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No subchronic toxicity of multiple herbicide-resistant soybean FG72 in Sprague-Dawley rats by 90-days feeding study

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ABSTRACT

The genetically modified (GM) soybean FG72 contains two exogenous genes: p-hydroxyphenylpyruvate dioxygenase (*hppd*) and double mutant 5-enol pyruvylshikimate-3-phosphate synthase (*2mepsps*), endowing the FG72 with the glyphosate and isoxaflutole herbicides resistant abilities for presence of the 2mEPSPS and HPPD W336 proteins. A food safety assessment of GM soybean FG72 was evaluated by a 90-days feeding study using three different dietary concentrations (7.5%, 15%, or 30% w/w) of the GM soybean or its corresponding non-GM cultivar Jack fed to Sprague-Dawley rats. In our study, no biologically significant differences on animal daily clinical signs, body weights, clinical observations, hematology, clinical chemistry, histopathology on selected organs were observed within the GM soybean groups and among the GM soybean groups, the non-GM soybean groups and the control group. The results of the 90-days subchronic feeding study demonstrated that the GM soybean FG72 is as safe as the conventional non-GM soybean Jack.

1. Introduction

Soybean, one of the most important commercial crops, is widely planted all over the world. Soybean is an important commercial crop for its abundant protein and oil, it is also used as material of animal fodder. In China, over 80 million tons of soybean were imported for the use of oil manufacture and feedstuff in 2016. Soybean oil constitutes approximately 54% of American vegetable oils consumption in the year of 2016 (Soystats, 2017), and is currently the second largest source of vegetable oil worldwide (USDA, 2017). However, the output and quality of soybean is influenced by fast-growing weeds, which competed with crops for water and nutrients, making a serious threat to the production of soybean. Herbicides are effective to control the growing of weeds, while the crops are also sensitive to them. Genetic modification technology provides a method to introduce exogenous DNA into plants, endowing the target plants with a specific characteristic and contribute to most current commercialized herbicide-resistant crops (Duke, 2005). The application of the transgenic technique in soybean is one of the most successful cases. According to the data reported by International Service for the Acquisition of Agri-biotech Applications (ISAAA), genetically modified (GM) soybean accounted half of all the biotech crop hectare all over the world in 2016 (ISAAA, 2017).

A new GM soybean line FG72 with tolerance to two herbicides, glyphosate and isoxaflutole, has been grown by some countries in recent years(CERA, 2017a). The multiple herbicide-resistant soybean contains two exogenous genes, *hppd* and *2mepsps*, which are introduced into the non-GM publicly available cultivar Jack, using direct DNA integration of a linear DNA fragment isolated. The *2mepsps* gene is generated by introducing mutations into the wild-type *epsps*gene from maize, leading to a modified EPSPS protein with two amino acid substitutions (2mEPSPS). This modification confers a decreased binding affinity of the protein for glyphosate, allowing it to maintain sufficient enzymatic activity in the presence of the herbicide (Lepping et al., 2013). Therefore, the plants expressing the 2mEPSPS protein are tolerant to glyphosate herbicides. The *hppd* gene is isolated and cloned from *Pseudomonas fluorescen* and it is modified to *hppdW336* when inserted into GM soybean FG 72. The expressed protein, HPPD W336, is tolerant to isoxaflutole (IFT) and has 99.5% of autoploid with the soybean native HPPD protein.

To insure the food safety including potential toxicity, allergenicity, antinutritional effects and other unexpected or unintended effects, such as nutritional compositional analysis, toxic and allergenic assessment of new expressed proteins, and unintended effect analysis, it have come a consensus that each of GM crops should be underway to a series of food

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safety evaluations before put into market (EFSA, 2008). The *2mepsps* gene has been widely used in the genetic modification of a number of crop species (CERA, 2017b), while the *hppd* gene was first inserted into the crop (CERA, 2017a). The safety assessment study showed that the 2mEPSPS enzyme does not possess any of the properties associated with known toxins or allergens, including lack of amino acid sequence similarity to known toxins and allergens, rapid degradation in simulated gastric and intestinal fluids, and no adverse effects in mice in acute toxic test (Herouet-Guicheney et al., 2009). To verify if there is potential toxicity of FG 72 and test if the GM soybean is as safe as the non-GM soybean, it is necessary to perform related food safety assessment such as a 90-day feeding study compared the GM soybean FG72 to that of the non-GM soybean Jack. The 90-day subchronic toxicology study is considered to be the gold standard for hazard exploration (He et al., 2008). To identify any possible hazard associated with GM soybean FG72, a 90-day subchronic toxicology study was carried out using Sprague-Dawley rats following the Chinese standard (Chinese Agricultural Standard NY/T 1103-2006) and the guidelines for *repeated dose 90-day oral toxicity study in rodents* (OECD Guideline 408, 1998).

2. Materials and methods

2.1. Plant materials

The GM soybean FG72 and non-GM soybean Jack were grown in parallel in the same field trial under the same field management and harvesting and processing treatment. The analysis of main nutrient contents, including moisture determination, protein, fat, crud fiber, ash, fatty acids, vitamins, amino acids, mineral elements and anti-nutrients, were conducted according to the recommended methods of the Standards of the People's Republic of China.

2.2. Diet formulation and experimental design

The GM soybean FG72 or non-GM soybean Jack was formulated into the basic diets at the inclusion rate of 7.5%, 15%, or 30% (w/w), individually. The 30% inclusion rate is the highest level that would still allow proper nutritional balance. The diets were also been fortified with other ingredients such as bean pulp, wheat flour, fishmeal, and yeast powder to ensure a balanced diet for the rats. The nutritional contents of diets were conformed to Chinese Standard (Chinese standard GB14924.3–2010). All of diets were vacuum-packed and irradiated with ^{60}Co by KeAoXie Li Feed Co. Ltd (Beijing, China), then diets were kept in 4–8 °C before feeding to the rats. Finally, the main nutritional levels of diets were analyzed according to Chinese Standard (Chinese standard GB 5009.3-5-2010; Chinese standard GB 5009.6–2003; Chinese standard GB/T 5009.10–2003; Chinese standard GB/T 5009.87–2003; Chinese standard GB/T 5009.92–2003).

2.3. Animals and management

The rats were fed with special processing diets for 90 days in the specific pathogen free (SPF) animal laboratory of the Supervision and Testing Center for Genetically Modified Organisms (GMOs) Safety, Ministry of Agriculture, with license number SYXK (Beijing) 2010-0036. The temperature and humidity of the animal room was ranged from 20 to 24 °C and 40–70%, respectively. A 12 h light/dark cycle and air exchanges 15 times/h were used to keep the environment inhabitable for the rats.

70 male and 70 female SPF Sprague-Dawley rats were obtained from Vital River Laboratories, Inc. (Beijing, China) with the license number SCXK (Beijing) 2012-0001. All rats were 4-week old, weighing 80–100 g. After 5 days of acclimation, rats were randomly divided into 7 groups based on body weight with 10 male and 10 female rats in each group, and then 5 rats were housed in steel cages randomly. The first group was fed with a basic rodent diet (basic control group). Six treatment groups were fed with diets containing 7.5%, 15%, and 30% (w/w) GM soybean or non-GM soybean, respectively. Water and diet were supplied *ad libitum*. The animal study and housing procedures were carried out in compliance with the OECD Principles of Good Laboratory Practice. The animal study was approved by the Animal Experimental Welfare and Ethical Inspection Committee (No. 140032-033) in the Supervision and Testing Center for GMO Food Safety, Ministry of Agriculture (Beijing, China).

2.4. Clinical observation, body weight gain, and feed utilization

The rats were observed daily for their behavior, clinical signs including skin, fur eyes, secretion, excretion, posture, gait and movements were carefully recorded if abnormal signs happened. Feed consumption and body weight were recorded weekly.

2.5. Hematology

On day 91st, the rats were fasted overnight and nearly 1.5 mL blood samples were collected from the orbital sinus under anesthesia using the EDTA•K_2 as an anticoagulant. After that, 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 volume distribution (RDW), blood platelet count (PLT), and mean platelet volume (MPV) were measured using an automatic animal blood cell counter HEMAVET 950FS (Drew Scientific, Inc., Dallas, TX, USA).

2.6. Serum chemistry

On day 91st, the rats were fasted overnight and nearly 1.5 mL blood samples were collected from the orbital sinus under anesthesia. The samples were centrifuged at $4000 \times g$ for 15 min to separate the serum. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), alkaline phosphatase (ALP), glucose (GLU), blood urea nitrogen (BUN), creatinine (CREA), calcium (Ca), potassium (K), cholesterol (CHO), triglyceride (TG), lactate dehydrogenase (LDH), chlorine (Cl), and magnesium (Mg) in the serum were measured with an Automatic Biochemical Analyzer 7020 (HITACHI, Tokyo, Japan).

2.7. Necropsy and histopathology

At the end of the experiments, the rats were euthanized by decapitation after anesthesia, and then a gross necropsy was performed on all animals to check if there were any macroscopical pathology changes to tissues or organs. Selected organs, including heart, spleen, lungs, kidneys, adrenal glands, brain, liver, thymus, and testes or ovaries were separated and weighed, and the relative organ weight (organ weight/body weight in percent) was calculated. After that, the tissue sections from these organs as well as stomach and intestinal were immersed in a 10% neutral buffered formalin solution and embedded with paraffin, and then stained with hematoxylin and eosin (H&E). Pathologists from the College of Veterinary Medicine, China Agricultural University, conducted histopathological examination on the organs.

2.8. Statistical analysis

For the final statistical analysis of this study, each dose of GM soybean group was compared to its non-GM counterpart, and each soybean-containing group was compared to the basic control group. The data for body weight gain, feed consumption, organ weight, hematology, and serum chemistry between GM soybean FG72 groups, non-GM soybean groups and basic control group were analyzed with a one way analysis of variance (ANOVA) using statistical software

Table 1
The basic nutritional ingredients of the GM and non-GM soybean diets used in this study.

	Control diet	Non-GM soybean diet			GM soybean diet		
		7.5%	15%	30%	7.5%	15%	30%
Moisture (g/100 g)	5.00	4.50	4.14	5.97	4.41	3.44	6.12
Ash (g/100 g)	6.27	7.40	7.51	6.99	7.76	7.33	6.72
Protein (g/100 g)	20.2	20.0	19.9	19.0	20.4	20.2	18.0
Fat (g/100 g)	4.87	7.72	7.67	6.97	8.02	7.83	6.82
Crude fiber (g/100 g)	4.69	5.39	5.05	4.25	4.64	5.26	3.88
Calcium (mg/100 g)	1400	966	1074	1048	1032	1029	887
Phosphorus (mg/100 g)	671	649	748	688	765	709	538

Statistical Product and Service Solutions (SPSS) v19.0 (SPSS Inc., Chicago, IL, USA). All the data mentioned were presented as a mean value ± standard deviation (Mean ± SD) and the significance statistical level was set at p value < 0.05.

3. Results

During the 90-day trial, no toxicity symptoms were observed on any rats and no death happened.

3.1. Nutritional analysis of soybean and diets

The nutritional composition of GM soybean FG72 and non-GM soybean Jack were analyzed and the nutritional ingredients of the finished diet fed for the animals were tested and shown in Table 1.

3.2. Body weight gain

The body weight gains of the rats during the 90-day period are shown in Fig. 1. The body weight gains of the non-GM soybean groups and the GM soybean groups were almost all significantly lower ($p < 0.05$) than the data of the basic control group. Since there was no significant difference of the body weight gain in the GM soybean groups when compared with the corresponding non-GM soybean groups, indicating that these differences were not related to the GM soybean added into the fodder. Furthermore, as in the male rat groups, no differences were found in female rats between the GM soybean groups and the corresponding non-GM soybean groups on body weight gains The food consumption are also shown in Fig. 2.

3.3. Relative organ weight

The data of the organ/body ratio were shown in Table 2. The values of the brain and testis in GM male groups and non-GM male groups, together with the values of the kidney in GM male groups and 30% non-GM male group, were all higher ($p < 0.05$) than those in the basic control group. However, no significant differences were found in the values of brain or kidney between GM soybean groups and non-GM soybean groups, demonstrating that these differences were not related to the presence of GM soybean FG72 in the feed. The value of the testis in GM soybean groups and non-GM soybean groups were all higher ($p < 0.05$) than that in the control group, while this value in 30% GM soybean group was also lower ($p < 0.05$) than that in the 30% non-GM soybean group, which means that the testis of 30% GM soybean group was between that of 30% non- GM group and the control group. The reason of this phenomenon would be the addition of soybean to the animal fodder not the GM soybean. Besides, neither macroscopic pathological changes nor histopathological changes were found in testis of the 30% GM soybean group. The heart value of in 30% GM soybean group was lower ($p < 0.05$) than that of the 30% non-GM soybean group, but no significant difference was observed between the values of 30% GM soybean group and the basic control group, so it was not caused by the GM soybean.

In female groups, the value of adrenal in 30% GM soybean group was significantly higher ($p < 0.05$) than that in 30% non-GM soybean group, which was thought to be not related to the GM soybean, because no significant difference was found between the 30% GM soybean group and the basic control group. Meanwhile, when compared to the basic control group, the value of liver in 7.5% and 15% GM soybean group, the value of lung in 30% GM soybean group and the value of thymus in 15% GM soybean group were found to be significantly changed ($p < 0.05$), but these differences were not related to the GM soybean for the reason that no significant differences were found when the data were compared to their non-GM counterparts.

3.4. Hematology

The data of hematology were shown in Table 3, and several differences in some parameters were observed. In the male groups, compared with basic control group, the values of RBC, HGB, HCT and RDW in the 7.5% and 15% GM soybean groups were significantly higher ($p < 0.05$), which were not considered to be dosage-related because no significant differences were found in the high-dosage 30% GM soybean groups. Additionally, the values of PLT in all of the soybean groups were significant higher than that of the basic control group, while no difference was observed between GM groups and non-GM groups. On the contrary, the above differences were not observed in the female

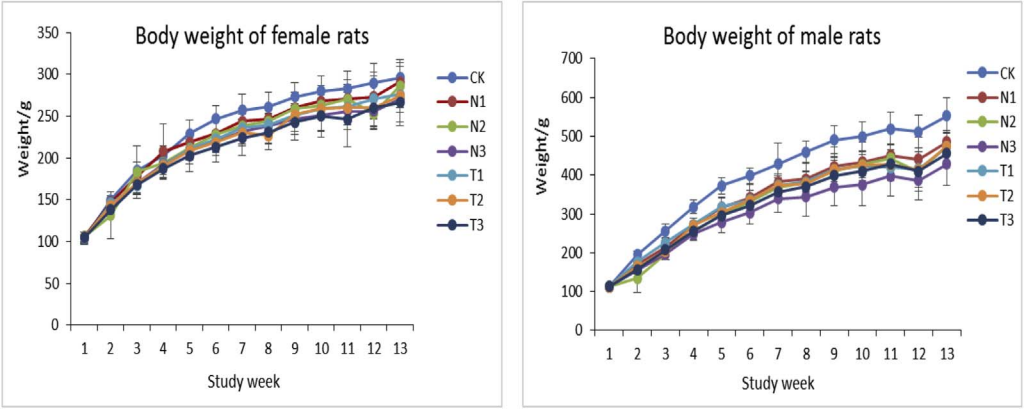

Fig. 1. Mean weekly body weight of rats. CK: control group; N1: 7.5% non-GM soybean group; N2: 15% non-GM soybean group; N3: 30% non-GM soybean group; T1: 7.5% GM soybean group; T2: 15% GM soybean group; T3: 30% GM soybean group.

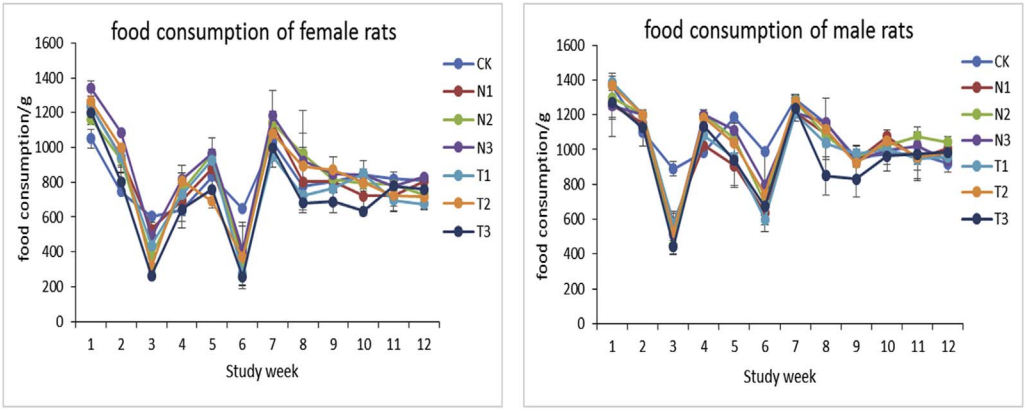

Fig. 2. Mean weekly food consumption of rats. CK: control group; N1: 7.5% non-GM soybean group; N2: 15% non-GM soybean group; N3: 30% non-GM soybean group; T1: 7.5% GM soybean group; T2: 15% GM soybean group; T3: 30% GM soybean group.

Table 2
Relative organ weights of rats fed with different diets (n = 10/group; mean ± SD).

	Control group	Non-GM soybean			GM soybean		
		7.5%	15%	30%	7.5%	15%	30%
Males							
Brain	0.38 ± 0.04	0.43 ± 0.05[a]	0.44 ± 0.03[a]	0.47 ± 0.05[a]	0.43 ± 0.04[a]	0.44 ± 0.03[a]	0.44 ± 0.04[a]
Liver	2.47 ± 0.54	2.61 ± 0.34	2.46 ± 0.17	2.64 ± 0.28	2.60 ± 0.16	2.62 ± 0.22	2.61 ± 0.59
Spleen	0.16 ± 0.02	0.13 ± 0.05	0.15 ± 0.02	0.16 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.03
Heart	0.32 ± 0.03	0.35 ± 0.04	0.35 ± 0.05	0.38 ± 0.04[a]	0.35 ± 0.03	0.36 ± 0.04[a]	0.34 ± 0.04[b]
Lung	0.40 ± 0.08	0.46 ± 0.08	0.46 ± 0.07	0.50 ± 0.10[a]	0.43 ± 0.04	0.45 ± 0.10	0.44 ± 0.09
Thymus	0.08 ± 0.04	0.08 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
Kidney	0.56 ± 0.06	0.59 ± 0.22	0.67 ± 0.16	0.62 ± 0.06[a]	0.67 ± 0.04[a]	0.64 ± 0.04[a]	0.66 ± 0.06[a]
Adrenal	0.013 ± 0.003	0.011 ± 0.002	0.015 ± 0.004	0.013 ± 0.004	0.013 ± 0.002	0.014 ± 0.002	0.013 ± 0.003
Testis	0.63 ± 0.11	0.75 ± 0.12[a]	0.78 ± 0.09[a]	0.85 ± 0.06[a]	0.74 ± 0.06[a]	0.78 ± 0.09[a]	0.76 ± 0.09[a,b]
Females							
Brain	0.66 ± 0.05	0.66 ± 0.05	0.62 ± 0.22	0.69 ± 0.04	0.68 ± 0.06	0.68 ± 0.06	0.70 ± 0.11
Liver	2.68 ± 0.33	3.04 ± 0.43	2.87 ± 0.25	2.75 ± 0.16	2.64 ± 0.17[a]	2.98 ± 0.29[a]	2.67 ± 0.35
Spleen	0.16 ± 0.02	0.16 ± 0.03	0.17 ± 0.03	0.15 ± 0.02	0.15 ± 0.02	0.17 ± 0.02	0.17 ± 0.03
Heart	0.35 ± 0.04	0.39 ± 0.16	0.39 ± 0.06	0.37 ± 0.05	0.36 ± 0.03	0.38 ± 0.03	0.38 ± 0.08
Lung	0.49 ± 0.09	0.54 ± 0.09	0.56 ± 0.24	0.52 ± 0.05	0.52 ± 0.06	0.57 ± 0.12	0.57 ± 0.08[a]
Thymus	0.14 ± 0.03	0.13 ± 0.02	0.12 ± 0.02	0.13 ± 0.03	0.12 ± 0.03	0.12 ± 0.01[a]	0.14 ± 0.03
Kidney	0.71 ± 0.09	0.69 ± 0.06	0.75 ± 0.22	0.64 ± 0.04[a]	0.67 ± 0.04	0.78 ± 0.21	0.74 ± 0.20
Adrenal	0.024 ± 0.010	0.025 ± 0.008	0.027 ± 0.005	0.020 ± 0.008	0.024 ± 0.004	0.029 ± 0.006	0.029 ± 0.004[b]
Ovary	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01[a]	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01

[a] $p < 0.05$ compared to the control group.
[b] $p < 0.05$ compared to the corresponding non-GM soybean groups.

groups.

The value of MCV in the 7.5% GM soybean female group was significantly lower ($p < 0.05$) than that in the 7.5% non-GM soybean group, but this difference was not found in the 15% and 30% GM soybean groups, which was considered not to be dosage-related. The values of RBC, MCV, MCH and PLT in 30% GM soybean group and the value of RDW in 7.5% GM soybean groups were found to be significantly different ($p < 0.05$) from the corresponding data in the basic control group. These differences were not related to the GM soybean FG72 added to the fodder because no significant differences were found between the GM groups and the non- GM groups. All of these differences were not considered to be related to the formulation of GM soybean since no dose- or gender-related changes were observed in this results.

3.5. Serum biochemistry

The data presented in Table 4 showed that, in most cases, there were nearly no significant differences on serum biochemistry parameters between the GM groups and the non-GM groups. In male groups, the data of ALT, BUN and LDH in the soybean groups were significantly lower ($p < 0.05$) than those in the basic control group, but no significant differences were found between the GM groups and the non-GM groups, which indicated that these changes were not related to the GM soybean FG72 added to the feed. Although the value of CREA in 15% GM soybean group and TG in 7.5% GM soybean group were different in statistics ($p < 0.05$) than their counterpart data of that in the non-GM groups, no differences were observed between the high-dosage 30% GM soybean groups and the corresponding non-GM soybean groups, indicating that there were no dosage-related relationships. Some other different values ($p < 0.05$) in soybean groups compared to the basic control groups, such as the values of AST, ALB, GLU, Ca, TG, Cl, Mg, were not thought to be related to GM soybean, based on the fact that these differences did not occur in the comparison between the GM soybean groups and the non-GM soybean groups, and they were not dosage-related.

In the female groups, the significant differences in the values of ALT, P and Mg ($p < 0.05$) in 7.5% GM soybean groups, as well as the value of TG in 15% GM soybean group compared with their corresponding non-GM soybean groups were not considered to be dosage related, because no significant difference was found in 30% GM soybean group compared with non-GM soybean group. The value of ALP in

Table 3
Terminal hematology of rats fed with different diets on the 91st day (n = 10/group; mean ± SD).

	Control group	Non-GM soybean			GM soybean		
		7.5%	15%	30%	7.5%	15%	30%
Males							
WBC(10^9/L)	9.27 ± 1.60	8.27 ± 2.67	9.62 ± 2.45	8.88 ± 1.43	8.59 ± 2.79	8.20 ± 2.15	9.67 ± 2.71
RBC(1012/L)	8.38 ± 1.37	10.18 ± 0.74a	9.54 ± 0.58a	8.60 ± 0.54	10.07 ± 1.05a	9.91 ± 0.67a	8.86 ± 0.43
HGB(g/L)	146 ± 24	172 ± 15[a]	166 ± 12[a]	151 ± 11	175 ± 21[a]	172 ± 11[a]	152 ± 5
HCT (%)	50.6 ± 7.7	58.9 ± 4.9[a]	57.4 ± 3.7[a]	53.0 ± 3.1	59.8 ± 6.0[a]	59.7 ± 3.4[a]	53.5 ± 2.2
MCV(fL)	60.6 ± 3.1	57.8 ± 1.5[a]	60.1 ± 1.4	61.6 ± 1.9	59.5 ± 2	60.3 ± 1.8	60.5 ± 3.3
MCH(pg)	17.4 ± 0.8	16.9 ± 0.5	17.4 ± 0.6	17.6 ± 0.7	17.4 ± 1.0	17.4 ± 0.7	17.2 ± 0.7
MCHC(g/L)	287 ± 9	292 ± 9	289 ± 5	285 ± 7	293 ± 7	289 ± 7	285 ± 5
RDW(fL)	14.5 ± 0.9	16.0 ± 0.7[a]	15.4 ± 0.6[a]	15.9 ± 0.8[a]	16.2 ± 1.3[a]	15.9 ± 0.7[a]	15.2 ± 0.9
PLT(10^9/L)	1092 ± 163	1256 ± 114[a]	1495 ± 132[a]	1394 ± 165[a]	1353 ± 222[a]	1388 ± 116[a]	1390 ± 61[a]
MPV(fL)	5.91 ± 0.46	6.10 ± 0.30	5.94 ± 0.27	5.93 ± 0.32	6.00 ± 0.38	5.94 ± 0.13	6.10 ± 0.51
Females							
WBC(10^9/L)	7.65 ± 2.18	8.57 ± 1.78	7.50 ± 2.88	5.82 ± 1.83	8.14 ± 2.14	5.84 ± 1.76	7.33 ± 2.48
RBC(10^{12}/L)	7.79 ± 0.22	7.81 ± 0.34	7.74 ± 0.25	8.20 ± 0.60	7.92 ± 0.63	7.39 ± 1.09	8.41 ± 0.34[a]
HGB(g/L)	142 ± 6	140 ± 7	139 ± 6	140 ± 8	141 ± 13	142 ± 10	145 ± 7
HCT (%)	49.1 ± 1.7	49.6 ± 2.4	47.3 ± 5.7	48.5 ± 2.3	48.9 ± 4.1	47.7 ± 6.7	49.2 ± 2.5
MCV(fL)	63.0 ± 1.7	63.6 ± 1.3	63.1 ± 2.0	59.3 ± 4.0[a]	61.8 ± 2.0[b]	64.6 ± 2.0	58.5 ± 3.5[a]
MCH(pg)	18.2 ± 0.6	18.0 ± 0.4	17.8 ± 0.5	17.1 ± 1.0[a]	17.8 ± 0.7	18.3 ± 0.8	17.2 ± 1.1[a]
MCHC(g/L)	289 ± 7	283 ± 8	283 ± 6[a]	288 ± 8	288 ± 6	284 ± 11	294 ± 10
RDW(fL)	13.1 ± 0.5	13.4 ± 0.3	13.5 ± 0.7	13.9 ± 0.7[a]	14.1 ± 1.2[a]	13.4 ± 0.4	14.1 ± 1.8
PLT(10^9/L)	1356 ± 97	1380 ± 183	1376 ± 391	1426 ± 120	1369 ± 288	1334 ± 209	1438 ± 40[a]
MPV(fL)	6.32 ± 0.32	6.16 ± 0.45	6.27 ± 0.25	6.29 ± 0.26	6.39 ± 0.42	6.42 ± 0.24	6.45 ± 0.36

[a] $p < 0.05$ compared to the control group.
[b] $p < 0.05$ compared to the corresponding non-GM soybean groups.

Table 4
Terminal serum biochemistry of rats fed with different diets on the 91st day. (n = 10/group; mean ± SD).

	Control group	Non-GM soybean			GM soybean		
		7.5%	15%	30%	7.5%	15%	30%
Males							
ALT (U/L)	53.2 ± 7.5	39.6 ± 6.4[a]	40.8 ± 4.4[a]	45.3 ± 8.9[a]	44.3 ± 8.0[a]	44.3 ± 9.8[a]	40.8 ± 7.2[a]
AST (U/L)	290 ± 54	225 ± 32[a]	210 ± 34[a]	237 ± 47[a]	213 ± 40[a]	241 ± 50	209 ± 51[a]
TP (g/L)	57.9 ± 3.0	56.8 ± 5.4	54.8 ± 3.9	55.8 ± 4.4	57.0 ± 3.7	55.3 ± 2.6	54.2 ± 4.7
ALB (U/L)	33.3 ± 1.8	33.7 ± 3.5	32.3 ± 3.1	33.6 ± 2.7	33.3 ± 1.6	31.7 ± 1.3[a]	31.8 ± 2.8
ALP (U/L)	112 ± 20	111 ± 20	130 ± 23	125 ± 26	133 ± 27	127 ± 33	125 ± 33
GLU (mmol/L)	5.98 ± 1.31	4.46 ± 0.95[a]	5.19 ± 1.30	4.90 ± 0.69[a]	4.94 ± 1.16	4.97 ± 1.36	5.15 ± 1.17
BUN (mmol/L)	9.56 ± 1.35	8.18 ± 1.10[a]	7.55 ± 1.18[a]	7.71 ± 0.69[a]	7.72 ± 1.0[a]	8.21 ± 0.73[a]	7.81 ± 0.99[a]
CREA (umol/l)	80.9 ± 5.1	77.9 ± 4.0	77.1 ± 3.3	79.7 ± 6.7	78.9 ± 6.6	82.8 ± 7[b]	77.3 ± 7.9
Ca (mmol/l)	2.51 ± 0.11	2.42 ± 0.17	2.39 ± 0.15[a]	2.45 ± 0.18	2.47 ± 0.15	2.39 ± 0.12[a]	2.42 ± 0.18
P (mg/dl)	2.69 ± 0.26	2.53 ± 0.17	2.54 ± 0.22	2.57 ± 0.20	2.60 ± 0.38	2.57 ± 0.19	2.61 ± 0.25
CHO (mg/dl)	1.45 ± 0.37	1.57 ± 0.39	1.59 ± 0.37	1.53 ± 0.27	1.56 ± 0.23	1.59 ± 0.30	1.59 ± 0.38
TG (mmol/l)	0.37 ± 0.09	0.38 ± 0.14	0.26 ± 0.10[a]	0.28 ± 0.07[a]	0.25 ± 0.06[a,b]	0.23 ± 0.07[a]	0.31 ± 0.13
LDH (U/L)	2258 ± 494	1545 ± 473[a]	1309 ± 514[a]	1643 ± 462[a]	1273 ± 444[a]	1554 ± 498[a]	1341 ± 600[a]
Cl (mmol/l)	105.1 ± 2.5	109.4 ± 2.9[a]	108.3 ± 3.6[a]	108.3 ± 3.3[a]	108.5 ± 3.3[a]	108 ± 2.9[a]	107.3 ± 4.2
Mg (mmol/L)	1.00 ± 0.08	0.92 ± 0.09[a]	0.91 ± 0.07[a]	0.94 ± 0.07	0.94 ± 0.17	0.93 ± 0.09	0.97 ± 0.17
Females							
ALT (U/L)	40.5 ± 10.6	35.6 ± 3.8	32.1 ± 4.0[a]	34 ± 7.4	29.5 ± 5.2[a,b]	33.8 ± 5.9	33.3 ± 8.3
AST (U/L)	204 ± 33	215 ± 60	210 ± 30	202 ± 62	174 ± 37	197 ± 39	222 ± 52
TP (g/L)	63.9 ± 6.1	64.9 ± 3.6	65.5 ± 4.4	64.3 ± 4.0	63.0 ± 6.0	63.6 ± 4.9	62.7 ± 6.6
ALB (U/L)	38.4 ± 4.1	40.7 ± 2.6	40.8 ± 3.7	40.8 ± 2.9	39.7 ± 4.1	40.1 ± 4.5	37.8 ± 3.6
ALP (U/L)	53.2 ± 11.2	53.6 ± 23.4	51.8 ± 6.4	50.6 ± 18.5	49.3 ± 13.1	56.2 ± 20.0	67.0 ± 16.6[a]
GLU (mmol/L)	5.97 ± 1.56	4.70 ± 1.46	4.73 ± 1.14	4.66 ± 1.91	5.42 ± 1.06	4.40 ± 1.10[a]	3.71 ± 1.17[a]
BUN (mmol/L)	10.74 ± 2.46	8.50 ± 1.05[a]	8.58 ± 1.60[a]	8.07 ± 1.30[a]	8.51 ± 1.70[a]	8.10 ± 1.30[a]	9.18 ± 1.65
CREA (umol/l)	86.6 ± 10.9	84.2 ± 3.4	91.2 ± 8.3	84.5 ± 5.7	86.6 ± 8.8	86.0 ± 6.1	84.2 ± 5.1
Ca (mmol/l)	2.57 ± 0.18	2.58 ± 0.12	2.54 ± 0.16	2.49 ± 0.13	2.49 ± 0.19	2.40 ± 0.20	2.36 ± 0.25
P (mg/dl)	1.97 ± 0.61	2.01 ± 0.21	1.86 ± 0.40	1.68 ± 0.19	1.80 ± 0.19[b]	1.70 ± 0.20	1.81 ± 0.32
CHO (mg/dl)	1.56 ± 0.35	1.80 ± 0.36	1.81 ± 0.28	1.70 ± 0.49	1.83 ± 0.26	1.90 ± 0.50	1.65 ± 0.55
TG (mmol/l)	0.30 ± 0.13	0.27 ± 0.09	0.30 ± 0.07	0.24 ± 0.05	0.25 ± 0.07	0.20 ± 0.10[b]	0.22 ± 0.09
LDH (U/L)	1215 ± 367	1347 ± 692	1361 ± 347	1200 ± 652	1011 ± 480	1145 ± 499	1493 ± 497
Cl (mmol/l)	103.7 ± 3.3	105.6 ± 2.6	106.7 ± 3.4	105.8 ± 1.5	105.3 ± 1.9	107.1 ± 2.5[a]	105.7 ± 2.9
Mg (mmol/l)	1.09 ± 0.15	0.93 ± 0.08[a]	0.96 ± 0.12	0.82 ± 0.07[a]	0.85 ± 0.07[a,b]	0.90 ± 0.10[a]	0.83 ± 0.10[a]

[a] $p < 0.05$ compared to the control group.
[b] $p < 0.05$ compared to the corresponding non-GM soybean groups.

Table 5
Summary of anatomic pathology findings.

		Male			Female		
		Control group	30% non-GM soybean group	30% GM soybean group	Control group	30% non-GM soybean group	30% GM soybean group
Heart	Focal inflammation	0/10	1/10	0/10	0/10	1/10	0/10
Kidney	Chronic progressive nephropathy	1/10	1/10	1/10	2/10	1/10	1/10
	Renal tubule, inflammation	0/10	0/10	1/10	1/10	0/10	1/10
Thyroid gland	Exfoliation of follicle epithelial cell	1/10	0/10	0/10	0/10	0/10	1/10
Adrenal gland	Central edema	0/10	1/10	0/10	1/9	0/10	0/10
Liver	Fatty liver	1/10	2/10	1/10	1/10	0/10	0/10
	Focal inflammation	0/10	1/10	0/10	1/10	0/10	1/10

The number in front of "/" is on behalf of the case of change.
The number behind of "/" is on behalf of the case of examination.

30% GM soybean group, GLU in 15% and 30% GM soybean group, BUN in 7.5% and 15% GM soybean group and Cl in 15% GM soybean group were significantly different ($p < 0.05$) from those in the basic control group, but not different from those in their non-GM counterparts. Therefore, these changes were not related to the GM soybean FG72 added to the fodder. All of the serum biochemistry data illustrated that the significant differences were not related to the dosage or gender.

3.6. Macroscopic observation and histopathology

No abnormal macroscopic observations were found during the dissection of the rats. Further pathology inspection results of the basic control group, 30% GM soybean group, and 30% non-GM soybean group are shown in Table 5, there was no distinct lesions founding in the selected organs, including heart, spleen, lungs, kidneys, adrenal glands, brain, liver, thymus, and testes, ovaries, stomach or intestinal. In most cases, the findings observed in the 30% GM soybean group were present with similar incidence compared with the 30% non-GM soybean group and the basic control group. All of these findings were randomly found in groups and genders. The incidence and severity of them were usually found in this strain and age of rats. Therefore, they were considered to be incidental or spontaneous, and it was concluded that the histopathologic findings was not related to the incorporation of GM soybean FG72 in the diet.

4. Discussion

Genetically modified technology is used in a broad range of crop farming, giving GM plants new properties, like obtaining resistance to herbicides or insects (Gaxiola et al., 2011) and producing improved nutrition or vaccines (Ahmad et al., 2012), which meets multiple needs of farmers and consumers nowadays. From the year 1996–2016, grown GM crops have been successfully increased from 1.7 million hectares to 185.1 million hectares(ISAAA, 2017). Twenty-eight countries have approved the use of GM crops in feed and food. Among this, GM soybean reaches 83% of the total 111 million hectares soybean, and the main trait of GM soybean is herbicide resistance. FG72 is a kind of GM soybean containing two stable genes (*2mepsps* and *hppd*), which provides farmers with options for weed control by using glyphosate herbicide and IFT herbicide.

The risk assessment of GM crops has been put on the agenda for agricultural biotechnology (EFSA, 2008; Xu et al., 2005). In many countries, like the United States, Japan, and European Union, regulatory systems have been developed and carried out (McComas et al., 2014). Among the food safety assessment strategies, the 90-day feeding test is considered an appropriate way to verify the toxicology in food testing (Knudsen and Poulsen, 2007). A 90-day subchronic feeding study was carried out on Sprague-Dawley rats to evaluate the food safety of a genetically modified soybean MON 87708 in the year of 2016. The researchers found statistically significant differences in some parameters including body weight, feed consumption/utilization, hematology, serum biochemistry etc. But these differences were considered to be attributed to incidental and biological variability (Wang et al., 2016).

According to the results of this 90-day feeding study, there were no treatment related or adverse effects in clinical observations, body weights, food consumption, organ weights, hematological parameters, serum biochemistry, and histopathology when comparing the GM groups to the non-GM soybean groups. There were some significant differences between both GM and non-GM soybean groups and the basic control group, which were especially obvious found in male groups, such as the values of the brain and testis in relative organ weight, the values of ALT, BUN and LDH in serum biochemistry, as well as the PLT level in hematology. Particularly necessary to point out that all of the changing values were in our lab historical reference ranges. Furthermore, these differences were not found between GM and non-GM groups, which were not considered to be caused by the consumption of GM soybean FG72. These differences may be related to the changing of composition materials of diets, and it seems that male animals are more sensitive. Such incidental differences, while having no toxicological significance, had also been found in other 90-day feeding studies (Yuan et al., 2013; Zeljenkova et al., 2014; Zou et al., 2016).

5. Conclusion

In conclusion, no observed toxicological effects for diets with up to 30% inclusion rates of GM soybean FG72 for Sprague-Dawley rats in this 90-day feeding study were found. This result demonstrated that soybean FG72 is as safe as the non-GM soybean Jack in this 90-day feeding study.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2018.02.004.

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