



Conservation Characteristics and Nutritive Value of Sunflower Silages as Affected by The Maturity Stages and Fibrolytic Enzymes[#]

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ABSTRACT

Silage quality and nutritive value of sunflower silages ensiled with different level of fibrolytic enzymes at blooming, milk and dough stages were investigated. Fibrolytic enzyme complex (Viscozyme® L, V2010 Sigma Novozyme) was applied 0, 1, 1.5 and 2.5 ml/kgDM at ensiling. The dry matter (DM) yield increased with each increment of the maturity stage. Dry matter (DM), ether extract (EE) and crude cellulose (CC) contents of silages increased also in each delay in harvesting, while crude protein (CP), NDF and ADF contents of silages were the highest in blooming stage. The pH was higher in sunflower silage harvested at dough stage when compared to blooming stage, while acetic, propionic and butyric acid concentrations were all higher in blooming stage, although there were no differences in lactic acid concentrations among harvesting periods. Although silage structural carbohydrate composition was not significantly affected by any enzyme dose, Dose III enzyme treatment at the dough was associated with the highest concentration of lactic acid. Overall, there was no profound effect of enzyme supplementation on nutritive value and silage characteristics of sunflower silage.

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Introduction

Sunflowers have a short production cycle, are resistant to cold, efficiently extract groundwater and are highly adaptable to different soil conditions, especially in comparison to other forage crops such as corn and sorghum (Gonçalves and Tomich, 1999; Tomich et al., 2003). The deep root system of the sunflower plant enables it to use approximately 92 percent of groundwater at a depth of 2 m, compared to only 64 percent for the sorghum plant. Therefore sunflower has a viable option for producing forage sources for ruminants where a condition is not suitable for producing other forage crops. Alternative fodder silages are needed in places with and during periods of low rainfall or water (Bremner et al., 1986), and recent studies have focused on the use of sunflower seed husks (Marx, 1977), sunflower herbage (Lloveras, 1990), sunflower seed meal (Drackley et al., 1985) and sunflower silage (McGuffey and Schingoethe, 1980; Rodrigues et al., 2001; Hill et al., 2003; Bueno et al., 2004; Demirel et al., 2006; Rezende et al., 2007) in animal nutrition.

Harvesting stage is the most important affecting silage quality and digestibility. Dry matter content increases as harvest date is delayed, but as harvest date is delayed silage quality and digestibility which is the major factor effecting feed value declines. In order to obtain high-quality sunflower silage, plants should be harvested

during the last bloom, as early harvest results in losses due to high water content (Gregoire, 1999; Gonçalves et al., 1999). Ideally, ensiled sunflower should have a dry matter content of 30-40 percent; however, excessive delay in harvesting has the disadvantage of increasing fiber content.

The treatment of forage with fibrolytic enzymes has been proposed to improve fiber digestibility and to increase the amount of water-soluble carbohydrate (WSC) available as a substrate for lactic acid bacteria (LAB) (McDonald et al., 1991; Weinberg et al., 1995). Enzymes that are able to degrade cell wall components could add to ensiling to improve fermentation and animal performance (McDonald et al., 1991). In theory, degrading cell wall components to simpler molecules should provide silage bacteria with a more fermentable substrate (McDonald et al., 1991) and should increase the rate and extent of silage digestion in the rumen (Weinberg et al., 1995). Enzyme additives have been shown to improve fermentation characteristics in cases where limited substrate is able for fermentation (Fredeen and McQueen, 1993) and to reduce silage fiber content (Stokes, 1992).

This study aimed to identify the effect of different levels of fibrolytic enzyme added to sunflower herbage harvested at various stages on silage fermentation quality and dry matter yield.

Material and Methods

The present study examined silages from green herbage sunflower harvested at blooming, milk and dough stages with the addition of Viscozyme® L (V2010 Sigma-Novozyme), a commercially available multi-enzyme product comprised of cellulase, hemicellulase, xylanase and beta glucanase.

After soil preparation with different tillage machines, the sunflower seeds with 350 g for weight of 1000 seeds were sown by pneumatic seeder with row spacing of 70 cm and a tractor with seed spacing 25 cm. During sowing 8 kg N and 8 kg P fertilizer were given per decare. 8 kg N fertilizer was given after hoeing.

In each harvest stage, average 5 m² area was harvested. In order to eliminate row edge effect one row was discarded. Crops obtained from each row were immediately weighed. Ten plants from each row were chosen to determine their plant, leaf, table and stalk weight and plant height. Samples taken from harvested plants were chopped and dried (60°C) and dry matter yield calculated.

Green herbage of sunflower cut with silotrack at each stage and liquid fibrolytic enzyme complex added at ensiling, as follows: Blooming: Control 0 (no enzyme), Blooming-Dose I (1 ml/kgDM), Blooming-Dose II (1.5 ml/kgDM); Milk: Control 0 (no enzyme): Milk-Dose I (1 ml/kgDM), Milk-Dose II (1.5 ml/kgDM), Milk-Dose III (2.5ml/kgDM); Dough: Control 0 (no enzyme): Dough-Dose I (1 ml/kgDM), Dough-Dose II (1.5 ml/kgDM), Dough-Dose III (2.5 ml/kgDM). The chopped sunflowers were tightly filled in 9 plastic (120 l) barrels for each silage groups. Then, barrels were turned upside down and placed approximately 20 cm in the soil for incubation. Barrels were opened 90 days later.

Silage samples were taken from upper, central and bottom of each barrel. Silage pH was determined with a glass electrode after homogenization of 25 g of silage with 100 ml of distilled water for 2 min in a blender (Hart and Horn, 1987). Then the remaining silage fluid was filtered through Whatman 54 paper, centrifuged and stored at -20°C. Lactic, acetic, propionic and butyric acids in silage fluids were analyzed using gas chromatography (Madrid et al., 1999). All of silage samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), and ash (AOAC, 1990), acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Van Soest and Robertson, 1979). PROC GLM in SAS/STAT (SAS,

2007) was used for all data analysis. Mean treatment differences were determined by Duncan's multiple range tests with a level of statistical differences of 5%.

Results and Discussion

Yield Properties

Green herbage yields (kg/da) of sunflower harvested at blooming, milk and dough stages were 3278.6, 7921.1 and 8166.3 kg/da, respectively. Green herbage yield increased when harvest was delayed from blooming to milk stage, but no significant increase was found between green herbage yield harvested at milk and dough stages ($P<0.05$; Table 1). But, dry matter yields increased linearly with delays in harvesting, from 466.9 kg/da at blooming to 1693.5 kg/da at milk and 1980.3 kg/da at dough stages. Mean plant height at blooming was 2.3 m, which was significantly lower than at milk (2.7 m) and dough (2.7 m) stages ($P<0.05$). Sunflower head ratios increased and leaf and stalk ratios decreased with maturity. Head ratio at blooming (18.1%) was significantly lower when compared to milk (44.0%) and dough (37.7%) stages ($P<0.05$). Leaf ratio at blooming (24.3%) was significantly higher than at milk (19.0%) and dough (18.79%) stages ($P<0.05$), and stalk ratio was also significantly higher at blooming (57.6%) than at milk (36.9%) and dough (53.5%) stages ($P<0.05$).

The agronomic characteristics of sunflower green herbage harvested at different vegetation periods reported here are similar to those reported by earlier studies (Gonçalves and Tomich, 1999; Tomich et al., 2003; Demirel et al., 2006; Rezende et al., 2007). Green herbage and dry matter yields of different varieties ranged from 1280 kg to 2910 kg/da and from 360 kg to 770 kg/da respectively, whereas mean sunflower leaf, stalk and head ratios were reported as 19.7%, 33.7% and 46.7%, respectively (Tomich et al., 2003).

Nutrient Composition

Dry matter contents of silages increased with a delay in harvesting, from 19.63% in silage harvested at blooming to 22.94% in silage harvested at milk and 31.34% in silage harvested at the dough stage (Table 2a, 2b; $P<0.05$). Enzyme addition at blooming resulted in an increase in dry matter; however, similar effects on DM were not observed ($P>0.05$) at milk and dough stages. The highest DM content was obtained at dough stage ($P<0.05$).

Table 1 Yield properties of sunflower herbage harvested at different stages

Variables*	Harvesting Stages			
	N	Blooming Stage	Milking Stage	Dough Stage
Herbage yield, kg/da	3	3278.6±287.12 ^b	7921.1±287.12 ^a	8166.3±287.12 ^a
Dry matter yield kg/da	3	466.9±65.40 ^c	1693.5±65.40 ^b	1980.3±65.40 ^a
Leaf ratio, %	3	24.3±1.37 ^a	19.0±1.37 ^b	18.8±1.37 ^b
Head ratio, %	3	18.1±0.80 ^c	44.0±0.80 ^a	37.7±0.80 ^b
Stalk ratio, %	3	57.6±1.34 ^a	36.9±1.34 ^c	53.5±1.34 ^b
Plant height, m	3	2.3±0.08 ^b	2.7±0.08 ^a	2.7±0.08 ^a

a, b, c: Values with different superscripts in the same line differ significantly ($P<0.05$).

Table 2a Nutrient composition of sunflower silage with different amounts of fibrolytic enzyme supplements and harvested at different stages (%)

Enzyme Doses	n	DM	OM	CA	EE	
Harvesting stage		**	NS	NS	**	
Blooming	9	19.6±0.51 ^c	86.4±0.17	7.8±0.14	5.6±0.23 ³	
Milking	12	22.9±0.45 ^b	85.9±0.15	7.8±0.12	13.0±0.20 ²	
Dough stage	12	31.3±0.45 ^a	86.2±0.15	7.5±0.12	15.2±0.20 ¹	
Doses		*		*		
Control	9	23.0±1.74	86.0±0.17	7.8±0.13 ^a	11.8±1.36	
Dose I(1ml/kgKM)	9	25.4±1.74	86.3±0.17	7.7±0.13 ^{ab}	10.8±1.36	
Dose II (1.5ml/kgKM)	9	25.3±1.74	86.4±0.17	7.3±0.13 ^b	11.4±1.36	
Dose III (2.5ml/kgKM)	6	27.3±2.13	86.1±0.21	7.9±0.16 ^a	14.0±1.66	
Harvesting stage×dose		*	**	**	*	
Blooming	Control	3	17.3±0.51E ^b	86.1±0.12CDEF ^b	8.0±0.17AB	5.5±0.18G ^{ab}
	Dose I	3	20.6±0.51D ^a	86.8±0.12AB ^a	7.9±0.17AB	5.2±0.18G ^b
	Dose II	3	21.0±0.51D ^a	86.3±0.12BCDE ^b	7.5±0.17BC	6.0±0.18G ^a
Milking	Control	3	21.7±0.72CD	85.3±0.22G ^b	7.8±0.21AB ^a	13.6±0.46DE
	Dose I	3	23.3±0.72C	85.6±0.22FG ^b	8.0±0.21AB ^a	12.9±0.46EF
	Dose II	3	23.5±0.72C	86.9±0.22A ^a	6.9±0.21D ^b	12.5±0.46F
	Dose III	3	23.3±0.72C	86.0±0.22DEF ^b	8.2±0.21A ^a	13.2±0.46EF
Dough Stage	Control	3	30.1±0.75B	86.5±0.14ABCD ^a	7.7±0.10ABC ^a	16.1±0.21A ^a
	Dose I	3	32.4±0.75A	86.5±0.14ABC ^a	7.2±0.10CD ^b	14.3±0.21CD ^c
	Dose II	3	31.5±0.75AB	85.8±0.14EFG ^b	7.5±0.10BC ^{ab}	15.56±0.21AB ^{ab}
	Dose III	3	31.4±0.75AB	86.2±0.14CDEF ^{ab}	7.6±0.10BC ^a	14.9±0.21BC ^{bc}

**P<0.01; *P<0.05; ^{a, b, c}:The values with different number between harvesting periods, enzyme doses and doses of each harvesting periods in the same column differ significantly (P<0.05).; A, B, C, D, E, F, G: The values with different superscripts in the same column differ significantly (P<0.05).

Table 2b Nutrient composition of sunflower silage with different amounts of fibrolytic enzyme supplements and harvested at different stages (%)

Enzyme Doses	n	CP	CC	NDF	ADF	
Harvesting stage		*	**	*	**	
Blooming	9	12.3±0.23 ^a	35.4±0.55 ^c	59.1±1.13 ^a	42.6±0.73 ^a	
Milking	12	11.5±0.20 ^b	41.9±0.47 ^b	55.9±0.98 ^b	39.2±0.63 ^b	
Dough stage	12	11.9±0.20 ^{ab}	44.9±0.47 ^a	56.5±0.98 ^{ab}	40.0±0.63 ^b	
Doses		*		*		
Control	9	12.2±0.23 ^a	41.5±1.39	56.5±1.20	39.9±0.85	
Dose I(1ml/kgKM)	9	11.4±0.23 ^b	40.5±1.39	57.2±1.20	40.7±0.85	
Dose II (1.5ml/kgKM)	9	11.9±0.23 ^{ab}	40.1±1.39	58.2±1.20	41.4±0.85	
Dose III (2.5ml/kgKM)	6	12.0±0.29 ^{ab}	43.7±1.70	55.6±1.47	39.5±1.04	
Harvesting stage×dose		*	NS	NS	*	
Blooming	Control	3	13.1±0.34A ^a	36.0±0.89D	57.4±2.04ABC	40.6±1.09BCD
	Dose I	3	11.8±0.34BC ^b	34.5±0.89D	60.9±2.04A	44.2±1.09A
	Dose II	3	12.0±0.34BC ^{ab}	35.7±0.89D	59.0±2.04ABC	42.9±1.09AB
Milking	Control	3	11.9±0.17BC ^a	42.5±0.96BC	53.6±2.14C	37.9±1.49D
	Dose I	3	11.2±0.17C ^b	42.1±0.96BC	54.4±2.14BC	38.1±1.49D
	Dose II	3	11.5±0.17BC ^{ab}	40.7±0.96C	60.2±2.14 AB	42.2±1.49ABC
	Dose III	3	11.5±0.17BC ^{ab}	42.2±0.96BC	55.4±2.14ABC	38.7±1.49CD
Dough Stage	Control	3	11.7±0.43BC	46.0±1.07A	58.5±1.38ABC	41.0±0.52ABCD ^a
	Dose I	3	11.3±0.43C	44.9±1.07AB	56.2±1.38ABC	39.7±0.52BCD ^{ab}
	Dose II	3	12.0±0.43BC	43.7±1.07ABC	55.5±1.38ABC	39.0±0.52CD ^b
	Dose III	3	12.6±0.43AB	45.2±1.07AB	55.9±1.38ABC	40.3±0.52BCD ^{ab}

The different nutrient composition of silage as affected by the forage type, amount and activity of enzyme added, and harvesting period was reported by the Harrison et al. (1994). Sneddon et al. (1981) found a DM content of 25.1% in sunflower silage harvested 120 days after sowing. Camara et al (1999a) reported that DM content of sunflower harvested at 5 different physiological stages (65, 81, 94, 108 and 121 days) had a DM content of 10.9%, 14.7%, 16.1%, 22.5% and 35.1%,

respectively. Our values are similar to those reported in other studies (Valdez et al., 1986; Henrique et al., 1998; Rezende et al., 2001; Tomich et al., 2003; Rodrigues et al., 2005; Pereira et al., 2005; Ko et al., 2005).

Ether extract content of silages increased (P<0.05) from 5.6 % at blooming to 13.0% at milk and to 15.2% dough stage, respectively. Application of enzyme level did not affect (P>0.05) ether extract ratios (Table 2a, 2b). Previous studies (Valdez et al., 1988; Henrique et al.,

1998) have also reported that ether extract content of sunflower increased gradually from blooming to dough stages. Camara and Monterio (1999) reported that ether extract contents of sunflower silages at blooming and maturity stages were 11.8% and 18.88%, respectively. The ether extract content of sunflower silage in this study was similar to other studies (Hill et al., 2003; Demirel et al., 2006; Camara et al., 1999b).

Crude protein (CP) content of silages ensiled at blooming (12.3%) was higher ($P<0.05$) than the CP levels at milk and dough stages. Although the addition of enzymes (Dose I) at dough stage had no effect on CP levels, CP was significantly lowered when enzymes were added (Dose I) at the blooming and milk stages ($P<0.05$) (Table 2a, 2b). In general, CP levels of silages were over than 10%, which is similar to values reported by Sneddon et al. (1981), Tomich et al. (2004), Rezende et al. (2007) and Fassio et al. (2007).

Crude cellulose (CC) content of silages at blooming (35.4%) was significantly lower ($P<0.05$) than the silages ensiled at milk (41.9%) and dough (44.9%) stages ($P>0.05$); the addition of enzymes did not affect CC levels at any maturity stage of ensiling (Table 2a, 2b). A previous study by Henrique et al. (1998) reported CC levels of silages with a range from 25.6%-26.3%. Hill et al. (2003) found that CC content of sunflower silage increased with progressive maturity from the blooming to the dough period. Camara et al. (1999b) harvested sunflower at four different growth period (56, 68, 94 and 103 days) and reported increased CC content with maturity (26.36%, 26.75%, 27.77% and 30.56%, respectively) but found enzyme doses had no effect on CC levels.

The NDF content at blooming (59.1%) was significantly higher than at milk (55.9%) and dough (56.5%) stages ($P<0.05$). The addition of enzymes did not affect NDF content of silages (Table 2a, 2b). The ADF content at blooming (42.6%) was significantly higher than ADF content measured at milk (39.2%) and dough (40.0%) stages ($P<0.05$). Although the addition of enzymes at blooming and milk stages had no effect on ADF levels, ADF levels decreased when enzymes (dose II) were added at the dough stage ($P<0.05$; Table 2a, 2b). Several studies suggest that cell-wall degrading enzymes-for example, cellulases can improve silage fermentation or alter the fiber content of silages (Kung et al., 1991; Almeida et al, 1995). These values are consistent with the findings in other studies (Valdez et al., 1998; Tomich et al., 2004; Yıldız et al., 2010) and enzyme doses did not affect the concentration of NDF and ADF (Gwayumba, 1997).

Fermentation Quality

Silage fermentation characteristics (pH, lactic acid, acetic acid, propionic acid, butyric acid) are given in Table 3. Silage pH values at blooming (4.1) and milk stages were similar (4.3), but the pH was significantly higher at the dough stage (4.4) ($P<0.05$). Enzyme

application at blooming and milk stages had no positive effect on silage pH; however, pH was significantly lowered as a result of enzyme application at the dough stage ($P<0.05$), which had a positive effect on fermentation. Overall, the blooming stage control (3.9) and Dose II (4.0) silages had the lowest pH levels, whereas the dough stage control silage had the highest pH level ($P<0.05$) in line with the other studies (Schingoethe et al, 1980; Tomich et al., 2004; Pereira et al., 2005; Demirel et al., 2006, Mafakher et al., 2010). This may be explained by the higher lactic acid concentration and DM content of silages as reported by Rezende et al. (2007) who stated that fermentation was limited by the lower levels of water-soluble carbohydrates and higher dry matter contents of silages at the dough stage when compared to earlier stages of development.

Harvesting period was not found to have a significant effect on lactic acid concentrations. The highest concentration (88.3 g/kg DM) was observed at blooming. Enzyme supplements had varied effects on lactic acid concentrations. At the blooming stage, enzyme supplements had no positive effect on lactic acid concentrations, but negatively affected the LA concentration. However, at the milk stage, significant differences were found between Dose I (96.6 g/kg DM) and Dose II (75.3 g/kg DM) ($P<0.05$), at the dough stage, Dose I and Dose II had no significant effect on lactic acid concentrations; however, the highest dose (Dose III) resulted in significantly higher lactic acid concentrations (91.6 g/kg DM) ($P<0.05$). This resulted in a decrease in pH of silage at dough stage. Some earlier studies have reported the addition of fibrolytic enzymes to increase lactic acid concentrations (Zhu et al., 1999). In the present study, lactic acid levels were similar to those reported by Demirel et al. (2006), Pereira et al. (2005), Ko et al. (2005) and Tomich et al. (2004).

The acetic, propionic and butyric acid concentrations decreased with maturity ($P<0.05$). Acetic acid concentrations of sunflower silage decreased when harvesting was delayed from the blooming stage (36.0 g/kgDM) to the milk (28.0 g/kgDM) and dough stages (23.5 g/kgDM) ($P<0.05$; Table 3). Enzyme supplements at the blooming and dough stages did not significantly affect acetic acid concentrations; however, at the milk stage, higher levels of supplements (Dose II) resulted in a significantly higher acetic acid when compared to lower levels of supplements (Dose I) ($P<0.05$). Propionic and butyric acids concentrations of sunflower silage decreased when harvesting was delayed from the blooming stage to the milk and dough stages ($P<0.05$; Table 3).

In conclusion, the dry matter yield of sunflower increased, but fiber content was similar with maturity. Moreover, increasing EE level with maturity suggests sunflowers could harvest at dough stage due to a similar silage fermentation patterns.

Table 3 Fermentation quality of sunflower silage with different amounts of fibrolytic enzyme supplements and harvested at different stages

Enzyme Doses		n	pH	Lactic Acid g/kgDM	Acetic Acid g/kgDM	Propionic Acid g/kgDM	Butyric Acid g/kgDM
Harvesting Stage			**		**	*	*
Blooming		9	4.1±0.07 ^b	88.3±4.54	36.0±2.36 ¹	21.0±1.17 ^a	1.0±0.25 ^a
Milking		12	4.3±0.06 ^{ab}	85.8±3.94	28.0±2.05 ²	17.2±1.01 ^b	0.2±0.21 ^b
Dough Stage		12	4.4±0.06 ^a	76.7±3.94	23.5±2.05 ²	17.0±1.01 ^b	0.2±0.21 ^b
Doses			*			*	
Control		9	4.3±0.08 ^{ab}	86.2±4.62	31.2±3.11	16.3±1.14 ^b	0.4±0.27
Dose I (1ml/kgKM)		9	4.3±0.08 ^{ab}	80.3±4.62	29.2±3.11	21.1±1.14 ^a	0.7±0.27
Dose II (1.5ml/kgKM)		9	4.3±0.08 ^b	77.7±4.62	28.3±3.11	16.6±1.14 ^b	0.3±0.27
Dose III (2.5ml/kgKM)		6	4.5±0.10 ^a	91.4±5.66	26.4±3.81	18.7±1.40 ^{ab}	0.1±0.33
Harvesting stages			**	**	*	**	*
Blooming	Control	3	3.9±0.04G ^b	104.4±9.05A	43.78±6.99A	16.7±1.33B ^b	0.6±0.77B
	Dose I	3	4.5±0.04BC ^a	73.2±9.05CD	33.1±6.99ABCD	27.7±1.33A ^a	2.0±0.77A
	Dose II	3	4.0±0.04G ^b	87.5±9.05ABCD	37.1±6.99AB	18.6±1.33B ^b	0.4±0.77B
Milking	Control	3	4.2±0.05EF ^b	80.2±5.28BCD ^{ab}	25.7±1.78BCD ^{bc}	15.4±1.62B	0.4±0.09B
	Dose I	3	4.3±0.05DE ^{ab}	96.6±5.28AB ^a	34.1±1.78ABC ^a	18.8±1.62B	0.1±0.09B
	Dose II	3	4.2±0.05EF ^b	75.3±5.28CD ^b	22.3±1.78CD ^c	15.4±1.62B	0.3±0.09B
	Dose III	3	4.4±0.05BCD ^a	91.2±5.28ABC ^{ab}	30.±1.78BCD ^{ab}	19.2±1.62B	0.2±0.09B
Dough Stage	Control	3	4.7±0.06A ^a	73.9±2.84CD ^b	24.2±2.06BCD	16.7±0.92B	0.3±0.12B
	Dose I	3	4.1±0.06F ^c	71.0±2.84D ^b	20.4±2.06D	16.8±0.92B	0.1±0.12B
	Dose II	3	4.3±0.06CDE ^b	70.4±2.84D ^b	25.9±2.06BCD	15.9±0.92B	0.2±0.12B
	Dose III	3	4.5±0.06B ^b	91.6±2.84ABC ^a	23.5±2.06CD	18.2±0.92B	0.1±0.12B

**P<0.01; *P<0.05; ^{a, b, c}: The values with different number between harvesting periods, enzyme doses and doses of each harvesting periods in the same column differ significantly (P<0.05). A, B, C, D, E, F, G: The values with different superscripts in the same column differ significantly (P<0.05).

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