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Effect of ensiling pumpkin *Cucurbita maxima* with the addition of inoculant or without it on chemical composition and quality of silages

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Abstract: *Effect of ensiling pumpkin Cucurbita maxima with the addition of inoculant or without it on chemical composition and quality of silages*. The aim of the studies was to determine the effect of ensiling the pumpkin on chemical composition and quality of silages. The silages were produced from pumpkin of Justynka variety. Before ensiling, the disintegrated pumpkin fruits were mixed with the dried beet pulp in ratio equals 80 : 20. Two variants of silages were prepared: with the inoculant and without it. In the silages, the following basic chemical composition was determined: the content of dry matter, crude ash, crude protein, crude fat, crude fibre, NDF, ADF and ADL. The indicators, being an evidence of the run of ensiling process and the quality of the obtained silages, were also determined, i.e. pH, lactic, acetic and butyric acids, ammonia nitrogen, ethanol and aerobic stability. In the obtained silages, as compared to the material before ensiling, the lower content of crude fibre and ADF was found whereas in the silage with inoculant, NDF level was also lowered. The silages with the inoculant were characterized by higher content of lactic and acetic acids and lower level of nitrogen, ammonia and ethanol. The silages with the inoculant had also higher aerobic stability. The conducted studies indicate that the application of inoculant has affected the improvement of the quality of the obtained silages.

*Key words: s*ilage, pumpkin, dried beet pulp, inoculants, chemical composition

INTRODUCTION

Pumpkin *Cucurbita maxima* and especially its new varieties with a higher content of dry matter and carotenoids may become a valuable feedstuff for farm animals. The fruits of pumpkin, containing many carbohydrates, are easily digested by animals and they are rich in mineral, carotenoids and vitamins (Danielenko 2000; Korzeniewska et al. 2004; Niewczas et al. 2005). The pumpkin may be utilized in nutrition of animals in a form of fresh fruits, dry solids and silage. The seasonal availability of fresh fruits causes that their ensiling may become a good solution for their preservation. Owing to the increased content of dry matter, fruits of new varieties of pumpkin reveal better suitability for ensiling and the obtained feed may possess a high nutritional value. It may become especially valuable feedstuff for the dairy cows, having a positive influence on palatability of the ration and improvement of dietetic values of the obtained milk (Kuczyńska 2011). The discussed feed may constitute an interesting proposal for organic farms. The increase of dry matter content from few to several percent (Korzeniewska et al. 2004) increases the suitability of the pumpkin for ensiling but it does not give the possibility of ensiling it alone. If we want to ensure the correct run of fermentation and obtain the feed with a high nutritional value, we must ensile the pumpkin together with the dry substance, e.g. with straw (Hashemi and Razzaghzaden 2007). The ensiling of the pumpkin with dry sugar beet root pulp seems to be an interesting solution. The high level of easily-fermenting carbohydrates in the pumpkin and the beet pulp, as mixed altogether, should ensure the correct fermentation run. The improvement of the ensiling process and the silage quality may be also obtained *via* application of the additives which facilitate the ensiling, e.g. inoculants (Raczkowska-Werwińska et al. 2008; Kilic and Saricicek, 2010; Alves et al. 2011; Selwet 2011).

The aim of the studies was to evaluate the nutritional value and quality of the silage, obtained from the pumpkin, ensiled with the dried beetroot pulp in variants with inoculant and without it.

MATERIAL AND METHODS

The study used the Justynka variety of pumpkin (*Cucurbita maxima* D.), which has an increased content of dry matter (14–20%) and carotenoids (8–12 mg per 100 g fresh matter). Pumpkin was grown in an experimental field at the Department of Plant Genetics, Breeding and Biotechnology, Warsaw University of Life Sciences – SGGW. Fruits were harvested from the field in late September and used to prepare silages at the Department of Animal Nutrition and Biotechnology, Warsaw University of Life Sciences – SGGW in October.

Silages were prepared in mini-silos, made of 20-L plastic bags. To increase the dry matter content of the ensiled material, ground pumpkin fruits were mixed with dried sugar beet pulp at 80 : 20. The material was compressed in an automated press and packed into mini-silos. The ensilage treatments were: with a bacterial-enzyme inoculant, and no inoculant. The bacterial-enzyme inoculant contained *Lactobacillus plantarum* bacteria, endo-1,4-beta-glucanase, xylanase, and glucoamylase. The preparation was applied at 0.2% of the ensiled material. Ten mini-silos were prepared for each ensiling treatment.

Mini-silos were stored in the laboratory for 10 weeks in shaded conditions (19–23°C). After 10 weeks mini-silos were opened and their contents were sampled for analysis.

Samples for analyses were collected from fresh pre-ensiled material and from silages. Fresh material samples were collected from 10 mini-silos (5 from inoculant treatment and 5 from control treatment) before sealing them up. After collection, the samples were frozen at –30°C until chemical analyses. Silage samples were collected from all the mini-silos.

The chemical composition of the fresh material and silage was determined according to AOAC (2005): DM by drying at 104°C for 24 h, ash by incineration at 550°C for 6 h, crude protein (N × 6.25) by using the micro-Kjeldahl technique (Kjeltec System 1026 Distilling Unit, Foss Tecator, Sweden) and crude fat after extraction with petroleum ether by the Soxhlet method. NDF was determined according to Van Soest et al. (1991) and expressed as the ash free residue after extraction with boiling neutral solutions of sodium lauryl sulfate and EDTA in a Tecator apparatus. The silage pH was determined using a CP-315 ELMETRON pH-meter equipped with a replaceable electrode. The concentrations of L-form lactic acid, L-form acetic acid and D-3-hydroxybutyric acid were determined spectrophotometrically using a commercial enzymatic test combination (Boehringer Mannheim/R-Biopharm, Darmstadt, Germany).

The content of ammonia nitrogen ($N-NH_3$) in the silage was determined by Conway method. Ethanol was determined by oscillometric method (PB-ZF/GS--11). The aerobic stability of the silages was determined by measurement of temperature, using thermometer (automatic, electronic, multi-channel thermometer LB-711), performing the measurements of temperature each hour during the successive 7 days. The measurements were conducted under the standardized conditions – in air-conditioning room, at constant temperature of 21°C.

The effects of ensiling and silage additive on chemical composition of the silages were evaluated by using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Statistical analyses were carried out, using Statgraphics 6.0 Plus.

RESULTS

In Table 1, the basic chemical composition of the studied silages has been given.

When we compared the basic composition of the silages and the material before the ensiling, we found a significant decrease of crude fibre content in the silages (for the silage without the inoculant $p \geq 0.05$; for the silage with the inoculant

TABLE 1. Effect of ensiling on chemical composition of the silages

Component	Fresh material	Silages		p-value	SE
		without inoculant	with inoculant		
Dry matter ($g \cdot kg^{-1}$)	289.56	303.31	298.01	0.083	4.140
Crude ash ($g \cdot kg^{-1}$ DM)	71.13	70.50	68.59	0.194	0.998
Crude protein ($g \cdot kg^{-1}$ DM)	114.69	109.72	108.97	0.131	1.873
Crude fat ($g \cdot kg^{-1}$ DM)	46.58	41.43	42.33	0.109	1.754
Crude fibre ($g \cdot kg^{-1}$ DM)	189.24^{Bb}	168.80^{a}	162.01^{A}	0.003	3.994
WSC $g \cdot kg^{-1}$ DM)	57.83^{B}	60.95^{A}	61.71^{A}	0.005	0.616
NDF ($g \cdot kg^{-1}$ DM)	340.85^{B}	314.39^{A}	307.32^{A}	0.001	6.502
ADF ($g \cdot kg^{-1}$ DM)	257.74^{B}	213.34^{A}	215.42^{A}	0.001	8.426
ADL ($g \cdot kg^{-1}$ DM)	51.35	45.51	45.32	0.147	2.366
UFL (kg^{-1} DM)	1.07	1.05	1.08	0.321	0.034
PDIN ($g\ kg^{-1}$ DM)	83.72	81.87	82.26	0.725	2.925
PDIE ($g\ kg^{-1}$ DM)	123.87	127.12	128.32	0.436	2.391

AB – differences between the selected rows ($P \leq 0.01$); ab – differences between the selected rows ($P \leq 0.05$); WSC – water-soluble carbohydrates; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid neutral lignin; UFL – unit of energy for milk production; PDIN – protein digested in the small intestine depending on rumen degraded protein; PDIE – protein digested in the small intestine depending on rumen-fermented organic matter.

$p \geq 0.01$), NDF ($p \geq 0.01$) and ADF ($p \geq 0.01$). The content of WSC in silages was significantly higher compare to fresh material ($p \geq 0.01$). On the other hand, the comparison of the basic composition of two studied variants of the silages did not reveal any statistically significant differences (Table 1). Any significant differences between the variants of ensiling in respect of pH of the silages were not found (Table 2). The silages produced with the addition of inoculant were characterized by significantly higher acetic acid ($p \geq 0.01$) and of lactic acid ($p \geq 0.05$) content (Fig. 1). The content of butyric acid in both variants of ensiling was recorded on the similar, very low level.

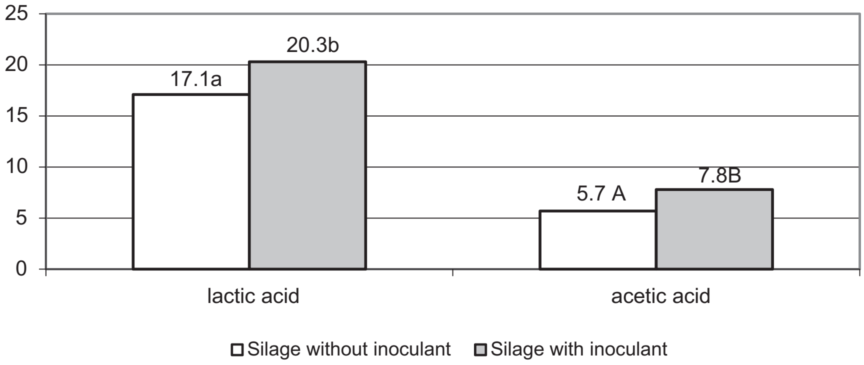
Statistically significant differences between the silages were found in the content of ammonia nitrogen ($N\text{-}NH_3$), the higher concentration of which occurred in the silage without the participation of the additive, facilitating the ensiling process ($p \geq 0.01$). The discussed silage was also characterized by the higher content of ethanol ($p \geq 0.01$), resulting from the alcohol fermentation (Table 2).

Aerobic stability of the silage is the important parameter, indicating the susceptibility of the silage to the secondary fermentation. There was found a positive effect of the employed inoculant on aerobic stability of the examined silages, i.e. significantly higher stability of the silages produced with the addition of inoculant as compared to the silages without the mentioned additive ($p \geq 0.01$).

TABLE 2. The effect of the ensiling method on the parameters, determining quality of the silages

Quality parameters of silage	Silage without inoculant	Silage with inoculant	SE	p-value
pH	4.5	4.4	0.123	0.548
Ammonia nitrogen ($g \cdot kg^{-1}$ DM)	11.06^{A}	8.23^{B}	0.421	<0.001
Ethanol ($g \cdot kg^{-1}$ DM)	9.72^{A}	7.13^{B}	0.512	0.003
Aerobic stability (h)	21.63^{A}	44.25^{B}	5.201	0.002

AB – differences between the selected rows ($P \leq 0.01$); ab – differences between the selected rows ($P \leq 0.05$).



AB – different letters indicate significant differences ($P \leq 0.01$);
ab – different letters indicate significant differences ($P \leq 0.05$).

FIGURE 1. The mean content of volatile fatty acids in the silages and the statistical analysis ($g \cdot kg^{-1}$ DM)

DISCUSSION

Freshly disintegrated fruits of pumpkin are characterized by a low content of dry matter; they are the material which is difficult to be ensiled. Due to this fact, it is recommended to enrich the ensiled biomass with the additional components, increasing the content of dry matter in the ensiled material. In the conducted experiment, dried beet pulp was the additional source of dry matter and organic substance, containing simple sugars. The application of the dried beet root pulp increased the dry matter content in the ensiled material and additionally, it increased the level of easily fermenting carbohydrates. The mentioned additives were the factors favourable for ensiling process and the obtained silage was characterized by high dry matter content and a high nutritional value. The addition of the beet root pulp could constitute a specific stimulator of fermentation in the initial stage of lactic fermentation (Bodarski et al. 2004). Hashemi and Razzaghzadeh (2007) ensiled pumpkin with the wheat straw (in the quantity of 28.6%) and also, added molasses to the ensiled material with the aim to improve the conditions of lactic fermentation.

The ensiling process, irrespectively of the examined variant, did not have any effect on the dry matter content in the silages as compared to the fresh material. Kilic and Saricicek (2010) when ensiling beet root pulp with different additives, including the bacterial ones, did not find any effect of ensiling on the content of dry matter in the silages. In the studies of Kilic and Saricicek (2010), the effect of the application of the inoculant on the crude protein content in the silages was also not found. In our studies, the content of crude protein in the silages and also, of the crude fat was similar as the content of the mentioned components in the fresh material. Any effect of the inoculant on the content of the discussed components in the silage was also not recorded. The application of the inoculant did not affect the content of crude protein in the grass silages in the studies of Jalč et al. (2009). On the other hand, the cited authors stated the decline of fiber content when the bacterial preparations were employed. In the studies of Kilic and Saricicek (2010) on the ensiling of beet root pulp, any effect of inoculant on fiber content in the obtained silages was not found. In our studies, the decrease of the crude fiber content and ADF in the silages vs. the fresh material was recorded and in the silages with inoculant, also NDF decline was stated. On the other hand, any differences in the fiber content (crude fiber, NDF, ADF and ADL) between the examined silages were not stated. In the pumpkin as well as in the beet pulp, there is a high content of sugars and pectins, which are greatly decomposed by fermentation bacteria. Perhaps in the case of the mentioned raw materials, the intensive fermentation, even without the participation of bacteria from inoculant, affects the decomposition of a considerable part of structural carbohydrates, being present in the discussed raw materials. Hence, there is a lack of distinct effect of the inoculant application whereas the effect of ensiling process is visible.

Any significant differences in respect of pH value between the examined variants of silages were not found. On the basis of the above-mentioned fact, we may state that the process of ensiling in the both cases was equally efficient and it was favourable for lowering of pH of the ensiled biomass. In the both silages, the pH value was found within the limits of 4.0–4.5. Such value of pH inhibits development of putrefying bacteria and fungi and activity of proteolytic vegetal enzymes, so it constitutes a preserving factor (Bodarski et al. 2004).

The bacterial-enzymatic preparation, as being employed in the experiment contained as follows: *Lactobacillus plantarum* bacteria, endo-1,4-beta-glucanase, xylanase and glucoamylase. *Lactobacillus plantarum* bacteria belong to the group of lactic acid bacteria and their addition should support fermentation towards lactic acid. The enzymes which are present in the preparation, i.e. endo-1,4--beta-glucanase, xylanase and glucoamylase, increase the decomposition of crude fiber in the ensiled material what increases the production of simple sugars, being a substrate for growth of lactic acid bacteria (Kowalik and Michalski 2006).

The content of ammonia nitrogen ($N-NH_3$) in the silage is an indicator, i.a. of putrefaction processes which run in the silage usually due to the activity of butyric acid bacteria. Therefore, we may usually observe a positive correlation between the butyric acid and ammonia nitrogen content in the silage (Kowalik and Michalski 2006). In the examined silages, significantly lower $N-NH_3$ content was recorded in the silage with inoculant. The discussed silages were characterized by a higher content of lactic and acetic acid. The higher level of the mentioned acids could limit the development of proteolytic bacteria, decomposing protein to ammonia.

The examined silages differed significantly in respect of aerobic stability – the variants of the silages without inoculant were characterized by its lower value. Certain studies indicate that the silages with a higher content of lactic acid are more sensitive to the secondary fermentation as compared to the silages with the higher level of acetic acid (Szyszkowska et al. 2010). We may, therefore, assume that the silage produced from pumpkin with the higher content of lactic acid will be characterized by lower aerobic stability. Any such relationship was not, however, stated and the silage produced with the inoculant with higher lactic acid content had also higher aerobic stability. In the case of the examined silages in variant with the inoculant, apart from the higher level of lactic acid, there was also stated higher content of acetic acid which limits the development of secondary fermentation-inducing bacteria. It was stressed in the studies of Pyś et al. (2008), who analyzed the effect of *Lactobacillus buchneri* bacteria on the process of ensiling the maize.

We should pay the attention to the fact that the water-soluble simple sugars as contained in the ensiled material are the main substrate for development of bacteria, yeasts and moulds. Aerobic stability of the silage is decreasing proportionally to the increase of their content in the silage after completion of fermentation (Szyszkowska et al. 2010). The addition of beet root pulp to the ensiled pumpkin had a favourable effect on the ensiling process and nutritional value of the obtained silage. It constituted, however, a certain threat to its quality. The higher content of water-soluble carbohydrates in the silage increased significantly its sensitivity to aerobic decomposition. Raczkowska-Werwińska et al. (2008) inform that a limited development of undesirable bacteria (*Clostridium* sp., *Coli* sp.) and fungi in the silages is obtained by the application of chemical and microbiological additives. It was confirmed in our studies, i.e. in lower content of ethanol and ammonia nitrogen in the silage with inoculant, and its higher aerobic stability.

CONCLUSIONS

The obtained results of the studies indicate that mixing the disintegrated pumpkin fruits and dried pulp before ensiling allows obtaining the feed with a high nutritional value which may be employed, first of all, in nutrition of ruminants but also of monogastric animals. The application of inoculant during ensiling process increases the quality of the silage and improves its aerobic stability.

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Streszczenie: *Wpływ zakiszania dyni olbrzymiej z dodatkiem lub bez dodatku inokulanta na skład chemiczny i jakość kiszonek.* Celem badań było określenie wpływu zakiszania dyni na skład chemiczny i jakość kiszonek. Kiszonki przygotowano z dyni odmiany Justynka. Przed zakiszeniem rozdrobnione owoce dyni wymieszano z suszonymi wysłodkami w proporcji wynoszącej 80 : 20. Przygotowano dwa warianty kiszonek z preparatem bakteryjno-enzymatycznym i bez preparatu. W kiszonkach oznaczono podstawowy skład chemiczny: zawartości suchej masy, popiołu surowego, białka ogólnego, tłuszczu surowego, włókna surowego, NDF, ADF, ADL. Oznaczono również wskaźniki świadczące o przebiegu procesu zakiszania i jakości uzyskanych kiszonek: pH, zawartość kwasów mlekowego, octowego i masłowego, azotu amonowego, etanolu oraz stabilność tlenową. W uzyskanych kiszonkach w porównaniu do materiału przed zakiszaniem stwierdzono mniejszą zawartość włókna surowego, ADF, a w kiszonce z inokulantem również NDF. Podstawowy skład chemiczny oraz pH w obu wariantach zakiszania były podobne. Kiszonki z inokulantem charakteryzowały się większą zawartością kwasów mlekowego oraz octowego, natomiast mniejszą azotu amoniaku i etanolu. Kiszonka z inokulantem miała również wyższą stabilność tlenową. Badania wskazują, że zastosowanie inokulanta wpłynęło na poprawę jakości uzyskanych kiszonek.

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