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Nutritive and Dietetic Value of Genetically-Modified Tomatoes Expressing Thaumatin Gene

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Genetically-modified (GM) tomatoes, carrying thaumatin gene encoding sweet-tasting protein may be a component of diet with high sensory values, constituting a valuable source of nutrients and substances with a health-promoting role. Good utilization and a lack of the effect on animal growth, value of hematological parameters, concentration of immunoglobulins and most of chemical blood parameters of laboratory rats were demonstrated in the nutritional studies on fruits of tomato GM plants. The biological response of the rats receiving GMO or its isogenic equivalent in the diet was recognized as similar. However, the unfavourable effect of the diets containing addition of tomatoes with the recombined thaumatin on the degree of oxidative degradation of DNA of rats liver was recorded. At the same time, the discussed dietary component had no effect on values of the remaining parameters of the oxidative status of tissue of the above mentioned organ and its histological image.

INTRODUCTION

In human nutrition tomatoes are a valuable source of mineral compounds, and biologically-active substances, including *e.g.*: carotenoids, flavonols, ascorbic acid, chlorogenic acid, tocopherol and rutin [Fanasca *et al*., 2006]. There are many studies indicating the relationship between consumption of tomatoes and reduced risk of the incidence of cancer diseases [Giovannucci, 2002; Campbell *et al*., 2004] and diseases of blood circulation system [Petr & Erdman, 2005]. The health-promoting effect of tomatoes is probably a result of the synergetic action of many components with anti-oxidative activity as well as of microelements, being contained in the tissues of tomato fruits, such as copper, iron and chromium [Gundersen, 2001] which may participate in redox reactions, preventing cell damage. In spite of the fact that a high dietetic value of tomatoes was confirmed in many experiments, their consumption in developed countries as well as consumption of other valuable fruits and vegetables is still decreasing and is not sufficient to cover demands of a human body for phytonutrients [Martin *et al*., 2011]. Genetic modification may induce intensification of biosynthesis and by this, an increase in the concentration of desirable, bioactive and health beneficial components of tomatoes, *e.g.* beta carotene [Romer *et al*., 2000], flavones and flavonols [Schijlen *et al*., 2006], anthocyanins [Butelli *et al*., 2008], ascorbic acid [Zhang *et al*., 2011] or lycopene [Neily *et al*., 2011]. It may also affect the improvement of organoleptic traits of fruits [Davidovich-Rikanati *et al*., 2007].

Modification, leading to the expression of a gene encoding a sweet–tasting protein – thaumatin – is an example of successful genetic transformation of tomato fruit. Fruits of GM plants may become valuable components of diet, increasing its taste values. Due to the possibility of occurrence of unintended effects of transgenesis, the suitability of fruits for this process requires experimental confirmation of their nutritive and dietetic values and evaluation of their biosafety. Thaumatin, the protein the expression of which was induced in GM plants is 2000–3000 times sweeter than saccharose and has a characteristic flavour. Thaumatin, originating from fruits of natural host of African plant ketamfe (*Thaumatococcus daniellii* Benth), was admitted to use in the European Union countries as a sweetener in food products (E-957) and on the ground of Food and Drug Administration (FDA) decision in the USA, it received GRAS (Generally Recognized as Safe)

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status. It is also utilized as a feed additive, registered in accordance with the Regulation EC No 1831/2003. Its recommended intake of 1–5 mg/kg of feed is recognized as completely safe for all animal species [EFSA, 2011]. Tomatoes containing thaumatin could be a valuable component of human diets, and by-products from processing of such tomatoes could constitute dietary additives to the feeds for selected groups of animals, medicated feeds and petfoods.

Dietary administration of GM plants affords a hypothetic possibility of changing the degree of nutrients conversion, modifying consumer's metabolism, inducing disorders in cellular processes, in functions of tissues and key organs. There is a need of eliminating the threat, causing the concern of consumers, connected with the possibility of immunomodulating, allergenic, carcinogenic or gene-toxic effects of components of the modified plant.

The aim of this study was, therefore, to determine the influence of diets, containing addition of fruits of GM tomato plant, revealing the expression of thaumatin gene, on the degree of nutrients digestion, as well as on the growth, development, metabolic functions and health status of model animals (rats).

MATERIAL AND METHODS

Plant materials – transformation and cultivation

The experimental material included fruits of GM and non-GM tomato plants – (*Solanum lycopersicum L.)*. The following plant materials were used for transformation of the plants and as non-GM tomatoes in this study,:

- Beta cultivar – obtained as a result of crossing "Jantarny" cultivar and line LD160. Its plants are dwarf, with stiff stems, and determinate growth: they have dark green leaves of intermediate type between ordinary and potato type, fruits are medium-sized, with heel; ripen fruit is of brick-red colour;
- Nor cultivar – breeding line carrying non-ripening mutation (nor) indeterminate growth, possessing dark green leaves of tomato type, fruits are yellow-green with green heel, small; they do not ripen, they can be stored for a long time [Seroczyńska *et al*., 1998].

Transformation of the plants by mediation of *Agrobacterium tumefaciens* strain LBA4404::pTiAch5::pRUR528 [Szwacka *et al*., 2001] was performed at the Department of Genetics of Breeding and Biotechnology, Warsaw University of Life Sciences (SGGW) according to Bartoszewski *et al*. [2003].

In this study, we used independent transgenic tomato lines (N446 and B310), containing 35S-pre-prothaumatin II transcriptional fusion from the *A. tumefaciens* binary vector pRUR528, bearing two chimearic genes:

- T. *danieli* pre-prothaumatin II cDNA under control of 35S CaMV promoter and *nos* terminator. Promoter 35S CaMV derives from virus of cauliflower mosaic. *Nos* terminator comes from wild strain *A. tumefaciens*. Expression of cDNA of pre-prothaumatin gene II should lead to the production of sweet-tasting protein thaumatin in all cells of transformants.
- nptII gene which provides kanamycin resistance and serves as a selectable marker, coding resistance to antibiotics from neomycin group, driven by *nos* promoter and *nos* terminator Promoter and terminator were derived from a wild strain of *Agrobacterium tumefaciens*. Coding sequence of gene nptII comes from *Escherichia coli.* Expression of nptII gene leads to generation of protein neomycin phosphotransferase in all tissues and organs of the receiver.

As a result of transformation, the T-DNA fragment of plasmid pRUR528, bearing gene nptII and cDNA of thaumatin II were transferred to nuclear genome of the receivers – tomato cultivars Beta and Nor, and several transgenic lines carrying T-DNA insertion (confirmed by Southern blot analysis) were developed, including line B310 (Beta background) and N446 (Nor background) used in this study. Western-blot analysis showed that biosynthesis of recombined thaumatin occurred in leaves and fruits of GM plants [Bartoszewski *et al*., 2003].

GM plants of tomato and the non-GM plants of the same cultivar were cultivated in glasshouse under the conditions of light regime: 16 h of light (temperature of 22 ± 5^{o}C) and 8 h in dark (temperature of 15 ± 5^{o}C) and were poured with water every day. Fruits were collected successively, after obtaining maturity, and then they were frozen and lyophilized. After grinding, fruit lyophilizate was subject to chemical analyses and stored at temperature of -20^{o} C until production of diet with its preparation for the rats. Fruits of GM plants of tomatoes of Beta cultivar (GM Beta) and Nor cultivar (GM Nor) were compared, at each stage of the experiment, with the fruits of non-GM plants of the same cultivar – their isogenic equivalents, cultivated in the same conditions.

Comparative analysis of chemical composition of fruits of GM and non-GM plants

Ground lyophilizate of fruits of GM plants and of their isogenic equivalents (non-GM) was subject to analysis in respect of the equivalency of their composition. Dry matter and chemical components of fruits: crude protein, crude fat, crude fiber, nitrogen-free extractives and Van Soest fibers (NDF – neutral-detergent fiber; ADF – acid-detergent fiber, ADL – acid-detergent lignin) and crude ash content were determined by standard methods, acc. to AOAC [1996], with the application of methodical applications and set of Tecator equipment. Mineral components (Ca, Mg, Zn, Mn, Cu, Na, K, P, Fe) – were determined by the method of flame absorption atomic spectrometry FAAS (Shimadzu AA 660), after mineralization of the sample in a microwave device (Milestone 1200 Mega). Anions Cl^{-}, SO_4^{2-} and HPO_4^{2-} were determined by HPLC (Waters) with a conductometric detector. Vitamin C – sum of ascorbic acid and dehydroxy-ascorbic acid – was determined by HPLC (Waters), with a UV/VIS and fluorescent detector with the application of a chromatograph by Waters company. Mineral compounds, anions and vitamin C were determined in accordance with the procedure approved by Polish Accreditation Centre and system of quality management (PB 1–2,6,7,8; PB 3–1, AB 439). Lycopene and beta-carotene were determined by the method of column chromatography [Saniewski & Czapski, 1983].

The analyses of chemical composition of lyophilized tomatoes of the same production cycle were repeated three times.

Animals and nutrition

Growth experiment

Wistar rats from outbreed flock with the initial body weight of *ca*. 120 g were classified into 5 experimental groups, 10 individuals in each group. The animals were kept in individual maintenance cages for 4 weeks, in the room with the stable controlled environmental conditions – light/darkness cycle 12 h/12 h; temperature of 21°C, and humidity 60%. The rats were fed *ad libitum* with the iso-protein, iso-caloric, semi-synthetic diets with the equalized fiber content, containing 30% of lyophilizate of GM tomatoes (GM Beta, GM Nor) or non-GM tomatoes (isogenic, Beta and Nor). The additional control group included rats receiving a standard synthetic diet. All diets were balanced according to nutrient requirements of animals [NRC, 1996]. Composition and nutritive value of diets for rats are given in Table 1.

Throughout the experiments, the health status of the rats was observed; all individuals were weighed once a week; the quantity of the consumed mixtures was controlled. The body weight (daily and total) gain and feed conversion per 1 g of body weight gain were determined.

TABLE 1. Composition and nutritive value of standard diet for rats, and diets with lyophilizate of genetically-modified tomato Beta cultivar (GM Beta) and Nor cultivar (GM Nor) and non-modified tomatoes of the same (Beta and Nor) cultivars.

Component	Standard	Tomatoes			
		Beta	GM Beta	Nor	GM Nor
Tomato lyophilizate (g/kg)	0	300	300	300	300
Casein (g/kg)	204	151	156	156	157
Cellulose (g/kg)	45	8	6	18	14
Soy oil (g/kg)	40	40	40	40	40
Mineral mixture* (g/kg)	35	35	35	35	35
Vitamin mixture** (g/kg)	10	10	10	10	10
Choline chloride (g/kg)	2	2	2	2	2
Dl-methionine (g/kg)	1	1	1	1	1
Maize starch (g/kg)	663	453	450	438	441
Nutritive value					
Crude protein (%)	15.62	15.65	15.61	15.64	15.62
Crude fiber (%)	4.5	4.5	4.6	4.5	4.6
Crude fat (%)	2.7	2.8	2.7	2.6	2.6
Crude ash (%)	4.1	4.3	4.4	4.3	4.2

* Mineral mixture, AIN 93G, ICN Biomedicals, Composition in g/kg: calcium carbonate 35.7; potassium phosphate 19.60; potassium citrate 7.078; sodium chloride 7.40; potassium sulphate 4.66; magnesium oxide 2.40; ferric citrate 0.606; zinc carbonate 0.165; magnesium carbonate 0.063; copper carbonate 0.03; potassium iodide 0.001; sodium selenate 0.00103; ammonium molybdate 0.000795; sodium (meta)silicate 0.145; potassium-chromium sulphate 0.0275; lithium chloride 0.00174; boric acid 0.008145; sodium fluoride 0.00635; nickel carbonate 0.00318; ammonium vanadate 0.00066; saccharose 22.1.

** Vitamin mixture, AIN 93 G, ICN Biomedicals. Composition g/kg: nicotinic acid 3.0; calcium panthotenate 1.6; pyridoxine 0.7; thiamine 0.6; riboflavin 0.6; biotin 0.02; cyanocobalamin 2.5; tocopherol 30; retinol palmitate 1.6; cholecalciferol 0.25; phytonadione 0.075; saccharose 958.855.

Digestibility experiment

Wistar rats with body weight of *ca*. 200–220 g, divided into 5 nutritional groups (10 individuals in each), kept in the individual balance cages for 7 days were fed the mixtures with the composition identical as in the growth experiment. Individual feed intake by the rats was determined and the quantitatively collected feces from individual animals and the representative samples of the administered mixtures were subject to chemical analysis. Coefficients of apparent digestibility of nutrients – crude protein, crude fiber, crude fat, crude ash N-free extractives – contained in the mixtures were calculated.

Collection and analysis of animal material

After completion of the growth experiment, the rats were killed by overdosing isoflurane, administrated by inhalation. Blood was collected from the heart and morphological, biochemical, immunological and associated with oxidative stress parameters were determined. The organs (liver, brain, kidneys, heart, spleen and small intestine) were prepared and weighed. Fragments of liver were cooled down and stored at temperature of -70°C until the time of determining the parameters of oxidative status.

After isolation and hydrolysis of DNA of liver tissue, the concentration of 8-oxo-2'deoxyguanosine (8-oxo-2'dG) and 2'-deoxyguanosine (2'dG) was determined using a Dionex HPLC with electrochemical (at 350 mV) and UV (at 254 nm) detectors and a 250×4.6 mm Supelcosil LC-18-S column (5μm grain). The amount of 8-oxo-2'dG in DNA was calculated as the number of 8-oxo-2'dG molecules per 10^6 unmodified 2'dG molecules. Thiobarbituric acid reactive substances (TBARS) in liver tissue were measured with 1.2.3.3-tetraethoxypropane (TEP) as the standard by spectrophotometric method (UNICAM 5625 UV/VIS, readout at wavelength 532 nm). The content of nitrites and nitrates (NO_2^- and NO_3^-) in the same tissue was determined using HPLC (Waters) with a conductometric detector.

The blood collected to test tubes with EDTA anticoagulant was directly subjected to morphological analyses. Red blood cells (RBC), haematocrite (HCT); haemoglobin (HGB); mean cell volume (MCV), blood plates (PLT) and red blood cells (WBC) were determined by standard laboratory methods, using a hematological analyzer ABACUS, Diatron. The blood intended for biochemical tests and for immunoglobulins concentration IgE, IgG, IgA, IgM (antibodies of class E, G, A, M) determination was collected in test tubes with anticoagulant and then separated (4500 r.p.m., 10 min). Serum was frozen at temperature of -70°C and stored until the moment of analysis. Total protein (TP), albumins (ALB), urea N (BUN), asparagine transferase (AST) and alanine transferase (ALT), glucose (GLU), cholesterol (CHOL), high density lipoproteins (HDL), triacylglycerols (TG) and creatinine (CREA) in blood serum were determined by spectrometric method, using a Vitros analyzer with Ektachem DT-60-II with DT, DTE and DTSC module (Johnson & Johnson Clinical Diagnostics, USA). Concentration of immunoglobulins

IgE, IgA, IgM and IgG was determined by the chemiluminescent method using an Immulete 2000 analyzer.

Statistical analysis

Results were developed in Excel for Windows 7. The data were subject to statistical analysis by one-way variance analysis ANOVA with the application of Duncan test, using Statgraphics Plus software v. 6.0. Two-way variance analysis was also done taking into consideration the influence of tomato cultivar, modification (presence of the transgene) and interaction between the two factors on the level of evaluated parameters (data not shown in the tables).

RESULTS

Chemical composition of fruits

Fruits of GM tomatoes were characterized by concentration of nutritive components and bioactive substances similar to their content in the fruits of isogenic plants (non-GM). Genetically-modified tomatoes with recombined thaumatin contained less crude fiber, NDF and ADF but a higher level of acid-detergent lignin ADL was recorded in their lyophilizate. GM tomatoes contained less lycopene (by *ca*. 9 %) in comparison to the fruits of non-GM tomatoes. The differences in the content of mineral components and ions in tissue of genetically-modified and traditional tomatoes were relatively small (Table 2).

TABLE 2. The content in dry matter of basic nutrients, fiber fractions, lycopene, beta-carotene and vitamin C, ions Cl^-, SO_4^{2-}, HPO_4^- and mineral compounds Ca, Mg, Zn, Mn, Cu, Na, Fe, K and P of genetically-modified tomato cultivar Beta and Nor (GM Beta and GM Nor) and non-modified tomatoes of the same cultivar (Beta and Nor).

Component	Beta	GM Beta	Nor	GM Nor
Dry matter (%)	82.54	82.52	82.57	82.56
Crude ash (%)	10.82	11.69	12.46	11.29
Crude protein (%)	19.27	21.02	20.61	20.26
Crude fat (%)	1.52	1.57	1.93	2.20
Crude fiber (%)	10.44	9.22	8.99	10.19
NDF (%)	12.21	11.13	9.10	10.00
ADF (%)	10.49	10.17	8.50	8.20
ADL (%)	3.58	4.13	2.82	3.40
Lycopene (mg/100 g)	2.26	2.05	1.81	1.60
Beta-carotene (mg/100 g)	2.30	2.10	2.40	2.50
Vitamin C (mg/100 g)	6.20	6.50	6.30	6.40
Ca (mg/kg)	720	747	755	767
Mg (mg/kg)	1715	1908	1730	1828
Zn (mg/kg)	41.6	28.0	36.2	30.1
Mn (mg/kg)	29.3	31.9	29.9	30.3
Cu (mg/kg)	11.0	11.5	11.3	11.2
Na (mg/kg)	801	856	814	835
K (%)	4.90	4.93	5.01	4.99
P (%)	0.374	0.428	0.383	0.408
Fe (mg/kg)	33.9	28.5	32.1	30.2
Cl^- (mg/kg)	11967	9287	10997	9879
SO_4^{2-} (mg/kg)	5843	6793	6143	6603
HPO_4^- (mg/kg)	9159	9878	9222	9612

Digestibility of nutrients

There were no statistically significant difference in values of the coefficients of apparent digestibility of nutrients between diets with fruits of GM and non-GM tomatoes. Crude protein, crude fiber and crude fat of the standard synthetic diet, containing casein, were better digested than the fat from diets with tomato lyophilizate but the differences were statistically insignificant (data not shown).

Indicators of rats growth

In the experiment, deaths of the animals were not recorded. When comparing the values of growth parameters of the rats receiving the diets with GM tomatoes or their non-modified equivalents, no significant differences were found. The type of administered iso-protein mixture, GMO content did not have any significant effect on relative weight of the organs of experimental rats, being prepared after completion of the experiment – liver, brain, kidneys, heart, spleen and small intestine (Table 3).

Hematological and biochemical parameters and concentration of antibodies in blood

Feeding the rats with the diets with lyophilizate of GM tomatoes with thaumatin or their non-transgenic equivalents did not cause any statistically significant differences in values of hematological parameters of rats blood (Table 4).

In blood of the animals fed the mixtures with addition of lyophilizate of Beta and GM Beta tomatoes, the activity of liver enzymes (AST and ALT) was higher than in the blood of the rats from control group which did not receive tomatoes. The mentioned difference was not, however, confirmed statistically. The intake of Nor and GM Nor tomatoes was connected with significantly lower concentration of GLU in blood in relation to the value of the discussed parameter in rats fed the diets with Beta and GM Beta tomatoes. Blood serum of the animals, receiving the mixtures with tomatoes contained less TG and more CHOL as compared to the serum of the rats which received diet without tomatoes. The content of TG in blood serum of the rats fed the diet with GM Beta tomatoes turned out to be statistically significantly lower as compared to the analogical indicator in blood serum of the rats, receiving non-GM tomatoes of the same cultivar (Table 4).

The composition of the diet was not a factor determining differences in the concentration of antibodies of E, A, G and M class in blood of the animals (Table 4).

Oxidative status and histological evaluation of liver

Oxidative status of the liver was determined based on TBARS (indicator of oxidative degradation of lipids) in blood serum of the animals, the content of 8-oxo-2'deoxyguanosine (indicator of DNA degradation) and of nitrites and nitrates – NO_2^-, NO_3^- (indicators of proteins' degradation) in liver tissue. Intake of the diet containing GM Beta and Nor tomatoes affected negatively the content of DNA adducts in rat

TABLE 3. Indicators of rats growth, diet intake/body weight gain and relative weights of the selected internal organs of animals receiving standard diet or diets with lyophilizate of genetically-modified tomato Beta cultivar (GM Beta) and Nor cultivar (GM Nor) and non-modified tomatoes of the same (Beta and Nor) cultivars.

Parameter	Standard	Tomatoes				SEM	p
		Beta	GM Beta	Nor	GM Nor		
Initial body weight (g)	121.62	120.25	120.13	120.12	120.12	2.1547	0.9839
Final body weight (g)	196.87	212.75	207.5	197.375	203.375	7.3834	0.5098
Diet intake/body weight gain (g/g)	3.37	3.31	3.47	3.41	3.42	0.2564	0.9620
Liver (g/100 g of b.w.)	2.81	2.73	2.73	2.75	2.74	0.0451	0.3611
Brain (g/100 g of b.w.)	0.60	0.62	0.59	0.60	0.61	0.0193	0.5103
Kidneys (g/100 g of b.w.)	0.64	0.66	0.64	0.64	0.65	0.0109	0.4790
Heart	0.33	0.33	0.32	0.33	0.33	0.0199	0.8313
Spleen (g/100 g of b.w.)	0.17	0.15	0.17	0.16	0.16	0.0159	0.6750
Small intestine (empty) (g/100 g of b.w.)	1.16	1.20	1.15	1.19	1.18	0.0534	0.8012
Liver (g/100 g of b.w.)	2.81	2.73	2.73	2.75	2.76	0.0461	0.3611

Average values of the parameters did not differ significantly statistically (P≤0.05).

liver. The concentration of 8-oxo-2'deoxyguanosine in the tissue of the organs sampled from the animals from the discussed groups occurred to be highly statistically significantly (p≤0.01) higher than in the remaining groups. In the liver of rats fed the diet containing tomatoes (GM and non-GM of both cultivars) significantly lower degradation of lipids (TBA-RS) was found when compared to the rats fed the standard diet. The degree of proteins degradation as being determined by NO_2^-, NO_3^- concentration, did not differ statistically significantly between the groups, although it was somewhat lower in the case of the rats receiving the mixtures with tomato lyophilizate (Figure 1).

Histopathological evaluation of the liver of the rats receiving 30% of tomato lyophilizate in the diet did not reveal

TABLE 4. Hematological and biochemical parameters and concentration of antibodies in blood serum of the rats receiving standard diet or diets with lyophilizate of genetically-modified tomato Beta cultivar (GM Beta) and Nor cultivar (GM Nor) and non-modified tomatoes of the same (Beta and Nor) cultivars.

Parameter	Standard	Tomatoes				SEM	P
		Beta	GM Beta	Nor	GM Nor		
RBC (T/L)	6.95	6.89	7.53	7.44	7.10	0.3459	0.6072
HGB (g/L)	129.50	127.80	132.30	136.50	131.25	4.7543	0.7480
WBC (G/L)	6.96	5.87	5.77	4.06	6.62	1.7306	0.7925
HCT (%)	38.32	39.43	39.85	41.3	41.85	1.8776	0.6827
MCV (fl)	57.50	56.00	55.00	56.25	55.25	0.7583	0.2057
PLT (G/L)	577.00	470.70	387.00	733.30	492.00	122.61	0.3730
TP (G/L)	57.33	54.17	58.17	53.16	53.39	1.8886	0.2191
ALB (G/L)	36.20	34.50	36.10	33.70	33.80	0.8802	0.1381
BUN (mmol/L)	6.97	7.10	8.17	6.38	5.74	0.5889	0.0766
AST (U/L)	233.00	304.50	512.00	279.50	179.70	119.867	0.3658
ALT (U/L)	46.67	147.0	210.0	54.83	33.80	67.3809	0.2983
GLU (mmol/L)	7.75^{AB}	8.70^{B}	8.43^{B}	7.15^{A}	6.56^{A}	0.4398	0.0094
CHOL (mmol/L)	1.87^{a}	2.22^{b}	2.42^{b}	2.26^{b}	2.17^{ab}	0.1103	0.0231
CHOL-HDL (mmol/L)	1.15	1.37	1.37	1.35	1.51	0.0979	0.1627
TG (mmol/L)	2.07^{C}	1.69^{BC}	1.13^{A}	1.53^{AB}	1.37^{AB}	0.1746	0.0087
CREA (μmol/L)	32.00	30.67	29.50	29.00	31.90	1.4024	0.4494
IgE total (IU/mL)	< 1	< 1	< 1	< 1	< 1	-	-
IgA (IU/mL)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	-	-
IgG (IU/mL)	0.50	0.68	0.71	0.58	0.67	0.0912	0.3686
IgM (IU/mL)	0.11	0.12	0.11	0.11	0.10	0.0079	0.8951

The means in the rows marked with letters A, B, C differ significantly for P≤0.01; a, b, for P≤0.05.

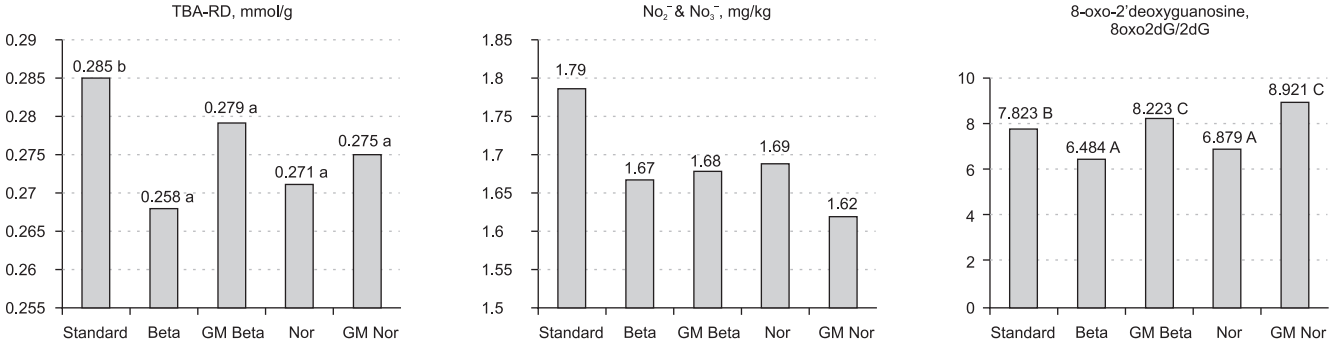

FIGURE 1. Concentration of the products of oxidative degradation of lipids (TBA-RS), proteins (NO_2, NO_3) and DNA (8-oxo-2'deoxyguanosine) in liver tissue of rats receiving standard diet or diets with lyophilizate of genetically-modified tomato Beta cultivar (GM Beta) and Nor cultivar (GM Nor) and non-modified tomatoes of the same (Beta and Nor) cultivars. The means marked with letters A, B, C differ significantly at P≤0.01; and these with a, b, at P≤0.05.

statistically significant differences in the number of normal cells compared to the control rats. The microscopic examination of hepatocytes in the liver of the rats also did not show any statistically significant differences in the number of dead and di-nuclear cells (Table 5).

Two-way analyses of variance demonstrated a significant (p=0.0113) influence of tomato cultivar on the concentration of glucose. There was no statistically significant influence of cultivar, modification or interaction between the two factors on any of the parameters evaluated in the experiment.

DISCUSSION

Differences in the content of nutrients, biologically-active substances or minerals in GM plants and their isogenic equivalent may become an unexpected effect of transgenesis of the plants with nutritional significance. When taking into consideration a relatively low concentration of dry matter (*ca*. 3–6 %) in fresh consumed fruits, we should not overestimate nutritional importance of the recorded differences in the content of the examined substances, micro- and macro-elements in tomato lyophilizate (Table 2). The potential exposure of consumers to GMO components is relatively small. It has been recognized that the observed variation in the composition of tomatoes is not greater than the differences found between the cultivars of the same species, or differences caused by conditions of cultivation, processing and storage [Dobromilska *et al*., 2008; Hernández *et al*., 2005; Hallmann & Rembiałkowska *et al*., 2007; George *et al*., 2011; Guil-Guerrero & Rebolloso-Fuentes, 2009]. The range of the recorded variations in contents of the above dietary constituents, significant for the consumer, did not suggest therefore a negative evaluation of the nutritive value of the fruits.

The nutritive value of fruits is determined, however, not only by their composition but also by the availability of nutrients. The susceptibility of dietary proteins to digestion is one of the indicators of intolerance and potential allergenicity. Sensitization of immunological system of the consumer occurs most frequently *via* gastro-intestinal tract, so the limited effectiveness of digestion may be directly a result of allergenic reaction to food components [Moreno, 2007]. An easily digested protein of diet does not provoke a negative reaction of the immunological system [Taylor, 2001]. Thaumatin, as being a transgenic protein, synthesized by the foreign gene, is recognized as easily digestible protein and should not deteriorate diets digestibility in the conducted studies. Good digestion and degradation of thaumatin into amino acids was confirmed by Higginbotham *et al.* [1983]. Twardowska [2003] showed a high susceptibility of thaumatin to the activity of trypsin and chymotrypsin in the conditions of *in vitro* digestion. Nutritional tests with the application of GM cucumbers with thaumatin expression excluded the presence of transgenic protein in the feces of rats [Kosieradzka *et al*., 2010], which confirms its complete degradation in the intestines.

An unintended effect of transgenesis is likely to occur, the symptom of which would include synthesis of anti-nutrients not identified at the stage of *in vitro* analyses. In such case, differences in the composition of fruits of GM plants as induced by transgenesis could affect the dynamics of growth and development of the rats – young, growing model animals. The development of animals receiving diets with fruits of GM tomato with thaumatin was recognized as undisturbed and proportional not differing from that observed in the rats receiving iso-protein mixtures with the conventional tomatoes (Table 3).

The influence of thaumatin on the consumer body was researched in just a few scientific studies, results of which were summarized in the monography of JECFA [1987]. The ex-

TABLE 5. Microscopic evaluation of the liver of rats receiving standard diet or diets with lyophilizate of genetically-modified tomato Beta cultivar (GM Beta) and Nor cultivar (GM Nor) and non-modified tomatoes of the same (Beta and Nor) cultivars.

Hepatocytes	Standard	Tomatoes				SEM	P
		Beta	GM Beta	Nor	GM Nor		
Natural	73.78	66.78	71.00	72.28	68.17	2.0667	0.1500
Di-nuclear	6.83	9.94	9.39	8.17	9.38	1.0086	0.1986
Dead	19.39	24.39	19.61	20.17	22.44	1.9517	0.4102

Average values of the parameters did not differ significantly statistically (P≤0.05).

periments with rodents revealed no negative effect of thaumatin addition not only on growth but also on reproduction and functioning of reproductive organs, however variations were observed in organs (liver, thyroid) weight depending on the diet fed to the animals. In spite of the change in thyroid gland weight, connected with the intake of thaumatin, its effect on hormonal activity (thyroxin and tri-iodothyronine) was not experimentally confirmed in the mentioned studies. In the experiments of Hagiwara *et al*. [2005] in which the rats received 3% addition of pure thaumatin, there was either no negative influence of thaumatin addition on survivability, body weight, diet intake and weight of internal organs.

The lack of the effect of GMO with thaumatin expression on the growth and development of animals and conversion of dietary components were recorded in the studies by Kosieradzka *et al*. [2001] where the experimental material included GM cucumber fruits.

Due to the necessity of eliminating the risk of allergenic reaction, the control of immune system response and examination of GMO effect on the activity of the immunological system are one of the basic elements in studies on the safety of food and feeds components [EFSA, 2004]. Administration of GM tomatoes, containing thaumatin, did not increase the level of immunoglobulins synthesized in the organism as a response to allergen presence in the diet (protein, inducing sensitiveness) (Table 4). In the experiments of Kosieradzka *et al*. [2004], 15% addition of GM cucumbers with thaumatin expression in diets for the rats had the immunomodulating effect; it affected differences in values of non-specific immunity parameters.

When interpreting the results of the experiment in which the animals received the diets containing natural components, we should remember that the response of the immunological system may be affected by substances which are different than transgenic protein and the concentration of which in the diet was not specified. The effect of interactive action of a few components of the mixture is also possible.

The lack of the allergenic effect of thaumatin was confirmed by the Committee of Experts of FAO/WHO for Food Additives on the grounds of the tests with rats and volunteers [JECFA, 1987]. Allergenic reaction of organism may be, however, determined by individual predispositions of people; from the studies, it is followed that the animals of different species may react in a different way to the presence of thaumatin [INCHEM, WHO Food Additives].

It was observed that the intake of tomato lyophilizate in the mixture caused a decrease in the concentration of TG in each case when compared to the control group, fed the standard mixture. Not all differences in TG content in blood of the rats fed the mixture with tomatoes in relation to the standard diet occurred to be statistically significant but the noticeable tendency includes undoubtedly the effect of biologically-active substances with anti-oxidative nature, present in the lyophilizate (*e.g.* carotenoids, vitamins C, E and flavonoids) (Table 4).

The differences in the activity of liver enzymes occurred to be statistically insignificant and did not have any relationship with the GMO presence in the diet. The rise in their concentration in the blood of the rats fed the mixtures with lyophilizate could be caused by intake of anti-nutrients present in tomato fruits (*e.g.* glycoalkalloid – tomatine) [Friedman *et al*., 2000]. Such relationship (statistically insignificant) was however observed only in relation to tomatoes of Beta cultivar.

In the studies of Hagiwara *et al*. [2005], even 3% addition of pure thaumatin in diet for rats did not affect the value of hematological indices. Similarly, the studies reviewed in the JECFA monograph [1987] and the experiments of Higginbotham *et al*. [1983] did not reveal any effect of sweet protein on the values of most of hematological and biochemical parameters, indicating the effectiveness of metabolic processes.

Synthesis of a potentially-toxic substance the concentration of which has not been determined at the stage of evaluation of GMO chemical composition may be the unintended and unexpected effect of transgenesis, leading to modification of plants metabolism and change in the concentration of metabolites. The intake of a substance with anti-nutritive, potentially-toxic nature may affect the intensity of reactive oxygen species (ROS) generation, and disturb the equilibrium of oxidation-reduction processes in the consumer's organism [Dybing *et al*., 2002]. The ROS react with the molecules, proteins, lipids and nucleic acids, that are significant for cellular metabolism, which leads primarily to damages of cellular membranes and mitochondria, and affects the activity of certain enzymes, having a key role in metabolism [Gajewski *et al*., 2005]. No effect of diet on values and differences in oxidative status indicators, being an evidence of experimental animals exposure to stress [Benito *et al*., 2004], may be interpreted as a lack of toxic, carcinogenic and gene-toxic effect. The increased concentration of TBARS, products of lipids oxidation, may indicate degradation of cellular membranes, irreversible changes, occurring due to the accumulation of the mentioned products in the cell, leading to damages of its different components [Halliwell & Gutteridge, 1989]. The content of TBARS in the liver of the rats receiving mixtures with tomatoes was however lower compared to control rats receiving standard mixture; therefore, the only statistically significant difference in analytical results was probably connected with the intake of antioxidants present in tomato lyophilizate (GM and non-GM). A similar effect of administration of diets with 10% addition of tomato powder was received by Alshatwi *et al*. [2010]. The components of tomatoes, especially lycopene, reveal capacity of protecting the cells from oxidative damage [Heber & Lu, 2002]. It increases the activity of antioxidative enzymes: superoxide dismutase, glutathione peroxidase and glutathione reductase, thereby decreasing the oxidative stress [Bose & Agrawal, 2007].

In our study, we have observed a negative reaction of rats organism to the presence of tomatoes with thaumatin in the diet, being expressed by a significantly increased content of 8-oxo-2'deoxyguanozine in the liver (Figure 1). The increased concentration of 8oxo in the bodies of experimental rats was also found in nutritional studies of some transgenic lines of potato with expression of protein 14–3-3 [Kosieradzka *et al*., 2008]. Diet composition is one of the factors which may affect the concentration of 8-oxo-2'deoxyguanozine in the consumer's organism [Chen *et al*., 1999]. Its synthesis is a natural consequence of cellular metabolism but an increase in its concentration indicates an increased number of oxida-

tive damages of DNA, being a result of the activity of reactive oxygen species. 8-Oxo-2'deoxyguanozine is a marker of damages of cellular structures, resulting, *i.a.*, from toxicity of diet although its concentration may increase in various pathological states [Dragsted *et al*., 2002] and its changes not always lead to carcinogenesis [Cooke *et al*., 2003]. Microscopic analysis of the image of liver tissue of the rats did not confirm the GMO-induced risk to the function of the discussed organ, being important for metabolism. No necrotic lesions were found in the tissue (Table 5) and values of biochemical parameters and relative weight of the discussed organ did not indicate disorders of its functions (Table 3 and 4). A considerable quantity of a substance with antioxidant activity (*e.g.* carotenoids, lycopene) as contained in tomato lyophilizate could have played a role of substance protecting DNA of rats liver from degradation. In relation to chemically-pure thaumatin, the toxicity, gene-toxicity or teratogenicity were not found [JECFA, 1987; Higginbotham *et al*., 1983]. The results of own experiments may, therefore, result from the interaction of various substances contained in GMO tissue. In the opinion of Foksiński *et al*. [2007], antioxidants may affect the level of potentially-mutagenic oxidative damages of DNA but their activity is dependent on bioavailability which may be determined genetically.

CONCLUSION

On the ground of comparison of the composition and digestibility of the fruits of tomato plants of Beta and Nor cultivars with the expression of gene, coding sweet protein – thaumatin, and on the basis of the nutritional experiments with model animals receiving diets with 30% addition of tomato fruit lyophilizate it was found that genetic transformation did not change significantly their nutritive and dietetic value. The content of modified tomatoes (or isogenic lines) in the diet of the rats was considerably higher than a typical probable consumption of the discussed fruits by humans [Sznajder *et al*., 2005]. Exposure and risk of the potential consumer to effect of factors connected with the intake of the examined GMO in the diet is considerably lower and by this, the probability of negative consequences is very limited. Habitual consumption of the examined GMO will probably have the influence on the health status of consumers in the way similar to that caused by the intake of conventional tomatoes.

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