Food and Chemical Toxicology 57 (2013) 314–321

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Safety assessment of meat from transgenic cattle by 90-day feeding study in rats

Shan Liu [a], Chen-Xi Li [a], Xiao-Lian Feng [a], Hui-Ling Wang [a], Hai-Bo Liu [a], Yuan Zhi [a], Gui-Ying Geng [a], Jie Zhao [b], Hai-Bin Xu [a,*]

[a] Key Laboratory of Food Safety Risk Assessment of Ministry of Health, China National Center for Food Safety Risk Assessment, Beijing 100021, PR China
[b] China Agricultural University, Beijing 100193, PR China

ARTICLE INFO

Article history:
Received 16 January 2013
Accepted 3 April 2013
Available online 10 April 2013

Keywords:
Meat
Transgenic cattle
Human lactoferrin
Rat
90-Day feeding study

ABSTRACT

The study was carried out to evaluate the subchronic toxicity of meat derived from human lactoferrin gene-modified cattle in male and female Wistar rats. Rats were fed 5% or 10% transgenic meat diet, 5% or 10% conventional meat diet, or AIN93G diet for 90 days. During the study, body weight and food consumption were weighed weekly and clinical observations were conducted daily. At the end of the study, urinary examination, hematology and blood biochemistry examination, macroscopic and microscopic examinations were performed. There were no biologically significant differences in these factors between the rat groups fed transgenic meat diet and conventional meat diet. Therefore, the present 90-day rodent feeding study suggests that meat derived from the transgenic cattle is equivalent to meat from conventional cattle in use as dietary supplements.

1. Introduction

Since the birth of the lamb "Dolly" in 1996, somatic cell nuclear transfer (SCNT) has been successfully applied to produce clone goat (Keefer et al., 2002), clone cattle (Cibelli et al., 1998; Yang et al., 2005), clone pig (Onishi et al., 2000) and so on. Meanwhile, research investigations on the safety of food products from clone animals have been continually performed. In January 2008, the Food and Drug Administration (FDA) of USA released its report "Animal Cloning: A Risk Assessment" and concluded that consumption of edible products from clone progeny would not pose any additional food consumption risk(s) relative to consumption of similar products from sexually-derived animals (FDA, 2008). A similar conclusion was drawn by the European Food Safety Authority (EFSA) (EFSA, 2008, 2010).

Transgenesis is another important modern biotechnology developed rapidly in recent years. It provides a shortcut for organisms to exhibit new or altered expression of traits and has been applied to some food animals including rabbits, sheep, pigs and cattle (Chan et al., 1998; Hammer et al., 1985). In 2008, Codex Alimentarius Commission (CAC) issued "Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals". In 2012, EFSA launched its scientific opinion on "Guidance on the risk assessment of food and feed from genetically modified animals and on animal health and welfare aspects". As mentioned in these guidelines, the concept of substantial equivalence is a key step in the safety assessment process, which is used to identify similarities and differences between the new food and its conventional counterpart.

Using transgenic technology and SCNT technology, China Agriculture University has produced transgenic cattle that secrete recombinant human lactoferrin (rhLF) on an industrial scale in milk (Yang et al., 2008). Human lactoferrin (hLF) is a natural iron-binding glycoprotein of the transferrin family and it is involved in many physiological processes, such as inflammatory regulation (Baveye et al., 1999), host defense (Nuijens et al., 1996), and growth promotion (Naot et al., 2005). Previously, several studies on the milk derived from the the female offspring of this transgenic cattle clone and rhLF purified from the milk have been performed (Hu et al., 2012; Liu et al., 2011; Wang et al., 2011, 2012; Yu et al., 2011; Zhou et al., 2011). These studies indicate that rhLF was similar to wild-type human lactoferrin in both structure and function, and there was no evidence of toxicity of rhLF or transgenic milk. But the male offspring of the cattle clone cannot produce rhLF and could enter

Abbreviations: A/G, albumin/globulin ratio; ALP, alkaline phosphatase; ALT, Alanine aminotransferase; APTT, activated partial thromboplastin time; BUN, blood urea nitrogen; CK, creatine kinase; EFSA, European Food Safety Authority; FDA, Food and Drug Administration; RBC, red blood cell count; rhLF, recombinant human lactoferrin; SCNT, somatic cell nuclear transfer; WBC, white blood cell count; AST, aspartate aminotransferase; ChE, cholinesterase; LDH, lactate dehydrogenase; PT, prothrombin time; TP, total protein.

* Corresponding author. Tel./fax: +86 10 67779019.
E-mail addresses: liushan78@hotmail.com (S. Liu), hbxu1231602@vip.sina.com (H.-B. Xu).

0278-6915/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.04.003

into the food chain. The safety of meat products from these cattle should be considered. In the present study, the safety of meat from progeny of the transgenic cattle clone was evaluated by a 90-day rodent feeding study.

2. Materials and methods

2.1. Preparation of meat powder

Three half-sib hLF gene-modified bulls and three wild-type conventionally-bred bulls were selected for the study. The hLF bulls are from the same clone line and the second generations of Xiang (Yang et al., 2008). Meat samples derived from eight parts (longissimus dorsi muscle, trapezius muscle, rectus abdominis, musculus pectoralis profundus, ventral saw muscle, longissimus lumborum, Semitendinosus, gluteus medius muscle) of each animal were collected independently. After removing excess fat and connective tissue, 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. The dry matter was passed through 40-mesh screen and mixed well.

Table 1
Nutritional analysis of meat powder derived from conventional cow and transgenic cow.[a]

	Meat powder derived from conventional cow	Meat powder derived from transgenic cow
Macronutrients		
Water content (g/100 g)	3.52	3.46
Ash content (g/100 g)	2.7	2.4
Crude protein (g/100 g)	90.4	91.1
Crude fat (g/100 g)	3.3	3.0
Carbohydrate (g/100 g)	0.1	0.0
Minerals		
Cu (mg/kg)	2.1	2.7
Zn (g/kg)	0.17	0.16
Mg (mg/100 g)	59.91	59.82
Fe (mg/100 g)	9.15	8.80
Mn (mg/100 g)	0.03	0.05
K (mg/100 g)	370.21	335.99
Na (mg/100 g)	116.16	99.31
Ca (mg/100 g)	12.75	10.88
P (g/100 g)	0.48	0.47
Se (mg/kg)	0.529	0.504
Vitamins		
A (μg/100 g)	4.79	1.75
B_1 (mg/100 g)	0.095	0.077
B_2 (mg/100 g)	0.20	0.22
B_6 (mg/100 g)	0.19	0.20
B_{12} (μg/100 g)	5.6	4.7
E (mg/100 g)	0.236	2.35
Niacin (mg/100 g)	8.73	10.75
Pantothenic acid (mg/100 g)	0.49	0.36
Folic acid (μg/100 g)	10.1	11.9
Free biotin (μg/100 g)	1.8	1.8
Amino acid compositions (g/100 g)		
ASP	9.18	9.01
THR	4.32	4.27
SER	3.70	3.80
GLU	15.86	15.18
GLY	4.36	4.58
ALA	5.61	5.56
VAL	3.97	3.91
MET	2.91	2.78
ILE	3.66	3.59
LEU	8.00	7.80
TYR	3.97	3.72
PHE	3.50	3.33
LYS	8.44	8.29
HIS	2.44	2.56
ARG	6.41	6.20
PRO	3.65	3.79
TRP	0.61	0.40
CYS	1.31	1.25
Energy (kJ/100 g)	1660	1660

[a] Two replicate meat powder samples were analyzed and mean amounts were listed here.

The samples were prepared as two pooled powders, transgenic meat powder and conventional meat powder. Then the nutritional values of each pooled powder were analyzed. As shown in Table 1, the items included macronutrients (water content, ash content, crude protein, crude fat and carbohydrate), minerals (Cu, Zn, Mg, Fe, Mn, K, Na, Ca, P and Se), vitamins (A, B_1, B_2, B_6, B_{12}, E, niacin, pantothenic acid, folic acid and free biotin) and amino acid analysis.

2.2. Preparation of test diets supplemented with meat powder

The diet was supplemented with 5% or 10% (w/w) each pooled meat powder. According to the result of nutrition analysis, the other ingredients were adjusted to meet the level of AIN93G purified diets for laboratory rodents (Philip et al., 1993), as shown in Table 2. The four test diets (5% conventional meat powder, 10% conventional meat powder, 5% transgenic meat powder and 10% transgenic meat powder) and one meat-free diet (AIN93G) were irradiated with ^{60}Co (25 kGy) and stored at −20 °C.

2.3. Animals and dosing

The study was conducted at Laboratory Animal Institute, Chinese Academy of Medical Sciences (SYXK (JING) 2010-0029). SPF weaning Wistar rats (50–60 g) were obtained from Laboratory Animal Center, Academy of Military Medical Sciences(SCXK-(JUN)2007-004). Animals were quarantined 3 days before study start and then were randomly assigned into five groups of approximately similar initial mean body weights. Male and female rats (n = 10/group/sex) were fed either AIN93G or one of the four test diets as described above for 13 weeks with water *ad libitum*. Each rat was kept in an individual stainless steel mesh cage in an animal room with a barrier system, controlled temperature (20–26 °C) and humidity (40–70%), ventilation about 15 times/h and a 12-h light/dark cycle. Also, the protocol for the study was reviewed and approved by Animal Experimental Welfare & Ethical Inspection Committee of Chinese Center for Disease Control and Prevention.

2.4. Observation and examination of rats

2.4.1. Clinical observation

Every rat was observed daily for clinical signs, and was weighed at the beginning of the study, weekly during the feeding period, and then at necropsy. The food consumption of each rat was also measured weekly.

2.4.2. Urinary examination

At the end of the study, urine was collected and analyzed for leukocyte, ketone, nitrite, urobilinogen, bilirubin, protein, glucose, specific gravity, pH, occult blood, creatinine, calcium and microglobulin by dry chemistry test paper method. Urit-55 urine analyzer and Urit-13G test strip were used for the urinalysis.

2.4.3. Hematology and blood biochemistry examination

At the end of the study, the fasting rats were anesthetized and blood was collected from the abdominal aorta. Red blood cell count (RBC), hemoglobin, platelet count, white blood cell count (WBC), percentage of lymphocyte, percentage of neutrophil and percentage of other leukocyte (including monocyte, eosinophil and basophil) were analyzed using an automatic blood analyzer (MEK-6318K, Japan). Immunophenotyping of leukocyte populations including B lymphocyte (CD3-CD45RA+), T lymphocyte (CD3+CD4+) and NK cell (CD3-CD161a+) percentage) were analyzed using flow cytometer (BD FACS Calibur, USA). Prothrombin time (PT) and activated partial thromboplastin time (APTT) were analyzed using a blood coagulation analyzer (KHB202-4, PRC). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), cholinesterase (ChE), total protein (TP), albumin, albumin/globulin ratio (A/G), globulin, total cholesterol, triglyceride, glucose, blood urea nitrogen (BUN), creatinine, inorganic phosphorus, chloride, sodium, potassium and calcium were analyzed using an automatic chemical analyzer (Hitachi 7080, Japan).

2.4.4. Examination of rat organs and tissues

At necropsy, brain, heart, liver, spleen, kidneys, testes, epididymides, thymus, adrenals were isolated and weighed. Histopathological examinations were performed on cerebrum, cerebellum, pituitary gland, eye ball, Harderian gland, salivary gland (sublingual and submandibular glands), parotid gland, thyroid, parathyroid, thymus, trachea, lung, bronchus principalis, aorta,heart, liver, pancreas, kidney, esophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, mesenteric lymph node, bladder, spleen, adrenal gland, skin, mammary gland, sternum, femur, bone marrow, vertebra, spinal cord, skeletal muscle, ovary, oviduct, uterus, vagina, testis, epididymis, prostate and seminal vesicle.