Ankara Univ Vet Fak Derg, **68**, 161-166, 2021
DOI: 10.33988/auvfd.753418

The effects of different storage conditions and periods on mould-yeast, aflatoxin, *E. coli* and *E. coli* O157 in wet sugar beet pulp

Pınar TATLI SEVEN[1,a], İsmail SEVEN[2,b], Nurgül BİRBEN[3,c], Kerem KARAKUŞ[3,d], Aslıhan Sur ARSLAN[4,e], Ahmet CEYLANER[1,f]

[1]Fırat University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Elazığ; [2]Fırat University, Vocational School of Sivrice, Department of Plant and Animal Production, Elazığ; [3]Institue of Veterinary Control of Elazığ; [4]Balıkesir University, Vocational School of Kepsut, Department of Veterinary, Balıkesir, Turkey
[a]ORCID: 0000-0002-0067-4190; [b]ORCID: 0000-0001-9489-8074; [c]ORCID: 0000-0002-7704-0591; [d]ORCID: 0000-0003-0341-6354; [e]ORCID: 0000-0002-3692-5510; [f]ORCID: 0000-0002-4185-1528.

✉Corresponding author: aslihan.arslan@balikesir.edu.tr
Received date: 18.06.2020 - Accepted date: 02.10.2020

Abstract: This study was designed to determine the effects of storage condition and period on mold-yeast, aflatoxin, *E. coli* and *E. coli* O157 in the wet sugar beet pulp (WSBP). For this aim, samples were taken from the first production of WSBP from the sugar factory (Control) and the newly packaged sacks from the packaging company (Packaged Control). Afterwards, additional two samples were taken from WSBP stored as packaged (Packaged) and as surrounded by a nylon cover outside near the barn (Non-packaged) from the predetermined farms with one-month interval. The pH value of the Packaged group was significantly lower than the Non-packaged group at the end of the 1st and 2nd months (P<0.01). The yeast-mold count of Non-packaged and Packaged groups at the end of the 1st and 2nd months was determined to be 4.61, 1.60 and 4.83, 1.26 log10 CFU/g, respectively (P<0.01). However, aflatoxin (B1, B2, G1 and G2) was not detected in groups. The *E. coli* was detected as 1.48 (in the 1st month) and 1.53 (in the 2nd month) log10 CFU/g in only Non-packaged group. It was worked for the identification of *E. coli* O157 from *E. coli* positive samples of Non-packaged group, but it was not detected. Consequently, it was determined that the use of packaged WSBP was better than the Non-packaged in terms of microbiological analysis.
Keywords: Aflatoxin, *E. coli*, mold-yeast, storage conditions, wet sugar beet pulp.

Yaş şeker pancarı posasında farklı depolama koşulu ve periyodunun küf-maya, aflatoksin, *E. coli* ve *E. coli* O157 üzerine etkileri

Özet: Bu araştırma, yaş şeker pancarı posasında (WSBP) küf-maya, aflatoksin, *E. coli* ve *E. coli* O157 üzerine depolama koşulu ve süresinin etkilerini belirlemek amacıyla planlanmıştır. Bu amaçla, fabrikadan WSBP'nın ilk üretimi (Kontrol) ve paketleme işletmesinin yeni paketlediği çuvallardan (Paket Kontrol) örnekler alınmıştır. Daha sonra, birer ay aralıklarla önceden belirlenmiş çiftliklerden paketlenmiş (Paketlenmiş) ve ahırın yakınında dışarıda bir naylonla çevrili olarak saklanan (Açık) WSBP'ndan iki numune daha alınmıştır. Paketlenmiş grubun pH değeri, 1. ve 2. ayların sonunda açık grubunkilerden önemli derecede daha düşüktü (P<0,01). 1. ve 2. ayların sonunda açık ve paketlenmiş grupların maya-küf sayısı sırasıyla, 4,61; 1,60 ve 4,83; 1,26 log10 CFU/g olarak belirlenmiştir (P<0,01). Bununla birlikte, gruplarda aflatoksin (B1, B2, G1, G2) tespit edilmemiştir. *E. coli* sadece açık grupta, 1,48 (birinci ayda) ve 1,53 (ikinci ayda) log10 CFU/g olarak belirlenmiştir. Açık grubun pozitif *E. coli* örneklerinde *E. coli* O157 identifikasyonuna çalışılmış, ancak tespit edilmemiştir. Sonuç olarak, mikrobiyolojik analizler açısından paketlenmiş WSBP kullanımının paketlenmemişten daha iyi olduğu belirlenmiştir.
Anahtar sözcükler: Aflatoksin, depolama koşulları, *E. coli*, küf-maya, yaş şeker pancarı posası.

Introduction

A great majority of livestock enterprises operate in the structure of small family-owned enterprises in Turkey. The enterprises should give importance to roughage production by using their equity capital in order to earn an economic income from animal husbandry. Since our farmers have started to produce particularly cereals (wheat, barley) and industrial crops, the high-quality roughage problem has been getting bigger (21). It is important to use the cheap feed resources efficiently in the animal feeding. Wet sugar beet pulp (WSBP), a by-product of sugar industry, is a cheap and safe diet component. It does not cause metabolic diseases since it has a high amount of pectin and a high level of

digestibility and is rich in cellulose (11). However, it may spoil rapidly if it is not stored under good conditions (13). The nutritive value of the spoiled feed decreases and several acute or chronic diseases develop in the animals. Feed is exposed to a waiting period from production stage until consumption stage. Even if several metabolic changes are observed in feed during this period mainly, microorganisms are responsible for feed spoilage. The types and amount of these microorganisms in feed vary based on the resource and type of feed. Also, they may be also affected by the factors such as the applications performed to prevent from formation of microorganism and the storage conditions of feed (6, 14). There are many microorganisms causing contamination in cereal kernels, feed, and vegetable substances. Mold and yeast can reproduce in 2-9 pH range, in the environments at 10-35^{o}C and with a water activity of 0.85 and above (17). As a result of fecal contamination, feeds may be contaminated by *E. coli*, *Listeria* and *Salmonella* species in the fields or Aspergillus species in humid environments depending on storage conditions (15). Some species of *E. coli* are dangerous. Especially, *E. coli* O157 which is known the most dangerous species may be formed in the environments at 37^{o}C and pH 7.2 (30). This species is found in cattle and, spreads through feces. Especially, the fertilizers contaminated by *E. coli* O157 carry the bacteria to the agricultural lands, and contaminate the products in these lands. Also, it may be seen in feed due to the irrigation of the cultivation areas with the contaminated water (8). The feed contaminated by aflatoxins negatively affects the health of animals. As aflatoxins are durable, they cause problems not only in the cultivation stages of products but also in their storage, transport and packaging stages. The toxicity of aflatoxins varies based on their species and, the most toxic ones are B1, G1, B2 and G2, respectively (25).

To determine which method is better in this study, samples were collected from businesses stored WSBP in packaged form or in open area at two different times (at one-month intervals). The samples were examined in terms of yeast-mold, aflatoxin, *E. coli*, *E. coli* O157 and pH.

Materials and Methods

The ethics committee approval was obtained based on the decision (04.05.2017 -08/01) of Fırat University Noninvasive Ethics Committee for this study.

Feed material and sample groups: For the first method, the samples were collected from the enterprises storing WSBP on the ground or covering with a tarpaulin or nylon cover. For the another method, the samples were collected from the enterprises providing of WSBP in the air-tight sacks. In order to compare of these two methods, the samples were taken from the same enterprises with one-month intervals. For this purpose, the samples were taken from the first production of WSBP in the sugar factory (Control with 5 pieces) and from the sacks newly packaged by the packaging enterprise (Packaged Control with 5 pieces). Two more times with one-month intervals, the samples of WSBP were taken from the enterprises storing in packages (Packaged; 10 enterprises, 10 samples) or from the enterprises surrounded with a nylon cover next to barn (Non-packaged; 10 enterprises, 10 samples). Since the number of the enterprises using package was few, the number of samples was limited to 10. The sampling method was applied based on the feed sampling regulation (4).

pH measurements of the sample groups: Twenty-five grams of WSBP samples and about 100 ml distilled water were placed in glass jars and, stirred in the mixer for about 10 minutes. Then, the pH of WSBP was measured with the calibrated digital pH meter (Orion star A111).

Microbiological analyses:

Yeast and mold count: The inoculation was performed using the pour plaque method in a dual-parallel manner. The samples were incubated at 25±1^{o}C for 5 days by using Dichloran Rose Bengal Chloramphenicol (DRBC) (Merck, Darmstadt, Germany) Agar. At the end of the incubation period, all the colonies developing in the media were counted as total yeast-mold. The results were given in CFU/g (3).

Aflatoxin analysis: Service procurement from a private company (Nanolab) working with a Shimadzu Prominence HPLC device for the determination of aflatoxin was realized. All the chemicals and the mobile phases in the analyses were HPLC grade. In accordance with the method, 25 grams of each sample were taken for aflatoxin analysis (B1, B2, G1 and G2) and, processed for HPLC analysis. The final solution was taken to the 1.5 ml HPLC vial. Afterwards, 100 µl of the solution was injected into the HPLC system whose sensitivity was increased by Kobra Cell (set to 100 µA). By using HPLC column (Spherisorb ODS-3, 250x4.6 mm, 5 µm), the samples were read in turn by using the fluorescence detector adjusted to 1 ml/minute mobile phase containing 350µ 4M Nitric acid. Then, 119 mg KBr added Methanol/ Acetonitrile/Water (20:30:50) in 1 liter (excitation:362 nm and absorption: 425) (5).

Before reading the samples, the HPLC device was calibrated by drawing the standard curves. The peaks of the extracted sample in their chromatogram were compared with the retention time of the standard peak. In case of determination of toxin in the sample, the amount of aflatoxin (B1, B2, G1 and G2) in the injected sample was calculated based on the following formula in the standard graphic (5).

Formula C = A / (M/Vext) x (Vcol / Vfin) x Vinj.

A= The amount calculated in the calibration graphic of the peak of the sample extraction (ng), M= Sample amount (g), Vext= Extraction volume (ml), Vcol= Solvent volume passing through the immunoaffinity column (ml), Vinj= Volume of the injected sample (µl), Vfin= The last volume at which the obtained eluate is dissolved (ml).

Escherichia coli count: It was taken twenty-five gr of each sample brought to the laboratory for *E. coli* count, and dilutions were prepared up to 10^{-2} and 10^{-3} from the dilution of 1:10 in the 225 ml peptone water. Each dilution was inoculated to TBX agar. After 24-hour incubation at 44ºC, it was counted (16).

Escherichia coli* O157:H7 *isolation: In the feed samples, FDA method (16) was used for *E. coli* O157:H7 isolation. 25 gr of each feed sample was subjected to enrichment (for 24 hours at 37ºC) in Tryptone Soya Broth containing 225 ml Novobiocin (TSB+n). The dilutions were prepared in Peptone Water up to 10^{-3}. 100 µl of each dilution was inoculated in Sorbitol Mac Conkey Agar containing Cefixime-Tellurite (CT-SMAC) by using spread plate method. After the inoculation, the petri dishes were incubated at 35-37ºC for 24-48 hours. Then, it was taken at least 10 typical colonies (colorless-transparent) from the petri dishes in which growth took place and, put in Tryptic Soya Agar with Yeast Extract (TSAYE) agar and, incubated at 35-37ºC for 18-24 hours. Those with indole test positive among the growing colonies were transferred into Levine's Eosin Methylene Blue (L-EMB) agar and, incubated at 35-37ºC for 18-24 hours. After the incubation, the metallic green or dark red brown colonies were inoculated in the 100 mg/L in SMAC agar containing 4-methylumbelliferone glucuronide (MUG) by using the spot-on lawn method and, incubated at 35-37ºC for 18-24 hours. Afterwards, the colonies not reflecting blue color (MUG negative) were taken under 365 nm UV light source and they were subjected to *E. coli* O157:H7 latex test. The agglutination strains were sought (16).

***Statistical analyses*:** SPSS software was used to determine the differences between the pH levels of the study groups (20). In the data assessment, the normality analysis was performed, then One Way Anova test was applied for pH analysis. The T-test was applied for mold-yeast analysis. Duncan multiple comparison test was applied for determining the significance between the pH levels. T-test was used for determining the pH, mold-yeast and *E. coli* density between 1st and 2nd months. In the statistical assessment of the results, the significance was accepted as $P<0.05$.

Results

Table 1 shows the pH levels of the groups measured at the end of the 1st and 2nd months. The pH levels of the Control and the Packaged control groups were measured as soon as the samples were obtained. The pH levels of the experimental groups at the end of the 1st and 2nd months were compared with the baseline pH levels of the Control groups. The pH values of the Packaged group significantly decreased compared with the other groups at the end of the 1st and 2nd months ($P<0.01$). The pH level of the Non-packaged group was significantly lower than the Control groups at the end of the 2nd month ($P<0.01$; Table 1). No difference was found between the pH results of the Non-packaged and Packaged groups in the 1st and 2nd month.

Table 2 shows the mold-yeast analyses of the experimental groups. Mold and yeast were not found in the control groups. When the examination of the mold-yeast analyses of the experimental groups, it was found that the mold-yeast levels of the Non-packaged group at the end of the 1st and 2nd months were significantly higher than the Packaged group ($P<0.01$). No difference was found between mold-yeast results of the Non-packaged and Packaged groups in the 1st and 2nd month. No aflatoxin (B1, B2, G1 and G2) was detected in all groups.

Table 1. pH levels of experimental groups (mean±SE).

Storage period, month	Control	Packaged Control	Non-packaged	Packaged	P
1	4.06±0.03^{a}	4.00±0.09^{ab}	3.84±0.05^{b}	3.41±0.06^{c}	P<0.01
2	4.06±0.03^{a}	4.00±0.09^{a}	3.80±0.06^{b}	3.37±0.07^{c}	P<0.01
t	1.36	0.388	0.539	0.445	
P	NS	NS	NS	NS	

$^{a-c}$: Mean values with different superscripts within a row differ significantly. NS: non-significant.

Table 2. Mold-Yeast count (log10 CFU/g) of the experimental groups (mean±SE).

Storage period, month	Control	Packaged Control	Non-packaged	Packaged	t	P
1	ND	ND	4.61±0.29	1.60±0.09	9.70	P<0.01
2	ND	ND	4.83±0.36	1.26±0.30	7.69	P<0.01
t			-0.472	1.11		
P			NS	NS		

ND: Not detected; NS: non-significant.

Table 3. *E. coli* and *E. coli* O157 counts (log10 CFU/g) of the experimental groups (mean±SE).

Storage period, month	Control	Packaged Control	Non-packaged	Packaged
1	ND	ND	1.48±0.13	ND
2	ND	ND	1.53±0.08	ND
t			-0,292	
P			NS	
E. coli O157			ND	

ND: Not detected; NS: non-significant.

Table 3 shows the results of *E. coli* analysis in the groups. In the analyses of the aforementioned groups, *E. coli* was found in the Non-packaged group. At the end of both 1st and 2nd months. *E. coli* counts were performed, but no significant difference was determined between the storage periods. *E. coli* O157 was not identified in the *E. coli* bacteria in the Non-packaged group.

Discussion and Conclusion

The pH values of the Non-packaged and Packaged groups at the end of the 1st and 2nd months were determined to be 3.84, 3.41 and 3.80, 3.37, respectively (P<0.01). Çerci et al. (10) determined that the pH value of WSBP silage was 4.36. Owing to the pH of the main material in the present study was low, the pH values of the Non-packaged and Packaged groups were deceased slightly (P<0.01; Table 1). This situation was attributed to the fact that easily soluble carbohydrate content of WSBP is high and therefore, an acidic environment was formed due to the fermentation in feed. In another study (29), it was reported that the pH value of WSBP was 4.36 and, decreased pH values of the silages prepared with different additives (P<0.05). In another study (31), the pH value of WSBP silage was determined to be 3.76. Especially, the pH is prime importance in terms of aflatoxin and some microorganisms in feed. Aflatoxins grow within 3.5-8 pH range (27, 28). In the present study, pH values in both Non-packaged and Packaged groups did not show significant differences to change the growth of microorganisms, compared with the main material. However, a significant decrease was observed in pH value of the Packaged group depending on a better fermentation, compared with the other groups. In the present study, the mold-yeast rate of the Packaged group was found to be significantly lower compared with the Non-packaged group (P<0.01). This was associated with the fact that WSBP is contacted with air much and is exposed to rain in the open environment (19). When the animal breeders store the pulp taken from a factory in bulk, 40-60% of the nutrients of pulp get lost due to the undesired fermentation events (2). The nutrient loss in this study was related to the storage conditions. It has been reported that some yeast species and, to a lower extent, some mold species cause a thermal breakdown in the structure of WSBP as they produce hemicellulosic enzymes (24). Similarly, it has been reported that the storage duration and storage method of sugar beet affects mold growth (19). In the present study the mold yeast counts at the end of the 2nd month did not have a significant difference in both the Non-packaged and Packaged groups. This indicated that factors such as contact with air increasing the mold-yeast counts emerged in the 1st month and, did not cause a statistically significant effect in the following period. Indeed, it is known that aerobic fermentation forms in the earlier stages in silage production, oxygen consumption occurs in the first day in normal fermentation, and the mold-yeast counts are affected by the contact of feed with air (14).

As a result of mold-yeast growth, aflatoxin risk rises in feed. But, as a result of the analyses performed in this study, aflatoxin was not detected in all the groups. As known, aflatoxins are produced at 12-40°C and pH 3.5-8.0 (27, 28). It was observed that the pH values of the groups varied between 4.06 and 3.37 in the present study. Those pH values were within the limits required for the development of aflatoxin. However, low pH values of the study may be effective in preventing aflatoxin production. In a study conducted to determine the levels of aflatoxin contamination in corn silage, Karakaya and Atasever (22) reported that aflatoxin B1 was found in 95.84% of the examined silage samples, but none of them had aflatoxin B1 greater than the maximum tolerance limit. If anaerobic conditions are maintained well, the pH remains around 4 and mold growth is no problem. However, if there is any change in anaerobic conditions (e.g. air and water entering the silo), molds can grow in the silage and, cause aflatoxin formation (22). It has been reported that mold and yeast grow in feed under inappropriate storage conditions and, form toxin (1, 6). The fact that aflatoxin was not found in all the groups in the present study indicated that the storage conditions of the Non-packaged and Packaged groups were not very bad. The Non-packaged group was more likely to mold growth due to air and water contact but this mold growth did not cause aflatoxin production. Indeed, in a study (23) in which a simple guideline was published for the identification of some common mold spoilage, it was reported that the white mold growth did

not produce toxin, but the yellow-green, pinky-red and purple, brown-black mold growth is an indicator of toxin production. In this study, the absence of aflatoxin production was associated with the mold growth in white color (23). However, absence of aflatoxin production did not eliminate the negative effects caused by mold growth. Mold growth causes losses especially at the energy level of feed (6, 23).

In the present study, *E. coli* was found in Non-packaged group. However, *E. coli* O157 was not found. In a study conducted in Diyarbakır region to determine the presence of *E. coli* in feed (6), it was determined to be $6x10^2$, $1.6x10^2$, $3.3x10^2$ and $1.4x10^2$ CUF/g in the feeds of dairy cattle, beef cattle, calf and lamb, respectively. As seen in the results of this study, *E. coli* was not found in the mixed feed types. The *E. coli* was not detected in the Control and Packaged groups in the present study. This was indicated that there was no contamination. However, *E. coli* was found in the Non-packaged group even if not detected at a high rate. This revealed the presence of contamination in the environment. However, *E. coli* O157 was not found at the end of the 1st and 2nd months. It has been reported that *E. coli* O157 has an acid resistance mechanism although it is known to grow in 7.2 pH under normal conditions (12, 18, 30). If *E. coli* O157 is present in the environment with 4.5-5 pH for a long time (18 hours), it is able to gain acid resistance and, may be resistant in more acidic environments (pH 3-3.5) (7, 9). Also, it has been reported that *E. coli* O157 can continue its activity during cold storage (26). In the present study, it was observed that *E. coli* O157 did not grow in the storage environment under cold winter conditions.

It was determined that the Non-packaged and Packaged groups, when compared with the control groups, were cleaner than expected in terms of the bacteria examined microbiologically. This, the pH levels under both storage conditions were associated with the fact that it was quite lower than the optimal pH conditions for the growth of the examined microorganisms.

Consequently, the growth of mold and yeast may be reduced to very lower levels by preventing air inlet in the outside storage. Also, contamination of *E. coli* may be prevented by attention to contamination from the environment such as feces. It was determined that packaging was partially better than the conventional method (Non-packaged group). However, hydration may occur in case of long-term storage. Therefore, packaged pulp is recommended to be consumed in maximum 2 months and stored in cold environments.

Acknowledgments

This article was summarized from Ahmet CEYLANER's master thesis project with the same name.

Financial Support

This study was funded by Fırat University Scientific Research Projects (FUBAP) Coordination Unit (Project No: VF.17.14).

Ethical Statement

This study was approved by the Fırat University Noninvasive Ethics Committee (04.05.2017 and 08/01).

Conflict of Interest

The authors declared that there is no conflict of interest.

References

1. **Akkaya MR, Bal MA, Duman AD** (2005): Yemlerde ve Yem Hammaddelerinde Görülen Mikotoksinlerin Hayvan Sağlığı Üzerine Etkileri ve Analiz Yöntemleri. 459-462. III. Ulusal Hayvan Besleme Kongresi, Adana, Türkiye.
2. **Altaçlı S, Deniz S** (2013): *The tetermination of in vivo and in vitro digestibility and energy contents of sugar beet pulp silages produced in different ways.* Van Vet J, **24,** 9-13.
3. **Anonymous** (2008): ISO 21527-1. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds (first edition; 2008.07.01). Available at: https://www.sis.se/api/document/preview/910016/(Accessed June 03, 2020).
4. **Anonymous** (2020): Regulation on sampling and analysis methods for official control of feeds. Available at: https://www.resmigazete.gov.tr/eskiler/2011/12/20111227-13.htm(Accessed June 03, 2020).
5. **AOAC** (2003): International, AOAC Official Method 2003.02.
6. **Baran MS, Erkan ME, Vural A** (2008): *The nutrient values and microbiological quality properties of mixed feeds used in ruminant nutrition in Diyarbakir province.* J Fac Vet Med İstanbul Univ, **34,** 9-19.
7. **Bearson S, Bearson B, Foster JW** (1997): *Acid stress responses in enterobacteria*. FEMS Microbiol Lett, **147,** 173-180.
8. **Bell C** (2002): *Approach to the control of Entero-haemorrhagic Escherichia coli (EHEC).* Int J Food Microbiol, **78,** 197- 216.
9. **Buchanan RL, Edelson RL** (1999): *pH-dependent stationary- phase acid resistance response of enterohaemorrhagic Escherichia coli in the presence of various acidulants*. J Food Prot, **62,** 211-218.
10. **Çerçi İH, Çiftçi M, Güler T, et al** (2005): Silolamada Yoncaya Şeker ve Arpaya Alternatif Olarak Pazarlanamayan Elmanın Katılma Olanağının Araştırılması. III. Ulusal Hayvan Besleme Kongresi. Adana, Türkiye.
11. **Ceylaner A, Tatli Seven P, Seven I, et al** (2020): *The effects of two different storage condition and duration on nutrient level and in vitro digestibility of wet sugar beet pulp.* FÜ Sağ Bil Vet Derg, **34,** 7-12.
12. **Coia JE** (1998): *Clinical, Microbiological and epidemiological aspects of Escherichia coli* O157: H7 *Infections*. FEMS Immunol Med Microbiol, **20,** 1-9.

13. **Erdoğan Z, Aslantaş Ö** (2004): *A survey on the microbiological quality of mixed feeds and feedstuffs used in Hatay province*. Vet Bil Derg, **20,** 33-38.
14. **Ergün A, Tuncer ŞD, Çolpan İ, et al** (2016): Yem Hijyeni ve Teknolojisi. Ankara Üniversitesi Veteriner Fakültesi Ders Kitabı, Ankara, Türkiye.
15. **FAO** (2013)*:* Quality assurance for animal feed analysis laboratories. FAO Animal Production and Health Manual No. 16. Rome, Italy.
16. **Feng P, Weagant SD, Jinneman K, et al** (2018): Diarrheagenic Escherichia Coli. In: Bacteriological Analytical Manual.
17. **Gürsoy A** (2018): Nutrition and Development of Microorganisms. Available at: http://cv.ankara.edu.tr/duzenleme/kisisel/dosyalar/03112017135411.pdf (Accessed July 8, 2020).
18. **Halkman AK, Noveir MR, Doğan HB** (2001): Escherichia coli O157:H7 Serotipi. Sim Matbaacılık Ltd. Şti, Ankara, Türkiye.
19. **Huijbregts T, Legrand G, Hoffmann C, et al** (2013): Long-term storage of sugar beet in North-West Europe. COBRI, Report no. 1-2013.
20. **IBM SPSS** (2013): IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA.
21. **İptaş S, Yılmaz M, Öz A, et al** (1997): Tokat Ekolojik Şartlarında Silajlık Mısır, Sorgum Tür Ve Melezlerinden Yararlanma Olanakları. 287. Türkiye Birinci Silaj Kongresi. Hasad Yayıncılık, İstanbul, Türkiye.
22. **Karakaya Y, Atasever M** (2010): *Aflatoxin B1 in corn silage and its probability passing in milk.* Kafkas Univ Vet Fak Derg, **16,** 123-127.
23. **Kaya Ş** (2017): *Practical guide for some aerobic spoilage in silage.* MKÜ Ziraat Fak Derg, **22,** 127-134.
24. **Kubadinov N, Hollaus F, Braunsteiner W** (1984): *Beiträge zur Aufklärung mikrobiologischer und chemischer Zusammenhänge bei der Pressschnitzelsilierung. 3. Mitteilung.* Veränderung der chemischen Zusammensetzung von Pressschnitzeln während des Silierprozesses. Zuckerindustrie, **109,** 38-45.
25. **Kumar P, Mahato DK, Kamle M, et al** (2017): *Aflatoxins: A global concern for food safety, human health and their management*. Front Microbiol, **7,** 2170.
26. **Lin J, Smith MP, Chopin KC, et al** (1996): *Mechanisms of acid resistance in enterohaemorrhagic Escherichia coli.* Appl Environ Microbiol, **62,** 3094-3100.
27. **Öksüztepe G, Erkan S** (2016): *Mycotoxins and their importance in terms of public health.* Harran Univ Vet Fak Derg, **5,** 190-195.
28. **Sweeney MJ, Dobson ADW** (1998): *Mycotoxin production by Aspergillus, Fusarium and Penicillium species*. Int J of Food Mic, **43,** 141-158.
29. **Şahin K, Sarı M** (1996): *Elazığ yöresinde yaygın olarak kullanılan yemlerin bakteri ve mantar florası üzerine bir araştırma*. F Ü Sağ Bil Derg, **10,** 251-258.
30. **Tosun H, Gönül ŞA** (2003): *E. coli* O157:H7'nin *aside tolerans kazanması ve asidik gıdalarda önemi.* Orlab On-Line Mikrobiyoloji Derg, **1,** 10-17.
31. **Ülger İ, Kaliber M, Büyükkılıç Beyzi S, et al** (2015): *Yaş şeker pancarı posasının bazı meyve posaları ile silolanmasının silaj kalite özellikleri, enerji değerleri ve organik madde sindirilebilirlikleri üzerine etkisi*. Alınteri, **29,** 18-25.