Slovak J. Anim. Sci., 43, 2010 (4): 210-214

ISSN 1337-9984

Short communication

DIGESTIBILITY OF NUTRIENTS BY TRANSGENIC AND NON-TRANSGENIC RABBITS

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ABSTRACT

The aim of this work was to compare digestibility of nutrients between transgenic and non-transgenic groups of rabbits from different lines. The experiment was carried out on a population of transgenic and non-transgenic rabbits. The population of transgenic animals (n=5) was presented by the New Zealand White rabbit F1 offspring that were born after breeding of transgenic founders (WAP-hFVIII gene construct) with non-transgenic rabbits of the same breed. Non-transgenic animals were represented by four different populations of meat rabbits. The faeces were collected individually during 4 consecutive days (every 2 hours) according to the European reference method for rabbit digestion trials. Chemical analysis of the diets and faeces for dry matter, crude protein, crude fibre, crude fat, nitrogen free extract, ash, organic matter, ADF and NDF was carried out according to the same method. The population of transgenic rabbits in our experiment showed the lowest coefficients of digestibility of crude fibre, ash, ADF and a higher digestibility of crude protein, crude fat and organic matter, but the differences were not statistical significant comparing with non-transgenic rabbits. This study showed that the integration of the WAP-hFVIII gene into rabbit genome does not affect significantly the digestibility of nutrients in commercial rabbit diet.

Key words: rabbit; transgenic; nutrient; digestibility

INTRODUCTION

Gene manipulations on the level of an embryo are directly proportionate to growing number of genomic data enabling targeted and controlled production of transgenic organisms (Chrenek *et al.*, 2006). Transgenic animals are able to produce human recombinant proteins (Fan and Watanabe, 2003; Chrenek *et al.*, 2005, 2007; Chrenek, Makarevich, 2008). Expression of recombinant molecules of proteins is directed mostly at the mammary gland that enables their purification from milk (Lubon *et al.*, 1996). One of such proteins is a human factor VIII (antihaemophilic globuline A), the lack of which causes haemophilia of A type in man (Tuddenham *et al.*, 1991).

Despite more than 100 reports on transgenic rabbit generation there are at disposal minimum data that indicate not only quality of obtained recombinant proteins but also possible effects of transgenesis on the digestibility of nutrients by 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 the human activity. Possible interactions of integrated

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Received: August 17, 2010
Accepted: October 8, 2010

genes of transgenic organisms with the existing genotype are one of the intensive discussed problems. Emigration of integrated foreign gene from the experimental population into production herds can markedly affect performance with possible consequences in food chain. Therefore, a better knowledge of relations between performance indexes and expression of integrated genes gives precondition of objective evaluation of application benefits of transgenesis.

Exact definition of relations between the different rabbit genotypes and digestibility of nutrients is very important. Definition of these relations will contribute to a better knowledge of processes inspection of degradation and utilization of nutrients in rabbit digestive tract. Especially estimation of the coefficients of digestibility of basic nutritive in transgenic rabbits will serve to better knowledge of eventual influence of incorporated foreign gene on the ability of feed utilization. On the other hand, the cost of feeding presents more than 50% of total production costs, therefore, the optimal feed utilization is one of the basic premises of the proficient rabbit production (Rafay, 1993).

The process of rabbit digestion of basic nutrients – proteins and fats is very similar as in other non-ruminants. Digestion of crude fibre is microbiological and digestion of sugars and starch is enzymatic (Lebas *et al*., 1997).

Correct examination of feed nutritional value is very important. The nutritional value is determined by chemical analysis of feed, content of water and basic organic nutrients (crude fibre, crude fat, and crude protein) and ash. Digestibility is the utilization ratio of feed components and it is determined by a coefficient of Integrated gene construct (WAP-hFVIII) should cause expression of recombinant protein in the mammary gland only. The aim of the present work was to collect more information about the influence of different genotype and foreign transgene integration on digestibility of nutrients.

MATERIAL AND METHODS

Animals

In our experiment, five rabbits (males) at the age of 70 days and 2200g±100g of live body weight from each of genotypes were used.

Transgenic rabbits

Transgenic New Zealand White (NZW) rabbit F1 offspring carrying mWAP-hFVIII gene construct was used (Chrenek *et al*., 2005). Detection of the transgene integration in F1 generation was done by PCR method, where a total genomic DNA was isolated from an ear tissue of newborn rabbits (Chrenek *et al*., 2005).

Non-transgenic rabbits

Four different genotypes of non-transgenic meat rabbits: line P91, line M91, NZW and hybrid Hycole were used. The animals of NZW breed were half sibling to transgenic rabbits. Another three lines were commercial hybrids.

Housing and nutrition

The rabbits were housed individually in metabolic wire cages that allowed the faeces separation. A cycle of 16 h of light and 8 h of dark was used throughout the

Table 1: Ingredients and chemical composition of commercial diet

Ingredients	%	Chemical composition	%
Lucerne meal	29	Dry matter	90.27
Wheat bran	16	Crude protein	16.41
Sunflower meal	14	Crude fat	2.86
Oat	13	Crude fibre	17.94
Dried sugar beet pulp	14	Nitrogen free extract	46.00
Soybean	6	Ash	7.05
Mix minerals and vitamins	1	Organic matter	83.27
MDCP	0.6	ADF	20.47
$CaCO_3$	0.9	NDF	35.39
NaCl	0.3	-	-
Wheat flour	0.1	-	-
Methionine, lysine, soya-bean oil	0.1	-	-
Malt sprout	5	-	-

MDCP – mono-dicalcium phosphate, ADF – acid detergent fibre, NDF – neutral detergent fibre experiment. Temperature and humidity in the building were recorded continuously by a digital thermograph positioned at the same level as the cages. Heating and forced ventilation systems allowed the building temperature to be maintained within 18±4˚C throughout the experiment. Relative humidity was about 70±5%. The rabbits were fed with a commercial diet (pellets of 3 mm in diameter, Lebas, 1980). The ingredients and chemical composition of this diet is presented in Table 1. All animals were given access to the feed *ad libitum*. The adaptation period for this diet was 14 days. Drinking water was provided with nipple drinkers *ad libitum*.

Digestibility study

The faeces were collected individually during 4 consecutive days according to the European reference method for rabbit digestion trials (Perez *et al*., 1995). Taking of faeces samples was realized every 2 hours. Faeces were frozen daily and subsequently dried and ground for analysis. Chemical analyses were conducted according to AOAC (1995) with the considerations mentioned by EGRAN (2001) for dry mater (DM), crude protein (CP), crude fibre (CF), crude fat, nitrogen free extract, ash and organic matter. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed sequentially (Van Soest *et al*., 1991) with a thermo stable amylase pre-treatment.

Coefficients of digestibility of nutrients were determined according to the formula:

$$\frac{[(\text{quantity of feed} \times \text{nutrients of feed}) - (\text{quantity of faces} \times \text{nutrients of faces})] \times 100}{(\text{quantity of feed} \times \text{nutrients of feed})}$$

Statistical analysis

The statistical analysis was performed for all monitored traits. A linear model and a one-way analysis of variance were used to analyze the data. Least square mean estimates with standard errors of the estimates were produced. Differences among least square means were estimated and tested using Scheffe's multiple range tests. The statistical package SAS 9.1 (SAS, 2003) was used for the analysis.

Table 2: Digestibility of nutrients by transgenic and non-transgenic rabbits

Nutrients	Genotype				
	Hycole n=5	**M91 n=5**	**P91 n=5**	**NZW n=5**	**Transgenic rabbits n=5**
	$\bar{x}$ ± sd	$\bar{x}$ ± sd	$\bar{x}$ ± sd	$\bar{x}$ ± sd	$\bar{x}$ ± sd
Dry mater (%)	68.27±0.98	68.65±0.47	68.23±1.32	71.47±3.96	68.54±4.24
Crude protein (%)	78.48±1.29	80.23±1.26	77.40±2.31	80.03±4.80	79.87±4.91
Crude fat (%)	84.38±0.95	85.03±1.31	85.23±1.96	89.45±4.77	87.05±5.03
Crude fibre (%)	38.95±1.58	37.71±1.30	38.92±1.89	36.41±10.13	33.82±12.00
Nitrogen free extract (%)	83.99±11.06	75.92±0.38	76.06±0.97	81.54±3.98	78.35±4.47
Ash (%)	64.85±1.79	66.31±1.77	63.50±2.27	61.05±6.50	60.13±6.61
Organic matter (%)	68.56±0.98	68.85±0.50	68.64±1.25	72.37±3.79	70.98±5.89
ADF (%)	37.46±3.79	37.99±1.84	39.96±2.24	34.65±7.42	30.38±11.78
NDF (%)	47.10±2.84	47.78±1.20	49.36±1.91	50.61± 5.90	47.48±10.77

$\bar{x}$ – average, sd – standard deviation, ADF – acid detergent fibre, NDF – neutral detergent fibre

RESULTS

The digestibility of nutrients by transgenic and non-transgenic rabbits is presented in Table 2. The population of transgenic rabbits in our experiment showed lowest, but not significant differences in coefficients of digestibility for crude fibre, ash and ADF compared to other evaluated breeds of rabbits. Coefficients of digestibility of crude fibre and its fractions were lowest in a population of NZW rabbits and NZW-derived populations (line M91 and transgenic rabbits). For other nutrients (crude protein, crude fat and organic matter) we recorded higher coefficients of digestibility in population of transgenic and non-transgenic NZW rabbits.

DISCUSSION

The determination of digestibility of nutrients in feedstuffs is very important in assessing their nutritional value. Digestibility refers to the fraction of the feed or individual components that is digested and absorbed. The chemical composition of feeds gives an indication of the potential nutrient supply, but determination of digestibility provides an estimate of the nutrients available to the animal. The digestibility of nutrients in different rabbit genotypes was studied by several authors. Kustos and Hullár (1992) followed the heritability of digestibility in NZW rabbits. In their experiment the authors determined low ($h^2 = 0.19$) heritability values for the coefficients of digestibility. Halga (1977) determined the influence of the genotype on digestibility. This author found out higher coefficients of digestibility in the case of Chinchilla and the Termonde white breed. Lebas (1973) in the NZW breed determined 4% better coefficients of digestibility for dry matter and organic matter than in the Californian rabbits, these coefficients of digestibility correspond to our results.

The contribution of crude fibre is optimized up to the level of 14-16% in rabbit mixture. Crude fibre is digested by a microbial fermentation and main place of this fermentation is caecum (Volek, 2005). Rafay (1993) specified these values of digestibility of basic nutrients: crude protein - 75%, crude fat - 65% and crude fibre - 20-35%. Coefficients of digestibility of crude protein and crude fibre in our experiment were higher than those published by Tůmová et al. (2004) and Skřivanová et al. (2004). These authors carried out a balance experiment on meat rabbits and their values of digestibility of present nutrients were 77.2% vs. 72.6% and 10.7% vs. 15.7%. Our values of digestibility of nutrients for crude protein were in the range of 78.48- 80.23%, which was similar to the data of Battaglini and Grandi (1988). The values of crude fibre digestibility (33.82-38.95%) and crude fat (84.38-89.45%) were higher when compared to Bielaňski and Niedžwiadek (1993). Pascual et al. (2008) recorded different coefficients of digestibility for dry matter, organic matter and gross energy ($P < 0.05$) between two different groups of rabbit does selected for a litter size and longevity. Al-Dobain (2010) followed the effect of the diet on digestibility of four rabbit breeds. The author observed that all digestibility coefficients were significantly ($P < 0.01$) affected by the interaction dietary treatments × genetic groups.

CONCLUSION

The results of this study show that the digestibility of nutrients in the followed diet was not significantly influences by different breeds of meat rabbits. Similar results were obtained with population of transgenic rabbits. The integration of WAP-hFVIII into rabbit genotype does not affect the digestibility of nutrients; however the comparison of non-transgenic and transgenic population of meat rabbits brought original results in this field of research.

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