IMPACT OF FLOWERING STAGE ON NUTRITIVE VALUE, PHYSICAL QUALITY AND DIGESTIBILITY OF SILAGES MADE FROM CEREAL FODDERS

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Abstract. The objective of current study was to investigate the effects of flowering phase (maturity) i.e. early bloom (20% flowering), mid bloom (50% flowering) and full bloom (100% flowering) on chemical and physical characteristics of silages, made from *Zea mays* L., *Sorghum bicolor* (L.) Moench and *Avena sativa* L. cereal fodders grown in subtropical conditions. The three fodders were harvested at three different maturity stages, chopped and ensiled in laboratory silos for 30 days fermentation period. The analysis of variance revealed that the increase in maturity from early to full bloom significantly (P < 0.05) increased dry matter (DM %) in maize (18.82 ± 0.02 to 25.80 ± 0.05), sorghum (20.65 ± 0.01 to 29.47 ± 0.01) and oat (17.68 ± 0.01 to 26.54 ± 0.01) silages, and decreased crude protein in all. The NDF and ADF increased with increase in maturity for sorghum and oats silage, while it decreased for maize silage. Lactic acid increased linearly (P < 0.05) with increasing maturity while pH, ME and in vitro dry matter digestibility (*IVDMD*) decreased. Stage of maturity also had significant effect on physical characteristics (*color, smell and structure*) of silage in all fodder crops. The highest flieg score was recorded in full bloom stage of maturity (100% flowering) due to low pH and higher DM recovery in all cereal silages. It was concluded from the current study that quality silages can successfully be made from cereal fodders grown in subtropical conditions.

Keywords: cereals maturity, bloom stages, lactic acid, physical quality, flieg score

Introduction

The shortage of good quality fodder, round the year, is the major constraint for livestock production in Pakistan (Khan et al., 2011). The seasonal fodder production poses a great challenge for livestock farmers to feed their animals when fodder supply is limited especially during summer (May - July) and winter (November – January) months (Rasool et al., 1996). The conservation of fodder in the form of silage is a viable solution to ensure its supply during those lean periods (Khan et al., 2011).

The quality of silage is dependent on the availability of fermentable substrates (McDonald et al., 1981) energy density, and water content in plants (Bal et al., 1997). As the plant matures, the water-soluble carbohydrates decrease, thereby

decreasing the fermentation activity of bacteria (Jhonson et al., 2003). Too early or too late harvesting stage not only impairs the energy density of whole plant but also affects the optimum moisture level required for good silage preservation (Bal et al., 1997). Therefore, optimum stage of maturity is important to harvest maximum nutrients for livestock feeding.

Optimal harvesting stage to increase yield and silage quality of maize, in literature, varied from tasseling stage (Fu et al., 2011), one-third milk line (Johnson et al., 2002), late dough stage (Vecchiettini et al., 2003) to two-thirds milk line stage (Fariani et al., 1994). When sorghum was harvested at late-milk, late-dough and hard-grain stages of maturity, higher nutritive values were noticed at late milk stage silages (Sonon and Bolsen, 1996). Also, reported that advancing development (maturity) of corn from 30.0 to 42.0% (i.e. black layer stage) dry matter (DM) at ensiling did not affect DM intake, milk production and composition in dairy animals (Khan et al., 2012). Despite the difference in optimal harvesting stage for silages, it is also worth mentioning that there are even very few studies investing the effects of maturity stage on silage quality in subtropical environment as in Pakistan. Under such scenario, the studies are needed to tackles the issue of silage production for maximum feed supply to the livestock.

The objective of current study was to examine the effect of varying maturity stages of three different fodders (*Zea mays L., Sorghum bicolor* (L.) Moench and *Avena sativa L.*) on fermentation characteristics and nutritive value of silages under local environmental conditions.

Materials and Methods

Sowing and harvesting of cereal fodder crops

The three fodder crops i.e. Zea mays L. (maize), Sorghum bicolor (L.) Moench (sorghum) and Avena sativa L. (oats) were used for silage making. The maize, sorghum and oats were planted during the month of June, July and November, respectively on agriculture field of Dairy Animals Training and Research Center, University of Veterinary and Animal Sciences, Ravi Campus Pattoki, Pakistan ($31^{\circ}1'0''$ North, $73^{\circ}50'60''$ East with an altitude of 186 meters (610 ft.). Each fodder crop was harvested at three different stages of maturity. The maturity stages were based on the flowering in the field and categorized as; 1) 20 % flowering (20% of plants in the field had shown flowers), 2) 50% flowering, 3) 100 % flowering. The detail of planting and harvesting at different maturity stages has been presented in *Table 1*. At each harvesting time, the respective fodder was randomly cut during a clear day from four different parts of the field and chopped by mechanical chopper (Fimax, V-Belt Driven, MC10X and Turkey) with a chop size of about 2 cm.

		Date of harvest				
Fodder type	Date of sowing	Early	bloom	Mid bloom	Full bloom	
		(20%		(50%	(100%	
		flowering)		flowering)	flowering)	
Maize	15 July	25 September		1 st October	6 th October	

 Table 1. Date of sowing and harvest stages of cereal fodders

Sorghum	15 June	15 th August	25 August	4 th September
Oats	15 November	25 February	7 th March	12 th March

Ensiling of chopped fodder in bag silos

The chopped forage taken from different parts of the field was mixed to make a representative sample for silage making. The mixed sample was packed in laboratory silos (transparent thick polyethylene bags) with capacity of 40 kg having dimensions 80×40 cm. The chopped materials were packed in the bag silo step by step. To fill the bag, about 3-6 kg of chopped forage was placed in the bag each time and pressed manually for compaction to remove air. The procedure was repeated till the bag was full and then sealed immediately. All the bags were labeled and stored under shed at room temperature for fermentation.

Physical quality evaluation of silages

After 30 days of fermentation period, the bags were opened and samples were taken for physical and chemical analysis. For physical analysis, the quality of silages was determined by color, smell, and structure along with total flieg score described by Kilic (1986). For color evaluation the scale 1-4 was used on the basis of change in green color from dark brown, dark green to pale yellow, for smell the scale 1-7 was used on the basis of repugnant putrid smell to acidic sweet pleasant smell, for structure the scale 1-4 was used on the basis of softness of leaves and stem as well as its ability to remain intact after squeezing the silage tightly in hand and then opening from breaking into small pieces to break into two or three pieces. The same person scored the silages for smell, color and structure to avoid any bias. Flieg score was calculated using a formula (Flieg Score = 220 + (2x Dry Matter% - 15) - 40 x pH) reported by Kilics (1986). The flieg score with value 81-100, 61-80, 41-60, 21-40 and 0-20 represented the silage quality a very good, good, medium, low and poor respectively. The overall silage quality was classified into categories as poor, medium, good and very good on the basis of cumulative score obtained from color, smell and structure flieg score.

Chemical quality and pH analyses of silages

Chemical analyses were done to determine pH, lactic acid, dry matter content (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Approximately 25g sample was taken from each bag immediately after opening. The sample silage was adulterated with 100 ml of distilled water (Hart and Horn., 1987). After hydration for 10 min using blender, the diluted material was then filtered through cheese cloth and then pH was determined by using a digital pH meter. The liquid obtained was further filtrated through Whatman 54 filter paper, centrifuged and kept at 20° C for lactic acid determination by high pressure liquid chromatography (Muck and Dickerson., 1988). Also, approximately 250g sample (in triplicate) was taken from each bag dried in a hot-air oven (Memmert, Beschickung-Loading Model 100-800, Germany) at 60°C for 72 hours (for DM%) then ground through hammer mill (Wiley laboratory Mill, Standard Model No. 2, Arthur H. Thomas Company, USA) making particle size of about 0.5

to1mm and stored in pre labeled bottles for further laboratory analyses. Nitrogen (N) contents of samples were determined by procedure AOAC (1990) using Kjeldahl apparatus (ID 984.13) and then multiplying the N concentration by a factor 6.25 to calculate CP content. The NDF and ADF contents were determined according to Van Soest et al. (1991). The gross energy of the silage samples was determined through the IKA C-2000 Bomb Calorimeter, while metabolizable energy (ME) was calculated as 63% of the gross energy (Mandal et al., 2003).

In-vitro dry matter digestibility of silages

The in vitro dry matter digestibility (IVDMD) trials were conducted at the University of Sydney, Camden. The dried samples were taken from Pakistan to Camden by air cargo. For IVDMD study, rumen liquor (inoculant) was collected from rumen of cannulated lactating Holstein cows managed on pasture and cerealbased concentrate (9kg DM/cow/day), at Corstorphine farm, University of Sydney. The collected rumen liquor was filtered through various layers of cheese cloth and mixed with buffered minerals solution in 1:2 ratio and placed at 39° C under O₂ free environment. Dry matter digestibility (DMD) was determined in vitro by batch incubation of samples in rumen liquor (Wang et al., 1999). All the dried samples from respective cereal silages were incubated in duplicate using ANKOM filter bags technology (New York, USA). The open side of the bag (having 0.5g ground sample) was sealed with heat sealer impulse and then put into a 50ml dark bottle. All the bottles were injected with 25ml of buffer solution under anaerobic condition then sealed with rubber lid. Bottles including blank (inoculums only) were incubated in rotary incubator for 48hrs at 39°C and rotated 90 times per minute. After 48 h of incubation the bags having digested sample were removed from the flasks, washed under running tap water then dried in oven at 60°C for 48 hours. The IVDMD percent (%) was calculated from the difference of the dry weight of sample and residues remained in the bag after 48 h of digestion divided by weight of sample×100 (Wang et al., 1999).

Statistical analyses

The data were analyzed by analyses of variance, using General Linear Model's procedures of SAS (SAS 9.1.3). Differences of means among main effects were compared by Fisher's least significant difference test (Steel et al., 1997).

Results

Effect of maturity on chemical composition of cereal silages

Chemical composition of three silages (maize, sorghum and oats) harvested at three different stages of maturity i.e. early bloom (20% flowering), mid bloom (50% flowering) and full bloom (100% flowering) has been presented in *Table 2*. DM content % increased with increasing maturity from early to full bloom (P < 0.05) in maize (18.82±0.02 to 25.80±0.05), sorghum (20.65±0.01 to 29.47±0.01) and oats (17.68±0.01 to 26.54±0.01) silages respectively, but in contrast CP and ME contents were significantly (P<0.05) decreased in all cereals silages with increasing maturity (*Table 2*). However, with advance maturity increasing pattern

of NDF and ADF were also observed in sorghum and oats silage with progressed age form early to full bloom stage, but in contrary tended to be decreased NDF and ADF in maize silage with advancing maturity.

Maturity stages						
Silages	Parameters	Early bloom	Mid bloom	Full bloom		
	DM%	$18.82 \pm 0.02^{\circ}$	22.38±0.03 ^b	$25.80{\pm}0.05^{a}$		
Maiza	CP%	9.84±0.02 ^a	$8.60{\pm}0.01^{b}$	$7.89{\pm}0.02^{c}$		
IVIAIZE	NDF%	66.26 ± 0.01^{b}	66.83 ± 0.01^{a}	$62.3 \pm 0.01^{\circ}$		
	ADF%	$33.25{\pm}0.03^{b}$	34.76 ± 0.02^{a}	31.6±0.03 ^c		
	ME(Mcal/kg)	$2.92{\pm}0.003^{a}$	$2.87{\pm}0.001^{b}$	2.85 ± 0.02^{b}		
	DM%	20.65±0.01 ^c	26.56±0.03 ^b	29.47±0.01 ^a		
	CP%	$7.45{\pm}0.31^{a}$	$7.00{\pm}0.33^{ab}$	6.19 ± 0.32^{b}		
Sorghum	NDF%	$62.42{\pm}0.04^{b}$	$56.50 \pm 0.08^{\circ}$	$64.68 {\pm} 0.14^{a}$		
	ADF%	$33.68{\pm}0.01^{b}$	$29.38{\pm}0.01^{\circ}$	$35.25{\pm}0.05^{a}$		
	ME (Mcal/kg)	$2.83{\pm}0.009^{a}$	$2.79{\pm}0.005^{b}$	$2.78{\pm}0.009^{b}$		
	DM%	$17.68 \pm 0.01^{\circ}$	23.61 ± 0.03^{b}	26.54±0.01 ^a		
Oats	CP%	$8.86{\pm}0.02^{a}$	$7.40{\pm}0.02^{b}$	$6.82{\pm}0.01^{\circ}$		
	NDF%	52.70 ± 0.36^{c}	63.31 ± 0.11^{b}	$65.33{\pm}0.03^{a}$		
	ADF%	$33.42{\pm}0.03^{b}$	33.41 ± 0.03^{b}	$34.26{\pm}0.02^{a}$		
	ME (Mcal/kg	$2.80 \pm .003^{a}$	$2.79{\pm}0.005^{a}$	2.77 ± 0.002^{b}		

Table 2. Effect of maturity stages on chemical composition of cereal silages

Means within each row followed by different superscripts are significantly different (p<0.05).

Fermentation characteristics and IVDMD of silages

The results showed that with the increase in maturity stage of silages, fermentation characteristics significantly got better (*Table 3*). The pH with advancing maturity linearly decreased (P < 0.05) in maize 4.29±0.005, 4.24±0.005 and 3.94±0.008, sorghum 3.95±0.01, 3.83±0.008 and 3.62±0.017 and oats silage 4.04±0.008, 3.95±0.02 and 3.71±0.008 from early to full bloom stage respectively (*Table 3*). Whereas, lactic acid (LA) concentration significantly (P<0.05) increased with advance maturity. However, IVDMD decreased (P < 0.05) with increasing maturity for sorghum and oats but not or maize (*Table 3*).

Maturity stages						
Silages	Parameters	Early bloom	Mid bloom	Full bloom	P-V	
	pН	4.29 ± 0.005^{a}	4.24 ± 0.005^{b}	$3.94{\pm}0.008^{\circ}$	<.0001	
Maize	LA%	$4.03 \pm 0.04^{\circ}$	$4.46{\pm}0.08^{b}$	$7.06{\pm}0.05^{a}$	<.0001	
	IVDMD%	$68.80{\pm}0.17$	$67.53 {\pm} 0.26$	$67.00{\pm}0.76$	0.087	

Table 3. pH, LA, and IVDMD of cereal silages at different stage of maturity

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Sorghum	pН	$3.95{\pm}0.01^{a}$	$3.83{\pm}0.008^{b}$	3.62 ± 0.017^{c}	<.0001
	LA%	$4.67 \pm 0.09^{\circ}$	5.76 ± 0.09^{b}	6.18 ± 0.13^{a}	0.0002
	IVDMD%	$65.30{\pm}0.90^{a}$	$63.03{\pm}0.78^{a}$	$60.26{\pm}0.49^{b}$	0.009
Oats	pН	$4.04{\pm}0.008^{a}$	3.95 ± 0.02^{b}	$3.71 \pm 0.008^{\circ}$	<.0001
	LA%	3.22 ± 0.067^{c}	$3.74{\pm}0.19^{b}$	$4.91{\pm}0.07^{a}$	0.0002
	IVDMD%	$60.60{\pm}0.49^{a}$	57.46 ± 0.76^{b}	$55.33 \pm 0.41^{\circ}$	0.002

Means within each row followed by different superscripts are significantly different (p<0.05).

Physical quality of silages

Effect of maturity on physical characteristics including color, smell and structure and flieg score has been shown in *Table 4*. Maturity had non-significant (P>0.05) effect on color of the silages in all cereals. However, the score for smell and structure positively increased with the increase in maturity for maize, sorghum and oat silages from early bloom to full bloom (P<0.05). Numerically higher flieg score was observed in full bloom followed by mid bloom and early bloom stage of maturity for all silages respectively. The cumulative effect of all physical traits indicated that silages had highest quality at 100 % maturity stage of flowering (full bloom) in all cereal silages.

Maturity stages						
Silages	Parameters	Early bloom	Mid bloom	Full bloom	P-V	
	Color	3.66±0.08	3.50 ± 0.05	3.70±0.05	0.172	
	Smell	3.76 ± 0.14^{b}	4.77 ± 0.14^{a}	$5.27{\pm}0.40^{a}$	0.018	
Maize	Structure	$2.63 \pm 0.08^{\circ}$	$3.43{\pm}0.12^{b}$	$3.90{\pm}0.05^{a}$	0.000	
	Sensory score	10.05	11.7	12.87		
	Flieg score	71.04	80.16	99.00		
	Quality class	Good	Very good	Very good		
	Color	3.36±0.08	3.66 ± 0.08	3.36±0.08	0.083	
	Smell	4.31 ± 0.09^{b}	$4.40{\pm}0.09^{ab}$	4.91 ± 0.21^{a}	0.056	
	Structure	2.80±0.11 ^c	$3.37{\pm}0.17^{b}$	$3.88{\pm}0.04^{a}$	0.002	
Sorghum	Sensory score	10.47	11.43	12.15		
	Flieg score	78.76	94.22	109.68		
	Quality class	Good	Very good	Very good		
	Color	4.53±0.14	4.10±0.20	4.70±0.32	0.26	
	Smell	$3.33{\pm}0.08^{b}$	$3.40{\pm}0.05^{b}$	$3.90{\pm}0.05^{a}$	0.002	
	Structure	$2.80{\pm}0.15^{c}$	$3.36{\pm}0.08^{b}$	$3.83{\pm}0.08^{a}$	0.002	
Oats	Sensory score	10.66	10.86	12.43		
	Flieg score	88.3	104.92	119.14		
	Quality class	Good	Very good	Very good		

Table 4. Effect of maturity stages on physical quality of silages

Means within each row followed by different superscripts are significantly different (P<0.05)

Discussion

Chemical composition and nutritive value

The finding of increased DM and decreased CP and ME with increasing maturity in our study was consistent with previous studies (Khorasani et al., 1997; Khan et al., 2011). Corn silage DM also increased quadratically and CP% tended to decrease as maturity increased (Row, 2015). The current results are in agreement to Khan et al. (2011) who reported that NDF and ADF contents in maize silage decreased with increasing maturity while it increased in other cereal silages like sorghum and millets. But, in contrast to Row (2015) who presented that NDF contents increased quadratically in corn silage as maturity increased. The increased DM and lower CP and ME with increasing age could be attributed to increased lignification and decreased content of leafy part of plant. The decreased NDF and ADF in maize silage with increasing maturity could be due to the deposition of starch into grains. Similarly, Khan et al. (2012) who found that difference in maturity at harvest during grain filling had a major effect on the carbohydrate structure (starch:NDF ratio) and fatty acid (FA) content of corn silages.

Fermentation characteristics

The linear decrease in pH values of maize, sorghum and oats silages from early to full bloom stage of maturity was in agreement with the findings of Sarwatt et al. (1989) who described that pH values of maize silages decreased with advancing growth of fodder. Similarly, Khan et al. (2011) also reported that ensiled fodder (maize, sorghum and millet) at initial stage of growth did not decrease pH quickly. The decrease in pH is mainly due to the accumulation of lactic acid as a result of fermentation. Lactic acid contents in the our study were supported by the findings of Khan et al. (2011) who ensiled maize, sorghum and millet fodder at pre-heading, heading and milk stage of maturity and concluded that lactic acid contents increased with the advancement of age of fodder. Similarly, Bal et al. (1997) and Harrison et al. (1998) reported that maize silage harvested at milk stage of maturity have highest concentration of lactic acid. The pre-requisite for the development of the lactic acid bacteria during the early stages of ensilage are the contents derived from the plant juices (water soluble carbohydrates) released by plasmolysis as a result of plan cell wall breakdown (McDonald, 1981). Khan et al. (2011) reported that ensiled fodder (maize, sorghum and millet) at initial stage of growth had low level of available water soluble carbohydrates (WSC). Bergen et al. (1991) reported that WSC was greater in silages made from barley, wheat and oats fodders harvested at milk stages. The range of pH values in current study ranging between 3.62 and 3.94 at full stage of maturity (100% flowering) in all cereals silages were consistent with the reports of McCullough (1978) who observed that pH value less than 4.2 was indicative of good quality preserved silage.

In-vitro dry matter digestibility

The decreased IVDMD in sorghum and oats silages, but unchanged in maize silage with advancing maturity was supported by Edmisten et al. (1998) who reported that IVDMD of small grain cereals (barley, oat, rye, and wheat) silages harvested at six growth stages, decreased from vegetative to the milk stage and then remained similar or declined marginally to the hard dough stage. The decline in IVDMD from growing to

the boot stage was probably due to the consistent small increase in lignification of stems. Russell et al. (1992) also investigated the effect of growth on IVDMD harvested at 0, 14 and 28 days intervals after physical maturity and suggested that later harvest did not affect IVDMD of the maize forages. Though, digestibility of NDF decreased linearly as maturity advanced (Row, 2015).

Effect of maturity proceeding to physical quality of cereals silages

The improvement in physical traits of silages with increasing age was in agreement to previous studies (Khan et al., 2011; Khan et al., 2012). Higher values for color, smell, structure and flieg score in full bloom followed by mid bloom and lower in early bloom stage of maturity could be due to low pH and higher DM as well as higher lactic acid concentration across all crops silages in advanced maturity stage. Türemiş et al. (1997) reported that low pH and acetic acid contents resulted in high physical quality scores.

Conclusion

It was concluded from the current study that quality silages can successfully be made from cereal fodders grown in subtropical conditions.

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