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Caecal fermentation in rats fed diets containing transgenic potato tubers

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ABSTRACT

Caecal fermentation in rats fed diets with 40% autoclaved potato tubers was examined. The potato tubers of the conventional cultivar, Irga, somaclone Irga, and four transgenic lines with genetically improved resistance to a necrotic strain of potato virus Y (PVY^N) were compared. As regards the analysed indices, tubers of transgenic clone R1F (truncated gene coding PVY^N polymerase in *sense* orientation), R2P (truncated gene coding PVY^N polymerase in *antisense* orientation), and NTR1.16 (non-translated regions of the PVY^N genome in *sense* orientation) were similar to tubers of the conventional cultivar Irga. Tubers of transgenic clone NTR2.27 (non-translated regions of PVY^N genome in *antisense* orientation) increased the amount of caecal digesta and production of SCFA compared with tubers of the conventional cultivar and other transgenic clones.

KEY WORDS: genetically modified potato, caecum, short-chain fatty acids, rat

INTRODUCTION

The first Polish transgenic clones resistant to a necrotic strain of potato virus Y (PVY^N) were obtained in the last decade (Chachulska et al., 1997). The results of our earlier studies indicate that genetically modified potato did not differentiate animal growth and feed utilization (Zduńczyk et al., 2004a) as well as serum enzymes and indices of non-specific defence of rats (Zduńczyk et al., 2004b), compared with non-transgenic tubers. The purpose of this study was to evaluate the potential influence of the transgenic potato on the fermentation processes in the caecum of rats.

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MATERIAL AND MTHODS

The transgenic lines of potato that were transformed with viral genome sequences in order to improve their resistance to a necrotic strain of potato virus Y (PVY^N) were prepared at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences (Chachulska et al., 1997). Four transgenic clones: R1F and R2P (transgenic lines with a truncated gene coding PVY^N polymerase in *sense* or *antisense* orientation, respectively), NTR1.16 and NTR2.27 (transgenic lines with non-translated regions of PVY^N genome in *sense* or *antisense* orientation, respectively) were compared with the conventional cultivar Irga and a non-transgenic somaclone (Irga *w.t.*). The autoclaved (121°C, 1013 hPa, 15 min) and dried (40°C) potato tubers were introduced in a high amount (40%) to diets, which were used in a 3-week feeding experiment on rats. Each diet contained about 9.3% crude protein in air-dry matter (5.3% of casein and 3.6% potato protein), potato and maize starch (about 27 and 36%, respectively), and standard mineral and vitamin mixtures. Samples of fresh caecal digesta obtained from 8 animals in each group were analysed. The caecal pH was measured using a microelectrode and a pH/ION meter (model 301, Hanna Instruments). The content of short-chain fatty acids (SCFA) was determined by gas chromatography (Shimadzu GC-14A with a 2.5 m × 2.6 mm glass column, containing 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb W AW, column temperature 110°C, detector FID temperature 180°C, injector temperature 195°C). The results were statistically evaluated using one-way ANOVA and Duncan's multiple range test.

RESULTS

The weight of caecal digesta of rats fed diets with tubers of transgenic clone NTR2.27 was significantly higher than in the groups receiving Irga *w.t.* and R1F (Table 1). The lowest hydration of caecal digesta was found in groups R2P and NTR1.16, while the highest was in group R1F. The highest amount of dry matter in the caecum was found in group NTR2.27. The caecal pH in group NTR1.16 increased, especially in comparison with non-transgenic Irga *w.t.*

TABLE 1

Caecal parameters of rats fed diets containing conventional and transgenic potato tubers

	Irga	Irga *w.t.*	Irga - transgenic clone				SEM
			R1F	R2P	NTR1.16	NTR2.27	
Caecal digesta, g	0.98^{ab}	0.90^{b}	0.87^{b}	0.95^{ab}	0.99^{ab}	1.09^{a}	0.02
Dry mater, %	18.6^{ab}	18.7^{ab}	20.5^{a}	17.6^{b}	18.0^{b}	19.3^{ab}	0.29
Dry matter, g	0.178^{b}	0.171^{b}	0.179^{b}	0.170^{b}	0.178^{b}	0.204^{a}	0.01
pH of digesta	6.99^{ab}	6.89^{b}	7.03^{ab}	7.08^{ab}	7.21^{a}	6.99^{ab}	0.04

^{a,b} - values within each row with the same superscript do not differ at $P \leq 0.05$

The lowest concentration of SCFAs in the caecal digesta was found in group R2P, and the highest one, in group Irga *w.t.* (Table 2). A similar distribution of SCFA concentrations was also found for C_2, C_4 and C_5 acids. No significant differentiation was observed in the concentrations of C_3 acids in particular groups. The lowest values of the total SCFA pool size and of the major acids (C_2-C_4) were found in the R2P group, while the highest values of the total SCFA pool and individual acids were determined in rats fed a diet supplemented with the NTR2.27 line.

TABLE 2

SCFA concentration (μmol/g) and SCFAs pool (μmol/100 g BW) in the caecal digesta

	Irga	Irga *w.t.*	Irga - transgenic clone				SEM
			R1F	R2P	NTR1.16	NTR2.27	
SCFA concentration							
total	66.07^{ab}	71.61^{a}	60.67^{b}	53.05^{c}	59.51^{bc}	67.82^{ab}	1.76
acetate - C_2	47.63^{ab}	52.48^{a}	43.71^{bc}	38.31^{c}	42.65^{bc}	49.05^{ab}	1.04
propionate - C_3	8.83	8.64	8.39	7.45	8.48	8.71	0.19
isobutyrate - C_{4i}	0.81^{ab}	0.94^{a}	0.94^{a}	0.65^{b}	0.78^{ab}	0.89^{a}	0.02
butyrate - C_4	6.47^{a}	6.84^{a}	5.46^{ab}	4.75^{b}	5.32^{ab}	6.62^{a}	0.22
isovalerate - C_{5i}	0.93^{ab}	0.98^{ab}	1.00^{a}	0.78^{b}	0.89^{ab}	0.94^{ab}	0.03
valerate - C_5	1.40^{bc}	1.73^{a}	1.17^{cd}	1.11^{d}	1.39^{bc}	1.61^{ab}	0.05
SCFA pool							
total	64.75^{ab}	64.45^{ab}	52.78^{bc}	50.40^{c}	58.91^{ab}	73.92^{a}	2.23
acetate - C_2	46.68^{ab}	47.23^{ab}	38.03^{bc}	36.39^{c}	42.22^{ab}	53.46^{a}	1.62
propionate - C_3	8.65	7.78	7.30	7.08	8.40	9.49	0.34
isobutyrate - C_{4i}	0.79^{ab}	0.85^{a}	0.82^{ab}	0.62^{b}	0.77^{ab}	0.97^{a}	0.03
butyrate - C_4	6.34^{ab}	6.16^{ab}	4.75^{bc}	4.51^{c}	5.27^{abc}	7.22^{a}	0.28
isovalerate - C_{5i}	0.91	0.88	0.87	0.74	0.88	1.02	0.04
valerate - C_5	1.37^{ab}	1.56^{a}	1.02^{b}	1.05^{b}	1.38^{a}	1.75^{a}	0.06

^{a,b,c,d} - values within each row with the same superscript do not differ at $P \leq 0.05$

DISCUSSION

The results of a few *in vivo* experiments of other authors (Hashimoto et al., 1999; Rogan et al., 2000; Zduńczyk et al., 2004a,b) indicate that tubers of potato with genetically improved virus- or insect-resistance were nutritional equivalents of their conventional counterparts. In the presented study tubers of the conventional cultivar Irga and transgenic clones R1F, R2P and NTR1.16 exerted a similar influence on the fermentation processes in the caecum of rats. However, a diet with tubers of transgenic clone NTR2.27 significantly increased the amount of caecal digesta, dry matter content and total SCFA pool. This may point to decreased utilization of nutrients (mainly starch) in the upper part of the gastrointestinal tract and/or to an increase in the activity of caecal microflora in

this group of rats. The results obtained in a relatively short feeding experiment (3 weeks) are insufficient to explain the reasons for the determined differences: increased bulk of caecal digesta and total SCFA pool in group NTR2.27, as well as the highest hydration of caecal digesta in group R1F.

CONCLUSIONS

As regards their influence on caecal fermentation processes in rats, tubers of transgenic clone R1F, R2P, and NTR1.16 were similar to tubers of the conventional cultivar Irga. In contrast, tubers of transgenic clone NTR2.27 increased the bulk of caecal digesta and production of SCFA.

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STRESZCZENIE

Fermentacja w jelicie ślepym szczurów żywionych dietami z bulwami ziemniaków linii trangenicznych

Porównano wpływ diet z zawartością 40% autoklawowanych bulw ziemniaków konwencjonalnej odmiany Irga, bulw z hodowli *in vitro* oraz czterech klonów transgenicznych o genetycznie zwiększonej odporności na nekrotyczny szczep wirusa Y ziemniaków (PVY^N) na procesy fermentacyjne w jelicie ślepym szczurów. Pod względem analizowanych kryteriów bulwy klonu R1F i R2P (z wstawką niekompletnego genu kodującego PVY^N polimerazę, odpowiednio w orientacji *sens* i *antysens*) oraz klonu NTR1.16 (z wstawką fragmentu cDNA wirusa PVY^N w orientacji *sens*) były podobne do bulw konwencjonalnej odmiany Irga. Bulwy klonu NTR2.27 (z wstawką fragmentu cDNA wirusa PVY^N w orientacji *antysens*) zwiększały ilość treści jelita i produkcje LKT w porównaniu z bulwami odmiany konwencjonalnej.