

## IN VIVO MEASUREMENT OF BODY PARTS AND FAT DEPOSITION IN RABBITS BY MRI

KÖVÉR Gy.\*, SZENDRÓ Zs.\*, ROMVÁRI R.\*, JENSEN J.F.\*\*\*, SØRENSEN P.\*\* , MILISITS G.\*

\*Pannon Agricultural University, Faculty of Animal Science, 7400 KAPOSVÁR, P.O. Box 16. - Hungary

\*\*Danish Institute of Animal Science, 8830 TJELE P.O. Box 39. - Denmark

\*\*\*Institute of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 FREDERICKSBERG C, - Denmark

**ABSTRACT :** An experiment using Magnetic Resonance Imaging (MRI) tomography were done involving 87 rabbits of four genotypes, 12 or 16 weeks old. MRI was applied on the day before slaughter. The slices were taken in three orthogonal planes. The resulting pictures indicate that MRI provides very detailed slices. The volume of the fat deposit around the kidneys, the total body fat volume of the body and the muscle of the hind part was collected from the MRI pictures. Correlation coefficients were computed between the volume of the

perirenal fat and its weight, the muscle volume of the hind part and its weight, the total body fat volume and the crude fat content. The correlation values were found to be very high (0.77 to 0.94) in the group of age 16 proving that the MRI tomograph is an excellent *in vivo* method to determine the volumes of fat and muscle. The lower correlation values in the group of age 12 (0.39 to 0.76) indicate that the MRI tomography is sensitive to the digital sampling errors.

**RESUME :** Mesure *in vivo* de différentes parties du corps et des dépôt graisseux des lapins par imagerie en résonance magnétique.

Un total de 87 lapins appartenant à 4 génotypes âgés de 12 à 16 semaines ont été étudiés par imagerie en résonance magnétique (IRM). Les lapins ont été analysés par IRM la veille de l'abattage. Les "coupes" ont été réalisées dans 3 plans orthogonaux. Les images obtenues pour chaque coupe sont très détaillées. Le volume de la masse adipeuse périrénale, de l'ensemble des masses adipeuses du corps et celui des masses musculaires de l'arrière de l'animal ont été

déterminées par analyse de coupes. Les coefficients de corrélation entre ces volumes déterminés par IRM et leur poids mesuré par dissection après abattage sont de 0,77 à 0,94 chez les lapins de 16 semaines. Ceci atteste que l'IRM est une bonne méthode pour la mesure *in vivo* des volumes de tissu adipeux ou musculaire. Les valeurs beaucoup plus faibles des corrélations obtenues chez les lapins de 12 semaines, (0,39 à 0,76) montrent la limite de l'IRM pour ce type d'étude (faible masse des tissus adipeux) en raison de sa sensibilité aux erreurs d'analyse des images informatiques numérisées.

### INTRODUCTION

As in other fields of animal research, determining the whole body composition using *in vivo* methods has great importance also for the rabbit breeders. There are more and more strict animal protection regulations, limitations in experimenting, not to mention the losses caused by slaughtering valuable animals.

The earlier studies (LÖHLE and HAUBOLD, 1980) using the outside measurements of the animals, resulted in very low values of correlation. Ultrasonic device was used to diagnose the pregnancy in the rabbit by RINCK *et al.* (1993). With the help of the Near Infrared Reflectance Spectroscopy the changes in the muscle composition caused by stress was demonstrated (MASOERO *et al.*, 1992a). Some papers direct attention to the TOBEC method (FEKETE and BROWN, 1992; MILISITS *et al.*, 1997).

It has been pointed out in SZENDRÓ *et al.* (1992) earlier studies, that the X Ray Computer Tomograph (CT) system is a suitable method to estimate the amount of the muscle *longissimus dorsi* ( $r=0.8$ ), the muscle content of the intermediate part of the carcass, but not acceptable to estimate the muscle content of the hind part and of the hind legs. The methods of using the CT system is summarised by ROMVÁRI (1996).

The fat content of the body seems to be important in two aspects. The high fat content is objectionable to the customers and increases the feeding cost of the production. In the case of the rabbit does, the appropriate fat deposit supply ensures the long term productivity.

Thus it is interesting to study not only the overall development of fat deposits during growth, but also the relative development of the adipose deposits with different locations (VEZINET and PRUD'HON, 1975). The total fat content of the body and the carcass fatness can be estimated by the volume of the kidney fat (OUHAYOUN, 1978; BRUN and OUHAYOUN, 1988; ROMVÁRI, 1996). The signals of body mobilisation are indicated in fat depots (MASOERO *et al.*, 1992b). The perirenal fat content decreased during the first pregnancy of does (XICCATO, 1996; MILISITS *et al.*, 1996, 1997). The weight of the fat in loin and hind part were correlated with the weight of the fat in the carcass ( $r=0.69$  and  $0.63$  respectively; NIEDZWIADK, 1980). A significant positive relationship ( $r=0.59$ ) was determined between fat deposit content and intramuscular fat content by MAERTENS and DE GROOTE (1992).

*In vivo* measurement of the perirenal fat gives a good opportunity to follow the change of fat in rabbits

during the fattening period, pregnancy and lactation. The first results of using Magnetic Resonance Imaging (MRI) determining *in vivo* the amount of the adipose deposits were published by KÖVER *et al.* (1996). With the help of CT scanning prediction equations were constructed to estimate body fat content by ROMVÁRI (1996). The R2 values varied between 0.71 and 0.93.

In the present study the MRI tomography was used to estimate the muscle volume of the hind part, the volume of the perirenal fat and the total fat content of the empty body. The effect of the genotype, sex and age of rabbits on the data produced by the MRI tomograph was also examined.

## MATERIALS AND METHODS

Magnetic Resonance Imaging tomography is the most important method for analysing the body structure and composition of living humans and animals. This procedure of measurement is non-invasive and highly sensitive in visualising the body structure in subtle details (STARK and BRADLEY 1992). MRI is based on the  $T_1$  and  $T_2$  relaxation process of the of the excited proton ( $H^1$ ) in a strong magnetic field. A radio pulse of a specific frequency is used to induce transitions between energy states by causing certain hydrogen atoms to absorb and transfer energy. Various measures of the time required for the material to return to a baseline energy state. The protons in different bounding have different relaxation process and relaxation times ( $T_1$ ,  $T_2$ ). The relaxation times are translated by a computer program to a visual image on the computer screen. The parameters of the MRI image are set by a selection of a pulse sequence. The applied sequence in the study is a  $T_1$  weighted sequence (spin echo sequence). On  $T_1$  weighted images substances causing high signal (i.e. bright white pixels) include fat. Other soft tissues have intermediate level of signal (i.e. pixels with grey shading).

The MRI tomography represents a big chance in animal body composition analysis, similar to the revolution caused by the MRI in human radiology.

In this present experiment 87 rabbits of 4 different genotypes :

DD = Danish White pure-bred,  
DP = Danish White x Pannon White crossbreed,  
PD = Pannon White x Danish White crossbreed,  
PP = Pannon White pure-bred

were scanned *in vivo* in a Siemens Magnetom SP 63 1.5 Tesla whole body MRI scanner (Figure 1.). The distribution of the rabbits according to genetic groups, sex and age (12 or 16 weeks) can be found in table 1. The average live weight of the animals was 3198 g and ranged from 2280g to 4010g. The breeding conditions and the zootechnical performance of the rabbits used in

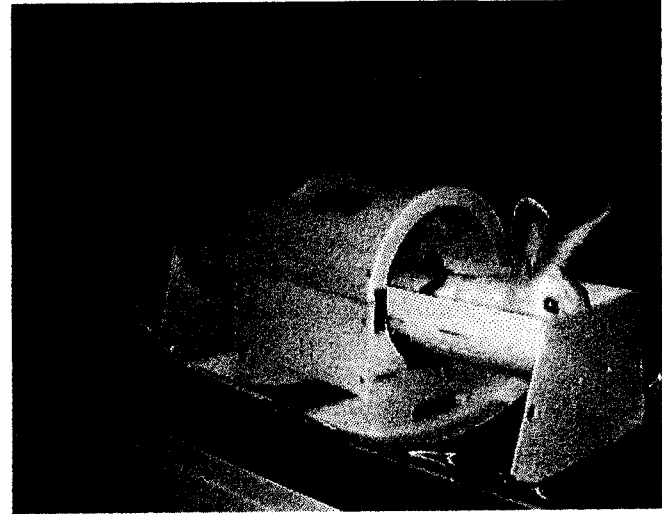


Figure 1 : The rabbit in the knee coil before anaesthetisation.



Figure 2 : Localising MRI image in the axialplane using gradient echo sequence

this experiment were described by SZENDRŐ *et al* (1996).

Before putting the rabbits in the MRI tomograph they were starved for ten hours and anaesthetised using Rompun (containing Xylasin, 0.1 ml/kg of body weight) in order to reduce the motion artefacts on the MRI images. The rabbits were fixed in a special cradle lying on the belly, the legs were tied and straightened forward and backward (Figure 1.).

After using a gradient echo sequence in the axial plane for localisation (Figure 2.) spin echo sequence was used to take images in the coronal plane (Figure 3.). All the images were taken with a knee coil using  $T_1$  weighted spin echo sequence. The slices were 6, 8, 10 mm thick in the coronal, sagittal and transversal plane respectively, covering the whole body. The other parameters of the applied sequence were:



**Figure 3 : The area of the kidney fat and the hind part are measured on the spin echo images in the coronal plane.**

- number of excitations : 3
- time of repetition : 400-800 ms, depending on the number of slices
- field of view : 400 mm
- time of echo : 15 ms

The total time of acquisition depended on the number of slices necessary to cover the body. In one plane the total series could take about 5 - 6 minutes. As shown in Figure 3, T<sub>1</sub> weighted spin echo sequence results in a very good picture quality with high details of the soft tissues.

The MRI picture consists of 256x256 pixels, each with a numeric value. High pixel values are represented by white on the MRI pictures and mean fat tissues. Pixels with lower values are represented with grey scale and mean other soft tissues, e.g. muscle.

With the help of the MRPC (computer program, Pannon University, Diagnostic Centre, Kaposvár, by

KÖVÉR and BERÉNYI, 1995) on a graphic work station the area of the fat tissues from a whole picture, or from a part of a picture can be taken.

The MRI picture is very rich in details on muscle and fat. That is why it is possible to draw around the fat deposit of the kidney or the muscles of the hind part. In order to measure the area of the perirenal fat and the muscles of the hind part, the coronal plane was selected. The volume of the perirenal fat and the muscles of the hind part can be approximated by summing these fat and muscle areas separately and multiplying the sum by the slice thickness (6 mm). The two parts of the perirenal fat deposit are clearly seen on some pictures of the coronal series. Due to the motion artefacts there are some incomplete cases. The total volume of the body fat was also collected from the scans in the coronal plane.

After slaughter, a few more rabbit traits were added to the MRI dataset. In the slaughterhouse the carcasses were cut into three parts using the method described by BLASCO *et al.* (1993). The perirenal fat and the hind part of the carcass were weighed. The slaughter was followed by a chemical analysis of the whole empty body where the crude fat content was determined with Soxhlet extraction after hydrochloride acid digestion. Using the crude fat percentage and the empty body weight (weight of rabbits after 24 hours of starving excluding the weight of the gut) the total weight of the body fat can be calculated.

Mean and standard deviation were calculated in all rabbit subgroups. Analysis of variance was used to test the significant effect of the sex, genotype and age on all the measured or calculated variables. Pearson's correlation coefficient was calculated in the two age groups of rabbits between the volumes estimated by the MRI tomography and the weight of the perirenal fat, the muscle of the hind part, and the computed total body fat weight based on the chemical analysis.

## RESULTS AND DISCUSSION

During the process of evaluating the MRI images, the scans from the coronal plane proved to be the best one of the three orthogonal planes. The perirenal fat can be seen very clearly on some pictures (Figure 3). The hind part is totally covered with a few scans opposite to the large number of the axial scans. The means and standard deviations of the dataset can be found in Table 1.

The analysis of variance shows that the genotype and sex have no significant effect on any of the variables. The age has significant effect on every measured and computed rabbit traits. Nor two-way neither three-way interactions are significant among the groups. The correlation between the data provided by the MRI and the data from slaughter and from chemical

**Table 1 : Measured traits, means and standard deviations grouped by genotype, age and sex.**

		N	Perirenal fat [g]	Perirenal fat (MRI) [cm <sup>3</sup> ]	Hind part [g]	Hind part (MRI) [cm <sup>3</sup> ]	Total fat [g]	Total fat (MRI) [cm <sup>3</sup> ]
DD	mean	15	38	58	531	451	286	380
	SD		20	35	81	65	109	212
DP	mean	29	36	76	557	548	317	401
	SD		22	49	89	97	141	225
PD	mean	11	32	61	589	560	290	385
	SD		22	39	98	78	125	190
PP	mean	32	34	60	558	528	261	320
	SD		19	44	94	90	111	162
12 weeks	mean	31	21	34	455	432	188	235
	SD		7	11	34	36	42	88
16 weeks	mean	56	42	83	614	595	343	438
	SD		21	45	55	51	118	203
M	mean	52	35	68	555	525	289	382
	SD		21	47	89	91	119	212
F	mean	35	34	59	561	533	286	341
	SD		19	36	94	86	131	172
All	mean	87	35	65	557	528	288	365
	SD		20	43	91	93	123	197

Age has significant effect on all the six variables ( $P < 0.05$ ). The genotype and sex have no significant effect on any of the rabbit traits.

analysis were computed separately in the two age groups.

The correlation values in the case of the group at the age of 16 weeks proves that the MRI scanning is an excellent *in vivo* method to determine the volumes of fat and muscle as it can be seen in Table 2. The coefficients of correlation are in the range from 0.77 to 0.94. These results are similar to the measurement of muscle and fat

very high (0.86).

The  $r$  values were lower (0.39 - 0.76) on all the variables measured at the age of 12 weeks compared to the  $r$  values of the group of the age of 16 weeks. The characteristics of the digital sampling explains the low values. The same unified imaging protocol was applied to scan all the rabbits. The value of the "field of view" parameter determines the resolution of the MRI tomograph.

In present case 400 mm was represented by 256 pixels. Digital pictures, like the MRI scans are created by digital sampling. Digital sampling always means errors in the sampling process. The smaller the rabbit or the lower the fat content the higher the relative error of the sampling. The low correlation coefficients are explained by the higher relative error of the collected MRI data.

**Table 2 : Coefficients of correlation in the subpopulations.**

	N	Perirenal fat [g] & MRI vol	Hind part [g] & MRI vol.	Total fat [g] & MRI vol.	Perirenal fat (MRI) vol. & Tot. fat (MRI) vol.
12 weeks	31	0.74	0.55	0.39	0.76
16 weeks	56	0.94	0.86	0.77	0.90

The lower values in group age 12 are explained by the higher relative errors of the digital sampling

In future experiments the "field of view" parameter of the MRI tomography should be optimised to reduce the partial errors caused by the digital sampling.

Besides the very good quality of the MRI picture the MRI has no ionising side effect during the imaging process. The CT scanners have the disadvantages of working with harmful X rays. The MRI gives us a powerful non-invasive method to measure the body composition of the rabbit *in vivo*. The development of the various tissues and organs can be followed in the same animal.

The validation of the collected MRI volumes can be done through experimental slaughter and direct chemical analysis. According to the results the MRI is a suitable *in vivo* method to measure the fat and muscle content of rabbits affected by biological, zootechnical and environmental factors.

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