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NUTRITIONAL EVALUATION OF GENETICALLY MODIFIED MAIZE CORN PERFORMED ON RATS

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The aim of this study was to determine the composition and nutritional value of conventional and transgenic, so-called Roundup Ready (RR) maize with an introduced gene of glyphosate resistance. Crude protein, crude fibre, ash, fat, starch, sugar, amino acids, fatty acid and macroelement levels were determined by chemical analysis. In both maize lines a low level of Ca ($0.15\,g{\cdot}kg^{-1}$ DM) and of the essential amino acids lysine and tryptophan (2.6 and $1.7\,g{\cdot}kg^{-1}$ DM, respectively) were observed. In the biological experiment carried out on rats the tested maize lines were the only dietary sources of nitrogen, thus, the experimental diets contained 9% CP in dietary dry matter. In the feeding experiment no significant differences in the protein efficiency ratio (PER) were observed between groups receiving conventional or transgenic maize (1.51 and 1.41, respectively). Also almost equal results were obtained in the balance experiments. Both maize lines revealed a high nitrogen digestibility (84.9 and 84.5%, respectively) and the net protein utilization amounted to 63.5 and 63.2%, respectively. From these results can be concluded that regarding nutrient composition and utilisation, genetically modified (RR) maize is equivalent to isogenic maize.

Keywords: Genetically modified feeds; Roundup Ready maize; Substantial equivalence; Rats; Nitrogen digestibility; Nitrogen utilization

1. INTRODUCTION

With the increasing population and the decreasing area of land available for food production the development and use of genetically modified crops is considered as an important tool to ensure global food security. The Global Review of Commercialised Transgenic Crops (James, 1999) states that between 1996 and 1999 the area of genetically modified (GM) crops grown globally increased from 2 to 40 million ha at adoption rates which are unprecedented and the highest for any new technology in agriculture. While in 2000, the area cultivated had increased, the rate of increase has slowed down due to the controversial nature of the technology. While North America and Argentina were responsible for the vast majority of the area grown, in the European Union the cultivation of transgenic feed crops on large areas is limited. The same is true for the Slovak Republic. In spite of these facts nowadays 60 to 70% of foodstuffs come into contact with gene technologies (Phipps and Beever, 2000). While the so-called red gene techniques used in medicine has been accepted, discussions on the

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so-called green gene techniques used in agriculture and in food industry are still controversial and emotional (Hodges, 1999, 2000). The genetical modifications of plants are aimed at herbicide tolerance (29.6%), insect resistance (23.8%), increase of product quality (20.2%), viral resistance (9.9%), fungal resistance (4.7%) and improvement of agronomical properties (4.8%) (Payne, 1998).

The primary objective in animal nutrition is to assess if that plants modified for herbicide tolerance crops are nutritionally equivalent to unmodified commercial varieties and if they present a risk compared to their conventional counterparts.

The aim of this study was to compare the nutritive value of transgenic and conventional maize by chemical and biological methods.

2. MATERIALS AND METHODS

Experiments were carried out on conventional (*Zea Mays* L.) and genetically modified Roundup Ready maize grain (RR maize) of the same line (MONSANTO, USA). The transgene of glyphosate (N-phosphonomethyl)glycine, produced under the technical name Roundup, inhibits the activity of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme (Cox, 1998). This transgene encodes an analogous enzyme by origin in *Agrobacterium tumefaciens*, sp. Strain, which is not blocked by glyphosate.

2.1 Chemical methods

The analytical methods of STN 467092 (1981) were used to estimate crude protein, crude fibre, crude fat and crude ash contents. The Van Soest analysis (Lutonská and Pichl, 1983) was used to determine NDF and ADF. Starch was determined by polarimetric method (STN 46 7092, 1981) on Polarimeter ADP 220 (Bellingham & Stanley Ltd., UK) and both total and reducing sugars by Luff-Schoorl method (STN 46 7092, 1981). Higher fatty acids were determined as methyl esters by gas chromatography on GC 800 TOP (CE Instruments ThermoQuest, Italia S.p.A.) using wide bore columns with an ID of 0.53 mm and a length of 30 m with a stationary phase (50% cyanopropylphenyl – methylpolysiloxane). After dry mineralization the mineral elements (Ca, Mg, Na and K) were determined on an AAS Unicam 939 (Cambridge, UK), P by spectrophotometry using a molybdovanadate agent on a Spekol 11 (Carl Zeiss, Jena), amino acids after acid hydrolysis with 6M HCl, sulphuric amino acids after oxidation hydrolysis and tryptophan after alkalic hydrolysis on the AAA 400 (Ingos Prague, Czech Republic). Metabolizable energy was calculated (according to Hoffmann and Schiemann, 1980).

2.2 Biological methods

Digestibility of crude protein, the biological protein value (BPV), net protein utilization (NPU), utilizable protein (UP), protein efficiency ratio (PER) and feed consumption per 1 g of weight gain were estimated on Wistar rats of SPF husbandry (Velaz Prague) with an initial weight of approximately 75 g. The animals were weighed before preliminary and experimental period, at beginning and end of balance experiment and end of feeding experiment, respectively. Each group consisted of 10 animals. The animals were kept individually in balance cages. The course of the whole experimental period (29 d) was divided into a preliminary period (8 d) and a experimental period (21 d; feeding and balance

TABLE I Composition of experimental diets [% DM]

Ingredient	*Conventional maize*	*Transgenic maize*
Maize	94.1	93.8
Oil	3.7	3.8
Mineral mixture[1]	1.3	1.4
Vitamine mixture[2]	0.9	0.9

[1] 15 g $CaCO_3$, 14 g $Ca_3(PO_4)_2$, 10 g K_2HPO_4, 8 g NaCl, 7 g Na_2HPO_4, 5 g $MgSO_4 \cdot 7H_2O$, 0.48 g Fe citrate, 0.45 g $MnSO_4$-$4H_2O$, 0.0195 g $CuSO_4 \cdot 5H_2O$, 0.0005 g KI, 0.01 g NaF, 0.04 g $ZnCO_3$

[2] 5000 IU vit. A, 500 IU vit. D_3, 50 mg vit. E, 1 mg vit. K_3, 20 mg vit. C, 20 mg vit. B_1, 20 mg vit. B_2, 10 mg vit. B_6, 50 mg Ca pantotenate, 50 mg nicotinic acid, 1000 mg choline·HCl, 2 mg folic acid, 100 mg inositol, 200 mg p-aminobenzoic acid, 30 mg vit. B_{12}, ad 20 g starch.

experiment; balance experiment lasted from day 8 to 14 of this period). Urine and feces were collected quantitatively once daily. Urine was collected into 5% v/v sulfuric acid. Daily feces samples were frozen at −20°C. Afterwards total amounts of feces and urine collected were homogenised and analysed for nitrogen and DM content. Throughout the preliminary and balance periods each animal received 150 mg N daily. Feed was offered once a day. Maize grain was the only nitrogen source in the experimental diets (Table I). Because the content of CP in maize was only 9.76% and 9.66% for conventional and transgenic maize, respectively, it was impossible to compose experimental diets with 10% CP as suggested by Eggum (1973) and Heger *et al.* (1990). The individual components were calculated according to AOAC (1975). The content of nitrogen in the experimental diets with conventional and transgenic maize was 1.42 and 1.39 g N per 100 g original matter. PER values were estimated for a period of 21 d (feeding experiment) and BPV for 7 d (balance experiment). UP was calculated by multiplying the crude protein content and NPU. NPU was calculated by multiplying the BPV and protein digestibility (Heger *et al.*, 1990). The maintenance requirement of N (MRN) and metabolic N of the faeces (MNF) were calculated from the regressioN equations (Metodické pokyny, 1986):

$$\mathrm{MRN} = 0.222x + 22.93$$

$$\mathrm{MNF} = 0.081x + 3.01$$

where x is the mean weight of the laboratory rat in the balance period.

The rat experiments were carried out according to the method of Eggum (1973) and Heger *et al.* (1990). We also observed the health state of the rats, which were killed after completion of the experiment and their internal organs subjected to examination. Visual inspection did not reveal any pathological changes.

3 RESULTS AND DISCUSSION

Chemical analyses (Tables II, III and IV) revealed that, by its composition, genetically modified maize did not differ from conventional unmodified maize. From this follows that a substantial equivalence is given between both maize varieties. There exist only very slight

TABLE II Comparison of nutrient contents [g·kg^{-1} DM] and metabolizable energy [MJ kg^{-1} DM] in conventional and transgenic maize (n = 4)

	Conventional maize	Transgenic maize
Crude protein	97.3	96.6
Crude fibre	17.2	15.3
NDF	268.0	264.3
ADF	27.9	26.2
Hemicellulose	240.1	238.1
Crude Fat	39.5	41.0
Crude Ash	13.9^{a}	12.7^{b}
Starch	729.0	735.8
Sugar	37.0	31.2
Reducible sugar	9.0	8.9
Nitrogen-free extract	834.2	831.6
Organic matter	986.1	987.3
Ca	0.15	0.15
P	1.4	1.8
Mg	1.0	0.9
Na	0.01	0.02
K	3.3	3.0
Metabolizable energy	16.54	16.77

Means with different superscript letters are significantly different ($P<0.01$)

differences between the basic components (Table II), which were except for crude ash statistically not significant. Both maize varieties have a high starch contents (729 g·kg^{-1} DM and 736 g·kg^{-1} DM) and thus also a high NFE content (830 g·kg^{-1} DM). The fat and CP levels reached 40 g·kg^{-1} and 97 g·kg^{-1} DM, respectively, whereas the proportion of fibre was low

TABLE III Amino acid analysis of RR-maize grains and conventional control (n = 3)

	Conventional maize		*Transgenic maize*	
	[g·kg^{-1} DM]	*[g·16g^{-1} N]*	*[g·kg^{-1} DM]*	*[g·16g^{-1} N]*
Aspartic acid	5.7	5.85	5.6	5.84
Threonine	3.0	3.11	3.0	3.12
Serine	4.1	4.20	4.0	4.16
Glutamic acid	16.5	16.98	15.6	16.20
Proline	8.1	8.35	7.6	7.92
Glycine	3.4	3.45	3.4	3.49
Alanine	6.6	6.78	6.5	6.78
Valine	4.0	4.10	4.3	4.45
Isoleucine	2.9	2.98	3.2	3.31
Leucine	11.3	11.64	11.6	12.03
Tyrosine	3.4	3.50	3.5	3.62
Phenylalanine	4.3	4.40	4.4	4.54
Histidine	2.9	2.95	2.9	3.01
Lysine	2.6	2.69	2.6	2.74
Arginine	3.7	3.83	3.7	3.81
Cysteine	1.9	1.97	2.1	2.19
Methionine	2.3	2.41	2.3	2.40
Tryptophan	1.7	1.75	1.7	1.76

Means do not differ significantly ($P>0.05$)

TABLE IV Fatty acid pattern of RR-maize grains as compared to that of the corresponding conventional control [percent of total fatty acids] (n = 3)

		Conventional maize	*Transgenic maize*
Lauric acid	$C_{12:0}$	0.07	0.05
Myristic acid	$C_{14:0}$	0.07	0.09
Palmitic acid	$C_{16:0}$	13.78	15.80
Palmitoleic acid	$C_{16:1}$	0.11	0.10
Stearic acid	$C_{18:0}$	1.80	1.40
Oleic acid	$C_{18:1}$	29.46	32.65
Linoleic acid	$C_{18:2}$	52.57	47.78
Linolenic acid	$C_{18:3}$	0.90	1.25
Arachidic acid	$C_{20:0}$	0.46	0.40
Arachidonic acid	$C_{20.4}$	0.22	0.12
Behenic acid	$C_{22:0}$	0.45	0.34
Eruic acid	$C_{22:1}$	0.11	0.03

(17 and 15 g·kg^{-1} DM). Also regarding the content of metabolisable energy no significant differences were observed (16.5 and 16.8 MJ ME·kg^{-1} DM). One of the typical insufficiencies of maize is its low level of minerals, mainly Ca. The existing studies, which compare the substantial equivalence between conventional and transgenic plants, are focused on soybean (Padgette *et al.*, 1995; Taylor *et al.*, 1999). Some studies compared also conventional and Bt maize (Flachowsky and Aulrich, 2001a, b; Aulrich *et al.*, 2001a and b) but also other crops like sugar beet (Bartsch and Pohl-Orf, 1996; Böhme *et al.*, 2001). In none of the aforementioned papers statistically significant differences were observed between isogenic and transgenic crops.

The amino acid composition of transgenic and conventional maize reveals, similarly to other cereal feeds, a lack of two essential amino acids, lysine and tryptophan (0.26 and 0.17% in DM; Table III). Although the gene inserted into RR maize acts by inhibiting the activity of the enzyme which is part of the synthetic path of aromatic amino acids (phenylalanine, tryptophane and tyrosine), no differences were observed for the content of these amino acids. Non-significant differences in the contents of nutrients between transgenic and conventional soybean were also confirmed by Harrison *et al.* (1996) and Padgette *et al.* (1996). Evaluation of conventional and transgenic Bt maize was similar (Aulrich *et al.* 2001). Padgette (1996), who compared the amino acid levels in genetically modified glyphosate-tolerant soybean also indicated the differences to be non significant.

The fatty acid patterns of both maize lines (Table IV) demonstrated similar proportions and coincide with the results of Aulrich *et al.* (2001b). Certain differences could be observed to range within the standard interval of the contents of these substances in maize grain. In the published literature no significant differences between transgenic and isogenic crops were reported.

The biological experiments conducted with rats (Table V) proved that there were no statistically significant differences between the animals fed mixtures containing isogenic or transgenic maize. In both hybrids high digestibility of nitrogen was observed (84.9% and 84.5%, respectively) and the difference between the PER values was nonsignificant (1.51 and 1.41, respectively). BPV, NPU and UP values were almost identical. Similarly other authors (Fuchs *et al.*, 1996, Flachowsky and Aulrich, 2001a, b) did not observe any significant differences in balance experiments with other animal species. Brake and Vlachos (1998)

TABLE V Results of testing conventional and transgenic maize in experiments on rats (n = 10)

	Conventional maize	Transgenic maize
Weight gain per feeding period (21 d) [g]	35.5 ± 4.90	34.2 ± 2.40
Feed consumption [g/g BWG]	8.13 ± 1.36	8.24 ± 0.94
PER	1.51 ± 0.15	1.41 ± 0.14
BPV [%]	74.06 ± 3.96	75.11 ± 5.53
Digestibility of CP [%]	84.92 ± 1.94	84.50 ± 1.82
NPU[%]	63.53 ± 4.44	63.24 ± 4.97
UP [%]	12.88 ± 0.09	12.82 ± 1.01
Retained N from N received [%]	42.46 ± 4.10	42.26 ± 5.07
Retained N from N digested [%]	50.32 ± 4.40	50.68 ± 6.12

All differences are not significant ($P > 0.05$)

examined the effects of feeding mixtures that contained more than 60% conventional and transgenic Bt maize to broiler chicks. Flachowsky and Aulrich (2001b) performed numerous experiments (poultry, pigs, ruminants) which dealt with the substantial equivalence of transgenic products with conventional source lines. So far, results published have not revealed any significant differences. Similar data were reported by Donkin *et al.* (2000) who examined the effect of feeding Roundup Ready corn silage and grain on feed intake, milk production and milk composition in lactating dairy cattle. The diets fed contained 62% corn silage and 17% corn maize from the RR and ISO lines, respectively. The data obtained demonstrated the performance of lactating cows fed RR corn and its conventional counterpart to be similar.

4. CONCLUSION

It can be concluded that the genetically modified (RR) maize used in our experiments revealed equivalence in chemical composition and nutritional value compared to conventional maize. Only small differences were found between the individual components which were statistically non significant and corresponded with the results of other studies.

Furthermore, genetically modified (RR) maize, which was included in diets of rats at an amount of 94% had no effects on production, consumption and digestion of nutrients or on the health status of the animals.

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