



Short communication

Subchronic toxicity study *in vivo* and allergenicity study *in vitro* for genetically modified rice that expresses pharmaceutical protein (human serum albumin)



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ABSTRACT

Genetically modified (GM) crops that express pharmaceutical proteins have become an important focus of recent genetic engineering research. Food safety assessment is necessary for the commercial development of these crops. Subchronic toxicity study *in vivo* and allergenicity study *in vitro* were designed to evaluate the food safety of the rice variety expressing human serum albumin (HSA). Animals were fed rodent diets containing 12.5%, 25.0% and 50.0% GM or non-GM rice for 90 days. The composition analysis of the GM rice demonstrated several significant differences. However, most of the differences remained within the ranges reported in the literature. In the animal study, a range of indexes including clinical observation, feed efficiency, hematology, serum chemistry, organ weights and histopathology were examined. Random changes unrelated to the GM rice exposure, within the range of historical control values and not associated with any signs of illness were observed. The results of heat stability and *in vitro* digestion of HSA indicated no evidence of potential allergenicity of the protein. Overall, the results of these studies suggest that the GM rice appears to be safe as a dietary ingredient when it is used at up to 50% in the diet on a subchronic basis.

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1. Introduction

Rice is one of the most important staple foods in the world and has great advantages and significance as a bioreactor to produce pharmaceutical proteins. Some studies have shown that the genetically modified (GM) rice promotes human health and is environmentally safe (He et al., 2008b). For example, human lysozyme and lactoferrin were successfully expressed in rice seeds (Nandi

et al., 2005; Yang et al., 2003). The former has antimicrobial functions and the latter is useful as nutritional supplements.

Human serum albumin (HSA) is widely used clinically to treat hemorrhagic shock, serious burn injuries, hypoproteinemia, fetal erythroblastosis and ascites (Belgacem et al., 2007; Xie et al., 2008). Annual worldwide use of HSA approaches 500 metric tons at a cost of over \$1.5 billion (Daniell, 2003). Presently, HSA is primarily produced for commercial purposes by extraction from human plasma, which is costly, highly regulated and poses a potential risk of the transmission of blood-derived infectious pathogens such as hepatitis and HIV (Chamberland et al., 2001; Erstad, 1996). Fortunately, the accurate expression of a high level of HSA in rice with a productivity rate of 2.75 g/kg brown rice was achieved by Wuhan University, China (He et al., 2011). This production rate meets the needs of large-scale commercial production. Many experiments related with “substantial equivalence” between rice-extracted HSA (rHSA) and plasma-derived HSA (pHSA) were performed by He et al., 2011. Amino acid sequence and crystal structure of rHSA were exactly the same with pHSA. Both rHSA and pHSA have equivalent biological characteristic

Abbreviations: GM, genetically modified; HSA, human serum albumin; rHSA, rice-extracted human serum albumin; pHSA, plasma-derived human serum albumin; OECD, Organization for Economic Cooperation and Development; PCR, polymerase chain reaction; SD, standard deviation; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; SPF, specific pathogen-free.

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including efficiency of promoting cell growth and immunogenicity *in vitro* and *in vivo* (He et al., 2011). Although the GM rice will not be sold in the food market, some concerns for the food safety of the GM rice must be addressed before large-scale applications. This is of particular concern for the potential release of GM forms of staple crops such as rice, wheat, corn, and potatoes into conventional plant populations (Halford and Shewry, 2000).

A relatively mature safety assessment system for GM crops was established based on “substantial equivalence” (Millstone et al., 1999) proposed by the Organization for Economic Cooperation and Development (OECD). A 90-day rodent feeding study using the whole GM food is required to assess potential unintended effects of toxicological and/or nutritional relevance and to establish whether the GM food is as safe and nutritious as its traditional comparator (EFSA, 2008). In such a case, it is indispensable to assess the safety and nutritional quality of GM crops using the 90-day toxicity study in rodents (Kuiper et al., 2001). In addition, the Scientific Committee on Food and FAO/WHO has recommended the use of the 90-day rodent feeding study as the sentinel study for safety testing of GM food (FAO/WHO, 2000; SCF, 1996).

Nutritional data, toxicological data, and data concerning allergenic potential need to be considered for novel GM foods (Kuiper et al., 2001). In this study, a comprehensive analytical characterization of the rice materials was conducted before the subchronic toxicity study *in vivo*. In addition, heat stability and simulation digestibility *in vitro* in stimulated gastric and intestinal fluids were performed evaluating allergenicity of rHSA protein. The animal experiment was performed in accordance with (A) the Chinese Toxicology Assessment Procedures and Methods for Food Safety (Chinese standard GB15193.13-2003) and (B) the Organization for Economic Co-operation and Development Guidelines for the Testing of Chemicals at the Experimental Animal Center, Supervision and Testing Center for GMOs Food Safety, Ministry of Agriculture (Beijing, China).

2. Materials and methods

2.1. Crop materials

The GM rice 4-114-7 and its non-transgenic isogenic control “Taipei 309” were supplied by Wuhan University, China. Both of the rice materials were planted in Taoyuan City, Hubei Province, China under identical climate conditions in the growing season of 2010. The rHSA protein (extracted from the GM rice 4-114-7) was also provided by Wuhan University with the purity of 99.9%.

The identification of the GM and non-GM rice was conducted utilizing a specific polymerase chain reaction (PCR) assay for the presence of the HSA gene. The results demonstrated that the gene was present in the 4-114-7 rice but was not detected in the non-GM rice Taipei 309 (data not shown). The rHSA accumulates at up to 4.93 g/kg brown rice (He et al., 2011).

2.2. Composition analysis and preparation of the rodent diets

The nutritional proximates (moisture, protein, fat, crude fiber, ash), amino acids, minerals and anti-nutritional factors (trypsin inhibitor and phytic acid) of both brown rice materials were measured using validated standard methods (Supplemental Information Table S1). The rodent diets similar to the nutrient composition of the AIN93G diet (Reeves et al., 1993) but containing concentrations of 12.5%, 25%, and 50% (wt/wt) of each of the rice strains were prepared by Ke Ao Xie Li Feed Co. Ltd. (Beijing, China). A minor adjustments in diet formulations due to differences in composition between GM and non-GM rice were made to eliminate nutritional differences. A commercial rodent diet was included as a vehicle control. The formulation of all the rodent diets is shown in Supplemental Information Table S2. All diets were vacuum packed and sterilized.

2.3. Animals and experimental design

The experimental design was approved by Animal Ethics Committee of China Agricultural University, Beijing, and the approval ID of this study is 20120011.

Five-week-old Sprague–Dawley male and female rats free from specific pathogens were obtained from Vital River Laboratories Inc. (Beijing, China) with the license number SCXK (Beijing) 2012–0004. After acclimatization for five days, seventy male and seventy female healthy rats were selected on the basis of general

health and body weight within $\pm 20\%$ of the mean within the same sex. The selected rats were randomly distributed by weight into 7 experimental groups (10 rats/sex/group) utilizing a stratified randomization procedure. The animals were administered balanced rodent diets with 12.5%, 25%, 50% (wt/wt) GM rice (T1, T2, T3, respectively), non-GM rice (N1, N2, N3, respectively) or commercialized rodent diet (CK). The animals were housed (5 rats/cage) in a specific pathogen-free (SPF) animal laboratory of the Supervision & Testing Center for GMOs Food Safety, Ministry of Agriculture (Beijing, China) with the license number SYXK (Beijing) 2010-0036 under standard laboratory conditions (adequate fresh air exchange, temperature 20–25 °C and relative humidity 40–70%). A 12-h light/dark automatic cycle of artificial illumination was used. All animals were provided sterile drinking water.

Heat stability and *in vitro* digestion of rHSA protein were performed in accordance with published articles (Xu et al., 2009; Cao et al., 2010). A range of indexes including clinical observation, feed efficiency, hematology, serum chemistry, organ weights and histopathology were examined (Qi et al., 2012; Yuan et al., 2013) as regulated. More details are shown in Supplemental Information Materials and Methods S1, S2, S3, S4 and S5.

2.4. Statistical analysis

The mean value and standard deviations (SD) of all quantitative data from both male and female rats were calculated using IBM SPSS Statistics 20.0 software (IBM SPSS Inc., USA). One-way analysis of variance was used to determine homogeneity of the variances within a group and by gender as well. SPSS was used to perform the multiple comparisons among the GM groups, non-GM groups and the control group (Dunnett's test). Statistically significant differences were defined as $p < 0.05$.

3. Results

3.1. Composition analysis

Comprehensive analytical characterization of both brown rice materials was performed. The compositional data were compared with data reported for brown rice (Juliano, 1993; Liu et al., 2005; OECD, 2004; USDA, 2004). Nutritional proximates, anti-nutritional factors and minerals of the GM and non-GM rice are shown in Table 1. The levels of amino acids (Table 2), reflecting protein content, were also analyzed.

No statistically significant differences were observed in the levels of moisture, ash, crude fiber, fat, phytic acid, vitamin B1 and most of the minerals between GM rice and non-GM rice. However, some components of the GM rice exhibited statistically significant differences ($P < 0.05$) compared with the non-GM rice. Most of the differences remained within the ranges reported in the literature (Juliano, 1993; Liu et al., 2005; OECD, 2004; USDA, 2004).

Table 1
Composition analysis.

	Non-GM rice	GM rice	Literature data ^b
Moisture (g/100 g)	13.99 $\pm$ 0.01	14.05 $\pm$ 0.03	9.1–14.1
Ash (g/100 g)	1.63 $\pm$ 0.02	1.36 $\pm$ 0.02	1.0–1.5
Protein (g/100 g)	7.11 $\pm$ 0.14	8.05 $\pm$ 0.23 ^a	7.1–8.3
Crude fiber (g/100 g)	1.57 $\pm$ 0.06	1.71 $\pm$ 0.02	0.6–3.5
Fat (g/100 g)	2.23 $\pm$ 0.03	2.6 $\pm$ 0.04	1.6–2.8
Trypsin inhibitor (TIU/g)	104.69 $\pm$ 1.03	174.54 $\pm$ 3.15 ^a	100–2000 ^c
Phytic acid (mg/g)	7.89 $\pm$ 0.03	8.49 $\pm$ 0.07	6.45–11.1
Calcium (mg/100 g)	9.28 $\pm$ 0	9.16 $\pm$ 0.06	1–5
Zinc (mg/kg)	39.08 $\pm$ 0.43	27.45 $\pm$ 0.23 ^a	6–28
Copper (mg/kg)	4.8 $\pm$ 0.07	4.72 $\pm$ 0.03	1–6
Manganese (mg/kg)	3.06 $\pm$ 0.02	2.79 $\pm$ 0.03	2–37
Potassium (mg/kg)	246.76 $\pm$ 1.47	236.79 $\pm$ 0.06	60–280
Phosphorus (mg/100 g)	274.06 $\pm$ 4.54	234.93 $\pm$ 5.31 ^a	170–430
Vitamin E (mg/100 g)	1.16 $\pm$ 0.02	0.95 $\pm$ 0 ^a	0.9–2.5
Vitamin B1 (mg/100 g)	0.11 $\pm$ 0	0.14 $\pm$ 0	0.29–0.61
Vitamin B2 (mg/100 g)	0.03 $\pm$ 0	0.05 $\pm$ 0 ^a	0.04–0.14

^a Significant differences between GM rice and non-GM rice ($P < 0.05$).

^b Reference range based on previously reported values (Juliano, 1993; Liu et al., 2005; OECD, 2004; USDA, 2004).

^c Data collected by the Supervision, Inspection and Testing Center of Genetically Modified Organisms, Ministry of Agriculture, Beijing, China.

Table 2
Amino acid composition analysis of both brown rice materials.

	Non-GM rice	GM rice	Literature data ^b
Alanine (g/100 g)	0.42 ± 0.02	0.55 ± 0.02 ^a	0.46–0.58
Arginine (g/100 g)	0.63 ± 0.01	0.74 ± 0.01 ^a	0.44–0.91
Aspartic acid (g/100 g)	0.69 ± 0.01	0.87 ± 0.03	0.75–0.87
Glutamic acid (g/100 g)	1.38 ± 0.02	1.56 ± 0.05	1.52–1.76
Glycine (g/100 g)	0.35 ± 0.02	0.41 ± 0.01	0.39–0.49
Histidine (g/100 g)	0.22 ± 0.03	0.24 ± 0.01	0.23–0.25
Isoleucine (g/100 g)	0.27 ± 0	0.31 ± 0.02	0.26–0.44
Leucine (g/100 g)	0.58 ± 0.01	0.7 ± 0.05	0.50–0.85
Lysine (g/100 g)	0.34 ± 0.02	0.48 ± 0.03	0.10–0.42
Methionine (g/100 g)	0.17 ± 0.02	0.18 ± 0.01	0.05–0.31
Phenylalanine (g/100 g)	0.45 ± 0.04	0.5 ± 0.06	0.30–0.55
Proline (g/100 g)	0.33 ± 0.01	0.39 ± 0.01 ^a	0.37–0.40
Serine (g/100 g)	0.37 ± 0	0.42 ± 0.01 ^a	0.41–0.50
Threonine (g/100 g)	0.28 ± 0.01	0.34 ± 0.01	0.19–0.62
Tyrosine (g/100 g)	0.15 ± 0.02	0.2 ± 0.05	0.21–0.47
Valine (g/100 g)	0.45 ± 0.01	0.52 ± 0.02	0.48–0.63

^a Significant differences between GM rice and non-GM rice ($P < 0.05$).

^b Ranges from minimum to maximum reported values (Juliano, 1993; OECD, 2004; USDA, 2004).

3.2. Heat stability

On sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), the rHSA protein was clearly visible at about 66.5 kDa at time zero (Supplemental Information Fig. S1). When incubated at 100 °C for 60 min, the rHSA persisted and was still detectable. Therefore, it was not degraded or modified in a way that would affect its migration on SDS–PAGE after exposure to 100 °C for 60 min.

3.3. Rapid degradation in digestive fluids

SDS–PAGE was used to detect the digestion of the rHSA in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). The result showed that rHSA was rapidly digested within 15 s in both the SGF and the SIF (Supplemental Information Fig. S2).

3.4. Survival and clinical observations

There were no test article-related mortalities during the feeding trial. All of the test animals appeared to be healthy, and no treatment-related signs of adverse effects in clinical appearance were observed (data not shown).

3.5. Body weight gain and food consumption

In the males, the mean body weight (Fig. 1A) of T1 group was lower ($P < 0.05$) than that of CK group. Some individual rats in T1 group were observed wasting feed, which is a habit for some rodents. This may account for the higher feed use and lower body weight for this group. Furthermore, this significant difference was not considered adverse or related to the addition of GM rice in the rodent diets because the mean body weight in T2 and T3 groups was not different from CK group. The mean body weight of the female rats (Fig. 1B) in the GM group was comparable with both the control group and the non-GM group. No significant differences in total food consumption or food efficiency were observed (Supplemental Information Fig. S3).

3.6. Hematology examination

A few statistically significant differences between the control group and other treatment groups were found in some hematology parameters on the 45th and 90th day (Supplemental Information

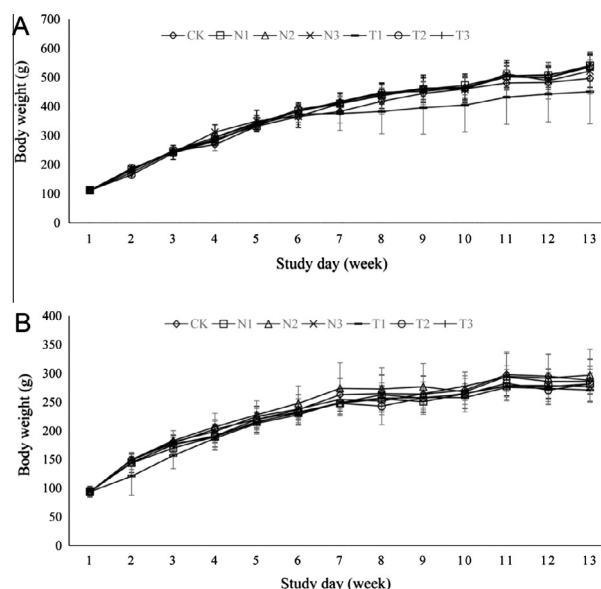


Fig. 1. Mean weekly body weights of male and female rats. A, male groups. B, female groups. The values are presented as means ± standard deviation (10 rats/sex/group).

Tables S3 and S4). Majority of these variations are within the historical control data (More details in Supplemental Information Results S1). Any changes observed were sporadic, non-dose related and considered incidental.

3.7. Serum chemistry

Although some statistically significant differences were observed in a few serum chemistry parameters on the 45th and 90th day (Supplemental Information Tables S5 and S6), there were no treatment-related biologically adverse effects. All the variations are within the historical control data (More details in Supplemental Information Results S2) and considered as incidental and toxicologically insignificant due to lack of dose-dependency and corresponding changes in histopathology.

3.8. Organ/body weight

The relative organ weights of all groups are summarized in Supplemental Information Table S7. The mean relative weight of the brains of the male rats that consumed 25% GM rice was slightly lower ($P < 0.05$) than that of the non-GM group and the CK group. However, the change was within historical control data and considered incidental because no significant differences were observed in the female rats, and the microscopic histology was normal. Any changes noted in organ weights were incidental and lack of corresponding changes in histopathology.

3.9. Pathology

At necropsy, there were no gross or microscopic anatomic pathological changes that were considered to be test article related (data not shown).

4. Discussion

Subchronic toxicity study plays an important role in the toxicological evaluation of GM crops. However, in the past decade, most of the modified genes focused on commercial traits such as

insect-resistance (Chen et al., 2011; He et al., 2008a), herbicide-resistance (Hammond et al., 2004; Zhu et al., 2013) and modified product quality (Qi et al., 2012). The rice material used in this study expresses HSA, a pharmaceutical protein. A 90-day subchronic toxicity study *in vivo* and an allergenicity study *in vitro* were designed to assess the food safety of the GM rice. The results of the present animal toxicity study show that the administration of balanced diets (made from GM rice) to male and female rats caused no adverse effects as evaluated by clinical observations, growth, feed consumption, hematology, serum chemistry, organ weights and histopathology. The results of heat stability and *in vitro* digestion of rHSA indicated no evidence of potential allergenicity of the protein.

Although the two rice varieties were cultivated under identical environmental and climatological conditions, statistical analyses indicated that several parameters of the GM rice were significantly different from the isogenic non-GM variety. In this case, additional field trials are required to confirm whether the differences measured are due to the genetic modification process or biological variability in the field. Most of the compositional parameters of the GM rice were within the ranges presented in the literature (Juliano, 1993; OECD, 2004; USDA, 2004), except for calcium, lysine, tyrosine, and vitamins B1 and B2. However, the existing food composition database do not necessarily reflect the full range of natural variation (Burlingame, 2004). Amino acids, especially alanine, arginine, proline and serine, in the GM rice were all higher compared with the isogenic non-GM line. This probably reflects the increased level of protein caused by the high expression of HSA in the GM rice. The expression of the HSA protein did have some influences on the composition of the rice. However, relevant unintended side effects could not be determined from the analyses of specific compounds or intermediates in important nutritional and anti-nutritional pathways (Kok and Kuiper, 2003).

Three dietary formulations containing a maximum of 50% GM and non-GM brown rice were incorporated into the rodent diets as a range from 40% to 70% concentration were selected in previous study (Domon et al., 2009; Schröder et al., 2007; Zhou et al., 2011). Furthermore, some unintended effects on animal health may be observed when experimental animals are treated with test materials at exaggerated doses and unbalanced with respect to nutrients (Monamy, 2009). The formulated rodent diets used in the present study were balanced to ensure an adequate supply of macro- and micro-components. A little biological variations were made considering composition differences between GM and non-GM rice as well as different concentrations of added rice materials. These differences guaranteed the nutrition-balanced diets for rats in different groups and provided rats the same nutrition level.

Some parameters of hematology, serum chemistry and organ weights in the GM groups demonstrated statistically significant differences compared with the control group and/or the non-GM groups. In the male rats, a lower level of WBC, an indication of hyp immunity, was detected only in the 25% GM group. This change was amplified by individual rats in this group, but no histopathological symptoms in the liver or thymus were observed. The absolute and relative decrease of ALT and AST were observed only in the male rats. This change was not considered to be clinically significant to the health of the animals (Anderson et al., 2000). It may actually reflect eneficial effects such as membrane stabilization in hepatocytes (Lee et al., 2005). The HGB and HCT values decreased in 12.5% GM male group and this symptom is often related with anemia. Some individual rats of the group were observed wasting feed resulting in the higher feed use and lower body weight. Anemia is often following dietary restriction (Chow and Lee, 1964), so low mean HGB and HCT values of some individuals may be associated with anemia. Hence, these changes were considered biologically insignificant, incidental, and unassociated

with the test article administration. These results were similar to previous subchronic toxicity studies for other GM rice materials (Cao et al., 2011; Poulsen et al., 2007; Schröder et al., 2007). For instance, Poulsen conducted a 90-day safety study in Wistar rats fed GM rice expressing snowdrop lectin. Several components, including protein, ash, fiber and sugars, of the GM rice were significantly different from the non-GM traditional isogenic line. Some statistically significant differences were also observed in blood biochemistry, hematology, immunological parameters and histopathology between the GM groups and non-GM groups, but none of these differences were considered to indicate adverse effects on the animal health.

Allergenicity assessment is indispensable for the food safety assessment of GM crops (Aalberse, 2008) apart from toxicology evaluation. Food allergens are considered to be able to exert systemic allergic reactions only after they have been absorbed through the intestinal mucosa (Tlaskalová-Hogenová et al., 2002). In addition, the resistance of a protein toward digestion in the human gastrointestinal tract could indicate its potential allergenicity. (Cao et al., 2010). In this study, heat stability and *in vitro* digestion of the rHSA protein were performed to assess potential allergenicity of the pharmaceutical protein. Although the rHSA protein was stable for heat under 100 °C, it could be digested rapidly in both SGF and SIF within 15 s. Therefore, the results indicated no evidence of potential allergenicity of the protein.

Plasma-derived HSA has been widely used clinically for a long time and there is a significant history of safe human exposure (Anderson and Anderson, 2002). “Substantial equivalence” between rHSA and pHSA was certified through a series of experiments. Physical and biochemical characterization of rHSA revealed it equivalent to pHSA (He et al., 2011). Recombinant protein production in plants to provide botanical therapeutics is responsible for launching a new generation of improvements in human health in the twenty-first century (Raskin et al., 2002). Research on pharmaceutical GM crops is an exciting field of genetic engineering with great advantages, such as low cost, high safety and facilitated mass production (Fischer et al., 2004; Ma et al., 2003). However, food safety assessment for pharmaceutical GM crops is necessary prior to the development of their commercialization. In this study, experiments both on toxicology and allergenicity assessment were performed. The results indicate no evidence of potential allergenicity of the protein and the GM rice 4-114-7 has no adverse or toxic effects on the test animals. The GM rice appears to be safe as a dietary ingredient when it is used at up to 50% in the diet on a subchronic basis.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The [Transparency document](#) associated with this article can be found in the online version.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fct.2014.07.030>.

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**Subchronic toxicity study *in vivo* and allergenicity study *in vitro* for
genetically modified rice that expresses pharmaceutical protein
(human serum albumin)**

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Running Title: Subchronic toxicity and allergenicity studies of GM rice expressing the
pharmaceutical protein HSA

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Number of figures: 3

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No figures in color

Materials and Methods

S1. Heat stability study

The rHSA protein were dissolved in 20 mM Tris-HCl and 5 mM EDTA buffer at a concentration of 0.1 mg/ml in 1.5 ml microcentrifuge tubes. They were tested for stability at temperatures 100 °C for periods of 10, 20, 30, and 60 min in a temperature-controlled heating block. The assay was terminated by placing the sample tubes on ice and adding SDS-sample buffer (50 mM Tris-Cl, 8% sucrose, 2% SDS, with 5% 2-mercaptoethanol, and 0.02% bromophenol blue). Control samples subjected to no incubation (kept at 4 °C) were also prepared. The resulting proteins were analyzed by SDS-PAGE.

S2. *In vitro* digestion of rHSA

The concentrations of rHSA in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) (Sigma, USA) were 0.25 mg/mL and 0.1 mg/mL, respectively. The ratio of the rHSA (5 g/L for SGF, 2 g/L for SIF) and enzyme was 1:19. The mixture was incubated at 37 °C, and the samples were taken out at intervals of 0, 15s, 2, 30 and 60 min. According to the results of SDS-PAGE, the stability of the protein to be digested in SGF/SIF was observed. The experiments were also performed for positive control soybean trypsin inhibitor (STI) and negative control bovine serum albumin (BSA) or α -casein for SGF or SIF, respectively, as the method above.

S3 Clinical examination, body weight gain and feed consumption

Regular observations were conducted every day during the animal experiment to evaluate behavior, fur condition, eyes, ingestion, excretion and clinical signs of toxicity. The body weights and food intake of all animals were recorded every week for the calculation of food efficiency. All animals were fasted overnight for 12 h prior to blood collection from the inner canthus.

S4 Hematology and serum chemistry

Hematological analyses for white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT) and mean platelet volume (MPV) were performed using the Blood Cell Counter HEMAVET950 (Drew Scientific, Dallas, USA).

Serum chemistry analyses for alanine aminotransferase (Chamberland et al.), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), alkaline phosphatase (ALP), glucose (GLU), blood urea nitrogen (BUN), creatinine (CREA), calcium (Ca), phosphorus (P), total cholesterol (CHO), lactate dehydrogenase (LDH), chlorine (CL) and magnesium (Mg) were performed using an RA-1000 autoanalyzer (Technicon, Tarrytown, NY, USA).

S5 Anatomic pathology

Gross anatomic pathology was evaluated by visual inspection after the animals were killed by decapitation, and the major organs, including brain, liver, spleen, heart, lung, thymus, kidney, adrenals, stomach, intestine (jejunum, ileum, duodenum), and ovaries or testes, were collected. Weights for all organs, except for the gastrointestinal organs, were measured. The various organs were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned, and the tissue sections were stained with hematoxylin-eosin. These procedures were conducted at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences (Beijing, China), where microscopic examination of the sections was performed by pathologists.

Results

S1 Hematology examination

The values of HGB and HCT in the 12.5% male GM groups were somewhat lower ($P < 0.05$) than the control group on the 45th day, but this difference was not observed in the 25% and 50% male GM groups. The level of WBC in the 25% GM male group was significantly different ($P < 0.05$) from the control group and the non-GM group on the 45th day; however, this change was not found in the male 50% GM group. The values of HGB, HCT and WBC were all normal on 90th day. Small proportion of groups including 25% GM male groups (45 day), 12.5% non-GM female groups (45 day) and 50% non-GM male groups (90 day) showed lower ($P < 0.05$) mean values of PLT, which was contributed by some individual rats. This phenomenon was sporadic and most likely related to unexpected coagulation of the blood samples. The female groups demonstrated less variable values compared with male groups on the 45th day. And there were less significant differences on 90th day.

S2 Serum chemistry

A slightly lower level ($P < 0.05$) of ALT and AST were observed in the male 25% GM group both on the 45th and 90th day; however, they appear to be normal compared with the historical control data. And a relative low level of ALT and AST may actually reflect beneficial effects. A changed BUN/CREA ratio are always following glomerulocystic kidney disease. Although elevated levels of BUN and CREA were observed in the female 12.5% GM group on the 45th day, BUN/CREA ratio was similar to the other groups. In the terminal measurement, statistically significant differences were observed in several parameters including ALP, GLU, calcium, Chlorine, CHO, LDH, phosphorus and magnesium.

Figure S1.

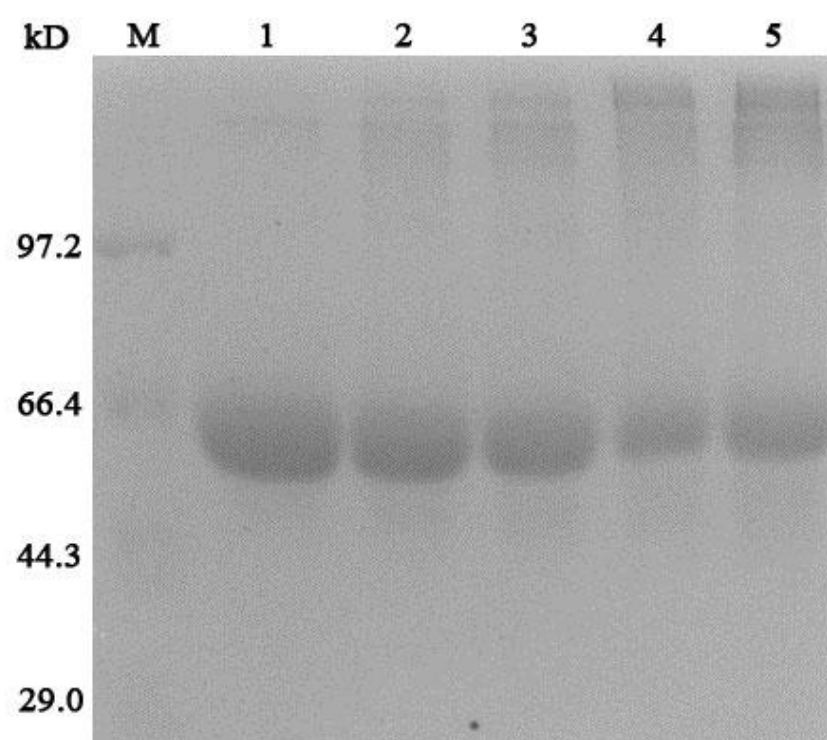


Figure S2.

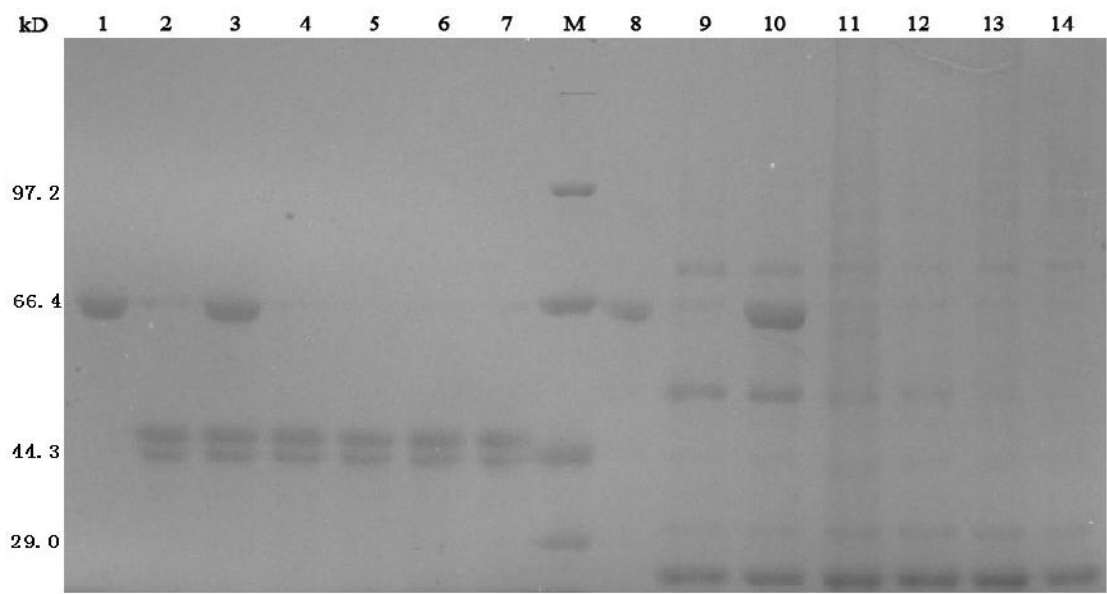


Figure S3.

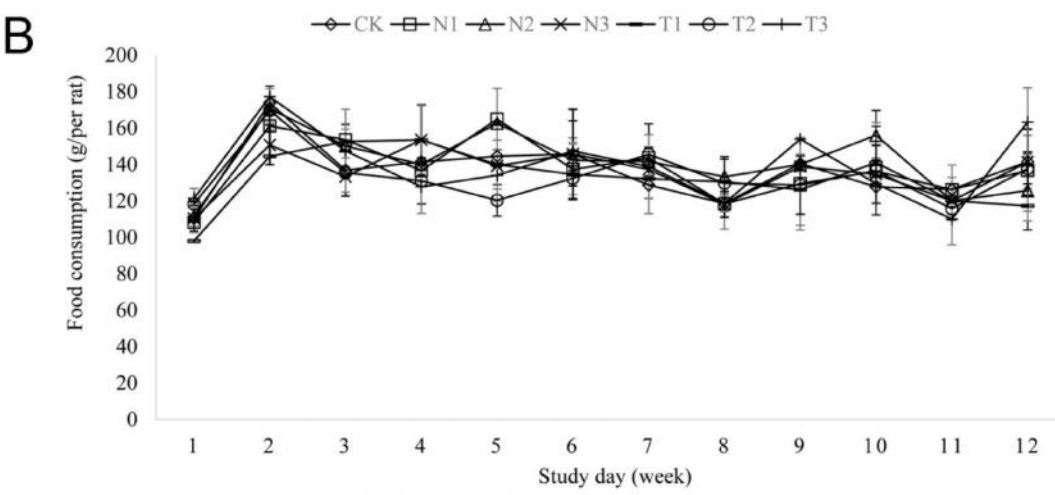
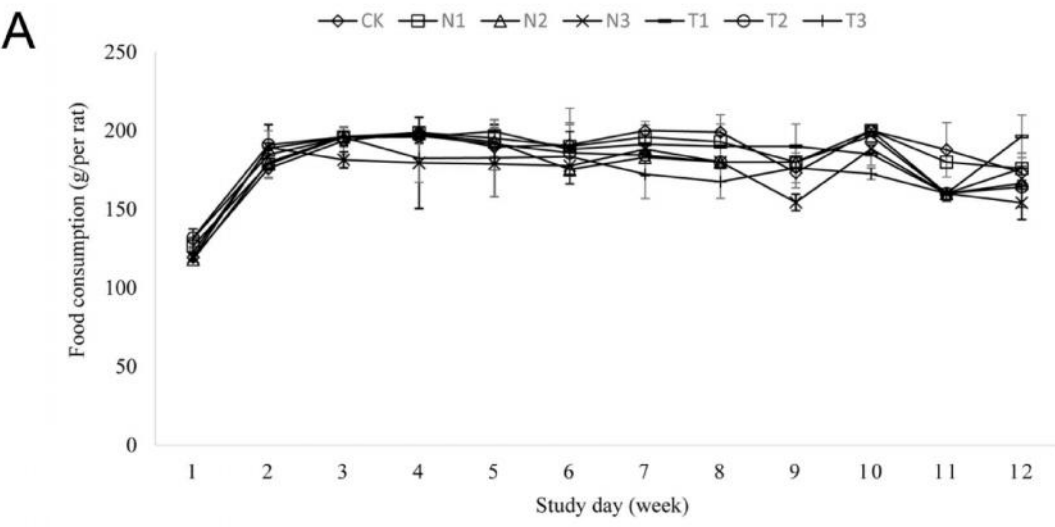


Table S1. Methods for Composition Analysis

Composition parameters	Method
Moisture	Chinese standard GB 5009.3-2010. 2010. National food safety standard: Determination of moisture in foods. Standards Press of China, Beijing, China.
Ash	Chinese standard GB 5009.4-2010. 2010. National food safety standard: Determination of ash in foods. Standards Press of China, Beijing, China.
Protein	Chinese standard GB 5009.5-2010. 2010. National food safety standard: Determination of protein in foods. Standards Press of China, Beijing, China.
Crude Fiber	Chinese standard GB/T 5009.10-2003. 2003. National food safety standard: Determination of crude fiber in vegetable foods. Standards Press of China, Beijing, China.
Fat	Chinese standard GB/T 5009.6-2003. 2003. National food safety standard: Determination of fat in foods. Standards Press of China, Beijing, China.
Trypsin inhibitor	Chinese Agricultural Standard NY/T 1103.2-2006. 2006. Safety assessment of genetically modified plant and derived products Part 2: assay of anti-nutrients pancreatic typsin inhibitor. The Ministry of Agriculture of the People's Republic of China, Beijing, China.
Phytic acid	Chinese Agricultural Standard NY/T 1103.1-2006. 2006. Safety assessment of genetically modified plant and derived products Part 1: assay of anti-nutrients phytate,gossypol and erucic acids. The Ministry of Agriculture of the People's Republic of China, Beijing, China.
Calcium	Chinese standard GB/T 5009.92-2003. 2003. National food safety standard: Determination of calcium in foods. Standards Press of China, Beijing, China.
Zinc	Chinese standard GB/T 5009.91-2003. 2003. National food safety standard: Determination of zinc in foods. Standards Press of China, Beijing, China.
Copper	Chinese standard GB/T 5009.13-2003. 2003. National food safety standard: Determination of copper in foods. Standards Press of China, Beijing, China.
Manganese	Chinese standard GB/T 5009.90-2003. 2003. National food safety standard: Determination of iron,magnesium

	and manganese in foods. Standards Press of China, Beijing, China.
Potassium	Chinese standard GB/T 5009.91-2003. 2003. National food safety standard: Determination of potassium and sodium in foods. Standards Press of China, Beijing, China.
Phosphorus	Chinese standard GB/T 5009.87-2003. 2003. National food safety standard: Determination of phosphorus in foods. Standards Press of China, Beijing, China.
Vitamin E	Chinese standard GB/T 5009.82-2003. 2003. National food safety standard: Determination of retinol and tocopherol in foods. Standards Press of China, Beijing, China.
Vitamin B1	Chinese standard GB 5413.11-2010. 2010. National food safety standard: Determination of vitamin B1 in foods for infants and young children, milk and milk products. Standards Press of China, Beijing, China.
Vitamin B2	Chinese standard GB 5413.12-2010. 2010. National food safety standard: Determination of vitamin B2 in foods for infants and young children, milk and milk products. Standards Press of China, Beijing, China.
Amino acids	Chinese standard GB/T 5009.124-2003. 2003. National food safety standard: Determination of amino acids in foods. Standards Press of China, Beijing, China.

Table S2. Nutritional analysis of the rodent diets.

Rice	Diets with Non-GM rice			Diets with GM rice			Commercial diet
	12.5%	25.0%	50.0%	12.5%	25.0%	50.0%	
Producing method (kg/50kg)							
Test sample	6.25	12.50	25.00	6.25	12.50	25.00	0
Maize	15.70	12.70	0	16.00	12.50	0	14.35
Wheat meal	5.00	3.00	3.00	5.00	3.50	3.50	7.50
Wheat middling	5.00	3.00	3.00	5.00	3.50	3.50	10.00
Fishmeal	2.00	2.00	2.00	2.00	2.00	2.00	0.75
Beet pulp	1.50	1.25	1.25	1.50	1.00	1.00	2.25
Bean pulp	11.00	12.00	12.00	10.75	11.45	11.30	11.50
Plant oil	0.70	0.70	0.90	0.65	0.65	0.80	0.80

Calcium hydrophosphate	0.85	0.85	0.85	0.90	0.90	0.90	0.90
Mountain flour	0.75	0.75	0.75	0.70	0.75	0.75	0.70
Yeast powder	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Additive	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Nutrition level							
Dry matter (%)	88.40	88.60	89.30	88.10	88.10	88.10	88.40
Crude protein (%)	18.90	19.00	18.90	18.90	18.90	18.90	20.50
Crude fiber (%)	2.70	2.70	2.00	2.60	2.60	2.60	2.80
Crude fat (%)	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Crude ash (%)	6.70	6.80	6.90	6.60	6.70	6.70	6.30
Calcium (%)	1.15	1.15	1.14	1.13	1.16	1.16	1.08
Phosphorus (%)	0.74	0.74	0.74	0.75	0.75	0.74	0.70

Table S3 Hematology mean value±SD (n-10) on 45th day.

	Control group	Non-GM groups			GM groups			Historical control data
		12.50%	25%	50%	12.50%	25%	50%	
Male								
WBC (10 ⁹ /L)	8.04±1.82	8.07±2.14	8.75±1.97	8.16±2.2	6.35±1.37	5.19±1.55 ^{ab}	6.83±1.98	6.9-14.5
RBC (10 ¹² /L)	6.26±1.69	6.45±0.64	5.93±1.03	5.82±1.48	6.00±1.17	6.52±1.43	5.90±0.76	5.09-8.33
HGB (g/L)	109±30.8	116±12.7	106±25.7	104±29.5	88.4±23.5 ^a	98.1±26.7	103±18.3	99.0-161.5
HCT (%)	37.3±10.6	34.2±4.05	35.5±6.05	34.7±9.57	29.5±7.09 ^a	36.4±8.54	34.9±5.75	33.1-52.2
MCV (fl)	59.6±1.4	60.7±1.55	59.7±2.15	59.4±2.38	59.3±3.9	58.4±1.26	58.9±2.58	56.9-70.1
MCH (fl)	17.4±0.87	17.9±0.49	17.9±1.95	17.7±0.87	17.9±0.79	17.3±0.67	17.4±1.13	16.1-23.9
MCHC (pg)	292±11.6	295±11.5	310±12.3 ^a	299±10.3	302±10.2	297±7.89 ^b	295±13.8	236.3-406.7
RDW (%)	15.1±0.53	15.8±0.55	14.7±1.91	15.2±1.88	14.6±0.9	14.2±0.76	14.8±0.5	13.5-15.5

PLT (10 ⁹ /L)	481±174	580±190	655±80.6 ^a	567±140	502±104	346±97.2 ^b	572±175	551-927
MPV (fl)	6.83±1.58	6.24±0.98	6.38±0.73	6.26±1.37	6.46±0.9	7.71±1.41 ^b	7.06±1.05	5.41-7.22
Female								
WBC (10 ⁹ /L)	7.61±3.08	7.87±3.60	7.54±3.08	7.16±2.77	7.38±2.63	7.16±3.64	6.76±3.50	4.73-11.31
RBC (10 ¹² /L)	5.83±1.28	5.36±1.1	5.45±1.09	5.71±1.03	5.58±0.62	4.66±1.63	5.18±1.66	4.77-8.09
HGB (g/L)	107±26.6	90.6±14.7	98.3±23.5	106±19.6	99.5±13.1	93.9±24.8	94.4±32.4	96.7-153.3
HCT (%)	34.8±8.37	31.2±7.04	32.2±7.08	34.3±6.07	33.5±4.05	27.9±9.43	30.3±9.93	30.84-51.96
MCV (fl)	59.4±2.1	57.9±2.48	58.8±3.33	60.1±2.32	60.1±1.91	60.2±3.79	58.2±2.12	58.85-69.95
MCH (fl)	18.2±0.98	17.9±0.57	17.9±1.13	18.6±0.73	17.82±1.05	18.2±1.18	18±1.08	14.15-25.03
MCHC (pg)	307±9.82	310±11.5	305±9.71	309±5.83	297±15.9	304±18.8	309±12.2	217-395
RDW (%)	13.1±0.35	14±1.2 ^a	13.2±0.83	13.9±1.43	14±0.94 ^a	15.4±4.86	13.3±0.67	12.16-15.0
PLT (10 ⁹ /L)	695±217	448±98.7 ^a	562±221	710±216	605±250	546±236	538±117	297-831
MPV (fl)	6.97±1.15	7.18±1.09	6.74±0.61	6.88±1.08	6.82±0.71	7.16±0.78	7.36±1.18	5.17-6.89

^a Significant differences between rice diets groups and the control diet group (P < 0.05).

^b Significant differences between GM and non-GM group (P < 0.05).

^c Data collected by the Supervision, Inspection and Testing Center of Genetically Modified Organisms, Ministry of Agriculture, Beijing, China.

Table S4 Hematology mean value±SD (n-10) on 90th day.

	Control group	Non-GM groups			GM groups			Historical control data ^c
		12.50%	25%	50%	12.50%	25%	50%	
Male								
WBC (10 ⁹ /L)	6.03±1.5	6.21±1.9	7.13±2.96	5.59±1.12	5.71±2.05	6.35±1.02	6.55±0.96	3.28-9.8
RBC (10 ¹² /L)	7.69±0.82	7.72±0.6	7.35±0.65	7.4±0.52	7.58±0.7	7.44±0.37	7.73±0.63	5.52-9.04
HGB (g/L)	129±16.7	125±9.8	125±17.9	125±11.8	126±7.63	125±5.96	134±11.2	110.3-161.4
HCT (%)	46.4±4.9	46.7±3.46	44.4±5.34	44.2±3.7	45.2±2.33	44.7±1.95	45.9±3.63	32.95-53.85
MCV (fl)	60.4±1.27	60.5±2	60.2±2.42	59.8±1.77	59.8±3.98	60.1±2.2	59.4±2.96	54.56-65.64

MCH (fl)	16.7±0.75	16.2±0.73	17±1.28	16.9±0.68	16.6±0.82	16.8±0.47	17.3±0.71	13.02-24.26
MCHC (pg)	277±11.3	277±9.52	282±16.6	283±8.22	278±10.7	280±9.75	282±12.4	212.5-425.8
RDW (%)	15±0.6	14.7±0.68	14.3±0.46 ^a	15.4±0.5	15.9±1.67	14.7±0.67	15.7±0.69	12.68-17.26
PLT (10 ⁹ /L)	704±170	741±149	738±142	537±129 ^a	732±130	733±50.4	828±97.6 ^b	318.6-825.6
MPV (fl)	5.87±0.51	6.01±0.66	5.66±0.35	5.79±0.55	5.69±0.43	5.47±0.34	5.81±0.27	5.31-6.67
Female								
WBC (10 ⁹ /L)	3.55±1.41	3.26±1.16	3.72±1.19	3.33±1.14	3.18±0.94	3.91±1.58	4.29±0.59	2.13-7.59
RBC (10 ¹² /L)	6.58±1.85	7.18±0.4	7.39±0.54	7.13±1.05	7.09±0.73	6.87±0.42	6.58±0.36	5.12-8.14
HGB (g/L)	121±34.5	127±6.77	128±7.77	129±17.8	128±13.2	124±10.9	119±5.8	107.5-154.6
HCT (%)	42.6±7.44	44±2.27	44.6±3.28	43.8±6.45	44.2±4.81	42.4±3.49	40.7±2	31.79-50.91
MCV (fl)	61.2±1.61	61.3±2.21	60.4±2.94	61.5±2.15	62.4±1.89	61.7±1.54	61.8±1.2	56.94-68.68
MCH (fl)	18.1±1	17.6±0.58	17.4±1.42	18.1±1.07	18.1±0.77	18.1±0.98	18.1±0.95	14.89-25.41
MCHC (pg)	296±12.5	288±13.5	287±14.5	295±12.4	290±11.5	293±18	293±13	275.4-778.6
RDW (%)	13.4±0.6	13.2±0.29	13.5±0.52	13.4±0.5	13.2±0.58	13.5±0.59	13.6±0.56	11.25-16.57
PLT (10 ⁹ /L)	635±184	691±65.2	723±86.6	573±151	659±142	700±82.9	635±102	422-731
MPV (fl)	5.42±0.28	5.67±0.38	5.57±0.42	5.54±0.32	5.57±0.35	5.62±0.44	5.59±0.38	5.08-6.8

^a Significant differences between rice diets groups and the control diet group (P < 0.05).

^b Significant differences between GM and non-GM group (P < 0.05).

^c Data collected by the Supervision, Inspection and Testing Center of Genetically Modified Organisms, Ministry of Agriculture, Beijing, China.

Table S5 Serum chemistry mean value±SD (n-10) on 45th day.

Serum chemistry mean values (n=10) on 13 day								
	Control group	Non-GM groups			GM groups			Historical control data
		12.50%	25%	50%	12.50%	25%	50%	
Male								
ALT (U/L)	46.7±3.7	44.8±5.5	44.2±4.2	43.4±6.7	44.6±6.6	38.6±6.1 ^{ab}	44.25±6.0	31.7-48.7
AST (U/L)	274±54.1	281±43.0	263±47.7	260±60.8	226±48.5 ^b	200±67.7 ^{ab}	270±37.4	181.9-318.1

TP (g/L)	65.4±2.51	67.7±3.36	63.3±16.3	66.9±3.73	66.8±3.24	65.2±1.96	66.6±3.4	61.9-74.5
ALB (g/L)	29.1±1.03	29.1±2.14	28.9±1.65	28.2±1.52	28.9±1.43	29.9±0.88	28.7±1.6	31.9-42.5
ALP (U/L)	186±24.3	159±26.0 ^a	174±32.0	176±35.5	210±84.2	181±41.0	186±29.6	128.9-236.3
GLU (mmol/L)	5.14±1.46	4.74±0.86	3.91±1.28	4.37±1.24	4.96±0.93	4.33±0.98	3.64±0.89 ^a	4.3-7.9
BUN (mmol/L)	7.26±1.23	7.07±1.34	7.25±1.49	6.37±1.07	7.01±1.83	7.12±0.91	6.38±0.71	5.5-9.3
CREA (μmol/L)	46.9±5.93	43.3±3.55	40.4±10.35	39.9±6.89 ^a	43.2±8.26	40.0±1.56 ^a	45.5±4.11	37.8-66.8
Calcium (mmol/L)	2.50±0.10	2.57±0.05	2.55±0.10	2.55±0.14	2.53±0.08	2.53±0.09	2.49±0.09	1.99-2.61
Phosphorus (g/L)	3.59±0.64	3.69±0.19	3.43±0.44	3.28±0.51	2.80±0.37 ^{ab}	3.18±0.30	3.43±0.45	2.31-4.01
CHO (g/L)	1.52±0.23	1.67±0.19	1.54±0.34	1.47±0.23	1.63±0.15	1.50±0.23	1.56±0.23	1.46-2.16
LDH (U/L)	2818±814	303±530	2581±570	2604±603	2577±823	2575±801	2979±622	2108-3873
Chlorine (mmol/L)	103±1.37	102±2.04	103±1.85	104±1.68	105±2.13	104±1.32	105±1.28	101.5-111.1
Magnesium (mmol/L)	1.21±0.17	1.24±0.08	1.11±0.13	1.08±0.19	1.14±0.09	1.12±0.19	1.20±0.16	0.89-1.29
Female								
ALT (U/L)	35.8±5.0	33.7±3.0	33.8±2.7	32.2±4.6	33.11±4.0	34.7±3.5	30.8±4.4	27.0-44.6
AST (U/L)	196±46.2	231±48.4	209±40.7	205±68.6	192±34.6	199±38.1	221±39.4	156.1-284.7
TP (g/L)	74.1±3.64	73.7±3.05	77.5±3.10 ^a	74.9±3.11	78.1±3.78	75.5±5.95	74.7±4.96	61.2-85.2
ALB (g/L)	43.3±3.01	44.3±2.51	43.5±4.04	43.0±4.32	41.2±4.11	43.1±3.17	44.8±7.01	35.4-47.2
ALP (U/L)	100±17.5	105±28.2	96.4±23.1	103±20.7	102±15.0	93.8±16.5	107±32.0	76.6-164
GLU (mmol/L)	4.76±1.18	4.99±1.63	4.37±0.56	5.18±0.85	5.22±0.99	4.41±1.06	5.23±1.80	3.7-7.2
BUN (mmol/L)	7.28±1.12	7.53±0.66	7.65±1.10	6.88±1.34	8.50±1.44 ^a	7.46±1.10	7.15±1.19	6.2-10.2
CREA (μmol/L)	44.9±6.97	50.0±9.37	50.5±5.98	49.3±7.59	54.2±7.68 ^a	48.3±7.38	45.6±8.35	41.2-86.4
Calcium (mmol/L)	2.72±0.10	2.66±0.09	2.76±0.07	2.63±0.13	2.71±0.13	2.64±0.15	2.70±0.08	1.96-2.74
Phosphorus (g/L)	2.63±0.30	2.56±0.23	2.62±0.24	2.48±0.30	2.39±0.24	2.40±0.21	2.51±0.29	1.96-3.11
CHO (g/L)	1.87±0.31	1.73±0.43	1.91±0.43	1.50±0.17 ^a	2.03±0.40	1.74±0.34	1.72±0.31	1.46-2.32
LDH (U/L)	2453±850	2622±500	2401±540.3	2113±691	2017±602 ^b	2130±552	2457±527	1662-3783
Chlorine (mmol/L)	105±1.88	102±4.24	103±2.31	103±2.47	106±10.3	105±2.93	105±2.28	101.4-117

Magnesium (mmol/L)	1.09±0.10	1.03±0.11	1.08±0.08	1.03±0.09	1.09±0.07	1.06±0.10	1.10±0.05	0.82-1.32
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^a Significant differences between rice diets groups and the control diet group (P < 0.05).

^b Significant differences between GM and non-GM group (P < 0.05).

^c Data collected by the Supervision, Inspection and Testing Center of Genetically Modified Organisms, Ministry of Agriculture, Beijing, China.

Table S6 Serum chemistry mean value±SD (n-10) on 90th day.

	Control group	Non-GM groups			GM groups			Historical control data
		12.50%	25%	50%	12.50%	25%	50%	
Male								
ALT (U/L)	44.5±9.1	46.9±8.9	44.1±7.7	43.8±7.0	41±5.1	36.9±3.9 ^a	43.9±6.4	32.6-57.4
AST (U/L)	248±44.4	306±46.8	281±74.5	250±41.4	265±53.4	215±63.8 ^b	227±38.0	161.7-292.9
TP (g/L)	61.5±4.06	65.3±4.32	64.6±2.77	62.6±5.45	63.1±3.57	64.3±3.31	63.5±3.17	60.7-78.5
ALB (g/L)	34.3±0.84	34.6±0.68	34.3±0.64	34.2±0.69	34.2±0.73	34.4±0.31	34.6±0.62	26.2-40.6
ALP (U/L)	115±19.3	95.7±16.4 ^a	104±16.1	110±17.5	133±56.4	113±36.8	117±27.7	72.4-158.4
GLU (mmol/L)	8.40±3.01	7.34±1.82	6.79±1.44	8.49±3.97	8.65±1.33	10.0±2.55 ^b	8.34±1.17	5.5-9.7
BUN(mmol/L)	6.55±1.16	8.01±1.34 ^a	7.36±1.15	7.80±1.64	7.00±1.69	8.70±2.23 ^a	7.42±0.77	5.8-9.2
CREA (μmol/L)	72.2±9.73	69.8±12.5	76.2±16.0	65.3±9.82	68.3±10.8	73.9±12.3	73.2±6.77	46.2-84.2
Calcium (mmol/L)	2.54±0.19	2.74±0.26	2.64±0.10	2.59±0.34	2.54±0.08 ^b	2.59±0.15	2.59±0.13	2.02-2.8
Phosphorus (g/L)	2.71±0.4	3.86±1.57 ^a	3.02±0.60	3.64±1.35	2.98±0.51	3.16±0.55 ^a	2.80±0.23	2.26-3.46
CHO (g/L)	1.60±0.17	1.66±0.28	1.51±0.31	1.40±0.24 ^a	1.49±0.09	1.45±0.26	1.55±0.25	1.22-2.2
LDH (U/L)	2543±513	2823±708	2122±693	1786±655 ^a	1991±732 ^b	1671±511 ^a	2171±335	1151-2971
Chlorine (mmol/L)	101±3.43	102±1.60	102±1.58	103±1.91	104±2.28 ^a	103±1.35	103±1.91	99.2-111.6
Magnesium (mmol/L)	1.22±0.09	1.34±0.22	1.27±0.28	1.16±0.25	1.26±0.29	1.28±0.36	1.12±0.21	0.88-1.26
Female								
ALT (U/L)	33.8±13.4	32.9±3.3	33.1±4.6	36.5±11.1	36.22±13.1	32.2±6.0	33.33±5.9	24.9-55.9
AST (U/L)	197±25.8	187±34.3	185±23.3	189±30.2	195±45.4	183±37.3	198±38.7	121.8-271

TP (g/L)	70.5±5.97	71.7±3.46	69.3±3.20	71.9±4.49	72.2±5.64	71.0±4.33	72.1±4.42	63.2-96.8
ALB (g/L)	45.7±3.01	45.8±2.89	44.2±2.47	46.1±3.39	45.4±4.25	46.1±3.55	45.6±3.04	33.6-47.8
ALP (U/L)	74.8±27.1	52.3±15.6 ^a	58.8±15.1	61.8±7.9	61.9±17.2	53.4±7.8 ^a	68.2±19.6	37.2-88.6
GLU (mmol/L)	6.77±2.44	5.17±1.34	6.10±0.8 ^a	5.49±1.59	7.95±2.13 ^b	6.56±0.72	5.89±1.01	5.6-10.2
BUN(mmol/L)	11.8±2.46	10.1±2.15	9.97±1.34	11.0±2.86	11.5±2.34	9.30±1.82 ^a	9.37±2.19 ^a	6.1-11.1
CREA (μmol/L)	77.4±9.21	77.8±10.6	84.0±19.7	70.5±11.1	91.1±12.1 ^{ab}	80.2±13.0	83.8±9.52 ^b	56.4-99.4
Calcium (mmol/L)	2.66±0.11	2.66±0.09	2.58±0.06	2.61±0.11	2.66±0.14	2.65±0.08 ^b	2.71±0.09	1.99-2.93
Phosphorus (g/L)	2.03±0.20	2.12±0.67	2.10±0.45	1.82±0.37	2.41±0.46 ^a	2.29±0.54	2.31±0.26 ^{ab}	2.07-3.0
CHO (g/L)	2.75±0.78	2.22±0.50	2.18±0.84	1.94±0.20 ^a	2.30±0.33	2.22±0.55	1.72±0.55 ^a	1.31-2.52
LDH (U/L)	1897±557	1473±437	1436±191 ^a	1803±289	1538±459	1434±404 ^a	1768±369	645-1993
Chlorine (mmol/L)	104±2.02	103±0.97	104±1.66	106±1.51 ^a	105±2.31	102±1.04 ^{ab}	103±1.59 ^b	100.3-113.5
Magnesium (mmol/L)	1.62±0.39	1.55±0.28	1.47±0.37	1.48±0.23	1.57±0.23	1.57±0.35	1.29±0.20 ^a	0.87-1.39

^a Significant differences between rice diets groups and the control diet group (P < 0.05).

^b Significant differences between GM and non-GM group (P < 0.05).

^c Data collected by the Supervision, Inspection and Testing Center of Genetically Modified Organisms, Ministry of Agriculture, Beijing, China.

Table S7 Organ/body weight mean value±SD (n-10).

	Control group	Non-GM groups			GM groups			Historical control data ^c
		12.50%	25%	50%	12.50%	25%	50%	
Male								
Brain	0.417±0.039	0.404±0.039	0.389±0.052	0.394±0.036	0.425±0.104	0.336±0.018 ^{ab}	0.379±0.038 ^a	0.335-0.444
Liver	2.217±0.166	2.124±0.794	2.116±0.783	2.508±0.650	2.378±0.950	2.283±0.136	2.192±0.166	2.045-2.664
Spleen	0.144±0.026	0.142±0.021	0.138±0.052	0.149±0.017	0.150±0.029	0.138±0.019	0.137±0.017	0.111-0.174
Heart	0.307±0.032	0.329±0.047	0.340±0.066	0.314±0.036	0.339±0.209	0.338±0.051	0.343±0.044	0.267-0.373
Lung	0.357±0.051	0.382±0.071	0.380±0.046	0.384±0.067	0.402±0.095	0.363±0.044	0.372±0.056	0.301-0.482
Thymus	0.101±0.025	0.108±0.045	0.103±0.017	0.105±0.017	0.122±0.049	0.095±0.027	0.094±0.035	0.067-0.133
Kidney	0.623±0.061	0.674±0.105	0.623±0.065	0.664±0.089	0.694±0.134	0.592±0.068	0.597±0.038	0.501-0.762
Adrenals	0.015±0.006	0.013±0.003	0.012±0.005	0.015±0.003	0.016±0.005	0.014±0.005	0.015±0.002	0.014-0.022
Testis	0.656±0.075	0.663±0.089	0.653±0.049	0.634±0.060	0.811±0.196 ^{ab}	0.627±0.081	0.645±0.071	0.545-0.733
Female								
Brain	0.715±0.084	0.727±0.051	0.689±0.086	0.719±0.041	0.735±0.087	0.695±0.047	0.707±0.049	0.545-0.734
Liver	2.513±0.337	2.375±0.122	2.399±0.167	2.467±0.174	2.550±0.237	2.485±0.180	2.646±0.305	2.078-2.783
Spleen	0.152±0.054	0.164±0.017	0.170±0.029	0.154±0.056	0.154±0.065	0.167±0.031	0.162±0.015	0.131-0.182
Heart	0.353±0.027	0.388±0.033 ^a	0.351±0.047	0.368±0.064	0.361±0.040	0.355±0.029	0.370±0.050	0.321-0.442
Lung	0.615±0.154	0.518±0.064	0.538±0.082	0.525±0.061	0.534±0.075	0.523±0.045	0.552±0.062	0.401-0.643
Thymus	0.132±0.021	0.127±0.020	0.119±0.026	0.141±0.033	0.146±0.046	0.127±0.026	0.168±0.109	0.081-0.161
Kidney	0.660±0.082	0.661±0.057	0.630±0.045	0.630±0.132	0.759±0.163	0.651±0.085	0.643±0.113	0.589-0.773
Adrenals	0.031±0.005	0.032±0.005	0.028±0.005	0.030±0.005	0.035±0.005	0.027±0.010	0.032±0.006	0.021-0.035
Ovaries	0.059±0.007	0.057±0.010	0.055±0.024	0.051±0.020	0.057±0.010	0.056±0.014	0.059±0.007	0.031-0.072

^a Significant differences between rice diets groups and the control diet group (P < 0.05).^b Significant differences between GM and non-GM group (P < 0.05).^c Data collected by the Supervision, Inspection and Testing Center of Genetically Modified Organisms, Ministry of Agriculture, Beijing, China.

Figure legends

Fig S1. Heat stability of rHSA. M, marker; 1- 5, rHSA was tested for stability at temperatures 100°C for periods of 0, 10, 20, 30, and 60 min.

Fig S2. Simulative digestive experiment in vitro for rHSA with pepsase and trypsin. 1 and 8, rHSA control; 2, pepsase control; 3-7, rHSA was digested by pepsase in the time points of 0s, 15s, 2min, 30min and 60min; M, marker; 9, trypsin control; 10-14, rHSA was digested by trypsin in the time points of 0s, 15s, 2min, 30min and 60min.

Fig S3. Weekly mean food consumption per rat. A, male groups. B, female groups. The values are presented as means±standard deviation (10 rats/group).