The effect of aerobic exposure on nutritive value and fermentation parameters of maize silage

Vplyv aeróbnej expozície na nutričnú hodnotu a fermentačné ukazovatele kukuričnej siláže

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Abstract

The objective of the study was to investigate the effect of 24 hours aerobic exposure on the nutritive value and fermentation parameters of maize silage under operating conditions in autumn. The maize silage was treated with the addition of granulated biological silage additive (*Lactobacillus plantarum*, *Lactobacillus buchneri* and *Pediococcus pentosaceus*) in a 0.25 $kg*t^{-1}$ dose of matter. The maize silage was stored in unsheltered, impassable 36x23x6m silo, covered with thin translucent underlying sheet and black upper sheet with tires laid on the top at the University farm, Ltd. Kolíňany – large scale dairy farm Oponice. Sampling of the silage from silo was realized by block cutter on 5th of October 2011 at 4 a.m. (before feeding) and subsequently, maize silage was stored at the feeding table in dairy cow's stall. The sampling of the maize silage happened immediately after collection (H0) and after 24 hours of storage at the feeding table (H24). The air temperature in dairy cow's stall was 13.5 °C (H0) and after 24 hours of storage (H24) 15 °C. The temperature of the silage in 30cm depth was 28 °C (H0) and 40.3 °C (H24). After 24 hours of aerobic exposure, the content of dry matter, starch, hemicelluloses and neutral detergent fiber in maize silage was statistically significantly ($P < 0.05$) increased. Our results indicate a reduction *in vitro* organic matter digestibility and a decrease in energy and nitrogen values during aerobic exposure. We found statistically significant ($P < 0.05$) decrease in lactic acid content, the degree of proteolysis and an increase in acetic acid (by 41%) in maize silage with *Lactobacillus plantarum, Lactobacillus buchneri* and *Pediococcus pentosaceus* during 24 hours aerobic exposure.

Keywords: aerobic exposition, maize, nutrients, silage

Abstrakt

Cieľom štúdie bolo v prevádzkových podmienkach zistiť vplyv 24 hodinovej aeróbnej expozície v jesennom období na výživnú hodnotu a fermentačné ukazovatele kukuričnej siláže. Kukuričná siláž bola ošetrená prídavkom granulovaného biologického silážneho aditíva (*Lactobacillus plantarum, Lactobacillus buchneri* a *Pediococcus pentosaceus*) aplikovaného v dávke 0,25 kg*t^{-1} hmoty. Kukuričná siláž bola uskladnená na Vysokoškolskom poľnohospodárskom podniku s.r.o. Kolíňany - Veľkokapacitná farma dajníc Oponice v nezastrešenom, neprejazdnom silážnom žľabe s rozmermi 36x23x6m, zakrytá tenkou priesvitnou podkladovou fóliou a čiernou hornou krycou fóliou, ktorá bola zaťažená pneumatikami. Odber siláže so silážneho žľabu sa realizoval blokovým vykrajovačom 5.10. 2011 ráno o 4:00 hod. (pred skrmovaním) a následne bola kukuričná siláž uskladnená na kŕmnom stole v ustajňovacom priestore dojníc. Odber vzoriek kukuričnej siláže bol realizovaný ihneď po vybratí zo silážneho žľabu (H0) a po 24 hodinách skladovania na kŕmnom stole (H24). Teplota vzduchu v ustajňovacom priestore dojníc bola 13,5 °C (H0) a po 24 hodinách (H24) 15 °C. Teplota siláže v 30 cm hĺbke od povrchu bola 28 °C (H0) a 40,3 °C (H24). Po 24 hodinovej aeróbnej expozícii sa štatisticky preukazne ($P < 0,05$) zvýšil obsah sušiny, škrobu, hemicelulózy a neutrálnedetergentnej frakcie vlákniny. Naše výsledky naznačujú zníženie *in vitro* stráviteľnosti organickej hmoty, pokles energetickej a dusíkatej hodnoty počas aeróbnej expozície. Zistili sme štatisticky preukazné ($P < 0,05$) zníženie obsahu kyseliny mliečnej, stupňa proteolýzy a zvýšenie obsahu kyseliny octovej (o 41%) v kukuričných silážach s *Lactobacillus plantarum*, *Lactobacillus buchneri* a *Pediococcus pentosaceus* počas 24 hodinovej aeróbnej expozície.

Kľúčové slová: aeróbna expozícia, kukurica, živiny, siláž

Detailný abstrakt

Cieľom štúdie bolo v prevádzkových podmienkach zistiť vplyv 24 hodinovej aeróbnej expozície v jesennom období na výživnú hodnotu a fermentačné ukazovatele kukuričnej siláže. Kukuričná siláž bola ošetrená prídavkom granulovaného biologického silážneho aditíva (*Lactobacillus plantarum, Lactobacillus buchneri* a *Pediococcus pentosaceus*) aplikovaného v dávke 0,25 kg*t^{-1} hmoty. Kukuričná siláž bola uskladnená na Vysokoškolskom poľnohospodárskom podniku s.r.o. Kolíňany - Veľkokapacitná farma dajníc Oponice v nezastrešenom, neprejazdnom silážnom žľabe s rozmermi 36x23x6m, zakrytá tenkou priesvitnou podkladovou fóliou a čiernou hornou krycou fóliou, ktorá bola zaťažená pneumatikami. Odber siláže so silážneho žľabu sa realizoval blokovým vykrajovačom 5.10. 2011 ráno o 4:00 hod. (pred skrmovaním) a následne bola kukuričná siláž uskladnená na kŕmnom stole v ustajňovacom priestore dojníc. Odber vzoriek kukuričnej siláže bol realizovaný ihneď po vybratí zo silážneho žľabu (H0) a po 24 hodinách skladovania na kŕmnom stole (H24). Teplota vzduchu v ustajňovacom priestore dojníc bola 13,5 °C (H0) a po 24

hodinách (H24) 15 °C. Teplota siláže v 30 cm hĺbke od povrchu bola 28 °C (H0) a 40,3 °C (H24). Vo vzorkách kukuričných siláží sme stanovili obsah živín a parametre fermentačného procesu. Obsah sušiny sme stanovili sušením pri teplote 103 °C, obsah popolovín spálením v muflovej peci pri teplote 550 °C. Obsah vlákniny, neutrálnedetergentnej frakcie vlákniny (NDV), acidodetergentnej frakcie vlákniny (ADV) a lignínu bol stanovený pomocou prístroja Ankom 200 Fiber Analyzer (Ankom Technologies, Macedon, NY) ako hmotnostný zvyšok po extrakcii. Obsah hemicelulózy sme vypočítali ako rozdiel medzi NDV a ADV a obsah celuózy ako rozdiel medzi ADV a lignínom. Obsah dusíkatých látok sme stanovili ako celkový obsah dusíka (Kjeldahlovou metódou) vynásobený faktorom 6,25. Obsah tuku bol stanovený extrakčnou metódou a obsah škrobu polarimetrickou metódou. Obsah bezdusíkatých látok výťažkových (BNLV) a organickej hmoty (OH) sme vypočítali (BNLV = sušina – dusíkaté látky – vláknina – tuk – popoloviny, OH = sušina – popoloviny). Koeficient stráviteľnosti organickej hmoty bol stanovený s použitím *in vitro* pepsín-celulázovej metódy (PEPCEL) na prístroji DAISY^{II} incubator (ANKOM Technology, 2004). Hodnotu netto energie laktácie sme vypočítali podľa Prílohy č. 8 Výnosu č. 39/1/2002-100. Pre výpočet (NEL_1) sme použili koeficient stráviteľnosti organickej hmoty podľa Petrikoviča et al. (2000) a pre výpočet (NEL_2) sme použili in vitro koeficient stráviteľnosti organickej hmoty. Obsah fermentačných kyselín (mliečnej, octovej, maslovej) bol stanovený metódou iónovej elektroforézy na analyzátore EA 100 (VillaLabeco). Obsah alkoholov a NH_3 sme stanovili mikrodifúznou metódou, kyslosť vodného výluhu alkalimetrickou titráciou na hodnotu pH 8,5 a aktívnu kyslosť (pH) elektrometricky. Obsah fermentačných produktov sme vypočítali podľa Sommera et al. (1994). Stupeň proteolýzy (%) sme stanovili výpočtom: $N\text{-}NH_3$ / celkový N * 100. Po 24 hodinovej aeróbnej expozícii sa štatisticky preukazne ($P < 0,05$) zvýšil obsah sušiny, škrobu, hemicelulózy a neutrálnedetergentnej frakcie vlákniny. Naše výsledky naznačujú zníženie *in vitro* stráviteľnosti organickej hmoty, pokles energetickej a dusíkatej hodnoty počas aeróbnej expozície. Zistili sme štatisticky preukazné ($P < 0,05$) zníženie obsahu kyseliny mliečnej, stupňa proteolýzy a zvýšenie obsahu kyseliny octovej (o 41%) v kukuričných silážach s *Lactobacillus plantarum, Lactobacillus buchneri* a *Pediococcus pentosaceus* počas 24 hodinovej aeróbnej expozície.

Introduction

The conservation of forages as silage is an important source of nutrients for livestock nutrition in many countries of the world, because it enables crops to be available for use either throughout the year or in periods of restricted seasonal availability of pastures for the grazing animal (Wilkinson, 2011). Ensiling is a common preservation method for most forage crops. It is based on lactic acid bacteria converting watersoluble carbohydrates into organic acids, mainly lactic acid, under anaerobic conditions (McDonald et al., 1991). Maize silage provides an important source of energy in the form of starch and fibre fractions for dairy cows nutrition (Juráček et al., 2012a, Mlyneková and Čerešňáková, 2013). Anaerobic conditions are not always achieved in silos on individual farms, especially in the outer layer of a silo, because of the difficulty of sealing it efficiently (Borreani et al., 2007). Silage is often exposed to air when it is still in the silo because sealing is not hermetic, and spoilage might start as early as this stage (Honig et al., 1991). During unloading, silage is normally fully exposed to air, which could result in an increase in temperature and spoilage (aerobic deterioration). Many factors affect the aerobic stability of silages. Oxygen is detrimental to silage quality because it enables aerobic spoilage microorganisms such as yeasts and moulds to grow (Woolford, 1990). Silage may be exposed to air during storage and unloading for feeding, and so it is susceptible to spoilage (Ashbell et al., 2002). The stability of silage in the presence of oxygen is a very important factor in determining its quality and value (Weinberg and Ashbell, 1994). The aerobic deterioration of silages is a significant problem for farm profitability and feed quality throughout the world (Borreani and Tabacco, 2010). The present experiment was designed to study the effect of aerobic exposure on nutritive value and fermentation parameters of maize silage.

Materials and methods

Changes of nutrient values and fermentation parameters of maize silage after aerobic exposure was carried out at the University farm, Ltd. Kolíňany - large scale dairy farm Oponice. Whole crop maize (*Zea mays* L.) hybrid with FAO number 450 was harvested at milk-wax maturity of grain. The maize was treated with addition of granulated biological silage additive (*Lactobacillus plantarum, Lactobacillus buchneri* and *Pediococcus pentosaceus*, applied in a dose 0.25 kg*t^{-1} of mass). The maize silage was stored in unsheltered, impassable 36x23x6m silo, covered with thin translucent underlying sheet and black upper sheet with tires laid on the top. Sampling of silage from silo was made by block cutter SILOCUT 150 (Manatech Ltd. Humpolec, CZ) on 5th of October 2011 at 4 a.m. (before feeding) and subsequently, maize silage was stored at the feeding table in dairy cow's stall. The sampling of maize silage was immediately after collection (H0) and after 24 hours of storage at the feeding table (H24). The air temperature after placing on stabling alley was 13.5 °C and after 24 hours of storage (H24) 15 °C. Temperature of silage in 30cm depth was 28 °C (H0) and 40.3 °C (H24). Temperature was measured using thermometer TRONIC. Aerobic stability was evaluated by changing of nutrient values and fermentation parameters.

The content of nutrients was detected in the average samples according to the regulation no. 2145/2004-100. Each samples were dried, ground to pass a 1 mm screen in a mill and analyzed for the content of nutrients. Chemical analyses were performed on triplicate samples. Dry matter (DM) content of the samples were determined by oven drying at 103 °C for 4 hours. Ash content was obtained in a muffle oven after 12 h at 550 °C. Crude fiber (CF), Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and lignin were determined gravimetrically as the residue remaining after extraction using the Ankom 200 Fiber Analyzer (Ankom Technologies, Macedon, NY). Hemicellulose was calculated as the difference between NDF and ADF and cellulose as the difference between ADF and lignin. Crude protein (CP) was determined by using the Kjeldahl's method, for N and multiplication by 6.25, fat by extraction and starch by polarimetric method. The content of nitrogen free extract (NFE) and organic matter (OM) was calculated (NFE = dry matter-crude protein-crude fiber-fat-ash, OM = dry matter-ash). Organic matter digestibility (OMD) was evaluated by the pepsin-cellulase (PEPCEL) *in vitro* method using the DAISY^{II} incubator (ANKOM Technology, 2004). Net energy for lactation was calculated according to Annex no. 8 of Decree no. 39/1/2002-100 with using organic matter digestibility according to Petrikovič et al. (2000) (NEL_1) and net energy for lactation (NEL_2) using *in vitro* organic matter digestibility.

Contents of fermentation acids (lactic, acetic, butyric) were detected on analyzer EA 100 (VillaLabeco) by electrophoretic method. Content of alcohols and NH_3 were determined by microdiffusion method, acidity of water extract by alkalimetric titration to pH 8.5 and active acidity (pH) by electrometric method. Fermentation products were calculated according to Sommer et al. (1994). The degree of proteolysis was calculated according following formula: degree of proteolysis (%) = N-NH_3 / total N x 100.

Significant differences between means were identified by using the Tukey's range test, which $P < 0.05$ was declared as significant. These analyses were performed with the statistical package SPSS 16.0 (SPSS Inc., 2007, Chicago, IL, USA).

Results and discussion

The effects of aerobic exposition on the content of nutrients and nutritive value of maize silage is shown in Table 1. The level of dry matter at which forage crop is ensiled has profound effects on silage fermentation and nutritive value (Rajcakova et al., 2012). According Kudrna et al. (2006), for successful production of maize silages, it is necessary to collect the matter in milk - wax ripeness of grain, that occurs when the whole plant dry matter range between 280-340 g*kg^{-1} . DM content in analyzed silage was 293.25 (H0) and after aerobic exposition significantly increased ($P < 0.05$). CP content of the maize silage is generally low. In our experiment, the CP content was 85.25 (H0) and 83.85 (H24) g*kg^{-1} DM, difference were insignificant ($P > 0.05$). Decrease of crude protein content during 2-day aerobic exposure also found Gerlach et al. (2013). Gálik et al. (2005) detected CP content in maize silages from 81.8 to 87.7 g*kg^{-1} DM. The content of carbohydrates in the form of nitrogen free extract (NFE) increased during aerobic exposition ($P > 0.05$). Juráček et al. (2012b) reported similar values of NFE in different hybrids of maize silages. The content of OM from analyzed maize silages decreased non-significantly. Starch is a quantitatively important nutrient for high-yielding dairy cows (McDonald et al., 1995). Mitrík (2010) recommends the minimum content of starch for maize silages of 1st class quality 275 g*kg^{-1} DM. We found statistically significant higher value ($P < 0.05$) of starch in silage H24 compared to H0 by 18.85 g*kg^{-1} DM. The same increase in starch content during 2-day aerobic exposure were also found by Gerlach et al. (2013). ADF content in maize silage decreased during aerobic exposition, differences were insignificant ($P > 0.05$). Podkówka and Podkówka (2011), also Mitrík (2009) reported higher average content of ADF in maize silage. The content of NDF significantly ($P < 0.05$) increased from 352.95 (H0) to 370.15 g*kg^{-1} DM (H24), which is in agreement with results of Gerlach et al. (2013). According to Adesogan (2006) is desired value of NDF in maize silage ≥ 470 g*kg^{-1} DM. Lignin form a bond and fills the space between the fibers of cellulose, branched structure of hemicelluloses and pectin in the cell wall. Its quantity and localization directly related to the digestibility of the cell walls of plant tissue (Mitrík and Vajda, 2006). The content of lignin was 21.7 (H0) and 18.10 g*kg^{-1} of DM (H24) ($P > 0.05$). Martin et al. (2008) reported range of lignin content in maize silages 15.7-23.3 g*kg^{-1} DM. The content of hemicelluloses during aerobic exposure significantly increased ($P < 0.05$). The value of net energy for lactation (NEL_1) calculated with using digestibility coefficient of organic matter 71% (Petrikovič et al., 2000) was identical in samples H0 and H24 ($P > 0.05$). Also Gerlach et al. (2013) not detected changes in energy content during the 2 days of aerobic exposure. Petrikovič et al. (2000) reported higher average value of NEL (6.35 $MJ*kg^{-1}$ DM) in maize silage in comparison to our results. We found, that with aerobic exposure non-significant ($P > 0.05$) decrease the *in vitro* digestibility of organic matter. De Boever et al. (1997) reported in maize silages average organic matter digestibility of 74.7%, which are comparable with our results (76.88 and 76.15%). Poštulka and Doležal (2010) found higher organic matter digestibility in maize silages: 78.8, 80.1 and 81.1%. Majlát et al. (2013) also had higher OMD in maize silages with low temperature than silages with high temperature, this author suggest that with increasing temperature is statistically significant decrease the organic matter digestibility. Larger differences between energy values were in NEL_2 calculated with using *in vitro* digestibility coefficient of organic matter. Maize silages after 24 hours aerobic exposition had non-significantly lower NEL_2 value (6.85 vs. 6.94 $MJ*kg^{-1}$ DM). These values are comparable with Barriere et al. (1996) who found the content of NEL in range 5.82-6.95 $MJ*kg^{-1}$ DM. The evaluation of the content of nitrogenous substances in ruminant nutrition is done by the PDI system (crude protein digestible in the intestine). This system takes into account the mainly separate intake of crude protein for the body of animal and microflora in the digestive tract (Pajtáš et al., 2009). The lower value of PDIN (nitrogen) was detect in silage H24 (50.85 $g*kg^{-1}$ DM), compare to H0 (51.70 $g*kg^{-1}$ DM) ($P > 0.05$). Higher value of PDIN (53.44 $g*kg^{-1}$ DM) reported Machačová and Loučka (2001) 53.44 kg^{-1} DM. The value of PDIE decreased during aerobic exposition (65.05 vs. 64.80 $g*kg^{-1}$ DM) ($P > 0.05$). Sommer et al. (1994) found in identical phenological phase of harvesting higher average content of PDIE (81 $g*kg^{-1}$ DM).

Table 1. The effects of aerobic exposition on the content of nutrients and nutritive value

Tabuľka 1. Vplyv aeróbnej expozície na obsah živín a výživnú hodnotu

n=3		Maize silages			
		H0		H24	
Parameters	Unit	$\bar{x}$	SD	$\bar{x}$	SD
DM	a	293.25*	0.78	302.55*	0.07
CP	b	85.25	0.64	83.85	0.64
Fat	b	29.50	0.71	32.15	0.07
Ash	b	48.00	0.14	48.20	0.28
NFE	b	663.90	2.83	672.70	2.83
OM	b	952.00	0.14	951.80	0.28
Starch	b	367.25*	1.63	386.10*	2.26
ADF	b	194.90	0.85	192.85	0.07
NDF	b	352.95*	1.91	370.15*	1.20
Lignin	b	21.7	1.98	18.10	0.42
Cellulose	b	171.85	4.88	174.75	0.49
Hemicellulose	b	158.10*	0.99	177.25*	1.34
NEL_1	c	6.26	0.01	6.26	0.01
OMD *in vitro*	d	76.88	0.65	76.15	0.06
NEL_2	c	6.94	0.07	6.85	0.01
PDIN	b	51.70	0.42	50.85	0.35
PDIE	b	65.05	0.07	64.80	0.14

a: $g*kg^{-1}$, b: $g*kg^{-1}$ DM, c: $MJ*kg^{-1}$ of DM, d: %, H0: maize silage after collection, H24: maize silage after 24 hours of exposure to air, SD: standard deviation, v: coefficient of variability DM: dry matter, CP: crude protein, NFE: nitrogen-free extract, OM: organic matter, ADF: acid detergent fiber, NDF: neutral detergent fiber, NEL_1: net energy for lactation with using organic matter digestibility according to Petrikovič et al. (2000), NEL_2: net energy for lactation with using in vitro organic matter digestibility, OMD: in vitro organic matter digestibility, PDIN: protein digested in the small intestine when rumenfermentable nitrogen is limiting; PDIE: protein digested in the small intestine when rumen-fermentable energy is limiting, *the values with identical superscripts in row are significantly different at P < 0.05

The effects of aerobic exposition on fermentation parameters of maize silages is shown in Table 2. We found higher content of lactic acid than 10 $g*kg^{-1}$ of original matter in all samples of silage. Lactic acid content decreased during 24 hours of aerobic exposition ($P < 0.05$). Maize silages H0 had higher level of acetic acid. Our results confirm previous findings that the addition Lactobacillus buchneri resulted in increased acetic acid concentration (Basso et al., 2012; Hu et al, 2009; Reich and Kung, 2010). Acetic acid content increased significantly ($P < 0.05$) in maize silages during aerobic exposition. We didn't find undesirable butyric acid in any sample. No effect of 24 hours aerobic exposition on pH value, content of NH3 and fermentation products was observed (P 0.05). This is in agreement with Ashbell et al. (2002) reported stable pH in maize silages after 3 and 6 days exposure to air. Content of ethanol decreased during aerobic exposition. This is supported by the findings of Gerlach et al. (2013). The degree of proteolysis and titration acidity of water extract in silages H24 were higher compared to silages H0 (P < 0.05).

Table 2. The effects of aerobic exposition on fermentation parameters

Tabuľka 2. Vplyv aeróbnej expozície na fermentačné ukazovatele

n=3		Maize silages			
		H0		H24	
Parameters	Unit	$\bar{x}$	SD	$\bar{x}$	SD
Lactic acid	a	59.55*	0.64	54.50*	0.57
Acetic acid	a	38.40*	1.13	54.15*	0.21
Butyric acid	a	nd	-	nd	-
pH value	-	3.83	0.01	3.85	0.01
NH_3	a	1.30	0.01	1.19	0.03
DP	b	7.82*	0.04	7.31*	0.06
Ethanol	b	17.30*	0.28	4.70*	0.14
AWE	c	1849.00*	4.24	2104.00*	56.57
FP	b	115.25	1.77	113.35	0.35

a: $g*kg^{-1}$ DM, b: %, c: mg KOH per 100 g silage, nd: non-detected, DP: degree of proteolysis, AWE: acidity of water extract, FP: fermentation products, *the values with identical superscripts in row are significantly different at P < 0.05

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