Effects of four organic acids known as key intermediates in citric acid cycle on fermentation and biochemical composition of alfalfa silage

W. C. Ke, Z. T. Ding, J. Bai, F. H. Li, D. M. Xu, Y. X. Zhang, X.S. Guo†

Probiotics and Biological Feed Research Centre, Lanzhou University, Lanzhou, P. R. China

Email: kewc12@lzu.edu.cn

Keywords: alfalfa silage, fermentation quality, proteolysis

Introduction Previous studies have confirmed that some organic acids, such as fumaric, malic, citric, and succinic acids, could stimulate the growth of microoganisms in the rumen and ultimately improve animal performance. It is supposed that addition of these four acids may not only have effects on silage fermentation but also indirectly provide animals with feed additives after ingestion of organic acid–treated silages. In addition, different dry matter contents (DM) also have effects on the fermentation quality of silage. Thus, this study aimed to investigate the effects of fumaric, malic, citric, and succinic acids on fermentation quality, proteolysis of alfalfa silage ensiled at two different DM contents, and to evaluate the utilization efficiency of these four organic acids in ensiled alfalfa after fermentation.

Material and Methods Alfalfa from the first-cut was harvested in April 2018 by using a field mower. After wilted to approximately DM contents of 330 (normal DM) and 400 (high DM) g/kg of fresh weight, the forage was chopped into lengths of 1-2 cm. The chopped forages were mixed thoroughly and randomly divided into 20 sub-samples and four of each were treated with each additive and ensiled singly. The Chopped alfalfa was ensiled with treatments of distilled water alone (control); (2) 0.5% malic acid (MA); (3) 0.5% citric acid (CA); (4) 0.5% sodium succinate (SS); (5) 0.5% sodium fumarate (SF). Each treatment was ensiled in four replicates and kept at ambient temperature for 60 d.

Results and Discussion The effects of four organic acids on the fermentation quality of alfalfa after 60 d are shown in Table 1. Compared with the control group, MA and CA-treated silages had lower pH at two different DM contents. This might be due to the acid properties of MA and CA. However, sodium succinate and sodium fumarate are both alkaline compounds, and the application of these two additives resulted in a higher pH in treated groups. This may be the reason for the similar pH in SS and SF treatments. As expected, greater pH was observed in MA and CA-treated silages with a high DM versus a normal DM. It was probably due to the lack of moisture in silages ensiled at a high DM. Because of additional fermentation substrates provided by these four additives, greater contents of WSC were observed in treated silages when compared to the control group. As the fermentation is closely related to the moisture contents of forage, the WSC were greater in silages with a high versus a normal DM. In the present research, treatments of MA and CA reduced the concentrations of NPN and NH_3-N relative to the control silage. It was probably due to the lower pH in MA and CA treated silages as plant enzymes are

quickly inactivated with a decrease in pH. Compared with silages ensiled at a normal DM, silages ensiled at a high DM had lower concentrations of NPN and NH_3-N. This is consistent with the observation from Cavallarin et al. (2005) where a significant decrease in proteolysis was found in alfalfa with increasing DM levels. In addition, lower NDF and ADF were observed in MA and CA-treated when compared to the control silage at two different DM contents. It was probably due to the lower pH in MA and CA-treated silages. The application of SS and SF reduced the contents of NDF in treated silages with a high DM, but it may due to the randomness of samples.

Table 1 The effects of four acids on the fermentation quality of alfalfa silage after 60 d

Variable	Forage	Treatments (T)					SEM	*P*-value		
	DM	Control	MA	CA	SS	SF		DM	T	DM × T
pH	Normal	4.92	4.58	4.67	4.89	4.99	0.013	<0.001	<0.001	0.005
	High	4.95	4.73	4.79	5.00	4.99				
WSC, g/kg DM	Normal	10.8	18.1	18.9	22.0	19.0	0.055	<0.001	<0.001	<0.001
	High	12.6	20.3	23.7	25.1	19.2				
NPN, g/kg TN	Normal	793	670	670	747	740	1.087	<0.001	<0.001	<0.001
	High	704	599	578	639	621				
NH_3-N, g/kg TN	Normal	161	69.8	80.4	94.2	107	0.536	<0.001	<0.001	<0.001
	High	91.8	28.0	32.2	36.7	38.8				
NDF, g/kgDM	Normal	32.0	27.5	29.6	32.5	32.0	1.191	0.500	<0.001	<0.001
	High	33.7	28.5	30.2	30.3	29.8				
ADF, g/kg DM	Normal	23.1	21.1	21.6	23.2	23.6	0.972	0.002	<0.001	0.042
	High	23.7	22.3	23.2	23.8	23.2				

MA, malic acid; CA, citric acid; SS, sodium succinate ; SF, sodium fumarate .

SEM, Standard error of the mean; DM, dry matter; WSC, water soluble carbohydrates; TN, total nitrogen; NPN, non-protein N; NH_3-N, ammonia N; NDF, neutral detergent fiber; ADF, acid detergent fiber.

Conclusion Application of these four acids could effectively improve silage fermentation quality with greater WSC and limited proteolysis during ensiling. Addition of MA and CA reduced the pH value and the contents of NDF and ADF at two different DM while the effects of SS and SF were quite small. Malic and citric acids are suitable additives in alfalfa silage fermentation.

Reference

Cavallarin, L., S. Antoniazzi, G. Borreani, and E. Tabacco. 2005. Effects of wilting and mechanical conditioning on proteolysis in sainfoin (Onobrychis viciifolia Scop) wilted herbage and silage. J. Sci. Food Agric. 85:831–838.