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LACTATION AND GROWTH INTENSITY OF TRANSGENIC RABBITS

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ABSTRACT

Live weight growth and milk production in selected age categories of transgenic and non-transgenic rabbits were evaluated. The transgenic rabbits originated from descendants of two female founders who were applied the gene construct WAP-hFVIII into the pronucleus by microinjection. The studied animals were kept in standard breeding conditions. On day 1, 2, 5, 10, 20 and 30 post partum had the transgenic animals higher live weights without statistical significance of differences in arithmetic means for the growth intensity. Higher milk production was noticed with transgenic females on day 10, 15, 20 and 30 post partum. However, the difference was not statistically significant.

Key words: transgenic rabbits, growth, lactation.

INTRODUCTION

The development in transgenesis techniques enables to produce more and more organisms with integration of foreign genes. The method using mammary glands to express the products of exogenous genes is best one used nowadays. It is based upon easy availability of milk and on the fact that many biologically active substances in milk are in advanced stage of clinical tests.
Factor hFVIII is one of such substances, it plays an important role in blood clotting, and its non-functional form causes A haemophilia. It is possible to transfer a functional gene into the genotype of rabbit by means of transgenesis techniques and to detect the presence of clotting factor hFVIII in milk.
A potential side effect of the transgenic bioactive molecule on livability or performance traits of such animals rise a practical question in connection with the production of transgenic animals.
The study was conducted to obtain basic data on milk production and growth intensity during the first stages of ontogenesis in transgenic rabbits with human clotting factor VIII-hFVIII, their descendants and crosses with non-transgenic animals.

MATERIAL AND METHODS

Transgenic rabbits were produced by microinjection of WAP-hFVIII gene construct into male and female pronuclei of fertilized eggs from superovulated New Zealand white or California rabbits (CHRENEK *et al*., 2004). The gene construct was kindly provided by Dr.H.Lubon (American Red Cross, Maryland, USA). The transgenic experiments and observations of traits were performed in Research Institute of Animal Production at Nitra (Slovakia).

Two females with positive integration of gene analysed by PCR and Southern blot method (founder) were obtained by the method mentioned above. Following, transgenic founders were mated with non-transgenic rabbits of the same breed to obtain F1 and F2 generation. Animals with positive analysis of integration (P) were evaluated in one group and compared with animals with negative analysis (N).

After mating the founders with non-transgenic females 14 animals were obtained (6 out of them were positives). Out of the generation of founders' progeny, positive siblings were mated and progeny of F1 generation was obtained (14 individuals, out of them 7 positive). Generation F2 arose by mating the selected positive siblings (17 descendants, 17 positive ones).

Totally were used 45 descendants of two founder females to evaluate the effect of WAP-hFVIII gene integration on growth intensity. Live weight at birth, on Day 1, 2, 5, 10, 20 and 30 (w1, w2, w5, w10, w20, w30) in grams were studied. Live weight of transgenic animals (30 individuals) was compared with live weight of animals without gene integration (15 individuals). Transgenic and non-transgenic animals came from common litters.

First and second lactations were observed together in 6 transgenic females (2 founders, 4 positive daughters). The lactation in females was measured as the difference in weight of female before suckling and after suckling by young on Day 10, 15, 20 and 30 post partum (L10, L15, L20, L30) in kilograms. Milk production was compared with milk production of 12 non-transgenic females of the same age and lactation order.

The animals were bred in iron cages on grates in a breeding hall in the Research Institute for Animal Production at Nitra, too. The average temperature was 17±5^{o} C, air humidity 70±10 %. The animals were fed pelleted feed (crude protein 16.5 – 17.5 %, crude fibre 14 – 16 %, fat 2.5 – 3.0 %, ME 11.0. MJ) ad libitum.

Basic statistical characteristics (means, standard error) were processed from the data of live weight growth and lactation. The influence of integration on the studied traits was evaluated by the factor analysis of variance.

RESULTS AND DISCUSSION

Data, the basic statistical evaluation of which is in Table 1, were obtained by the settled method.

Live weights of young with positive integration were higher in all age categories, with the tendency to decrease the relative difference /W1- 9.35 g (15.0 %), W2 - 8.50 g (12.5 %), W5 – 10.12 g (9.9 %), W10 – 4.08 g (2.3 %), W20 – 34.4 g (11.3 %), W30 – 1.49 g (0.3 %)/. The differences between transgenic and non-transgenic rabbits were statistically non-significant in the rest of measured age categories. It is evident also from the table of the analysis of variance (Table 2) in which F values do not reach the level of likelihood

Table 1. Basic statistical characteristics of live weight and lactation

	N	Pos	N	Neg	d
Day		$\bar{x} \pm s_x$		$\bar{x} \pm s_x$	$\bar{x}_{Pos} - \bar{x}_{Neg}$
W1 (g)	30	65.710 ± 2.510	15	56.360 ± 4.010	9.35
W2 (g)	30	72.140 ± 2.550	15	63.640 ± 4.070	8.5
W5 (g)	30	106.790 ± 3.550	15	96.670 ± 5.910	10.2
W10 (g)	30	179.640 ± 6.720	15	175.560 ± 11.860	4.08
W20 (g)	30	321.070 ± 19.790	15	286.670 ± 34.910	34.4
W30 (g)	30	487.400 ± 27.080	15	488.890 ± 47.760	-1.49
L10 (kg)	12	0.173 ± 0,019	12	0.165 ± 0.019	0.008
L15 (kg)	12	0.178 ± 0.042	12	0.169 ± 0.042	0.009
L20 (kg)	12	0.233 ± 0.051	12	0.214 ± 0.051	0.019
L30 (kg)	12	0.136 ± 0.007	12	0.123 ± 0.007	0.013

Pos=positive integration; Neg=negative integration; N=number of animals; d=diferences Pos - Neg

0.05. Difference on the level of significance (P = 0.056) in W1 was caused by a larger error of measurement at low weights of young. Average live weights of born young on Day1 correspond with standard weights in breeds used for the experiments (New Zealand White, California rabbits). From the comparison of absolute weights in further age stages it is obvious that the young in both studied groups achieved live weights on lower level of the standard valid for New Zealand and California rabbits in age categories of 10, 20 and 30 days (VZORNÍK PLEMIEN KRÁLIKOV, 1999).
Milk production was higher in females with WAP-hFVIII gene integration on all days of lactation. The highest difference in milk production between transgenic and non-transgenic animals was on Day 20 (0.019 kg) without statistical significance. F-test values in the table of the analysis of variance confirm the non-significant influence of hFVIII gene integration on milk production.

CONCLUSION

The evaluation of growth intensity and milk production in transgenic and non-transgenic rabbits shows that at the studied age stages were observed neither statistically significant differences in arithmetic means nor significant influence of WAP-hFVIII gene integration on inter-population variability of the studied groups of rabbits. It is necessary to verify these conclusions on a larger statistical set because of low numbers of animals in the studied groups.

Table 2. Analysis of variance for influence of integration

Day		integration	residuum	P
W1	MS	690.5095	176.7638	0.0556
	F	3.9060		
W2	MS	571.4619	181.7831	0.0845
	F	3.1440		
W5	MS	697.3938	314.5918	0.1455
	F	2.2170		
W10	MS	113.7816	1266.2472	0.7693
	F	0.0900		
W20	MS	8061.8726	10967.6530	0.4062
	F	0.7351		
W30	MS	20.7636	20527.4380	0.9751
	F	0.0010		
L10	MS	0,0000416	0.0042	0.7600
	F	0.1000		
L15	MS	0.0715	0.0213	0.0800
	F	3.3580		
L20	MS	0.0850	0.0310	0.1140
	F	2.7060		
L30	MS	0.0011	0.000623	0.2044
	F	1.7110		

P= probability; MS= mean square; F= F test; 0,05 ≥P $^{+}$

REFERENCES

CHRENEK P., VASICEK D., MAKAREVICH A., JURCIK R., SUVEGOVA K., BAUER M., RAFAY J., BULLA J., HETENYI L., ERICKSON J. PALEYANDA R. K. 2004 : Integration rates of exogenous DNA into the rabbit genome using single and double pronuclei microinjection. Transgenic Research, February, abstract.

VZORNÍK PLEMIEN KRÁLIKOV (Standards of rabbit breeds) 1999, SZCH Bratislava, Slovak Republik