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Article

Effect of the cutting date and the use of additives on the chemical composition and fermentative quality of sunflower silage

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Abstract:

The aim of this study was to evaluate cutting dates and the use of additives on the silage quality of the entire sunflower plant. The forage variety (Rumbosol-91) was harvested in weeks 1, 3 and 5 post-flowering (F1, F2 and F3, respectively) and treated with the following

additives: 1) 1.5 × 105 cfu of g^{-1} forage inoculant, based on homofermentative lactic acid bacteria *Enterococcus faecium*, *Pediococcus pentosaceus* and *Lactobacillus plantarum* (INOC), 2) 3 ml kg^{-1} forage of an 85% formic acid solution (FORM) and 3) without additive (Control); following a 3x3 factorial design with five replications. Effluent production and total dry matter (DM losses decreased, from 282 and 134 g kg^{-1} on D + 1 to 96 and 87 g kg^{-1} on D + 5 as a result of the high moisture content of the forage close to flowering. NIRS analysis of the silage samples showed that the protein, fiber and digestibility contents decreased significantly with the maturity of the plant; the rapid accumulation of oil in the DM made the energy concentration higher in the most advanced phenological state. The fermentative quality of the silages was satisfactory, regardless of the cutting moment and the use of additive. It is concluded that the cutting moment of the plant is better at five weeks post-flowering, when an acceptable fermentation is expected without the need to use preservatives.

Key words: Sunflower, Maturity, Digestibility, *in vitro*, Preservatives.

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Introduction

The sunflower (*Helianthus annuus* L.) is an annual plant, with many genotypes or subspecies cultivated for ornamental, oilseed and forage purposes. Official data indicated that, in 2006, the surface area of the species cultivated in Mexico[(1)] was approximately 2,000 ha —well below its potential—, allocated to oil production, mainly in irrigated areas[(2).] On the other hand, it has been demonstrated that the cultivation of sunflower as silage forage is a viable option in the feeding of ruminants, given its characteristics of rapid development, tolerance to low temperatures and little demand for moisture and fertilization; therefore, it is considered a good option for rain-fed areas, being an alternative to the cultivation of corn for use in animal feed[(3)].

The use of sunflower as green or summer silage was popular in the United States at the beginning of the 20th century, but today it has been surpassed by corn[(4)]. It can be established as a monoculture or combined with corn[(5)], and it is used as silage in crops when its establishment was late or when it has been damaged by the climate[(6)].

Its use as silage is subject to controversy, since the optimal time of use has not been determined. On the one hand, some studies present a harvest point around flowering, based upon maximum digestibility and protein values[7], and on the other hand, other authors recommend more advanced stages, when the seeds are well formed[8] or even close to the physiological maturity of the plant [9], in order to avoid high effluent production and reduction of silage losses.

The low dry matter content of sunflower, a moderate carbohydrate content and a relatively high buffer capacity, throughout its cycle are factors that can compromise the quality of fermentation and its conservation in the silo[10,11]; despite this, various authors have demonstrated the possibility of obtaining well-preserved silages[12]. The development in the use of additives to control fermentation and reduction of losses in the silage process was one of the most relevant technological advances of the last century. Today, inoculants, based on lactic acid bacteria are the most widely used additives in Europe and America[13]; these are added to the forage with the aim of controlling the bacterial populations that cause silage losses from the inefficient fermentation of sugars[14]. In addition, there is evidence of the usefulness of inoculants in forages with high moisture content, although results are inconsistent [15]. In this situation, direct acidification of the forage with organic acids may be an alternative. There is extensive literature on the effectiveness of formic acid on improving the quality of fermentation in animal production when high moisture forage is used[15-18]. Applied in the form of a commercial 85% solution and doses between 2 to 5 L t^{-1} of fresh forage, reduces pH immediately, favors the action of lactic bacteria against enterobacteria and clostridia and restricts the intensity of fermentation, which minimizes the risk of the presence of undesirable metabolites in the silage that may limit its chemical composition[17]. Faced with these advantages, the use of formic acid in high moisture forages can, on certain occasions, increase the production of effluence and the level of total losses, compared to the control without additives[15]. Therefore, the present work had the objective of assessing the effect of the cutting date and the use of additives on the level of losses, effluent production, chemical composition and fermentative quality of sunflower silage.

Material and methods

The study was accomplished during the summer-fall 2016 period at the Mabegondo Agriculture Research Center (CIAM, Spanish acronym) —located on the northwestern Atlantic coast of Galicia (Spain), at 43º 14’ 18.45’’ N and 8º 15’ 59.60’’ W, at 100 m asl —, in humid rain-fed areas. Three cutting dates and three additives were assessed following a

factorial design with five replications. The assay lasted for 108 d, from sowing to the last harvest. During cultivation, the mean temperature was 18.2 °C and the accumulated precipitation was 137 mm, somehow lower than the usual amount in the location. The onset of flowering took place 72 d after sowing, according to the Schneiter and Miller scale[18] corresponding to the final R4 phase (opening of the flower buds, the yellow ligulate flowers beginning to be visible). The forage variety Rumbosol-91, sown in late June 2016, was harvested in wk 1, 3 and 5 after flowering (treatments D + 1, D + 3, and D + 5, respectively). On each cutting date, about 50 kg of chopped forage was collected at 2-3 cm, homogenized and divided into three sub-samples and the additives were assigned: i) an inoculant (INOC) based on homofermentative lactic bacteria *Enterococcus faecium*, *Pediococcus pentosaceus* and *Lactobacillus plantarum* (SILOSOLVE F100, 3F Technology) at the manufacturer's recommended dose (1.5 x 105 cfu g^{-1} of forage); ii) an 85% formic acid solution (FORM) at a dose of 3 mL kg^{-1} of forage and, iii) a control without additive (CONTROL). For each combination (cutting date and additive), five laboratory silos were produced in polyethylene bags (40x10 cm), inside a 2.2L PVC pipe with a useful capacity equipped with an effluent drainage system[19,20].

Chemical analysis

The net weight of the forage of each silo and the weight of the effluent produced by it were determined at the time of its preparation and immediately before its opening at 60 d, using a 0.1 g precision balance (AND, model HR-202). From each sample taken at the time of filling each silo, the dry matter (DM) content was determined by drying it in a forced air oven at 80 ºC for 16 h. The spectra of the dried and ground samples at 1 mm were obtained using a Foss NIRSystem 6500 monochromator spectrophotometer (Foss NIRSystem Silver Spring, Washington, USA), and their composition in organic matter (OM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), water soluble carbohydrates (WSC), *in vitro* digestibility of organic matter (OMdv) and ethereal extract (EE) were determined using the calibrations developed at CIAM[19]. The buffer capacity (BCNaOH) was determined by reference methods[21], expressed as meq of NaOH kg^{-1} DM. The forage fermentability coefficient was estimated[22] according to the equation FC = (DM + 80 x WSC) / (BCNaOH x 0.127 - 0.3), where DM represents the % content of DM, WSC represents the concentration of soluble CHO in water expressed as % of DM and BCNaOH is the buffer capacity expressed in milliequivalents of alkali per 100 g of DM. FC values higher than 45 indicate ease of ensiling, while values lower than 35 are indicative of a high probability of bad fermentation.

Fermentative analysis

The silos opened after 60 d. In a fresh silage sample, the DM content was determined and subsequently corrected for loss of volatiles for the fermentation products during drying[23]. The values of OM, CP, ADF, NDF, OMdv and EE of the dry and ground samples at 1 mm were determined using NIRS calibrations developed at CIAM. The concentration in net energy value for lactation (NEl) of the silage was calculated[24] according to the NEl expression (Mcal kg^{-1} DM) = (178 x OMdv x MO + 0.008 x $OMdv^2$ x MO^2) x 10^{-6}, where OMdv is expressed in %, and OM, in % of DM. The net energy corresponding to the oil of the samples was added to this, considering an average value of 4.9 Mcal of NEl kg^{-1} of oil[25] for EE values above 4 % DM, since the NIRS calibrations for estimating *in vitro* digestibility with ruminal fluid[20] were obtained with degreased samples when this EE value was exceeded, in order to avoid the depressor effect of the samples' oil on the activity of ruminal microorganisms[26]. A second aliquot of silage was frozen at -18 ºC until the time when the fermentative analysis was performed, determining the pH by using a pH meter fitted with a combined electrode, ammonia nitrogen (N-NH3) with a selective electrode (Orion) and soluble nitrogen (Soluble N) by macro-Kjeldahl digestion. Fermentation acids (lactic, acetic, propionic, butyric, valeric, caproic, isobutyric and isovaleric) and alcohols (ethanol, butanol, propanol) were determined by gas chromatography[27]. The total volatile fatty acids (VFA) value was calculated as the sum —expressed in mmoles kg^{-1} DM— of the concentrations of the fermentation acids, excluding lactic acid.

The total loss values, effluent production, chemical composition and fermentation quality parameters were analyzed by analysis of variance (ANOVA) using the GLM procedure of SAS (SAS Institute 2009 v. 9.2) according to the model:

$$y_{ijk} = \mu + \alpha i\ F + \beta j\ A + (\alpha\beta)_{ij}\ FxA + \gamma_k + \varepsilon_{ijk}$$

Where the cutting date (D, i = 3) and the additive (A, j = 3) were considered fixed factors, and the replication (R, k = 5), a random factor, and FxA represents the interaction. The separation of means of the variables when the F test in the ANOVA was significant was performed using the Duncan HSD test ($\alpha = 0.05$).

Results and discussion

Crop development and dry matter production

On the first cutting date (D + 1) the phase was R5.5 (50 % of the tubular flowers in anthesis or post-anthesis); the phase R6 (complete flowering, with the seeds formed and the wilted ray florets) was reached on D + 3, and the phase R7 (lower part of the chapter is pale yellow in color, with thickened seeds, in a milky-pasty state) was attained on D + 5. The yield on these three dates was estimated[28] at 9.1, 10.1, and 11.5 t DM ha^{-1}, respectively. The highest unit production obtained at the latest cutting date coincides with that observed in other researches carried out at CIAM with the same forage variety[29], as well as in previous reports with various genotypes[8.30], according to which the most suitable phase for ensiling is when plants have yellow-green structures at the base and the seeds are well formed.

The chemical composition, buffer capacity, fermentability coefficient and estimation of net energy for lactation of the sunflower in the fresh state, are shown in Table 1. The cutting moment significantly affected (P<0.001) the content of DM, the chemical composition (except for the ADF content), and the *in vitro* digestibility and net energy for lactation values of sunflower at the time of ensiling. The DM of the crop averaged 16 % in all the cuttings and increased with the age of the plant from a value of 12.1 % on D + 1 to 18.6 % on D + 5, at a rate of 1.53 % units per week. The OM content showed a quadratic trend, with a value on D + 3 (95.5 % DM) higher than that of the other two cutting dates (90.9 and 90.5 % DM). Sunflower maturity decreased the protein, cell wall, sugars and digestibility contents, with values of 9.4, 9.2 and 8.6 for CP; 41.8, 40.5, and 36.8 for NDF; 16.9, 15.3 and 10.6 for WSC, and 67.0, 65.7 and 58.4 for OMdv on D + 1, D + 3, and D + 5, respectively, in contrast with the DM values. The concentration of EE increased from 2.7 to 17.6% with advancing maturity as a consequence of the conversion of non-structural carbohydrates to oil in the seeds, which was especially evident on the last date of the silage. The energy concentration, which increased from NEl of 1.38, 1.57, and 1.83 Mcal kg^{-1} DM on D + 1, D + 3, and D + 5, respectively, exhibited the same behavior. The buffer capacity and the fermentability coefficient were not affected ($P \geq 0.05$) by the cutting date, with the values of BCNaOH ranging between 320 and 346 meq NaOH kg^{-1} DM and FC between 45.7 and 38.6, although the latter exhibited a trend ($P = 0.10$) that suggests a greater ensilability of the plant in earlier stages of development, due to the higher sugar content, compared to later dates.

Table 1: Effect of the cutting date on the DM content, the buffer capacity, the fermentability coefficient, and the nutritional composition of fresh sunflower

	Cutting date				
	D+1	**D+3**	**D+5**	**SEM**	***P***
Dry matter (DM), %	12.1 [c]	14.1 [b]	18.6 [a]	0. 098	***
BC (meq NaOH kg^{-1} DM)	320	346	335	6.94	NS
Fermentability coefficient	45.7	42.5	38.6	2.02	NS
Chemical composition (% DM):					
OM	90.90 [b]	95.50 [a]	90.50 [b]	0.35	***
CP	9.40 [a]	9.20 [a]	8.60 [b]	0.12	***
NDF	41.80 [a]	40.50 [b]	36.80 [c]	0.23	***
ADF	34.10	33.40	33.80	0.26	NS
EE	2.70 [c]	6.90 [b]	17.60 [a]	0.18	***
WSC	16.90 [a]	15.30 [b]	10.60 [c]	0.31	***
OMdv, %	67.00 [a]	65.70 [b]	58.40 [c]	0.43	***
NEl, Mcal/kg MS	1.38 [c]	1.57 [b]	1.83 [a]	0.018	***

n= number of observations; SEM: standard error of the mean; BC= buffer capacity; OM= organic matter; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; EE= ethereal extract; WSC= water soluble carbohydrates; OMdv= organic matter digestibility *in vitro*.
(*** $P<0.001$; ** $P<0.01$; * $P<0.05$; NS: non-significant $P>0.05$);

Several studies carried out in Brazil and Argentina with the Rumbosol-91 forage variety show the variation in the chemical composition with the maturity of the plant. In the first country, in a harvest carried out between 4 and 7 wk after flowering, CP values were obtained between 9.9 and 7.0 on a dry matter basis[31], while, another research with cuttings between 97 and 112 d after planting (Phases R7 to R9) obtained CP values between 10.0 and 9.3 and EE values from 9.9 to 14.3 on a dry matter basis, respectively[32]. In another study, with a cutting date of 97 d since planting (DSP), a wide range of variation was obtained in the CP contents (9.4 to 14.5 % DM) and in NDF (40.6 to 48.7 % DM)[33]. Values of 9.6 and 8.3 % DM were obtained for CP, of 37.9 and 40.1 % DM for NDF, and of 15.1 and 13. 4 % DM for EE were obtained for the same Runbosol-91 variety at phases R7 and R9[34].

Two researches performed in the Rumbosol-91 variety at the CIAM experimental farm in different years and on different cutting dates[29,30] allowed comparing the cuttings from wk 2 to wk 6 after flowering; in this interval, the fresh forage contents were observed to increase in DM (from 15.6 to 22.4 %), in OM (from 89.9 to 85.8 % DM), in EE (from 2.3 to 17.0 % DM), and in NEl (from 1.34 to 1.61 Mcal kg^{-1} DM), while exhibiting a decrease in those of soluble carbohydrates (from 22.4 to 8.4 % DM) and digestibility (from 66.4 to 52.7 % DM). Studies carried out in France at the beginning of the last third of the XX^{th} century[32] indicate

digestibility values of 70 to 75 % at the onset of flowering, and 60 to 75 % for the plant at a fodder grain phase. However, more recent researches by Italian authors in the rainforests of the Po valley[35] report OMdv values close to 60 % for the plant in full bloom, which is more in line with the results obtained in the present work.

According to the coefficients estimated on D1, D2 and D3, the ensilability of the sunflower at these three phases of development can be rated good to medium. Despite the fact that studies carried out in Germany[10] assign low values to this species, an extensive review by other authors[36] indicates that fresh sunflower usually has a sugar content and a buffer capacity that can be considered as average, of 120-200 g kg^{-1} DM and 350-550 meq NaOH kg^{-1} DM, like Italian ryegrass or forage peas, consistently with the results shown the present paper.

The losses of dry matter, production of effluent, and nutritional composition of the silages are shown in Table 2. The cutting date had a strong significant effect (P<0.001) on the production of effluent, which was very high, especially in the first two cuttings (28.2 % of the fresh weight initially ensiled on D + 1, and 17.4 % on D + 3) due to the high moisture content of the forage, which subsequently decreased to 9.6 % in the last harvest. Thus, total DM losses in the first two cuttings (13.4 % in the first, and 12.5 % in the second) were significantly higher (P<0.001) than those of the last date (8.7 %). Studies evaluating the effect of harvest DM content on silage losses indicate that, with a DM content of 30 % or more, respiration and fermentation losses should not exceed 5 to 8 % of ensiled DM, while in the case of crops harvested under conditions of high moisture (DM <25 %), the losses are usually greater, due to a higher fermentation intensity and, above all, to the losses caused by the effluent[13,37].

Table 2: Effect of the cutting date and of the use of additives on the effluent production, total loss level and nutritional composition of sunflower silage

	Main effects									Interaction
	Cutting				Additive					
	D+1	D+3	D+5	*P*	TES	INOC	FORM	P	SEM	*P*
n	15	15	15		15	15	15			
Dry matter losses (%DM)										
DML	13.4 [a]	12.5 [a]	8.7 [b]	*	8.8 [b]	10.9 [b]	14.8 [a]	**	1.14	NS
Effluent (% initial fresh matter)										
EFL	28.2 [a]	17.4 [b]	9.6 [c]	***	16.8 [b]	17.50 [b]	20.9 [a]	***	0.666	**
Dry matter (%)										
DM	14.8 [b]	15.3 [b]	19.0 [a]	***	16.3	16.3	16.5	NS	0.227	NS
Chemical composition (%DM)										
OM	89.3 [b]	90.3 [a]	89.0 [b]	***	90.1 [a]	90.1 [a]	88.5 [b]	***	0.154	NS
CP	11.4 [a]	11.3 [a]	9.3 [b]	***	10.7	10.7	10.6	NS	0.057	***
NDF	45.5 [a]	43.9 [b]	38.9 [c]	***	42.1 [b]	42.6 [b]	43.5 [a]	**	0.259	**
ADF	37.5 [a]	35.7 [b]	32.3 [c]	***	34.3 [b]	34.8 [b]	36.4 [a]	***	0.161	***
EE	2.7 [c]	7.8 [b]	18.0 [a]	***	9.1 [b]	9.7 [a]	9.7 [a]	***	0.094	***
in vitro Digestibility										
OMdv (%)	53.6 [a]	53.3 [a]	46.4 [b]	***	49.5 [b]	49.6 [b]	54.2 [a]	***	0.414	***
Net energy for lactation (Mcal kg^{-1} MS)										
NEl	1.04 [c]	1.23 [b]	1.56 [a]	***	1.23 [c]	1.25 [b]	1.34 [a]	***	0.009	**

n= number of observations; SEM= standard error of the mean; OM= organic matter; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; EE= ethereal extract; OMdv= *in vitro* organic matter digestibility.

(*** $P<0.001$; ** $P<0.01$; * $P<0.05$); NS= non-significant $P>0.05$;

[ab] Values with different superscripts on the same row for each main effect are different ($P<0.05$).

Comparison between the mean values of the fresh sunflower and the resulting silage evidences an increase in the content in DM (+1.4 %) and in the concentration (in % DM) of CP (+1.6 units), ADF (+3.0 units), NDF (+1.4 units), and EE (+0.4 units), as well as a sharp decrease in the value of OMdv (-12.6 %) and NEl (-0.31 Mcal kg^{-1}DM), which can be attributed to the high production of effluent in all cuttings.

As some studies indicate, an increase in the DM of silage is expected when the DM content of green forage placed in the silo is less than 23-25 %[38]. Also, there will be little variation in the content of ash and total nitrogen when the corresponding values of the resulting silage are expressed on a dry matter basis corrected for the losses of volatile substances that take place during the drying process in the kiln[22], while the fiber content is usually significantly

affected by the silage, due to the passive increase caused by losses of dry non-cellulosic matter in a solid form in the effluent, or as gas during fermentation[39]. On the other hand, although it is generally assumed that the digestibility of the silage is equal to or slightly lower than that of the original forage[40], there is evidence of a significant decrease in digestibility in the case of ensiled forages with low dry matter content, because the effluent contains highly digestible nutrients[41]. An average decrease of 7.0 percentage points in the digestibility values of the DM[42] has been indicated for silages with dry matter contents close to 16%, a figure that was exceeded widely in our study, probably due to a higher moisture content.

The cutting date significantly affected the DM content, the chemical composition, the digestibility, and the energy concentration of the silage. In general, the variation observed in the quality of the silage in the different cuttings was similar to that observed for the original fresh forage. Neither the values of total losses of DM or the DM or CP content were affected by the use of additive ($P>0.05$), whose effect on the characteristics of the silages was comparatively less than that exerted by the cutting date. Notably, formic acid significantly increased the production of effluent ($P<0.001$) and the level of total DM losses ($P<0.01$) in relation to the inoculant and to the control without additive, which exhibited values of 20.9 *vs* 17.2 and 16.8 % of the fresh silage weight for the effluent and 14.8 *vs* 10.9 and 8.8 % for the level of losses, respectively. In addition, the silages treated with formic acid showed significantly lower concentrations of OM (88.5 *vs* 90.1 and 90.1% DM) and higher concentrations of NDF (43.5 *vs* 42.6 and 42.1 % DM), of ADF (36.4 *vs* 34.8 and 34.3 % DM), and, above all, of OMdv (54.2 *vs* 49.6 and 49.5 % DM) and NEl (1.34 *vs* 1.25 and 1.23 Mcal kg^{-1} DM). The results obtained agree with the observations by other authors who point out the increase in the production of effluent and losses when formic acid is applied to high moisture forages[39], and an improvement in digestibility due to the lower expenditure of non-structural carbohydrates during fermentation[15]. The effect of the use of inoculants on the level of DM losses in silage varies: certain reports show a negative effect, with DM recovery values below those of a control without additive[43]; but generally its effect on high moisture forages is low[44], as observed in this study.

Fermentative quality of silages

The harvesting date and the use of additives significantly affected the main parameters that define the fermentative quality (Table 3). The pH values increased with the cutting date, being different from each other in the three uses (D + 1: 3.77, D + 3: 3.94 and D + 5: 4.04, $P<0.001$). The acetic contents, VFA, soluble N, and N-NH_3 were lower ($P<0.001$) on the first cutting date than on the two subsequent dates, which did not differ from each other

(acetic: 1.71 *vs* 2.54 and 2.41 % DM; VFA: 289 *vs* 426 and 405 mmol kg^{-1} DM; soluble N: 32.8 *vs* 45.2 and 46.7% of the total N; N-NH_3: 3.87 *vs* 7.03 and 7.68 % of the total N). The contents of lactic acid and ethanol evolved in a contrary way, being higher in the earliest cuttings compared to the later ones (lactic: 9.06, 7.66 and 6.36 % DM; ethanol: 4.17, 4.92 and 3.39 % DM; D + 1, D + 3, and D + 5 respectively). The butyric content, propionic and longer chain VFA, as well as butanol and propanol, were very low at all cutting dates.

Table 3: Effect of the cutting date and use of additives on the fermentative quality of sunflower silage

	Main effects									**Interaction**
	Cutting				**Additive**					
	D+1	**D+3**	**D+5**	***P***	**TES**	**INOC**	**FORM**	***P***	**SEM**	***P***
n	15	15	15		15	15	15			
pH	3.77 ^{c}	3.94 ^{b}	4.04 ^{a}	***	3.8 ^{b}	3.77 ^{c}	4.18 ^{a}	***	0.007	***
Fermentation products (% DM)										
Lactic	9.06 ^{a}	7.66 ^{b}	6.36 ^{b}	***	11.2 ^{a}	11.48 ^{a}	0.35 ^{b}	***	0.481	NS
Acetic	1.71 ^{b}	2.54 ^{a}	2.41 ^{a}	***	3.05 ^{a}	2.61 ^{b}	1.00 ^{c}	***	0.086	*
Propionic	0.013	0.016	0.021	NS	0.021	0.016	0.012	*NS*	0.003	NS
Butyric	0.015 ^{a}	0.002 ^{b}	0.003 ^{b}	***	0.007	0.006	0.008	*NS*	0.001	NS
Valeric	0.002 ^{b}	0.00 ^{b}	0.005 ^{a}	*	0.003	0.002	0.001	*NS*	0.000	NS
Caproic	0.004	0.001	0.002	NS	0.005 ^{a}	0.002 ^{b}	0.001 ^{b}	*	0.000	NS
Isobutyric	0.003 ^{a}	0.001 ^{ab}	0.0 ^{b}	*	0.002	0.001	0.001	*NS*	0.000	NS
Isovaleric	0.002 ^{b}	0.0 ^{b}	0.005 ^{a}	**	0.003	0.002	0.001	*NS*	0.000	NS
Butanol	0.010 ^{a}	0.011 ^{ab}	0.007 ^{b}	*	0.009	0.009	0.01	*NS*	0.000	NS
Ethanol	4.17 ^{a}	4.92 ^{a}	3.39 ^{b}	**	1.85 ^{b}	1.95 ^{b}	8.68 ^{a}	***	0.261	***
Propanol	0.18	0.15	0.09	NS	0.13	0.17	0.12	*NS*	0.041	NS
Volatile fatty acids (mmoles kg^{-1} MS)										
VFA	289 ^{b}	426 ^{a}	405.7 ^{a}	***	513.6 ^{c}	437.9 ^{b}	169.1 ^{a}	***	14.9	*
Soluble and ammoniacal N (% N total)										
Soluble N	32.8 ^{b}	45.2 ^{a}	46.7 ^{a}	***	45.2 ^{a}	43.3 ^{a}	36.2 ^{b}	***	0.707	NS
N-NH_3	3.87 ^{c}	7.03 ^{b}	7.68 ^{a}	***	8.18 ^{a}	6.84 ^{b}	3.57 ^{c}	***	0.161	***

n= number of observations; SEM= standard error of the mean; VFA= mmoles kg^{-1} DM of acetic, butyric, isobutyric, propionic, valeric and isovaleric.

(*** $P<0.001$; ** $P<0.01$; * $P<0.05$; NS: non-significant $P>0.05$);

^{abc} Values with different superscripts on the same row for each main effect are significantly different ($P<0.05$).

According to the criteria of the French INRA or the German DLG[45], a good fermentation quality of high moisture silages is defined by values of pH ≤4.0, absence or traces of butyric

and propionic acids, acetic acid content <2-3 % DM, a N-NH3 content ≤5-8 % of the total N, and a soluble N content equal to 50 % of the total N, with VFA content below 600 mmol kg^{-1} DM. In regard to these criteria, the results obtained in this study reflect an acceptable or good quality of sunflower fermentation throughout the growth cycle considered and suggest that, despite the higher moisture, the greater availability of sugars at the earliest dates favors a better fermentation quality compared to a later use, confirming the fermentability coefficients registered in the fresh state.

The control silage showed values of pH (3.80), VFA (513 mmol kg^{-1} DM), soluble N (45.2 % of the total N), and N-NH3 (8.1 % of the total N), which is indicative of an acceptable fermentative quality, although it had a somewhat high concentration of acetic acid (3.05 % DM). Compared with the control group, the inoculant significantly (P<0.001) reduced the pH (3.77), and the contents of acetic acid (2.61 % DM), VFA (437 mmol kg^{-1} DM), and N-NH3 (6.84 % of the total N), slightly improving the fermentation. The results obtained with the application of inoculant to the sunflower agree with those of the researches that show a positive effect of its use on the fermentation quality[43,46], its effectiveness being especially interesting, even on high moisture forages. As in in the present study, this effect is consistently observed with silage forages where the DM content is close to 30 % or higher[47].

The addition of formic acid reduced the intensity of fermentation in the silage, evidenced by a higher average pH value (4.18) and lower average contents of lactic acid (0.35 % DM), acetic acid (1.0 % DM), VFA (169 mmol) kg^{-1} DM), soluble N (36.2 % total N), and N-NH_3 (3.5 % of the total N), differing significantly (P<0.001) from those of the other two additive treatments. Another typical feature of the formic activity is the elevation of the ethanol content, compared to the inoculant and the control (8.68 *vs* 1.85 and 1.95 % DM), which is attributed to a higher activity of the yeasts, particularly tolerant to the action of formic acid[48], linked to the greater availability of sugars in less intense fermentation. In agreement with these observations, various studies have shown that the application of formic acid produces silages with low values of lactic and acetic acids, and a lower lactic: acetic acid ratio, as well as a lower ratio of ammoniac N over total N, as a consequence of the reduction of the intensity of the proteolytic processes caused by the additive[15,49].

The effect of the different additives on the fermentation parameters was relatively homogeneous on the three cutting dates, as evidenced by the low quantitative importance of the interactions with a significant effect on pH and the contents of acetic, ethanol, VFA and N-NH_3. While the effect of formic acid was similar at the three cutting dates of sunflower, the positive effect of the inoculant in improving fermentation is more evident when the plant is harvested in the vicinity of flowering, probably due to the greater amount of the sugary substrate available to the lactic bacteria added to the forage, which are effective despite the

higher moisture at this time, consistently with the anticipated coefficient of fermentability for fresh forage.

To the evident improvement in fermentation induced by the use of formic acid, it must oppose, on the one hand, the difficulty of its application due to its strong, potentially corrosive acid character, and, on the other, the increased production of effluent, already high in the control treatment, caused by its application. From this point of view and taking into account the high polluting power of the effluent[50], it would not be advisable to use formic acid versus the inoculant and the control without additives. On the other hand, given the good fermentative quality of preservative-free sunflower silage, the justification of the expense in the addition of inoculant is subject to controversy, and, despite the improvement in the expected fermentative quality, its use should be compared, in economic terms, with improving the productivity of animals fed with different types of silage —an aspect that lies outside the scope of the objective.

Conclusions and implications

Sunflower silage has a good energy content and a moderate amount of protein. The cutting date affects dry matter and energy increasing as the plant becomes older, but the percentage of the first cutting is minimized. Because the use of additives provides a low margin of advantage in terms of silage quality, it is should be subjected to cost-benefit analyses. Due to its concentration of nutrients, its chemical composition values and fermentative characteristics, sunflower silage can be a complementary forage in the nutrition of dairy cattle; however, its high oil content near the optimal harvest time may represent a limitation to its use in the diet.

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