INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–1382/2020/23–2–253–258
DOI: 10.17957/IJAB/15.1283
http://www.fspublishers.org

*Full Length Article*

# Harvesting at Milking Stage along with Urea and Molasses Addition Improved the Quality and Fermentation Characteristics of Corn Silage

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*Received 09 September 2019; Accepted 30 September 2019; 16 January 2020*

## Abstract

Use of corn silage has become very promising approach to provide long-term high quality and economical plant-based feed for ruminants. Therefore, this two-year study was performed to determine the effect of harvesting stages and silage additives on the quality of corn silage. The study contained different harvesting stages ($H_1$: milking and $H_2$: dough) and variable silage additives ($S_1$: control $S_2$: 0.4% urea $S_3$: 4% molasses and $S_4$: urea (0.4%) + molasses (4%). Interaction between harvesting stages and silage additives had significant impact on plant characteristics, composition and fermentation quality of corn silage. Harvesting at milking stage with combined urea + molasses addition resulted in maximum crude protein (CP) (10.85%, 10.95%) and lowest fiber fractions; acid detergent fiber (ADF) (25.4 and 25.0%), and neutral detergent fiber (NDF) (43.6 and 42.9%) in both years. Likewise, the harvesting at milking stage with urea + molasses addition also resulted in lower cellulose (18.8 and 18.1%) and hemi-cellulose (18.0 and 18.1%) contents and maximum lactic acid concentration in both years. Interestingly, CP was negatively correlated with all the fiber fractions (ADF, NDF, cellulose and hemi-cellulose) and pH, whilst it had positive association with lactic acid. Moreover, all the fiber fractions (ADF, NDF, cellulose and hemi-cellulose) had the positive relationship with each other. In conclusion, harvesting at milking stage combined with urea (0.4%) + molasses (4%) addition improved the quality and fermentation characteristics of corn silage. 

**Keywords:** Corn silage; Crude protein; Fiber; Harvesting maturity; Molasses; Urea

## Introduction

Mushrooming human population urges to use the livestock resources to meet the needs of animal protein. However, it is not possible without proper fodder availability. In Pakistan, availability of lower quality fodder along with the reduction in area under fodder production Substantially affecting the animal production. It is expected that in future, ruminants will depend more on forages, owing to fact the increasing population will have direct competition with livestock for the edible grains. The fodder production in Pakistan is less than 55–60% to meet requirements of livestock (GOP 2018). One of the imperative ways to tackle this problem is the introduction of new high yielding genotypes and adaptation of modern technologies to preserves the fodder to meet livestock needs.

The main purpose of silage making is to covert soluble carbohydrates into organic acids through stablizing fermentation process, preferably lactic acid which help to decrease silage pH with the minimum fermentation period (Jalč *et al.* 2009). On-farm silage production has become common means of high-quality feed preparation for ruminant livestock. The high quality of fermentation plays a vital role in feed-intake, digestibility and lactation of livestock (Jalč *et al.* 2009) which can be affected by several factors: type of crop, harvesting time and method of silage preparation.

Among green fodders, corn (*Zea mays*) fodder is commonly used as a winter silage for livestock industries because of its good agronomic characteristics, high yield and nutritive profile with low work-force and machinery requirements (Demirel *et al.* 2003). However, it has a shortcoming of low crude protein on dry matter (DM) basis which is usually around 70 to 80 g/kg DM (Geren *et al.* 2008). The silage quality can be enhanced by addition of additives which have different objectives while making silage. The main objectives of using additives are to

**To cite this paper:** Zamir SI, IU Haq, MU Chattha, MU Hassan, I Khan, MB Chattha, N Saeed, MM Iqbal, MA Ayub, A Rehman, 2020. Harvesting at milking stage along with urea and molasses addition improved the quality and fermentation characteristics of corn silage. *Intl J Agric Biol* 23:253–258

improve the quality of silage. As the addition of additives increases lactic acid fermentation, and assisting to prevent secondary fermentation and butyric acid accumulation that results in a well-preserved silage (Aksu *et al.* 2004). The previous investigations showed that addition of urea and molasses (Demirel *et al.* 2003) and minerals improved the crude protein (CP), lactic acid and pH of corn silage.

In addition, the timing of harvest is an important management practice for maximum nutritional silage production. Stage of harvesting is an imperative factor, which fundamentally affects the quality of fodder, digestibility and consumption (Ball *et al.* 2001). In general, fiber fractions (acid detergent fiber (ADF), neutral detergent fiber (NDF), cellulose and heme-cellulose) increases, whilst, protein contents and digestibilty of biomass decreases with advancing maturity (Hassan *et al.* 2019). Moroever, advancing maturity also decreases the fermentation characteristices of corn silage owing to substantial increase in fiber contents (Ghanbari-Bonjar and Lee 2003). Thus, it was hypothesized that stage of harvesting and addition of additives may improve the quality and fermentation characterizes of corn silage. A little information is available concerning the effects of urea and molasses on the quality and fermentation characteristics of corn silage under warm semi-arid conditions. Thus, this study was performed to determine the influence of silage additives (urea and molasses) and harvest time (dough and milking stage) on the quality of corn silage.

## Materials and Methods

### Experimental site and climate

The present study was conducted for two years (2013 and 2014) at Agronomic Research Area University of Agriculture Faisalabad to determine the effect of silage additives (urea and molasses) and growth stages (milking and dough) on the quality of corn silage. The study site had warm, and sub-tropical climate, further details of climatic conditions during the growing season are given in Fig. 1.

### Experimental treatments

The experiment consisted of two factors; i) harvesting at different stages *i.e*., milking stage and dough stages and ii) various silage additives; control, 0.4% urea, 4% molasses and urea + molasses. The harvested plants were chopped and prepared for four treatment groups: control (none urea and molasses), 0.4% urea, 4% molasses and combined 0.4% urea and 4% molasses. These silage samples were kept into glass jars (size=1 kg) and pressed. The jar lids were pressed, and afterward all the jar was placed in upside-down position, in order to drain out the excessive water. All the jars remained closed for 50 days and after that they were open to determine the chemical composition and fermentation characteristics of silage.

### Crop husbandry

Prior to sowing, the soil was cultivated twice followed with planking to prepare seed bed. The hybrid seeds of maize (Monsanto DK-6789) were purchased from Monsanto Pakistan (Pvt.) Ltd. and were treated with a fungicide (Benlate® at a rate of 2 g $kg^{-1}$) to protect from seed borne diseases. The seeds were sown in 60 cm spaced rows by hand drill with using seed rate of 125 kg $ha^{-1}$. Moreover, the plant to plant distance was kept 20 cm by removing the extra plants at 4 leaf stage. The weeds were removed by hand-weeding throughout the research period. The Furadan (3-G) (20 kg $ha^{-1}$) was applied at the four-leaf stage to protect the crop from borer and shoot fly. The nitrogen (N), phosphorus (P) and potassium (K) fertilizers were applied at 250:125:125 kg NPK $ha^{-1}$ at sowing using urea, di-ammonium phosphate (DAP) and sulphate of potash (SOP). The total amount of P, K and one-third N was applied at sowing time and rest of N applied in two splits; 1 spilt after 20 days of sowing and remaining spilt at tasselling. All other recommended agronomic operations were practised to grow healthy crop.

### Measurement of plant characteristics and chemical analysis of silage

Ten plants from each treatment were selected and plant height and stem diameter were measured and leaves per plant were counted and averaged. The Kjeldahl method was used to determine total N and crude protein content in given samples. The neutral and acid detergent fibre was measured to follow the procedure of Goering and Vansoest (1970). The silage pH value was determined by pH meter. The concentration of lactic acid in the silage was sort out by using hydroxy biphenyl calorimetry method as described by the Schmidt and Kung (2010).

### Experimental design and statistical analyses

The randomized complete block design (RCBD) with the factorial arrangement having three replications was used for study. The analysis of variance (ANOVA) was performed by Statistix 8.1 software and treatment means were compared by LSD test at 5% probability (Steel *et al.* 1997).

## Results

### Plant characteristics

Harvesting stages had significant impact on the stem diameter and leaves per plant; however, harvesting stages had no impact on the plant height (Table 1). The maximum number of leaves per plant during both years were recorded when crop was harvested at milking stage (Table 1). Likewise, the maximum stem diameter in both years was recorded with harvesting at milky stage compared with maize harvested at dough stage (Table 1).

**Table 1:** Effect of harvesting stages on the plant characteristics

| Harvesting stages | Plant height (cm) | | Number of leaves per plant | | Stem diameter (cm) | |
|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| Milking ($H_1$) | 169 | 167 | 13.3A | 12.7A | 1.3A | 1.2A |
| Dough ($H_2$) | 171 | 169 | 10.0B | 9.7B | 1.1B | 1.0B |
| LSD value at ≤ 0.05 | NS | NS | 1.43 | 2.48 | 0.02 | 0.05 |

Means with different letters, within a column, differ significantly from each other at $P \leq 0.05$

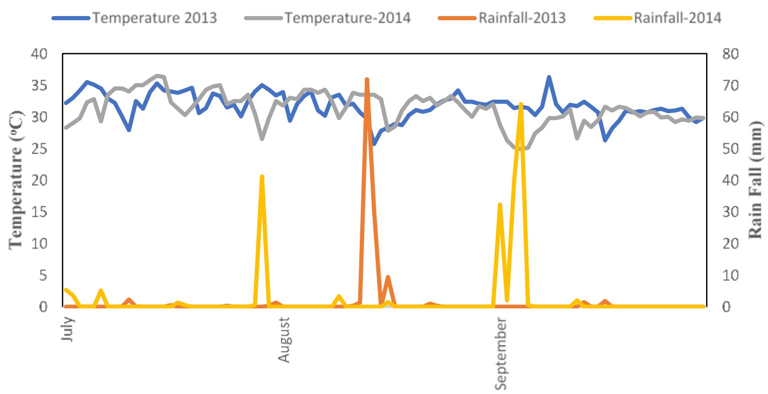


**Fig. 1:** Climatic conditions during the growing season
Source: Agro-met bulletin, agricultural metrology cell, department of Agronomy, U.A.F.

### Chemical composition of corn silage

Harvesting stages and silage additives had significant impact on the composition characters of corn silage (Table 2 and 3). The maximum crude protein (CP) (12.45%, 12.46%) was recorded when crop was harvested that milking, with addition of urea + molasses, that remained same with crop harvested at milking with addition of 4% molasses. Moreover, the lowest CP (6.18%, 6.41%) was noticed in silage made by harvesting crop at dough stage without addition of silage additives (Table 2). Likewise, harvesting stages and addition of silage additives also substantially affected the fiber fractions including the acid detergent fiber (ADF) and neutral detergent fiber (NDF). Harvesting at milking stage with combined urea + molasses resulted in lowest ADF (25.40%, 25%) and NDF (43.56%, 42.90%) followed by harvesting at milking stage with only molasses addition (Table 1). Moreover, delaying in harvesting led to increase in the both ADF and NDF and the maximum ADF (35%, 34.60%) and NDF (62.84%, 62.575) was recorded when crop was harvested at dough stage without addition of urea and as well as the molasses (Table 2).

The interactive effects of harvesting stage and silage additives had significant impact on the cellulose and hemi-cellulose contents (Table 3). The maximum cellulose (25.36%, 26.43%) and hemi-cellulose (27.80%, 27.94%) in the corn silage was noted when crop was harvested at dough stage without urea and molasses addition in both years (Table 3). Conversely, lowest cellulose (18.83%, 18.10%) and hemi-cellulose (18.02%, 18.05%) was noticed in corn silage made from crop harvesting at milking stage with addition or urea + molasses (Table 3).

### Fermentation characteristics of corn silage

The fermentation properties of silage were significantly affected with harvest maturity and silages additives: type and their levels. Similarly, interactive effects of harvesting times and silage additives also had the significant impacts on the silage pH and lactic acid concentration (Table 3). The minimum silage pH (4.06, 4.10) was noticed when silage was made from the crop harvested at the dough stage with combined urea + molasses addition, whereas the maximum silage pH (5.13, 5.20) was noticed in silage made from crop harvested at milking stage without addition of urea and molasses in both years (Table 3). The maximum lactic acid concentraion (794 ppm, 791 ppm) (794, 791) was recorded in silage made from the crop harvested at milking stage with molasses addition and minimum lactic acid was noted in silage made from crop harvest at dough stage without any additive (Table 3).

### Interrelationship between different traits

The correlation analysis has been taken to measure the degree of relationship among harvesting maturity and silages additives on silage attributes. The CP crude protein content showed highly positive relation with lactic acid (0.94) whereas it showed negative relation with NDF (-0.94), ADF (-0.83), cellulose (-0.90) and pH (-0.44). In addition, NDF, ADF and cellulose showed the negative relation with lactic acid (-0.88) whereas individually NDF, ADF and cellulose have positive relation with each other (Table 4).

**Table 2:** Effect harvesting stages and silage additives on crude protein and acid and neutral detergent fiber contents of corn silage

| Treatments | Crude protein (%) | | Acid detergent fiber (%) | | Neutral detergent fiber (%) | |
|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| Harvesting stages (H) | | | | | | |
| Milking ($H_1$) | 10.8A | 10.9A | 28.4B | 27.8B | 48.4B | 48.3B |
| Dough ($H_2$) | 7.9B | 7.9B | 31.0A | 30.8A | 56.7A | 56.3A |
| LSD ≤ 0.05 | 0.34 | 0.38 | 0.88 | 0.50 | 0.83 | 0.69 |
| Silage additive (S) | | | | | | |
| Control ($S_1$) | 7.1D | 7.4C | 32.4A | 32.1A | 58.2A | 58.3A |
| 0.4% Urea ($S_2$) | 9.2C | 9.3B | 30.3B | 30.4B | 54.2B | 54.2B |
| 4% Molasses ($S_3$) | 10.2B | 10.3A | 28.9B | 28.4C | 51.0C | 50.3C |
| Urea + Molasses ($S_4$) | 10.9A | 10.9A | 26.5C | 25.9D | 46.9D | 46.7D |
| LSD ≤ 0.05 | 0.65 | 0.74 | 1.68 | 0.97 | 1.59 | 1.32 |
| Interaction | | | | | | |
| $H_1 \times S_1$ | 8.0d | 8.3cd | 29.8bc | 29.5c | 53.6c | 54.0c |
| $H_1 \times S_2$ | 10.9b | 11.0b | 28.4c | 28.7c | 49.0d | 49.5d |
| $H_1 \times S_3$ | 11.9ab | 12.1ab | 28.4c | 27.9cd | 47.7d | 47.0e |
| $H1 \times S_4$ | 12.5a | 12.5a | 25.4d | 25.0e | 43.6e | 42.9f |
| $H_2 \times S_1$ | 6.2e | 6.4e | 35.0a | 34.6a | 62.8a | 62.6a |
| $H_2 \times S_2$ | 7.5d | 7.5de | 32.2ab | 31.9b | 59.4b | 58.9b |
| $H_2 \times S_3$ | 8.5cd | 8.5cd | 29.4bc | 28.8cd | 54.3c | 53.6c |
| $H_2 \times S_4$ | 9.4c | 9.3c | 27.4cd | 27.9d | 50.3d | 50.2d |
| LSD value at ≤ 0.05 | 1.12 | 1.27 | 2.89 | 1.66 | 2.78 | 2.26 |

Means with different letters, within a column, differ significantly from each other at $P \leq 0.05$

**Table 3:** Effect harvesting stages and silage additives on cellulose, hemicellulose, pH and lactic acid contents of corn silage

| Treatments | Cellulose (%) | | Hemicellulose (%) | | pH | | Lactic acid (ppm) | |
|---|---|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| Harvesting stages (H) | | | | | | | | |
| Milking ($H_1$) | 21.5B | 20.8B | 20.9B | 20.4B | 4.55A | 4.5A | 786.2A | 781.8A |
| Dough ($H_2$) | 24.0A | 23.3A | 25.5A | 25.7A | 4.38B | 4.4B | 764.8B | 762.1B |
| LSD ≤ 0.05 | 0.41 | 0.40 | 0.42 | 0.45 | 0.13 | 0.08 | 1.39 | 0.89 |
| Silage additive (S) | | | | | | | | |
| Control ($S_1$) | 24.6A | 23.9A | 25.8A | 26.2A | 4.9A | 5.0A | 766.2D | 761.9D |
| 0.4% Urea ($S_2$) | 23.8A | 23.1A | 23.8B | 23.8B | 4.7A | 4.7B | 772.3C | 769.5C |
| 4% Molasses ($S_3$) | 22.0B | 21.3B | 22.1C | 21.9C | 4.1B | 4.2C | 778.7B | 775.2B |
| Urea + Molasses ($S_4$) | 20.6C | 19.9C | 20.4D | 20.8D | 4.2B | 4.0C | 784.7A | 781.3A |
| LSD ≤ 0.05 | 0.79 | 0.78 | 0.81 | 0.87 | 0.25 | 0.16 | 2.68 | 1.72 |
| Interaction | | | | | | | | |
| $H_1 \times S_1$ | 23.9b | 23.2b | 23.8c | 24.4b | 5.1a | 5.2a | 779.1c | 773.5d |
| $H_1 \times S_2$ | 23.2bc | 22.5bc | 20.5d | 20.7d | 4.7ab | 4.8b | 784.7b | 779.1c |
| $H_1 \times S_3$ | 20.2d | 19.5d | 19.2de | 19.1e | 4.7bc | 4.8b | 794.0a | 791.3a |
| $H_1 \times S_4$ | 18.8e | 18.1e | 18.0e | 18.1e | 4.8ab | 4.7b | 787.0b | 783.3b |
| $H_2 \times S_1$ | 25.4a | 24.6a | 27.8a | 27.9a | 4.2cd | 4.1d | 752.3g | 750.3g |
| $H_2 \times S_2$ | 24.5ab | 23.8ab | 27.2a | 26.9a | 4.1d | 4.3c | 764.0f | 759.9f |
| $H_2 \times S_3$ | 23.1b | 23.1b | 24.8b | 24.7b | 4.1d | 4.0cd | 769.3e | 767.0e |
| $H_2 \times S_4$ | 22.4c | 21.7c | 22.8c | 22.5c | 4.1d | 4.1cd | 773.7d | 771.3d |
| LSD ≤ 0.05 | 1.35 | 1.33 | 1.40 | 1.49 | 0.44 | 0.28 | 2.35 | 2.95 |

Means with different letters, within a column, differ significantly from each other at $P \leq 0.05$

**Table 4:** Pearson correlation among different traits

| Correlation | ADF | CP | Cellulose | Hemicellulose | Lactic acid | NDF |
|---|---|---|---|---|---|---|
| CP | -0.83** | | | | | |
| Cellulose | 0.84** | -0.90** | | | | |
| Hemi-cellulose | 0.87** | -0.97** | 0.90* | | | |
| Lactic acid | -0.91* | 0.94* | -0.88* | -0.95* | | |
| NDF | 0.95** | -0.94** | 0.90* | 0.97** | -0.96* | |
| pH | 0.59* | -0.44* | 0.60* | 0.46* | -0.37* | 0.54* |

ADF: Acid detergent fiber, CP: Crude protein, NDF: Neutral detergent fiber, *: $P \leq 0.05$, **: $P \leq 0.01$

## Discussion

The delayed harvesting results in reduction in leaves per plant and stem diameter during both years. The reduction in the leaves per plant and stem diameter with delaying harvesting was due to onset senescence, which leads to reduction in leaves per plant and stem diameter (Chattha *et al.* 2017; Oyier *et al.* 2017). The stage of harvesting and silage additives had significant impact on the composition attributes of corn silage. The early stage of harvesting resulted in maximum CP, however, advancement in maturity led to reduction in protein contents (Table 1). The reduction in CP (-37%, -38%) with maturation from milking to dough stage may be due to loss of plant leaves and in increase in the concentration of fiber contents (Atis *et al.* 2012; Chattha *et al.* 2017). The fiber factions including the ADF, NDF, cellulose and hemi-cellulose increased substantially from milking to dough stage. The fiber fractions are the part of plant cell walls, however, with advancing the maturity the cell wall becomes thickened and, therefore, the concentration of fibers increases owing to conversion of carbon skeleton of cell contents into fibers and lignin (Atis *et al.* 2012; Sher *et al.* 2016; Hassan *et al.* 2019).

The harvesting of maize at milking stage significantly reduced the pH value owing to increase in rate of respirations which dropped the water-soluble carbohydrates and caused quick growth of lactic acid bacteria which were responsible for the drop in silage pH (Shaver 2003; Bal 2006). The more mature corn silage showed the significant lower concentration of lactic acid in silage. The mature silage showed reduction in lactic and acetic acid concentrations probably due low water and bacterial activity and higher fiber fractions (Filya 2004).

The various additive types and levels had significant impact on fermentation characteristices and chemical composition of silage (Table 1 and 2). The silage treated with urea and molasses has significant higher crude protein content compared to other treatments. The 95% of urea nitrogen was recovered in silage which increased CP content by adding urea and molasses (Moselhy *et al.* 2015). The addition of additives (urea + molasses) reduces silage pH which in turns increases the protein sparing activities owing to inactivation of plant proteolytic enzymes. The activities of these enzymes is maximum at 5–6 pH, however, it decreases as pH reached to 5 and completely stopped at pH from 3.8–4.5 (Sharp *et al.* 1994; Bilal 2009). The addition of additivities, enhanced the protein contents, and reduces the fiber fractions thus lead to improvement in the silage quality and digestibility (Nursory *et al.* 2003; Wanapat *et al.* 2013).

The addition of urea and molasses improved the composition of corn silage by increasing the concentration of CP and decreasing the fiber fractions including the ADF, NDF, cellulose and hemi-cellulose (Table 1 and 2). The addition of urea and molasses increases the number of an-aerobic bacteria which triggered the degradation of fiber fractions including the ADF and NDF (Islam *et al.* 2001; Guney *et al.* 2007; Wanapat *et al.* 2013). The results of current study also disclosed that addition of both urea and molasses enhanced the lactic acid production which improved the digestibility of the corn silage. The addition of urea and molasses improved the fermentable carbohydrates in silage which increased lactic acid bacteria in medium. The increase in bacteria helped to convert the sugars into lactic acid and decreases the pH of silage to discontinue the bacterial activities (Yunus *et al.* 2000; Niekerk *et al.* 2007). The reduction in pH owing to addition of urea and molasses is the desirable attribute of well-preserved silage.

## Conclusion

Harvesting stages and silage additives had significant impact on the silage quality. The harvesting at milking stages with addition of urea (0.4%) + molasses (4%) efficiently improved fermentation characteristics and chemical profile of corn silage by increasing crude protein contents and lactic acid production along with reduction in fibre contents.

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