



## The Yield and Composition of Milk from Transgenic Rabbits

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**ABSTRACT :** Basic objective of this research was to compare the milk yield and composition of New Zealand White transgenic rabbit females expressing recombinant human factor VIII (hFVIII) in mammary gland during lactation with that of non-transgenic rabbit females of the same age during 30 days of lactation. Transgenic founders were generated by the microinjection of foreign DNA (mWAP-hFVIII gene construct) into the egg. F1, F2 and F3 generations of transgenic rabbits were obtained after mating of transgenic founder rabbits with non-transgenic rabbits. The amount of milk rejected was measured by weight-suckle-weight method at 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day of lactation. Quality of milk (content of fat, protein, lactose, dry ash, and some minerals) from transgenic and non-transgenic rabbit was also determined. Comparison of milk yield, determined by weight-suckle-weight method, showed significantly higher ( $p < 0.05$ ) milk production at day 20 of first lactation in non-transgenic females, but on the same day of second lactation higher milk yield was measured in transgenic ones. Significantly higher ( $p < 0.05$ ) content of milk fat and protein was determined in transgenic milk whilst higher content of lactose was found in non-transgenic milk. The content of minerals (calcium, phosphorus, magnesium and sodium) did not differ in both experimental and control groups. Our results showed that milk yield and composition of transgenic rabbit females (mammary specific transgenic over-expression of hFVIII) over several generations is only slightly and transiently different from milk yield of non-transgenic females, which had no significant consequence on the litter size and viability. (**Key Words :** Rabbit, Transgenic, Milk Yield, mWAP-hFVIII Gene)

### INTRODUCTION

The use of transgenic animals as bioreactors ("pharmaceutical farming") is cost-effective and variability in post-translational modification is an alternative to cell culture methods (Garber, 2000). Animals automatically supplement their body fluids with fresh nutrients, remove waste products, reliably regulate their internal temperature and pH and resist pathogens. By targetting the expression of the transgene product, so that the secretory cells of lactating mammary gland produce it, "farmers" may collect and process body fluids with minimal effort. The mammary gland probably 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. Repercussion of that can be also irregular secretion and alteration of milk composition.

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 (Fraga et al., 1989; Chrastinova et al., 1997), number of kids suckling and their age of weaning (Lukafahr et al., 1983; Taranto et al., 2003) and pregnancy during lactation (Lukafahr et al., 1983). Intensive recombinant human protein production by 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 investigation was to compare the milk quantity and quality between transgenic (expressing recombinant human factor VIII into the milk) and non-transgenic rabbit females.

### MATERIALS AND METHODS

#### Biological material

Two lines (single microinjection, SM and double microinjection, DM) of New Zealand White transgenic

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**Table 1.** Basic statistical parameters of live weight growth on the first lactation (kg)

Day	n	Transgenic	n	Non-transgenic
1 d	90	0.063±0.001	61	0.058±0.002 <sup>sd</sup>
2 d	90	0.071±0.001	61	0.068±0.002
5 d	88	0.107±0.003	61	0.108±0.004
10 d	88	0.171±0.005	61	0.167±0.005
20 d	88	0.352±0.006	61	0.347±0.010
30 d	88	0.577±0.009	61	0.584±0.013

n: Number of animals, data are mean±SEM.

sd: Significantly different,  $p < 0.05$ .

rabbits (F1, F2 and F3 generations) were obtained after breeding of transgenic founder rabbit females (F0 generation) that were derived after microinjection with a mWAP-hFVIII transgene (Chrenek et al., 2005a). The animals of about 4-5 months old at 3.5-4 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*.

### Milk production

Rabbit pups' milk consumption and litter growth were used to assess lactation performance. Measurements were realized in transgenic (n = 11 females) and non-transgenic (n = 7 females) animals during first and second lactation. Body weight of pups was determined on day 1, 2, 5, 10, 20, 30 *post partum*. It is known, that rabbit pups are nursed only for about 3 min once every 24 h. Milk consumption on 1<sup>st</sup> and 2<sup>nd</sup> lactation was assessed on day 10, 20 and 30 *post partum* using the weight-suckle-weight method. Just before suckling, body weight of removed pups from their mother (24 h prior suckling) was controlled and immediately after suckling body weight of pups was again controlled. The difference between body weights reflected daily milk consumption (yield).

### Rabbit milk analysis

Milk samples were collected (per 10 ml) from each lactating transgenic and non-transgenic females on day 21<sup>st</sup> using "home made air vacuum pump" from several parts (nipples) of 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,

**Table 2.** Basic statistical parameters of live weight growth on the second lactation (kg)

Day	n	Transgenic	n	Non-transgenic
1 d	93	0.065±0.001	58	0.060±0.002 <sup>sd</sup>
2 d	93	0.072±0.001	58	0.069±0.003
5 d	93	0.110±0.004	58	0.107±0.003
10 d	93	0.199±0.005	58	0.195±0.005
20 d	93	0.362±0.006	58	0.357±0.011
30 d	93	0.579±0.009	58	0.580±0.012

n: Number of animals, data are mean±SEM.

sd: Significantly different,  $p < 0.05$ .

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 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

### Rabbit milk production

Basic statistical characteristics of live weight growth intensity on the first and second lactation are shown in Table 1 and 2. Low level of variability in all age categories in both groups is obvious from values of standard of the mean error. It was manifested by statistically significant estimation of influence of the integrated gene on live weight at birth in spite of the fact that absolute difference of arithmetical means of transgenic and control groups was only 0.005 kg (0.063±0.001 vs. 0.058±0.002 or 0.065±0.001 vs. 0.060±0.002). The difference of arithmetical means was not statistically significant in other age classes. From the point of absolute growth reached animals in both groups average values in individual age categories that correspond to normal growth intensity in this group of genotypes, were not different. The pups mortality as one of the indicators of milk quality and quantity was not considered in our study, except 2 pups from 10 of female 36 (first lactation), which were dead at born or they were trampled down during birth.

Results of milk yield recorded during the first lactation (Table 3) showed that there was significant differences

**Table 3.** Milk yield of transgenic and non-transgenic rabbits on the first lactation (kg)

Females	Average litter size	10 day of lactation	20 day of lactation	30 day of lactation
Transgenic average (n = 11)	8.2	0.156±0.008	0.234±0.004 <sup>sd</sup>	0.131±0.008
Non-transgenic average (n = 7)	8.7	0.161±0.004	0.258±0.004	0.133±0.007

sd: Significantly different,  $p < 0.05$ . Data are mean±SEM.

**Table 4.** Milk yield of transgenic and non-transgenic rabbits on the second lactation (kg)

Females	Average litter size	10 day of lactation	20 day of lactation	30 day of lactation
Transgenic average (n = 11)	8.5	0.192±0.004	0.315±0.008 <sup>sd</sup>	0.153±0.007
Non-transgenic average (n = 7)	8.3	0.189±0.002	0.285±0.005	0.148±0.004

sd: Significantly different,  $p < 0.05$ . Data are mean±SEM.

**Table 5.** Milk composition of transgenic and non-transgenic rabbits on the first lactation

Females	Fat (g/100 g)	Protein (g/100 g)	Lactose (g/100 g)	SNF (g/100 g)
Transgenic average (n = 11)	11.48±0.25 <sup>sd</sup>	9.35±0.25 <sup>sd</sup>	1.89±0.03 <sup>sd</sup>	12.78±0.24
Non-transgenic average (n = 7)	10.4±0.22	8.38±0.15	2.26±0.03	12.09±0.23

sd: Significantly different,  $p < 0.05$ . Data are mean±SEM.

**Table 6.** Milk mineral content of transgenic and non-transgenic rabbits on 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 average (n = 11)	2.02±0.02	0.38±0.01	0.33±0.03	0.04±0.02	0.12±0.02
Non-transgenic average (n = 7)	1.88±0.02	0.41±0.01	0.31±0.04	0.04±0.02	0.11±0.03

( $p < 0.05$ ) between transgenic and non-transgenic females on day 20 of lactation (0.234±0.004 vs. 0.258±0.004 kg). During the second lactation significant differences ( $p < 0.05$ ) between transgenic and non-transgenic rabbit milk yield (Table 4) were found (0.315±0.008 vs. 0.285±0.005 kg), respectively.

#### Rabbit milk composition

To analyze of transgenic and non-transgenic rabbit females milk composition the content of fat, protein, lactose, SNF and minerals was determined (Table 5). Significant differences ( $p < 0.05$ ) in content of milk fat (15.80±0.25 vs. 13.64±0.22 g/100 g), milk protein (9.35±0.25 vs. 8.38±0.15 g/100 g) and lactose (1.92±0.06 vs. 2.24±0.05 g/100 g) between transgenic and non-transgenic milk composition were found. No significant differences in mineral content of milk between transgenic and non-transgenic animals were found (Table 6).

## DISCUSSION

We present here a new results about milk yield and composition of transgenic (human factor VIII gene) rabbit females over several generations. Mammary gland specific transgenic over-expression of hFVIII can be obtained without any strong influence on rabbit milk yield in different lactations, indicating that this technology can be applied to "pharmaceutical farming". Generally, rabbit milk yield shows gradual increase until 20<sup>th</sup> day of lactation, afterwards it decreases by next 10 days (Lukafahr et al., 1983; Chrastinova et al., 1997). This observation is in agreement with our data on transgenic and non-transgenic rabbit milk yield in both of the investigated lactations. The peaks of milk production were at 234 g and 258 g for transgenic and non-transgenic females respectively on first

lactation, and 304 g and 271 g for transgenic and non-transgenic females respectively on the second lactation. These results are consistent with previous reports, where 250-300 g yields for New Zealand White non-transgenic rabbits (Sanchez et al., 1985) or 225-250 g yields for New Zealand White crossbred with Californian rabbits (Partridge et al., 1986) were obtained. Our present observation is similar to our previously published report (Dragin et al., 2004), where we compared milk yield of NZW transgenic rabbit females with mammary gland specific over-expression of hPC (human protein C) and non-transgenic females. Similar result of non-transgenic rabbit milk yield on day 10, 20 and 30 was also published by Schraner, (1993). The difference obtained in milk yield between transgenic and non-transgenic rabbit females at I. and II. lactation may be explained by lower number of analyzed females and also by different number of kids per litter, however we analyzed females which had from 7 to 9 newborn pups. This agrees with the fact, that higher milk yield was obtained in non-transgenic females at first lactation on day 20, but on the second lactation higher milk yield was found in transgenic females on same day. It is important to note that rabbit milk yield in this study was assessed according to milk consumption of pups by weight-suckling-weight method and not by mechanical milk extraction from mammary gland. An advantage, especially for rabbits, is that rabbit pups are nursed only for about 3 min once every 24 h. Based on this fact a positive correlation between milk intake and pups' weight was found (Bautista et al., 2005). The above study, however, included a variety of diet, sex, geographical regions and times of year, any of which can alter production of rabbit milk (Fraga et al., 1989; Yalcin et al., 2006).

During this study, an individual body weight control of rabbit kids on day 1, 2, 5, 10, 15, 20, and 30 postpartum

was also done. It was reported, that rabbit pups with higher birth weight grew faster, and weight hierarchies became increasingly stable over the 3 weeks (Drummond et al., 2000). According to the aim of this study, where we compared body weight all pups before and immediately after suckling to assess milk yield, we found low level of variability in all age categories in both groups.

Rabbit milk composition varies depending on various factors, such as breed, nutrition, lactation stage or number of pups (Lukafahr et al., 1983; Chrastinova et al., 1997). To investigate difference in milk composition between transgenic and non-transgenic rabbit females, basic analysis of milk was performed at the same conditions. Preliminary results of analysis of transgenic rabbit milk samples have showed that all transgenic females tested in this work produced lower or higher concentration of rhFVIII ranged within 5 to 1,170 µg/ml depending on transgenic females with confirmed biological activity (Chrenek et al., 2005a, 2005b), and significant differences were found in the content of milk fat, protein and lactose. The higher variability of rhFVIII concentration may be explained by different copies of integrated gene, the site of transgene insertion or its genomic environment, what could 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 mammary gland of transgenic animals did not automatically result 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% (Jenness, 1982; 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 recent histological analyses of mammary gland tissues of lactating transgenic rabbit (carrying hFVIII transgene) showed no changes, when compared to non-transgenic mammary gland tissues (Dragin et al., 2006).

Ultrastructural measurements of cellular organelles showed no difference in cellular structure of mammary tissue, but significant differences in relative volume of mitochondria and vacuoles between transgenic and non-transgenic ultrastructure of mammary gland epithelium were observed (Dragin et al., 2006).

## CONCLUSION

Our results showed that mammary gland specific transgenic over-expression of 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-hFVIII gene construct has only minor and transient effect on milk yield, which had no significant consequence on litter growth and viability.

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## REFERENCES

- Barash, I., A. Faerman, M. Richenstein, R. Kairi, G. M. Damary, M. Shani and M. J. Bissell. 1999. *In vivo* and *in vitro* expression of human serum albumin genomic sequences in mammary epithelial cells with β-lactoglobuline and whey acid protein promoters. *Molec. Reprod. Devel.* 52:241-252.
- Bautista, A., M. Mendoza-Degante, G. Coureaud, M. Martinez-Gomez and R. Hudson. 2005. Scramble competition in newborn domestic rabbits for an unusually restricted milk supply. *Anim. Behaviour* 70:1011-1021.
- Chrenek, P., D. Vasicek, A. V. Makarevich, R. Jurcik, K. Suvegova, M. Bauer, V. Parkanyi, J. Rafay, A. Batorova and R. K. Paleyanda. 2005a. Increased transgene integration efficiency upon microinjection of DNA into both pronuclei of rabbit embryos. *Transgenic Res.* 14:417-428.
- Chrenek, P., V. Kaufmann, A. V. Makarevich, M. Bauer, L. Ryban, P. Uhrin, R.K. Paleyanda and B. R. Binder. 2005b. Production of recombinant human clotting Factor VIII in mammary gland of two transgenic rabbit lines. 49<sup>th</sup> GTH meeting in Mannheim, February, poster p. 118.
- Chrastinova, L., A. Sommer, J. Rafay and M. Svetlanska. 1997. Avotan exploitation in rabbit nutrition. II. Nutrient digestibility and lactation performance of does rabbit. *J. Farm Anim. Sci.* 30:80-86.
- Davies, M. 1983. The composition of milk. *Biochem. Lactation*, T.B. Mepham. Elsevier, pp. 71-117.
- Dragin, S., A. Bozic and P. Chrenek. 2004. Effect of transgenesis on F2 and F3 rabbit offspring generations. 5th. scientific conference of PhD. students, University of Constantine

- Philosophie, Nitra, Slovakia, pp. 28-32.
- Dragin, S., J. Pivko, P. Massanyi, N. Lukac, A. Makarevich and P. Chrenek. 2006. Ultrastructural morphometry of mammary gland in transgenic and non-transgenic rabbits. *Anat. Histol. Embryol.*, 35:in press.
- Drummond, H., E. Vazquez, S. Sanchez-Colon, M. Martinez-Gomez and R. Hudson. 2000. Competition for milk in the domestic rabbit: Survivors benefit from littermate deaths. *Ethol.* 106:511-526.
- Fraga, M. J., M. Lorente, R. M. Carabano and J. C. De Blas. 1989. Effect of diet and of remaining interval on milk production and milk composition of the doe rabbit. *Anim. Prod.* 48:459-465.
- Garber, K. 2000. RhFVIII deficit questioned. *Nat. Biotechnol.* 18:133-134.
- Jenness, J. P. 1982. Inter-species comparison of milk proteins. *Dev. Dairy Chem.* 1:87-114.
- Lukefahr, S., W. D. Hohenboken, P. R. Cheeke and N. M. Patton. 1983. Doe reproduction and preweaning litter performance of straightbred and crossbred rabbits. *J. Anim. Sci.* 57:1090-1096.
- Lukefahr, S., W. D. Hohenboken, P. R. Cheeke, N. M. Patton. 1983. Characterization of straightbred and crossbred rabbits for milk production and associative traits. *J. Anim. Sci.* 57:1100-1107.
- Monaco, M. H., D. E. Gronlund, G. T. Bleck, W. L. Hurley, M. B. Wheeler and S. M. Donovan. 2005. Mammary specific transgenic over-expression of insulin-like growth factor-I (IGF-I) increase pig milk IGF-I and IGF binding proteins, with no effect on milk composition or yield. *Transgenic Res.* 14:761-773.
- Palmer, C. A., H. Lubon and L. McManaman. 2003. Transgenic mice expressing recombinant human protein C exhibit defects in lactation and impaired mammary gland development. *Transgenic Res.* 12:283-292.
- Partridge, G. G. 1986. Meeting the protein and energy requirements of the commercial rabbit for growth and reproduction. *Proc. 4<sup>th</sup> World congress of Animal Feeding.* Madrid, Spain, pp. 271-277.
- Salvo-Garrido, H., S. Travella, L. J. Bilham, W. A. Harwood and J. W. Snape. 2004. The distribution of transgene insertion sites in barley determined by physical and genetic mapping. *Genet.* 167:1371-1379.
- Sanchez, W., P. R. Cheeke and N. M. Patton. 1985. Effect of dietary crude protein level on the reproductive performance and growth of New Zealand White rabbits. *J. Anim. Sci.* 60:1029-1039.
- Taranto, S., C. Di Meo, G. Stanco, G. Piccolo, M. P. Gazaneo and A. Nizza. 2003. Influence of age at weaning on caecal content characteristics and post-weaning performance and health of rabbits. *Asian-Aust. J. Anim. Sci.* 16(10):1540-1544.
- Schranner, S. 1993. Untersuchungen zum maschinellen Milchentzug beim Kaninchen als Grundlage zur Bestimmung von Laktationsleistungen und Milchhaltsstoffen. Inaugural-Dissertation, Ludwig-Maximilians-Universität München, Germany, pp 142.
- Van Cott, E. K., H. Lubon, F. C. Gwazdauskas, J. J. Knight, W. N. Drohan and W. H. Velander. 2001. Recombinant human protein C expression in milk of transgenic pigs and effect on endogenous milk immunoglobulin and transferrin levels. *Transgenic Res.* 10:43-51.
- Wilde, C. J., A. J. Clark, A. M. Kerr, C. M. Knight, M. McClenaghan and J. P. Simons. 1992. Mammary development and milk secretion in transgenic mice expressing the sheep beta-lactoglobulin gene. *Biochem. J.* 284:717-720.
- Yalcin, S., E. E. Onbasilar and I. Onbasilar. 2006. Effect of sex on carcass and meat characteristic of new Zealand white rabbits aged 11 weeks. *Asian-Aust. J. Anim. Sci.* 19(8):1212-1216.