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RESEARCH ARTICLE

Nutrient Composition and Mycotoxin Residues in the Hay Stored as Stack Forms During the Storage Period, and Aflatoxin M_1 in the Milk of the Cows Fed by Them

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Summary

The objectives of the study were to determine nutrient composition and mycotoxin contamination of the pasture samples at harvesting time and their hay forms which were dried in ground and stored as stacks form during the storage period, and to reveal the aflatoxin M_1 (AFM_1) residues in the milk samples of the cows fed by these hay samples. Ten pasture grass and their hay samples stored as stack form were obtained from 10 different pasture areas and family farms in Kars district, respectively. Upon taking the hay samples from both the inner and outer parts of the stacks, they were analyzed for dry matter (DM), crude ash (CA), organic matter (OM), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), nitrogen free extract (NFE) and total aflatoxin, aflatoxin B_1 (AFB_1), and zearalanone residues in harvesting time, and then 6 times with 45 days of interval during the storage period. The concentrations of DM, CA, OM, CP, CF, NDF, ADF, EE and NFE of pasture at harvesting time were found to be 307.9, 93.3, 906.7, 118.0, 313.2, 509.7, 351.3, 29.1 and 446.4 g/kg DM basis, respectively. Total aflatoxin, AFB_1 and zearalenone residues were determined in all the fresh grass samples examined at average 24.10, 15.21 and 14.97 ppb, but they were within the acceptable limits. During the storage period of the hay; DM content fluctuated, CA, OM and NDF contents unchanged, CF content increased, CP decreased in the inner part, and ADF contents increased in the inner part at the beginning of the storage, as compared with at the end of the storage period. During the storage period, mycotoxin residues were also within the acceptable limit except for the total aflatoxin and AFB_1 levels in the 4^{th} period samples. Except for the one milk sample, AFM_1 residues were within the acceptable limit. It is concluded that, depending on the longer storage period, generally nutrient composition of the hay unchanged substantially, mycotoxin residues also unchanged and generally not exceed the acceptable limits.

Keywords: *Hay, Nutrient composition, Aflatoxin, Zearalenone, Aflatoxin M_1*

Yığın Şeklinde Depolanan Çayır Kuru Otlarının Depolama Süresince Besin Kompozisyonu ve Mikotoksin Rezidüleri ile Bu Otlarla Beslenen Süt İneklerinin Sütlerinde Aflatoxin M_1 Rezidüsü

Özet

Bu çalışmanın amacı, çayır otlarının biçim zamanında ve yerde kurulup yığın şeklinde muhafaza edildikleri sürede besin madde kompozisyonu ve mikotoksin kirlilikleri ile bu otlarla beslenen süt ineklerinin sütlerinde aflatoksin M_1 (AFM_1) kalıntısının belirlenmesi idi. Kars bölgesindeki 10 farklı aile işletmesine ait çayırlardan çayır otu ve aynı çayır otlarından yığın şeklinde muhafaza edilen kuru ot örnekleri alındı. Çayır kuru otu örnekleri alınırken yığının dış ve iç kısmından örnekler alındı. Alınan örneklerde kuru madde (KM), ham kül (HK), organik madde (OM), ham protein (HP), ham selüloz (HS), neutral detergent fiber (NDF), acid detergent fiber (ADF), ham yağ (HY), azotsuz öz madde (NÖM) ile total aflatoksin, aflatoksin B_1 (AFB_1) ve zearalenon rezidüsü analizleri biçim zamanında ve depolama süresince 45 günlük aralıklarla 6 defa yapıldı. Biçim zamanında çayırların KM, HK, OM, HP, HS, NDF, ADF, HY ve NÖM konsantrasyonu kuru madde bazında sırasıyla 307.9, 93.3, 906.7, 118.0, 313.2, 509.7, 351.3, 29.1 ve 446.4 g/kg olarak bulundu. İncelenen taze çayır otlarının tümünde total aflatoksin, AFB_1 ve zearalenon miktarları kabul edilebilir sınırlar içinde ve sırasıyla 24.10, 15.21 ve 14.97 ppb idi. Depolama süresince çayır kuru otlarının KM içerikleri yığınların iç kısmında dalgalı bir seyir gösterirken; HK, OM ve NDF içeriklerinin değişmediği, HS içeriklerinin arttığı, HP içeriklerinin ise azaldığı görüldü. ADF içerikleri depolama başına göre depolama sonunda arttı. Depolama süresince dördüncü analiz döneminde total aflatoksin ve AFB_1 hariç tüm mikotoksin rezidüleri ile incelenen süt örneklerinden birisi hariç tümünün AFM_1 rezidüsü kabul edilebilir sınırlar içerisinde idi. Sonuç olarak; depolama süresi uzadıkça çayır kuru otlarının besin madde kompozisyonunda önemli ölçüde bir değişiklik olmadığı, mikotoksin rezidüsünün değişmediği ve kabul edilebilir sınırları aşmadığı kanaatine varıldı.

Anahtar sözcükler: *Çayır kuru otu, Besinsel kompozisyon, Aflatoksin, Zearalenon, Aflatoksin M_1*

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INTRODUCTION

As far as the pasture is concerned, the North East Anatolia (Kars, Ardahan, Iğdır, Ağrı) is one of the richest regions in Turkey. The pasture hereby grows naturally, and has not yet been cultured. During the grazing period, feeding ruminants, particularly common in the region, depends mostly on the low quality pasture left and on the preserved hay in the in-door period. Natural pastures at medium-high quality, which have not been grazed and used in hay making for years, is cut and dried on the ground within the July, as the most common conversation method. Most of the dried hay is stored as large stack forms over open areas.

A significant amount of quality and quantity losses may occur in the hay during the periods of cutting, drying and storage in stack form. These losses can be related to the botanical composition of the pasture, drying conditions, bleaching, mechanical losses during the drying and transportation periods, storage conditions, leaching losses due to rain, and microbial activity [1,2].

Enoh et al.[3] have determined the changes in the levels of nutrient losses of different pasture during the process of harvesting, hay making, and storage over a period of 20 weeks under the subtropical environmental conditions, and have found that crude protein (CP) and nitrogen free extract (NFE) contents decrease, while crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents increase. The nutrient losses in the hay stored as stack or bale also differ in the inner and outer parts. It has been reported that CP and ADF contents of the inner and outer parts of the alfalfa hay stored at open areas as round bales are 189 and 194, and 386 and 458 g/kg, respectively [4].

In the cases where the hay moisture content is not maintained below 150 g/kg and inadequate drying, fungal activity is seen, in general, along with nutrient losses. Some fungi may produce mycotoxins as secondary metabolites. In relation with the fungus production, CF and NDF contents of the hay increase. This then decreases the palatability and voluntary intake, leading to a further decrease in digestibility [5]. Recent studies have indicated that aflatoxin is found commonly in the roughage [5-8]. Aflatoxin may have several adverse effects (i.e. decrease in appetite, feed intake and feed efficiency, depressed immune response, hepatotoxic, carcinogenic, mutagenic, and teratogenic) on animals, depending on the age and gender, and the level and type. Four related aflatoxin may occur in animal feed-stuffs: B_1, B_2, G_1 and G_2. Aflatoxin B_1 (AFB_1) is the most important and common type. It is metabolized and its derivative aflatoxin M_1 (AFM_1) is excreted in animal products such as milk and egg. Their residue can negatively affect human health [9,10]. It was reported that 6 out of 12 hay samples obtained from different region of Turkey contaminated with aflatoxin at the levels of 2.74-17.45 ppb [8]. Demirel and Yıldırım [7] determined on the levels of the aflatoxin in the hay stored at open areas within June, September and February, found that they were within the acceptable limit. Another mycotoxin present in hay is zearalenone which induces reproductive disturbances and depresses feed intake through affecting hormonal balance in animals [6,9].

There has been very limited research done so far on the hay and its nutrient changes and mycotoxin residues during the period from harvesting and drying throughout the consumption. Therefore, the objectives of this study were to assess the level of nutrient contents [dry matter (DM), crude ash (CA), organic matter (OM), CP, CF, NDF, ADF, and ether extract (EE)] and mycotoxin residues (total aflatoxin, AFB_1, and zearalenone) of the pasture in harvesting time and 6 times with forty five days of interval during the storage period of the hay at open areas as stack forms, and to determine AFM_1 residues in the milk's of the dairy cattle fed by in Kars, Turkey.

MATERIAL and METHODS

Sample collection

The study started at 10 different pasture areas, which belonged to different family farms in Kars district, located in North East of Turkey. The grass samples were obtained from each pasture areas where 8 to 10 different parts of 1.5 m^2 areas was cut at a height of 4-5 cm with a scythe at 8th July, which is the averagely accurate harvesting time in this area. They grow naturally, and have been preserved for hay making for years.

The family farms that the samples were obtained from were those which dried pasture grasses on ground, transported to near their barns, and stored as stack form at open areas. The rest of the study was performed on these stacks. The samples of the study were obtained within 45 day of intervals (23rd August 2007, 6th October 2007, 21st November 2007, 5th January 2008, 19th February 2008, and 4th April 2008) both from the inner and outer parts of the stacks, in accordance

with the method of Ergün et al.[11]. Additionally, milk samples were taken four times from the cows of the 9 farms (milk samples were not collected from the one farm since there was no cow in lactation period), which were fed by the hays, on 21st November, 5th January, 19th February, and 4th April. An amount of 500 ml milk samples were obtained from each farm after the milk of the all cows in the related family farm had been mixed. 10 ml of each sample was centrifuged in a cooled centrifuge at 3500 g for 10 min, skimmed milk was acquired, and 5 ml of which was stored at -18ºC.

The average climatic data of the research period was displayed in *Table 1*.

Table 1. *Mean temperature ,relative humidity, rain-snow fall and snow depth from June 2007 to March 2008 in Kars (Reference, Devlet Meteoroloji İşleri Genel Müdürlüğü)*

Tablo 1. *Haziran 2007 ile Mart 2008 tarihleri arasında Kars'taki ortalama sıcaklık, nispi nem, yağmur-kar yağışı ve kar kalınlığı (Kaynak, Devlet Meteoroloji İşleri Genel Müdürlüğü)*

Months	Temperature (°C)	Humidity (%)	Monthly total rain-snow fall (mm)	Snow depth (cm)
June	14.7	65.5	51.9	-
July	17.6	62.9	56.7	-
August	18.0	62.2	49.8	-
September	15.7	58.7	1.1	-
October	9.3	67.1	55.6	-
November	0.1	72.6	80.8	2.3
December	-9.0	76.9	31.7	6.3
January	-16.9	77.5	30.6	20.9
February	-12.4	74.5	16.1	43.4
March	2.0	66.8	29.8	20.4

Analytical procedures

Fresh pasture grass and hay samples were dried at 60ºC and then ground to pass through a 1 mm screen. Concentrations of DM, CA, CP, CF, NDF, ADF and EE were determined using the methods described by A.O.A.C. [12]. Concentrations of NDF and ADF were determined by methods of Van Soest and Robertson [13]. Total aflatoxin, AFB_1, zearalenone and AFM_1 residues were measured by competitive ELISA (StatFax 3200 Reader), according to procedure described by the manufacturer [14] using RIDASCREEN® Aflatoxin Total-Test Kits (Art. No.: R4701), RIDASCREEN® Aflatoxin B_1 30/15-Test Kits (Art. No.: R1211), RIDASCREEN® Zearalenone-Test Kits (Art. No.: R1401) and RIDASCREEN® Aflatoxin M_1 30/15-Test Kits (Art. No.: R1111). The sensitivities of the commercial mycotoxin kits used were 1.75, 1.00, 1.75, ppb and 5 ppt, respectively.

Statistics

Data obtained were subjected to analysis of variance using one-way ANOVA procedures [15]. Where significant values obtained, Duncan Multiple Range test was performed. Statistical significance was presented at a probability of less than 5%.

RESULTS

Nutrient compositions and mycotoxin residues of fresh grasses in harvesting time

The average concentrations of DM, CA, OM, CP, CF, NDF, ADF, EE, and NFE were 307.9, 93.3, 906.7, 118.0, 313.2, 509.7, 351.3, 29.1, and 446.4 g/kg, in dry matter basis at harvesting time, respectively *(Table 2)*.

Total aflatoxin, AFB_1 and zearalenone residues were found in all the fresh pasture samples to be means of 24.10, 15.21, and 14.97 ppb, respectively *(Table 3)*.

Changes on the nutrient compositions and mycotoxin residues in hay during the storage period

Dry matter ratios in the inner and outer parts of the stacks were ranged from 829.5 to 922.0 g/kg and from 774.5 to 917.2 g/kg, respectively throughout the storage period, showing significant differences ($P<0.001$) depending on the storage period *(Table 2)*. Dry matter ratios in the inner parts of the stacks at 21st November and 5th January, and in the outer parts of the stack at 21st November, 5th January and 19th February, were significantly lower than those in the other months ($P<0.001$). There were no significant differences on the concentrations of CA and OM both in the outer and inner parts of the hay stacks throughout the storage period ($P>0.05$). There were no substantial changes in the concentrations of the CP in the outer part of the stacks during the storage period. However, CP concentrations in the inner part of the stack were higher on the 23rd August than on the 6th October, 21st November and 4th April ($P<0.001$), yet were stable after the 6th October. The concentrations of the CF, NDF and ADF gradually increased till 21st November, and were then substantially stable. However, CF concentrations of the inner and outer parts of the stacks at the end of the storage period were significantly higher as compared to those at the beginning of the storage ($P<0.001$) even though those of the NDF were not. Similarly, ADF concentrations were higher in the inner part of the stacks at the end

Table 2. *Mean nutrient concentrations of fresh pasture grasses in the harvesting time and in the 6 periods with 45 days of interval during the storage period of the hay at open areas as stack forms, g/kg on the basis DM, mean ± standard error of means*

Tablo 2. *Çayır otlarının biçim zamanındaki ve açık havada yığın şeklinde kuru ot olarak depolandığı süreçte 45 günlük aralıklarla 6 kez belirlenen ortalama besin madde konsantrasyonları, g/kg*

Date		DM	CA	OM	CP	CF	NDF	ADF	EE	NFE
8th July	Harvesting time	307.9±15.4e	93.3±3.4	906.7±10.9	118.0±5.2a	313.2±4.7e	509.7±12.8d	351.3±6.0f	29.1±0.8a	446.4±6.4a
23rd August	IPS [1]	890.1±7.5ab	90.3±3.8	909.8±3.8	108.8±3.0b	328.0±3.9de	541.3±11.8c	378.0±6.3e	25.4±0.9b	447.6±4.0a
6th October	IPS	922.0±3.8a	87.4±2.9	912.6±2.9	104.2±2.5bcd	338.3±5.6cd	565.1±10.2bc	396.0±6.8de	25.1±1.0b	445.0±4.4a
21st November	IPS	838.9±9.0c	94.9±3.7	905.1±3.7	98.4±2.2cd	352.7±8.0bc	583.3±11.5ab	408.3±6.5bcd	24.7±0.7b	429.3±7.8abc
5th January	IPS	829.5±17.0c	91.7±3.0	908.3±3.0	100.3±3.5bcd	351.8±7.9bc	571.9±10.3bc	401.2±7.0bcd	24.9±0.9b	431.2±7.4abc
19th February	IPS	867.6±13.0bc	88.8±3.8	911.2±3.8	101.7±2.4bcd	351.7±6.1bc	568.0±10.1bc	399.4±6.7cde	25.2±0.9b	432.6±6.7abc
4th April	IPS	906.6±9.7ab	89.6±3.6	910.4±3.6	99.3±1.9cd	354.1±5.7abc	574.3±10.2bc	402.8±6.8bcd	24.9±0.6b	432.2±7.1abc
23rd August	OPS[2]	901.3±10.0ab	88.8±3.0	911.2±3.0	105.3±2.1bc	340.0±5.3cd	562.1±11.6bc	404.0±7.8bcd	21.8±1.2cd	444.1±5.2abc
6th October	OPS	917.2±4.6a	89.5±3.5	910.5±3.5	100.0±1.5bcd	355.4±6.9abc	596.8±7.8ab	416.8±8.1abcd	21.8±1.1cd	433.3±6.9abc
21st November	OPS	774.5±23.0d	92.8±3.5	907.2±3.5	95.1±2.2d	372.5±6.7a	612.3±8.5a	437.0±7.3a	21.0±1.1d	418.5±8.5c
5th January	OPS	827.0±23.9c	94.6±4.3	905.4±4.3	97.5±2.6cd	363.0±6.3ab	591.9±9.7ab	422.2±9.4abc	22.5±0.9bcd	422.4±7.9bc
19th February	OPS	843.5±25.4c	94.2±3.3	905.8±3.3	98.3±3.1cd	363.9±5.3ab	587.4±9.8ab	415.2±9.4abcd	24.1±0.7bc	419.5±7.1c
4th April	OPS	911.7±9.7ab	91.3±3.3	908.8±3.3	98.1±2.6cd	366.5±5.6ab	591.2±10.6ab	424.8±9.0ab	22.8±1.0bcd	421.3±8.8c
	Significance [3]	***	NS	NS	***	***	***	***	***	*

DM: *Dry matter,* ***CA:*** *Crude ash,* ***OM:*** *Organic matter,* ***CP:*** *Crude protein,* ***CF:*** *Crude fiber,* ***NDF:*** *Neutral detergent fiber,* ***ADF:*** *Acid detergent fiber,* ***EE:*** *Ether extract,* ***NFE:*** *Nitrogen free extract*
[1] ***IPS:*** *Inner part of the stacks,* [2] ***OPS:*** *Outer part of the stacks,* [3] *Significance: Means in the same columns with different letters significantly differ (****NS:*** *Non significant,* ****:*** *P<0.05,* ******:*** *P<0.001)*

Table 3. *Mean mycotoxin residues of pasture grasses in the harvesting time and in the 6 periods with forty five days of interval during the storage period of the hay at open areas as stack forms, ppb in 88 % DM, mean ± standard error of means*

Tablo 3. *Çayır otlarının biçim zamanındaki ve açık havada yığın şeklinde kuru ot olarak depolandığı süreçte 45 günlük aralıklarla 6 kez belirlenen ortalama mikotoksin rezidüleri, ppb % 88 KM bazında, ortalama ± standart hata*

Date		Mycotoxin		
		Total Aflatoxin	Aflatoxin B1	Zearalenone
8th July	Harvesting time	24.10±3.87B	15.21±2.88C	14.97±3.56
23rd August	IPS [1]	16.64±1.19B	10.43±0.90C	8.70±1.40
6th October	IPS	19.35±1.51B	12.92±1.10C	11.25±2.43
21st November	IPS	22.02±2.68B	14.12±1.51C	13.68±6.74
5th January	IPS	45.48±7.67A	29.03±6.11B	10.54±4.43
19th February	IPS	18.82±1.38B	12.98±1.72C	9.48±1.47
4th April	IPS	23.07±1.68B	18.05±1.43C	14.81±1.93
23rd August	OPS [2]	21.03±4.73B	16.63±4.23C	9.80±2.15
6th October	OPS	20.01±2.40B	12.70±1.50C	35.67±20.63
21st November	OPS	20.12±2.37B	14.27±1.89C	16.62±7.47
5th January	OPS	54.23±5.69A	38.63±4.10A	34.98±23.57
19th February	OPS	17.00±3.00B	12.79±2.77C	66.80±44.77
4th April	OPS	16.86±2.00B	11.90±1.52C	14.48±3.09
	Significance [3]	***	***	NS

[1] ***IPS:*** *Inner part of the stacks,* [2] ***OPS:*** *Outer part of the stacks*
[3] *Significance: Means in the same columns with different letters significantly differ*
*(****NS:*** *Non significant,* ******:*** *P<0.001)*

Table 4. *Aflatoxin M1 residues in the milk samples of the dairy cows fed by hay stored as stack forms, ppt*

Tablo 4. *Yığın şeklinde muhafaza edilen çayır kuru otlarıyla beslenen süt ineklerinin sütlerindeki aflatoksin M1 rezidüleri, ppt*

Farm	Date			
	21st November	5th January	19th February	4th April
1	ND*	ND	ND	ND
2	5.9	ND	ND	ND
3	ND	ND	ND	ND
4	ND	ND	ND	ND
5	6.4	ND	ND	ND
6	ND	ND	8.10	ND
7	ND	ND	ND	ND
8	ND	7.2	ND	ND
9	32.2	20.7	64.90	17.70

* ***ND:*** *Non detected*

of the storage period than at the beginning of the storage (P<0.001). There were no substantial changes in the concentrations of the EE of the inner and outer parts of the stacks during the storage period. NFE concentrations of the inner and outer parts of the stacks were stable during the storage period but there was a significant difference between the first two periods of the inner and last four periods of the outer parts (P<0.05).

Total aflatoxin and AFB_1 residues in the inner and outer parts of the stacks were ranged from 16.64 to 45.48, 16.86 to 54.23 and 10.43 to 29.03, and 11.90 to 38.63 ppb, respectively, throughout the storage period *(Table 3)*. Total aflatoxin and AFB_1 residues in the inner and outer parts of the stacks at 5th January were statistically higher than those in the other months (P<0.001). Zearalenone residues in the inner and outer parts of the stacks were ranged from 8.70 to 14.81 and 9.80 to 66.80 ppb, respectively; yet, there were not statistical differences throughout the storage period (P>0.05).

Comparison of nutrient compositions and mycotoxins residues of fresh grasses and hay

As average nutrient concentrations of the fresh pasture grasses were compared with their hay form, CA and OM in the fresh material and hays were similar, but CP and EE were higher in the fresh material than hays (P<0.001), while CF, NDF and ADF were lower (P<0.001). Thus, NFE of the fresh grasses at harvest differ from that of the outer part of the stack samples of the last four periods (P<0.05).

Total aflatoxin and AFB_1 residues of the outer and inner parts of the stacks at 5th January were higher then those of the fresh grasses at harvest *(Table 3)*. There were no statistical differences on the zearalenone residues of the hays and fresh grass at harvest.

Aflatoxin M_1 residue in the milk samples

Aflatoxin M_1 residues above the detectable limit (>5 ppt), were determined in the 8 out of 36 milk samples, ranging in-between 5.9 and 64.90 ppt *(Table 4)*.

DISCUSSION

Nutrient compositions and mycotoxin residues of the fresh grass in harvesting time

Mean concentrations of the DM, CA and OM of the fresh grass samples used in the study are similar with that of Karslı et al.[16] and lower than that of Koç et al.[17]. Likewise, the CF concentration was lower than that of Koç et al.[17], and the NDF and ADF concentrations were lower than that of Karslı at al.[16]. Overall, discrepancies in the nutrient compositions of the samples between this study and other studies can be related to the geographical locations of the research areas, climatic conditions, differences on the vegetation period and harvesting time, and botanical composition of the pastures examined. Şenel [18] has indicated that a good pasture should include 80-120 g/kg CP and 255-285 g/kg CF. Accordingly, we have concluded from our results that pastures in Kars district are of medium-good quality.

The total aflatoxin and AFB_1 residues have been determined in all the fresh grass samples examined in this study. Ministry of Agriculture and Rural Affairs of Turkey [19] and European Union [20] indicate the acceptable limit for the AFB_1 level in feedstuffs to be 20 ppb. Moreover, Oruç et al.[21] have reported the acceptable limit for the total aflatoxin level to be twofold of the acceptable AFB_1 limits, based on the European Commission Regulation [22] and Turkish Food Codex [23]. The total aflatoxin and AFB_1 levels in this study were well below the acceptable limits. A previous study conducted on fresh pasture grass by the thin layer chromatography found no detectable amount of AFB_1 residues [7]. This might be due to either the fact that there was indeed no mycotoxin residues in the samples because of the climatic and geographic factors or the extraction type and sensitivity of the analysis method used in that study. The zearalenone residue was also present in all the examined fresh grass samples used in the study. Yet, European Union [24] mentions the acceptable limit for zearalenone to be 500 ppb in the feedstuffs. The mean zearalenone level determined in this study is also below this limit. Reed et al.[25] found the zearalenone residue in 93% of the 29 pastures with an average of 1.67 in 1999, and in 74% of the 58 pastures averaging 1.08 ppb in 2000. However, the results of this study are far below these levels, as well as that of Towers [26]. This may be related to the higher rainfall rate in Kars district.

Changes on the nutrient compositions and mycotoxin residues in hay during the storage period

Minimum DM content of the hay for safe storage is recommended to be at least 800-850 g/kg [1,2]. The DM contents detected in this study are occasionally below

this limit during the storage period. This was the case particularly between November and February, specifically in the samples from the outer parts of the stacks. This is in fact due to the higher humidity in this period of the year (*Table 1* and *2*). Previous studies found the DM contents of the hay stored as stacks at open areas to be ranging from 910 to 926 g/kg [27], to be 916.9 g/kg [28].

The CA and OM concentrations found in this study are in accordance with the literature reports [27-29].

The CP concentration in the inner part of the stacks was lower in the last sample than that in the first sample. As far as the results of the outer parts were concerned, there was, however, no significant difference. As can be drawn from the *Table 2*, there were 68.4 and 87.3 g/kg decrease in the CP concentrations in the outer and inner parts of the stacks, respectively, as the results at the beginning and at the end of the storage were compared. The proportionally lower decrease in the outer parts is in fact a relative decrease because mycotoxin produced more in the outer part of the stacks is at protein nature; thus, somehow contributes to the higher overall protein levels determined. Moreover, there was probably a relative increase in the CP due to a proportional decrease in the DM because of the longer storage period and microbial activity occurring in the hay. Therefore, we think that this proportional decrease in the CP concentration in the outer part should be indeed more than we have measured. The higher mycotoxin residues determined in the outer parts of the stacks is supported this idea *(Table 3)*. It was also found that CP concentrations in the inner part of the stacks were lower than the outer parts of the bales of the alfalfa hay [4]. A decrease in the CP of the hay due to the longer storage period was also reported by Enoh et al.[3]. The decrease in the concentrations of CP in the hay during the longer storage period can be related to the rain damage and humidity. The fact that proteases in the plants break down proteins to amino acids, which are water soluble [5], also explains the CP losses due to rain damage. The CP values found in this study were similar to the results of Gündüz and Deniz [28], but were higher than the results of others [27,29].

Depending on the prolonged storage period, significant increases were observed in the concentrations of the CF both in the inner and outer parts of the stacks, and concentration of the ADF in the inner parts *(Table 2)*. These increases may be related to the mechanical losses such as leaf shattering, environmental and climatic factors. Obviously, there is a negative correlation between these increases and the decreases in digestibility, voluntary intake and palatability. The results of our study on the CF, NDF and ADF concentrations are in accordance with the results of previous studies [27-29]. Similarly, Enoh et al.[3] determined increases in the CF, NDF and ADF concentrations in the hay due to a prolongation in the storage time. Ensminger et al.[2] have recommended the level of 250-350 g/kg CF for the hay to be medium quality. Accordingly, our results hereby have shown that the hay of the Kars district is within the limit of the medium quality.

Storage period did not affect concentration of the EE in the both inner and outer parts of the stacks. Mean EE concentrations in this study are similar with the results of Kara and Deniz [29] but higher than those of Gündüz and Deniz [28].

Storage period did not affect concentrations of the NFE in the both inner and outer parts of the stacks, but higher in the inner parts at 23rd and 6th October than in the outer parts of the last four periods. A gradual decrease was reported on the concentration of NFE of the hay, depending on the prolonged storage period [3].

Except for the 5th January, total aflatoxin residues in the inner and outer parts of the stacks were below the indicated acceptable limit. Higher total aflatoxin residues on the 5th January can be related to the high humidity in the weather *(Table 1)* or sampling condition. The total aflatoxin residue levels found in this study were higher than that of Naicker et al.[30]. These differences may be because of the geographic and climatic factors of the two study areas.

The AFB_1 levels in the inner and outer parts of the stacks on the 5th January were statistically higher than those of the other months and their levels were also higher than the acceptable limits. Yıldız [8] found AFB_1 residues in the 6 out of the 12 hay samples, however, they were below the acceptable limits. Like wise, Prior [31] found AFB_1 residue in 1 sample out of the 108, which was also higher than acceptable limit.

Zearalenone was found in all the samples in this study but its levels were below the acceptable limit. Thus, our results indicated that zearalenone levels increased numerically in the outer part of the stacks but decreased in the inner part as storage period lasted *(Table 3)*. This can be related to the fact that environmental and climatic factors affect the outer part of the stacks more than the inner part. Our results

were lower than the results of the Naicker et al.[30]. Other reports also determined no zearalenone residues in the hay [31,32].

As a whole, our results have suggested that mycotoxin is mainly originated from the fresh material and there are no substantial changes occurring during the storage period except for the total aflatoxin and AFB_1 on the 5th January. In the cases of the relative humidity higher than 75%, total aflatoxin and AFB_1 levels exceed the acceptable limits. On the other hand, Naicker et al.[30] have reported that aflatoxin is associated with storage while zearalenone is associated usually with crops while under cultivation in the field. To prevent animals from the adverse affects of the mycotoxin in the case of the weather humidity exceeding 75%, routine mycotoxin analyses need to be performed and preventive measures should be taken against the fungi production. Additionally, to minimize the adverse affects from the environmental and climatic factors, it is of essential that the hay be stored at a covered place, using hay preservatives such as organic acids (propionic acid, lactic acid, etc). If this is not possible, the stack can be enclosed by proper materials to avoid from environmental damages. The moldy, darker and rotten hay should not be given to animal, at least, they should be diluted with the feedstuffs that contain no mycotoxin or they can be used as bedding.

Aflatoxin M_1 residue in the milk samples

In this study, AFM_1 residue was determined to be higher than the detectible limit in the 8 out 36 milk samples *(Table 4)*. The acceptable limit for the AFM_1 residues in the milk is considered as 50 ppt in both Turkey [23] and European Union [22]. Except for the one sample, the results were below the acceptable limit. Naicker et al.[30] detected no AFM_1 in the milk samples, suggesting that it was due to the low concentration of AFB_1 in the grass.

Comparison of nutrient compositions and mycotoxin residues of fresh grass and hay

The differences in the nutrients and mycotoxin residues between the fresh pasture grass and hay are due to the several factors including drying method, mechanical losses during the drying and transport, rain damage, and climatic and environmental factors. To minimize the losses, instead of drying on ground, drying can be applied on the frames and tripods/ tables or at a covered area, artificial drying can also be used. On the storage of the hay, the stack can be enclosed by proper materials to avoid from the harms of environmental conditions or it can be stored in a covered place.

It is concluded from our results that during the storage period of the hay stored as stack form in open area; concentrations of the dry matter, crude protein, crude fiber, neutral detergent fiber, acid detergent fiber, ether extract, and nitrogen free extract change, but concentrations of the crude ash and organic matter unchanged. Total aflatoxin, aflatoxin B_1 and zearalenone levels of the hay have not exceeded the acceptable limit, except for the one period for total aflatoxin and aflatoxin B_1. Like wise, aflatoxin M_1 levels of the milk have not exceeded the acceptable limit, except for the one sample.

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