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Farklı Sodyum Bentonit Seviyelerinin Balkabağı Atığı Silajının Kimyasal Bileşimi, Mikrobiyolojik Kompozisyon ve Laktik Asit Seviyeleri Üzerine Etkisi

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ÖZ

Balkabağı atığı silaj olarak hayvan beslemesinde kullanılabilmekte ancak düşük kuru madde içeriği nedeniyle silolanma işlemi olumsuz etkilenmekte ve mikotoksinlerin üremesine neden olabilmektedir. Sodyum bentonit (SB), yüksek su absorpsiyon kapasitesine sahip ve ruminantlar ile tek mideli hayvanlar için pelet yem üretiminde kullanılan bir kil mineralidir. Bu çalışmanın amacı, balkabağı atığı silajına iki farklı SB seviyesinin eklenmesinin silaj örneklerinin kimyasal bileşimi, silaj kalitesi, mikrobiyota ve laktik asit seviyeleri üzerine etkisinin incelenmesidir. Balkabağı atıkları, %0, %1 ve %2 oranlarında SB ilave edilerek üç ay boyunca silolanmıştır. Analizlerin sonucunda, SB ilavesinin ham protein, suda çözünür karbonhidrat, Fleig skoru ve laktik asit seviyelerini önemli ölçüde azalttığını, ancak ham kül, kuru madde içeriği ve pH değerlerini arttırdığı görülmüştür. Maya-küf ve laktik asit bakterileri koloni sayıları en yüksek seviyede kontrol grubunda bulunmuştur. Bunula birlikte, en düşük laktik asit bakteri ve maya-küf koloni seviyeleri %1 SB ilave edilen silajlarda görülmüştür. Bu nedenle, SB'nin silaj katkısı olarak kullanılmasının maya ve küf üremesini azaltabildiği, ancak aynı zamanda silajın besin değerini ve laktik asit seviyesini de düşürdüğü belirlenmiştir. Sonuç olarak, çalışmadan elde edilen bulgulara dayanarak balkabağı atığının silolanmasında kullanılan farklı düzeylerdeki SB'nin silajda maya-küf hariç diğer önemli parametreleri negatif olarak etkilediğinden dolayı kullanımı önerilmemektedir. İleride yapılacak araştırmalar, farklı silaj tipleri için optimal SB seviyesini belirleyerek kalite ve raf ömrünü arttırmayı inceleyebileceği düşünülmektedir.

The Effect of Different Sodium Bentonite Levels on Chemical Composition, Microbialogical Composition and Lactic Acid Levels of Pumpkin Waste Silage

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ABSTRACT

Pumpkin waste could be used as silage in animal nutrition, but due to its low dry matter content, it negatively affects the ensiling process and may cause the growth of mycotoxins. Sodium bentonite (SB) is a clay mineral with high water absorption capacity and is used in the production of pellet feed for ruminants and monogastric animals. The aim of this study was to investigate the effect of adding two different SB levels to pumpkin waste silage on the chemical composition, silage quality, microbiota and lactic acid levels of silage samples. Pumpkin wastes were ensiled for three months by adding SB at 0%, 1% and 2%. The results of the analyses show that the addition of SB significantly decreased crude protein (CP), water soluble carbohydrate (WSC), Fleig score and lactic acid levels, but increased crude ash (CA), dry matter content (DM) and pH values. The highest colony counts of yeast-mold and lactic acid bacteria were found in the control group. However, silages supplemented with 1% SB had the lowest levels of lactic acid bacteria and yeast-mold colony levels. Therefore, it was determined that the use of SB as a silage additive can reduce yeast and mold growth, but it also reduces the nutritional value and lactic acid level in silage. In conclusion, based on the findings of the study, the use of different levels of SB in pumpkin waste ensiling is not recommended because it negatively affects other important parameters except yeast-mold in silage. Future research could investigate the optimal level of SB for different silage types to improve quality and shelf life.

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1. Introduction

The escalating costs of production, growing concerns over environmental degradation, and other factors have resulted in animal nutritionists and producers turning their attention towards the utilization of agricultural by-products and waste materials to meet the nutritional needs of livestock (Ganji Jamehshooran et al., 2022). Pumpkin (*Cucurbita moschata Duschene*) fruit is a rich source of easily digestible carbohydrates, minerals, carotenoids, and vitamins, making it a valuable food for human consumption (Halik et al., 2014). Nonetheless, a significant quantity of pumpkin remains in the fields after the harvest and separation of seeds (Ulger et al., 2020). The leftover material, pumpkin waste, has a high nutritional value and could be utilized as animal feed in both fresh and preserved forms, such as through ensiling, to prolong their shelf life and enable their use in animal diets over extended periods (Łozicki et al., 2015; Ganji Jamehshooran et al., 2022). However, one of the primary challenges associated with ensiling pumpkin waste is its low dry matter content, which can adversely affect the ensiling process and promote mycotoxin production in the resulting silage (Pirinç et al., 2020). To increase dry matter in pumpkin silage, several absorbents were used in early studies such as dried sugar beet (Ülger et al., 2020), pomegranate pulp (Ülger et al., 2020), maize fodder (Ülger et al., 2020), wheat straw (Pirinç et al., 2020; Konca et al., 2021) and alfalfa hay (Ülger et al., 2020). The materials used to increase dry matter in pumpkin silages in previous research were rich in cellulose, which is a natural polymer that facilities hydrogels with the water content of feed with its hydroxyl groups (Winarti et al., 2018).

Clay minerals, particularly bentonite and montmorillonite, have been commonly employed as binders in pellet feed manufacturing for ruminants and monogastric animals (Damato et al., 2022). Bentonite is commonly used for its high water-absorbing capacity, which is attributed to its surface charge and surface area. Owing to the negative charges on its surface, bentonite exhibits strong attraction to positively charged water molecules, allowing it to hold a significant amount of water (Murray, 2006). However, a very limited number of studies (Woolford et al., 1983; Khorvash et al., 2006) have been carried out to determine the effects of sodium bentonite as an adsorbent in silage production.

Although most cellulose-rich feedstuffs have been used to increase the dry matter content of pumpkin silage and improve silage quality in previous studies, no study has evaluated the effects of inorganic materials on pumpkin silage quality. In this study, we aimed to investigate the effects of adding two different concentrations (1% and 2%) of sodium bentonite (SB) to pumpkin waste silage on its chemical composition, silage quality, microbiota, and lactic acid levels.

2. Material and Methods

Approximately 12 kg of pumpkin waste, which includes the fleshy parts of the skin, seeds, fibrous strands, and some pulp parts of the fruit, was procured from local producers in Aksaray, Türkiye. This waste was brought to the laboratory in 2 h, chopped into pieces measuring 2-4 cm and divided into three parts. One part of the waste was ensiled without any supplementation, whereas the other two parts were ensiled with 1% and 2% SB supplementation, respectively. The silage additive doses were determined based on Fransen and Strubi (1998). All samples were ensiled in polyethylene vacuum bags and the experiment was conducted in four replicates.

Table 1. Chemical structure (DM basis) of fresh pumpkin waste before ensiling

Components	%
Dry matter	9.39
Crude ash	11.31
Soxhlet extract	10.56
Crude fibre	19.17
Crude protein	23.56
Water soluble carbohydrates	5.65

Table 2. Chemical structure of sodium bentonite

Components*	%
Silicon dioxide (SiO_2)	58.53
Aluminum oxide (Al_2O_3)	14.73
Calcium carbonate ($CaCO_3$)	9.95
Iron (III) oxide (Fe_2O_3)	5.09
Magnesium oxide (MgO)	3.18
Sodium oxide (Na_2O)	1.76
Potassium oxide (K_2O)	1.25
Sulphur trioxide (SO_3)	0.92
Titanium dioxide (TiO_2)	0.72
Other Minerals	3.87
Total	**100.00**

*Provided from manufacturer company

The chemical composition of the pumpkin waste is presented in Table 1, and the SB content is listed in Table 2. The experimental silages were stored for three months. After the bags were opened and 40 g silage samples from each bag were diluted with 360 ml distilled water and filtered with Whatman No.1 paper. The pH of the filtrate was determined using a pH glass electrode (HI 1230 B, Hanna Instruments). The filtrate was also utilized for the analysis of water-soluble carbohydrates, whereas for lactic acid analysis, it was diluted by a factor of 1/100. The Silage Fleig score was calculated using the following equation: $220 + (2 \times DM\%–15) – (40 \times pH)$. Water-soluble carbohydrate levels of the silages were determined using the method described by Dubois et al. (1951). The WSC values were multiplied by 10 and divided by the DM. Similarly, the lactic acid content was determined following the method described by Barnett (1951). The obtained values were multiplied by 10 and divided by the DM of the silage samples to determine the lactic acid content (g/kg DM).

Upon opening the silage samples, a 10 g portion was diluted with 90 ml of peptone water for microbiological cultivation using the spread plate technique. The medium was poured into Petri dishes, according to the manufacturer's instructions. Yeast mold, enterobacteria, and lactic acid bacteria were quantified in the samples. The detection of yeast mold, enterobacteria, and lactic acid bacteria colonies was established according to Sırakaya, 2023, while clostridium colonies were determined based on the method provided in   The resulting colonies were then counted. Colony-forming units were counted at the end of the incubation period using the 'ImageJ 1.53k' program, and the results are reported in the table on a logarithmic basis.

The chemical compositions of the fresh material and silage were determined according to AOAC (2000). After drying the silage samples at 105°C for 24 h to obtain the dry matter content, the remaining pumpkin waste silages were ground and dried in an oven at 60°C until a constant weight was reached (approximately 48 h). The ground samples were then placed in Ziplock bags for chemical analyses. The samples were then subjected to ash analysis by ashing them in a 550°C ash furnace (NÜVE). The Soxhlet extract was extracted from the samples using N-hexane in a Soxhlet apparatus. The nitrogen (N) content of the samples was determined using Kjeldahl's method, and the resulting N content was multiplied by a factor of 6.25 (N × 6.25) to obtain the crude protein (CP) content. Crude fiber levels were determined according to the method described by Karabulut and Canbolat (2005).

All obtained data were subjected to analysis of variance (ANOVA) using the General Linear Model of SPSS. Significant differences between individual means were identified using Tukey’s multiple-range test. Differences were considered significant if $p<0.05$ or $p<0.001$. Polynomial contrasts were conducted for wilting duration to assess the linear and quadratic responses of wilting duration on the traits of ensiled oats. Data were analyzed using SPSS (version 22.0, IBM Corp., Armonk, NY, US).

3. Results and Discussion

Table 1 listed the chemical contents of fresh pumpkin waste. According to the analysis, the DM of fresh pumpkin waste was 9.39%, which is acceptable as very low for the silage-making process. Similar to our results, Ülger et al. (2020) found 8.98% DM in fresh pumpkin residues and Konca et al. (2021) detected 4.70% DM in fresh pumpkin waste. The dry matter content of the fresh material to be ensiled is an important factor during the ensiling process. A high moisture content (above 75 %) is generally considered unfavorable for ensiling because it creates a favorable environment for the growth of *Clostridium* bacteria. Fresh pumpkin has very low dry matter, and most studies on pumpkin silages have focused on reducing the amount of water and increasing DM to improve silage quality (Pirinç et al., 2020; Konca et al., 2021).

The chemical composition of the silage samples was shown in Table 3. No significant differences were found in terms of crude fiber or ether extract (p>0.05). However, significant differences were observed in terms of crude protein, crude ash, and WSC content between pumpkin waste silage prepared with different ratios of SB (p<0.05). The highest CP and WSC values were observed in silages supplemented with 0% SB. SB supplementation in pumpkin waste silage significantly reduced CP content. In particular, pumpkin waste silage supplemented with 1% SB had the lowest CP level. Similar to our findings, Fransen and Strubi (1998) suggested that, 1% SB supplementation in perennial grass silage slightly reduced CP content. In the present study, the development of *Clostridium* spp. increased in silage samples supplemented with 1% SB. Certain strains of clostridia are known to exhibit high proteolytic enzyme activity, which could potentially result in a reduction in soluble nitrogen levels in silage (Kung et al., 2018). This might explain the lower CP levels observed in pumpkin waste silages supplemented with 1% SB.

In the present study, the incorporation of SB also resulted in an elevation of the crude ash proportion in pumpkin waste silages. Likewise, Woolford et al. (1983) found an increase in the ash content of ryegrass silage with SB supplementation. Another study reported that incorporating 100 g/kg zeolite as a silage additive increased the ash ratio in forage silage (Herremans et al., 2019). Bentonite clays are composed of various minerals including silicon, aluminum, calcium, and sodium (Murray, 2006). The minerals in bentonite can be incorporated into the silage material, contributing to its overall ash content. In the current study, increasing the percentage of SB supplementation led to a significant decrease in the WSC levels in pumpkin waste silage. Theng (2012) reported that the basal hydroxyl surface found in clay minerals has the capability of establishing potent hydrogen bonds with polar groups present in polysaccharides and absorbing them thoroughly.

Table 3. Mean values and analysis of variance results of chemical composition of silage groups (DM basis)

Parameters	SB Supplementation (%)			SEM	p**		
	0	1	2		Linear	Quadric	Combined
Crude protein, %	23.17[a]	13.05[b]	19.66[ab]	1.67	0.309	0.011	0.028
Soxhlet extract, %	11.94	10.95	12.00	0.84	0.979	0.614	0.874
Crude fiber, %	16.69	16.37	15.84	0.23	0.834	0.164	0.352
Crude ash, %	13.41[b]	15.91[a]	16.32[a]	0.47	0.003	0.134	0.007
WSC, %	3.75[a]	3.00[ab]	2.35[b]	0.23	0.006	0.888	0.018

*WSC: Water soluble carbohydrates
**Data were analyzed using combined (C), linear (L), and quadratic (Q) regression models of SPSS
a-b Means in the same row with no superscript letters after them or with a common superscript letter are not significantly different ($p<0.05$).

The DM, pH, and Fleig scores of the pumpkin waste silages were given in Table 4. The results indicated that DM ratio was significantly affected by the SB ratio ($p<0.05$), with the highest DM content observed in silages prepared with 2% SB. Dry matter level in silage is an important indicator of silage quality. Studies have used different additives to increase the dry matter content to improve the silage quality of pumpkin waste, which contains high amounts of water. Pirinç et al. (2020) and Konca et al. (2021) used straw to increase the dry matter content of pumpkin silages. Additionally, Adamović et al. (2020) reported that the inclusion of 1% pyrophyllite could increase the DM levels in maize silage. Bentonite is utilized in various industries as a water binding agent, owing to its remarkable water retention capacity. This property is attributed to the expansion of the interlayer space between its clay sheets through cation exchange, which enables the material to accommodate more water molecules, thereby enhancing its absorption into the clay structure (Barbanti et al., 1997).

Table 4. Mean values and analysis of variance results of dry matter, pH and Fleig scores of silage groups

Parameters	SB Supplementation (%)			SEM	p*		
	0	1	2		Linear	Quadric	Combined
Dry matter, %	11.70[b]	12.99[ab]	13.65[a]	0.33	0.090	0.556	0.025
pH	4.02[b]	4.40[a]	4.17[b]	0.05	0.079	0.001	0.020
Fleig score	67.48[a]	54.97[b]	65.36[a]	2.09	0.557	0.004	0.012

*Data were analyzed using combined (C), linear (L), and quadratic (Q) regression models in SPSS.
a-b Means in the same row with no superscript letters after them or with a common superscript letter are not significantly different ($p<0.05$).

Moreover, the lowest pH levels were recorded in pumpkin waste silages supplemented with 0% and 2% SB; however, the silages prepared with 1% SB had the highest pH levels in the current study ($p<0.05$). Similarly, Fleig scores were higher in the silage samples with 0% and 2% SB, whereas the silages prepared with 1% SB had lower Fleig scores (p<0.05). Bentonite is renowned for its capacity to buffer pH via cation exchange and is utilized to regulate ruminal pH in ruminants (Wallace and Newbold, 1991). The production of lactic acid by bacteria is closely linked to the presence of soluble polysaccharides in feed materials (Adamović et al., 2020). Bentonite may establish a strong bond with certain soluble carbohydrates in the plant material, thus reducing their availability for fermentation. As a result, the fermentation process may be slowed, leading to reduced production of lactic acid. Additionally, the pH of pumpkin waste silage supplemented with 2% SB was similar to that of the control group. This could potentially be attributed to the capacity of bentonite to adsorb ammonia on exchange sites (Redding, 2013).

Effects of the inclusion of increasing rates of SB on microbial counts were provided in Table 5. Enterobacteria were not detected in any of the tested silage samples. Similarly, no clostridium colony-forming units were observed in the 0% and 2% SB-supplemented samples. However, silage samples supplemented with 1% SB had a slightly higher count of clostridium colony-forming units. Moreover, mold colonies were not detected in the SB-supplemented silage samples in the current study. Bentonite is known for its mold and mycotoxin absorption ability owing to its high surface area and cation exchange capacity, which enables it to adsorb toxins from feed materials (Murray, 2006; Ghofrani Tabari et al., 2018). In the same study, lactic acid bacterial population decreased linearly with SB supplementation in pumpkin waste silage. It is unlikely that bentonite supplementation would directly inhibit lactic acid bacterial production in pumpkin waste silage. However, as mentioned previously, bentonite absorbs carbohydrates that are essential for lactic acid fermentation (Theng, 2012), which could ultimately lead to a decline in the bacterial population.

Table 5. Effect of SB supplementation on microbiological values of pumpkin waste silage, log cfu g^{-1}

	SB Supplementation (%)		
Parameters*	**0**	**1**	**2**
Clostridia	ND	2.42	ND
Enterobacteria	ND	ND	ND
Lactic acid bacteria	3.68	0.54	ND
Yeast	3.51	2.98	3.49
Mold	0.15	ND	ND

*ND: Not detected

The lactic acid content of the pumpkin waste silage samples was represented in Figure 1. Compared with the control group, the amount of lactic acid in DM was reduced linearly by the addition of SB to pumpkin waste silage (p<0.05). Ensiling preserves forage through anaerobic lactic acid fermentation by epiphytic LAB, which converts water-soluble carbohydrates to lactic and acetic acids, leading to decreased pH and inhibition of spoilage microorganisms (Weinberg and Muck, 1996). The nutritional quality of silage is largely influenced by its lactic acid content, as higher levels of lactic acid are generally desirable because of their inhibitory effect on undesirable microbial growth (Kung et al., 2018). In our study, supplementation of pumpkin waste silages with SB led to a linear decrease in the lactic acid content. This finding is consistent with the results reported by Herremans et al. (2019) who observed a reduction in lactic acid levels in ryegrass and red clover silages supplemented with zeolite, as a silage additive. Similarly, Adamović et al. (2020) reported a decrease in the lactic acid content in maize plant silage with the addition of pyrophyllite. However, it is worth noting that the effects of silage additives on lactic acid production vary. Koljajic et al. (2003) reported an increase in lactic acid production in silage samples when zeolite was added during the ensiling of beet pulp. The population of lactic acid bacteria and lactic acid production are closely associated with the amount of WSC present in silage material (Astuti et al., 2013). In our study, WSC content decreased linearly with increasing SB supplementation in pumpkin waste silages. It has also been reported that aluminosilicates such as zeolites can inhibit certain fermentation processes (Herremans et al., 2019). Moreover, lactic acid content was detected in pumkin waste silage samples supplemented with 2% sodium bentonite; however, no lactic acid bacteria were detected in the medium. Some filamentous fungi and alkaliphilic lactic acid-producing microorganisms (*e.g., Marinilactibacillus psychrotolerans and Alkalibacterium psychrotolerans) can* also produce lactic acid from carbohydrates along with lactic acid bacteria (Calabia et al., 2011). This result might be related with this situation.

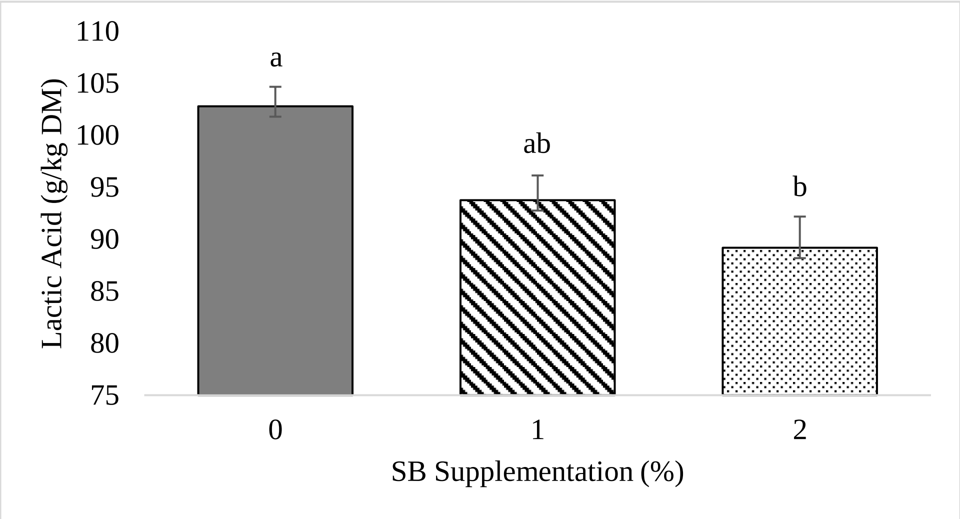

Figure 1. Effect of SB supplementation on lactic acid content of pumpkin waste silage

a-b Means in the same row with no superscript letters after them or with a common superscript letter are not significantly different ($p<0.05$).

4. Conclusion

The results of this study indicated that the addition of sodium bentonite to pumpkin waste silage affects various aspects of fermentation. Increasing the amount of SB improved the crude ash and dry matter content in pumpkin waste silage. Moreover, supplementation with 1% SB resulted in higher pH values and lower Fleig scores, likely because of the buffering capacity of bentonite. Furthermore, the adsorption of soluble polysaccharides by bentonite reduced their availability for fermentation, thus slowing down the lactic acid bacteria population and lactic acid production. In conclusion, sodium bentonite supplementation did not improve pumpkin silage quality. In future studies, it would be appropriate to investigate the use of sodium bentonite with different silage additives such as inoculants and water-soluble carbohydrate sources to increase lactic acid production.

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Conflict of Interest

The authors of the article declare that there is no conflict of interest.

Author Contributions Statement Summary

The authors declare that they contributed equally to this work.

References

Adamović M., Stojanović M., Harbinja M., Maslovarić M., Bočarov-Stančić, A., Pezo L. Efficiency investigation of the use of pyrophyllite in ensiling maize plant. Food and Feed Research 2020; 47(2): 109–118.

AOAC. Official Methods of Analysis of AOAC International 2003; 17th Edition. Maryland, USA.

Astuti WD., Widyastuti Y., Ridwan R., Yetti E. Quality of vegetable waste silages treated with various carbohydrate sources. Media Peternakan 2013; 36(2): 120–125.

Barbanti D., D'Orazio M., Versari A. The use of bentonite as a moisture regulating system 1. Study on some sorption properties of bentonites for their potential use in food technology. Journal of Food Engineering 1997; 33(1–2): 193–206.

Barnett AJG. The colorimetric determination of lactic acid in silage. Biochemical Journal 1951; 49(4): 527–529.

Calabia BP., Tokiwa Y., Aiba S. Fermentative production of l-(+)-lactic acid by an alkaliphilic marine microorganism. Biotechnology Letters 2011; 33(7): 1429-1433.

Damato A., Vianello F., Novelli E., Balzan S., Gianesella M., Giaretta E., Gabai G. Comprehensive review on the interactions of clay minerals with animal physiology and production. Frontiers in Veterinary Science 2022; 9: 889612.

Dubois M., Gilles KA., Hamilton JK., Rebers PA., Smith F. A colorimetric method for the determination of sugars. Nature 1951; 4265(168): 167–167.

Fransen SC., Strubi FJ. Relationships among absorbents on the reduction of grass silage effluent and silage quality. Journal of Dairy Science 1998; 81(10): 2633–2644.

Ganji Jamehshooran E., Jafari Khorshidi K., Bayat Kouhsar J. Chemical composition, aerobic stability and fermentation pattern of tomato pomace and pumpkin waste silage using fibrolytic enzymes and lactic acid bacteria. International Journal of Recycling Organic Waste in Agriculture 2022; 11(1): 47–59.

Ghofrani Tabari D., Kermanshahi H., Golian A., Majidzadeh Heravi R. In vitro binding potentials of bentonite, yeast cell wall and lactic acid bacteria for aflatoxin B1 and ochratoxin A. Iranian Jornal of Toxicology 2018; 12(2): 7–13.

Halik GD., Łozicki A., Koziorzębska A., Dymnicka M., Arkuszewska E. Effect of ensiling pumpkin *Cucurbita maxima* with the addition of inoculant or without it on chemical composition and quality of silages. Annals of Warsaw University of Life Sciences 2014; 53: 103–110.

Herremans S., Decruyenaere V., Beckers Y., Froidmont E. Silage additives to reduce protein degradation during ensiling and evaluation of in vitro ruminal nitrogen degradability. Grass and Forage Science 2019; 74(1): 86–96.

Khorvash M., Colombatto D., Beauchemin KA., Ghorbani GR., Samei A. Use of absorbants and inoculants to enhance the quality of corn silage. Canadian Journal of Animal Science 2006; 86(1): 97–107.

Koljajic V., Djordjevic N., Grubic G., Adamovic M. The influence of zeolite on the quality of fresh beet pulp silages. Journal of Agricultural Sciences, Belgrade 2003: 48(1): 77–84.

Konca Y., Beyzi SB., Kaplan M., Boga M., Kaliber M. Silage quality, aerobic stability, gas production and in vitro digestibility of pumpkin waste silages. 2021 [Preprint]. In review.

Kung L., Shaver RD., Grant RJ., Schmidt RJ. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. Journal of Dairy Science 2018; 101(5): 4020–4033.

Łozicki A., Koziorzębska A., Halik G., Dymnick M., Arkuszewska E., Niemiec T., Bogdan J. Effect of ensiling pumpkin (*Cucurbita maxima* D.) with dried sugar beet pulp on the content of bioactive compounds in silage and its antioxidant potential. Animal Feed Science and Technology 2015; 206: 108–113.

Murray HH. Chapter 6 Bentonite applications. In H. H. Murray (Ed.), Applied Clay Mineralogy 2020; Vol. 2: pp. 111–130.

Pirinç A., Özbilgin A., Kahraman O., Polat ES. Çekirdeklik kabak (*Cucurbita pepo* L.) artıkları silajının besin değerlerinin belirlenmesi. Turkish Journal of Agriculture - Food Science and Technology 2020; 8(3): 768–772.

Redding MR. Bentonite can decrease ammonia volatilisation losses from poultry litter: Laboratory studies. Animal Production Science 2013; 53(10): 1115.

Sırakaya S. Chemical, nutritive, fermentative and microbial composition of almond hull silage. Journal of Applied Animal Research 2023; 51(1): 17–23.

Theng BKG. Polysaccharides.Developments in Clay Science 2012; Vol. 4: pp. 351–390.

Ülger İ., Kaliber M., Büyükkiliç Beyzi S., Konca Y. Possible ensiling of pumpkin (Cucurbitapepo) residues. Turkish Journal of Veterinary and Animal Sciences 2020; 44(4): 853–859.

Wallace RJ., Newbold CJ. Effects of bentonite on fermentation in the rumen simulation technique (Rusitec) and on rumen ciliate protozoa. The Journal of Agricultural Science 1991; 116(1): 163–168.

Weinberg ZG., Muck RE.. New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiology Reviews 1996; 19: 53-68.

Winarti C., Kurniati M., Arif AB., Sasmitaloka K., Nurfadila. Cellulose-based nanohydrogel from corncob with chemical crosslinking methods. IOP Conference Series: Earth and Environmental Science 2018; 209: 012043.

Woolford MK., Wilkinson JM., Cook JE. Investigations on the effect of sodium bentonite and other moisture absorbents on the production of effluent from grass silage. Animal Feed Science and Technology 1983; 8(2): 107–118.