MYCOTOXINS AND BIOGENIC AMINES CONTENT AND THEIR CHANGES DURING STORAGES IN PRODUCED IN LITHUANIA IN MAIZE SILAGES

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Abstract. The aim of the current work was to evaluate mycotoxins and biogenic amines contents and their changes during storage in maize silages produced in Lithuania after 3 and 8 months of ensilage. Maize silages samples were collected from 20 conventional dairy farms in Lithuania.

Mycotoxins were quantified by direct competitive enzyme-linked immunosorbent assays (ELISA). The highest ZON, DON and OTA contents, respectively 880.04±60.62, 2600.0±260.0 and 29.15±5.6 µg/kg were found in silage samples after 3 months of storage. T-2/HT-2 (T-2 and HT-2 toxins) and aflatoxin total (AFL (total)) respectively 147.25±20.80, 20.05±5.33 µg/kg - after 8 months of storage. The amount of biogenic amines (BA) was determined by high-performance liquid chromatography. The highest biogenic amines content in maize silage samples were found in samples after 3 months of storage. The present study indicates that maize silage is an important source of mycotoxins and biogenic amines in the diet of cattle.

Keywords: mycotoxins, biogenic amines, volatile fatty acids, maize silage

Introduction

About 70 percent of all growing corn is used for the green fodder production. Maize silage is the main ruminant livestock feed in many European countries. The quality of raw milk and dairy products is related primarily to the quality of silage fed to cows (Purwin et al., 2006). Currently, silage quality is evaluated by chemico-fermentative parameters. However, the presence of bacteria, moulds, and/or some of their metabolites, i.e., mycotoxins, must be considered because of their effects on animal production and health (Chelia et al., 2013). Because the use of silage is increasing, risk assessment for mycotoxins becomes important as it concerns human and animal safety as well as animal performance (Pereyra et al., 2008). Little attention has been paid to the content of different protein end-products in silage and their effect on the animal organism (Olt et al., 2005). Biogenic amines (BA) are low-molecular-weight nitrogenous organic bases, which can accumulate in high concentration in food or feed due to microbial activity and cause toxic effects. In some fermented foods or feed it is difficult to prevent the accumulation of BA since the microbiological/chemical/physical conditions of the fermentation can not be easily modified The production of BA has been associated with yeast (*Debaryomyces hansenii*, *Yarrowia lipolytica*, *Pichia jadinii*, *Geotrichum candidum*) Gram-negative (*Escherichia coli*, *Hafnia alvei*, *Klebsiella pneumoniae*, *Morganella moorganii*, *Pseudomonas* spp. or*Serratia* spp.) and Gram-positive bacteria (lactic acid bacteria (LAB) (Alvarez et al., 2014). BA high levels were reported even in maize silages as amines are produced by decarboxylation of amino acids not only by enzymes of putrefactive bacteria but also of many species and strains of lactic acid bacteria (Křížek et al., 1993; Steidlová et al., 2002).

With the changes in agricultural practices towards all-year feeding of silages, silages are often 14 months or more old at the time of feeding. A better understanding of the long-term dynamics of silage is therefore important to optimize long-term storage, minimize fungal deterioration and decrease the risk of mycotoxins in silages (Storm et al., 2010). BA concentration in silages depends on the process of microbiological decarbonization of amino acids. The level of amine concentrations is mainly the result of the action of lactic fermentation bacteria and *Enterobacteriaceae* (Hernández-Orte et al., 2008).

This study evaluated mycotoxins and biogenic amines content and their changes during storage in maize silages produced in Lithuania after 3 and 8 months of ensilage.

Materials and methods

Maize samples were collected from 20 conventional dairy farms in Lithuania. The sampling sites located all over the country: from centre, west, south parts of country. From each silo were taken three samples from different places and made mixed sample. The samples were transported in polyethylene bags with minimum air content. Samples immediately were freeze at –20°C and kept until the beginning of the laboratory analyses. Samples of spoiled silage with visible fungal growth were not sampled. Silages samples from the same farms were sampled three times. Silage was preserved by spontaneous fermentation, without biological additives.

The dry matter (DM) was analyzed on the day of sampling. In order to determinate dry matter, the maize samples were chopped in 4 cm–diameter-particles and dried for 18 hours at 55ºC. After the air equilibration, the samples were

weighed and then dried again for 6 hours at 105°C. Crude protein (CP) were analyzed by Near Infrared Reflectance Spectroscopy (NIRS-6500)

The pH was measured in diluted silage with a pH-meter (WTW®inoLab pH 720, Germany) fitted with a glass electrode after homogenization of 10 g silage with 40 ml of distilled water. The content of ammonia nitrogen was determined by the Convay's micro-diffusion method.

The concentration of VFA, lactic acid and ethanol content were determined in silage extract by high performance liquid chromatography (HPLC) according to Kostulak-Zielińska and Potkański (2001), Gąsior (2002). The fresh maize silage samples were homogenized in a manual blender (Bosch), in an ice bath (for 2 min), pouring in water five times more than the weight of the given sample. The homogenate was filtered by straining through miller gauze; the filtrate was passed through a soft filter (Filtrak No. 388), deproteinised with 24% (w/v) metaphosphoric acid (FLUKA) and centrifuged (7 min., 10000 × g at 4ºC) in an MPW-350R centrifuge. The supernatant was filtered (0.22-µm PTFE syringe filter 30-SF-02 CHROMACOL LTD) and analysed by a SHIMADZU HPLC system, RP, column: METACARB 67H (ORGANIC ACIDS COLUMN, Varian), mobile phase: 0.002 M (v/v) sulphuric acid solution (95%, Sigma-Aldrich) in deionised water, flow rate 1 cm3/min., loop 20 µl, detector SDP-20A UV/Vis - 210 nm). The external-standard method was employed using the FLUKA lactic acid standard and the SUPELCO standards of acetic, propionic and butyric acids. A mixture of standards was prepared: lactic acid 3 mg/cm3, acetic acid 0.5 mg/cm3, propionic acid 0.495 mg/cm3, butyric acid 0.482 mg/cm3. The area of peaks from the sample was compared with the area of peaks from the standards.

The amount of biogenic amines was determined by high-performance liquid chromatography using the Shimadzu HPLC system according to Joosten and Olieman (1986), Gąsior and Brzóska (1999). The extraction of the amines: 50 g ground maize silage sample (robot coupe® Blixer® 3) were homogenized in a manual blender (Bosch), in an ice bath and 250.0 cm3 distilled water (for 2 min.). A portion of the filtrate (5 cm3) of this suspension was mixed with 0.5 cm3 55% (w/v) of trichloroacetic acid (TCA) and centrifuged for 10 min at 10 000 × g and 4ºC. After centrifuging the supernatant was filtered (0.22-µm PTFE syringe filter 30-SF-02 CHROMACOL LTD). High-performance liquid chromatography (HPLC) Shimadzu system (RP) with a column Nucleosil-C18 250/4, post-column derivatization with ninhydrin at the temperature of 145ºC, the phase of the carrier on the basis of DMSO, UV-VIS detector 546 nm, the patterns of biogenic amines SIGMA Mycotoxins analysis. Whole maize silage samples were air-dried, ground to pass a 1 mm screen and homogenized. The silage samples were determined by direct competitive enzyme-linked immunosorbent assays (ELISA) (AOAC, 2000). Contamination with total aflatoxins (AFL), deoxynivalenol (DON), zearalenone (ZEN) and T-2 toxin/HT-2, Ochratoxin A (OTA) toxin was tested. The Veratox test kits (Neogen Corporation, Scotland), approved by the AOAC Research Institute (Certificate No. 950702) were used for the analysis. OTA was tested with the RIDASCREEN test kits ('R-Biopharm AG', Germany). Mycotoxin extraction and tests were performed according to manufacturer's instructions. Extraction of samples was carried out in distilled water for DON, in methanol:water (70:30 v/v) for AFL, ZEN. Absorbance was determined using the micro well strip reader (Neogen, USA) at 650 nm. The measured absorbance was automatically converted to the mycotoxin concentration units – µg/kg. The results were estimated taking into account the lowest calibration curve's mycotoxin concentration value (LOD-limit of detection), which is for AFL – 2.0 µg/kg, ZEN – 10.0 µg/kg, DON – 100.0 µg/kg, T-2/HT-2 – 10.0 µg/kg, OTA – 5 µg/kg.

For determination of fungal colony-forming units per sample (CFU/g) 10 g of each sample was suspended into 90 ml of sterile water and shaken for 20 min. A dilution series (from 10^{-1} to 10^{-3}) was prepared from the obtained suspension. 1 ml of suspension from each dilution series was uniformly dispensed under the surface standard agar Czapek-Dox (Oxoid) supplied with chloramphenicol (50 mg/l) (Sigma) in Petri-dishes and incubated for 5-7 days at 26±2ºC in dark. For identification of lactic acid bacteria (LAB) count performed according LST ISO 15214:2009 „Microbiology of food and animal feeding stuffs - Horizontal method for the emumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 °C (ISO 15214:1998, identical)". Results of microbiological analysis of total fungi spores count in silage, using mathematical method of logarithm, transferred into log10 CFU/g.

Statistical analysis was carried out using SPSS software (version 12.0 for Windows, SPSS Inc., Chicago, IL, USA). The data were analyzed by analysis of variance (ANOVA). Specific means differences were identified with LSD's multiple range tests. Pearson correlation coefficients between silage fermentation quality parameters and the individual mycotoxins, biogenic amines contents were calculated. Values are presented as means ± standard error (SE) and were considered statistically significant when $P \leq 0.05$.

Results and Discussion

Findings of the present study revealed that all samples of maize silage were contaminated with mycotoxins and biogenic amines (BA) (Table 1).

A high incidence of co-occurrence of deoxynivalenol (DON) and zearalenone (ZEA) in maize silage is observed globally, while the incidence of other mycotoxins widely differs as a result of regional differences in environment conditions (Chelia, 2013). Virtually all toxins have been found in fresh forages and can also be detected in preserved forage. However, with increasing storage time, the toxin burden is often reduced due to acidic anaerobic fermentation activity that may metabolise some of the mycotoxins present at harvest (Fink-Gremmels, 2005). Skladanka et al. (2013) indicated that mycotoxins generally not degraded by the ensiling process. In the present study, 15% tested samples from fresh maize were positive for AFL (total). Eighty percent silage samples after 3 months of storage and 75% after 8 months of storage were positive for AFL (total). Mycotoxins levels between fresh and fermented silage samples showed

significant differences (P≤0.05). The maize plants arrive to the silo contaminated with *Aspergillus* strains could provide initial aflatoxin levels present in the silage. In the present study, only three fresh maize samples were positive for aflatoxin contamination. Pereyra et al. (2008) suggests that the prevailing environmental conditions could allow aflatoxin production by the potential aflatoxin producers initially present.

All samples were observed contaminated with *Fusarium* mycotoxins ZEA and DON. In the fresh cut material 45% samples and all fermented silage samples were found positive for T-2/HT-2 (T-2 and HT-2 toxins). The European Commission (EC) advisory guideline for DON is 5000 µg/kg of dry matter. In the present study DON content increased 36.92% (P≤0.05) in silage 3 months of storage and decreased 31.81% (P≥0.05) 8 months of storage. This result is in contrast with Storm et al. (2010) who found concentrations of DON higher (an average concentration - 1056 µg/kg) and T-2 lower (an average concentration - 2 µg/kg) than ours in maize silage samples 3 months after ensilage. The concentrations of DON have not exceeded the maximum allowed concentrations prescribed by EU. Despite the high toxicity of T-2 and HT-2 toxins, guidance or limiting values for feed do not yet exist. The guidance value for ZEA in Europe is 500 µg/kg. The highest ZEA content (37.5%), than EC advisory guideline, was determined in the silage samples 3 months of storage. In the study of Eckard et al. (2011) zearalenone in the maize silage before ensiling was found with a maximum level of 430 µg/kg. Ochratoxin A is frequent contaminant of maize in temperate regions, EC advisory guideline for OTA– 250 µg/kg of dry matter.

Histamine, tyramine, putrescine and cadaverine are reported as undiserable silage constituents, wide year-to-year variations (Duniere et al., 2013). In the current study the highest biogenic amines content in maize silage samples were found in samples after 3 months (P≥0.05) of storage where DM content was lower. Cadaverine content in the silages samples of 3 and 8 month of storage were higher than contents of other amines. Histamine was detected at lower content than contents of the other amines. In the study of Nishino et al. (2007) histamine was detected in the maize silages at low levels (<10 mg kg-1 DM), tyramine, putrescine and cadaverine were produced at about 400, 70 and 200 mg kg^{-1} DM, respectively. Steidlova and Kalac (2002) determined values of BA in 51 corn silage samples and BA composition was 145 mg/kg of tyramine, 136 mg/kg of putrescine, 96.2 mg/kg of cadaverine, 37.9 mg/kg of spermidine, 3 mg/kg of histamine, 2.8 mg/kg of spermine and 2.5 mg/kg of tryptamine. McDonald et al. 1991 related to the evidence that histamine is produced solely by an action of microbial decarboxylase, whereas tyramine, putrescine and cadaverine are also produced by plant enzymes (Nishino et al., 2007).

Table 1. **Parameters of maize silage**

Parameters	Fresh-cut material	After 3 months of storage	After 8months of storage
Dry matter	369.62±14.50^{a}	331.73±10.56^{b}	405.17±17.69^{a}
Crude proteins	79.12±1.47^{a}	78.83±1.43^{a}	71.29±2.39^{b}
pH	5.26±0.12^{a}	3.84±0.05^{b}	3.93±0.08^{b}
NH_3/N	-	10.55±0.53^{a}	11.06±0.55^{a}
Lactic acid	-	19.66±2.43^{a}	15.40±2.48^{a}
Acetic acid	-	2.34±0.24^{a}	1.32±0.19^{b}
Butyric acid	-	2.53±0.23^{a}	1.94±0.20^{a}
Propionic acid	-	0.70±0.33^{a}	2.16±0.49^{b}
Ethanol	-	0.99±0.34^{a}	0.71±0.38^{a}
AFL(total)	0.94±0.51^{b}	16.86±3.96^{a}	20.05±5.33^{a}
ZEA	206.88±31.52^{c}	880.04±60.62^{a}	380.42±19.20^{b}
DON	1640.0±40.0^{b}	2600.0±260.0^{a}	1118.3±160.35^{b}
T-2/HT-2	40.21±18.23^{b}	141.48±23.37^{a}	147.25±20.80^{a}
OTA	-	29.15±5.6^{a}	18.95±4.86^{a}
Histamine	-	13.41±4.12^{a}	8.98±5.07^{a}
Tyramine	-	114.10±20.14^{a}	81.58±27.13^{a}
Putrescine	-	191.38±26.65^{a}	149.91±37.65^{a}
Cadaverine	-	898.22±248.65^{a}	633.57±328.23^{a}
Fungi, $\log_{10}$ CFU/g	4.74^{b}	4.49^{b}	5.29^{a}
LAB, $\log_{10}$ CFU/g	7.09^{b}	7.28^{b}	7.56^{a}

Values in the same rows with different following letters are significantly different (P≤0.05), LSD's multiple range test. Values are the means ± SEM; LAB-lactic acid bacteria.

The combination of moisture, temperature, increasing pH and availability of nutrients and oxygen are among the important factors can have a significant proportionate influence on annual fluctuation in mycotoxin concentrations (Chelia, 2013). Formation of BA can be affected by several factors such a temperature, rapidity of pH decrease during the initial stage of fermentation, and oxygen availability (Steidlová et al., 2004; Duniere et al., 2013). Correlation coefficient between mycotoxins, amine contents and silage quality parameters are given in Table 2. According Driehuis et al. (2008)

production of DON and zearalenone in forage maize occurs during growth in the field and is affected, among other factors, by weather conditions. Neither of these mycotoxins is affected by ensiling. the concentrations detected in silage reflect the contamination levels at the time of harvesting. In the study of Krnjaja et al. (2013) a significant positive correlation has only been found between moisture content and DON (r=0.61), while for the other mycotoxins tested this correlation was negative for AFB1 (r=-0,07), ZON (r=-0.25). In the present study, weak not significant correlation was found between moisture and all tested mycotoxins contents in the fresh cut material samples and positive not significant correlation between moisture and mycotoxins was found in the silages samples 3 and 8 months after ensilage, while significant positive this correlation was found between moisture content and DON (r=0.67).

It were found strong negative correlations between pH and ZEN (P≤0.05) and T-2 (P≤0.05) toxins and no similar correlations were observed between BA and silage fermentation parameters in the all fermentation stages. The incidence of toxin metabolism under low pH conditions during the storage period on the toxin burden of mature silages depends on the pH sensitivity of different toxins. An increase, a decrease or no change was reported for the levels of different mycotoxins during ensiling (Cheli et al., 2013).

Hernández-Orte et al. (2008) maintain that together with the growing population of lactic bacteria, levels of biogenic amines also increase which can be connected with the growing concentrations of acetic and lactic acids. In the present study, the correlation cofficient between LAB and the different BA was weak, while histamine coefficient in maize silage samples after 8 month of ensilage was 0.68 (P≤0.05).

Table 2. **Correlation (Pearson coefficients) coefficient between mycotoxins (µg/kg DM), biogenic amines contents (mg/kg DM) and maize silage fermentation parameters**

Parameter	DM (g/kg)	pH	NH_3/kg N	Lactic acid (g/kg)	Acetic acid (g/kg)	Propionic acid (g/kg)	Butyric acid (g/kg)	Ethanol	Crude proteins (g/kg)	Fungi, log $_{10}$ CFU/g	LAB, log $_{10}$ CFU/g
Fresh-cut material											
AFL (total)	-0.11	-0.33	-	-	-			-	-0.11	-0.11	0.39
ZEA	-0.05	-0.56**	-	-	-			-	0.18	0.03	0.02
DON	-0.24	-0.15	-	-	-			-	0.10	-0.05	0.03
T-2/HT-2	-0.16	-0.53*	-	-	-				-0.13	-0.22	0.03
after 3 months of storage											
AFL (total)	0.08	-0.30	-0.68*	-0.14	-0.02	0.33	-0.25	0.25	-0.16	0.11	-0.11
ZEA	0.24	-0.50**	-0.21	0.15	-0.17	-0.02	0.05	-0.38	-0.05	0.44	-0.56**
DON	-0.003	-0.35	-0.11	-0.28	-0.30	-0.15	-0.65*	-0.21	-0.27	-0.17	-0.02
T-2/HT-2	0.02	0.26	0.57	-0.10	-0.05	-0.28	-0.46	-0.53	0.13	0.32	0.06
OTA	0.39	-0.02	-0.03	-0.06	-0.02	0.34	-0.47	0.32	-0.23	-0.21	-0.12
Histamine	-0.19	0.42	0.18	0.12	0.15	-0.39	-0.01	0.43	-0.31	-0.33	0.24
Tyramine	-0.64*	-0.03	0.28	0.30	-0.29	0.627*	-0.08	0.04	-0.42	-0.7	0.18
Putrescine	-0.57	0.002	-0.01	0.07	-0.06	-0.45	-0.04	0.24	-0.40	-0.22	0.01
Cadaverine	-0.26	0.07	0.37	0.11	0.15	-0.51	-0.16	0.13	0.01	-0.20	0.21
after 8 months of storage											
AFL (total)	0.21	0.10	0.37	0.24	0.24	0.66*	0.46	0.40	0.21	-0.48	0.30
ZEA	0.16	-0.71**	-0.11	0.58	-0.29	-0.35	-0.03	-0.20	0.04	-0.12	-0.07
DON	0.67*	-0.24	0.12	0.18	-0.24	-0.07	0.04	0.20	0.08	0.06	0.04
T-2/HT-2	0.07	-0.82**	0.12	0.73**	0.37	-0.34	0.73**	-0.10	0.38	0.27	0.20
OTA	0.07	-0.02	-0.03	0.11	-0.12	0.36	0.09	0.14	-0.15	0.13	0.76**
Histamine	-0.38	-0.01	0.03	0.36	0.10	0.38	0.34	-0.08	-0.08	-0.11	0.68*
Tyramine	-0.30	-0.05	0.39	0.30	-0.41	0.13	0.13	0.68*	0.26	-0.14	-0.38
Putrescine	0.33	0.17	0.23	0.14	-0.27	0.41	0.26	0.75**	0.57	-0.03	-0.04
Cadaverine	0.38	0.32	0.28	0.06	-0.48	0.54	-0.01	0.82**	0.46	-0.20	-0.03

*P ≤ 0.05; **P ≤ 0.05.

Nishino et al. (2007) reported that ammonia was positively strong (r=0.845) related with the BA contents in silage, in the present study weak not significant correlation was found between NH3-N and the different BA contents. The concentrations of NH_3-N and butyric acids represent good indicators for biogenic amines (r=0.67 and r=0.80,P≤0.05) there have been high concentrations of biogenic amines in the low-DM silages (Richardt et al., 2011). Commonly, amine content increased with decreasing dry matter level (Steidlová et al., 2004). In the current study were found strong negative correlations (r=0.64, P≤*0.05;* r=*-0.57,* P≥0.05*)* between dry matter and tyramine, putrescine respectively in silage samples after 3 month of ensilage. Macana et al. (2006) maintain that the activity of bacterial proteases and, consequently, development of biogenic amines is correlated with the following: content of amino acids, synergistic action of

microorganisms as well as with ensiling conditions.

Conclusions

The present results indicate that mycotoxins were generally not degraded by the ensiling process and the presence of biogenic amines in maize silage. Despite the significant presence of mycotoxins (DON, AFL (total), OTA) in the maize silage the concentrations has not exceed the maximum allowed concentrations prescribed by EU. Combined effects of toxic compounds as mycotoxins and biogenic amines are considered to limit palatability and influenced health of ruminants.

References

1. Alvarez M. A., Moreno-Arribas M. V. The problem of biogenic amines in fermented foods and the use of potential biogenic amine-degrading microorganisms as a solution. *Trends in Food Science and Technology*. 2014. 39(2). P. 146-155.

2. AOAC. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Gaithersburg, Maryland, International. 2005.

3. Cheli F., Campagnoli A., Dell'Orto V. Fungal populations and mycotoxins in silages: From occurrence to analysis. *Animal Feed Science and Technology*. 2013. 183. P. 1–16.

4. Commission Recommendation 2006/576/EC of 17 August 2006 on the presence of DON, ZEA, ochratoxin A, T-2 and HT-2 toxins, and fumonisins in products intended for animal feeding.

5. Driehuis F., Spanjer M. C., Scholten J. M., Te Giffel M. C.. Occurrence of mycotoxins in maize, grass and wheat silage for dairy cattle in the Netherlands. *Food Additives & Contaminants: Part B.* 2008. 1(1). P. 41-50.

6. Duniere L., Sindou J., Chaucheyras-Durand F., Chevallier I., Thevenot-Sergentet D. Silage processing and strategies to prevent persistence of undesirable microorganisms. *Animal Feed and Sciences Technology*. 2013. 182. P. 1–15.

7. Eckard S., Wettstein F. E., Forrer H. R., Vogelgsang S. Incidence of Fusarium Species and Mycotoxins in Silage Maize. *Toxins*. 2011. 3. P. 949-967.

8. Fink-Gremmels J. Mycotoxins in forages. In: Diaz, D.E. (ed.) *The Mycotoxin Blue Book*. Nottingham University Press, Nottingham, UK. 2005. P. 249–268.

9. Gąsior R. Oznaczanie lotnych kwasów tłuszczowych i kwasu mlekowego w kiszonkach i treści żwacza. Instytut Zootechniki, Balice, Kraków 2002. (In Polish)

10. Gąsior R., Brzóska F. The type and level of biogenic amines in alfalfa and red clover silage. Zeszyty Naukowe Akademii Rolniczej w Krakowie. 1999. Vol. 347. P. 89–94. (in Polish)

11. Hernández-Orte P., Lapeña A. C., Peña-Gallego A., Astrain J., Ferrer B., Cacho J., Ferreira V.: Biogenic amine determination in wine fermented in oak barrels: Factors affecting formation. *Food Res. Internat*. 2008. 41. P. 697-706.

12. Joosten H.M., Olieman C. Determination of biogenic amines in cheese and some other food products by HPLC in combination with thermo-sensitized reaction. *J. Chromatogr*. 1986. 356. P. 311–319.

13. Kostulak-Zielińska M., Potkański A. Quality of baled grass-clover silage ensiling with chemical additives. *Chemical composition. Annals of Animal Science*. 2001. 1. P. 153–165.

14. Křížek M., Kalăc P., Peterk, J. Biogenic amines in silage. 3. The occurrence of six biogenic amines in farm-scale grass and maize silages. *Archives of Animal Nutrition*. 1993. 45. P. 131–137.

15. Krnjaja V., Lević J., Stanković S., Petrović T., Tomić Z., Mandić V., Bijelić Z. Moulds and mycotoxins in stored maize grains. *Biotechnology in Animal Husbandry*. 2013. 29(3). P. 527-536. 16. Macana J., Turk R., Vukušić J., Kipčić D., Milković-Kraus S. Long-term follow-up of histamine levels in a stored fish meal sample. *Anim. Feed Sci. Technol*. 2006. 127. P. 169-174.

17. Nishino N., Hattori H., Wada H., Touno E. Biogenic amine production in grass, maize and total mixed ration silages inoculated with Lactobacillus casei or Lactobacillus buchneri. *Journal of Applied Microbiology*. 2007. 103 P. 325–332.

18. Olt A., Kärt O., Kaldmäe H., Ots M., Songisepp E., SmidtI. I. The effect of additive and dry matter content on silage protein degradability and biogenic amine content. *Journal of Agricultural Science*. 2005. Available at: http://agrt.emu.ee/pdf/2005_2_olt_i.pdf

19. Pereyra M.L., Gonza´lez Alonso V.A., Sager R., Morlaco M.B., Magnoli C.E., Astoreca A.L., Rosa C.A.R., Chiacchiera S.M., Dalcero A.M., Cavaglieri L.R. Fungi and selected mycotoxins from pre- and postfermented corn silage. *Journal of Applied Microbiology*. 2008. 104. P. 1034–1041.

20. Purwin C., Łaniewska-Trokenheim Ł., Warmińska-Radyko I., Tywończuk J. Jakość kiszonek – aspekty mikrobiologiczne, zdrowotne i produkcyjne. *Medycyna Wet*. 2006. 62(8). P. 865–869.

21. Richardt W., Wein R., Steinhöfel O., Pries M. Untersuchungen zurVeränderung der Proteinqualität während der Silierung von Grassila-gen unter Berücksichtigung biogener Amine. VDLUFA-Schriftenreihe. 2011. 67. P. 541–549.

22. Steidlová Š., Kalăc P. The effects of lactic acid bacteria inoculants and formic acid on the formation of biogenic amines in grass silages. *Archives of Animal Nutrition*. 2004. 58(3). P. 245–254.

23. Skladanka J., Adam V., Dolezal P., Nedelnik J., Kizek R., Linduskova H., Mejia J. E. A. Nawrath A. How Do Grass Species, Season and Ensiling Influence Mycotoxin Content in Forage? *Int. J. Environ. Res. Public Health.* 2013. 10. P. 6084-6095.

24. Steidlová Š., Kalăc P. Levels of biogenic amines in maize silages. *Animal Feed Science and Technology.* 2002. 102. P. 197–205.

25. Storm I.M.L.D., Kristensen N.B., Raun B.M.L., Smedsgaard J., Thrane U. Dynamics in the microbiology of maize silage during whole-season storage. *Journal of Applied Microbiology.* 2010. P. 1017–1026.

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