



Article

Subchronic Toxicological Evaluation of Xiushui 134Bt Transgenic Insect-Resistant Rice in Rats

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Abstract: In this study, Xiushui 134Bt, a highly insect-resistant rice transgenic material with stable expression of *CryIaC1* gene in rice plants and no significant changes in main agronomic traits, was obtained by means of *Agrobacterium*-mediated transformation method using japonica rice Xiushui 134 as receptor material. Biosecurity of the transgenic rice Xiushui 134Bt was assessed by rat feeding experiments. Wistar rats were fed a nutritionally balanced diet supplemented with 35% Genetically Modified (LGM) or 70% Genetically Modified (HGM) Xiushui 134Bt, respectively, for 90 days. Compared with the wild-type group (Xiushui 134), there were no significant differences in body weight, total food intake, food conversion rate, relative organ weight, blood routine, blood physiological or biochemical parameters, or histopathological examination between LGM and HGM Bt groups ($p > 0.05$). These results indicate that the transgenic rice Xiushui 134Bt is non-toxic in laboratory animals and provide guidance for the future commercial release of the transgenic rice Xiushui 134Bt in China.

Keywords: GM rice; safety assessment; insect resistance; *Bacillus thuringiensis*; toxicity



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1. Introduction

Rice is an important staple crop and is seriously vulnerable to pest infestations. Previous reports indicate that plant diseases and insect pests can cause global annual yield losses of up to 37% for crops, with 13% of these losses attributed to insects [1]. In order to address the global food crisis, it is necessary to promote the industrialization of the breeding of genetically modified organisms in an orderly manner. Various strategies, such as pesticides, have been proposed to perform crop protection against these pests. Applications of pesticides initially played a significant role in controlling the occurrence of diseases and insect pests; however, their abuse also results in environmental pollution and negatively affects food safety and human health [2]. Therefore, breeding new rice varieties with insect resistance is an important way to control rice insect pests, which is of great significance to ensure food security.

Bacillus thuringiensis (Bt) is a Gram-positive bacterium widely distributed in nature [3]. During the spore-forming phase, one or more companion crystals of different shapes are produced in the bacteria. Bt genes can be encoded with the insecticidal activity of specific protein toxins, which have specific insecticidal activity against a variety of insects, such as Lepidoptera, Diptera, and Coleoptera, as well as nematodes, waxes, and protozoa. With the development of biotechnology, Bt genes encoded into plants have many advantages, such as resistance to insects, strong selection, less investment, and quick effect, which greatly improve the economic benefit and environmental significance through reducing pesticide use [4]. As one of the most important economic crops, the cultivation of rice transgenic Bt crops has received increasing attention. For example, the genes of *cryIA(b)/cryIA(c)*, *cryIA(b)*,

cryIA(c), and *cryIC* have been successfully transferred and expressed in rice via various pathways, e.g., protoplast fusion, *Agrobacterium*-mediated, pollen tube channel, and gene bombardment [5–8]. GM rice Xiushui 134Bt is a transgenic line with a single copy *Cry1Ac1* gene derived from *Agrobacterium*-mediated genetic transformation using conventional rice variety Xiushui 134 as the acceptor material and the Bar gene as the marker gene, which has the advantage of being both insect- and herbicide-resistant (Supplementary Figure S1) [4]. The insertion site of both borders in Xiushui 134Bt was located at positions 20856153 to 20856229 of rice chromosome 2 (Supplementary Figure S2). It was not inserted into the coding region of known genes, and the major agronomic traits were not significantly altered by the survey and analysis of the agronomic traits in the field.

Food safety assessments are important for the early stages of commercial planting of GM crops. GM crops must be shown to be as safe as those derived from conventional crops, which have an established history of safe use [9]. This principle was originally referred to as substantial equivalence but is now more typically referred to as comparative safety assessment [10]. The assessments of GM crops are based mainly on safety evaluation according to the cultivation performance index of crops, the comprehensive analysis of known nutrients, anti-nutrients and toxic substances of the crop variety, and the analysis of crop-specific components. The comparison between GM crops and their most closely related traditional similar crops is based mainly on safety evaluation. The toxicological experiment in rats is an important method for safety evaluation of genetically modified crops and their products [10–14]. A number of studies have found that *Bt* transgenic crops are as safe as corresponding conventional crops, and no adverse effect was found in groups consuming diets containing fractions from GM crops as compared with those from non-GM *Bt* crops [15,16]. One example is transgenic rice with the Bar gene, which serves as one material to study the effects of transgenic rice on the life activities of mice, showing no significant abnormalities, indicating that the Bar gene was also relatively safe [17]. In this study, transgenic rice Xiushui 134Bt and its non-transgenic parent control Xiushui 134 were added to the formulated feed at rates of 35% and 70%, respectively. A 90-day feeding trial was conducted on Wistar rats to assess the potential toxicity of GM rice 134Bt using clinical manifestations, blood biochemistry indicators, blood routine, urine indicators, and organ pathology analysis. The aim was to assess the safety of the transgenic rice 134Bt.

2. Materials and Methods

According to the standards of the Ministry of Agriculture and Rural Affairs of China No. 323-27-2020 (Food safety detection of genetically modified plant and derived products 90-day feeding test in rats), the dose group was designed, and related indexes were determined. The experiment commissioned by the Shanghai Academy of Agricultural Sciences was conducted and completed in the Ministry of Agriculture and Rural Affairs Crop and Food Safety Supervision, Inspection and Testing Center (Tianjin), China, with license number TJCDCLL2021004.

2.1. Diet Formulation

The dietary level for experimental animal nutrition needs to conform to the standards listed in GB 14924-2010 (Chinese Standard methods). Based on nutrition balance, the maximum dosage in the feed was taken as the high dose group, and the dose in the low dose group was not lower than the expected intake of the human body. Rats were fed conventional basal diet Formula Design feed formula for the framework. The feed should be full of protein, fat, carbohydrates, vitamins and minerals, and other nutrients for the growth of the pods. Factors such as the variety and characteristics of transgenic plants and the proportion of transgenic plants in the dietary composition of the population were considered. Based on the results of the compositional analysis, transgenic 134Bt rice and xiushui 134 non-transgenic rice were added to the formulated feed in 35% (low dose group) and 70% (high dose group) proportions, respectively. The diet groups were defined as follows: (i) feeding with oral diet (the control); (ii) feeding with compound diets containing

35% non-transgenic Xiushui 134 (LP); (iii) feeding with compound diets containing 70% non-transgenic Xiushui 134 (HP); (iv) c (LT); and (v) feeding with compound diets containing 70% transgenic Xiushui 134Bt (HT). The level of the main nutrients is processed into pellet feed. After $^{60}\text{Co-}\gamma$ radiation sterilization, the feed can reach a clean level. The moisture, ash, fat, protein, crude fiber, and other components of the feed were measured to ensure that the feed of the transgenic group, the non-transgenic group, and the levels of nutrients in the basal diet were the same, all of which met the needs of growth and development of rats (Table 1).

Table 1. Nutritional composition of diets (%).

Item	Control	LP	HP	LT	HT	Detection Method
crude protein	18.67	18.77	18.1	18.4	18.06	GB/T6432-2018
crude fat	6.4	7	6.8	7.4	7	GB/T6433-2006
crude fibre	4.6	4.6	4.8	4.7	4.4	GB/T6434-2006
moisture	6.2	6.5	6	6.8	6.2	GB/T6435-2014
ash content	2.8	2.8	2.8	2.8	2.8	GB/T6438-2007

LP, feeding with compound diets containing 35% non-transgenic Xiushui 134. HP, feeding with compound diets containing 70% non-transgenic Xiushui 134. LT, feeding with compound diets containing 70% transgenic Xiushui 134. HT, feeding with compound diets containing 70% transgenic Xiushui 134Bt.

2.2. Animals and Housing

A total of 100 SPF (Specific Pathogen Free, SPF) healthy Wistar rats were divided into male and female in the barrier environmental animal laboratory of center for safety evaluation of traditional Chinese medicine (Tianjin, China) with license number TJCDCLL2021004. Animal conditions were maintained in the environment with a temperature of 20–25 °C, relative humidity of 40–70%, a 12 h light/12 h dark cycle, and air change of 15 times/h. The animals were provided with unlimited tap water throughout the study.

2.3. Experimental Design

All animals were acclimatized for 5 days prior to the start of the study; during that time, they were supplied food and filtered tap water. The animals were randomly divided into 5 groups according to sex and body weight, with 20 animals in each group and half male and half female. All rats were then provided with the corresponding diets and observed for 13 weeks. Body weight and food consumption were measured each week.

2.4. Clinical Evaluation, Body Weight Gain, and Food Consumption

During the experiment, the rats' daily activity levels (such as gait, posture, response to handling, rigidity or spasm activity, stereotyped responses, and abnormal behavior), coat condition, respiratory system, nervous system, autonomous activity (such as tearing, piloerection, pupil size, and abnormal breathing), food and excretion patterns, as well as the onset and duration of poisoning symptoms and death were observed. The rats' growth and development were monitored, and their food intake and body weight were recorded weekly. At the end of the experiment, the weekly food intake, body weight, food efficiency ratio, total food intake, and body weight gain were calculated. Ocular examinations (including cornea, lens, conjunctiva, and iris) were conducted on the high-dose group and control group experimental animals before and after the experiment.

2.5. Hematology and Serum Chemistry Assays

At the terminal experiment, the rats were anesthetized with 50 mg/kg·BW pentobarbital sodium after they fasted for 16 h. The blood samples were collected from the rat aorta abdominals, in which the parts in the presence of anticoagulant were used for evaluation for mean corpuscular volume (MCV), platelet count (PLT), red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HGB), hematocrit (HCT), prothrombin time (PT), activated partial thromboplastin time (APTT), neutrophils (NEU), monocytes (Mon), eosinophils (EOS), and basophils (BAS) with a Sysmex (Japan) XT-2000iv hematology analyzer.

For serum chemistry assays, following centrifugation, the blood samples without anticoagulant were used to examine alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), glucose (GLU), urea nitrogen (BUN), creatinine (CRE), cholesterol (CHO), triglycerides (TG), glutamyl transpeptidase (GGT), potassium (K^+), sodium (Na^+), and chloride (Cl^-) levels with a TOSHIBA (Japan) TBA-40FR Full-automatic biochemical analyzer.

2.6. Urinalysis Examination

Urinalysis examinations were conducted with the collected urine samples from metabolism cages and detected by using a DIRUI (China) H-500 H500 urine analyzer to determine the leukocyte (LEU), ketone bodies (KET), urobilinogen (UBG), bilirubin (BIL), specific gravity, occult blood (BLD), protein (PRO), glucose (GLU), nitrite (NIT), and pH.

2.7. Pathological Analysis

To perform a complete gross pathology inspection of all rats during the necropsy, the rats were examined for gross pathology by dissection, and the absolute weights of the brain, heart, thymus, adrenals, liver, kidneys, spleen, testes, epididymis, uterus, and ovaries were measured. Organ relative weight (organ-to-body ratio) was calculated. At the end of the experiment, the brain, pituitary gland, heart, liver, lungs, spleen, kidneys, prostate, adrenals, stomach and duodenum, jejunum, ileum, colon, cecum, rectum, pancreas, mesenteric lymph nodes, bladder, thyroid, thymus, testes and epididymis, ovaries, and uterus were dissected from the rats. For microscopic observation, after being fixed with 10% formalin, above paraffin-embedded tissues were sectioned to 5–6 μm thick and stained with hematoxylin and eosin (H&E) before a microscope examination.

2.8. Statistical Analysis

All statistical analyses were performed using SPSS 22.0 software package (Chicago, IL, USA), and all data were expressed as means $\pm$ standard deviations. The comparison between groups was tested for homogeneity of variances. If the variances had homogeneity, one-way ANOVA was conducted. If the variance was not uniform, a nonparametric test was performed. Each dose of the transgenic group was compared with the corresponding dose of the non-transgenic group, and each experimental group was compared with the basal diet group. Fisher's test, Chi-square test, and rank sum test were used for classification data. All differences were considered statistically significant if $p < 0.05$.

3. Results

3.1. Effects of GM on Clinical Observation, Body Weight, and Food Consumption in Rats

All investigated animals survived and were evaluated as possessing a healthy status throughout the trial duration. During the experiment, the animals in each group moved freely, the coat was glossy, and there were no abnormal secretions in the nose, eyes, or mouth. No abnormality was found in the examination, and no poisoning death occurred. No adverse signs of clinical effects were observed in behavior, activity, posture, or mental state, regardless of GM and/or gender.

The weekly body weight showed no statistically significant differences among rat groups ($p > 0.05$) (Figure 1). In addition, pairwise comparisons showed no remarkable differences in food consumption, including total body weight gain, total food intake, and food utilization rates (Table 2). Despite higher values of indexes being detected in male rats than in females, there was no tight association with the GM feedings (Table 2).

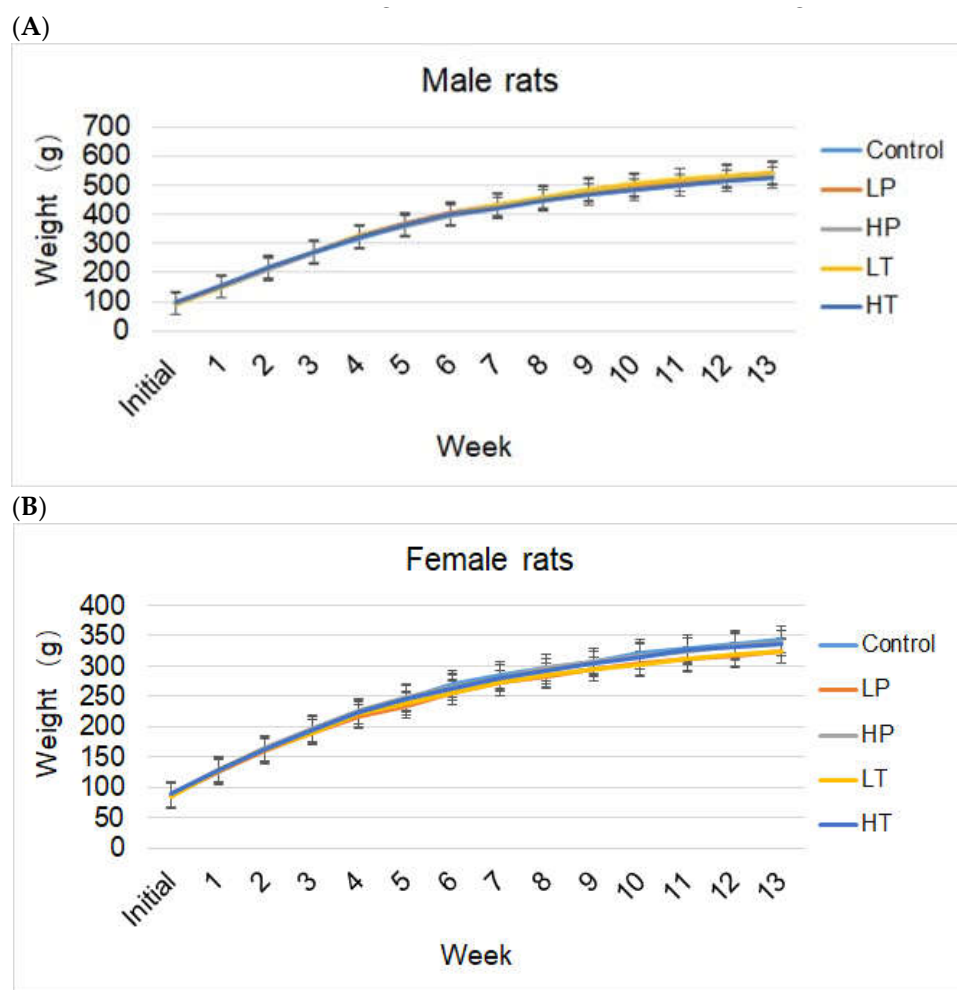


Figure 1. Changes in body weight of male (A) and female (B) rats.

Table 2. Total body weight gain, total food consumption, and food utilization rates in rats ($n = 10$).

Gender	Group	Total Increment (g)	Total Food Intake (g)	Food Utilization Rate (%)
Male	Control	442 ± 75	2069 ± 193	21.3 ± 2.0
	LP	446 ± 38	2111 ± 146	21.1 ± 0.8
	HP	434 ± 46	2009 ± 133	21.5 ± 1.3
	LG	450 ± 36	2057 ± 73	21.9 ± 1.3
	HG	430 ± 39	2018 ± 128	21.3 ± 1.3
	<i>p</i> -value	0.877	0.491	0.797
Female	Control	256 ± 48	1619 ± 171	15.8 ± 1.8
	LP	236 ± 30	1568 ± 147	15.0 ± 1.0
	HP	250 ± 29	1587 ± 113	15.8 ± 1.6
	LG	238 ± 28	1527 ± 112	15.6 ± 1.0
	HG	248 ± 37	1558 ± 145	15.8 ± 1.3
	<i>p</i> -value	0.694	0.662	0.695

3.2. Effects of GM on the Blood Routine

There were no significant differences detected in the hematology values, except that RBC and MON in both groups of HP, LT in males, and EOS in females' LT group showed differences as compared with the control (Table 3). RBC in HP, as well as MON in HP and LT, had a statistically remarkable decrease as compared with the control and the HT group ($p < 0.05$), but no noticeable changes were observed between the HT groups and the control group. Similarly, despite significantly higher EOS rates that were observed in

LT than in other groups, no difference was detected among the HT, LP, HP, or the control groups (Table 3). Therefore, these differences in both males and females are within the control range rather than associated with the feeding of genetically modified plants.

Table 3. Hematology values mean $\pm$ SD ($n = 10$).

Item	Control	LP	HP	LT	HT	<i>p</i> -Value
Males						
WBC ($\times 10^9$ /L)	5.28 $\pm$ 1.70	5.91 $\pm$ 1.52	6.26 $\pm$ 2.10	4.87 $\pm$ 1.26	6.28 $\pm$ 2.67	0.386
RBC ($\times 10^{12}$ /L)	8.48 $\pm$ 0.27	8.38 $\pm$ 0.35	8.08 $\pm$ 0.34 *	8.32 $\pm$ 0.30	8.29 $\pm$ 0.32	0.096
HGB (g/L)	146.0 $\pm$ 4.0	147.4 $\pm$ 6.7	143.4 $\pm$ 4.5	144.7 $\pm$ 3.8	146.0 $\pm$ 8.8	0.62
HCT (%)	40.4 $\pm$ 1.1	41.0 $\pm$ 1.7	40.3 $\pm$ 1.4	40.2 $\pm$ 1.0	41.0 $\pm$ 2.7	0.775
PLT ($\times 10^9$ /L)	1144.5 $\pm$ 139.2	1102.3 $\pm$ 93.6	1157.5 $\pm$ 137.1	1129.3 $\pm$ 59.0	1107.9 $\pm$ 107.4	0.687
PT (s)	9.0 $\pm$ 0.9	9.0 $\pm$ 0.3	8.5 $\pm$ 0.4	9.1 $\pm$ 0.6	8.7 $\pm$ 0.5	0.111
APTT (s)	26.6 $\pm$ 3.3	25.1 $\pm$ 3.2	24.3 $\pm$ 2.6	25.5 $\pm$ 3.2	26.2 $\pm$ 3.8	0.535
NEU (%)	19.8 $\pm$ 4.7	18.9 $\pm$ 4.8	18.4 $\pm$ 5.7	17.1 $\pm$ 3.9	16.4 $\pm$ 6.0	0.575
LYM (%)	73.2 $\pm$ 5.9	74.9 $\pm$ 5.8	76.0 $\pm$ 7.0	77.2 $\pm$ 4.2	77.5 $\pm$ 6.8	0.499
MON (%)	4.4 $\pm$ 0.9	3.8 $\pm$ 0.9	3.5 $\pm$ 1.5 *	3.2 $\pm$ 0.9 *	3.9 $\pm$ 0.8	0.095
EOS (%)	2.58 $\pm$ 1.43	2.54 $\pm$ 0.66	2.09 $\pm$ 0.53	2.50 $\pm$ 0.71	2.29 $\pm$ 0.72	0.691
BAS (%)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	/
Females						
WBC ($\times 10^9$ /L)	3.77 $\pm$ 1.38	3.25 $\pm$ 1.50	3.34 $\pm$ 0.93	3.02 $\pm$ 1.37	2.59 $\pm$ 1.38	0.386
RBC ($\times 10^{12}$ /L)	7.66 $\pm$ 0.37	7.44 $\pm$ 0.38	7.64 $\pm$ 0.33	7.54 $\pm$ 0.47	7.40 $\pm$ 0.36	0.456
HGB (g/L)	139.0 $\pm$ 6.4	136.1 $\pm$ 5.7	138.9 $\pm$ 4.7	138.4 $\pm$ 6.7	133.8 $\pm$ 7.9	0.302
HCT (%)	38.7 $\pm$ 1.4	38.2 $\pm$ 1.4	38.8 $\pm$ 1.2	38.6 $\pm$ 1.7	37.9 $\pm$ 1.9	0.406
PLT ($\times 10^9$ /L)	1078.0 $\pm$ 113.3	1015.6 $\pm$ 88.6	1113.3 $\pm$ 124.4	1024.3 $\pm$ 148.5	1052.4 $\pm$ 142.2	0.685
PT (s)	8.1 $\pm$ 0.2	8.0 $\pm$ 0.3	8.3 $\pm$ 0.3	8.1 $\pm$ 0.2	8.3 $\pm$ 0.5	0.175
APTT (s)	16.8 $\pm$ 1.7	16.6 $\pm$ 1.8	16.0 $\pm$ 1.7	16.7 $\pm$ 1.6	17.7 $\pm$ 1.9	0.319
NEU (%)	15.0 $\pm$ 3.7	19.3 $\pm$ 9.2	14.8 $\pm$ 3.3	18.8 $\pm$ 6.2	17.2 $\pm$ 5.0	0.297
LYM (%)	79.0 $\pm$ 5.3	76.3 $\pm$ 9.6	79.7 $\pm$ 4.7	75.0 $\pm$ 6.5	77.8 $\pm$ 5.8	0.504
MON (%)	3.5 $\pm$ 1.5	2.8 $\pm$ 0.7	3.2 $\pm$ 1.5	3.1 $\pm$ 1.2	2.5 $\pm$ 1.2	0.443
EOS (%)	2.52 $\pm$ 1.20	1.63 $\pm$ 0.98	2.28 $\pm$ 1.18	3.09 $\pm$ 1.17 ^b	2.47 $\pm$ 0.92	0.074
BAS (%)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.000 $\pm$ 0.000	0.00 $\pm$ 0.00	/

* Compared with the control group, $p < 0.05$; ^b Compared with the LP group, $p < 0.05$.

3.3. Effects of GM on the Blood Physiological and Biochemical Parameters

There were some statistical differences in blood physiological and biochemical parameters, such as Cl⁻ in males and ALT and K⁺ in females ($p < 0.05$) (Table 4). Although lower values of Cl⁻ in males and K⁺ in females were observed in HP and LP groups as compared to the control, respectively, statistically similar values existed in both LT and HT and the control ones (Table 4). In addition, the difference of ALT in females was observed in the LP and HP relative to the control (Table 4). Aside from these differences, no remarkable changes were detected in other serum chemistry values (Table 4). All these results support the conclusion of no adverse effects of GM on the serum chemistry values.

Table 4. Blood biochemical values mean $\pm$ SD ($n = 10$).

Item	Control	LP	HP	LT	HT	<i>p</i> -Value
Males						
ALT (U/L)	46.6 $\pm$ 4.2	44.7 $\pm$ 6.3	46.9 $\pm$ 7.8	47.2 $\pm$ 5.7	44.6 $\pm$ 5.1	0.778
AST (U/L)	67.1 $\pm$ 14.4	64.7 $\pm$ 15.0	71.7 $\pm$ 10.7	75.5 $\pm$ 10.6	65.2 $\pm$ 12.2	0.275
TP (g/L)	57.3 $\pm$ 2.5	57.8 $\pm$ 3.0	55.8 $\pm$ 2.2	55.7 $\pm$ 3.3	55.7 $\pm$ 2.6	0.278
ALB (g/L)	35.3 $\pm$ 1.2	35.6 $\pm$ 1.5	35.1 $\pm$ 1.7	34.3 $\pm$ 2.2	34.5 $\pm$ 1.9	0.431
GGT (U/L)	0.67 $\pm$ 0.37	0.52 $\pm$ 0.34	0.63 $\pm$ 0.27	0.82 $\pm$ 0.31	0.70 $\pm$ 0.42	0.339
ALP (U/L)	67.2 $\pm$ 13.1	72.4 $\pm$ 16.4	68.5 $\pm$ 13.7	69.3 $\pm$ 21.7	78.5 $\pm$ 14.9	0.549
GLU (mmol/L)	7.24 $\pm$ 0.9	7.88 $\pm$ 0.92	7.52 $\pm$ 0.95	7.79 $\pm$ 0.93	7.42 $\pm$ 0.37	0.454
BUN (mmol/L)	4.68 $\pm$ 0.44	4.78 $\pm$ 0.47	4.98 $\pm$ 0.61	4.89 $\pm$ 0.41	4.87 $\pm$ 0.51	0.693
CHO (mmol/L)	2.18 $\pm$ 0.51	2.27 $\pm$ 0.41	2.34 $\pm$ 0.18	2.00 $\pm$ 0.31	2.28 $\pm$ 0.34	0.285

Table 4. Cont.

Item	Control	LP	HP	LT	HT	p-Value
CRE ($\mu\text{mol/L}$)	27.2 ± 2.3	27.5 ± 2.7	27.1 ± 1.4	27.4 ± 3.5	26.6 ± 2.1	0.938
TG (mmol/L)	0.44 ± 0.13	0.48 ± 0.14	0.42 ± 0.09	0.38 ± 0.11	0.41 ± 0.09	0.396
Na^+ (mmol/L)	136.6 ± 1.4	136.3 ± 1.0	136.3 ± 0.9	137.1 ± 0.8	137.0 ± 0.6	0.251
K^+ (mmol/L)	3.96 ± 0.54	3.87 ± 0.65	3.94 ± 0.14	3.98 ± 0.30	3.93 ± 0.16	0.981
Cl^- (mmol/L)	106.8 ± 0.8	106.6 ± 0.9	$105.6 \pm 1.5^*$	106.6 ± 1.2	106.1 ± 1.3	0.191
Females						
ALT (U/L)	45.1 ± 6.7	$38.4 \pm 5.8^*$	$38.0 \pm 7.6^*$	41.5 ± 4.6	40.3 ± 9.8	0.187
AST (U/L)	59.0 ± 10.0	52.2 ± 7.7	59.5 ± 8.4	60.1 ± 16.7	62.6 ± 13.9	0.383
TP (g/L)	61.3 ± 3.1	59.6 ± 3.2	58.5 ± 3.9	59.4 ± 5.1	58.7 ± 3.1	0.498
ALB (g/L)	39.3 ± 1.8	38.9 ± 1.9	38.5 ± 2.6	38.2 ± 3.1	37.4 ± 2.9	0.526
GGT (U/L)	0.72 ± 0.38	0.71 ± 0.33	0.77 ± 0.46	0.65 ± 0.43	0.77 ± 0.55	0.973
ALP (U/L)	47.1 ± 11.8	43.6 ± 8.8	44.7 ± 7.3	40.3 ± 16.9	47.9 ± 12.0	0.628
GLU (mmol/L)	7.68 ± 1.30	7.95 ± 1.98	7.87 ± 1.14	7.82 ± 1.35	7.85 ± 1.56	0.997
BUN (mmol/L)	5.57 ± 0.94	4.91 ± 0.71	5.16 ± 0.78	5.62 ± 0.61	5.42 ± 1.03	0.29
CHO (mmol/L)	1.93 ± 0.24	1.94 ± 0.49	1.93 ± 0.65	1.83 ± 0.52	1.74 ± 0.33	0.846
CRE ($\mu\text{mol/L}$)	20.9 ± 1.6	20.2 ± 1.6	20.8 ± 2.8	20.7 ± 4.6	21.0 ± 5.3	0.989
TG (mmol/L)	0.46 ± 0.08	0.39 ± 0.09	0.42 ± 0.13	0.45 ± 0.20	0.39 ± 0.19	0.726
Na^+ (mmol/L)	136.3 ± 1.7	136.7 ± 0.7	136.7 ± 1.3	136.7 ± 1.9	136.8 ± 1.3	0.961
K^+ (mmol/L)	3.54 ± 0.17	3.40 ± 0.32	3.59 ± 0.25	$3.65 \pm 0.17^*$	3.50 ± 0.31	0.257
Cl^- (mmol/L)	105.7 ± 1.3	105.8 ± 2.7	106.2 ± 0.9	106.8 ± 1.8	107.1 ± 1.3	0.311

* Compared with the control group, $p < 0.05$.

3.4. Effects of GM on the Urinalysis

There was no statistical difference detected in the urinalysis profiles among all the groups, except that specific gravity in LT was lower than in the control for female rats. These differences were within the control range rather than associated with the feeding of genetically modified plants (Table 5).

Table 5. Urine index record table of rats (positive number/total number).

Item	Control	LP	HP	LT	HT	p-Value
Males						
Specific gravity	1.020 ± 0.004	1.018 ± 0.005	1.020 ± 0.0047	1.017 ± 0.003	1.012 ± 0.004	0.824
pH	7.30 ± 0.48	7.05 ± 0.83	7.10 ± 0.66	7.20 ± 0.54	7.00 ± 0.53	0.497
Female						
Specific gravity	1.023 ± 0.003	$1.018 \pm 0.005^*$	1.020 ± 0.005	$1.018 \pm 0.003^*$	1.019 ± 0.007	0.207
pH	7.10 ± 0.57	7.00 ± 0.33	6.90 ± 0.57	6.65 ± 0.53	6.85 ± 0.58	0.393

* Compared with the control group, $p < 0.05$.

3.5. Effects of GM on the Organ Weights and Histopathological Examinations in Rats

No statistically noticeable difference was detected on the relative organ weight ratios, except testicles in males and thymus in females (Table 6). Despite higher testicle and lower thymus ratios being observed in males and females, respectively, no statistical significance was related to the GM treatment (i.e., LT and HT), which had no significant difference with the control ones (Table 6).

Moreover, no adverse effects of treatment-related histopathology changes were observed in the investigated tissues, including brain, heart, thymus, liver, spleen, kidney, adrenal, testicles, epididymis, and so on (Table 7), suggesting no damage due to GM on the changes of histopathology and specific damages.

Table 6. Organ/body relative weight ratio mean $\pm$ SD ($n = 10$).

Items	Control	LP	HP	LT	HT	<i>p</i> -Value
Males						
Brain	0.422 $\pm$ 0.036	0.406 $\pm$ 0.050	0.417 $\pm$ 0.036	0.422 $\pm$ 0.032	0.437 $\pm$ 0.047	0.564
Heart	0.299 $\pm$ 0.024	0.296 $\pm$ 0.030	0.293 $\pm$ 0.024	0.298 $\pm$ 0.020	0.293 $\pm$ 0.014	0.973
Thymus	0.131 $\pm$ 0.038	0.129 $\pm$ 0.037	0.136 $\pm$ 0.031	0.124 $\pm$ 0.032	0.120 $\pm$ 0.023	0.811
Liver	2.471 $\pm$ 0.171	2.482 $\pm$ 0.163	2.583 $\pm$ 0.180	2.428 $\pm$ 0.139	2.437 $\pm$ 0.165	0.243
Spleen	0.196 $\pm$ 0.028	0.178 $\pm$ 0.023	0.226 $\pm$ 0.038	0.206 $\pm$ 0.043	0.205 $\pm$ 0.036	0.049
Kidney	0.662 $\pm$ 0.039	0.650 $\pm$ 0.061	0.633 $\pm$ 0.039	0.653 $\pm$ 0.045	0.643 $\pm$ 0.036	0.683
Adrenal	0.017 $\pm$ 0.006	0.016 $\pm$ 0.002	0.016 $\pm$ 0.005	0.018 $\pm$ 0.005	0.019 $\pm$ 0.003	0.512
Testicles	0.793 $\pm$ 0.067	0.727 $\pm$ 0.080	0.709 $\pm$ 0.255	0.761 $\pm$ 0.054	0.835 $\pm$ 0.120 *	0.260
Epididymis	0.308 $\pm$ 0.082	0.294 $\pm$ 0.027	0.292 $\pm$ 0.058	0.327 $\pm$ 0.136	0.320 $\pm$ 0.061	0.571
Females						
Brain	0.598 $\pm$ 0.085	0.620 $\pm$ 0.090	0.626 $\pm$ 0.053	0.630 $\pm$ 0.054	0.626 $\pm$ 0.058	0.859
Heart	0.337 $\pm$ 0.056	0.334 $\pm$ 0.038	0.334 $\pm$ 0.036	0.325 $\pm$ 0.030	0.342 $\pm$ 0.023	0.888
Thymus	0.152 $\pm$ 0.045	0.179 $\pm$ 0.039	0.180 $\pm$ 0.033	0.141 $\pm$ 0.020 *	0.151 $\pm$ 0.036	0.054
Liver	2.598 $\pm$ 0.288	2.501 $\pm$ 0.140	2.540 $\pm$ 0.280	2.526 $\pm$ 0.179	2.546 $\pm$ 0.141	0.892
Spleen	0.235 $\pm$ 0.048	0.224 $\pm$ 0.036	0.253 $\pm$ 0.030	0.246 $\pm$ 0.028	0.261 $\pm$ 0.049	0.247
Kidney	0.677 $\pm$ 0.093	0.651 $\pm$ 0.065	0.638 $\pm$ 0.055	0.649 $\pm$ 0.055	0.646 $\pm$ 0.058	0.734
Adrenal	0.029 $\pm$ 0.006	0.032 $\pm$ 0.007	0.031 $\pm$ 0.004	0.034 $\pm$ 0.006	0.032 $\pm$ 0.008	0.602
Uterus	0.186 $\pm$ 0.044	0.179 $\pm$ 0.035	0.201 $\pm$ 0.066	0.168 $\pm$ 0.027	0.200 $\pm$ 0.055	0.490
Ovary	0.065 $\pm$ 0.015	0.075 $\pm$ 0.018	0.072 $\pm$ 0.020	0.066 $\pm$ 0.011	0.068 $\pm$ 0.016	0.600

* Compared with the control group, $p < 0.05$.**Table 7.** Pathological nonspecific changes recorded in rats.

Organ	Lesion Type	Control	HT	HP
Heart	Myocardial interstitial inflammatory cell infiltration, vacuolar degeneration, and fibrous tissue hyperplasia	1/20	1/20	1/20
Liver	There was a small amount of inflammatory cell infiltration in the portal area	3/20	3/20	4/20
Kidney	Renal tubular dilatation, vacuolar degeneration, protein cast: renal tubular atrophy	2/20	2/20	0/20
	There was a small amount of inflammatory cell infiltration in the renal interstitium	5/20	4/20	4/20
Lung	Mild or focal interstitial pneumonia; perivascular inflammatory cell infiltration	3/20	3/20	2/20
Adrenal glands	The medullary organs were dilated	2/20	2/20	0/20
Thyroid gland	Follicular epithelial hyperplasia and vacuolization	1/20	0/20	0/20
Pituitary gland	Cystic change	2/20	0/20	0/20
Testicles	There were fewer or no sperm in the lumen	1/10	0/10	0/10

HT, feeding with compound diets containing 70% transgenic Xiushui 134Bt. HP, feeding with compound diets containing 70% non-transgenic Xiushui 134.

4. Discussion

With the increasing demand for food supplies, increasingly more strategies have been developed to increase crop productivity, such as the enhancement of cereal starch through GM lines [18]. Since first produced in 1983, the GM plants have gone through a history of nearly 40 years and gained rapid development up until the present, which shows potential in improvement of rice yields for alleviating the food crisis. Increasingly more attention has been paid to the safety of GM crops. At present, there have been many reports on the safety evaluation methods of GM crops [10,15,19,20]. Substantial homogeneity evaluation is currently the international standard for the safety evaluation of GM food [21]. The evaluation of the safety of GM crops and recipient parents on human health includes mainly toxicological safety evaluation, allergic studies, anti-nutritional factors, nutritional components,

and the impact on human and food safety, among others. [22]. The toxicological evaluation methods can be divided into acute toxicity tests, subchronic toxicity tests, reproductive genotoxicity tests, and other toxicity tests. The subchronic toxicity evaluation of GM crops is conducted mainly on rodents fed for 30 or 90 days. Most existing studies showed that there was no difference in the effects of transgenic rice and non-transgenic rice on experimental animals. In some studies, there were significant differences in individual indicators, but some researchers interpreted them as having no biological significance [9,23,24].

In GM rice Xiushui 134Bt, green tissue-specific promoter Rbcs3 (Os12g0291100) was used to induce the expression of the *Bt* gene, and the *Bt* toxin proteins were concentrated in the stem and leaf of rice, but it was reduced or not expressed in rice grains [4]. It has become a feasible strategy to use tissue-specific promoters or chemically induced promoters to drive the expression of target genes in specific tissues, organs, or periods to improve the biosafety of transgenic rice. The expression of *Bt* toxin protein in Xiushui 134Bt rice at different growth stages and tissues was studied by double-antibody sandwich enzyme-linked immunoassay (ELISA). Our previous results showed that *Bt* toxin protein was expressed in all tissues, but the expression levels were significantly different in different organs. The expression level of *Bt* toxin protein was the highest in leaves, followed by leaf sheath, and was relatively low in roots, stems, and spikelets, but hardly detected in mature seeds [25].

Herein, the aim is to evaluate the potential effect of Xiushui 134Bt on rats. The transgenic Xiushui 134Bt rice and Xiushui 134 non-transgenic rice were added to the formulated diet at 35% and 70%, respectively, and fed to rats for 90 days. During the experiment, the animals in each group were free in activities, with shiny coats, no abnormal secretions in nose, eyes, or mouth, and no poisoning or death. During the experiment, there were no significant differences in body weight, feed intake, total weight gain, total feed intake, or total food utilization ratio between groups and the control group ($p > 0.05$). The relative organ weight of some transgenic Xiushui 134Bt rice group was significantly different from that of Xiushui 134 non-transgenic rice group ($p < 0.05$), there was no dose–response relationship and no biological significance. There were no significant differences in organ weight or relative weight between the other groups and the control group ($p > 0.05$). Part of the blood biochemical indexes, blood routine, and urine indexes of the transgenic Xiushui 134Bt rice group and Xiushui 134 non-transgenic rice group had statistical significance as compared with those of the conventional basal diet group ($p < 0.05$). Moreover, some transgenic Xiushui 134Bt rice group and corresponding parental control group had statistical significance ($p < 0.05$), all of which were within the normal range of the detection unit, and there was no dose–response relationship or biological significance. In addition, no abnormality during animal dissection was observed. No typical histopathological changes or specific lesions were found in experimental animals caused by transgenic samples.

In general, the subchronic toxicity evaluation of *Bt* transgenic rice was primarily conducted on rodents fed for 30 or 90 days, and the existing research results did not find significant effects on half of the physiological characteristics of rodents fed on *Bt* transgenic rice. Our results are consistent with those of previous studies. Currently, studies on the edible safety of *Bt* rice focus mainly on small rodents, and scarce research has been revealed on primates with high genetic homology. Nonetheless, the safety of transgenic food is vital for human health, and therefore, subchronic toxicity studies on non-rodent animals should be increased to comprehensively investigate the safety of GM rice for mammals.

5. Conclusions

This study was conducted to investigate the safety of GM rice with the *Bt* gene and Bar gene. Our results showed that there were no adverse dose-related effects on the growth and development of Wistar rats consuming diets formulated with *Bt* GM rice Xiushui 134Bt as compared with the control, demonstrating that Xiushui 134Bt was as safe as conventional non-GM rice Xiushui 134.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13030826/s1>, Figure S1: T-DNA region of transformation construct pZTRT-Bt; Figure S2: Schematic diagram of the insertion site of the foreign T-DNA.

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