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Effect of Storage Time on Nutrient Composition and Quality Parameters of Corn Silage

Betul Zehra Saricicek*, Birgul Yildirim, Zahide Kocabas, Emel Ozgumus Demir

Department of Animal Science, Agriculture Faculty, Ankara University, 06110 Ankara, Turkey

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* Corresponding Author:
E-mail: zsaricicek@ankara.edu.tr

ABSTRACT

This study was carried out to determine the effects of storage duration on nutrient composition and silage quality parameters. Corn was used as silage material. Corn (31.41% dry matter) was harvested at the dough stage and fermented for 90, 104, 118, 132, 146, 160, 174, 188 and 202 days in three trench silos. The samples were brought to laboratory every 14 days. This process was repeated 9 times. After the 132th day, whereas silage crude protein (CP), ether extract (EE), ash and crude fiber (CF) contents decreased, nitrogen free extract (NFE) content increased. Lactic acid concentration of corn silage increased until the 118th day but decreased between the 118th and the 160th days. On the contrary, of decrease in LA concentration, acetic acid concentration increased depending on storage time. Ammonia nitrogen and CO_2 concentration of silage increased decreased with progressing time. Storage time had significant influence on Flieg scores. The lowest score was found between days the 104th-118th. In this research, it was observed that there was a change in silage nutrient contents and fermentation characteristics with increasing storage time.

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Mısır Silajının Besin Madde Kompozisyonu ve Silaj Kalite Parametreleri Üzerine Depolama Süresinin Etkileri

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* Sorumlu Yazar:
E-mail: zsaricicek@ankara.edu.tr

ÖZET

Bu çalışma; mısır silajının besin madde kompozisyonu ve silaj kalite parametreleri üzerine depolama süresinin etkisinin belirlenmesi amacıyla yapılmıştır. Silaj materyali olarak mısır kullanılmıştır. Mısır hamur olumu devresinde (%31.41 kuru madde) hasat edilmiş ve 90, 104, 118, 132, 146, 160, 174, 188 ve 202 gün 3 hendek siloda fermentasyona bırakılmıştır. Örnekler 14 günde bir alınarak analiz için laboratuvara getirilmiştir, işlem 9 kere tekrarlanmıştır. Silajın ham protein (HP), ham yağ (HY), ham kül (HK) ve ham sellüloz (HS) içeriğinde 120 günden sonra azalma, NÖM içeriğinde ise artış olmuştur. Silajın laktik asit (LA) konsantrasyonu 108 güne kadar artmasına rağmen 108-150. günlerde düşmüştür. Depolama süresine bağlı olarak LA içeriğinde azalmanın aksine asetik asit (AA) konsantrasyonunda artış olmuştur. Silajın amonyak azotu ve CO_2 konsantrasyonu depolama süresine bağlı olarak artış göstermiştir. Flieg puanı bakımından depolama süresinin etkisi önemli bulunmamıştır. Bu çalışmada, silajın depolama süresi uzadıkça besin madde içeriği ve fermentasyon özelliklerinde değişim gözlenmiştir.

Introduction

Recent studies have suggested that the high quality roughage feed consumption must be increased in order to get the highest performance from animals; thereby, silage is traditionally used as a roughage source in ruminant nutrition at dry seasons and winters. Silage consumption is so important to feed animals at winter and solve roughage problem. Silage reduces mixed feed cost by 50-60% as well as the ratio of feed expense in meat and milk costs. (Saricicek, 2007). Corn silage, which is an indispensable roughage source for ruminant feeding in many countries, is a major source of energy for livestock.

Pressing process is crucial to provide anaerobic environment during ensiling process. (Adesogan and Newman, 2010). After pressing process, silo is covered and plant enzymes use the remaining oxygen in silo. Then, lactic acid bacteria in anaerobic environment ferments sugar to lactic acid and acetic acid and consequently silage pH decreases.

Plant respiration is the most important process during silo filling, because it affects the silage quality. Dry matter loss occurs in this process as well. During the respiration, carbohydrates are broken down into lactic acid, acetic acid and CO_2 by using oxygen in the silo. Therefore, the inner temperature of silo increases. Processes during the ensiling can change the nutrient content depending on respiration, mechanic detriment and fermentation process (Toenjes and Marble, 1970).

After filling the silo, two unwanted processes are respiration and proteolysis. After harvesting of plants, plant proteolytic enzymes are responsible for the conversion of protein into peptides and amino acids; thereby silage protein level decreases. Proteolysis is a major factor for reducing the silage protein utilization and distinct from the catabolism of amino acids by certain micro flora in silage resulting in the production of ammonia, amides and amines (McDonald et al., 1991).

The losses occur during the aerobic period are the losses due to contacting plant material with oxygen during the period from filling to closing, hence, it increases in losses stem from extensionof this period. Recent studies suggest that approximately 3-4 weeks storage time is sufficient for silage fermentation (Kung and Der Bedroisan, 2010). However, Ward and De Ondarza (2008) notified that a complete fermentation for corn silage necessitates 4 months. Moreover, Schaadt and Johnson (1969) pointed out that after the 180th day of ensiling, proteolysis causes to decrease in true protein.

The storage and feeding period of silage varies depending on regions and enterprises. Depending on the silo capacity in large enterprises, it can take a long time to use silage for feeding animals. To provide silage with animals, the covered Bunker type silo can be opened and opened again. When the silos is reopened for feeding, the plant material contacts with oxygen, which causes to growth of aerobic microorganism and leads to changes in nutrient composition and fermentation characteristics, and/or to spoil the silage.

Although corn silage is widely used and can be conserved as a forage for long time, there is no sufficient information on as to change limits in nutrient loss and fermentation characteristics in large enterprises depending on long time storage. Therefore, the aim of this research was to determine the change in silage nutrients composition and the fermentation characteristics depending on storage time.

Material and Methods

Experimental Procedures

To investigate the changes in nutrient components and the characteristics of silage fermentation during silage storage, the experiment was conducted at the Research and Application Farm of Ankara University (altitude 700 m, 43^{o} 06' 44''S and 33 ^{o} 25' 43'' E). The corn (Zea Mays L. OSSK-644) was used as a silage material. The corn was planted on May 01 and 02, 2014. The corn was harvested at milk line maturity, (dry matter content is nearly 31.41%) on September 24, 2014. The harvested corn chopped at length of 2-3 cm using a forage harvester, and filled in three bunker silos (11 m length, 8 m wide and 3 m height). The corn compressed by tractor were covered with foil, and ensiled for 90, 104, 118, 132, 146, 160, 174, 188 and 202 days. At the end of 90 days, the silos were opened, and the first samples were randomly taken from the silage. Then, the silage was covered with foil again. The other samples were taken on 104, 118, 132, 146, 160, 174, 188 and 202th days of ensiling, each of which had three replications. Dry matter (DM) content, pH, water-soluble carbohydrate (WSC), organic acids (lactic acid, acetic acid and butyric acid), ammonia nitrogen and aerobic stability analyses for the fresh silage were performed in the laboratory. pH was measured with a digital pH-meter (Hanna HI 2211). Silage organic acids were analyzed according to Lepper method, (Akyildiz, 1984). WSC content was determined according to Dubois et al. (1956). Aerobic stability test was made according to Ashbell et al., (1991).

One third of the ensiled forage material from each sample was dried in oven at 45°C for 48 hours. The dried silage samples were grinded through a 1-mm screen.Nutrients composition (DM; crude protein, CP; ether extract, EE, crude fiber, CF and ash) (CP and NH_3-N were determined by the Kjeldahl procedure) was analyzed according to A.O.A.C (1990). NFE was determined by calculation. Flieg score was also calculated by using the following equation (Kilic, 2006):

$$FS = [220 + (DM - 15)] - 40 \times pH$$

FS = Flieg score
DM = 2% dry matter of silos feed

Statistical Analysis

The data was analyzed as the repeated measurement experiment, in which time had repeated measurements, by using ANOVA. Duncan's multiple comparison test was performed to investigate which times were significantly different in terms of nutrients from each other, if it is required according the results of ANOVA. SPSS statistical package was used to perform the analyses (Winer et al., 1991).

Results and Discussion

Nutrient composition of corn silage the different storage times are given in Table 1. The change to exhibit fluctuations in DM, CP and CF of silage, which are the most important components of silage, were plotted against storage times and presented in Figures 1a, b and c.

As seen in Figure 1a, even though there were fluctuations in DM of fresh-silage at the beginning of storage, it showed a tendency to increase after 118 days of storage, during which DM level reached the lowest level due to loss of water. The results of the ANOVA also clarified that there was a statistically significant difference in DM between the 118th day and the others ($P<0.05$). The DM content of the corn silage was 27.7% for the 90th day, and 31.98 % for the 202nd day ($P<0.05$). The increases in DM can be resulted from free-water deposition in silage. On the other hand, the decrease in the moisture content of corn silage can be attributed to the increase in ambient temperatures during storage period.

Regarding with CP, EE, ash, and CF of the silage during the later period of storage, there were, however, noticeable dicreases from the 118th day on, which were also significantly different from those being before the 118th day, ($P<0.05$), (Table 1).

With regard to CP, as seen in Figure 1b, the longer ensiling time of corn silage takes the more decreases in CP occurs. The CP content of the silage was determined as 8.52% at the 90th day of storage period, however it reached 6.63% at 202nd day of ensiling period, being significantly different from each other (P<0.05). These findings showed that CP content of silage decreases with increasing ensiling period. The lowest CP content (5.82%) was observed at the 160th day, which differed significantly from the others (P<0.05).

The results of the study proved that the EE content between the 104th and the 132nd day of ensiling periods were higher comparing with the remaining ensiling periods (P<0.05). The ash content of the silage varied from 6.96% to 6.00% during the ensilage period. Ensilage period gave rise to numerical decrease in ash content.

The increase in CF content of the corn silage for the period from the 90th day to 104th was not significant (P>0.05). Then, it started to decrease, reaching 23.02 % at the 202nd day of ensiling (P<0.05).

Moreover, the NFE content of silage inclined to increase starting from the beginning of storage, which was 57.31% for the 90th day and 62.10% for the 202nd day, and was significantly different from each other (P<0.05). The results of this study pointed out that the longer period of storage resulted in decreases in CP and CF contents even though DM and NFE tended to increase (P<0.05).

Table 2 shows organic acid concentrations of corn silage during storage. Moreover, acetic acid and lactic acid concentrations were plotted against storage time and presented in Figure 2.

The results of this study verified that the changes in AA and LA depend on each other. There was an abrupt reduction of lactic acid concentration from the 132th to the 160th days. LA reached the lowest level on the 146th days where AA content reached the highest level. After the 146th days, the fluctuations were observed for both acid contents (P<0.05). The lowest AA concentration was observed at the 118th day of ensiling (0.71%). Prolonging the ensiling time resulted in increase in AA concentration (P<0.05) (Table 2, Figure 2).

In this study, increments in LA concentrations of corn silage were not significant during the first 90, 104 and 118 days of ensiling (Table 2). These results clarified that LA had a quadratic decreasing trend with increasing ensiling time.

Furthermore, in this study, the quality characteristics of corn silage were studied. Table 3 shows some quality characteristics of corn silage for different storage times.

Figure 3a, b and c depict the variation in NH_3-N, CO_2 and WSC during the storage time.

As seen at Table 3 and Figures 3a, b and c, the quality characteristics of corn silage fluctuated throughout the ensiling period. The NH_3-N and CO_2 concentrations increased with increasing storage time. The lowest NH_3-N concentration was obtained at the 90th day of ensiling (8.62g/kg) while its highest value was determined at the 132nd day (20.46g/kg) (P<0.05).The CO_2 concentration of silage varied considerably among storage periods, ranging from 10.00 g/kg at 90th day to 18.17 g/kg at 202nd day (P<0.05).

WSC content of the silage changed between 45.2 and 75.1 g/kg throughout storage period. The lowest WSC content was observed after 146 days of ensiling period. The constant fluctuations in WSC were noticed during the storage in spite of the fact that the highest values of it were obtained at the 118th day (P<0.05).

Table 1 Nutrient composition of corn silage over 202-days period (% of DM)

Days	DM	CP	EE	Ash	CF	NFE
90	27.21±0.538^{bc}	8.52 ± 0.302^{a}	2.87 ± 0.126^{c}	6.96 ± 0.065	24.34±0.398^{abc}	57.31 ± 0.733^{d}
104	30.39± 1.44^{ab}	7.72 ± 0.289^{abc}	3.51 ± 0.145^{a}	6.64 ± 0.300	25.31±0.182 ^{a}	58.72 ± 1.950^{bcd}
118	26.00± 2.070^{c}	8.22 ± 0.282^{ab}	3.19 ± 0.184^{abc}	6.19 ± 0.392	24.98±0.285^{ab}	57.92 ± 0.599^{cd}
132	28.74±1.280^{abc}	7.46 ± 0.209^{bcd}	3.40 ± 0.098^{ab}	6.48 ± 0.397	23.94±0.328^{bcd}	58.38 ± 0.307^{bcd}
146	28.79±0.897^{abc}	7.02 ± 0.050^{cde}	2.96 ± 0.035^{bc}	6.29 ± 0.171	23.24±0.216^{cd}	60.50 ± 0.216^{abcd}
160	30.70± 1.070^{ab}	5.82 ± 0.138^{f}	2.86 ± 0.180^{c}	6.41 ± 0.117	23.35±0.223^{cd}	61.57 ± 0.519^{ab}
174	30.14± 1.130^{ab}	6.26 ± 0.161^{ef}	2.86 ± 0.155^{c}	6.45 ± 0.165	23.08±0.077^{d}	61.36 ± 0.232^{ab}
188	32.33± 1.070^{a}	6.72 ± 0.096^{de}	2.96 ± 0.023^{bc}	6.36 ± 0.364	23.34±0.345^{cd}	60.63 ± 0.430^{abc}
202	31.98± 0.243^{a}	6.63 ± 0.201^{def}	2.68 ± 0.086^{c}	6.00 ± 0.114	23.02±0.121^{d}	62.10 ± 0.281^{a}

DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract, Means within columns with different superscript letters differ (P<0.05).

Table 2 Organic acid concentration of corn silage over 202-days period

Days	Acetic acid	Lactic acid
90	1.09 ± 0.028^{de}	5.60 ± 0.061^{a}
104	0.81 ± 0.106^{de}	5.34 ± 0.165^{a}
118	0.71 ± 0.020^{e}	5.04 ± 0.039^{ab}
132	1.73 ± 0.030^{bc}	3.74 ± 0.106^{cd}
146	2.51 ± 0.287^{a}	2.73 ± 0.096^{e}
160	2.01 ± 0.047^{b}	2.58 ± 0.224^{e}
174	1.29 ± 0.089^{cd}	4.98 ± 0.388^{ab}
188	1.19 ± 0.051 ^{de}	4.72 ± 0.201^{bc}
202	1.81 ± 0.145^{b}	3.21 ± 0.335^{de}

Means within columns with different superscript letters differ (P<0.05)

a)
a)
c)

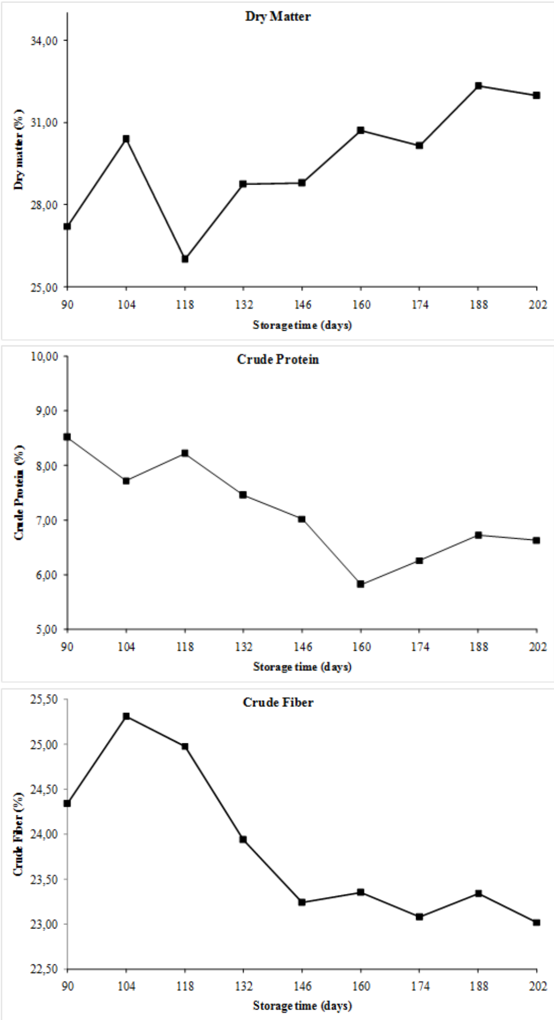

Figure 1 a)DM, b) CP, and c) CF content of silage over 202-days period

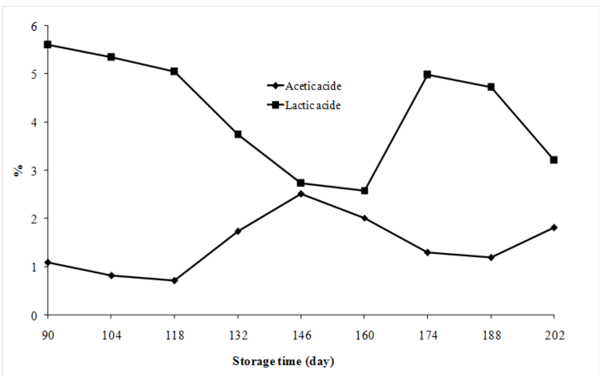

Figure 2 AA and LA concentration of silage over 202-days period

In this experiment, there was obvious evidence that the pH values of corn silage decreased during the ensiling period. The highest pH value of silage (4.42) was measured at 104^{th} day of ensiling period, which was significantly different from the other time points (P<0.05) (Table 3).

The Flieg score, which gives information on the quality of silage, was determined using DM and pH. The calculated Flieg scores ranged from 89.11 to 115.00, being adequately high. The Flieg score at the 118^{th} day significantly differed from the 132^{nd} day and the later period of storage (P<0.05). It steadily increased up to the end of storage because of the loss of water and due to increment in DM.

Discussion

The results of this study verified that extension of storage period resulted in increase in DM content of silage. This can be caused by uncovering of silage for feeding the animals increasing, silage temperature and evaporation of silage water. In addition to these reasons, movement of bottom-water of silage can cause increase in DM. The results obtained in this study contradicted with the results reported by Toenjes and Marble (1970). The decrease in CP, EE, and ash contents of silage were quietly high at the beginning of storage occurred from 132 days on. The decreases in these components indicate that the fermentation and proteolysis last, which results in reduce in CP content. Moreover, this could be because of the exposure to the air after opening the silo for feeding. Schaadt and Johson (1969) stated that decreases occur in protein depends upon proteolysis after 180^{th} days. Whereas, Newbold et al. (2006) declared that CP degradability increased after 10 months of ensiling.

The initial CF content of corn silage commenced to diminish after 118 days of ensiling. This can be attributed to degradation of CF cell wall by activity cellulose and hemicellulose enzymes in acidic condition of silos. Morrison (1979) declared that a 5% decrease in CF content occurred after 150 days but there was no change in lignin concentration.

The NFE value showed increase with the longer storage period. This increase is a result of the decrease in the other nutrient component.

In this study, while LA concentration decreased, AA concentration increased. Lindgren et al. (1990) reported that this could be because at the beginning of storage, microbial activity lasts in low pH, after several month, some changes in fermentation product starts and microorganism converts LA in carbohydrates to AA during later storage period. In addition, they emphasized that the higher pH and AA values and the lower LA values were achieved in later storage in comparison with the earlier storage period. Der Bedrosian et al. (2012) also reported similar results.

The findings of this study also clarified that NH_3-N and CO_2 concentration increased when the storage time increased. NH_3-N concentration of silage increased from 90^{th} to 202^{nd} day of ensiling periods, which might be attributed to degradation of soluble proteins by activity of bacterial enzymes and production of organic acids during fermentation.

The increment in NH_3-N shows that fermentation continues, which is the result of photolytic degradation in CP. Thus, the decrease in CP with longer time supports this assumption. According to McDonald et al. (1991), NH_3-N is a product of catabolism proteins and bacterial deamination of amino acids by certain microorganism in silage. Romero (2004) reported that NH_3-N concentration for excellent silage was 7-10 mg/kg DM, which agrees with our findings. Kleinschmit et al. (2006) showed that the highest NH_3-N obtained at the 361^{th} day.

Table 3 Some quality parameters of corn silage over 202-days period

Days	NH_3-N (g/kg)	CO_2 (g/kg DM)	WSC (g/kg)	pH	Flieg score
90	8,62 ± 0,953[c]	10,00 ± 1,530[d]	68,46 ± 0,033[bc]	3,90 ± 0,012[b]	103,41 ± 1,380[ab]
104	8,85 ± 0,129[c]	11,37 ± 0,344[cd]	73,53 ± 0,935[b]	4,42 ± 0,137[a]	89,11 ± 8,280[b]
118	10,49 ± 0,307[bc]	14,05 ± 0,443[bc]	75,10 ± 4,940[ab]	4,08 ± 0,115[b]	97,81 ± 5,320[ab]
132	20,46 ± 2,770[a]	13,17 ± 0,203[c]	65,43 ± 0,219[c]	3,86 ± 0,015[b]	108,34 ± 2,790[a]
146	8,87 ± 0,124[c]	12,28 ± 0,055[cd]	45,20 ± 0,200[d]	3,84 ± 0,030[b]	108,71 ± 2,720[a]
160	10,74 ± 0,390[bc]	16,62 ± 0,586[ab]	68,53 ± 0,318[bc]	3,79 ± 0,003[b]	114,79 ± 2,140[a]
174	15,14 ± 2,570[ab]	12,13 ± 0,038[cd]	71,77 ± 0,524[bc]	3,79 ± 0,003[b]	113,68 ± 2,270[a]
188	10,30 ± 0,445[bc]	18,17 ± 1,160[a]	72,50 ± 0,058[bc]	3,87 ± 0,007[b]	115,00 ± 1,970[a]
202	9,30 ± 0,282[bc]	17,91 ± 0,210[a]	82,33 ± 0,371[a]	3,89 ± 0,047[b]	114,96 ± 0,485[a]

WSC: Water soluble carbohydrate, Means within columns with different superscript letters differ ($P<0.05$)

a)

b)

c)

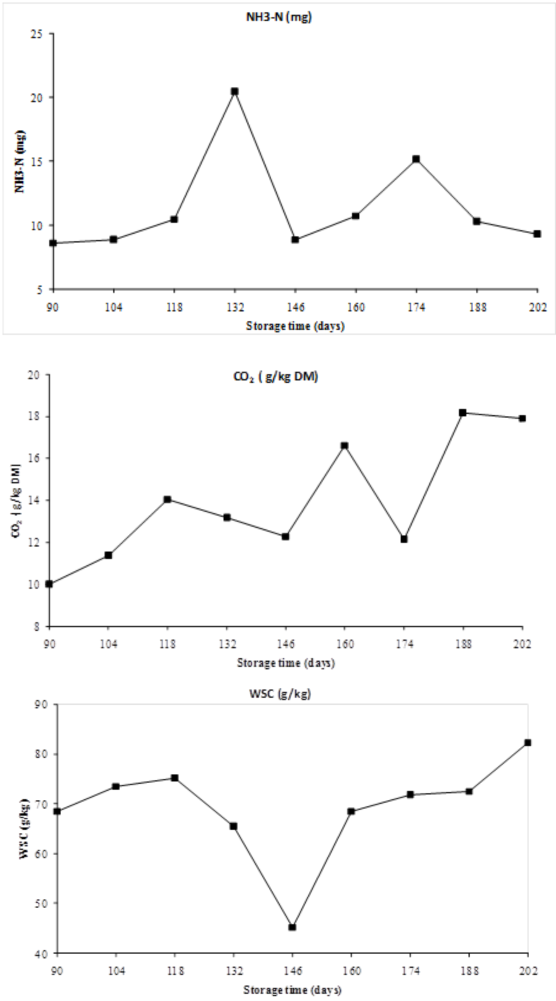

Figure 3.Some quality parameters of silage over 202-days period

In our study, the CO_2 production was generally low. CO_2 production (36.5 g/kg) reported by Weinberg (2002) for corn silage was higher than our finding.

In this study, pH value of silage at the 104[th] day of ensiling was high comparing with the other storage times. However, pH value in silage was within normal levels as reported in the literature for corn silage (Kung and Shaver, 2001; Henrichs and Ishler, 2010). Our findings are in accordance with Ashbell et al. (1998) who reported that the pH values in good silage must be between 3.90 and 4.60.

In conclusion, the findings of this study indicated that the prolongation of storage time results in the changes in nutrient composition of corn silage and silage quality parameters.

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