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A 90-day toxicology study of high-amylose transgenic rice grain in Sprague–Dawley rats

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ABSTRACT

A transgenic rice line (TRS) with high amylose level has been developed by antisense RNA inhibition of starch branching enzymes. Compositional analysis of TRS demonstrated that the content of resistant starch (RS) was significantly higher compared to conventional non-transgenic rice. High level of RS is an important raw material in food industry and has various physiological effects for human health. In order to provide the reliable theory basis for field release of TRS rice, we evaluated the potential health effects of long-term consumption of the TRS. The 90-day toxicology feeding experiment was conducted in Sprague–Dawley rats fed with diets containing 70% of either TRS rice flour, its near-isogenic rice flour or the control diet. The clinical performance variables (body weight, body weight gain and food consumption) were measured and pathological responses (hematological parameters and serum chemistry at the midterm and the completion of the experiment, urinalysis profile and serum sex hormone response at the completion of the experiment) were performed. Besides, clinical signs, relative organ weights and microscopic observations were also compared between TRS group and its near-isogenic rice group. The combined data indicates that high-amylose TRS grain is as safe as the conventional non-transgenic rice for rat consumption.

1. Introduction

Rice is an important source of food for over 3 billion inhabitants on earth, providing 21% of calories for the global population. In Asia, where more than 90% of the world's rice is grown and consumed, at least 30% of the daily caloric intake is from rice. China is the largest rice producer in Asia, accounting for 31% of the world rice production (Hareau, 2006; OECD, 2004).

Dietary starch has been an important source of energy for many human communities, and it has also clearly showed some specific health benefits (Fuentes-Zaragoza et al., 2010; Ratnayake and Jackson, 2008). Starch consists of two main components: a mostly linear amylose moiety and a highly branched amylopectin moiety (Gallant et al., 1997; Sharma and Yadav, 2008). Earlier studies discovered that the amylose content is proportional to the resistant starch (RS) (Lee et al., 1997). RS is the sum of starch and starch degradation products not absorbed in the small intestine due to their resistance to enzymatic digestion (Asp and Björck, 1992). High levels of RS have various physiological effects on the human body, such as an increase in the cecal and large intestinal contents, the promotion of short-chain fatty acid synthesis in the large intestine, alteration of microbial population, increase of satiety and reduction of energy intake, regulation of glycemic and insulinemic responses and alteration of the blood lipid profile (Fuentes-Zaragoza et al., 2010). These effects could positively correlate with the decreased incidence of cecal cancer, atherosclerosis and obesity-related complications in humans. In addition, RS is an important raw material for the food industry (Simsek et al., 2009). Surprisingly, although rice has the highest starch content among those major grain crops, its RS content is relatively low.

In this study, the transgenic rice line (TRS) with high amylose and RS contents, and its near-isogenic Teqing (TQ) were compared in the following toxicology study. TRS was generated from the *indica* rice cultivar TQ after transgenic inhibition of two starch branching enzymes (SBEs) (SBE I and SBE IIb) through antisense RNA technique, and it holds the homozygous transgene and shows the identical growth behavior and plant type as its near-isogenic TQ with the exceptions of the grain composition and starch structure (Wei et al., 2009; Zhu, 2009). Grains of the transgenic rice are rich in RS and have demonstrated significant potential in improving the large bowel health in rats (Li et al., 2008). The TRS rice has already undergone a compositional assessment in accordance

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with the principle of substantial equivalence. Results showed that the RS content in transgenic rice was significantly higher than the near-isogenic rice, while the insertion of the SBE gene did not affect its other nutrition constituents (Li et al., 2009). However, it is unclear whether unintended changes could have occurred in individual genetic modification (GM) events. To address this issue, animal tests are required, such as those long-term dietary exposure studies for soybean (Appenzeller et al., 2008; Delaney et al., 2008), maize (Appenzeller et al., 2009a,b; He et al., 2008, 2009) and rice (Schrøder et al., 2007; Poulsen et al., 2007a,b; Kroghsbo et al., 2008). Therefore, in this study, we conducted a rodent feeding experiment for a recommended period of 90 days (FAO/WHO, 2000) to examine the potential adverse effects of TRS rice. This paper presents the result from the subchronic dietary toxicity assessment of TRS rice in rats.

2. Materials and methods

2.1. Preparation of diet rice flour

TRS and TQ were simultaneously cultivated in the experiment field of Yangzhou University (Yangzhou, China). After harvest, the seeds were milled and processed to flour for animal diet. After comparing contents of the rice and standard rat diet, flours from TRS and TQ rice grains were formulated into complete feed, representing 70% of the dry weight. Both rice diets were supplemented with vitamins and trace minerals to ensure an adequate supply of nutrition comparable to the standard diet (the control diet). The rice diets were designed and processed by Ke Ao Xie Li Feed Co. Ltd. (Beijing, China), according to the standards of the People's Republic of China and then vacuum-packed with polyethylene bags and sterilized by ^{60}Co. The composition of the TRS diet and the TQ diet are summarized in Table 1, and the nutritional composition of these diets are presented in Table 2.

2.2. Animals and housing conditions

Sixty Sprague–Dawley male and female rats were obtained and housed from the Laboratory animal research center of Jiangsu University (Zhenjiang, China) under the license number SCXK (SU) 2009-0002 and SYXK(SU)2008-0024. Rats were approximately 4 weeks old upon arrival with an average body weight of 80 ± 20 g. Rats were housed singly in polycarbonate cages with stainless steel covers. Sterile poplar wood chips were used as the bedding. Animal rooms were maintained at temperature of 22 ± 2 °C and a relative humidity of 40–60%, and artificially illuminated (fluorescent light) on an approximate 12-h light/dark cycle. The air exchange was about 18 times/h. All rats were provided food and filtered tap water ad libitum.

Table 1
Composition of diets for rats in different test groups.

Ingredient (%)	TQ group	TRS group
Non-transgenic rice	70	–
Transgenic rice	–	70
Bean pulp	1.8	0.7
Wheat flour	–	3.2
Fishmeal	17.0	15.0
Grass powder	5.0	5.0
Yeast powder	2.0	2.0
Vegetable oil	1.9	1.4
Additive	1.0	1.0
Limestone	1.1	1.2
$CaHPO_4$	–	0.2
Salt	0.2	0.3

The control diet is made from corn, fish meal, soybean meal, milk powder, lysine, $CaHPO_4$, yeast powder, limestone, vegetable oil, salt, $CuSO_4$, $ZnSO_4$ $FeSO_4$, vitamin A, vitamin E, etc. Product license: SCXK (Beijing) 2009–0012.

Table 2
Nutritional composition of diets.

Components	Content (%)		
	Control group	TQ group	TRS group
Moisture	10	10	10
Crude protein	20	19.6	19.7
Crude fiber	2.9	2.1	2.0
Total calcium	1.1	1.1	1.2
Phosphorus	0.71	0.75	0.76
Crude fat	4.0	4.0	4.0
Crude ash	5.0	5.2	5.1

2.3. Experimental design

During the pretest period, all rats were allowed to acclimate to the housing conditions for 1 week with the control diet. Subsequently, rats were divided into 3 groups with 10 rats/sex/group following computerized randomization scheme based on body weight. All rats were then provided the corresponding diets and observed for 13 weeks. Body weight and food consumption were measured each week.

2.4. Clinical observations, body weight gain and food consumption

All animals were observed for signs of mortality or moribundity (the overt signs of toxicity), abnormal behavior and appearance at least once daily throughout the study. Body weight and food consumption were measured at weekly intervals. The average weekly food utilization rate was determined using the following calculation:

$$\text{Mean weekly food utilization rate}(\%) = (\text{weekly body weight gain})/(\text{weekly food consumption}) \times 100\%$$

2.5. Hematology

Rats were fasted at least 12 h prior to blood sample collection. Whole blood was collected from the rat tail vein at the midterm and from the rat orbital sinus at the terminal in the presence of anticoagulant, and evaluated for white blood cell count (WBC), red blood cell count (RBC), platelet count (PLT), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV) and platelet distribution width (PDW) measurements with a BC3000 Hematology Analyzer (Mindray Inc., China).

2.6. Serum chemistry

Rats were fasted at least 12 h prior to blood sample collection. Whole blood was collected from the rat tail vein at the midterm and from the rat orbital sinus at the terminal without the addition of anticoagulant. Following centrifugation, the sera were assayed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP), total protein (TP), albumin (ALB), globulin (GLOB), urea nitrogen (BUN), creatinine (CREA), glucose (GLUC), triglycerides (TRIG), cholesterol (CHOL), high-density lipoproteins cholesterol (HDLC), low-density lipoproteins cholesterol (LDLC) and inorganic phosphorus (IPHS) levels with an Olympus AU2700 Clinical Chemistry Analyzer (Olympus Inc., Japan).

2.7. Urinalysis

At the terminal experiment, urine was collected from metabolism cages to examine the pH, urine protein concentration (UMTP), level of ketones (KET), urine specific gravity (USG) and urobilinogen (URO) level by a Uritest-200A Automated Urine Chemistry Analyzer (Uritest Inc., China).

2.8. Serum sex hormone levels

Rats were fasted at least 12 h prior to blood sample collection at the terminal experiment. Whole blood from the rat orbital sinus was collected without the addition of anticoagulant. After centrifugation, supernatants of the sera were assayed for testosterone in male rats, estradiol and progestin in female rats, follicle stimulating hormone and luteinizing hormone in both genders with the rat sex hormone ELISA kit (Boston Biochem Inc., Australia).

2.9. Gross and anatomic pathology

All surviving rats were weighed before they were anesthetized with 10% chloral hydrate and sacrificed by exsanguination. All rats received a complete gross pathology examination during the necropsy. The following organs were trimmed of extraneous fat and weighed (paired organs were weighed together): brain, heart, liver, spleen, lung, kidneys, stomach, intestine, thymus and pancreas. The relative organ weight was calculated as a percentage of the total body weight. The above tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Embedded tissues were sectioned to approximately 5–6 μm in thickness, stained with hematoxylin and eosin (H&E) before they were examined under a microscope.