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# Safety assessment of genetically modified milk containing human beta-defensin-3 on rats by a 90-day feeding study

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*Abbreviations:*
AIN93G diet
Growth purified diet for rodents recommended by the American Institute of Nutrition
ALB
albumin
ALP
alkaline phosphatase
ALT
alanine aminotransferase
AST
aspartate aminotransferase
BUN
urea nitrogen
CAC
Codex Alimentarius Commission
FAO
Food and Agriculture Organization
GLO
globulin
GM
genetically modified
HBD3
human beta-defensin-3
HE
hematoxylin-eosin

ABSTRACT

In recent years, transgenic technology has been widely applied in many fields. There is concern about the safety of genetically modified (GM) products with the increased prevalence of GM products. In order to prevent mastitis in dairy cows, our group produced transgenic cattle expressing *human beta-defensin-3* (*HBD3*) in their mammary glands, which confers resistance to the bacteria that cause mastitis. The milk derived from these transgenic cattle thus contained HBD3. The objective of the present study was to analyze the nutritional composition of HBD3 milk and conduct a 90-day feeding study on rats. Rats were divided into 5 groups which consumed either an AIN93G diet (growth purified diet for rodents recommended by the American Institute of Nutrition) with the addition of 10% or 30% HBD3 milk, an AIN93G diet with the addition of 10% or 30% conventional milk, or an AIN93G diet alone. The results showed that there was no difference in the nutritional composition of HBD3 and conventional milk. Furthermore, body weight, food consumption, blood biochemistry, relative organ weight, and histopathology were normal in those rats that consumed diets containing HBD3. No adverse effects were observed between groups that could be attributed to varying diets or gender.



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OECD
Organization for Economic Co-operation and Development
TBIL
total bilirubin
T-CHOL
total cholesterol
TG
triglyceride
TP
total protein
WHO
World Health Organization
w/w
(weight/weight)

## 1. Introduction

Human beta-defensin-3 (HBD3) is a small, cationic, host defense peptide comprised of 45 amino acid residues, which was first isolated from human lesional psoriatic scales and cloned from keratinocytes by Harder et al. (2001). HBD3 possesses six conserved cysteine residues that facilitate both broad antimicrobial activity against many pathogenic microbes and diverse innate immune functions (Van Hemert et al., 2012). Previous studies have shown that HBD3 is effective against the majority of Gram-positive and Gram-negative bacteria, including a number of multiple antibiotic resistant strains (Maisetta et al., 2006; Zasloff, 2002). The antimicrobial effects of HBD3 differ for different strains of bacteria. In an *in vitro* study of oral cavity bacteria, aerobes were found to be more sensitive to HBD3 than anaerobes (Joly et al., 2004). HBD3 also has inhibitory effects against fungi and viruses (Dhople et al., 2006). Quinones-Mateu et al. (2003) reported that HBD3 can inhibit replication of the human immunodeficiency virus. In addition, HBD3 has chemotaxis effects on immature dendritic cells and memory T cells (Scudiero et al., 2010). Therefore, HBD3 plays an important role in the regulation of immunity. In the immune system, HBD3 acts as a bridge linking innate immunity and acquired immunity.

Mastitis is the most common disease related to milk production in dairy cows. Mastitis causes great economic losses due to a decrease in the quality and quantity of milk production and the increased cost of disease treatment (Kerro Dego et al., 2002; Sinha et al., 2014). Mastitis is an inflammatory response to pathogenic microorganisms entering through the teat canal and multiplying in the mammary gland (Oviedo-Boyso et al., 2007). Many different bacteria can cause mastitis, including contagious and environmental bacteria such as *Staphylococcus aureus, Escherichia coli*, and *Streptococcus dysgalactiae.* Antibiotics are the most common treatment for mastitis. However, antibiotics are not an ideal treatment as there are various different pathogenic bacteria species that could cause infection and the overuse of antibiotics also causes problems such as drug-resistant bacterial strains and milk containing antibiotic residues which is unfit for consumption.

Transgenic animals are animals where one or more genes from one organism has been transferred to another by using genetic engineering technologies (Hino, 2002). With the continued development of transgenic technology, genetically modified (GM) products are becoming more prevalent in daily life. When new GM products are developed, testing must be performed to determine whether the new trait will affect the nutritional value of the product or consumer health. The safety assessment of GM products focuses primarily on potential allergenic compounds in the food, the nutritional content of the food, possible expression of antibiotic selection markers, and transgene stability and inheritance (Domingo, 2016; Domingo and Gine Bordonaba, 2011; Nicolia et al., 2014). The current principles of the safety assessment for GM products, which are accepted by most nations and organizations, support the concept of substantial equivalence and the steps involved for the scientific evaluation of each GM product are formed in a case by case basis (Codex, 2008; OECD, 1993). Feeding studies are often used to assess the safety of food products, including GM products. Traditionally, a feeding study is conducted within 30 days or 90 days for a general health assessment. If the GM product is for a special population such as infants or the elderly, special parameters in addition to general health may need to be evaluated, and thus a feeding study may be conducted using animals of different ages over varying lengths of time (Malatesta et al., 2008). Many GM products have been the subject of feeding studies, including rice (Schroder et al., 2007; Tang et al., 2012; Yuan et al., 2013), soybeans (Appenzeller et al., 2008), tomatoes (Fares and El-Sayed, 1998), maize grain (He et al., 2008, 2009), and animal products such as meat and milk (S. Liu et al., 2013; Yamaguchi et al., 2007; Zhou et al., 2011).

In order to prevent mastitis in dairy cows, our group exploited the broad-spectrum antimicrobial activity of HBD3 and produced transgenic cattle expressing HBD3 specifically in their mammary glands to prevent colonization in this area by the bacteria that cause mastitis. Lactation in the transgenic cattle was comparable to healthy conventional cattle, and the milk from transgenic cows repressed the growth of both *S. aureus* and *E. coli.* (Yu et al., 2013).

A previous *in vitro* study showed the GM milk containing HBD3 was easy to digest, and did not cause any adverse effects on the general and gastrointestinal health of the mice in the study (Chen et al., 2016). Rat and mouse models are the commonly-used model animals in feeding studies. Here, we analyzed the composition of HBD3 GM milk and the general health of rats following consumption of HBD3 GM milk, which included analysis of body weight, food consumption, blood biochemistry, relative organ weight, and pathology. A 90-day feeding study was conducted in accordance with the *Chinese Toxicology Assessment Procedures and Methods for Food Safety* (Chinese standard GB 15193.13–2003).

## 2. Materials and methods

### 2.1. Test sample

The GM milk containing HBD3 was produced by transgenic cattle with *HBD3* inserted into a “safe harbor” in the bovine genome by phiC31 integrase. The concentration of HBD3 in the milk was measured during the lactation period using ELISA, and it ranged from 3.9 to 10.4 μg/ml (Yu et al., 2013). We selected the milk with the highest HBD3 concentration for use in this study.