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EFFECT OF INSECT-PROTECTED MAIZE SILAGE (BT-MON 810) ON FEEDING VALUE AND DIGESTIBILITY OF NUTRIENTS ESTIMATED WITH WETHERS

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ABSTRACT

The objective of this study was to compare the feeding value of conventional maize silage with its isogenic Bt form in digestibility trial on wethers. In 2006, two maize hybrids, MONUMENTAL (C) and Bt-MON 810 (Bt) were planted at the area of Ivanovice na Hané, Czech Republic under comparable conditions. Maize plants were harvested at soft-dough stage of maturity and ensiled into mini-silos (2 m^3). Silages were used in a digestibility trial with 10 Suffolk wethers with an average live weight of 45.3 $\pm$ 1.35 kg that were divided into two groups. The experiment was carried out in two periods of 14 days (7-d preliminary period and 7-d collection period). In the fresh maize forage the content of crude protein in C (74.0 g kg^{-1}DM) was higher than in Bt (71.0 g kg^{-1} DM, P<0.05) and the content of acid detergent fiber (ADF) was lower in C in comparison to Bt (205.8 vs. 210.1 g kg^{-1} DM, respectively, P<0.05). Content of nutrients in silages was unaffected by the maize hybrid (P>0.05). No effect of the maize hybrid on fermantation process was observed (P>0.05) except of ethanol content that was lower in C and acetic acid content that was higher in C compared to Bt (P<0.05). Intake of dry matter (DM) was similar in both groups (P>0.05). Digestibility of crude fiber and nitrogen-free extractives was lower in C (34.4 and 74.1 %) than in Bt (40.0 and 76.4 %, respectively, P<0.05). Haematological characteristics of animals were unaffected by the type of silage (P>0.05).

Key words: maize silage; Bt-MON 810; wethers; digestibility

INTRODUCTION

Maize silage has been the main preserved forage fed to ruminants in many countries. Due to the importance of maize silage for cattle feeding, there is a growing demand on the production of high-quality silage. Targets of maize breeding improvement include agronomic traits, such as whole plant yield and maturity, tolerance to abiotic traits, resistance or protection against diseases and pests and feeding value for ruminants. Technology of genetically modified (GM) crops is used to increase crop yield, improve food quality and reduce an environmental impact of agriculture, amongst other things fosters the use of less toxic agrochemicals (Phipps and Beever, 2000). Bt-maize is characterized by the introduction of a gene for a Bt-toxin from *Bacillus thuringiensis* which protects maize against the European maize borer (EMB). Except of above mentioned benefits, indirect effects associated with reduced insect damage of Bt hybrids, such as greater carotene and moisture content or lower mycotoxin concentrations, have been described (Faust, 1998).

Since introduced on the market, Bt maize has become an object of considerable research activity to consider the effect of gene insertion on the chemical composition of the resultant feed with specific reference to nutritional value. Studies in which Bt maize feeds have been compared with non-Bt feeds were performed

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on high-producing animals, such as beef and dairy cattle (forages) or lying hens, broilers and growing-finishing pigs (grains). Results of above mentioned experiments were recently reviewed by Flachowsky et al. (2007). No differences in feed intake, nutritional value or in animal performance have been detected. Additional digestibility experiments carried out on adult wethers confirmed the nutritional equivalence of GM maize to their near isogenic parental plants.

Not all Bt hybrids are the same because the trait that has been incorporated into hybrids is based on different transgenic events resulting in varying distribution of the endotoxin in various portions of the maize plant and thus different relative efficacy among these transgenic events. The effect of feeding Bt-MON 810 form to ruminants has not been investigated extensively (Calsamiglia et al., 2003). In the study of Donkin et al. (2003), the effect of feeding Bt-MON 810 maize silage and grain on feed intake, milk production, and DM digestibility in lactating dairy cows was studied.

The objective of this study was to compare the feeding value of maize silage produced from conventional maize (MONUMENTAL) with its isogenic Bt form (Bt-MON 810) in digestibility trial on wethers.

MATERIAL AND METHODS

Maize cultivation, harvest and ensiling

During the 2006 cropping year, two commercial maize hybrids, MONUMENTAL - C and Bt MON 810, (MONSANTO CR, s. r. o., Czech Republic) - Bt were planted at the area of Ivanovice na Hané, Czech Republic. This Bt hybrid produces a truncated version of the insecticidal protein, Cry1Ab that was derived from the transgenic event MON 810. The control non-Bt hybrid represents corresponding near-isoline developed by conventional breeding methods. Both hybrids were grown for harvest of silage in adjacent fields under comparable agronomic conditions. Maize plants were harvested at soft-dough stage of maturity, based on moisture content and visual observations. For each hybrid, the chopped maize (15 mm chop length) was ensiled in separate mini-silo (2 m^3, laminated container) according to standard farming practices. Digestibility trial began at earliest of 200 d after ensiling.

Sampling and analytical procedure

Samples of chopped forage were taken during ensiling, samples of silage were taken at the beginning of digestibility trial. Samples were analyzed for the following parameters. Dry matter (DM) was determined by drying at 103 °C for 4 h. Content of crude protein (CP), crude fiber (CF), ash, fat and starch were estimated according to AOAC (1984). Neutral detergent fiber (NDF, with α-amylase) and ash-free acid detergent fiber (ADF) were estimated according to Van Soest et al. (1991) and Goering and Van Soest (1970), respectively.

The parameters characterizing the ensilage process and silage quality were determined from the aqueous silage extract prepared according to Suzuki and Lund (1980). pH was determined using accurate pH meter. Aqueous silage extract acidity (titration acidity) was determined after titration with 0.1 M KOH to pH = 8.5. Analysis of amino acid N ($N\text{-}NH_2$) was conducted by formol titration with 0.1 M KOH after addition of HCHO. Ammonia content was determined by the Conway microdiffusion method.

The VFA and alcohols were determined from aqueous extract using gas chromatography on a CHROM-5 gas chromatograph fitted with a glass column, packed with 80/120 Carbopack B-DA/4% CARBOWAX 20 M. The temperature gradient program was held at 85°C for 2 min (methanol and ethanol) and increased up to 147°C at a rate of 7.5°C /min, then increased up to 180°C at a rate of 15°C/min for determination of other alcohols, volatile fatty acids and lactic acid. As an internal standard trimethylacetic acid was used, nitrogen was the carrier gas. Results were evaluated by the CSW 1.5 program method with an internal standard ISTD – 2.

From the obtained results a degree of proteolysis was calculated according to the following formula: degree of proteolysis (%) = $N\text{-}NH_3$ / total N

In vivo experiment

Animals and procedure

A group of 10 Suffolk wethers with an average live weight of 45.26 kg (SEM = 1.35 kg) was used in a balance trial to measure the digestibility of nutrient. Animals were divided into two groups according to body weight and housed individually in balance cages. The control group (C) was fed maize silage made from conventional hybrid MONUMENTAL while the experimental group (Bt) was fed silage prepared from near isogenic Bt hybrid MONSANTO (Bt-MON 810). The experiment was carried out in two periods of 14 days consisting from a 7-d preliminary period (adaptation to balance cages) followed by a 7-d collection period. In the first period 5 animals received a control diet (C) and the remaining 5 were fed the experimental diet (Bt). In the subsequent period the wethers were switched to the other treatment. Wethers were weighed at the beginning of each period.

During the experiment, wethers were fed individually twice daily (07:30 and 17:30 h) maize silage *ad libitum* in such a way that refusals did not exceed 10 % (Barriére et al., 2001). The nutrient content of the silage is given in Table 2. Only nitrogen (1.5% urea), minerals and vitamins were added to maize silage to balance the diet. This type of the diet (i.e. maize silage supplemented with urea, minerals and vitamins) was fed a month prior to the beginning of the experiment.

Sampling and analytical procedure

During the experiment feed intake and respective refusals were monitored daily, an aliquote of them was taken and analyzed for a basal chemical composition as described above.

Faeces were collected daily by total grab sampling; the daily collections were kept frozen at –20 °C until the end of the experiment. Then the faeces were pooled, homogenized and an aliquote was taken and analyzed in the same way as feeds.

On a last day of each period, blood samples were taken from the *vena jugularis* into the heparinized tubes (at 7.45 h) for determination of the haematological parameters using automated analyzer (ABX Micros ABC Vet, Horiba, United Kingdom).

Statistical analysis

Data obtained in the experiment were analyzed using GLM procedure of the Statgraphics 7.0 package (Manugistics Inc., and Statistical Graphics Corporation, Rockville, Maryland, USA). Nutrient composition and fermentation process was evaluated using a one-way ANOVA, digestibility trial was analyzed according to the following model:

$$Y_{ijk} = \mu + T_i + P_j + I_{ij} + \varepsilon_{ij}$$

where μ = general mean, T_i = treatment effect (i = 2), P_j = period effect (j = 2), I_{ij} = interaction between the treatment and period and ε_{ij} = error term.

RESULTS

Nutritional value of fresh and ensiled maize hybrids

Nutritional value of fresh forage of C and Bt maize is given in Table 1. Average content of dry matter, fat, starch, ash, crude fiber and NDF was similar in both hybrids (P>0.05). Content of a crude protein in C was higher than in Bt (P<0.05). Content of ADF was lower in C in comparison to Bt (P<0.05). Content of PDIN and PDIE as well as content of energy (NEL) were not influenced by the type of maize hybrid (P>0.05).

Nutritional value and characteristics of a fermentation process of C and Bt silage is compared in Table 2. No significant diferences were determined in studied parameters (P>0.05) except of starch content, that was higher in C than in Bt (P<0.05). pH values of both silages were similar being 3.71 in C and 3.68 in Bt (P>0.05). No effect of maize hybrid on titration acidity, ammonia content or degree of proteolysis measured as

Table 1: Nutrient composition (g kg^{-1}) of fresh forage from the control (C) and insect-protected (Bt) maize hybrids prior to ensiling (n=4)

Nutrients	Units	C [1]	Bt [2]	SEM
Dry matter	g kg^{-1}	306.9	303.7	1.45
Fat	g kg^{-1}	23.0	23.4	0.23
Ash	g kg^{-1}	43.7	43.4	0.62
Crude protein	g kg^{-1}	74.0 a	71.0 b	0.49
Starch	g kg^{-1}	286.5	274.9	1.96
Crude fiber	g kg^{-1}	186.3	190.6	2.22
NDF [3]	g kg^{-1}	413.8	417.7	1.32
ADF [4]	g kg^{-1}	205.8 a	210.1 b	1.04
PDIN [5]	g kg^{-1}	45.8	44.0	0.31
PDIE [5]	g kg^{-1}	75.7	75.1	0.05
NEL [6]	MJ kg^{-1}	6.3	6.3	0.01

[1]Control (MONUMENTAL); [2]Bt-MON 810; [3]neutral detergent fibre; [4]acid detergent fibre; [5]digestible protein in the intestine when rumen fermentable N supply or energy supply are limiting, respectively; [6]net energy of lactation

a, b - rows with different superscripts differ significantly (P<0.05)

Table 2: Nutrient composition (g kg^{-1}) and characteristics of fermentation process of silage made from control (C) and insect-protected (Bt) maize hybrids (n=4)

Nutrients	Units	C[1]	Bt[2]	SEM
Dry matter	g kg^{-1}	324.5	310.2	13.52
Fat	g kg^{-1}	31.2	29.5	0.89
Ash	g kg^{-1}	42.8	43.8	2.48
Crude protein	g kg^{-1}	86.2	84.6	2.37
Starch	g kg^{-1}	336.7 a	305.4 b	4.31
Crude fiber	g kg^{-1}	175.7	181.0	6.46
NDF [3]	g kg^{-1}	371.7	384.1	13.93
ADF [4]	g kg^{-1}	212.7	211.7	8.23
PDIN [5]	g kg^{-1}	53.0	52.0	1.46
PDIE [5]	g kg^{-1}	64.6	66.1	0.55
NEL [6]	MJ kg^{-1}	5.2	5.4	0.02
Fermentation process				
pH		3.71	3.68	0.01
Titration acidity	mg$_{KOH}$/100g	1745.7	1690.3	38.77
Formol titration	g kg^{-1} NH_3	1.1	1.0	0.04
Ammonia	g kg^{-1}	0.3	0.3	0.01
Degree of proteolysis	%	6.7	6.3	0.32
Methanol	g kg^{-1}	0.3	0.3	0.15
Ethanol	g kg^{-1}	2.1 a	4.3 b	0.63
Lactic acid	g kg^{-1}	26.7	27.5	2.52
Acetic acid	g kg^{-1}	2.8 a	2.0 b	0.18
Isovaleric acid	g kg^{-1}	0.2	1.3	0.83
Total VFA [7]	g kg^{-1}	3.1	3.4	0.97

[1]Control (MONUMENTAL); [2]Bt-MON 810; [3]neutral detergent fibre; [4]acid detergent fibre; [5]digestible protein in the intestine when rumen fermentable N supply or energy supply are limiting, respectively; [6]net energy of lactation; [7]butyric and propionic acids were not detected

a, b - rows with different superscripts differ significantly (P<0.05)

$N\text{-}NH_3$ (% of total N) was observed (P>0.05). Lactic acid, acetic acid and ethanol were predominant products of fermentation in both silages. Other volatile fatty acids and lower alcohols were not detected. The content of ethanol in C was significantly lower than in Bt (P<0.05). The content of acetic acid in C was higher compared to Bt (P<0.05).

***In vivo* experiment**

During the first period of the experiment one animal had to be removed due to health problems (leg injury). Thus the experiment was performed on nine wethers. Results of digestibility trial are summarized in Table 3. Intake of DM expressed in g kg^{-1} of body weight was similar in both groups (P>0.05). Digestibility of DM and crude fiber was lower in C in comparison to Bt (P<0.05). Digestibility of other nutrients was not influenced by the type of maize hybrid (P>0.05). Haematological characteristics of animals were within physiological ranges and were not affected by the type of silage (P>0.05, data not presented).

Table 3: Dry matter intake (g kg^{-1} of body weight) and digestibility of nutrients (%) from the control (C) and insect-protected (Bt) silage determined on wethers (n=9)

Nutrients	Units	C [1]	Bt[2]	SEM
Dry matter intake	g kg^{-1} BW	55.2	56.8	1.93
Digestibility				
Dry matter	%	61.0	63.1	0.80
Organic matter	%	63.9	66.0	0.71
Crude protein	%	36.2	29.9	3.62
Fat	%	78.5	78.7	0.89
Crude fiber	%	34.6a	40.2b	1.72
NDF[3]	%	38.2	41.1	1.38
ADF[4]	%	34.3	34.5	2.06
Nitrogen free extract	%	74.1 a	76.4 b	0.57

[1]Control (MONUMENTAL); [2]Bt-MON 810; [3]neutral detergent fibre; [4]acid detergent fibre

a, b rows with different superscripts differ significantly (P<0.05)

DISCUSSION

Nutritional value of fresh and ensiled maize hybrids

Generally, chemical composition of studied Bt maize hybrid ranged within values published in literature (e. g. Aumaitre, 2004, Donkin et al., 2003 or Faust and Splangler, 2000) and within the tolerance interval determined for commercial varieties, as described by AGBIOS (2005). Content of nutrients in fresh forage was not affected by the type of maize hybrid except of ADF, that was lower in C, and crude protein, that was higher in C than in Bt (P<0.05). This finding is in discrepancy with Barriére et al. (2001) who found higher CP content in Bt form (67 g kg^{-1}, event Bt 176) of hybrid Rh208 used in their study in comparison to isogenic control (60 g kg^{-1}). Faust et al. (2007) found higher DM content in maize forage of the Control (420.2 g kg^{-1}) in comparison to its near isogenic Bt form (375.8 g kg^{-1}, event TC 1507). This difference resulted in a higher DM content of maize silage from the Control compared to the TC 1507 maize (450.8 vs. 422.4 g kg^{-1} of DM) and presumably reflected greater EMB infestation of the Control forage. On the other hand, Calsamiglia et al. (2007) did not find any substantial differences between control and GM maize forage. Similarly, George et al. (2004) demonstrated in their study that chemical composition of GM maize (MON 863) was comparable to the nontransgenic control. Recently, based on the comprehensive compositional data, EFSA (2009) concluded that maize MON 810 is compositionally equivalent to the non-GM maize counterparts MON 820 and MON 818 and to conventional maize varieties except for the presence of the Cry1Ab protein.

In the present experiment no diferences (P>0.05) were determined in nutritional paremeters of silages. These findings are in agreement with Donkin et al. (2003) or with Faust and Splangler (2000) who found that nutrient composition of silages from Bt-MON 810 and its conventional near-isogenic form were similar throughout the year of harvest. Similarly, Folmer et al. (2002) reported no differences in chemical composition between the Bt and non-Bt hybrids from Bt11 transformation event. On the other hand, Barriére et al. (2001) found higher content of CP in silage made from the Bt hybrid (82 g kg^{-1} of DM) in comparison to conventional hybrid (74 g kg^{-1} of DM). Characteristics describing fermentation process correspond to values reported by Steidlová and Kalač (2002), who evaluated fermentation process in 113 samples of silages prepared from maize hybrids commonly grown in the Czech Republic in two subsequent years. They reported the mean pH between 3.56 and 3.64, content of lactic acid ranged from 18.5 to 21.3 g kg^{-1}, ethanol from 2.3 to 3.1 g kg^{-1} and ammonia from 35.6 to 39.6 mg $100g^{-1}$. In the present experiment no substantial differences in the fermentation process were found between Bt and C silages. Similar findings were also described by Barriére et al. (2001), who found that the fermentation parameters of conventional and Bt silages used in their experiment were almost the same (pH = 3.8, acetic, butyric, and lactic acid contents were 12.2, 0.15, and 50.0 g kg^{-1} DM, respectively).

***In vivo* experiment**

Results of a number of livestock feeding studies, as summarised in EFSA (2008), have shown that the bioavailability of a wide range of nutrients from a range of GM plants modified for agronomic input traits was comparable with those for near isogenic non-GM lines. EFSA (2008) also mentioned that some statistically significant differences that were noted in some studies, were generally small, inconsistent and not considered to be biologically meaningful. These results, in the case of silages, are not surprising because as mentioned above, nutritional value of Bt maize MON 810 is equivalent to its near isogenic conventional maize hybrids except for the presence of the Cry1Ab protein (EFSA, 2009). Furthemore, to date, no studies have demonstrated any adverse effects when the currently registered GM plants or products derived from them have been used as feeds (Alexander et al., 2007; Flachowsky et al., 2005). This is supported by the findings of Einspanier et al. (2004), who described the reduction in transgene concentration during ensiling to less than 0.03 of the starting quantity and Einspanier et al. (2001) who did not detect the transgenic DNA in organs of cattle given *ad libitum* access to Bt 176 silage. Similarly, no effect of feeding grain from genetically modified Bt176 maize on the health status and performance of sheep was observed by Trabalza-Marinucci et al. (2008) in their three-year longitudinal study.

The intake of DM in the present experiment did not differ significantly (P>0.05). Similar findings were reported by Barriére et al. (2001). Digestibility of basal nutrients was not influenced by the type of maize hybrid (P>0.05) except of digestibility of crude fiber and nitrogen-free extracts that was lower in C in comparison to Bt (P<0.05). This is in disagreement with Barriére et al. (2001), who did not find any differences in digestibility of basal nutrients. Recently, Rutzmoser and Mayer (2000) compared Bt forage maize silage with a non-Bt isogenic line using sheep to determine diet digestibility and lactating dairy cows to examine feed intake and milk yield and composition. No effects on digestibility were determined and neither milk yield nor milk fat, protein, and lactose contents were affected. Daenicke et al. (1999) examined Bt maize fed after ensiling to Holstein bulls in a comparison to non-Bt maize. Results of their study indicated no effects on daily live weight gain, carcass weights, or composition. Furthermore, they did not reveal any effects on apparent digestibility of the organic matter, fiber or nitrogen-free extractive fractions. Similarly, in studies of Aulrich et al. (2001), Faust and Splangler (2000) with MON 810 event or Folmer et al. (2002) with Bt 11 event, no effects on the digestibility of organic matter, fiber or nitrogen-free extractive fractions were noted, suggesting substantial equivalence between the conventional and Bt maize hybrids.

CONCLUSION

The results from the present experiment indicate the equivalence of the nutrient composition and feeding value between Bt-MON 810 maize and its respective near-isogenic control. The digestibility of crude fiber and nitrogen-free extracts of Bt silage determined on wethers was higher than that of the control silage. Digestibility of other nutrients was not influenced by the genetic modification of maize.

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