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A 90-day subchronic feeding study of genetically modified maize expressing Cry1Ac-M protein in Sprague–Dawley rats

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ABSTRACT

The *cry1Ac-M* gene, coding one of *Bacillus thuringiensis* (Bt) crystal proteins, was introduced into maize H99 × Hi IIB genome to produce insect-resistant GM maize BT-38. The food safety assessment of the BT-38 maize was conducted in Sprague–Dawley rats by a 90-days feeding study. We incorporated maize grains from BT-38 and H99 × Hi IIB into rodent diets at three concentrations (12.5%, 25%, 50%) and administered to Sprague–Dawley rats (n = 10/sex/group) for 90 days. A commercialized rodent diet was fed to an additional group as control group. Body weight, feed consumption and toxicological response variables were measured, and gross as well as microscopic pathology were examined. Moreover, detection of residual Cry1Ac-M protein in the serum of rats fed with GM maize was conducted. No death or adverse effects were observed in the current feeding study. No adverse differences in the values of the response variables were observed between rats that consumed diets containing GM maize BT-38 and non-GM maize H99 × Hi IIB. No detectable Cry1Ac-M protein was found in the serum of rats after feeding diets containing GM maize for 3 months. The results demonstrated that BT-38 maize is as safe as conventional non-GM maize.

1. Introduction

Maize is the world's third leading cereal crop, following wheat and rice. It is grown as a commercial crop in over 25 countries worldwide. In 1995–1997, 66% of all the maize produced worldwide was used for animal feed and 17% for human consumption. In the developing countries, 30% of the maize produced was used for human consumption and 57% for animal feed, whereas in Western Europe, North America and other high income countries, 4% was used for human consumption and 76% for animal feed during the same period (OECD, 2002). GM maize is one of the most extensively cultivated genetically modified organisms (GMO), with traits introduced in these lines being, basically, resistance to herbicides and increased tolerance to insects and pests (Hernández et al., 2004). GM insect-resistant maize with *Bacillus thuringiensis* (Bt) gene occupying 37.3 million hectares, was the second most frequently cultivated GM plant (James, 2011).

Different strains of *B. thuringiensis* express different crystal proteins with relatively high target specificity to target insects. For example, Cry41 proteins selectively resist *herbivorous lepidopteran larvae*, while Cry3 proteins selectively resist *Phytophagous coleoptera*, particularly the Western Maize rootworm (*Diabrotica* sp., Chrysomelidae) (Knecht and Nentwing, 2010). The gene of Cry1Ac conferring insect-resistance (*Helicoverpa armigera*) was introduced into plants widely, such as cotton (Gunning and Moores, 2010), maize (Ma et al., 2008), and rice (Han et al., 2011). The modified cry1Ac gene (cry1Ac-M) and the bar gene were introduced into the genome of H99 × Hi II B maize with microparticle bombardment method to obtain BT-38 GM maize, and then the transgenic cultivars were harvested through selecting the survivors after the glufosinate elixir spraying process. Crop pest was one of the main hazards to the maize production. BT-38 maize was conferred *H.* armigera (Lepidoptera: Noctuidae) resistant ability through the introduction of gene cry1Ac-M.

Plant transformation techniques used in modern agriculture to improve plant traits have the potential to generate biosafety issues

Abbreviations: ANOVA, one-way analysis of variance; Bt, *Bacillus thuringiensis*; EFSA, European Food Safety Authority; ELISA, enzyme-linked immune sorbent assay; EU, European Union; FAO, Food and Agriculture Organization of the United Nations; GM, genetically modified; GMOs, genetically modified organisms; GNA, *Galanthus nivalis*; ILSI, International Life Sciences Institute; OD, optical density; OECD, Organization for Economic Cooperation and Development; PHA-E, *Phaseolus* vulgaris lectin: agglutinin E; SD, standard deviation; SPSS, Statistical Product and Service Solutions; WHO, World Health Organization.

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by the presence of exogenous gene and selectable marker genes in transgenic plants (Filipecki and Malepszy, 2006). For the assessment of potential adverse effects of long-term exposure of foods derived from GM crops, ninety-day rodent feeding studies were recommended (FAO/WHO, 1996) and the sensibility of these studies to detect potential adverse effects has been documented (EFSA, 2008). Many GM events have undergone such studies including cotton seed (Dryzga et al., 2007), soybean (Hammond et al., 2008), rice (Zhou et al., 2011) and maize grain (He et al., 2009). The results from these studies showed no adverse effects resulting from the insertion of one or more transgenic proteins into plant seed germline except where they were expected (i.e., GM rice with PHA-E lectin).

In the current study, maize meal from BT-38 and its isogenic line H99 × Hi IIB maize crops was formulated into balanced basic control diets in complied with Chinese Standard GB14924.3, 2001 at concentrations of 12.5%, 25%, 50% (wt/wt). Another group of rats fed with a commercial diet were regarded as additional control. Body weight, feed consumption and toxicological response variables (hematological parameters, serum chemistry, absolute and relative organ weight) were measured, and gross as well as microscopic pathology were examined to detect the potential adverse effect of the GM maize on rat after long term exposure. Furthermore, Aris and Leblanc from Canada reported that the residue of *Cry1Ab* protein was detected in maternal and fetal blood samples, respectively, and in blood samples from non-pregnant women (Aris and Leblanc, 2011). The results of this study have received extensive attention and cause public anxiety. Therefore, the presence of insect resistant protein from genetically modified foods in human and animal blood should cause enough attention and detection of residual Cry1Ac-M protein in blood samples of rats consuming feed containing BT-38 maize was conducted in this study with an ELISA method.

This study was conducted in accordance with Chinese Toxicology Assessment Procedures and Methods for Food Safety (Chinese Standard GB5193.13, 2003) as well as guidelines of repeated dose 90-day oral toxicity study in rodents (OECD, 1998) and was conducted in compliance with OECD Good Laboratory Practice guidelines at The Supervision, Inspection and Testing Center of Genetically Organisms, Ministry of Agriculture (Beijing, China). The experiment operation obtained approval of Animal Ethics Committee of the center.

2. Materials and methods

2.1. Certification and compositional analysis of plant materials

Transgenic insect-resistant maize line (BT-38) and the near-isogenic non-GM maize line (H99 × Hi IIB) were provided by Professor Jinsheng Lai, College of Agriculture and Biotechnology, China Agricultural University (Beijing, China), which was both cultivated in the experimental field in adjoining plots under identical climate conditions in the growing season of 2010. Samples of the BT-38 and H99 × Hi IIB grains were evaluated for the existence of the *cry1Ac-M* and the *bar* gene with polymerase chain reaction (PCR) and the proteins encoded by Cry1Ac-M and *bar* genes with an antibody specific enzyme-linked immune sorbent assay (ELISA). As indicated by the results of the evaluation proceeded, the *cry1Ac-M* gene and protein were present in the BT-38 maize grain but not detected in the H99 × Hi IIB grain. Three random samples were selected from each type of maize for compositional analysis. Analysis of nutritional composition (ash, fat, crude fiber, moisture, crude protein, calcium and phosphorus) were conducted in accordance with Chinese Standard GB/T5009.3, 2003; Chinese standard GB/T5009.4, 2003; Chinese Standard GB/T5009.5, 2003; Chinese Standard GB/T5009.6, 2003; Chinese Standard GB/T5009.10, 2003; Chinese Standard GB/T5009.87, 2003; Chinese Standard GB/T5009.92, 2003). The composition of nutritional proximate of BT-38 and H99 × Hi IIB maize grain was presented in Table 1.

2.2. Diet formulation and compositional analysis

The maize grains were milled into 250 μm flour using a SFSP56x40 beater pulverizer (Muyang Group Co., Ltd., Yangzhou, Jiangsu Province, China), and then these flours were formulated into rodent diets at concentrations of 12.5%, 25% and 50% respectively in accordance with Chinese standard GB14924.3, 2001). All the diets were vacuum-packed with polyethylene bags and sterilized through irradiating by 60Co by Ke-Ao-Xie-Li Feed Co., Ltd., (Beijing, China). The components of all diets were presented in Table 2.

Table 1
Proximate concentration of the transgenic BT-38 and conventional non-GM H99 × Hi II B maize flour ($n = 3$)[a].

Nutrients	BT-38	H99 × Hi IIB
Moisture (%)	9.64	9.88
Ash (%)	1.70	1.39
Protein (%)	11.3	9.4
Crude fiber (%)	1.48	1.60
Fat (%)	4.49	3.96
Ca mg/100 g	21.8	20.8
P mg/100 g	105	94.8

[a] Wet weight.

2.3. Animals

One hundred and forty male and female SD rats, approximately five-weeks of age with an average body weight of 80 ± 20 g were obtained from Vital River Laboratories Co., Ltd., (Beijing, China). Every 5 rats were housed in a stainless steel with *ad libitum* access to filtered tap water and commercial feed in animal room of The Supervision and Testing Center for GMOs food safety, Ministry of Agriculture (Beijing, China) with the license number SYXK (Beijing) 2010–0036. Animal room was maintained at a temperature of 22 ± 2 °C, relative humidity of 40–70%, artificially illuminated (fluorescent light) with a 12 h light/dark cycle and air exchanges of 15 times/h.

2.4. Experimental design

Rats were acclimatized for 5 days with control diet and then divided into treatment groups with 10 rats/sex/group with computerized randomization scheme based on mean body weight which across each group did not vary more than 20%. Experimental groups were fed with diets formulated with 12.5%, 25% and 50% (wt/wt) BT-38 maize and corresponding control groups were fed with diets containing either 12.5%, 25% and 50% (wt/wt) H99 × Hi IIB maize. An additional control group was fed with commercially obtained diet.

2.5. Clinical evaluation, body weight gain and food utilization

Signs of mortality, morbidity and other noteworthy clinical signs of toxicity were recorded by the observation of all rats one a day throughout the whole 90 day feeding study. All rats were observed for 13 weeks. The body weight gain and feed consumption were measured each week. The calculation of the mean weekly food utilization rate was conducted as followed: Mean weekly food utilization rate (%) = (weekly body weight gain)/(weekly food consumption) × 100%. Hematology and serum biochemistry values were measured with blood obtained from all rats at the end of the experiment. Rats were fasted overnight (16 h) and

Table 2
Diet formulation (%).

	Control group	BT-38			H99 × Hi IIB		
		12.5%	25%	50%	12.5%	25%	50%
Corn	33.3	12.5	25	50	12.5	25	50
Corn starch	33.6	45.5	32.9	17.7	46	33.8	18.6
Casein	17.3	25.3	25.3	20.9	25.3	25.3	20.4
Grass	5.0	5.0	5.0	2.5	5.0	5.0	2.6
Dicalcium phosphorus	2.5	2.5	2.5	1.95	2.6	2.6	2.0
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Limestone	0.6	0.8	0.9	1.45	0.8	0.8	1.37
Soy oil	4.8	5.5	5.5	2.7	4.9	4.6	2.75
Premix[a]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Lysine	1.0	1.0	1.0	1.0	1.0	1.0	1.0
DL-methionine	0.4	0.4	0.4	0.3	0.4	0.4	0.35

[a] The premix contained vitamins (vitamin A, 7000 IU; vitamin B_{12}, 0.05 mg; vitamin C, vitamin D_3, 1500 IU; vitamin E, 60 mg; vitamin K, 3 mg; riboflavin, 10 mg; nicotinic acid, 45 mg; inositol, 400 mg; biotin, 0.2 mg; choline chloride, 1250 mg; thiamine, 10 mg; pyridoxine, 10 mg and D-calcium pantothenate, 20 mg) and minerals (Cu, 10 mg; Fe, 100 mg; Mn, 75 mg; Zn, 40 mg; Se, 0.2 mg; I, 0.5 mg; NaCl, 3.3 g; K,1.2 g and Mg, 0.5 g).