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SOME MEAT UTILITY AND QUALITY TRAITS OF TRANSGENIC RABBIT

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ABSTRACT

We studied the influence of the integration of human factor VIII (hFVIII) gene on complex of performance traits in rabbits of the inbred line of New Zealand White breed. Founders of the population of transgenic animals were two rabbits created by microinjection of DNA into pronuclei of zygote. Complex of growth and slaughter traits, meat quality and concentration of selected elements in muscular matter were evaluated in transgenic and non-transgenic animals of F_1 generation derived from identical litters.
Statistically significant effect ($p<0.05$) of the gene integration was noticed in following traits: live weight at birth of transgenic (0.063 ± 0.001 kg) compared to non-transgenic rabbits (0.058 ± 0.002 kg), in the weight of distal parts (0.062 ± 0.001 vs. 0.069 ± 0.001 kg), weight of the head without skin (0.119 ± 0.003 vs. 0.128 ± 0.003 kg), weight of thighs (0.405 ± 0.010 vs. 0.433 ± 0.009 kg), content of proteins in muscle (74.03 ± 0.26 vs. 74.84 ± 0.28 %), content of fat (3.66 ± 0.40 vs. 2.32 ± 0.44 %), content of energy (495.43 ± 11.81 vs. 458.07 ± 12.94 kJ) and water holding capacity (31.66 ± 0.84 vs. 35.63 ± 0.92 %). Effect of hFVIII gene integration was statistically non-significant in other studied traits.
Our results indicate no difference in the growth and slaughter traits between the transgenic and the non-transgenic rabbits. Interpretation of changes in meat quality traits requires a further study of muscle tissue on histological and biochemical levels.

Key words: transgenic rabbits; hFVIII; meat performance

INTRODUCTION

Progress of development in the sphere of genetic manipulations on the level of embryo is directly proportionate to growing number of genomic data enabling targeted and controlled production of transgenic organisms.

The most applied method of creation of transgenic farm animals is a microinjection of foreign DNA into the pronucleus of fertilized oocyte (Brem *et al*., 1985). Transgenic animals obtained in this way are able to produce recombinant human proteins (Fan and Watanabe, 2003, Chrenek *et al*., 2005). Expression of recombinant proteins is directed mostly at mammary glands that enable their purification from the milk (Lubon *et al.*, 1996). One of such proteins is the human factor hVIII (antihaemophilic globulin A), the lack of which causes A type haemophilia in man (Tuddenham *et al*., 1991).

Despite more than 50 reports on transgenic rabbit generation there are data at disposal minimum, that indicate not only quality of obtained recombinant proteins but also possible effects of transgenesis on meat, milk or reproduction performance of genetically modified animals.

The need of systematic study of biological properties in transgenic animals is a consequence of their spread and commercial utilization in various spheres of human activity. Possible interactions of integrated genes of transgenic organisms with the existent genotype are one of the intensive discussed problems. Emigration of integrated foreign gene from the experimental population into production herds can markedly affect performance

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with possible consequences in food chain. Therefore better knowledge of relations between performance indexes and expression of integrated genes gives a precondition for objective evaluation of benefits from transgenesis.

In practical conditions specialized rabbit lines are used for meat production, which maximize heterosis and complementary effects in generations of commercial hybrids within hybridisation schemes. Lines are created on the basis of multi-breed crossing and various types of selection. Results of such processes are populations of animals with fixed genes for the complex of maternal properties (maternal lines) and populations bred for intensive growth, dressing percentage and fattening capacity (paternal lines) (Mach *et al*., 2004). Harmonized criteria that enable comparison of parameters among various groups of animals are used to evaluate the level of performance.

The gene construct used in this study (WAP-hFVIII), in case of integration should ensure expression of recombinant protein in the mammary gland only. The objective of this work was to determine possible effect of the gene integration on 1) complex of growth and carcass traits, 2) qualitative meat parameters and 3) concentrations of selected constituents in rabbit meat.

MATERIAL AND METHODS

In the experiment we used New Zealand White transgenic rabbit offspring F1 generation that was obtained after breeding of transgenic founders (WAP-hFVIII gene construct) with non-transgenic rabbit of the same breed (Chrenek *et al*., 2005). Detection of the transgene integration in the offspring of F1 generation was done by PCR method. Total DNA was isolated from ear tissue (Chrenek *et al*., 2005) of newborn rabbits (F1 generation).

All studied animals were housed in wire-floor cages at the air temperature of 22 ± 3°C, humidity of 75 ± 5 %, ad libitum access to water and feed. They were fed with pellets (KKV, Anprokrmi Ltd. Slovakia) during the observation.

Tested animals were originated from identical litters and after testing on gene integration they were divided into two groups – with positive integration and without integration. The positively tested ones, without sex differentiation, were evaluated in one statistical group and compared with their non-transgenic siblings from identical litters.

In both groups of animals following parameters were observed: live weight growth on day 1, 2, 5, 10, 20 and 30 (1d, 2d, 5d, 10d, 20d, 30 d), carcass data, w – pre-slaughter weight, dw – weight after exsanguinations (dead weight), s – skin weight, sp – distal parts of hind legs weight, b – dressed carcass weight, hd – head weight without skin, fl – fore legs weight, t – thighs weight, r – ribs weight (chest), bk – back weight, ht – heart weight, ky – kidneys weight, l – lungs weight, lv – liver weight, git – stomach and guts with content weight, gite – empty stomach and guts weight, f – intramuscular fat weight, bt – bone weight in leg, mt – muscle weight in leg, bf – bone weight in fore leg, c – dressing percentage, meat quality of thigh meat (cw – water content, cp – crude protein content, cf – fat content, ve – energy value, pH, cc – colour, bw – water holding capacity) and microelements content in thigh meat (Cu – copper, Zn – zinc, Fe – iron, K – potassium, Na – sodium, Mg – magnesium, P – phosphorus, Ca – calcium). Carcass data were obtained using the method described by Rafay *et al*., (1995) taking into account the harmonizing criteria according to Pla and Dalle Zotte (2000).

Muscle of thigh (*m. biceps femoris*) was used for chemical analysis of meat sampled 1 hour after slaughter. Sample of muscle was wrapped into aluminium foil and stored at the temperature of 4°C for 24 hours. pH value was assessed by stab electrode and the Radelkis apparatus OP-109 24 hours p.m. Content of water, proteins and intramuscular fat in muscular substance was analysed by the Infratec 1265 apparatus 48 hours post-mortem. Meat colour was evaluated in the Spekol 11 apparatus as as a percentage of remission at the wavelength of 540 μm. Water holding capacity was determined by pressure method as described by Grau-Hamm in an apparatus modified by Hašek and Palanska (1976). Content of elements in thigh muscle was studied after dry mineralisation in atomic absorption spectrophotometer UNICAM 939 (Cambridge, UK). Content of phosphorus was measured spectrophotometrically by the SPECOL 11 apparatus.

Obtained values of all studied parameters were processed for basic variation-statistical characteristics (mean - $\bar{x}$, standard error – $s_{\bar{x}}$) and significances of mean differences were estimated by the t-test.

RESULTS

Basic statistical characteristics of live weight growth are in the table 1. Live weight growth in all age categories in both groups was characterized by low variability, as it is obvious from low values of standard mean error. It was proved by statistical estimation of live weight at birth in gene-integrated group, although absolute difference of arithmetical means of transgenic and control groups was only 0.005 kg (0.063 ± 0.001 vs. 0.058 ± 0.002 kg). Mean differences were not statistically significant in other age intervals. From the point of view of absolute growth reached by animals in both groups, average values in individual age categories correspond to normal growth intensity in this group of genotypes.

Table 1: Basic statistical parameters of live weight growth and their differences

day	n	Transgenic $\bar{x} \pm s_x$ (kg)	n	Control $\bar{x} \pm s_x$	mean differences and significance
1	90	0.063 ± 0.001	45	0.058 ± 0.002	0.005*
2	90	0.071 ± 0.001	45	0.068 ± 0.002	0.003
5	90	0.107 ± 0.003	42	0.108 ± 0.004	0.001
10	88	0.191 ± 0.004	42	0.187 ± 0.006	0.004
20	87	0.352 ± 0.007	42	0.347 ± 0.010	0.005
30	87	0.577 ± 0.009	42	0.584 ± 0.013	-0.007

(n – number of animals, significant difference at $p \geq 0.05$ *)

Table 2: Basic statistical characteristics of carcass traits and their differences

trait (kg)	n	Transgenic $\bar{x} \pm s_x$	n	Control $\bar{x} \pm s_x$	mean differences and significance
w	12	2.498 ± 0.044	15	2.576 ± 0.039	- 0.078
dw	12	2.422 ± 0.044	15	2.497 ± 0.039	- 0.075
s	12	0.385 ± 0.010	15	0.390 ± 0.009	- 0.005
sp	12	0.062 ± 0.001	15	0.069 ± 0.001	- 0.007*
b	12	1.355 ± 0.056	15	1.405 ± 0.050	- 0.050
hd	12	0.119 ± 0.003	15	0.128 ± 0.003	- 0.009*
fl	12	0.199 ± 0.004	15	0.207 ± 0.004	- 0.008
r	12	0.298 ± 0.008	15	0.301 ± 0.007	- 0.003
bk	12	0.266 ± 0.008	15	0.286 ± 0.007	- 0.020
t	12	0.405 ± 0.010	15	0.433 ± 0.009	- 0.028*
ht	12	0.015 ± 0.005	15	0.017 ± 0.005	- 0.002
ky	12	0.018 ± 9.275	15	0.019 ± 8.296	- 0.001
l	12	0.020 ± 0.002	15	0.022 ± 0.001	-0.002
lv	12	0.079 ± 0.004	15	0.079 ± 0.003	0.000
git	12	0.493 ± 0.017	15	0.520 ± 0.015	- 0.027
gite	12	0.178 ± 0.006	15	0.189 ± 0.005	- 0.011
f	12	0.023 ± 0.014	15	0.020 ± 0.012	- 0.003
bt	12	0.065 ± 0.006	15	0.057 ± 0.005	0.008
mt	12	0.264 ± 0.024	15	0.284 ± 0.022	- 0.020
bf	12	0.051 ± 0.010	15	0.054 ± 0.009	- 0.003
mf	12	0.134 ± 0.010	15	0.141 ± 0.009	- 0.007
c (%)	12	60.448 ± 1.882	15	60.637 ± 1.683	- 0.011

(significant difference at $p \geq 0.05$ *)

Data on carcass traits were characterized by low variability, as it is expressed by standard mean error values (table 2.). Statistically significant influence of the integrated gene was noticed in parameters of weight of distal parts (Sp) (0.062 ± 0.001 vs. 0.069 ± 0.001 kg), head weight (Hd) (0.119 ± 0.003 vs. 0.128 ± 0.003 kg) and weight of thighs (T) (0.405 ± 0.010 vs. 0.433 ± 0.009 kg) when compared to non-transgenic rabbits. In other characteristics of slaughter efficiency no statistically significant influence of the integrated gene was found.

Table 3: Basic statistical characteristics of chemical composition of meat (g.100^{-1}g) and their differences

Content of	n	Transgenic $\bar{x} \pm s_x$	n	Control $\bar{x} \pm s_x$	mean differences and significance
cw (%)	12	74.03 ± 0.26	10	74.84 ± 0.28	0.81*
cp (%)	12	21.45 ± 0.26	10	22.12 ± 0.29	- 0.67
cf (%)	12	3.67 ± 0.40	10	2.32 ± 0.44	0.35*
ce (kJ/100g)	12	495.43 ± 11.81	10	458.07 ± 12.94	37.36
pH	12	5.79 ± 0.10	10	5.48 ± 0.11	0.31
cc (%)	10	20.94 ± 1.58	10	25.44 ± 1.58	- 4.50
bw (%)	12	31.66 ± 0.84	10	35.63 ± 0.92	- 3.97*

(significant difference at $p \geq 0.05$ *)

Table 4: Basic statistical characteristics of element concentrations in meat and their differences

Element(mg.kg^{-1})	n	Transgenic $\bar{x} \pm s_x$	n	Control $\bar{x} \pm s_x$	mean differences
Cu	12	1.15 ± 0.21	10	0.86 ± 0.23	0.29
Zn	12	17.23 ± 4.43	10	18.23 ± 4.85	- 1.00
Fe	12	17.14 ± 6.02	10	20.99 ± 6.60	- 3.85
K	12	5.17 ± 0.32	10	5.44 ± 0.35	- 0.27
Na	12	0.80 ± 0.08	10	0.77 ± 0.09	0.03
Mg	12	0.40 ± 0.06	10	0.44 ± 0.07	- 0.04
P	12	2.30 ± 0.37	10	2.93 ± 0.40	- 0.73
Ca	12	0.30 ± 0.05	10	0.23 ± 0.06	0.07

(there are no significant differences)

Values of qualitative parameters of meat in leg are shown in table 3. The influence of the gene integration was statistically significant ($p<0.05$) in parameters of water content (cw) (74.03 ± 0.26 vs. 74.84 ± 0.28 %), fat content (cf) (3.67 ± 0.40 vs. 2.32 ± 0.44 %), energy value (495.43 ± 11.81 vs. 458.07 ± 12.94 KJ/100g) and water holding capacity (bw) (31.66 ± 0.84 vs. 35.63 ± 0.92 %). Statistically significant influences were not found in the other studied traits.

The highest variability in all studied parameters of muscular substance content was revealed in the group of non-transgenic animals (tab. 4). Mean differences in this trait were not statistically significant.

DISCUSSION

Values of studied characteristics of growth, carcass characteristics and meat quality mentioned in this work are comparable with values in zootechnical literature (Ludewig et al., 2003). Most of studied traits were in general not influenced by the presence of WAP-hFVIII gene in the genotype of rabbits. Several important differences were noticed only in meat quality characteristics.

In previous study (Rafay *et al*., 1995) the content of water in rabbit meat (*m. long. dorsii)* was in value of 74.37 ± 0.19 % which corresponded to the data obtained in muscle of leg in both groups in this study. Similarly, Szendrö *et al*. (1996) measured water content (73.8 ± 0.44%) in samples of muscle from hind legs of rabbits weighing 2.500 – 2.590 kg. Statistically significant difference in water content in muscles of legs between transgenic and non-transgenic animals (0.81 %), found in our work, is probably an artefact related to sample handling before analysis. Muscular fat consists of phospholipids of muscular contractile fibres, fibroblasts and membranes of adipocytes, glycerides located on adipocytes around fibres and free fatty acids. It stipulates for nutritive value and organoleptic properties of meat. Szendrö *et al*., (1996) reported an average value of fat in muscular substance of leg at 3.28 ± 0.56 %. We have found in transgenic animals a fat in muscular substance of leg at

average value of 3.67 ± 0.40 %. According to Lambertini *et al*. (1996) and Hernandez *et al*. (1998) differences in meat quality parameters among genotypes of rabbits are constant. In general, qualitative parameters of meat are improved with the increase in growth intensity. Content of fat correlates with the water content in muscle fibres (Battaglini et al., 1994).

It was found in some cases that in older rabbits glycolytical metabolism is increased whilst concentration of myoglobin and pH value are decreased (Hulot and Ouhayoun, 1999). Changes observed in content of water, fat, energy and water holding capacity are connected with changes in histological structure and level of metabolic processes. It is possible that these changes are caused by pleiotropic effect of the integrated gene. However, to clarify these changes a further study of muscle tissue of transgenic rabbits on histological and biochemical levels is required.

CONCLUSION

Our results indicate that the studied parameters of growth and slaughter traits in the transgenic rabbits were not changed compared to non-transgenic rabbits. Interpretation of changes in meat quality traits necessitates further study of muscle tissue on histological and biochemical levels.

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REFERENCES

BATTAGLINI, M. – CASTELLINI, C. – LATTAIOLI, P. 1994. Rabbit carcass and meat quality: effect of strain, rabittry and age. In: *Ital. J. Food Sci*., 1994, vol. 2, p. 157-166.

BREM, G. – BRENIG, B. – GOODMAN, H. M. – SELDEN, R. C. – GRAF, F. – KRUFF, B. – SPRINGMANN, K. – HONDELE, J. – MEYER, J. – WINNACHER, E. L. - KRAUSSLICH, H. 1985. Production of transgenic mice, rabbits and pigs by microinjection into pronuclei. In: *Zuchthygiene*, 1985, vol. 20, p. 251-252.

CHRENEK, P. – VASICEK, D. – MAKAREVICH, A.V. – JURCIK, R. – SUVEGOVA, K. – BAUER, M. – PARKANYI, V. – RAFAY, J. – BATOROVA, A. – PALEYANDA, R.K. 2005. Increased transgene integration efficiency upon microinjection of DNA into both pronuclei of rabbit embryos. In: *Transgenic Res.*, 2005, vol. 14, p. 417-428.

FAN, J. – WATANABE, T. 2003. Transgenic rabbits as therapeutic protein bioreactors and human disease models. In: *Pharmacol. Ther*., 2003, vol. 99, p. 261-82.

HASEK, A. – PALANSKA, O. 1976. Determination of water holding in the meat by apparatus with constant pressure. In: *Poultry Industry*, 1976, vol. 18, p. 228-233

HERNANDEZ, P. – PLA, M. – BLASCO, A. 1998. Carcass characteristics and meat quality of rabbit lines selected for different objectives: II. Relationships between meat characteristics. In: *Livestock Prod. Sci*., 1998, vol. 54, p. 115-123.

HULOT, F. – OUHAYOUN, J. 1999. Muscular pH and related traits in rabbits: a review. In: *World Rabbit Sci*., 1999, vol. 7, p. 15-36.

LAMBERTINI, L. – BERGOGLIO, G. – MASOERO, G. – GRAMENZI, A. 1996. Comparison between Provisal and Hyla rabbit strains. I. Slaughtering performances and muscle composition. In: *Proc. 6th WRSA Congres, Toulouse*, 1996, vol. 3, p. 195-199.

LUBON, H. – PALEYANDA, R. K. – VELANDER, W. H. – DROHAN, W. N. 1996. Blood proteins from Transgenic Animal Biorectors. In: *Transfusion Medicine Reviews*, 1996, vol. 10, p. 131-143.

LUDEWIG, M. – TREEL VAN, N. – FEHLHABER, K. 2003. Schlachtausbeute und Fleischqualität von Mastkaninchen in Abhängigkeit vom Alter. In: *Fleischwirtschaft,* 2003, vol. 3, p. 101-103.

MACH, K. – MAJZLIK, I. – DEDKOVA, L. – HERMANOVA, B. 2004. Growth and feed consumption of HYPlus broiler rabbits, final hybrids F_1, F_{11} and $F_{2(3)}$ - generation in operational conditions (in Czech republic). In: *Proc. XXII Conf. RIAP Nitra*, Nitra : VUŽV, 2004, p. 5-11.

PLA, M. – DALLE ZOTTE, A. 2000. Harmonisation of criteria and methods used in rabbit meat research. In: *World Rabit Sci*., 2000, vol. 8, p. 539-545.

RAFAY, J. – MOJTO, J. – PALANSKÁ, O. 1999. Characteristics of meat quality of domestic rabbit. In: *Agriculture*, 1999, vol. 45, p. 388-396.

SZENDRÖ, ZS. - RADNAI, I. - BIRÓ-NÉMETH, E. – ROMVÁRI, R. – MILISITS, G. 1996. Changes in water, protein, fat and ash content in the meat of rabbits between 2.2 – 3.5 kg live weight. In: *Proc. 6th WRSA Congres, Toulouse*, Tolouse : INRA Tolouse Research Centre, 1996, vol. 3, p. 269-272.

TUDDENHAM, E. G. D. – COOPER, D. N. – GITSCHIER, J. – HIGUCHI, M. – HOYER, L. W. – YOSHIOKA, A. 1991. Haemophilia A: database of nucleotide substitutions, deletions and rearrangements of the factor VIII gene. In: *Nucl. Acids Res*., 1991, vol.19, p. 4821-4833.

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