Krystyna J. ZIELIŃSKA, Agata U. FABISZEWSKA*, Barbara WRÓBEL**
Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology
* Warsaw University of Life Sciences
** Institute of Technology and Life Sciences
e-mail: krystyna.zielinska@ibprs.pl; agata_fabiszewska@sggw.pl; b.wrobel@itep.edu.pl

AN ASSESSMENT OF THE MICROBIOLOGICAL AND CHEMICAL QUALITY OF GRASS SILAGES FROM SELECTED ORGANIC FARMS

Summary

In order to assess the microbial and chemical quality of grass silages made of sward, without the addition of any inoculants, six organic farms were chosen, in which grass silages were the principal feed for dairy cows. In all farms silages were made from the second crop of sward and formed into bales. Dry matter content was from 45 to 57 %. Samples were collected for two consecutive years. Silages from all farms were characterized by good or satisfactory quality due to the low lactic acid content (below 0.6 %), high butyric acid content (of up to 0.2 %), whereas they were contaminated with moulds and mycotoxins. In grass silages made of the sward from grasslands fertilized with slurry the presence of fecal bacteria of the genus Salmonella and Escherichia coli was detected. Based on these results and the results of previous studies the authors found that in order to obtain silages of high quality, safe for health of animals, it is necessary to use bacterial preparations for ensiling, which have special abilities to lower significantly the contamination with moulds, and therefore substantially lower the level of mycotoxins. These bacteria have also abilities to reduce or eliminate from feed undesired bacteria of the genera Clostridium, Listeria and pathogenic bacteria Salmonella sp. and E. coli.
***Key words**: grass silages, microbiological and chemical quality, aflatoxins, ochratoxin A*

OCENA MIKROBIOLOGICZNEJ I CHEMICZNEJ JAKOŚCI KISZONEK W WYBRANYCH GOSPODARSTWACH EKOLOGICZNYCH

Streszczenie

Do badań dotyczących oceny mikrobiologicznej i chemicznej jakości kiszonek wyprodukowanych z runi łąkowej bez stosowania preparatów bakteryjnych, wybrano sześć gospodarstw ekologicznych, w których kiszonki stanowiły podstawową paszę dla krów mlecznych. We wszystkich gospodarstwach przez dwa kolejne lata kiszonki sporządzano, z drugiego pokosu przewiędniętej runi łąkowej o zawartości suchej masy od 45 do 57 %, w formie balotów. Kiszonki we wszystkich gospodarstwach charakteryzowały się co najwyżej dobrą lub zadawalającą jakością z powodu: niskiej zawartości kwasu mlekowego (poniżej 0,6 %), wysokiej zawartości kwasu masłowego dochodzącej do 0,2 %, ponadto porażone były pleśniami i skażone mikotoksynami. W kiszonkach pochodzących z gospodarstw, w których użytki zielone nawożono gnojowicą wykryto obecność bakterii fekalnych z rodzaju Salmonella oraz Escherichia coli. Na podstawie uzyskanych wyników i wyników wcześniejszych badań autorów stwierdzono, że w celu uzyskania kiszonek o wysokiej jakości, bezpiecznych dla zdrowia zwierząt hodowlanych, konieczne jest stosowanie do ich sporządzania preparatów bakteryjnych, zawierających szczepy bakterii o szczególnych zdolnościach hamowania rozwoju pleśni, a zatem znaczącego obniżenia poziomu mikotoksyn oraz obniżenia lub całkowitego zlikwidowania niepożądanych w paszy bakterii z rodzajów: Clostridium, sp. *Listeria* sp. *oraz patogennych z rodzaju Salmonella* sp. *i Eschericia coli.*
***Słowa kluczowe**: kiszonki, jakość chemiczna i mikrobiologiczna, aflatoksyny, ochratoksyna A*

1. Introduction

The permanent grasslands in organic farming constitute about 40% of agricultural lands. Sward collected from those meadows has similar composition to natural vegetation and comprises grasses, leguminous plants, herbs and weeds. The species composition of sward depends on the class of the soil, fertilization and it has a significant impact on the nutritional value of silages made from the sward [1, 2].

Maintaining the natural fertility of the soil of lands in organic farming is achieved by application of natural fertilizers. Manure is the best natural fertiliser, recommended for fertilising grasslands, which has beneficial effects for both soil and flora and which stimulates the growth of leguminous plants. Despite their many benefits, natural fertilisers can create a habitat for various pathogens, parasites and microorganisms which are undesirable in the silage fermentation process [4, 5, 6].

An important factor in the assessment of microbiological quality of silages is related to the presence of potentially pathogenic bacteria e.g. *Salmonella* genus and *Enterobacteriaceae* family, especially the presence of bacteria of the species *Escherichia coli*, which could be the cause of disorders in digestive system and inflammation of the mammary gland of cows fed with contaminated feed. Moreover, the presence of pathogenic microorganisms in silages poses a risk of their transfer to the food products of animal origin and endangers human health [7, 8].

Silages prepared without the addition of inoculants, which stimulate the process of lactic acid fermentation and inhibit the growth of moulds, can also be contaminated by species producing aflatoxins, commonly found in the environment and on plants, such as *Aspergillus flavus* , *A. parasiticus* and *A. nomius.* It can be also contaminated with moulds of the genus *Penicillium*, which are characterized by the ability to synthesize ochratoxin A. These mycotoxins pose a threat to the health of animals and following to humans [9, 10].

The presence of mycotoxins in feed for ruminants has been an ongoing and unsolved problem. Silages represent a basis of daily ratio in feeding of dairy cattle in organic farms, therefore the chemical and microbiological quality has a significant impact on the quality of the acquired milk. In the case of silage contamination with pathogenic bacteria, moulds and mycotoxins, especially aflatoxin B_1, the value of feed is disqualified. Aflatoxin B_1, served with feed, is metabolized by animals and excreted into milk in the form of aflatoxin M_1 - toxic to humans [11, 12].

A comprehensive assessment of the quality of silages comprises: the nutritional value, the pH, the content of lactic acid, acetic acid and butyric acid, the $N\text{-}NH_3$ to total nitrogen content ratio, the presence and value of the number of undesirable microorganisms, including moulds, bacteria of *Clostridium* and *Listeria* genera, *Enterobacteriaceae* family, especially of potentially pathogenic microbes and the degree of contamination with aflatoxins and ochratoxin A [13].

Silages in small organic farms are produced by farmers often without the addition of inoculants, a comprehensive evaluation of their chemical and microbiological quality can show the current status and may indicate the need to improve the quality of feed used to feed dairy cattle.

2. Aim of the study

The aim of the study was to assess the chemical and microbiological quality of grass silages from meadow sward of organic farms prepared without bacterial preparations, which stimulate processes of ensiling.

3. Materials and methods

The survey was carried out over a period of two years at six organic farms ranging from 5 to 10 ha, located in the Masovia voivodeship in Poland. Three of the sample farms fertilized their grasslands with manure, one with cattle slurry and two of them did not fertilize the grasslands.

In majority of organic farms grass silages were made from second crop of sward characterized with dry mass ranged from 45 to 57%. They were formed in bales weighted c.a. 500 kg. Ballots were formed using a crushing machine Sipma and then were double- wrapped with silage foil. After three months of ensiling samples from 10 bales from each farm were collected to evaluate their chemical and microbiological quality. The silage samples were analyzed for basic nutrients, organic acids content, aflatoxins, including aflatoxin B_1 and ochratoxin A concentration; the number of moulds and number of undesired bacteria, including potential pathogens, were also measured.

The applied chemical analysis methods:

- determining dry matter content using the gravimetric method in line with the PN-ISO 6496:2002 standard,
- determining the total protein content, raw fibre, ash, water soluble hydrocarbons and dry organic matter digestibility using near infrared spectroscopy with a Büchi NIRFlex N–500 spectrometer using presets for dry grasses created by INGOT,
- ammonia nitrogen concentration was determined by Convay method (Skulimowski, 1974),
- determination of pH by potentiometry,
- determination of L- and D-lactic acid, acetic acid and butyric acid concentrations using enzymatic methods (Boehringer Mannheim, Germany) (measurement error 0,15-0,03 $g \cdot dm^3$),
- the amount of total aflatoxins, aflatoxin B_1 and ochratoxin A using an immunoenzymatic method – ELISA tests and STAT FAX plate reader (coefficient of variation of less than 8%).

The study used the following microbiological methods:

- determining the number of *Salmonella* sp. bacteria using Rambach, a specialised agar medium produced by Merck (New Jersey USA),
- determining the presence of *Escherichia coli* bacteria and other bacteria in the coli group using Petrifilm Select *E. coli* and Petrifilm coliform/*E. coli* media made by 3M Heath Care Company (USA, Loughborough),
- determining the number of *Clostridium perfringens* bacteria using the Agar Base 9188 medium (Neogen, Lansing, Michigan, USA),
- determining the number of *Listeria* sp. using Chromocult Agar medium according to Ottoviani and Agosti, (Merck) in line with ISO 11290 (2004),
- determining the number of moulds according to ISO standard in PN ISO 21527-2: 2009 part 2.

The test results were subjected to statistical analysis using Statistica 12.0 software (Statsoft, Poland). Statistical analyses of repeated measurements were performed with one-way ANOVA followed by Tukey’s or NIR multiple comparison. P-values of $p \leq 0.05$ were considered to be statistically significant.

4. Results and discussion

In samples of grass silages made in organic farms nutritional value was evaluated at first and the results are presented in Table 1.

The average of dry mass of silages expressed in $g\ kg^{-1}$ ranged from 445.9 to 569.0 and did not differ significantly among farms. Digestibility of organic matter ranged from 46.7 to 57.0 % depending on the quality of the sward and method of fertilization on the farm.

It has been demonstrated a statistical significance between the protein content of the grass silages from farms no. 6 and silages of farms no. 1, 2 and 4. The total protein content in dry mass of grass silages totaled from 127.6 to 161.2 $g\ kg^{-1}$ DM and was the highest in silages made on farms no. 1 and 2, where the grasslands were fertilized with manure. The lowest total protein content was on farm no. 6, where the grasslands were not fertilized over a period of the experiment.

According to Brzóska [15] the bare minimum total protein content in fodder that guarantees the normal functioning of the digestive tract of dairy cattle is at least 150-170 $g\ kg^{-1}$ DM of fodder, while the raw fiber level should not exceed 300 $g\ kg^{-1}$ DM.

In the grass silages sampled from farms no. 5 and 6 the total protein content was lower than 150 $g\ kg^{-1}$ DM, but crude fiber content in silages for all farms did not exceed 300 $g\ kg^{-1}$ DM.

The average crude fiber content in grass silages sampled from farms no. 5 and 6 was the highest and ranged from 268.8 to 294.0 $g\ kg^{-1}$ DM respectively, but the difference between two farms was not significant. The crude ash content totaled from 54.8 to 69.1 $g\ kg^{-1}$ DM. Water soluble

Table 1. The chemical composition and digestibility of dry organic matter of grass silages made of second crop sward. Means were separated into statistically different groups marked with letters according to Tukey's test

Tab. 1. Skład chemiczny i strawność suchej masy organicznej kiszonek z runi łąkowej II pokosu. Średnie zostały pogrupowane na podstawie testu porównań wielokrotnych Tukey'a i oznaczone kolejnymi literami alfabetu

Farm, no.	Year of study	Dry matter, g kg^{-1}	Digestibility of organic matter, %	Content of nutritive components in dry mater of grass silages, g kg^{-1} DM				N-NH$_3$/ N-organic ratio, %
				Total protein, g kg^{-1} DM	Crude fiber, g kg^{-1} DM	Crude ash, g kg^{-1} DM	Water soluble sugars content, g kg^{-1} DM	
1*	I	498.4	50.08	147.1	259.2	61.6	50.9	15.2
	II	569.0	50.69	159.0	249.5	64.1	55.7	13.8
	average:	**533.7^{a}**	**50.39^{a}**	**153.1^{b}**	**254.4^{a}**	**62.9^{bc}**	**53.3^{cd}**	**14.5**
2*	I	460.6	48.78	156.4	256.6	57.1	49.3	16.1
	II	445.9	49.56	161.2	248.8	54.8	50.9	17.0
	average:	**453.3^{b}**	**49.17^{a}**	**158.8^{b}**	**252.7^{a}**	**56.0^{a}**	**50.1^{bcd}**	**16.6**
3*	I	582.5	49.45	147.4	238.2	62.8	42.1	15.0
	II	529.8	47.28	148.2	274.6	64.2	44.3	15.9
	average:	**556.2^{a}**	**48.36^{a}**	**147.8^{ab}**	**256.4^{a}**	**63.5^{bcd}**	**43.2^{ab}**	**15.5**
4**	I	492.8	49.28	144.5	272.2	70,6	58.0	15.8
	II	528.6	50,24	155.2	259.9	68.1	56.6	14.1
	average:	**510.7^{a}**	**49.76^{a}**	**149.9^{b}**	**266.1^{a}**	**69.4^{d}**	**57.3^{d}**	**14.9**
5	I	568.2	49.67	136.6	265.3	56.4	49.2	15.3
	II	560.0	47.60	141.5	272.2	59.0	46.8	13.5
	average:	**564.1^{a}**	**48.64^{a}**	**139.1^{ab}**	**268.8^{a}**	**57.7^{ab}**	**48.0^{bc}**	**14.4**
6	I	492.8	46.70	127.6	300.0	69.1	37.9	15.6
	II	514.6	49.78	130.3	288.2	68.2	39.0	17.1
	average:	**503.7^{a}**	**48.24^{a}**	**129.0^{a}**	**294.1^{a}**	**68.7^{cd}**	**38.5^{a}**	**16.4**

Source: Own work/ *Źródło:* Badania własne

** silages made of permanent grasslands fertilized with manure,*
*** silages made of permanent grasslands fertilized with slurry*

Table 2. Assesment of chemical quality of grass silages in organic farms

Tab. 2. Ocena jakości chemicznej kiszonek w gospodarstwach ekologicznych

No. farm	Year of study	Dry mass, g· kg^{-1}	pH	Organic acids content %			Quality according to Flieg-Zimmer scale	Quality according to N-NH$_3$/N-organic ratio
				lactic	acetic	butyric		
1	I	498.4	5.4	0.31	0.15	0.11	satisfactory	medium
	II	569.0	4.8	0.52	0.17	0.11	good	good
2	I	460.6	5.3	0.34	0.10	0.09	satisfactory	medium
	II	445.9	5.0	0.46	0.17	0.12	satisfactory	medium
3	I	582.5	5.0	0.49	0.15	0.10	good	good
	II	529.8	5.1	0.44	0.18	0.14	satisfactory	medium
4	I	492.8	5.0	0.46	0.17	0.10	satisfactory	medium
	II	528.6	5.1	0.49	0.12	0.09	good	medium
5	I	568.2	5.2	0.47	0.20	0.16	satisfactory	medium
	II	560.0	4.9	0.52	0.16	0.12	good	good
6	I	492.8	5.0	0.48	0.18	0.14	satisfactory	medium
	II	514.6	5.1	0.44	0.17	0.20	satisfactory	medium

Source: Own work/ *Źródło:* Badania własne

sugars content ranged from 37.9 to 58.0 and was higher in grass silages made of sward from grasslands fertilized with manure or slurry. The ammoniacal nitrogen to total nitrogen ratio is an indicator of the degree of decomposition of protein in feed and reflects the quality of silages; it ranged from 13.5% to 17.1% in the study.

According to Purwin [13] for silages characterized by the N-NH$_3$ to N-total ratio of not more than 15% its quality is assessed as good. If the value is within the range 15.1 - 17.5% the quality is characterized as average, above the level of 17.5 % silage quality is recognized as poor.

Table 2 shows the results of the chemical quality of silages produced in organic farms. Due to the pH, dry matter content and organic acids concentrations: lactic acid, acetic acid and butyric acid, and their share in the total acids content the quality of silages was rated according to the point assessment scale of Flieg and Zimmer. Based on the N-NH$_3$/ N-organic ratio (expressed in %) processes of protein decomposition were estimated, whose intensity correlates with the quality of fodder.

The examined samples of grass silages were characterized by pH, which valued from 4.9 to 5.4 and the dry matter content expressed in g · kg^{-1} of 445.9 to 569.0. The lactic acid content ranged from 0.31 to 0.52 %, acetic acid content ranged from 0.10 to 0.20 %, followed by high content of butyric acid from 0.10 to 0.20 %. Hence the assessment of chemical quality according to Flieg-Zimmer scale showed that majority of silages produced during two years of the study in the six organic farms, was of satisfactory quality. Only in four cases the quality could be evaluated as good. The grass silage quality assessed on the basis of ammonia nitrogen to the total nitrogen ratio was good or average. The quality of silages, according to various parameters accepted in both methods, was compatible.

Table 3. The number of undesirable and potentially pathogenic bacteria in dry matter of grass silages. Means were separated into statistically different groups marked with letters according to Tukey's test
Tab. 3. Stopień skażenia kiszonek niepożądanymi bakteriami, w tym potencjalnie patogennymi. Średnie zostały pogrupowane na podstawie testu porównań wielokrotnych Tukey'a i oznaczone kolejnymi literami alfabetu

No. farm	Year of study	Number of microorganisms in meadow sward, log CFU g^{-1} DM				
		Salmonella sp.	*Escherichia coli*	Coliform bacteria	*Clostridium perfringens*	*Listeria* sp.
1*	I	n.d.	n.d.	5.60	0.60	1.48
	II	n.d.	n.d.	5.00	0.90	1.60
	average:	**n.d. ^{a}**	**n.d. ^{a}**	**5.30 ^{a}**	**0.75 ^{a}**	**1.54 ^{a}**
2*	I	n.d.	n.d.	4.30	0.90	1.28
	II	n.d.	n.d.	4.60	1.00	1.38
	average:	**n.d. ^{a}**	**n.d. ^{a}**	**4.45 ^{b}**	**0.95 ^{a}**	**1.33 ^{a}**
3*	I	n.d.	1.00	5.80	1.48	1.60
	II	n.d.	0.90	5.70	1.00	1.48
	average:	**n.d. ^{a}**	**0.95 ^{ab}**	**5.75 ^{a}**	**1.24 ^{a}**	**1.54 ^{a}**
4**	I	1.48	1.95	4.60	0.50	3.88
	II	1.30	1.54	4.80	0.50	3.60
	average:	**1.39 ^{b}**	**1.75 ^{b}**	**4.70 ^{b}**	**0.50 ^{a}**	**3.74 ^{b}**
5	I	n.d.	0.90	4.00	0.90	2.48
	II	n.d.	1.60	4,70	1.00	1.60
	average:	**n.d. ^{a}**	**1.25 ^{ab}**	**4.35 ^{b}**	**0.95 ^{a}**	**2.04 ^{ab}**
6	I	0,50	1.00	4.30	0.25	3.00
	II	n.d.	n.d.	4.60	0.75	0.82
	average:	**0.25 ^{a}**	**0.50 ^{ab}**	**4.45 ^{b}**	**0.50 ^{a}**	**1.91 ^{ab}**

Source: Own work/ *Źródło:* Badania własne

** silages made of permanent grasslands fertilized with manure,*
*** silages made of permanent grasslands fertilized with slurry,*
n.d. – not detectable, a, b etc.- statistically different groups

Table 4. The content of CFU moulds in grass silages and its contamination with mycotoxins. Means were separated into statistically different groups marked with letters according to NIR test.
Tab. 4. Zawartość w kiszonkach j.t.k. pleśni i poziom ich skażenia mykotoksynami. Średnie zostały pogrupowane na podstawie testu porównań wielokrotnych NIR i oznaczone kolejnymi literami alfabetu.

No. farm	Year of study	Moulds, log CFU g^{-1} DM	Content of mycotoxins in silages, ppb***)		
			aflatoxin B_1	aflatoxins B_1, B_2, G_1, G_2	ochratoxin A
1*	I	5.66	4.88	12.40	4.90
	II	5.80	4.00	12.78	5.00
	average:	**5.73^{ab}**	**4.44^{ab}**	**12.59^{b}**	**4.95^{b}**
2*	I	5.98	5.76	13.90	5.10
	II	5.30	4.60	10.82	4.38
	average:	**5.64^{ab}**	**5.18^{b}**	**12.36^{ab}**	**4.74^{b}**
3*	I	5.30	4.82	10.92	5.10
	II	5.80	4.90	11.58	4.15
	average:	**5.55^{ab}**	**4.86^{ab}**	**11.29^{ab}**	**4.63^{b}**
4**	I	6.00	4.88	12.83	3.80
	II	5.30	4.00	10.70	4.20
	average:	**5.65^{ab}**	**4.44^{ab}**	**11.77^{ab}**	**4.00^{ab}**
5	I	6.30	5.38	12.26	4.19
	II	5.60	4.50	10.95	3.95
	average:	**5.95^{b}**	**4.94^{ab}**	**11.61^{ab}**	**4.07^{ab}**
6	I	4.60	3.60	9.78	3.29
	II	4.90	4.12	9.84	3.89
	average:	**4.75^{a}**	**3.86^{a}**	**9.81^{a}**	**3.59^{a}**

Source: Own work/ *Źródło:* Badania własne

** silages made of permanent grasslands fertilized with manure,*
*** silages made of permanent grasslands fertilized with slurry,*
**** ppb (μg·kg^{-1}DM), a, b etc. - statistically different groups*

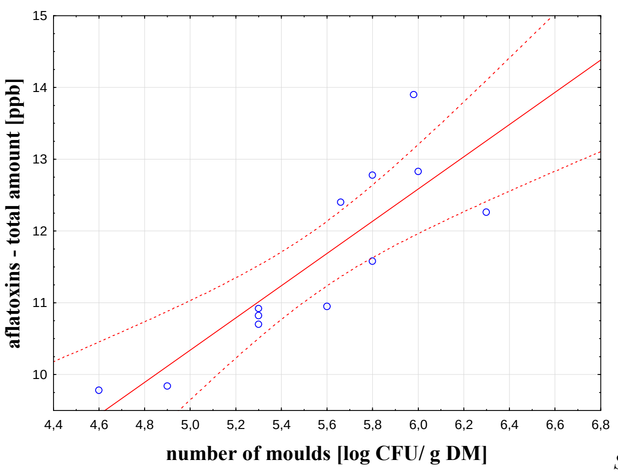
Table 3 presents of results for microbiological quality evaluation for examined silages. There was detected the presence of bacteria of the genus *Salmonella* in samples taken from farm no. 4, wherein the fertilizer used on grassland was slurry and from farm no. 6, where no fertilization was used. The presence of bacteria of the species *E. coli* was not detected in grass silages from farms no. 1 and 2, where grasslands were fertilized with manure. The number of *E. coli* was the highest for farm no. 4, where the grasslands were fertilized with slurry. The degree of grassland soil contamination by fecal bacteria that could be applied to the soil and plants from not fully fermented liquid manure, may translate into sward contamination, and therefore also contaminate silages made of it [6, 16].

Similar studies performed Davies and co-workers, who examined the presence of microorganisms of *Enterobacteriaceae* family and the genus *Clostridium*, which could have been introduced into the soil with organic fertilizers, and then were detected in plants and silages made of contaminated material [17, 18].

The samples of feed of all farms were found to be infested with coliform bacteria (4.00- 5.80 CFU g^{-1} DM), *Clostridium perfringens* (0.25-1.48 CFU g^{-1} DM) and *Listeria* sp. (0.82-3.88 CFU g^{-1} DM).

Contamination of silages with *Cl. perfringens* testifies to the butyric acid fermentation, protein disproportion and synthesis of ammonia. Silage of a low quality often contains butyric acid bacteria like *Clostridium* sp., as well as bacteria of the species *Listeria monocytogenes*. Infested feed, when fed by dairy cows, can cause microbial contamination of milk and its technological defects [17].

The results of research into ways of reducing microbial contamination of feed point that there is a possibility to inhibit the growth of bacteria and pathogenic fungi by biotechnological methods using lactic acid bacteria [19]. For this reason, it is important to use starter cultures of LAB species with antibacterial abilities for the improvement of ensiled feed [19, 20].



Source: Own work/ *Źródło:* Badania własne

Fig. 1. Number of moulds in dry matter of grass silages depending on the content of total aflatoxins (R = 0.8547)
Rys. 1. Korelacja liniowa pomiędzy liczbą pleśni a sumaryczną zawartością aflatoksyn (R = 0.8547)

Table 4 presents the results of analyzes on the contamination of silages with moulds and mycotoxins, including aflatoxin B_1, the sum of aflatoxins and ochratoxin A. The degree of contamination with moulds expressed in their number per gram of silage dry matter was high in grass silages taken from farms no. 1, 2, 3, 4 and 5, and ranged from 5.30 to 6.30 log CFU g^{-1} DM. Samples from farm no. 6 found to be less contaminated and during two years the number of fungi in those grass silages was 4.75 log CFU g^{-1} DM on average.

Values of the measured aflatoxin B_1 levels in surveyed grass silages ranged from 3.60 to 5.76 ppb. Total aflatoxin content was from 9.84 to 13.90 ppb and the concentration of ochratoxin A totaled from 3.29 to 5.10 ppb. The highest levels of mycotoxins were determined in grass silages originated from farm no. 2 produced in first year of the study and the lowest levels were found in silage samples from farm no. 6 also produced in the same year. The survey also showed a linear correlation between the number of moulds and the contamination with aflatoxins (Fig. 1). There should be pointed that the measured value of mycotoxin content was not so high, but being fed to dairy cows constantly, toxins can accumulate and as a consequence they can be detected in milk [11].

5. Conclusions

1. Grass silages made of the second crop of sward, without the addition of any inoculants, were characterized by the total protein content ranging from 127.6 to 161.2 g kg^{-1} DM, while the raw fiber level did not exceed 300 g kg^{-1} DM depending on the method and usage of grasslands fertilization.
2. The assessment of chemical quality of grass silages according to Flieg-Zimmer scale, based on lactic acid, acetic acid and butyric acid content, showed that in four cases the quality could be evaluated as good and for eight of averaged samples was only satisfactory. Respectively, the silages quality assessed on the basis of ammonia nitrogen to the total nitrogen ratio was good or average.
3. There was detected the presence of fecal bacteria of the genus *Salmonella* and *E. coli* species in samples taken from farm, wherein slurry was used as fertilizer. All surveyed grass silages were contaminated with coliform bacteria (4.00- 5.80 log CFU g^{-1} DM), *Clostridium perfringens* (0.25-1.48 log CFU g^{-1} DM) and *Listeria* sp. (0.82-3.88 log CFU g^{-1} DM).
4. High contamination with moulds occurred in all grass silage in examined farms and a correlation was found between the number of moulds and the level of contamination with the aflatoxins.
5. Number of moulds of dry matter of grass silages depended on the content of total aflatoxins, value R= 0.8547.

4. References

[1] Jankowska Huflejt H., Niczyporuk A. (2001): *Plonowanie, skład botaniczny i chemiczny runi oraz bilans potasu na łące nawożonej obornikiem i nawozami mineralnymi.* Zeszyty Problemowe Postępów Nauk Rolniczych, , Vol. 480, 233-243.

[2] Jankowska Huflejt H., Wróbel B. (2008): *Ocena przydatności pasz z użytków zielonych do produkcji zwierzęcej w badanych gospodarstwach ekologicznych.* J. Res. Appl. Agric. Eng., Vol 53(3), 103-108.

[3] Moraczewski R., Jankowska Huflejt H. (2007): *Niektóre problemy w wykorzystaniu trwałych użytków zielonych (TUZ) w produkcji zwierzęcej w gospodarstwach ekologicznych.* Wiad. Melior., Vol.2, 88-89.

[4] Zaleski K. J.,Josephson K. L., Gerba C. P., Pepper I. L.

(2005): *Survival, growth and regrowth of enteric indicator and pathogenic bacteria in biosolids, compost, soil and land applied biosolids*. J. Res. Sci. Technol., Vol. 2, 49-63.
[5] Holley R. A., Arrus K. M., Omiński K. H., Tenuta M. Blank G. (2006): *Salmonella survival in manure-treated soils during simulated seasonal temperature exposure*. J. Environ. Qual., Vol. 35, 1170-1180.
[6] Zielińska K. J., Stecka K., M., Kupryś M. P., Kapturowska A U., Miecznikowski A. H. (2011): *Ocena stopnia skażenia bakteriami potencjalnie patogennymi runi łąkowej i gleb nawożonych płynnymi nawozami organicznymi*, J. Res. Appl. Agric. Eng., Vol. 56(4), 212-215.
[7] Kwiatek K. (red.) (2007): *Bezpieczeństwo pasz dla bezpieczeństwa żywności.* Monografia. Państwowy Instytut Weterynaryjny – Państwowy Instytut Badawczy w Puławach, Puławy.
[8] Rozporządzenie (WE) nr 2160/2003 Parlamentu Europejskiego i Rady z dnia 17 listopada 2003 r. w sprawie zwalczania salmonelli i innych określonych odzwierzęcych czynników chorobotwórczych przenoszonych przez żywność.
[9] Oliveira C.A.F., Bovo F., Corassin C. H., Jager A. V., Reddy K. R.(2013): *Recent trends in microbiological decontamination of aflatoxins in food stuffs. In: Aflatoxins- recent advances and future prospects*, Razzaghi-Abyaneh M. (Ed.).
[10] Zielińska K., Stecka K. M., Suterska A., Miecznikowski A. (2007): *Wpływ ekologicznej technologii kiszenia runi łąkowej na hamowanie rozwoju pleśni wytwarzających mikotoksyny*., Problemy Inżynierii Rolniczej, Wydawnictwo IBMER, Warszawa, Vol. 1(55), 61-70.
[11] Prandini A., Tansini G., Sigolo S., Filippi L., Laporta M., Piva G. (2009): *On the occurrence of aflatoxin M_1 in milk and dairy products*, Food. Chem. Toxicol., Vol. 47, 984-991.
[12] Kapturowska A. U., Zielińska K. J., Stecka K. M. (2012): *Ocena jakości mleka surowego w powiązaniu z jakością kiszonych pasz objętościowych w wybranych gospodarstwach ekologicznych.* J. Res. Appl. Agric. Eng., Vol. 57(3), 194-197.
[13] Purwin C., Łaniewska-Trocenheim Ł, Warmińska-Radyko I., Tywończuk J. (1997): *Jakość kiszonek- aspekty mikrobiologiczne, zdrowotne i produkcyjne*. Med. Wet., 2006, 62, 865-869. Zielonki i ich konserwowanie. In: Pasze, Praca zbiorowa. Pub. Rozwój SGGW.
[14] Skulimowski J. (1974), Metody określania składu pasz i ich jakości. PWRL, Warszawa.
[15] Brzóska F. (2007): *Jakość pasz objętościowych i ich wykorzystanie w żywieniu zwierząt*. W: *Produkcja pasz objętościowych dla przeżuwaczy*, Conference 8-9 May 2007, Puławy: IUNG, PTA O/Puławy, 63-70.
[16] You Y., Rankin S. C., Aceto H. W., Benson C. E., Toth J. D., Dou Z. (2006): *Survival of Salmonella enterica serovar Newport in manure and manure-amended soils*. Appl. Environ. Microbiol., Vol. 72, 5777-5783.
[17] Meyer-Broseta S., Diot A., Bastian S., Rivicre J., Cerf O. (2003): *Estimation of low bacterial concentration: Listeria monocytogenes in raw milk*. Int. J. Food Microbiol, 1-15.
[18] Davies D.R., Merry R.J., Bakewell E.L. (1996): *The effect of timing of slurry application on the microflora of grass, and changes occurring during silage fermentation*; Grass Forage Sci., Vol. 51, 42-51.
[19] Zielińska K., Fabiszewska A., Stefańska I. (2015): *Different aspects of Lactobacillus inoculants on the improvement of quality and safety of alfalfa silage*, Chilean J. Agric. Res. Vol 75(3), 298-306.
[20] Zielińska K., Grzybowski R., Stecka K. M., Suterska A., Miecznikowski A. (2008): *Wpływ stosowania preparatu bakteryjno-mineralno-witaminowego w procesie kiszenia runi łąkowej na jakość mleka w gospodarstwach ekologicznych.* J. Res. Appl. Agric. Eng. 53(4), 153-158.