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The effect of harvest date on the yield and forage quality of ensiling safflower biomass

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Abstract
Safflower (*Carthamus tinctorius* L:) usually grown as a source of oil can be also used as fodder either for hay or ensiling purposes, particularly in semiarid regions. In 2002-2003 a research project was carried out in Southern Italy to evaluate the production and forage quality of safflower biomass cv Centennial (high oleic), harvested at three different stage: May 14 (at complete appearance of primary buds, PB), May 26 (at complete appereance of secondary and tertiary buds, STB), June 11 (at 25% of flowering stage, FS). For each stage of growth, 50% of the biomass was ensiled in 4-L glass jars without and with inoculation (*Lactobacillus plantarum*); the other 50% was field wilted for 24 hours before ensiling. DMY ranged from 4,5 t ha^{-1} (PB harvesting) to 11, 6 t ha^{-1} (FS harvesting). DM content varied from 129 g kg^{-1} (PB not wilted) to 630 g kg^{-1} (FS wilted). The following chemical parameters have been evaluated: pH, buffering capacity (BC), lactic acid, acetic acid, NH_3-N, crude protein content (CP), water soluble carbohydrates (WSC), NDF, ADF, ADL, ash and fat. The WSC concentration in the forage before ensiling without wilting ranged from 128 (PB stage) to 105 and 100 g kg^{-1} DM at STB and FS stage, respectively. The wilted safflower forage showed on the average a lower WSC (95.8 g kg^{-1} DM) than for forage which was not wilted (111.4 g kg^{-1} DM). The high sugar substrate allowed lactic acid fermentation and a good conservation quality in all the harvesting stages. Silages quality was strongly influenced by the treatment performed. Wilting practice as averaged effect increased DM, pH and NDF contents but reduced lactic acid, acetic acid and NH_3-N values. Inoculation reduced, on the average, DM, pH and NDF contents but increased lactic acid, acetic acid, CP and ash values. Wilting the forage for one day is very effective on early stage of harvesting because this practice will greatly increase DM, reducing at the same time the intensive fermentation and proteolysis processes of the silage. When harvesting is performed at the beginning of flowering stage wilting is not necessary.

Key words: *Carthamus tinctorius* L – silage – stage of growth – field wilting – lactic acid bacteria inoculation

Introduction

Safflower grown as an oil crop has been the object of several research studies conducted in Southern Italy in the last 30 years either as experimental trials (Corleto *et al.,* 1997) or as large crops at different farms (Corleto, 2001). The lack of the development of the crop for oil production in the Mediterranean environments is mainly due to the length of cropping cycle along with low seed yield potential (Corleto, 2008). However the safflower plant can be utilized as grazed forage or stored as hay or silage and its feed value and yield are similar to or better than oats or alfalfa (Smith, 1996). Safflower in the budding stage (end of May) showed good DMY around 7 t/ha, DMP 16-20%, CP around 9% and CF about 30% (Corleto *et al*., 2005). Weinberg *et al*. (2002) found that safflower silage has potential as alternative fodder in semi arid regions. The in vivo digestibility and the intake of green safflower fodder were higher in safflower than those of a vetch-oats mixture (Vonghia *et al.*, 1992), a very common fodder crop in Southern Italy. Landau *et al.* (2004) found that safflower silage has the potential for widespread adoption as a feed in Mediterranean countries, if special characteristics such as protein degradability are taken into account to optimize its inclusion in a total mixed ration. However there is little information available regarding the optimum time for harvesting. The overall aim of this study was to compare the yield, fermentation characteristcis and feeding value of safflower ensiled at different stages of growth and to determine whether the use of an

inoculant and the forage wilting technique influenced the fermentation and nutritional quality of the silage produced.

Materials and methods

Crop and ensiling

The trial was run in 2002-2003 at Gaudiano di Lavello (PZ), Basilicata Region at 180 m a.s.l. on a sandy clay textured soil (65% sand, 27% clay) characterized as sub-alkaline (pH 7.4), low in total nitrogen (0.98‰) and high in available phosphorus (127 ppm) and exchangeable potassium (662 ppm). Seeding was performed on December 12, 2002 using "Centennial", an high oleic type cv, at seed rate of 100 seeds m^{-2} in rows 30 cm apart on a plot area of 650 m^2. Complete seedling emergence was observed on January 12, 2003.

During the cropping cycle a mechanical weeding (March, 20) and nitrogen fertilization with 100 kg ha^{-1} of N as ammonium nitrate (March, 28) were performed while on May 5 a supplemental sprinkling irrigation with a water volume of 200 m^3 ha^{-1} was applied.

During the cropping cycle (December, 2002 – June, 2003) total rainfall was 378 mm and higher than the annual (?) mean (282 mm).

During the reproductive stages, the forage was cut at three different growth stage: at appearance primary buds (PB, May 14), at appearance secondary and tertiary buds (STB, May 26) and at 25% flowering stage (FS, June 11). The forage was manually harwested at a height of 50 mm and yield was determined by weighing the material cut from a sample area of 20 m^2 replicated at random, 3 times. Half the herbage was field wilted for a period of 24 h; the weather conditions were favourable, with no rainfall during three cuts. Unwilted and wilted herbage was chopped to ≈ 2 cm. For each wilting stage, half the forage was treated with an inoculant (Lactosil; CSL, Italy) applied at a rate of 10^6 colony forming units (CFU) of *Lactobacillus plantarum* per g fresh matter (inoculated), whereas the other half was ensiled untreated (control). Around 20 kg of forage was weighed accurately to the nearest gram, before being spread on a 3 m x 3 m polythene sheet. The inoculant was applied using a pressurized hand sprayer at a rate of 2 L t^{-1}. Control forage had a similar volume of water applied in the same way. The forage was then mixed thoroughly before being ensiled in sterile 4 L anaerobic glass jars. Silage was weighed immediately after filling and stored in the dark at 23 ° C for 120 days.

Analytical procedures

Dry matter (DM) of the silage samples was determined in a forced-draught oven set at 60°C for 48 h. Total nitrogen (TN) was determined by the Kjeldahl method, also expressed as crude protein (CP) (TN × 6.25), and ether extract (EE) was determined according to AOAC (1990). Fermentation losses were evaluated according to weight loss and expressed as gas loss (g/kg FW). Neutral detergent fiber (NDF, inclusive of ash), acid detergent fiber (ADF, inclusive of ash) and acid detergent lignin (ADL, inclusive of ash) were determined according to Van Soest *et al.* (1991). Ash was measured by igniting samples in a muffle furnace at 550°C for 16 h. Water-soluble carbohydrate (WSC) concentration was determined by the phenol–sulfuric acid method according to Dubois *et al.* (1956). The silage juice was extracted manually with a silage press. The silage pH was measured directly from the silage juice using a pH meter (Jenway 3010 pH meter; Kernick and Son, UK). The buffering capacity (BC) was determined in water extracts according to Playne and McDonald (1966). Ammonia nitrogen (NH3-N, g kg^{-1} of TN) determined using a specific electrode was quantified in the acid extract. The filtered and acidified silage extracts were analysed for D- and L-lactic acid and acetic acid by an enzymatic kit procedure, the acetic acid assay kit K-ACET and the D-/L-lactic acid kit K-DLATE, respectively, according to the manufacturer's manual (Megazyme Int. Ireland Co., Ltd., Wicklow,. Ireland). The sum of the L- and D-lactic acids was reported as the total lactic acid concentration. The fresh forage samples were analysed for DM, pH, BC, and WSC following the procedures described for silage.

Statistical analysis was applied to data following the split-plot design (fresh forage) and the split-split-plot design (silage); differences among mean values were tested by LSD test. ANOVA was performed using the Costat Software v. 6.2003 (Cohort Software).

Results

Safflower forage characteristics before ensiling

The results are reported in table 1. DMY ranged from 4.5 t ha^{-1} (PB, May 14) to 11.6 t ha^{-1} (FS, June 11). Wilting time reduced DMY only at the late harvest (10.8 vs 11.6 t ha^{-1}) (H x W interaction). The delay of the harvesting increased DM: 183 g kg^{-1} at PB stage, 229 g kg^{-1} at STB stage and 490 g kg^{-1} at FS stage. Wilting increased DM in all the 3 harvest dates. As average wilted forage showed a DM content higher (392 g kg^{-1} FW) than unwilted forage (209 g kg^{-1} FW). Differences in DM content between wilted and no wilted forage increased with the delay of the harvesting (H x W interaction).

Table 1. Effects of harvest dates (H) and wilting time (W) on the yield of dry matter (DMY, tonnes/ha), DM content (g/kg), pH, buffering capacity (BC, mequiv./100 g DM) and WSC (g/kg DM) in fresh safflower.

Harvest dates	Wilting time (h)	DMY	DM	pH	BC	WSC
PB (May 14)	0	4,5	129	6,07	52,0	128,4
	24	4,6	238	6,10	48,9	97,5
	Mean	*4,6*	*183*	*6,08*	*50,5*	*112,9*
STB (May 26)	0	6,5	149	6,10	54,5	105,8
	24	6,5	309	6,07	21,6	95,6
	Mean	*6,5*	*229*	*6,08*	*38,1*	*100,7*
FS (June 11)	0	11,6	351	6,10	28,8	100,0
	24	10,8	630	6,13	22,9	94,1
	Mean	*11,2*	*490*	*6,12*	*25,8*	*97,1*
Mean wilting time	*0*	*7,6*	*209,5*	*6,1*	*45,1*	*111,4*
	24	*7,3*	*392,0*	*6,1*	*31,1*	*95,8*
LSD (<0,05 P) mean effect H		0,3	23	NS	5,7	10,8
LSD (<0,05 P) mean effect W		0,2	19	NS	4,7	8,8
LSD (<0,05 P) (HxW)		0,4	32	NS	8,1	NS

The pH was not influenced by the treatments imposed (6.1 on the average). BC was strongly influenced by harvesting date: 50.5, 38.1 and 25.8 mequiv./100 g DM at PB, STB and FS stages, respectively. BC was reduced by forage wilting (31.1 *vs* 45.1 mequiv./100 g DM, respectively with and without wilting) and its influence resulted evident only at STB harvesting (21.6 mequiv./100 g DM with wilting vs 54.5 mequiv./100 g DM with no wilting) (H x W interaction). WSC showed the highest value at PB stage (112.6 g kg^{-1} DM) while the values were similar when the forage was harvested at STB stage (100.7 g kg^{-1} DM) and at FS stage (97.1 g kg^{-1} DM). The wilted safflower forage showed on the average a lower WSC (95.8 g kg^{-1} DM) than no wilting forage (111.4 g kg^{-1} DM). The ratio WSC/CP calculated on control plants at PB stage was 0.84, close to 1 that is considered in general the optimum ratio for good silage preservation.

Fermentation characteristics and feeding value of the silages

The data, reported in table 2, show the effects of three different harvesting dates, two wilting time and the bacterial inoculant on the fermentation characteristics and feeding value of the silages. All the silages were well preserved and controlled by a lactic acid fermentation even with DM content lower than 150 g kg^{-1} FW. Harvesting at PB stage without wilting produced a silage with low DM content (on the average 135 g kg^{-1} FW) characterized by an intensive fermentation with high lactic acid (78.5 g kg^{-1} DM) and acetic acid content (16 g kg^{-1} DM) but the ammonia-nitrogen resulted high (244 g kg^{-1} TN) suggesting that proteolisis occurred during ensiling. Inoculation with *Lactobacillus plantarum* decreased DM content (131 g kg^{-1} FW) and NH_3-N (193 g kg^{-1} TN) and increased lactic acid (82.3 g kg^{-1} DM) and acetic acid (17.5 g kg^{-1} DM). One day wilting almost doubled DM content that reached satisfying values (282 g kg^{-1} FW),consistently decreased the fermentation, lowering lactic acid (29.1 g kg^{-1} DM), acetic acid (4.8 g kg^{-1} DM) and NH_3-N (73 g kg^{-1} TN) contents of the ensiling. The inoculation showed the same trend observed with no wilting.

STB (May 26) harvest with no wilting still produced a silage with low DM content (on the average 153 g kg^{-1} FW) with a lower concentration of lactic acid (32.8 g kg^{-1} DM) but high NH_3-N content (207 g kg^{-1} TN). The high values of ammonia-nitrogen are related with high moisture content of the silage that will favour the proteolysis process. Also at this stage of harvesting the forage wilting for one day will affect silage fermentation by restricting the microflora activity, as shown by the decrease in the fermentation products as the DM content increased. The inoculation seems effective in reducing the ammonia-nitrogen content when wilting is not applied (207 *vs* 194 g kg^{-1} TN without and with inoculation, respectively) while with wilting the reverse was obtained (84 *vs* 103 g kg^{-1} TN without and with inoculation, respectively).

At FS harvesting (25% flowering; June, 11) the silage had a high DM content on the control jars (376 g kg^{-1} FW) that was increased by the inoculation (399 g kg^{-1} FW). Wilting increased the silage DM content (694 g kg^{-1} FW).

Table 2. Effects of harvest dates (H), wilting times (W) and inoculation (I) on the characteristics of silage from safflower. DM and gas losses are expressed in g/kg FW. Other chemical results are in g/kg DM.

Harvest dates	Wilting time (h)	Inoculation	DM	pH	Lactic acid	Acetic acid	WSC	NH_3-N	Gas losses	CP	NDF	ADF	ADL	Ash	FAT
PB (May 14)	0	Control	139	4,23	78,5	16,0	32,1	244	14,7	156	448	319	29	134	26
		Inoculant	131	4,32	82,3	17,5	30,4	193	9,0	148	437	322	32	118	19
	24	Control	282	4,74	29,1	4,8	55,3	73	10,4	137	463	335	43	113	13
		Inoculant	229	4,56	51,7	11,1	54,4	101	8,4	166	454	343	36	130	19
		Mean	*195*	*4,46*	*60*	*12,3*	*43,1*	*153*	*10,6*	*152*	*451*	*330*	*35*	*124*	*19*
STB (May 26)	0	Control	151	4,50	32,8	15,7	34,0	207	10,5	108	477	362	39	115	32
		Inoculant	156	4,27	39,3	14,9	36,6	194	5,4	116	484	368	33	116	22
	24	Control	333	4,60	22,0	4,8	59,0	84	6,0	125	495	345	49	112	21
		Inoculant	282	4,66	32,4	8,0	65,6	103	6,9	127	475	355	53	119	15
		Mean	*231*	*4,51*	*32*	*10,8*	*48,8*	*147*	*7,2*	*119*	*483*	*358*	*43*	*115*	*23*
FS (June 11)	0	Control	376	4,46	18,7	4,7	52,7	90	13,7	80	493	373	59	69	17
		Inoculant	399	4,15	29,2	5,7	49,7	116	6,7	86	512	376	61	98	20
	24	Control	694	4,75	7,4	1,1	31,9	37	8,3	81	683	396	72	88	20
		Inoculant	653	4,80	10,1	1,7	33,4	33	6,7	88	654	377	68	93	18
		Mean	*531*	*4,54*	*16*	*3,3*	*41,9*	*69*	*8,9*	*83*	*585*	*380*	*65*	*87*	*19*
Mean wilting time		0	*225*	*4,32*	*46,8*	*12,4*	*39,3*	*174*	*10,0*	*116*	*475*	*353*	*42*	*108*	*23*
		24	*412*	*4,69*	*25,4*	*5,2*	*49,9*	*72*	*7,8*	*121*	*537*	*359*	*53*	*109*	*18*
Mean inoculation		Control	*329*	*4,55*	*31,4*	*7,8*	*44,2*	*122*	*10,6*	*115*	*510*	*355*	*48*	*105*	*22*
		Inoculant	*309*	*4,46*	*40,8*	*9,8*	*45,0*	*123*	*7,2*	*122*	*503*	*357*	*47*	*112*	*19*
LSD (<0,05 P) mean effect H			14,0	NS	4,7	1,4	0,7	9	1,2	5,2	2,8	3,7	1,0	1,7	0,5
LSD (<0,05 P) mean effects W and I			11,5	0,06	3,8	1,1	0,6	7	*1,0*	4,2	2,3	3,0	0,8	1,4	0,4
LSD (<0,05 P) (HxW xI)			NS	0,14	9,3	NS	1,4	17	NS	10,4	5,5	7,4	2,1	3,5	1,1

On average the wilting practice increased DM content (225 *vs* 412 g kg^{-1} FW without and with wilting respectively), pH (4.32 *vs* 4.69), WSC (39.3 *vs* 49.9 g kg^{-1} DM), and NDF (475 *vs* 537 g

kg^{-1} DM) but reduced fermentation products as lactic acid (46.8 *vs* 25.4 *g kg^{-1} DM)*, acetic acid (12.4 *vs* 5.2 g kg^{-1} DM) and NH_3-N (174 *vs* 72 g kg^{-1} TN).

Inoculation with *Lactobacillus plantarum* reduced, on the average, DM (329 vs 309 g kg^{-1} FW), pH (4.55 *vs* 4.46) and NDF (510 *vs* 503 g kg^{-1} DM) but increased lactic acid (31.4 *vs* 40.8 g kg^{-1} DM), acetic acid (7.8 *vs* 9.8 g kg^{-1} DM), CP (115 *vs* 122 g kg^{-1} DM) and ash (105 *vs* 112 g kg^{-1} DM).

As to feeding value of the silages, CP content decreased from 152 g kg^{-1} DM averaged at PB harvesting stage to 119 g kg^{-1} DM at STB stage and 83 g kg^{-1} DM at FS stage. NDF values increased when harvesting was deferred (as average 451, 483 and 585 g kg^{-1} DM with PB, STB and FS harvesting stage, respectively). ADF and ADL were less influenced by harvesting date and by the other treatmets applied.

Discussion and conclusion

The knowledge of an appropriate conservation technology is fundamental when a new forage crop is introduced as animal feeding. Yields and quality of forage safflower depend on stage of maturation at harvest. Younger plants resulted in a lower DM yield but a higher nutritional value as already found by other authors (Weinberg *et al*., 2002). WSC and BC values found in fresh safflower at ensiling were higher in the earlier harvesting and resulted to be favourable in all the three harvesting time, to prevailing of lactic acid fermentation. Wilting the forage for one day is very effective on early stage of harvesting because this practice will greatly increase DM, reducing on the same time the intensive fermentation and proteolysis processes of the silage. When harvesting is performed at the beginning of flowering stage wilting is not necessary.

In conclusion it can be pointed out that safflower, a plant of the Compositae family, showed a high potential DMY ranging from 4.5 t ha^{-1} at PB stage, characterized by high feeding value of the forage, to 11.6 t ha^{-1} harvested at FS stage with 25% of flowering but lower feeding value. If the grower preferred forage quality instead of a high DMY he can harvest the forage at the PB stage, wilting the biomass for about 24 hours to reduce the high moisture content.

The present research performed in laboratory silos showed that safflower is suitable for conservation through ensiling and that the crop can be considered as an alternative or complement to the traditional Mediterranean forage resources. However, further research is needed in farm scale silos to define the most appropriate agronomic techniques to follow, along with conservation quality and animal performance.

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