

# The effect of transgenic cucumbers expressing thaumatin on selected immunity parameters in rats\*

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## ABSTRACT

The effect of a diet containing the fruits of genetically modified cucumber plants expressing thaumatin on the non-specific resistance parameters of the consumer was determined. For five weeks 24 laboratory rats allocated to 3 groups were fed freeze-dried isoproteinous diets containing 15% transgenic or non-transgenic cucumber fruits of the same variety, or a standard mixture. The phagocytic activity of neutrophils and monocytes was determined by flow cytometry (Phagotest and Bursttest). Feeding transgenic cucumber caused a statistically significant increase in neutrophilic granulocytes that activated oxidation processes after stimulation of PMA and fMLP. The fluorescence intensity of neutrophils was higher when *E. coli* was used as the chemotactic agent.

KEY WORDS: transgenic cucumber, thaumatin, rats, GMO safety

## INTRODUCTION

Introduction of the gene controlling the synthesis of a sweet protein, thaumatin (Szwacka et al., 2000), may considerably affect the diversification and feed use of plants. Synthesis of new protein may reveal allergenic or immunogenic activity and have unknown biological activities.

An unpredictable risk results from possible changes in the concentration of secondary metabolites and from interactions between the components of the diet

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(Conner and Jacobs, 1999). In the reported studies, the response of the immune system of rats receiving a diet containing transgenic cucumbers was assessed on the basis of non-specific resistance parameters.

## MATERIAL AND METHODS

The cucumber fruits of variety Borszczagowski, containing the gene coding the synthesis of thaumatin (Szwacka et al., 2000) were cultivated at the Department of Plant Genetics, Breeding and Biotechnology of Warsaw Agricultural University. The fruits of transgenic and non-transgenic plants were collected in similar growth and maturity phases, cut frozen (-30°C) and freeze dried. The transgenic cucumbers contained more crude protein and less crude fibre and somewhat less mineral compounds as compared with the non-transgenic fruits (Kosieradzka et al., 2001). The nutritional experiment lasted 5 weeks. Twenty-four male rats from the outbred IF<sub>2</sub>Jaz herd with a mean body weight of about 150 g were divided into 3 groups and kept in individual cages. The rats were fed a semisynthetic isoproteinous diet balanced according to NRC (1976). Freeze-dried transgenic and non-transgenic cucumbers constituted 15% of dry matter of the experimental diets. The control group received a diet without cucumbers. The diets were supplemented with a vitamin mixture (AOAC, 1975) and a mineral mixture (NRC, 1976), in diets with the cucumbers, the mineral supplement had the following composition: CaHPO<sub>4</sub>×2H<sub>2</sub>O, 139.44; maize starch, 12.15; K<sub>2</sub>SO<sub>4</sub>, 10.20; NaCl, 14.50; CaCO<sub>3</sub>, 3.15; Na<sub>2</sub>HPO<sub>4</sub>, 3.21; MgO, 3.75; mixture of trace elements, 2.70. After the completion of the nutritional experiment, and after 12 h of fasting, the rats were anaesthetized with ketamine; blood samples were collected from the heart into heparin-protected test tubes and cooled to a temperature of 4°C. The phagocytic activity of neutrophils and monocytes was determined by flow cytometry (Bohmer et al., 1992), using Phagotests and Bursttests (Orpegen Pharma, D-69115 Heidelberg, Germany). The results were analysed using the Statgraphics Plus 2.1 for Windows program. The significance of differences between the groups was compared by single-factor analysis of variance and Duncan's test.

## RESULTS AND DISCUSSION

Feeding transgenic cucumbers did not affect the number of phagocytising neutrophils and monocytes and fluorescence intensity related to the level of phagocytosis by neutrophils and monocytes (Table 1).

Higher activity of neutrophils, as stimulated by *E. coli*, and higher fluorescence intensity was found in the rats fed on transgenic cucumbers. A highly statistically significant difference was found between the control group and groups receiving non-

transgenic and GM cucumbers in the percentage of neutrophils capable of killing with oxygen-dependent mechanisms after fMLP and PMA stimulation (Table 2).

TABLE 1

Phagocytic activity of neutrophils and monocytes in peripheral blood (Phagotest)

Cells	Parameters	Groups			SEM	P
		control	non-transgenic	transgenic		
Neutrophils	% phagocytising cells	84.6	79.0	76.0	2.6	0.10
	mean fluorescence intensity	498.6	406.2	553.2	75.8	0.41
Monocytes	% phagocytising cells	86.6	85.2	87.8	2.8	0.21
	mean fluorescence intensity	762.6	674.6	882.2	108.5	0.92

TABLE 2

Oxidative burst activity of neutrophils in peripheral blood of the experimental rats

Stimulus	Parameters	Groups			SEM	P
		control	non-transgenic	transgenic		
<i>E. coli</i>	percent of oxidizing cells	94.0	94.4	90.0	2.126	0.306
	mean fluorescence intensity	20.5 <sup>a</sup>	22.5 <sup>a</sup>	29.8 <sup>b</sup>	2.703	0.042
fMLP <sup>1</sup>	percent of oxidizing cells	66.5 <sup>^</sup>	81.8 <sup>^</sup>	24.8 <sup>B</sup>	8.918	0.002
	mean fluorescence intensity	2.5	3.1	2.4	0.323	0.217
PMA <sup>2</sup>	percent of oxidizing cells	92.2 <sup>a</sup>	95.0 <sup>a</sup>	80.8 <sup>b</sup>	3.108	0.038
	mean fluorescence intensity	28.4	26.8	30.7	11.72	0.973

<sup>1</sup> fMLP-N-formyl-L-methionyl-L-leucyl-L-phenylalanine; <sup>2</sup> PMA-phorbol 12-myristate 13-acetate;

<sup>a,b</sup> - difference significant at  $P < 0.05$ , <sup>^,B</sup> - difference significant at  $P < 0.01$

The increase of non-specific immunity of the rats due to feeding the GM cucumber-containing diet may be a consequence of a change in the chemical composition of transgenic fruits inducing the immunological response of monocytes and neutrophils (Chandra and Sarchielli, 1993; Ferrante et al., 1996). However, earlier studies did not reveal any response of the experimental animals to the presence of thaumatin-producing cucumbers in the diet (Kosieradzka et al., 2003).

## CONCLUSIONS

The 15% addition of freeze-dried fruits of transgenic cucumbers overexpressing sweet protein to the rat diet caused an increase in the values of selected parameters of non-specific immunity, an increase in the number of neutrophils that activated oxidation processes after PMA and fMLP stimulation, and an increase in neutrophil activity, i.e. higher neutrophil fluorescence intensity when *E. coli* was used as the chemotactic factor.

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## STRESZCZENIE

**Wpływ transgenicznych ogórków wykazujących ekspresję taumatyny na wybrane wskaźniki odporności szczurów**

Określono wpływ diety zawierającej owoce genetycznie modyfikowanych roślin ogórka, wykazujących ekspresję taumatyny, na wskaźniki odporności nieswoistej organizmu. 24 szczury podzielone na 3 grupy żywiono do woli przez 5 tygodni izobiałkowymi dietami zawierającymi 15% liofilizatu z owoców ogórka transgenicznych lub nietransgenicznych tej samej odmiany lub dietą standardową. We krwi zwierząt oznaczono aktywność fagocytarną neutrofilii i monocytów metodą cytometrii przepływowej (Phagotest i Bursitest). Dodatek ogórków transgenicznych spowodował statystycznie istotny wzrost liczby granulocytów obojętnochłonnych, które uaktywniły procesy tlenowe przy stymulacji PMA i fMLP, większą intensywność fluorescencji neutrofilii przy zastosowaniu *E. coli* jako czynnika chemotaktycznego.