Effect of Crimped Maize Grain Ensiled with High Moisture Grains of Transgenic Bt Maize in Fattening Bulls

BACK

CHRENKOVÁ, M., POMIKALOVÁ, S., CHRASTINOVÁ, Ľ., POLAČIKOVÁ, M., FORMELOVÁ, Z., RAJSKÝ, M., MLYNEKOVÁ, Z.
National Agricultural and Food Centre, Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic, chrenkova@vuzv.sk

Abstract

The maize event MON 810 carries the novel gene *cry1Ab* from a naturally soil bacterium, *Bacillus thuringiensis* (Bt). Thus the transgenic Bt-maize produces the insecticidal *Cry1Ab* protein which provides resistance against the European corn borer. However, the increasing use of genetically modified plants in the production of animal feed has raised concerns about their safety. A feeding trial was carried out with 40 Holstein breed bulls with an initial live weight 298 kg which were randomly distributed into 4 groups with 10 bulls in each, housed in boxes. The feed consisted of maize silage, lucerne silage, meadow hay, wheat, rape extr. meal, minerals and crimped maize grain in two feeding TMR with content of isogenic maize and transgenic maize (event MON 810) intended for bulls. Bulls were fed TMR diet for *ad libitum* intake. Water was provided *ad libitum*. The TMR contained 139.84 $g.kg^{-1}$ crude protein, 6.45 $MJ.kg^{-1}$ NEV, 155.3 $g.kg^{-1}$ crude fibre, 264.47 $g.kg^{-1}$ starch, fat 28.8 $g.kg^{-1}$, Ca 9.21 $g.kg^{-1}$, P 3.67 $g.kg^{-1}$, Na 2.06 $g.kg^{-1}$, Mg 2.18 $g.kg^{-1}$ and K 10.735 $g.kg^{-1}$ in dry matter. The feeding trial was conducted with 2 x 20 bulls from average live weight 298 kg to the average slaughtering weight 620 kg in control and 622 kg in experimental group. The live weight gain was 1.248 $kg.d^{-1}$ and 1.255 $kg.d^{-1}$. The experiment lasted for 258 days. Data were processed by analysis of variance. The significance of differences was evaluated by the t-test. In fattening experiments there were studied live weight growth of bulls and consumption of feed mixtures per unit of live weight growth. Obtained results demonstrate minimal, statistically non-significant differences of individual parameters in tested groups. Bulls were slaughtered at the age of 586 days after achieving the live weight 620.5 kg. Feeding of TMR with proportion Bt transgenic and isogenic control maize to Holstein breed bulls did not influence zootechnical parameters, as well as it has no negative effect on feed conversion, growth performance, **meat quality** and health status.

***Key words**: high moisture grain; fermentation process; transgenic maize MON 810; Holstein bulls*

Introduction

Transgenic crops have been grown commercially for about 18 years, with a continuous increase of the cultivated area, and now they represent a significant proportion of the total world production for the major GM crops: soybean, maize, rapeseed and cotton. These crops are used directly or preserved in animal nutrition or as by-products from the processing industry such as distillers grain, extracted oil meal, sugar beet pulp, *etc*. Therefore, nutritional and safety assessments of feeds from GMP are one of the key questions. Most animal studies were conducted for the nutritional assessment of feeds. Flachowsky et al. (2005) and Chrenková et al. (2007) summarized the effects on the nutrients digestibility, feed intake, health and performance of animal as well as effects on the quality of food of animal origin. In the European context, Slovakia ranks among other 7 EU countries which have practical experience in Bt maize cultivation (Křistková, 2009; James, 2011). Maize MON 810 contains the Cry1Ab protein protecting the crop against certain lepidopteran insect pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia spp*.).

Crimped ensilage maize of rolled, moist grain is a suitable alternative to combine harvesting for grain intended to be used on-farm and when moist weather prevails during harvest. Farmers are recommended to apply additives during crimping (Pauly, 2015). Benefits of storage of high moisture grains in Nordic conditions have been already proven some decades ago (Palva *et al*. 2005; Jaakkola *et al*., 2005). No drying costs, less dependency on weather and an extended harvesting season are the main arguments that keep the method increasing in popularity. The method has become more common on farms with large herd sizes and total mixed ration (TMR) feeding.

The aim of this work was to verify substantial equivalence of maize MON 810 in fattening Holstein bulls from the investigation of fattening ability; carcass and consumer meat quality were obtained at the average age of 586 days.

Material and Methods

Experiment consisted of two variants (without preservative agents, control crimped isogenic maize grain (DKC 5143) and experimental variant transgenic maize MON 810. We preserved both variants into plastic sacks (2 meter diameter, 10 - 50 meter length) without any additives, volume of which were hermetically maintained. After five months of fermentation were the samples of silages determined for nutrient experiment opened and from average samples we evaluated parameters of nutritive value and fermentation process according to the AOAC (1995). Organic acids levels were determined by gas chromatography, alcohol by the microdiffusion method according to Conway (1962). Material for experiment was obtained from farm Mladějovice, Šternberk in the Czech Republic.

A feeding trial was carried out with 40 Holstein bulls, which were randomly distributed into 4 groups, with 10 bulls in each, housed in boxes. Bulls were fed TMR diet for *ad libitum* intake.

The diets were fed twice daily at 6.00 and 16.00 h in two equal doses. Water was provided *ad libitum*. The experiment lasted for 258 days. Detail dissection of right half carcass was done 24 hours after slaughtering to obtain weight and proportion of basic tissues (muscle, fat and bones). Weight of valuable cuts was calculated as a sum of weight of round (boneless round without back shank), shoulder (boneless shoulder without front shank), back (boneless *musculus longissimus thoracis* between 9th *thoracic vertebra* and 10th *thoracic vertebra*) and tender loin. The samples (approximately 500 g) were taken from MLTL (*musculus longissimus thoracis et lumborum*) and for the first time they were analysed as a fresh meat for physic-chemical meat quality after 48 hours of chilling.

Table 1 Content of nutrients and mycotoxins in crimped isogenic maize grain and crimped transgenic maize grain (g.kg^{-1} original matter)

Parameter	Crimped isogenic maize grain (DKC5143)	Crimped transgenic maize grain MON 810
Dry matter	642.2±0.3	616.6±0.1
Crude protein	68.4±1.7	65.7±1.5
Fat	33.4±0.5	25.3±1.1
Crude fibre	9.6±0.1	16.6±0.3
Starch	438.1±0.3	435.2±0.1
ADF	17.8±0.1	23.6±1.1
NDF	87.3±0.1	75.7±0.6
ME (MJ.kg^{-1} dry matter)	14.48	14.43
NEF (MJ.kg^{-1} dry matter)	6.45	6.45
Fumosidines (FUM) *	1250	trace
Results of fermentation process of high moisture corn silage		
pH	4.54	4.41
Acetic acid	0.89	1.01
Propionic acid	0.62	0.77
Butyric acid	0.03	0.08
Lactic acid	3.48	3.05
Total amount of acid	5.17	5.15
Alcohol	0.05	0.05

NEF = net energy of fattening, *Total were of quantification in μg.kg^{-1}

Table 2 Composition of the TMR diet

Ingredients (kg.d^{-1})	Daily feed intake	Ingredients (kg.d^{-1})	Daily feed intake
Maize silage	10.5	Rape meal	0.5
Lucerne silage	5.0	Vitamin- mineral mix*	0.2
Meadow hay	1.0	Crimped maize grain	2.8
Wheat	0.6		

*The supplement vitamin- mineral mix provided (per kg of additive): calcium carbonate, wheat, monocalcium phosphate, sodium chloride, zinc chelate and amino acid n-hydrate, sugar beet molasses, vit. A, D3, E, copper sulphate pentahydrate ($CuSO_4.5H_2O$), dioxide magnesia

Table 3 Nutritive value of TMR the diets

Parameters (g.kg^{-1} DM)	Control group	Experimental group	Parameters	Control group	Experimental group
Crude protein	139.8	138.6	Ca:P	2.51	2.63
NEF (MJ.kg^{-1})	6.4	6.4	K:Na	5.21	5.57
Crude fibre	155.3	162.4	Parameters (mg.kg^{-1})		
Starch	264.5	251.4	Zn	68.6	87.6
Fat	28.8	21.6	Cu	20.4	21.7
Ca	9.2	8.5	I	1.0	1.1
P	3.7	3.2	Se	0.4	0.5
Na	2.1	2.0	Parameters IU.kg^{-1}		
Mg	2.2	2.2	Vit. A	3 118	2 033
K	10.7	11.0	Vit. D	926	1 017

Chemical parameters of meat (proteins, fat and total water content) were analysed afterwards, when no more changes in chemical composition of meat are in progress. The devices Nicolet 6700 Spectrometer or Infratec 1265 with the application module for fat content assessment 1 - 10% were used. The pH meter Toledo with combined stab electrode was used to measure pH value. Water holding capacity was analysed by the Gramm Hama method. Meat colour (values L, a and b) was measured by the MiniScan XE plus.

The results were quoted as mean ± standard deviation (SD); statistical evaluation of the results was performed by the one-way ANOVA and Tukey test.

Results and Discussion

The diet consisted of maize silage, lucerne silage, meadow hay, wheat, rape meal, minerals and crimped isogenic maize grain or transgenic maize (Table 2). Water was provided *ad libitum*. The nutrient compositions of diets revealed no major differences. The TMR contained 138.60 - 139.84 g.kg^{-1} crude protein, 155.3 - 162.4 g.kg^{-1} crude fibre, 251.36 - 264.47 g.kg^{-1} starch, fat 21.62 - 28.8 g.kg^{-1}, Ca 8.552 - 9.21 g.kg^{-1}, P 3.242 - 3.67 g.kg^{-1} and energy value NEF (net energy of fattening) its 6.43 - 6.45 MJ in kg dry matter (is presented in Table 3; standard of analysis according to AOAC,1995).

Increased content of mycotoxins was observed in isogenic maize (1250 µg.kg^{-1}). All samples were contaminated by toxins under the EU limit (Table 1). By the valuation fermentative of the process at both of them silage from moist maize grain are found out that he course was he at both of them silage similar. Silage from grain GM maize had lower pH and by over little higher contents of acetic acid and propionic acid. The content of lactic acids was lower than at silage from grain izogenic maize.

The feeding trial was conducted with 2 x 20 growing bulls, Holstein breed, from average live weight 298 kg to the average slaughtering weight 620 kg in control and 622 kg in experimental group (Table 4). The live weight gain was 1.248 kg.d^{-1} and 1.255 kg.d^{-1}. Several existing studies, which compare the substantial equivalence between conventional and transgenic plants, applied usually to the production of fodder mixture for farm animals (Flachowsky et al., 2005; Sampietro et al, 2011; EFSA, 2008). Metabolism and performance data revealed no significant differences between the bulls that received the TMR with conventional, non-modified maize, and those that received the TMR with modified maize diets.

Table 4 Performances of bulls in response to dietary supplementation of isogenic maize and transgenic maize

Parameters (n=20)	Control group	Experimental group
Initial weight (kg)	298	298
Final weight (kg)	620	622
Daily weight gain (kg.day^{-1})	1.25±0.2	1.26±0.1

P >0.05; Means ±SD

The effect of addition of crimped isogenic maize grain or crimped transgenic maize grain to TMR is shown in figure 1. Average daily gains during the experiment in the control group were 1.25 kg.day^{-1} and the values ranged from 0.79 kg.day^{-1} to 1.60 kg.day^{-1}. Average daily gains in the experimental group were at 1.26 kg.day^{-1}, the range we have seen from 1.14 kg.day^{-1} to 1.39 kg.day^{-1}. In this group the variability was not significant. The health conditions are evaluated subjectively. Either in experimental or control group were not problems with the hygienic conditions of the animals, the weight gains were on adequate levels. No significant differences were found among experimental groups in feed intake and body weight in the fattening experiment (Table 4). It had no adverse effects on feed conversion, growth performance and health status. The transgenic maize in TMR has no deleterious or unintended effects.

Table 5 Physico-chemical characteristics of meat Holstein **bulls (M**usculus longissimus thoracis et lumborum - MLTL) 48h post mortem

Parameters	Unit	Control group			Experimental group		
		Minimum	Maximum	SD	Minimum	Maximum	SD
Total water content	g.100g^{-1}	74.7	75.1	0.2	75.0	75.7	0.15
Content of proteins	g.100g	21.7	21.6	0.05	21.2	21.5	0.15
Content of fat	g.100g	2.2	2.6	0.2	1.5	1.9	0.2
Energetic value	kJ.100g^{-1}	416.64	461.44	1.57	416.64	442.1	12.73
pH 24h		5.76	5.79	0.01	5.83	5.81	0.01
Colour L	%	23.62	24.37	0.37	25.48	25.44	0.02
Water holding capacity	g.100g^{-1}	33.07	34.12	0.52	36.56	35.74	0.41

An important parameter of meat quality is the end pH value, which affects water binding capacity, color of the meat and its tenderness, and thus plays a key role in sustaining meat quality during storage and the length of shelf life. As presented in Table 5, no differences between the pH values of the control and experimental groups were determined. Feeding GM maize had no influence on the other characteristics of meat quality.

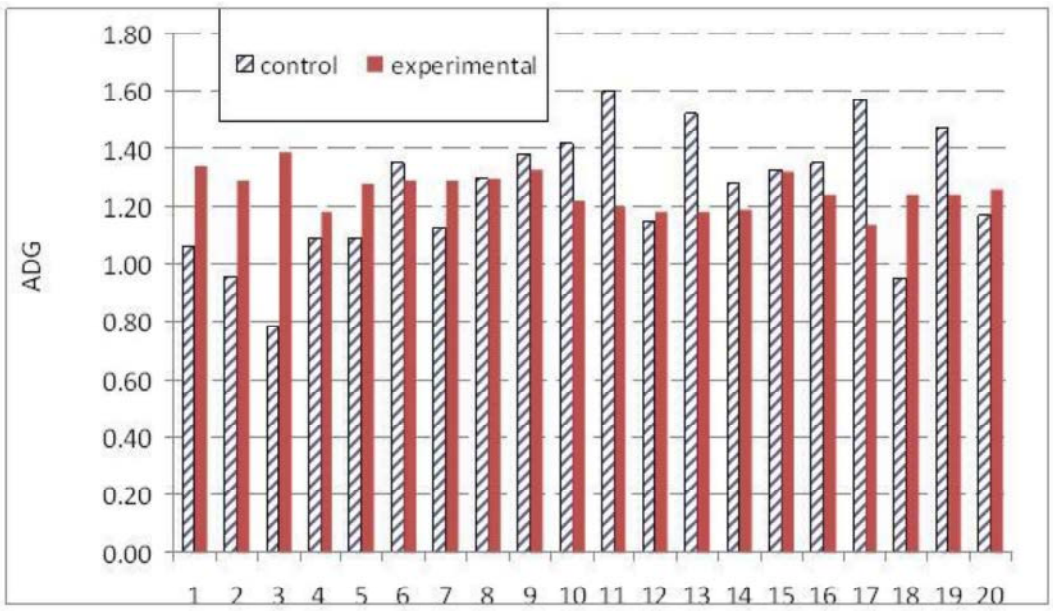

Figure 1 Comparison of daily weight gain in the fattening experiment; bulls (1-20) were fed TMR with content of isogenic maize (control group) or transgenic maize (experimental group).

Conclusion

Feeding TMR with GM maize MON 810 and isogenic maize to bulls did not influence biochemical and zootechnical parameters, as well as it had no negative effect on health status and growth performance of animals. No significant differences were found between the individual nutrients which corresponded with the results of live weight in slaughter bulls. There was no effect of diet on pH value, chemical composition of meat and its colour.

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