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Ensiling features of thistle (*Cynara cardunculus* L.) to be used for biogas production

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Introduction In recent years, there has been an increasing demand throughout the world for alternative energy crops for biogas. Over the last few years, a growing interest in thistle (*Cynara cardunculus* L.) has been observed, since it produces a large amount of biomass, even when the plants are provided with minimal inputs, which allows the crop to be grown on lands not usually used for cropping (Pari et al. 2017; Pesce et al. 2017). One critical point of alternative sources of biomass for biogas production, such as thistle, concerns their ability to well ferment and to be conserved as silage. The aim of this work was to evaluate the microbial and fermentation quality and the aerobic stability of thistle silage harvested at two different stages of maturity, and treated or not with a lactic acid bacteria (LAB) inoculum.

Material and Methods Two trials were conducted on thistle, harvested as a whole plant, at two different growing stages. The fresh forage was untreated (C) or treated with a mixture of *L. buchneri*, *L. plantarum*, and *L. casei* (L) [(theoretical application rate of 300,000 colony-forming units (cfu)/g fresh matter (FM)]. The forages were ensiled in 20-L plastic silos at a density of 716±24 and 378±20 kg/m^3 FM for stage I and II, respectively, and opened after 160 and 320 d of ensiling. At opening, the silages were analysed for dry matter (DM) content, pH, fermentative profile and microbial counts [lactic acid bacteria (LAB), yeast and mould]. The DM content was determined at 60°C for 72 h. The fermentative products were determined in the acid extract by HPLC. The microbial counts were determined using the pour plate technique on MRS and YGC agar, for LAB and for the yeast and mould, respectively. The weight losses due to fermentation were calculated as the difference between the weight of the forage placed in each plastic silo at ensiling and at the end of conservation, and were expressed on a DM basis. After each opening, silages were subjected to an aerobic stability test by continuously measuring the temperature during exposure to air. Aerobic stability was defined as the number of hours the silage temperature remained stable before increasing more than 2°C above room temperature. Data were analyzed for their statistical significance, via analysis of variance, using the GLM of SPSS (v. 24 for Windows, SPSS Inc., Chicago, IL). The data were analysed utilizing the treatments (T) and harvesting stage (S) as the fixed factor, with three replicates.

Results and Discussion The chemical and microbial characteristics of the thistle herbages, prior to ensiling, are reported in Table 1. The DM content at harvest was 24% (wet) and 45% (dry) for harvesting stages I and II, respectively. The buffering capacity was higher for the wet forages than for the dry ones, and no differences were found for the microbial counts. Fermentative profile, microbial counts, aerobic stability and weight losses of silages, after 160 and 320 d, are summarized in Table 2. The use of LAB inoculum did not influence many of the parameters at 160 or at 320 d of ensiling. The harvesting stage affected the fermentative profile to a great extent. Lactic acid was 7 and 10 times higher in wet silages than in dry ones for 160 and 320 d, respectively. Acetic acid was around 20 g/kg DM and 32 g/kg DM for wet and dry silages, respectively. This resulted in a higher lactic-to-acetic ratio than 3 in wet silages and lower than 1 in dry ones. Pari et al. (2017) found that the lactic acid ranged from 9 to 14 g/kg DM and acetic acid ranged from 10 to 19 g/kg DM in 35% DM thistle silages. The different lactic-to-acetic ratio determined the different yeast counts, which reached lower values in dry silages. A larger amount of ethanol (>43 g/kg DM) was found in wet silages than in the dry ones (<2 g/kg DM), with a significant interaction with the treatment, thus confirming a possible yeast activity during early phase of fermentation. The weight losses due to fermentation were influenced by the treatment and harvesting stage, and reached around 6% and 2% for wet and dry silages, respectively. During ensiling, the LAB count remained higher than 7 $\log_{10}$ cfu/g in all dry silage treatments, whereas it decreased as the ensiling duration increased in the wet silages. Despite the lower amount of LAB in wet silages, the fermentative products indicated a dominant lactic fermentation, probably because of the LAB fermented sugars in early stages of fermentation. The yeast count decreased during conservation, and this reduction improved aerobic stability in both wet and dry silages. Aerobic stability was 4 times higher in the dry than in the wet silages after 320 d. The higher the aerobic stability, the lower the yeast count, as previously reported for other forage crops (Kleinschmit and Kung, 2006).

Conclusion Thistle silage seems to represent a good opportunity for use as an energy crop for biogas production. The main factor that affects the fermentation quality appears to be the harvesting stage. Low DM content silages produced large amounts of fermentative products, but were characterized by high weight losses during fermentation and low aerobic stability. Dry thistle silages with low lactic-to-acetic ratios showed reduced yeast counts and improved aerobic stability. Further investigations are needed to evaluate the biogas production of these silages.

Table 1. The chemical and microbial characteristics of the thistle herbages prior to ensiling.

Parameters*	Stage I	Stage II
DM (%)	24.3 ± 0.15	45.2 ± 3.46
pH	6.06 ± 0.16	6.83 ± .012
Water activity (a_w)	0.996 ± 0.002	0.981 ± 0.003
Nitrate (mg/kg)	491 ± 177	578 ± 310
Buffering capacity (mEq/kg DM)	129 ± 8	71 ± 9
Yeast (log_{10} cfu/g)	6.43 ± 2.06	6.14 ± 0.13
LAB (log_{10} cfu/g)	7.84 ± 0.70	7.98 ± 0.47

* The values represent the mean of 6 replications ± SD. DM = dry matter, LAB = lactic acid bacteria.

Table 2. Fermentative profile, microbial count and aerobic stability of thistle silages.

Parameters*		Stage I		Stage II					
	d	C	L	C	L	*Treat*	*Stage*	*T*S*	SEM
DM (%)	160	20.6	22.1	44.7	43.1	NS	***	NS	3.44
	320	22.1	22.4	44.1	42.2	NS	***	NS	3.00
pH	160	4.13	4.11	4.90	4.78	**	***	NS	0.110
	320	4.14	4.07	4.74	4.72	NS	***	NS	0.091
Lactic acid (g/kg DM)	160	77.3	68.5	8.6	11.1	NS	***	NS	9.76
	320	55.4	61.3	6.6	6.2	NS	***	NS	7.59
Acetic acid (g/kg DM)	160	21.5	18.4	31.6	32.9	NS	***	NS	2.076
	320	18.4	19.5	31.7	35.2	NS	***	NS	2.26
Lactic-to-acetic ratio	160	3.7	3.7	0.3	0.4	NS	***	NS	0.521
	320	3.0	3.1	0.2	0.2	NS	***	NS	0.420
Ethanol (g/kg DM)	160	49.5	43.7	0.7	1.4	NS	***	NS	6.85
	320	58.5	55.4	0.6	1.7	*	***	*	7.94
LAB (log_{10} cfu/g)	160	5.58	5.34	7.21	7.61	NS	***	NS	0.305
	320	3.91	3.30	7.06	8.04	NS	***	*	0.841
Yeast (log_{10} cfu/g)	160	5.53	5.32	1.06	1.35	NS	***	NS	0.679
	320	3.03	1.50	1.73	1.46	NS	NS	NS	0.301
Aerobic stability (h)	160	64	69	54	125	NS	NS	NS	14
	320	113	134	391	>478	NS	***	NS	48
Weight losses (% DM)	160	6.05	5.87	1.97	1.95	NS	***	NS	0.604
	320	6.65	5.96	2.23	2.21	***	***	***	0.593

* d =day of ensiling; DM = dry matter; L = lactic acid bacteria inoculum; LAB = lactic acid bacteria.

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