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The Effects of Storage Time on Nutrient Composition and Silage Quality Parameters of Corn Silage Made in Plastic Mini Silo in Laboratory Conditions

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ABSTRACT: This study was carried out to determine the effects of storage duration on nutrient composition and silage quality parameters of corn silage made in plastic mini silo under laboratory conditions. Corn was used as a silage material. Corn (31.87% of dry matter) was harvested at the dough stage, and ensiled at laboratory conditions in PVC mini silos, for 90, 104, 118, 132, 146, 160, 174, 188 and 202 days. The dry matter, crude protein, ether extract, ash and crude fiber content of corn silage decreased with the longer storage time (P<0.05). However, nitrogen free extract (NFE) content showed an increase througout the storage time. Storage time didn't significantly lead to change on EE content of corn silage. Lactic acid (LA) concentration of corn silage increased until the 104th day, but it decreased between 104th and 188th days (P<0.05), after which it increased again. Prolongation of storage time gave rise to fluctuation of LA concentration. Contrary to the decrease in LA concentration, acetic acid (AA) concentration increased depending on storage time. Silage NH_3-N concentration was highest at the 146th day, CO_2 concentration increased until the 160th day, then, decreased in the other storage time (P<0.05). The lowest WSC concentration of corn silage was found at the 118th day, but the highest at the 202th day (p<0.05). Silage pH values were remained at normal levels. Flieg scores of corn silage were significantly affected by storage time. In this research, changes were observed in nutrient composition and silage fermentation characteristics with the prolonged storage time of corn silage made in mini silos in laboratory conditions.

Keywords: Mini silo, nutrient composition, silage, storage time, quality parameters

Laboratuvar Koşullarında Mini Siloda yapılan Mısır Silajının Besin Madde Kompozisyonu ve Silaj Kalite Parametrelerindeki Değişimi Üzerine Depolama Süresinin Etkisi

ÖZET: Bu çalışma; laboratuvar koşullarında plastik mini siloda yapılan mısır silajının, besin madde kompozisyonu ve silaj kalite parametreleri üzerine depolama süresinin etkisini belirlemek amacıyla yapılmıştır. Silaj materyali olarak mısır kullanılmıştır. % 31.87 Kuru madde (KM) içerikli Mısır hamur olumu devresinde hasat edilmiş, laboratuvar koşullarında PVC mini silolarda 90, 104, 118, 132, 146, 150, 164, 178, 182, 196 ve 202 gün silolanmıştır. Silajın depolama süresi uzadıkça kuru madde (KM) içeriğinde azalma görülmüştür. Silajın ham protein (HP), ham kül (HK) ve ham sellüloz (HS) içeriğinde depolama süresi uzadıkça azalma, NÖM içeriğinde ise artış olmuştur (P<0.05). Depolama süresi mısır silajının ham yağ (HY) içeriğinde önemli bir değişime neden olmamıştır. Silajın laktik asit (LA) konsantrasyonu depolama süresinin 104. gününe kadar artmış, 104-188. günler arasında düşmüş, 188. günden sonra tekrar artış göstermiştir (P<0.05). Depolama süresinin uzaması LA konsantrasyonunda dalgalanmaya neden olmuştur. Depolama süresine bağlı olarak AA konsantrasyonu, LA konsantrasyonunun tam tersi bir durum göstermiştir. Silajın NH_3-N konsantrasyonu 146. günde en yüksek değeri göstermiş, CO_2 konsantrasyonu 160. güne kadar artmış sonra azalmıştır (P<0.05). Silajın SÇK konsantrasyonu en düşük 118. günde, en yüksek ise 202 günde elde edilmiştir. Silajın pH değeri her depolama periyodu için normal sınırlar arasında kalmıştır. Mısır silajının Flieg puanı depolama zamanı ile etkilenmiştir. Bu çalışmada, laboratuvar koşullarında mini siloda yapılan mısır silajının, depolama süresi uzadıkça besin madde kompozisyonu ve fermentasyon karakteristiklerinde değişim olduğu görülmüştür.

Anahtar kelimeler: Besin madde kompozisyonu, depolama zamanı, kalite parametreleri, mini silo, silaj

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INTRODUCTION

The fiber materials have an important role in ruminant feeding to provide optimal rumen functions and physiology. Roughages which are cheaper compared to concentrate feeds, reduce the cost of ration and production. Silage is a conservation procedure applied to forages in anaerobic conditions and it is important for receiving wet roughage requirements of ruminants in winter feding when green forage is not available. The main reason of forages conservation is to provide of feed with maximum level of original dry matter (DM), nutrients and energy level in winter feeding (Kung and DerBedroisan, 2010). Therefore, the first process in ensiling provided removal of ambient air in silos. Otherwise, presence of air in silo can lead to a rise in yeasts and moulds, which can cause dry matter and other nutrients losses (Palic et al., 2011). Silage fermentation is mostly completed within 30-45 days, it can shorter, depending on harvest season and climatic conditions. Kung and DerBedrosian (2010) reported that 3-4 week time is enough for silage fermentation to reach a stable phase. Moreover, researchers stated that there were some factors affecting this process. Ward and Ondarza (2008) informed that they needed minimum 4 months for the whole fermentation with their research concerning silage fermentation changing. On the contrary, Jaster (1995) specified that lactic acid bacteria fermantation completed generally within 3 weeks.

Feeding period of ensiling feed extends in large enterprises. The changes of fermentation occur depending on climatic conditions in ensiling ambient, using material and mass size, and moisture content diversity (Kung and DerBedroisan, 2010). Furthermore, prolonged silage storage time gives rise to alteration of silage nutrient content. Making silage in large silos is both costly and hard for laboratory researches because of determination of roughage nutritive value, examination of silage quality parameters or in case of adding additives. Thus, laboratory type mini silos instead of large silos have been preferred by many researchers. Mini silos have been used in laboratory circumstances all over the world since the beginning of 20th century. Laboratory type mini silos enable researchers to investigate ensiling most silage samples, to make silage in controlled condition and to compare research on aerobic stability, weight loss or silage fermentation features. Besides, mini silos have some advantages, such as low cost and rapid response. For this reason, most researchers have been using many different types of mini silos in laboratory conditions; glass jar with lid (Autrey et al., 1947); glass cylinder (Archibald, 1946); vacuum polythene bag (Johanson et al., 2005); metal jars (Nevens, 1933); and recently polypropylene bags (Cabarkapa et al., 2010; Colovic et al., 2010).

There is no sufficient studies focusing on effects of silage made in mini silos in laboratory conditions on ensiling duration, silage quality and alteration of nutritive.

The aim of the current experiment was to investigate changes of the nutrients compositions and fermentation characteristics depending on ensiling duration of corn silage made in plastic mini silos in laboratory conditions.

MATERIALS AND METHODS

This experiment was conducted at the Research and Application Farm of Agricultural Faculty, Ankara University (altitude 700m above sea level, 43^{o}06’44’’ S and 33^{o}25’43’’ E).

Corn (Zea Mays L. OSSK-644) was used as a silage material. The corn was seeded on May 1st, 2014 in the Farm of Research and Application. The corn was harvested at the dough stage of maturity at 31.87% dry matter (DM) content in September 2014.

The herbage was chopped to a particle length of approximately 1.5-2.0 cm using a mechanical forage cutter. Immediately after cutting, the corn was ensiled in plastic jars with 3.5 L capacity (cylindrical shape with 35 cm height x 16 cm diameter) and each storage time group was prepared with three replication. Ensiling was done within two hours after harvesting. All jars were closed carefully with a lid, and jar tops were coil up with paraffilm to prevent entry of air. The jars were stored in dark room at ambient temperature of 20±2 ^{o}C for 90, 104, 118, 132, 146, 160, 174, 188 and 202 days.

Three jars were opened per two week during 202 days of storage period and pH, dry matter (DM), water soluble carbohydrate (WSC) (g kg^{-1} DM) and organic acids (lactic acid, acetic acid and butyric acid) (%), and aerobic stability, and ammonia nitrogen (g kg $^{-1}$ DM) analysis for the fresh silage were performed in the laboratory.

One third of the ensiled forage material from each sample was oven-dried at 45°C in a forced-air oven for 48 hours, then silage samples were grinded through a 1 mm screen, and analysed with two parallel per storage time. Nutrient composition (DM, crude protein (CP), ether extract (EE), crude fiber (CF), ash and NH3-N, were determined follow the procedure of Association of Official Analytical Chemist (AOAC,1990). Nitrogen Free Exract (NFE) matter was determined by calculation.

The pH value of silage was measured with digital pH meter (Hanna HI-2211). Water soluble carbohydrate (WSC) was determined by the phenol sulfuric acid method (Dubois et al., 1956). Aerobic stability test (for 5 day) was determined by the "bottle" system (Ashbell et al., 1991). Organic acids (lactic acid, acetic acid and butyric acid) concentration of silage was analyzed according to Lepper method (Akyildiz, 1984). Flieg point was calculated by using the following equation (Kilic, 2006);

Flieg point=220+(2x % dry matter of silos feed – 15) -40 x pH

Statistical Analysis

The data were evaluated by ANOVA according to the repeated measurement experiment. 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 analysis (Winer et al., 1991).

RESULTS AND DISCUSSION

The chemical composition of the ensiled corn is given in Table 1.

Table 1. The chemical composition of the ensiled corn**, % (**in %DM)

Days	DM	CP	EE	Ash	CF	NFE
90	28.02±0.246bc	8.95±0.413a	2.95±0.342ab	7.18±0.009a	24.32±0.283a	56.99±0.356d
104	31.95±0.478a	8.29± 0.302ab	2.86±0.72ab	7.03±0.153b	24.52±0.267a	57.30±0.722d
118	28.89±0.583b	7.51± 0.146bc	3.27±0.039a	6.68±0.184ab	23.78±0.587ab	58.43±0.887cd
132	26.38±0,889bc	7.06±0.241c	2.95±0.030ab	6.35±0.067bc	24.11±0.221a	59.20±0.241bc
146	25.78±0.504c	6.35±0.315c	2.73±0.091ab	6.69±0.234ab	23.86±0.240ab	59.38±0.372bc
160	26.41±0.150bc	6.54±0.334c	2.59±0,099b	6.35±0.044bc	23.29±0.198ab	60.23±0.212abc
174	27.43±0.293bc	6.55±0.263c	2.68±0.148ab	6.55±0.213ab	23.24±0.495ab	60.98±0.356ab
188	25.41±0.265c	6.73±0.117c	2.34±0.054b	6.51±0.179abc	22.64±0.448b	61.78±0.528a
202	27.23±0.934 bc	6.54±0.198c	2.43±0.100b	5.82±0.108c	23.19±0.086ab	62.02±0.324a

CP: Crude Protein; EE: Ether Extract; CF Crude fiber; NFE: Nitrogen free-extract
a,b,c,..: Means with different superscript within same column significantly differ ($p<0.05$)

Nutrient composition as it can be seen from the Table 1, the nutrient composition of corn silage changed with the longer storage time.

DM content of corn silage was affected by storage time. The highest DM content of the silage was determined at the 104 th day (31.95 %DM) of storage time but the lowest at the 188th d (25.41 % DM).

The storage time had significant effect on the nutrient composition of the silages ($P<0.05$). The highest CP, ash and CF content of silage was found for the 90th day as 8.95 %DM, 7.18%DM, 24.32%DM, respectively, and the lowest for the 202nd day as 6.54%DM, 5.82 %DM, 23.19%DM, respectively, the differences were statistically significant ($P<0.05$). During storage periods, the CP, EE, ash and CF contents of silages were significantly decreased, while the NFE was increased on the the 202nd day compared to day the 90th ($P<0.05$). (Table 1).

Organic Acid Compositions of Silage

Table 2 shows the organic acids (lactic acid, acetic acid and butyric acid) composition of the ensiled corn storage time.

Table 2. Organic acid concentrations of corn silages during the storage time**, %**

Days	Acetic acid	Lactic acid	Butyric acid
90	2.09±0.040bc	2.38±0.307cd	-
104	0.65±0.200d	5.16±0.239a	-
118	3.28±0.266a	4.21±0.170ab	-
132	2.47±0.216b	3.23±0.114bcd	-
146	1.71±0.149bc	2.17±0.248d	-
160	2.06±0.029bc	2.58±0.154cd	-
174	1.98±0.026bc	2.63±0.272cd	-
188	1.32±0.114cd	3.43±0.330bc	-
202	1.78±0.334bc	3.077±0.337cd	-

a,b,c,..:Means with different superscript within same column significantly differ (p<0.05)

In the current study, the lowest the acetic acid (AA) concentration was observed at the104th day of storage, and also the highest AA concentration was found for the 118th day (P<0.05). There was a statistically significant difference in AA between the 118th day and all the other days of storage period. As shown in Table 2, the lactic acid (LA) concentration increased during the first 104 days of the storage period, but after from the 104th day of storage periods, it decreased in disorder (P<0.05). Butyric acid (BA) was not determined in all corn silage.

Silage Fermentation Characteristics And Flieg Point of Silage

Effect of storage times of corn silage on some silage fermentation characteristics and Flieg point are shown in Table 3.

Table 3. Fermentation quality and Flieg point of corn silages, %

Days	NH_3-N (mg kg $^{-1}$ DM)	CO_2 (g kg^{-1} DM)	WSC (g kg^{-1} DM)	pH	Flieg point
90	9.65±1.47cd	2.46±0.190e	66.83±0.031cd	3.94±0.003bc	103.31±0.376b
104	9.26±0.368cd	6.62±0.319d	84.10±0.016b	3.84±0.003c	115.16±0.987a
118	14.19±0.100ab	9.27±1.600cd	34.63±0.014e	3.97±0.035b	103.97±1.97b
132	8.85±0.826cd	12.61±0.385b	69.60±0.015c	3.96±0.029b	99.50±0.877b
146	15.74±0.816a	17.92±0.885a	68.23±0.002cd	3.91±0.025bc	100.15±0.898b
160	11.69±0.538bc	19.15±0.523a	60.10±0.001d	3.88±0.048bc	102.49±1.65b
174	13.16±0.385ab	12.89±0.013b	64.10±0.005cd	3.98±0.003b	100.3±0.648b
188	8.31±0.385d	10.51±0.819bc	80.73±0.006b	4.63±0.003a	70.76±0.652c
202	8.96±0.263cd	13.76±0.321b	108.30±0.023a	3.91±0.009bc	103.19±1.99b

a,b,c,..:Means with different superscript within same column significantly differ (p<0.05)

The ammonia nitrogen (NH_3-N) concentration of corn silage ranged between 8.31- 15.74 g kg^{-1} DM during the storage period (from 90 to 202 day). The highest NH_3-N concentration was observed at the 146th day of ensiling periods (P<0.05), but decrease after the 146th days of ensiling was noted (P<0.05).

In the current study, after 5 days of aerobic exposure, the lowest CO_2 concentration of silage was determined at the 90th day (2.46 g kg^{-1} DM), and but the highest at the146 and the 160th days (17.92 and 19.15 g kg^{-1} DM, respectively). There are no significant differences between the 146th day and 160th day of ensiling for CO_2 concentration (P>0.05), but this two days of ensiling were different compared to the other days of ensiling (P<0.05).

While the WSC concentration of silage was 66.83 g kg^{-1} DM at the 90th day, it was 108.30g kg^{-1} DM for the 202nd day. The WSC concentration at the 202nd day was significantly higher than the other days of ensiling ($P<0.05$).

For corn silage, pH values ranged between 3.84 and 4.63. The highest pH value was obtained from the 188th day of storage period. This time was significantly different in pH value compared to all other storage periods ($P<0.05$).

The Flieg point which gives information on the quality of the corn silage was determined using pH and DM. Flieg point of the silage was highest for the 104th d of ensiling period, but was lowest for the 188th d. The 104th and 188th days are significantly different from the other days of ensiling ($P<0.05$). However, in this study Fleig point of the corn silage could be accepted as "supper or better class".

Nutrient Composition

DM content of silage decreased according to the fresh material (31.87%) during the storage period, except for the 104th day. DM content for 90 and 188 days of ensiling period were determined as 28.02 and 25.41%, respectively. This decrease in DM can be explained as a result of decrease in other nutrients. Buckmaster et al. (1989) reported that DM losses increased with prolonged storage time, which can be the reason of degradability to nutrients by microorganism during anaerobic fermentation. The CP, EE, ash and CF content of silage decreased during storage period, being from 90 to 202 d. Nutrients content of silage declined with prolongation of storage time. This reduction can be related to the degradation of some nutrients by micro-organisms in anaerobic condition of silages. These results show that CP content of silage decreased from 8.95% for 90 d. to 6.54% for 202 d. Naeini et al. (2014) reported that CP content of sorghum silage decreased from 54.9% to 53.5 for 90 and 120 d ensiling periods, respectively, which was in accordance with our results. But also our results are not consistent with results of the same researchers who determined that ash content increased with prolonged storage time. The differences among these studies may be related to the plant material and mini silo type used. According to our results, a change in the ether extract composition of corn silage due to prolonged storage in the silo has been observed. Decrease in EE content of silage was observed after 118 d of storage. This decrement could be due to an increase in the activity of plant enzymes and lipolysis. Moreover, Van Ranst et al., (2009) stated that lipolysis in the silage was greater when it had undergone extensive fermentation. The CF content of silage reduced between 90 and 220 days of ensiling. Changes in CF content with prolonged storage might be attributed to degradation of cell wall by bacterial enzymes activity and acidic condition in silage. Yahaya et al., (2001) supported that remarkable loss of the hemicellulose and pectin fractions occurred in alfalfa and orchard grass silage between fresh forage and ensiled materials. In contrast, prolonged ensiling resulted in a raise in the NFE content in corn silage in this experiment. The increase could be explained by reductions in the other nutrient

Organic Acids of Silage

The AA concentration in silages made in mini silos increased during the first 118 day of fermentation but after decreased until the 220th day. The LA concentration of silage fluctuated with prolonged storage time. Lindgren et al., (1990) determined that at the beginning of ensiling, microbial activity persists when pH is low, after several months, some changes occur in fermentation end products and lactic acids are converted to acetic acid from carbohydrates by micro-organism with prolongation in storage time. The same researchers reported that pH and acetic acid concentration were higher at the storage period later date when compared to the an earlier period, but lactic acid concentration was lower. Kleinschmit et al., (2006) reported that there were a decline of 15% in LA concentration, and an increase of 80% in AA when the corn silage stored in the mini silo from 14 d to 361 days. Our results are not consistent with the result of Der Bedrosian et al. (2012), who demonsrated that the AA concentration increased with prolonged storage time.

Silage Fermentation Characteristics and Flieg Score of Silage

In the present experiment, NH_3-N concentration increased from 9.65 to 15.74 g kg^{-1} DM for 90 and 146 days of ensiling, respectively. A similar finding was reported by Naeini et al., (2014), who found that NH_3-N concentration of sorghum silage increased steadily from 56.9 g kg^{-1} to 81.7 g kg^{-1} DM for 30-120 d storage time, respectively. Newbold et al., (2006) reported increasing amount of NH_3-N in ensiled 15 corn silages during 10 months, which was explained as a result of degradation of proteins in silage. According to the results of this study, the CO_2 concentration of corn silage (for 5 days) was the lowest in the first 90 days of ensiling while the highest concentration was obtained for the146th and 160th day (Table 3). The silage was stable for the first 90 and 104 days of ensiling, whereas, after the 118th days of ensiling, a decline in the stability was observed with the prolonged ensiling time. Weinberg et al. (2010) studied corn taken from two different location (central and south) in mini glass silo and stored for 5 months. At the end of 5 months, they reported that CO_2 concentrations (for 4 days) for central and south area were 3.3 and 38.3 g kg^{-1} DM, respectively. According to the results of this study and as reported in some literature, it can be concluded that aerobic stability can change due to storage time, exposure time to air of silage, region where plant material grow, and structure of silos. In the current experiment, WSC concentration of corn silage raised from 67 g kg^{-1} DM to 108 g kg^{-1} DM for the 90th and the 202th day. Weinberg et al., (2010) also reported that WSC concentration of corn silage in mini silos was 135.7 g kg^{-1} DM at the end of the 5 months. In our study, the pH value ranged from 3.8 to 4.63 for corn silage. Our results are consistent with results of many researchers (Kolver et al., 2001; Heinrichs and Ishler 2010). According to Ashbell et al., (1998), usually pH does not increase easily in silage which contains high corn level, because of high carbohydrate content and low buffer capacity of corn. Flieg point of corn silage was the lowest for the 188th d of ensiling compared to the remaining ensiling periods. This is because low dry matter content (25.41 %) and high pH values (4.63) of corn silage the 188th day.

CONCLUSION

Our study showed that nutrient composition of corn silage decreased with the prolonged storage time in mini silos at the laboratory condition. On the other hand, organic acids and silage fermentation parameters changed irregularly. Mini silos are necessary for investigation on the fermentation process. However, according to our findings, the storage of longer than 104 days was a disadvantage of plastic mini silos because after 104 days, fluactuations in nutrient composition and silage quality parameters were recorded. It is recommended that more studies should be undertaken on storage time since there are many factors affecting it.

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