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RESEARCH ARTICLE

Establishing the Optimum Cutting Date and Additives for Pasture Grass Silage and Its Mycotoxin Levels [1]

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Summary

This study was aimed at determining most suitable cutting date and additives for pasture grass for ensiling and their mycotoxin residues. Grass samples were obtained at 24th June; 1st and 8th July. No additives, 5% ground barley or molasses additives silages were made. Silages opened 134 day after ensiling, and analysed for dry matter (DM), crude ash (CA), crude protein (CP), crude fibre (CF), NDF, ADF, ether extract (EE), pH, lactic acid (LA), acetic acid (AA), butyric acid (BA), total aflatoxin, aflatoxin B_1 (AFB_1), zearalenone. There were no statistical differences in CA and organic matter contents among the silage; however, there were differences in DM, CP, NDF, ADF, EE, nitrogen free extract, pH, LA, AA and BA. Mycotoxins residues were found in all fresh grass and silage samples. Total aflatoxin and AFB_1 residues were generally above the acceptable limits in silages, but zearalenone residues were below. These findings suggest that the indicated times in our study are suitable for silage making, but the first two weeks of July is more proper due to the higher dry matter obtained, and best additive is 5% molasses provided that precautions against the fungi are taken in silages with molasses.

Keywords: *Grass, Silage, Silage fermentation products, Total aflatoxin, Aflatoxin B_1, Zearalenone*

Çayır Otu Silajları İçin En Uygun Biçim Zamanı ve Silaj Katkısının Belirlenmesi ve Silajlardaki Mikotoksin Düzeylerinin Belirlenmesi

Özet

Bu çalışma, çayır otlarından silaj yapılması için en uygun biçim zamanı ve silaj katısı ile silajlardaki mikotoksin rezidülerinin belirlenmesi için yapılmıştır. Çayır otu örnekleri 24 Haziran, 1 ve 8 Temmuz tarihlerinde alınmıştır. Çayır otlarından katkısız, %5 arpa, %5 melas katkılı silajlar yapılmıştır. Silajlar yapıldıktan sonraki 134. günde açılarak kuru madde (KM), ham kül (HK), ham protein (HP), ham selüloz (HS), NDF, ADF, ham yağ (HY), pH, laktik asit (LA), asetik asit (AA), bütirik asit (BA), total aflatoksin, aflatoksin B_1 (AFB_1), zearalenone yönünden analiz edilmiştir. Silajların HK ve organik madde içerikleri arasında istatistiksel farklılık görülmezken, KM, HP, NDF, ADF, HY, azotsuz öz madde, pH, LA, AA ve BA içeriklerinde farklılık gözlemlenmiştir. Tüm taze çayır otlarında ve silajlarında mikotoksin residüleri bulunmuştur. Silajlardaki total aflatoksin ve AFB_1 düzeyi genelde kabul edilebilir sınırların üzerinde iken, zearalenon düzeyi altında bulunmuştur. Bu çalışmada elde edilen bulgular söz konusu tarihlerinin tümünün silaj yapımı için uygun olduğunu, ancak elde edilecek kuru maddenin daha fazla olması bakımından Temmuz ayının ilk iki haftasının daha uygun olduğunu, en iyi katkının küflenmeye karşı gerekli tedbirler alınarak %5 melas olduğunu göstermiştir.

Anahtar sözcükler: *Çayır otu, Silaj, Silaj fermentasyon ürünleri, Total aflatoksin, Aflatoksin B_1, Zearalenone*

INTRODUCTION

Important nutrient losses occur in hay during drying, leaching, bleaching, transport and stack making, especially in rainy region. To minimise nutrient losses in hay, silage making may be an alternative especially in regions where heavy rainfall occurs during the harvesting and drying periods. Silage is produced by controlled

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fermentation of crop residues or forages with high moisture content [1]. Pasture grasses is moderately suitable for silage making [2]. Therefore, different additives such as ground grains, molasses, inoculants and organic acids is required to enhance silage quality by encouraging lactic acid fermentation, by inhibiting undesirable microbes or by improving its nutritional value.

For the silage making, determination of the most suitable cutting time of pasture and using additives are important factors. Silage has been made from timothy and meadow fescue combination pasture grasses harvested on 13th, 21st, 28th June and 4th July [3]. The best fermentation products and nutrient content of silage in this study were obtained from grass cutted at 13th and 21st June. In another research, no additives and formic acid-molasses additive silage made from pasture grasses cutted on 8th, 18th and 29th June [4]. Using the additives decreased the pH, NDF, ADF and butyric acid (BA) levels of silage, while increased the dry matter (DM), crude protein (CP), lactic acid (LA) and acetic acid (AA) levels.

During the growth, drying and storage period of roughage are at risk in contamination by a number of different fungi, some of them can produce mycotoxins, and they generate a wide range of toxic responses in animals and humans. One of the most common mycotoxin present in the roughage is aflatoxin including B_1, B_2, G_1 and G_2 form [5,6]. They induce several adverse effects (i.e. decrease in feed intake and feed efficiency, hemorrhagic enteritis, depressed immune response, hepatotoxic, nephrotoxic, carcinogenic, mutagenic, and teratogenic), depending on the age, gender and species of animals and their levels and types. Aflatoxin B_1 (AFB_1) is the most important type in the aflatoxins, it metabolised in organism and excreted in animal products such as milk, as aflatoxin form M_1 which can adversely affect human health [5]. Another mycotoxin present in roughage is zearalenone. It induces reproductive disturbances and depresses feed intake through affecting hormonal balance in animals [5,6]. It was reported that 19 out of 80 pasture grass silage contaminated at average of 10 ppb AFB_1, 35 out of 79 at average of 780 ppb zearalenone [7].

Considerable research has been carried out in animal feedstuffs about some single pure mycotoxins and their adverse affect on domestic animals. However, silages and especially grass silages have little attention. This study was designed to determine the most suitable cutting date and additives for pasture grass silage and to detect their mycotoxin residues.

MATERIAL and METHODS

Plant materials

Pasture grasses used in the experiment were obtained from 5 different pasture locations belonging to different family farms in Kars district in North East of Turkey. The grass samples were taken from each pasture locations where 8 to 10 different parts of 1.5 m^2 areas of the pasture was cut at a height of 4-5 cm by a scythe on three different dates for 7 days interval; first (24th June 2007=approximately pre-bloom), second (1st July 2007=approximately at the beginning of bloom) and third sampling (8th July 2007=approximately early bloom). Pasture grasses in Kars district are naturally growing and contains 64.2% Graminea, 22.8% Leguminoseae, and 13.0% other plant families [8]. Nutrient composition and mycotoxins residues of the grasses is given in *Table 1*.

Treatments

The obtained pasture grass samples were mixed homogeneously by hand and they ensilaged within the for 8 to 9 h after cut into 2-3 cm lengths. No additives, 5% ground barley, and 5% molasses as additive silages were made in plastic jars (1.5 L capacity, taking approximately 1000 g grass). After filling and squeezing by manually with a wood ramrod, the jars were sealed by a plastic lid. Five silage samples were made from every silage type at each vegetative period from each location, accounting for a total number of 225 silage samples. The jars were stored in a dark room at room temperature for 134 days. Samples for crude nutrients and mycotoxins were dried at 60°C in an oven and then ground to pass through a 1 mm screen. pH and CP in silage were determined immediately after opening the jars.

Chemical analyses

The DM, crude ash (CA), CP, crude fibre (CF) and ether extract (EE) analyses in the fresh grass and silage samples were made according to AOAC [9] procedures and concentration of neutral detergent fibre (NDF) and acid detergent fibre (ADF) was determined by the method of Van Soest and Robertson [10]. Silage pH was determined with a glass electrode after homogenization 3-4 min in a blender. After the filtered liquidised silage extracts by Whatman paper samples were taken for organic acids analyses and were stored at -18°C in a deepfreeze then analysed for LA, AA and BA with a GC (Agilent Technologies 6850) by the method of

Leventini et al.[11]. Total aflatoxin, AFB_1 and zearalenone residues of the samples were measured by competitive ELISA (StatFax 3200 Reader) according to procedure described by the manufacturer (R-Biofarm, 2008 [12]) by using RIDASCREEN® Aflatoxin Total-Test Kits (Art. No.: R4701), RIDASCREEN® Aflatoxin B_1 30/15-Test Kits (Art. No.: R1211) and RIDASCREEN® Zearalenone-Test Kits (Art. No.: R1401). The sensitivity of using commercial mycotoxins kits were 1.75, 1.00 and 1.75 ppb, respectively.

Statistical analyses

Data obtained from the fresh material was subjected to ANOVA (Analysis of Variance). Silages data was subjected to general linear model (GLM) and the differences among the means both fresh material and silages were determined using with Duncan's test at P<0.05. All statistical analyses were carried out by using the SPSS 10.0 [13].

RESULTS

Nutrient composition and mycotoxin residues of fresh pasture grasses

The sampling date did not effect concentrations of CA, OM, EE and NFE *(Table 1)*. The DM content of grass samples increased gradually depending on the sampling date and the third sampling was significantly higher than the first sampling (P<0.01). While the concentration of CP gradually decreased depending on the sampling date and it was lower in the second and third sampling than the first sampling (P<0.001). The NDF content of third sampling were also higher than the first sampling (P<0.05). The concentration of ADF was higher in the third sampling when compared to the first and second sampling (P<0.01).

Total aflatoxin, AFB_1 and zearelanone residues were found in all fresh grass samples, but their levels were not statistically different among the sampling date *(Table 1)*.

Nutrient composition, fermentation products and mycotoxin residues of silage

Nutrient compositions of the silage is given in *Table 2*. Sampling date did not affect CA and OM content of the silages. The DM and NFE content of the silages at second and third sample were higher than the first sample (P<0.001), and were lower for CP and EE content (P<0.05). The NDF and ADF contents of the third sample were higher than the first sample

Table 1. *Chemical composition and mycotoxin residues of the fresh pasture grasses at different cutting date*
Tablo 1. *Farklı tarihlerde biçilen taze çayır otlarının kimyasal bileşimi ve mikotoksin rezidüleri*

Chemical Composition	Cutting date			SEM	Significance
	24th June	1st July	8th July		
Dry matter, g/kg	264.8 ^{b}	303.2 ^{ab}	342.7 ^{a}	14.10	**
	--------------- g/kg of DM ----------------				
Crude Ash	91.5	87.0	90.2	4.50	NS
Organic matter	908.5	913.0	909.8	4.50	NS
Crude protein	134.9 ^{a}	119.0 ^{b}	109.3 ^{b}	3.40	***
Neutral detergent fiber	499.0 ^{b}	512.4 ^{ab}	523.4 ^{a}	7.50	*
Acid detergent fiber	340.0 ^{b}	350.6 ^{b}	367.0 ^{a}	3.80	**
Ether extract	26.3	2.68	2.88	1.20	NS
Nitrogen Free Extract	444.3	459.1	455.1	5.60	NS
	----------- 880 g/kg of DM, ppb ---------				
Total aflatoxin	18.99	24.96	22.35	4.20	NS
Aflatoxin B_1	9.96	19.62	12.31	3.50	NS
Zearalenone	10.36	14.67	11.94	2.30	NS

a, b: *Means within rows with different superscripts are significantly different,* ***NS:*** *Not significant, * P<0.05, ** P<0.01, *** P<0.001*

(P<0.001). Use of barley or molasses as additive increased DM and NFE contents (P<0.001), but decreased NDF and ADF contents (P<0.001). Addition of barley decreased CA (P<0.05) and EE (P<0.001) contents but increased OM (P<0.05), and addition of molasses decreased CP content of silages (P<0.001).

As seen in *Table 3*, pH and AA levels were higher the second sampling than the other sampling date (P<0.001). The highest LA level was found on the first sampling and it was statistically differ other sampling date (P<0.001). The BA level on the first and second sampling date were statistically lower than the third sampling date (P<0.001). Using the additives induced significant changes on the fermentation characteristics of the silages samples. Barley or molasses as additive significantly decreased the pH and BA levels when compared with the no additives (P<0.001), and using the molasses as additive was also more efficient lowering the pH and BA levels (P<0.001). Similarly using the both additives increased LA level when compared with the no additives (P<0.001) and the highest LA level was found in the molasses added silages. The AA level of the barley added silage was lower than the other silages (P<0.001).

Total aflatoxin, AFB_1 and zearalenone residues were found in all silage samples examined *(Table 3)*. Total aflatoxin residue in the second sampling date was higher than the other sampling date (P<0.001). The

Table 2. *Chemical compositions of the pasture grass silage making different cutting dates and additives, g/kg, (mean ± SEM)*

Tablo 2. *Farklı tarihlerde biçilen ve değişik katkılara kullanılarak yapılan çayır otu silajlarının kimyasal bileşimi, g/kg, (ortalama ± standart hata)*

Implication		DM, g/kg	In Dry Matter, g/kg						
			CA	OM	CP	EE	NDF	ADF	NFE
DATE		***	NS	NS	*	*	***	***	***
24th June		269.4±4.38b	98.1±1.86	901.9±1.86	148.5±1.59a	46.7±0.63a	527.1±3.96b	377.8±3.44b	372.3±5.23c
1st July		294.2±3.85a	96.0±0.92	904.0±0.92	124.4±1.43b	40.6±1.06b	529.9±3.16ab	379.8±2.47ab	407.3±3.45b
8th July		301.0±5.42a	98.0±1.55	902.0±1.55	116.4±1.32c	40.7±0.89b	534.9±3.50a	384.7±2.98a	420.4±3.36a
ADDITIVE		***	*	*	***	***	*	***	***
No additive		267.9±4.92c	99.0±1.69a	901.0±1.69b	134.0±2.62a	43.3±0.78a	562.7±1.76a	408.4±1.85a	370.5±4.44c
5 % Barley		304.9±4.60a	93.9±1.41b	906.1±1.41a	131.0±1.90a	40.5±0.75b	521.7±2.82b	367.9±1.99b	419.9±3.13a
5 % Molasses		291.8±4.00b	99.2±1.28a	900.8±1.28b	124.4±1.64b	43.6±1.15a	507.5±2.24c	366.0±2.06b	409.6±4.40b
DATE X ADDITIVE		***	NS	NS	***	***	***	***	***
24th June	No additive	250.3±7.01d	99.4± 3.76	900.6± 3.76	158.8±2.26a	45.4±0.71ab	562.9±3.76a	413.5±3.09a	339.7±8.41g
	5 % Barley	284.8±7.21bc	95.3±1.61	904.7±1.61	147.4±3.19b	44.1±1.01b	520.3±2.85bc	361.2±3.78de	401.9±469cd
	5 % Molasses	273.1±10.01c	99.5±3.11	900.5±3.11	139.3±2.25e	48.7±1.64a	498.0±2.43d	358.6±2.35e	375.2±5.15f
1st July	No additive	270.5±8.00cd	99.1±2.87	900.9±2.87	129.1±2.68d	38.6±0.66c	560.0±5.17a	402.9±3.22b	378.5±6.22ef
	5 % Barley	310.4±4.70a	92.4±1.69	907.6±1.69	126.8±1.80d	38.7±1.38c	520.0±4.28bc	366.2±2.48de	426.3±4.25ab
	5 % Molasses	301.7±9.11ab	96.6±2.68	903.4±2.68	117.5±2.23e	44.5±1.44ab	509.9±5.30c	370.3±3.91cd	417.1±3.64bc
8th July	No additive	282.9±6.21bc	98.4±3.01	901.6±3.01	114.0±1.71e	45.9±1.50ab	565.0±3.69a	408.9±3.15a	393.2±7.88de
	5 % Barley	319.4±5.14a	94.0±1.23	906.0±1.23	118.7±1.59e	38.5±2.56c	524.8±2.68b	376.3±1.56c	431.4±4.10ab
	5 % Molasses	300.7±7.81ab	101.6±2.01	898.4±2.01	116.3±2.36e	37.7±0.90c	514.7±4.41c	369.0±4.86cd	436.6±4.23a

DM: *Dry matter,* ***CA:*** *Crude ash,* ***OM:*** *Organic matter,* ***CP:*** *Crude protein,* ***EE:*** *Ether extract,* ***NDF:*** *Neutral detergent fiber,* ***ADF:*** *Acid detergent fiber,* ***NFE:*** *Nitrogen free extract*
a-g: *Means within the column with different superscripts are significantly different.* ***NS:*** *Not significant, * P<0.05, *** P<0.001*

Table 3. *The pH, organic acid concentrations (g/kg DM) and mycotoxin residues (88 mg/100g DM basis) of pasture grass silages making different cutting dates and additives (mean ± SEM)*

Tablo 3. *Farklı tarihlerde biçilen ve değişik katkılar kullanılarak yapılan çayır otu silajlarının pH, organik asit içerikleri (g/kg KM) ve mikotoksin residüleri (88 mg/100g KM bazında), (ortalama ± standart hata)*

Implication		pH	Lactic acid	Acetic acid	Butyric acid	Total Aflatoxin	Aflatoxin B_1	Zearalenone
DATE		***	***	***	***	***	***	***
24th June		4.87±0.08a	17.97±2.03a	11.33±0.65a	1.23±0.13b	53.60±5.30b	31.00±2.50a	33.25±1.52a
1st July		4.61±0.06b	7.82±0.69b	6.27±0.18b	1.08±0.14b	66.89±4.58a	33.97±3.09a	31.67±1.15a
8th July		4.87±0.06a	10.93±1.75b	10.31±0.99a	2.04±0.25a	47.89±2.66b	20.75±1.65b	27.03±0.79b
ADDITIVE		***	***	***	***	***	***	***
No additive		5.36±0.08a	4.20±0.95c	11.07±0.98a	2.48±0.95a	41.3±4.26b	21.15±2.66b	27.90±1.82b
5 % Barley		4.88±0.05b	7.69±0.97b	4.98±0.38b	1.72±0.76b	47.8±4.37b	19.54±2.29b	27.74±1.41b
5 % Molasses		4.12±0.03c	24.83±2.78a	11.86±1.04a	0.16±0.01c	84.4±7.71a	45.04±4.69a	36.31±2.08a
DATE X ADDITIVE		***	***	***	*	***	***	***
24th June	No additive	5.67±0.08a	5.94±1.09d	13.71±1.05a	1.88±1.09c	27.48±3.44c	22.52±3.11b	28.36±2.94c
	5 % Barley	4.91±0.03c	12.23±1.33c	6.25±0.56bc	1.82±1.33c	28.08±1.65c	18.13±1.75b	28.08±1.07c
	5 % Molasses	4.03±0.01f	35.73±3.85a	14.02±0.94a	-	105.23±8.88a	52.35±4.02a	43.31±2.28a
1st July	No additive	5.05±0.07c	5.13±1.13d	6.94±0.32bc	1.96±1.13b	50.10±4.83b	23.97±2.99b	29.79±1.28c
	5 % Barley	4.75±0.06d	5.80±0.93d	4.70±0.13bc	1.28±0.93c	56.29±7.28b	21.73±2.55b	27.23±1.77c
	5 % Molasses	4.04±0.02f	13.56±0.68c	7.18±0.20b	-	94.28±8.39a	56.22±6.47a	38.00±2.21b
8th July	No additive	5.36±0.07b	2.56±0.63d	12.57±1.56a	3.58±0.63a	46.19±4.52b	16.96±1.89b	25.55±1.24c
	5 % Barley	4.97±0.05c	5.01±0.65d	4.00±0.44c	2.06±0.65c	43.93±4.18bc	18.75±2.57b	27.92±1.38c
	5 % Molasses	4.28±0.06e	25.21±3.82b	14.35±1.98a	0.47±0.02d	53.56±5.86b	26.55±3.57b	27.60±1.74c

a-f: *Means within the column with different superscripts are significantly different. * P<0.05, *** P<0.001*

AFB_1 and zearalenone residues of the first and second sampling date were higher than the third sampling date (P<0.001). Significantly higher levels of total aflatoxin, AFB_1 and zearalenone were found in the molasses added silage than the other silage types (P<0.001).

DISCUSSION

Nutrient composition and mycotoxin residues of fresh pasture grasses

From the first to the third sampling concentrations of CA, OM, EE and NFE were not changed in fresh grass, while concentration of CP decreased and concentrations of DM, NDF and ADF increased *(Table 1)*. The observed changes in the some of nutrient concentrations may be related to increased fiber fractions and proportional changes in the plant's stem-twig-leaf due to the maturity. These results are in accordance with the previous studies [3,4,8].

The acceptable limit for the AFB_1 is 20 ppb in feedstuffs in both Turkey [14] and European Union [15]. Oruc et al.[16] reported that the maximum limit for total aflatoxin is twofold of the AFB_1 based on the European Commission Regulation [17] and Turkish Food Codex [18]. Acceptable limit for zearalenone is 500 ppb in the feedstuffs in European Union [19]. Total aflatoxin, AFB_1 and zearalenone residues were determined in all fresh grass samples examined *(Table 1)*, but their levels were within the acceptable limit. A previous study reported absence of detectable AFB_1 in grass at harvesting time [20]. The discrepancies between the studies may probably related to either AFB_1 was really absent in the grasses or differences in the extraction method and analytic sensitivity. Reed et al.[21] have found zearalenone residue in the 27 out of 29 pasture samples at an average level of 1.67 ppb in 1999, and in the 43 out of 58 pasture samples with an average amount of 1.08 ppb in 2000. These levels are lower than our results. The higher levels detected in this study may be related to higher rate of the rainfall and temperature in Kars district.

Nutrient composition, fermentation products and mycotoxin residues of silage

Dry matter contents of the silages ranged 250.3 to 319.4 g/kg (*Table 2*). Use of barley and molasses in silage as additive induced higher DM content when compared with control silage (except for the third sampling), as earlier studies have already reported that addition of molasses [4] and grain [22] to grass silage increased the DM content when compare to the silage with no additive. The higher content of DM in the additive silages may be related to the readily soluble carbohydrates concentration in the additives, thereby affecting the fermentation of the silage affirmatively. Addition of barley resulted in numerically higher DM content in the all silage when compared to molasses. This may be attributed to that barley contains higher DM content than molasses. Shaver [23] has reported that the higher the LA production occurs the lower the dry matter loses in the crop during the ensiling period. Our DM and LA results have supported this idea (*Table 2* and *3*). The DM content of the silage with no additive in this study was similar to some previous results [4,25,26] but lower than others [27-29]. These discrepancies between the studies may be related to differences in the harvesting time, botanical composition and state (naturally growing or cultivated) of the pasture.

The usage of an additive or no additive in the silage did not change the concentrations CA and OM. Our CA and OM results were in accordance with some previous studies [25,26] but higher than others [3,4,28,29].

Average concentrations of CP in the silages were significantly decreased depending on the sampling date. The decrease in the concentration of the CP in the silage was parallel with the decrease in the fresh grass samples in this study (*Table 1* and *2*). The concentration of CP in the silage with no additive and barley were greater than the silage with molasses at the first and second samples. Average CP concentration in the no additive silages in this study was in agreement with previous studies [4,24,26,27] but higher than the result of Kaya et al.[25]. Likewise, average CP concentrations in the barley and molasses additive silages in this study were in agreement with the results of grass silages with various additives [3,22,26,29]. In contrast, Baytok and Muruz [4] established that the CP concentration of the molasses added silage were higher than the silage with no additive at harvesting on 8th, 18th and 29th June.

Addition of barley and molasses in grass silage significantly decreased NDF and ADF concentrations as compared to silages with no additive. The higher concentrations of the NDF, ADF and BA, and the lower concentrations of the LA and NFE in the silages without any additive have indicated that many of the soluble nutrients have been degraded excessively. The data obtained for NDF and ADF in this study was accordance with Baytok and Muruz [4]. Overall silage with barley and molasses contained lower concentrations

NDF and ADF compared to the silage with no additive, this may be related to enhancement of fermentation by additives and to that barley has lower fiber matter than the grass and molasses do not have fiber fractions. The concentrations of EE in the silages are similar to that of Kaya et al.[25].

Barley and molasses addition as additive in silage significantly increased NFE concentration when compared to no additive silage. The increase may be resulted from that barley and molasses are rich in water soluble carbohydrates, so they enhanced the carbohydrate concentration in the silage material. Our NFE results are accordance with that of Kaya et al.[25].

Although pH has limited value for criteria of silage quality on its own, it is one of the important parameter for the determination of the silage quality. It is established that use of additives significantly decreased pH as compared no additives *(Table 3)*. On the other hand, molasses additive was more efficient than the barley additive in terms of attaining lower pH. The differences between the barley and molasses on the pH may be related to the diversity of their carbohydrate fractions. The positive affect using additives on the pH may be related to enhanced carbohydrate content of silage material and promoted fermentation during the ensiling process. The higher concentrations of the LA and lower concentrations of the BA in the additive silages *(Table 3)* have also supported this idea. Shaver [23] and Zimmerman [30] reported that the optimum pH for grass silage should be between 4.3 and 4.7, but McDonald et al.[1] and Ergun et al.[2] reported pH of 3.8 to 4.2 for good quality silage. In this view, the silage with only molasses attained desirable pH in this study. A high pH in the silages without any additive and in part in the barley additive silages has meant that the silages have not fermented well due to the lack of sufficient substrate for bacteria to make the acids, or an undesirable fermentation (e.g. *Clostridia*). The pH obtained from all silages with no additive was similar to Baytok and Muruz [4] but higher than those of some studies [23-25,27,31]. Our pH results, in the all barley additive silage were higher than the result of Kaya et al.[25], and molasses additive silage lower than that of Baytok and Muruz [4].

Lactic acid level is the single most important indicator of good silage fermentation and it dominates the good fermented silage and is mostly responsible for dropping silage pH and also minimizes the DM losses [1,2,23,30]. Molasses additives induced higher LA production than no additive and barley additive in silages of all sampling. The positive affect of the molasses might be due to the its carbohydrate fractions. Similar positive affect was also observed in barley additive but its affect was more partial. Shaver [23] and Zimmerman [30] reported that grass silage should have 6-10% of LA but the value obtained in our study is below this level. This situation may be related to that pasture grass is insufficient for silage making as good fermentation conditions are hard to attain. However, energy source supply such as molasses can affect lactic acid formation positively. In addition to the molasses use of inoculants containing homofermentative lactic acid bacteria can be useful for increasing the lactic acid production. The LA levels in silage with no additive in this study were lower than previous studies [4,24,26,31]. Similarly LA levels of the barley added silage in this study were also lower than the earlier findings [3,26,29,32,33]. Lactic acid levels in the molasses silage was similar to the results of some studies [29,33], but lower than others [3,4,26,32,34].

When LA production is not rapid as well as not enough during the silage fermentation, sugars are converted to AA by bacteria [1]. Except for the second sampling date, AA levels in the silages without additives and molasses were higher than those in the barley added silages. Shaver [23] and Zimmerman [30] have reported the desirable level of AA to be between 1 and 3% in the grass silages. The AA levels in the silage without additives in our study is similar to the results of Baytok and Muruz [4] and O'Brien et al.[31], but is higher than the results of Petit and Flipot [23]. Like wise, AA levels in the barley added silages is also in parallel with the findings of some previous research [29,33] while is lower than the data of others [3,4,26,34]. The AA levels in the silage with molasses in this study is similar to the results of Cai et al.[34], while is lower than those of Rinne et al.[3] and Kirkland and Patterson [26], and higher than those of others [24,33]. Marley et al.[29] have reported that the ideal ratio of LA:AA in silage should not be less than 3:1. The nearest data to this has been obtained from the silages with molasses additive in our study. The poor ratio of LA:AA in our silages may be due to the lack of an inoculant at ensiling.

The BA production is not desirable process in silages. A high concentration of BA indicates that the silage has undergone Clostridial fermentation, which is one of the poorest fermentations [1,23,30]. Our results have indicated that molasses addition to silages in the first two sampling has inhibited the BA production entirely and has also declined BA production significantly in the third sampling, as compared to other silage types. The lower pH level and CP concentrations and the higher LA levels in the molasses added silages (except the third sampling) may lead to think that pH drops very fast due to LA production, thus protecting the degradation of CP. Shaver [23] and Zimmerman [30] have reported that grass silage should have a BA level of less than 0.5-1.0%. Our results were below these levels, and were in accordance with the previous findings [3,4,24,29,33,34].

As in the fresh material, total aflatoxin, AFB_1 and zearalenone residues were determined in all silages. But mycotoxin levels were more excessive in the silage than fresh material (*Table 1* and *3*). Similarly, Sahin et al.[35] have found higher mould count in the silage of sugar beet pulp than in the pulp of the fresh sugar beet. Mycotoxins would not be expected in properly prepared and maintained silage, although some field mycotoxins such as zearalenone survive the ensilage [6,36]. The presence of mycotoxins in all silage samples can be related to the field contamination with mycotoxin *(Table 1)* and oxygen is successfully excluded in the silage medium. In general, it was noticed that BA concentrations increased in the silages as mycotoxin levels decreased *(Table 3)*. Like wise, Zimmerman [30] has reported that BA enriched silages are very stable, thus heating and molding are seen at very low level. Total aflatoxin and AFB_1 levels in this study *(Table 3)* were generally above the accepted limits (20 ppb for AFB_1) but zearelanone levels were below (500 ppb). In a previous study which comprised 10 maize, 3 wheat, and 3 wheat-common vetch silages, AFB_1 level was above the tolerable limit in the one wheat silage and one wheat-common vetch silage only [37]. In another study, AFB_1 were found in the 19 out of 80 grass silage samples and zearalenone in the 35 out of 79 [7] and in the 7 of 120 [28] samples, but all mycotoxin levels were within the acceptable limit. Although lower pH and higher LA concentrations were obtained from the molasses added silage, higher mycotoxin levels were also detected in this silage, which indicated that molasses promoted mycotoxin synthesis. To obtain lower mycotoxin levels in the silage, usage of inoculants, minerals and organic acids, and preservatives, alone or in combination, may be useful along with those additives enriching nutrient content of silage.

In a conclusion usage of ground barley and molasses as an additive improved the nutrient composition and fermentation peculiarities of grass silage, as compared to one without any additive. Even though silage fermentation was not optimal, adding molasses to the silage was more advantageous than the barley addition. Total aflatoxin, AFB_1 and zearalenone were present in all fresh grass and silage samples but mycotoxin levels were excessive markedly in silage samples. The total aflatoxin and AFB_1 levels in the silages were generally higher than the acceptable limit, while zearalenone levels were within the limit. In general, the data obtained hereby suggest that the three sampling dates evaluated in this study are suitable for ensiling, but the first two weeks of July is more advantageous since the dry matter amounts of the silages are higher, and the best additive is 5% molasses with precautions taken against the fungi.

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