive, silage quality, vetch, Turkey

INTRODUCTION

Animal food plant production assumes great importance in meeting the fodder needs of the animal entity. Vetch production ranks the second among the other animal food plants in Turkey. Vetch is classified under the groups of fodder which are difficult to ensile. Because vetches contain high levels of protein and low levels of carbohydrate it is reported that they must be cultivated in combination with cereals for fermentation to continue at desired levels. Moreover, it is reported that vetch-corn combinations could lead to quality silage.

It is further reported that ensiling of slashed barley which has low levels of dry material in combination with trefoil which has high levels of dry material and crude protein is harvested at the same time and is also difficult to ensile has positive effects on dry material consumption in sheep as well as on the digestion of crude food stuff. Leguminous fodder plants have high buffer capacities. Silage produced from such kind of fodder requires higher levels of acid formation for reduced pH. For such reasons, leguminous fodder plants are difficult to ensile (McDonald *et al*., 1995). Various fodder additives are used for facilitating the ensiling process in fodders which are difficult to ensile such as vetch, clover and trefoil. It is reported that many studies used organic acids as additives in production of silage (Baytok and Muruz, 2003; Adeyinka *et al*., 2008; Kamarloiy and Yansari, 2008; Arbabi *et al*., 2009; Paviz *et al*., 2011). Structures of organic acids are based on the carbon skeletal and such acids as malic, fumic and aspartic are commonplace in the nature as natural elements of animal and plant tissues (Indresh, 2007; Lehninger, 1975).

It is stressed that organic acids limit fermentation by rapidly decreasing pH in silage and consequently prevents warming and food stuff losses, principally protein losses. Furthermore, it is reported that anti-bacterial effects of organic acids prevent the development and growth of yeast, mould, Clostridia, Enterobacteriacea and other aerobic microorganisms in silage and therefore increase the aerobic stability and fodder and energy values of silage (Van Soest, 1994). In this context, this study aimed to explore the ability of vetch to be ensiled and the effects of malic acid on the quality of vetch silage given the positive aspects of food stuff content of the vetch as well as its difficulty to be ensiled.

Corresponding Author: Dilek Aksu Elmali, Department of Animal Nutrition and Nutritional Disease, Faculty of Veterinary Medicine, University of Mustafa Kemal, 31040 Hatay, Turkey

MATERIALS AND METHODS

Silage material: The vetch (*Vicia sativa* L.) which was cultivated in Mustafa Kemal University, Selam Husbandry Research and Implementation Farm was used in this trial as the silage material. Cultivated vetches were cut in a mean length of 1.5-2 cm. Then, groups M1-M5 were respectively added 1-5 g kg^{-1} of malic acid while control group received no addition of malic acid. Later on, malic acid was diluted with 5 mL of water in the groups and sprayed on the vetch. When mixed in a homogenous manner, the vetch was ensiled in jars of approximately 1 L. Five processes of ensiling were carried out in each group for 5 repetitions. After the end of ensiling period (on the 120th day) jars were opened with a view to analyzing the silage physically, chemically and microbiologically.

Physical analysis: As soon as the jars were opened silage samples were scored in terms of smell, outer view and color using silage evaluation survey reported by Alcicek and Ozkan.

Chemical analysis: Jars were opened at the end of 120 days. About 25 g of samples were taken from the silage and put in beakers. About 100 mL of pure water was added to the samples and the material was mixed using a mixer. Silage samples were measured for pH using pH meter (Orion 3 Star pH Benchtop) (Hart and Horn, 1987). Fresh fodder and silage material were analyzed for Dry Matter (DM), Crude Ash (CA), Crude Protein (CP), Crude Fiber (CF) and Ether Extract (EE) in accordance with AOAC (1999).

Microbiological analysis: The count of lactic acid Bacteria (LAB), yeast, mould, Enterobacteriaceae, sulfide reducing anaerobe and *Listeria* sp. in the silage was identified using the isolation and identification methods suggested by Harrigan (1998).

Statistical analysis: Variance analysis was used in the statistical evaluation of the data obtained in the trial and Duncan multiple comparison test was used in the identification of differences among the groups. Furthermore, data obtained in the physical analyses of the silage was identified in percentage and frequency values (SPSS Inc., 2002).

RESULTS AND DISCUSSION

Physical characteristics of the silage samples are shown in Table 1. Table 1 shows the frequency and percentage values of the physical characteristics of the silage samples. It is observed from Table 1 that only in the group which was added 5 g kg^{-1} of malic acid all the repetitions obtained the full score in terms of smell, outer view and color. Chemical characteristics of the silage samples are shown in Table 2. Groups M3-M5 demonstrated a significant increase in CP and EE values and a significant reduction in CF and CA values when compared to the other groups. Microbiological characteristics of the silage samples are shown in Table 3.

Table 1: Physical characteristics of the silage samples

	Smell			Outer view			Color		
Groups	Score	Frequency	%	Score	Frequency	%	Score	Frequency	%
C	14	1	20	4	1	20	2	2	40
	10	2	40	2	4	80	1	3	60
	4	2	40						
M1	14	5	100	4	5	100	2	3	60
							1	2	40
M2	14	4	20	4	5	100	2	4	80
	10	1	80				1	1	20
M3	14	3	40	4	4	80	2	4	80
	10	2	60	1	1	20	1	1	20
M4	14	4	20	4	5	100	2	4	80
	10	1	80				1	1	20
M5	14	5	100	4	5	100	2	5	100

Silage Evaluation Survey; Smell: 14 No butyric acid smell, lightly sour, aromatic smell; 10 Little butyric acid smell, strong, sour smell; 4 Moderate butyric acid smell, heating and mould smell; 2 Strong butyric acid smell, NH3-smell; 0 Strong mould smell, NH3 and decay. Outer view: 4 No distortion in the smell of leaves and stems; 2 A little distortion in the structure of leaves and stems; 1 Distortion in the structure of leaves and stems, mould and dirt; 0 decaying of the leaves and stems; Color: 2 preserves its color the way it was ensiled (brown in the faded silage); 1 A little change in the color (from yellow to brown) and 0 A totally different color (green as in mould)

Table 2: Chemical characteristics of the silage samples (DM)

Nutrients (%)	C	M1	M2	M3	M4	M5
pH	6.17±0.20^{a}	6.85±0.16^{a}	6.72±0.28^{a}	5.34±0.46^{b}	4.92±0.33^{b}	4.50±0.03^{b}
DM (natural)	9.60±0.18^{c}	10.49±0.18^{bc}	10.59±0.36^{bc}	11.41±0.79^{ab}	12.50±0.56^{a}	12.52±0.21^{a}
CP	17.92±1.44^{b}	14.69±0.48^{c}	16.70±0.89^{bc}	21.57±1.38^{a}	23.26±1.20^{a}	24.07±0.41^{a}
EE*	9.06±1.04^{a}	7.88±0.76^{ab}	7.00±0.50^{abc}	7.18±1.03^{abc}	5.44±0.37^{c}	5.95±0.47^{bc}
CF	36.06±1.71^{a}	40.18±1.57^{a}	36.02±1.81^{a}	26.72±0.85^{b}	27.49±1.95^{b}	27.15±0.62^{b}
CA	17.26±0.56^{a}	17.42±0.41^{a}	17.40±0.33^{a}	14.73±0.85^{b}	13.49±0.63^{b}	13.31±0.16^{b}
NFE	19.70±2.38^{b}	19.82±1.97^{b}	22.87±1.77^{b}	29.80±1.33^{a}	30.33±1.94^{a}	29.52±0.60^{a}

DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fiber; CA: Crude Ash; NFE: Nitrogen Free Extract; NFE = (100−CA)−(CP+CF+EE). The difference between the groups comprising different letters in the same line is statistically significant ($p<0.001$; $^{*}p<0.05$)

Table 3: Microbiological characteristics of the silage

Parameters	C	M1	M2	M3	M4	M5
LAB	<2.3	2.97±0.22[b]	4.04±0.22[a]	4.07±0.15[a]	4.64±0.18[a]	4.61±0.32[a]
Yeast	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
Mould	*	<2.3	<2.3	<2.3	<2.3	<2.3
Enterobacteriaceae	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
Sulfide reducing anaerobe	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
Listeria sp.	ND	ND	ND	ND	**	ND

ND: Not Detected; the difference between the groups comprising different letters in the same line is statistically significant ($p<0.001$), *The level of mould was found to be 2.3 cfu g^{-1} in two samples taken from the control group; ***Listeria* sp. was detected in one sample taken from Group M4

In this trial, silage was examined and scored in terms of smell, outer view and color as soon as the silage jars were opened. Only the Group M5 which contained 5 g kg^{-1} of malic acid obtained full score in smell, outer view and color parameters in all the five repetitions (Table 1).

In this trial, the group which did not receive any additives (control group) frequently showed low scores in physical characteristics. Furthermore, Demirel *et al.* report that smell and color are negatively affected by the increased ratios of vetch in the silage. One of the critical problems experienced by all the countries, principally warm countries which produce silage is aerobically unstable state of the silage following to the fermentation. For good quality silage fermentation aerobic requirements and reduced pH should be ensured. 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 pH value was higher in control group, M1 and M2 than in M3, M4 and M5 ($p<0.001$). pH value was identified to be 5.34, 4.92, 4.50 for M3-M5, respectively. Furthermore, it was observed that increased additions of malic acid to the silage decreased pH value (Table 2).

For example, pH value was reported to be in an interval of 5.12-5.36 in the timothy silage with no additions while it was reported to be in an interval of 4.51-4.62 in the timothy silage with organic acid addition (Baytok and Muruz, 2003). Such findings support this study. Baytok *et al.* (2005) report that organic acid has no impact on the pH value of the silage. On the other hand, a study suggests that addition of formic acid to the corn increases pH value (Rowghani and Zamiri, 2009). These differences reported in relation to the pH value of silage may have occurred as a result of the different levels of organic acid that was used. Another study suggests that addition of organic acid to the clover silage decreases pH value and therefore can be used as a contributory factor in better silage fermentation (Basole, 2010). In the relavant study, Basole (2010) suggest similar conclusions for pH value of the silage. Moreover, it was identified that increased levels of malic acid in the samples led to an increase in DM, CP and Nitrogen Free Extract (NFE) ratios and a reduction in EE, CF while CA levels (Table 2). It was further evaluated that CP and NFE levels were higher while CF and CA levels were lower in M3-M5 when compared to the remaining three groups ($p<0.001$). It is reported that DM, CA, OM and CP values are not significantly impacted by the organic acid addition to the silage (Baytok *et al.*, 2005). In this study, however, CP and DM values assumed differences in the groups with organic acid additions. In the relavant study, Baytok *et al.* (2005) suggest similar conclusions for CA of the silage. It was reported that DM and CP increase with the addition of organic acid to the corn silage (Rowghani and Zamiri, 2009). Such findings support this study. On the other hand, Rowghani and Zamiri (2009) report no significant difference in EE values among the groups. This may have been a result of the different levels of organic acid that was used. Furthermore, it is reported that CP and NFE values increase with the addition of organic acid to the silage.

Findings of such trials are similar to those of this study. In silage fermentation, lactic acid bacteria are the desired microorganisms while Clostridium, Enterobacteriacea family, yeast, acetic acid bacteria, mould fungi and Listeria are the undesired ones. High density of lactic acid bacteria in the silage indicates quality silage while high amounts of undesired microorganisms in the silage such as yeast, mould fungi and acetic acid bacteria indicate degradation. In this study, lactic acid bacteria in the silage were counted. No statistical analysis could be conducted on the control group as LAB amount was below the detection limit (<2.3) (Table 3). On the other hand, LAB was evaluated to be significantly higher in M2-M5 when compared to M1 ($p<0.001$). In this juncture, Basmacioglu and Ergul suggest that lactic acid bacteria can decompose such organic acids as citric acid and malic acid as well as carbohydrates. The relation between the increased amounts of malic acid in the silage and the increased numbers of lactic acid bacteria may have been triggered by the ability of such bacteria to decompose malic acid. In a similar study, it is suggested that addition of organic acid to the vetch-cereals silage significantly increases the count of LAB. Yeast is one of the most significant microorganisms which affect the quality of silage. Furthermore, some kinds of yeast may grow in an anaerobic way (Muck, 2010). In this trial, no statistical analyses could be conducted on yeast or microorganisms from Enterobacteriacea family or sulfide reducing anaerobes because they were under the detection limit (<2.3) (Table 3). Koc suggest that organic acid added to the vetch-cereals silage significantly increases the yeast levels in the silage excluding the silage which has a temperature of 20°C. Such findings about the effects of organic acid on the yeast levels do not support this study. Such findings may have been the result of the fact that Koc used as the fodder material, a mixture of vetch-cereals in their study.

Mould is classified under the groups of fibrous fungi and is absolutely aerobic. In relation to the other microorganisms, its growth in the silage is slower. The visual entity of mould indicates particularly low quality silage (Muck, 2010). In this trial, in an analysis conducted on two silage samples taken from the control group, mould level was identified to be 2.3 cfu g^{-1} (Table 3). On the other hand, mould level was identified to be under the detection limit in the other three samples taken from the same group. Furthermore, all the other samples of all the other groups demonstrated mould levels which were under the detection limit. Significantly higher pH levels in the control group when compared to M3-M5 may have been a reason for the bad quality silage. Similar to the findings of this study, it is suggested that addition of formic acid to the clover silage which is also difficult to ensile decreases pH value and contributes to regulating the fermentation process. Moreover, it is suggested that high pH levels may have negative effects on the microbiological characteristics of the silage. Koc report that addition of organic acid to the silage hampers the growth of mould. It is furthermore suggested that aerobic degradation occurs in vetch-cereals silage and in this case growth of mould may be hampered by the use of organic acid, especially at high temperatures. A similar study reports that organic acid decreases the amount of mould in the silage. Decreased pH value in the silage leads to reduced enzyme activity and prevents the growth of hazardous microorganisms, particularly Clostridia and Enterococci (Van Soest, 1994). In this study, Enterobacteriaceae and sulfide reducing anaerobes were identified to be below the detection limit in all the trial groups (<2.3). *Listeria* sp. requires oxygen and a pH value which is above 5.5 to be able grow in the silage. Sahin report that no *Listeria* sp. could be detected in the dry grass and the existence of *Listeria* sp. in the silage samples may be connected with the silage composition creating a good nourishing environment for microorganisms. In this trial, *Listeria* sp. was detected only in one sample which was taken from Group M4. It is considered that this sample may have picked it up from the soil. It is suggested that *Listeria* sp. may grow at a wide range of temperatures and it is more dominant in the silage with low DM content. Therefore, existence of this type of microorganism in the relevant group may have been connected with the low DM content in the silage.

CONCLUSION

In this study, it is evaluated that 5 g kg^{-1} of malic acid addition to the vetch silage has positive effects on the physical and chemical characteristics of the silage. Given the microbiological characteristics of the silage, it is concluded that addition of malic acid to the silage has positive effects on the ability of the vetch to be ensiled as well as the on the quality of the silage. Therefore, it is further concluded that malic acid may be used as an alternative silage additive in fodder which is difficult to ensile.

REFERENCES

AOAC, 1999. Official Methods of Analysis of the Association of Official Agricultural Chemists. 16th Edn., AOAC International, Gaithersburg, MD, USA.

Adeyinka, I.A., J.T. Amodu, M.S. Kallah, C.A.M. Lakpini and J.P. Alawa, 2008. The nutritive value of silages made from mixtures of pearl millet (*Pennisetum americanum*) and lablab (*Lablab purpureus*) as feed for yankasa rams. Asian J. Anim. Vet. Adv., 3: 78-84.

Arbabi, S., T. Ghoorchi and S. Hasani, 2009. The effect of delayed ensiling and application of an organic acid-based additives on the fermentation of corn silage. Aian J. Anim. Vet. Adv., 4: 219-227.

Basole, S., 2010. Effect of mechanical treatments and formic acid on quality of silage prepared from lucerne. Bioinfolet-Q. J. Life Sci., 7: 284-286.

Baytok, E. and M. Muruz, 2003. The effects of formic acid or formic acid plus molasses additives on the fermentation quality and DM and ADF degradabilities of grass silage. Turk. J. Vet. Anim. Sci., 27: 425-431.

Baytok, E., T. Aksu, M.A. Karsli and H. Muruz, 2005. The effects of formic acid, molasses and inoculant as silage additives on corn silage composition and ruminal fermentation characteristics in sheep. Turk. J. Vet. Anim. Sci., 29: 469-474.

Harrigan, W.F., 1998. Laboratory Methods in Food Microbiology. 3rd Edn., Academic Press, London.

Hart, S.P. and F.P. Horn, 1987. Ensiling characteristics and digestibility of combinations of turnips and wheat straw. J. Anim. Sci., 14: 1790-1800.

Indresh, H.C., 2007. Organic acids and plant extracts can be effective antibiotic alternatives. Feed Int., 28: 10-12.

Kamarloiy, M. and A.T. Yansari, 2008. Effect of microbial inoculants on the nutritive value of corn silage for beef cattle. Pak. J. Biol. Sci., 11: 1137-1141.

Lehninger, A.L., 1975. Biochemistry. 2nd Edn., Worth Publishers, New York.

McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan, 1995. Animal Nutrition. 5th Edn., Longman Singapore Publishers (Pvt.) Ltd., Singapore.

Muck, R.E., 2010. Silage microbiology and its control through additives. Proc. Rev. Brasileira De Zootecnia, 39: 183-191.

Paviz, M.M., T. Ghoorchi and F. Ghanbari, 2011. Effects of molasses and bacterial inoculant on chemical composition and aerobic stability of sorghum silage. Asian J. Anim. Vet. Adv., 6: 385-390.

Rowghani, E. and M.J. Zamiri, 2009. The effects of a microbial inoculant and formic acid as silage additives on chemical composition, ruminal degradability and nutrient digestibility of corn silage in sheep. Iranian J. Vet. Res. Shiraz Univ., 10: 110-118.

SPSS Inc., 2002. Statistical Package for Social Sciences (SPSS 11.5 for Windows). SPSS Inc., Chicago, IL., USA.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. 2nd Edn., Cornell University Press, Ithaca, New York, Pages: 373.