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COMPARISON OF MILK QUALITY OF TRANSGENIC RABBITS CARRYING DIFFERENT GENE CONSTRUCTS

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ABSTRACT

Basic objective of this research was to compare the milk quality of New Zealand White transgenic rabbit females expressing recombinant human protein C (hPC) or recombinant human factor VIII (hFVIII) in the mammary gland during first lactation with that of non-transgenic rabbit females of the same age. Transgenic founders were generated by the microinjection of foreign DNA (mWAP-hPC gene construct or mWAP-hFVIII) into the egg. Then several generations of transgenic rabbits were obtained after mating of transgenic founder rabbits with non-transgenic rabbits. Quality of milk (content of fat, protein, lactose, dry ash, and some minerals) from transgenic rabbits was determined.

Our analyses of transgenic rabbit milk samples showed that all the transgenic females (carrying hPC or hFVIII) tested in this work produced lower or higher amounts of recombinant human protein C or recombinant human FVIII, depending on transgenic females, with proved biological activity. No significant differences in the content of milk fat, protein, lactose and somatic cell counts were found.

Our results showed that milk performance of transgenic rabbits over several generations (using both gene constructs) did not differ significantly from those of non-transgenic animals.

Key words: transgenic rabbit; milk quality; mWAP-hPC and mWAP-hFVIII gene

INTRODUCTION

The use of transgenic mammary gland as bioreactors („pharmaceutical farming“) is cost-effective and variability in post-translational modification is an alternative to cell culture methods (Garber, 2000). The mammary gland is the most promising target tissue because it produces large amounts of protein in a temperature-regulated fluid that may be collected daily in a non-invasive fashion. Transgenic animals are not only cost-effective bioreactors, but, with the complex secretory cell types and organs of the mammalian organism, can perform much more complicated protein modifications than simply cultured cells. Transgenic animals used as bioreactors to produce human proteins represent new horizon in animal husbandry often followed by low viability of newborn animals (Lubon *et al*., 1996, Chrenek *et al*., 2007a).

Milk is usually the sole source of nourishment of young mammals, therefore offspring growth and development depends on milk. Rabbit milk yield may be affected by breed of doe (Lukafahr *et al*., 1983), nutrition (Chrastinová *et al*., 1997), number of kids suckling and their age of weaning (Taranto *et al*., 2003) and pregnancy during lactation (Lukafahr *et al*., 1983). Intensive recombinant human protein production by the mammary gland of transgenic rabbit necessitates the knowledge of the lactation curve and quality (composition) of milk as a possible effect of transgenesis on milk yield.

The objective of this research was to compare the milk quality of New Zealand White transgenic rabbit females expressing recombinant human protein C

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(hPC) or recombinant human factor VIII (hFVIII) in the mammary gland during first lactation with that of non-transgenic rabbit females of the same age.

MATERIAL AND METHODS

Biological material

Transgenic rabbit females from second generations were obtained after mating of transgenic rabbit females carrying the mWAP-hPC or mWAP-hFVIII transgene (Chrenek *et al*., 2002, 2005) with non-transgenic males.

The animals of about 4-5 months age at 3.5 - 4.0 kg of weight were used in this experiment. All females had from 7 to 9 pups per litter. They were housed in individual steel cages, with adjacent box for a possibility to separate litter during entire experiment, in controlled environment (constant temperature and light-dark regime). Food and water were supplied *ad libitum*.

Rabbit milk analysis

Milk samples (10 ml) were collected from each lactating transgenic and non-transgenic females on day 21st using "home made air vacuum pump" from several parts (nipples) of the mammary gland. In order to stimulate milk letdown, an intramuscular injection of 5 IU of oxytocin (Veyx Pharma, Germany) was given 10 min before milk collection.

Rabbit milk composition (content of fat, protein, lactose, dry matter as solids non-fat, SNF) was investigated by infrared absorption instrument (Instrument Milko-Scan FT 120 Foss Electric Denmark, according to the manufacturer's manual). Determination of minerals in ash after annealing was done by atomic absorption spectrometer (UNICAM 939 Solar STN 57 0532).

Statistics

Differences between the transgenic and non-transgenic groups were determined by ANOVA followed by Duncan´s multiple range test. Differences between groups at $p<0.05$ were considered as significant.

RESULTS AND DISCUSSION

To analyze milk composition from transgenic and non-transgenic rabbits, the content of fat, protein, lactose, SNF and minerals was determined (Table 1). There were no differences in the content of rabbit milk fat in the hPC transgenic rabbits (11.18 g/100 g) or hFVIII transgenic rabbits (11.48 g/100 g) compared to non-transgenic ones (10.40 g/100 g). Similarly, although the contents of rabbit milk protein (9.25 and 9.30 g/100 g respectively) and lactose (1.99 and 1.89 g/100 g respectively) were higher in the transgenic compared to non-transgenic (8.38 and 2.26 g/100 g respectivelly) rabbits, the differences were not significant. Also, there were no differences in mineral content (ash, Ca, P, Mg, Na) of milk between transgenic and non-transgenic animals (Table 2).

We present a results about milk composition of transgenic (carrying human protein C or human factor VIII gene) rabbit females at first lactation. Specific transgenic over-expression of hPC or hFVIII in the mammary gland can be obtained without any strong influence on rabbit milk yield in different lactations, indicating that this technology can be applied to "pharmaceutical farming"

Table 1: Milk composition of transgenic and non-transgenic rabbits at the first lactation

Females	Fat g/100 g	Protein g/100 g	Lactose g/100 g	SNF g/100 g
Transgenic (hPC) average (n=8)	11.18±0.24	9.25±0.25	1.99±0.33	12.08±0.24
Transgenic (hFVIII) average (n=8)	11.48±0.24	9.30±0.25	1.89±0.03	12.04±0.24
Non-transgenic average (n=8)	10.40±0.22	8.38±0.15	2.26±0.03	12.09±0.23

(Dragin *et al*., 2005, Chrenek *et al*., 2007a).

Rabbit milk composition varies depending on various factors, such as breed, nutrition, lactation stage or number of pups (Chrastinova *et al*., 1997; Lukafahr *et al.*, 1983). To investigate difference in milk composition between transgenic and non-transgenic rabbit females, basic analysis of milk was performed at the same conditions.

Our previous results on transgenic rabbit milk samples showed that all tested transgenic females produced either lower or higher concentrations of rhFVIII ranged from 5 to 1170 µg/ml, with variations among individual transgenic females (Chrenek *et al*., 2005, 2007b). Significant differences were found in the content of milk fat, protein and lactose.

The higher variability in rhFVIII concentration may be explained by different copies of integrated gene, the site of transgene insertion or its genomic environment, what influence its expression (Salvo-Garrido *et al*., 2004). Higher protein content in transgenic rabbit milk samples may be explained by the production of recombinant human factor VIII, although an expression of recombinant protein into the mammary gland of transgenic animals may not be automatically resulted in any increase in total milk protein content (Wilde *et al.*, 1992). Significant differences obtained in our milk samples are in agreement with the physiological range of variability, where an average of fat content is 10-15 g/100 g, protein is 8-12.5 g/100 g, lactose 1.0-2.0 g/100 g and ash is 2-3 % (Davies, 1983).

Palmer *et al.* (2003) recently reported that transgenic mice expressing recombinant human protein into mammary gland, under mouse WAP promoter, exhibit defects in lactation and impaired mammary gland development. The WAP promoter was shown to be less efficient than the β-lactoglobulin promoter at driving the over-expression of recombinant human protein into milk (Barash *et al*., 1999). On the other hand, Van Cott *et al.* (2001) concluded that transgenesis and recombinant human protein secretion in milk was not connected with any abnormality concerning milk production, such as mastitis or other mammary gland disorders of transgenic pig. Mammary gland specific over-expression of IGF-I did not have also any impact on lactation performance in swine (Monaco *et al*., 2005).

Our results showed that mammary gland specific transgenic over-expression of hPC or hFVIII can be obtained without any strong influence on rabbit milk yield and composition in several generations and at different lactations. Mammary gland specific over-expression of mWAP-hPC or mWAP-hFVIII gene constructs has only minor and transient effect on milk composition. These differences are in agreement with the physiological range of variability for rabbit milk composition.

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Table 2: Milk mineral content of transgenic and non-transgenic rabbits at the first lactation

Females	Ash g/100 g	Ca g/100 g	P g/100 g	Mg g/100 g	Na g/100 g
Transgenic (hPC) average (n=8)	2.12±0.02	0.33±0.01	0.31±0.01	0.04±0.02	0.11±0.01
Transgenic (hFVIII) average (n=8)	2.02±0.02	0.38±0.04	0.33±0.01	0.04±0.02	0.12±0.02
Non-transgenic average (n=8)	1.88±0.02	0.41±0.01	0.31±0.04	0.04±0.02	0.11±0.03

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