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A 90-day subchronic study of rats fed lean pork from genetically modified pigs with muscle-specific expression of recombinant *follistatin*

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ABSTRACT

Because cardiovascular disease incidence has rapidly increased in recent years, people are choosing relatively healthier diets with low animal fat. A transgenic pig with low fat and a high percentage of lean meat was created in 2011; this pig overexpresses the *follistatin* (FST) gene. To evaluate the safety of lean pork derived from genetically modified (GM) pigs, a subchronic oral toxicity study was conducted using Sprague–Dawley rats. GM pork and non-GM pork were incorporated into the diet at levels of 3.75%, 7.5%, and 15% (w/w), and the main nutrients of the various diets were subsequently balanced. The safety of GM pork was assessed by comparison of the toxicology response variables in Sprague–Dawley rats consuming diets containing GM pork with those consuming non-GM pork. No treatment-related adverse or toxic effects were observed based on an examination of the daily clinical signs, body weight, food consumption, hematology, serum biochemistry, and organ weight or based on gross and histopathological examination. The results demonstrate that GM pork is as safe for consumption as conventional pork.

1. Introduction

Pork is one of the main sources of animal protein for humans and is rich in animal fat with concentrations of 15–30%. Animal fats largely consist of saturated fatty acids, consumption of which may increase the risk of cardiovascular disease (Britton et al., 2013). Therefore, low-fat pork or other lean meats are popular among consumers. To decrease the fat content of pork, many methods have been applied in the past, such as use of the steroid clenbuterol. However, this technique is forbidden in China (Aresta et al., 2008; Pulce et al., 1991).

Biotechnology is an important modern technology that has developed rapidly in recent years. Compared to time-consuming traditional breeding methods, biotechnology offers greater efficiency in animal breeding. For example, biotechnological applications have enhanced the disease resistance of pigs, improved the feed utilization rate, and improved the lean meat percentage in a short time and with more precision. Genetic modification has been widely applied to several edible animals, including rabbits, cattle, pigs and sheep (Houdebine, 2000; Miao, 2013). Transgenic pigs have been under development for many years, with the research primarily focused on disease resistance or nutrition improvement. Transgenic pigs with increased nutrient content were typically genetically modified with various types of *growth hormone* (GH), *insulin-like growth factor I* (IGF-I) and *myostatin* genes (Gannon et al., 1990). Hammer et al. (1985) successfully integrated the *MT-hGH* gene into rabbits and pigs by microinjection (Hammer et al., 1985). In 1989, Pursel et al. cultivated one transgenic pig expressing bovine growth hormone (bGH) with low fat accumulation, rapid growth, a high feed utilization rate and low incidence of gastric ulcers, arthritis, cardiomegaly and renal disease (Pursel

Abbreviations: FST, Follistatin; OECD, Organization for Economic Cooperation and Development; GM, genetically modified; GMOs, genetically modified organisms; CAC, Codex Alimentarius Commission; BW, body weight; ANOVA, one-way analysis of variance; SD, standard deviation; EFSA, European Food Safety Authority.

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et al., 1989). Solomon et al. created a GM pig that yielded leaner meat than control pigs, and the meat lipids contained less saturated fatty acids and more unsaturated fatty acids (Pursel et al., 1997). In 2004, the transgenic pigs produced by with a lower rate of fat accretion (Pursel et al., 2004) directly expressed an *IGF-I* gene specifically in striated muscle but did not display any major changes in growth performance. Lai et al. generated a transgenic pig rich in *omega-3* fatty acids, which are beneficial to human health. The hfat-1 transgenic pigs produced high levels of *n-3* fatty acids from *n-6* analogs, and their tissues have a significantly reduced ratio of *n-6*/*n-3* fatty acids (Lai et al., 2006).

Follistatin (FST) is known to be a myostatin-binding protein, which was initially investigated for the suppression of follicle-stimulating hormone (FSH) secretion from the anterior pituitary. Additionally, it is known that FST plays an important role in the development and growth of body tissues and organs and is expressed in many tissues in mammals. FST is one member of the TGF β family and is one type of potential antagonist. In muscle tissue, FST has been shown to naturally antagonize myostatin (MSTN) function (McPherron et al., 1997). The overexpression of the *FST* gene in mouse muscle significantly enhance muscle mass and inhibit the accumulation of fats (Lee, 2004; Reisz-Porszasz et al., 2003). Therefore, the *FST* gene is valuable for economical breeding of nutritionally improved animals. Li et al. successfully developed a GM pig by overexpression of the *FST* gene in 2011 (Rui, 2011). The pigs had no obvious fat tissue on the back and abdomen. The average area of fat cells in transgenic pigs was remarkably lower than that of wild type pigs.

Considering consumer concerns, before the pork from GM pigs can become commercially available, a comprehensive food safety assessment must be performed. GM crops have been commercialized for approximately twenty years and made up an area of approximately 1.752 billion hectares around the world in 2013 (James, 2014). Meanwhile, the safety of genetically modified organisms (GMOs) has been a topic of interest for more than twenty years. The FAO/WHO has initiated the harmonization of food safety evaluation of GMOs, and the Codex Alimentarius Commission (CAC) has established *The guidelines for conduct of food safety assessment of foods derived from recombinant-DNA animals* (CAC/GL 68-2008), considering that GM animals were likely to appear on the market in the future.

In the present study, we evaluated the safety of lean pork from GM pigs via a 90-day sunchronic feeding study in Sprague–Dawley rats in accordance with the Chinese standard *thirty and ninety days feeding test* (Chinese standard GB 14924.3-2010) as well as guidelines of the *repeated dose 90-day oral toxicity study in rodents* (OECD guideline 408, 1998).

2. Materials and methods

This trial was conducted at the SPF animal laboratory of the Supervision & Testing Center for GMO Food Safety, Ministry of Agriculture (Beijing, China), with license number SYXK (Beijing) 2010-0036. Rats were obtained from Vital River Laboratories, Inc. (Beijing, China) with the license number SCXK (Beijing) 2012-0001. These rats were acclimatized for one week in cages containing five rats each. The temperature was maintained at 22 ± 2 °C, with relative humidity between 40 and 70% and a 12-h light/dark cycle. Food and water were provided *ad libitum*. During the acclimatization period, animals were fed with a commercial diet.

2.1. Pork material and preparation of meat powder

The Duroc pig powers were provided by Pro.Ning Li of The Key Laboratory of Agricultural Biotechnology. Pork sample collection and meat powder preparation were conducted as described by Liu et al. (Liu et al., 2013). Meat samples driven from eight parts of each animal were collected independently. Each meat sample was cut into chunks and boiled for 0.5 h at atmospheric pressure. Then it was minced and vacuum freeze dried for 15 h. One kilogram of meat powder was produced from 5.0 kg of fresh pork in this study. The dry matter was passed through 40-mesh screen and mixed well. The main nutritional ingredients of the GM meat powder and non-GM meat powder were measured, including moisture, protein, fat, fiber and ash.

2.2. Diet administration and experimental design

The GM pork and non-GM pork were incorporated into the basic rodent diet by Ke Ao Xie Li Feed Co. Ltd. (Beijing, China) at 3.75%, 7.5% and 15% (w/w), respectively. The highest test dose was selected as the highest possible dose that could be given without disturbing the nutritional balance. According to the result of nutritional analysis, all ingredients were adjusted to meet the growth and development requirement of the rodents (Chinese standard GB 14924.3-2010). All diets were vacuum-packed and sterilized by ^{60}Co to keep them germ-free. The nutritional components of the test diets, including ash, fat, crude fiber, moisture, crude protein and carbohydrates, were measured.

2.3. Animals and management

One hundred and forty Sprague–Dawley rats (70 male and 70 female) that were approximately five weeks old with an average body weight of 100 ± 20 g were included in this study. Five rats per gender were housed together in a stainless steel cage for acclimation. The animal experiment and housing procedures were carried out in compliance with the *OECD Good Laboratory Practice* guidelines. The animal study was approved by the Animal Experimental Welfare & Ethical Inspection Committee (No.120005), the Supervision & Testing Center for GMO Food Safety, Ministry of Agriculture (Beijing, China). Animals were cared for according to the Guide for the Care and Use of Laboratory Animals (Bayne, 1996), and the committees approved the protocols.

Following one week of acclimatization, the rats were randomly divided into seven groups (10 rats/sex/group) based on body weight such that there were no statistically significant differences in weight between the groups. Six treatment groups were fed with diets containing 3.75, 7.5 and 15% (w/w) GM pork or non-GM pork, and the seventh group was fed with the rodent basic diet. The maximum dose corresponds to 10.5 g meat powder/52.5 g meat per kg body weight of rats.

2.4. Detection of indexes

2.4.1. Clinical evaluation, body weight gain and food utilization

Clinical observation was performed daily to detect rat behavior and appearance. Coat condition, skin, eyes, excretion, mentality, mortality and other clinical signs of toxicity were recorded. Body weight gain and feed consumption were measured weekly. Feed utilization was determined using the following calculation:

$$\text{Feed utilization}(\%) = (\text{body weight gain}/\text{feed consumption}) \times 100\%.$$

2.4.2. Hematology and serum chemistry examination

Blood samples were collected twice, on days 45 and 90. The blood samples were collected from the orbital sinus under

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