ESTIMATION OF MILK PRODUCTION OF RABBIT DOES BY CROSS SECTIONAL DIGITAL IMAGING

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ABSTRACT

Several researchers have measured the milk yield of rabbit does. Daily milk yield can easily and accurately be measured by determining the weight difference of the doe before and after nursing; there is an estimation possibility of milk yield based on the kits' growth during the suckling period. Computer Tomography (CT) is used for in vivo body composition analysis in rabbits. The aim of this study was to develop an indirect computer tomography-aided method to predict the milk production of lactating does. Five lactating does were weighed by weight-suckle-weight method four times at days 7, 14, 21, 28 of lactation. At the same time they were scanned by spiral CT before and after suckling to estimate their milk yield. During the examination, the animals were fixed with belts in a specially designed cradle without using anaesthetics. Spiral CT scans were adjusted to acquire 10 mm thick overlapping slices from the whole body. Data collection was performed on the basis of whole mammary gland tissues. To determine the rabbit milk density, some milk samples were collected for digital imaging. The density interval of milk was approximately +20-60 Hounsfield Unit (HU). The mammary gland density range was 0-60 HU; the mean density slightly changed before and after nursing. The linear regression between measured and mammary gland density based CT estimated milk yield showed the highest coefficient of determination (R^2=0.963; SE 17.03; $P<0.01$). The estimated milk yields of the first, second, third and fourth pair of glands were 20, 33, 25 and 22%, respectively. The applied *in vivo* CT examination method seems to be suitable in estimating milk yield depending on pairs of mammary glands.

Key words: Rabbit, Milk yield, Computer tomography.

INTRODUCTION

Several researchers have measured the milk yield of rabbit does. The direct measurements are used in large animals. For rabbits, milking machines were developed and described (Lebas, 1970; Schley, 1975; Marcus *et al*., 1990). However, in rabbit research this methodology is not used to determine the milk yield during the whole lactation period. As a consequence of the nursing behaviour, daily milk yield can easily and accurately be measured by determining the weight difference of the doe before and after nursing (Lebas, 1968). This "weight-suckle-weight" method is widespread for research purposes and has an advantage over weighing the kits. Kits' weighing is more difficult because they are nervous while the accuracy is lower because kits show some urine loss even during the suckling event (Lebas, 1971). There is an estimation possibility of milk yield based on the kits' growth during the suckling period (Lebas, 1969). Computer Tomography (CT) is used for in vivo body composition analysis in rabbits (Romvari *et al*., 1996), accordingly, it was advisable to use for estimation of milk yield. The aim of this study was to develop an indirect (CT-aided) method to predict the milk production of lactating does.

MATERIALS AND METHODS

Animals and experimental design

The analyses were conducted at the University of Kaposvár using 5 Pannon White does for 28 days of lactation. The lighting period was 16 hours and the temperature was between 16-24°C. The rabbits were fed ad libitum with a commercial pellet (energy=11 MJ DE/kg, crude protein=17%, crude fibre=15.5%) and drinking water was also available *ad libitum* from nipple drinkers. The does were kept in cages made of spot-welded wire having a basic area of 275 × 600 mm (measured without the feeder and the nest). The nest tray, sunk into the 550 × 240 mm nest area within the cage, was bedded with wood shavings. The door on the wall separating the cage and the nest area could be closed. Eight pups of average body weight were placed into each litter. The litter size did not change during the experiment.

Cross sectional digital imaging

The does were scanned by SIEMENS Somatom Emotion 6 multislice CT scanner at the Institute of Diagnostic and Oncological Radiation of the Kaposvár University. At days 7, 14, 21 and 28 of the lactation period the does and their pups were transported from the rabbits house to the preparation room of the institute. In the previous afternoon, the doors of nest boxes were closed at 16:00 p.m., thus the does did not visit their pups for suckling purpose. Before and after suckling the does were weighted and also evaluated by serial spiral CT scanning. During the examination, the animals were fixed with belts in a specially designed cradle without using anaesthetics. Spiral CT scanner was adjusted to acquire 10 mm thick overlapping slices from the whole body (slice thickness: 10 mm, feed: 10 mm, pixel spacing: 0.3515 x 0.3515 mm, tube voltage: 130 kV, dose: 95 mAs). The picture forming pixels of the images are in fact small prisms (voxel) with a defined volume (approx. 1.24 mm^3) and a characteristic X-ray density value on the Hounsfield (HU) scale. All CT data were stored in a DICOM file format. The images were analysed by Medical Image Processing V1.0 software developed by our institute. Data collection was performed on the basis of whole mammary gland tissues (Figure 1). To determine the rabbit milk density, some milk samples were collected for digital imaging. Indirect volumetric estimation was performed to determine the milk production of the mammary gland.

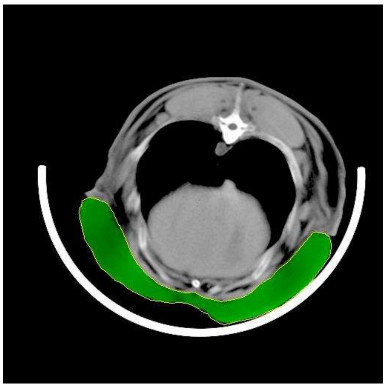

Figure 1: Rabbit mammary gland tissue (green area) on a cross sectional image at the level of 8th thoracal vertebrae.

Statistical Analysis

The milk yield was estimated by the difference of the volumetric data before and after suckling using correlation and linear regression, and the mean of densities were tested using paired samples t-test with SPSS for Windows 10.0 (1999) software.

RESULTS AND DISCUSSION

The calibration process was based on cross-sectional imaging, and consists of two independent data sets originating from the image analysis and from measured milk yield. A previous study reported the HU ranges of muscle, fat tissues of the rabbit (Romvari *et al*., 1996). The basic concept was to find out whether the milk yield can be estimated better using the proper range. The estimation method was based first on the standard water density interval (between -20 and +20 HU), but the correlation between the reference weight-suckle-weight milk yield and the CT estimation gave low value (Table 1). The latter approach was the determination of milk density range. CT scans were performed on samples of milk taken from several lactating does which were milked following the same lactation period as of the CT examined does. The density interval of milk was approximately +20-60 HU at all of the collection times. The difference of the water and milk density may be caused mainly by the protein content of rabbit milk, because the protein has higher while the fat has lower HU values than the water HU range. The composition of the rabbit milk is almost consistent during the 1-4 weeks of lactation period (Maertens *et al*., 2007), therefore the same HU value range was used during this study. The milk density based correlation was much better than the water based one (Table 1).

Table 1: Correlation of the measured and CT estimated milk yield

Density range	HU values	Correlation (r)	P
Water	-20 to 20	0.373	0.13
milk	20 to 60	0.978	<0.01
mammary gland	0 to 60	0.981	<0.01

The next concept was to base milk yield estimation mainly on the volumetric changes of the whole mammary gland, since the examined density range was almost equal for the rabbit milk and the mammary gland tissue. The mammary gland density slightly changed before and after the suckling (30.3±3.2 and 29.8±3.2; P=0.081) as Figure 2 shows.

The best calibration result arose using the mammary gland density interval (R^2=0.963; SE 17.03; P<0.01), as Figure 3 shows.

There were some problems with the adaptation of this method for estimation of does milk yield. First of all, the element unit of the CT examination is the voxel which was approx. 1.24 mm^3, therefore it may contain different histological objects like mammary gland tissue, milk canal with secreted milk or fat tissue, etc. The HU density value of a voxel represents the average density of all included objects. For this reason the milk was inseparable from the other tissues of the mammary gland based on HU differences (Figure 4). The second problem was that the rabbit muscle density range is close to the mammary gland density range limiting the conspicuisness of the border between these tissues. It was especially difficult in the thoracic region of the body.

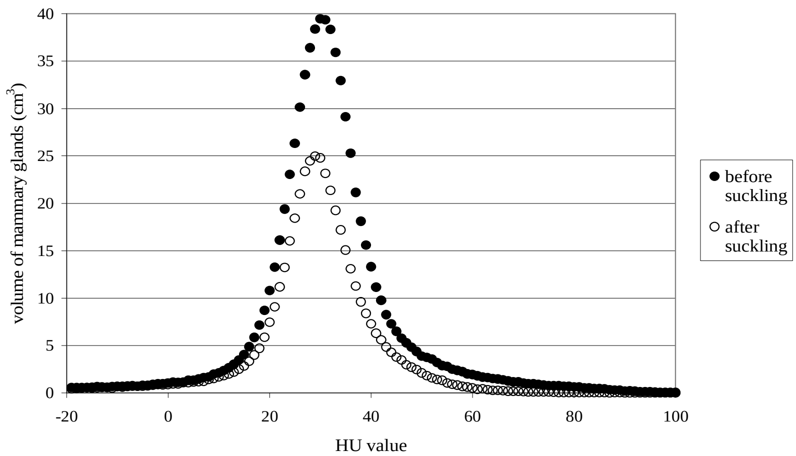

Figure 2: Volume of mammary glands of a doe before and after suckling estimated by CT at the second week of the lactation

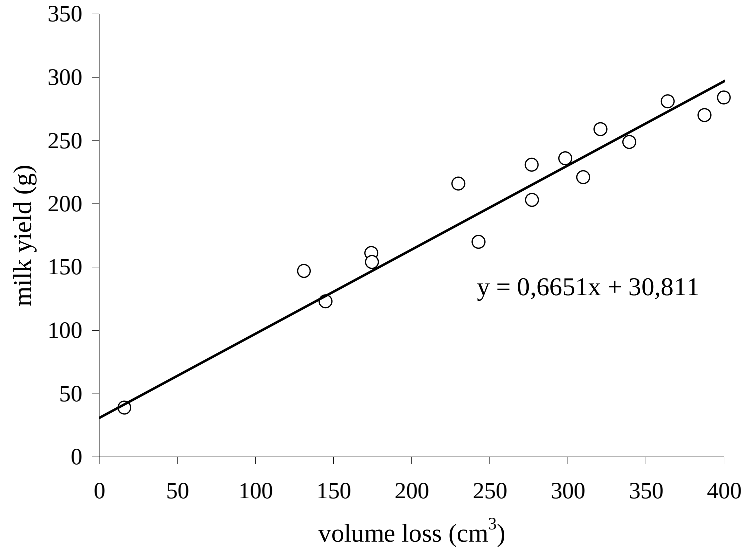

Figure 3: Linear regression between measured and the CT estimated milk yield based on the density of the mammary gland

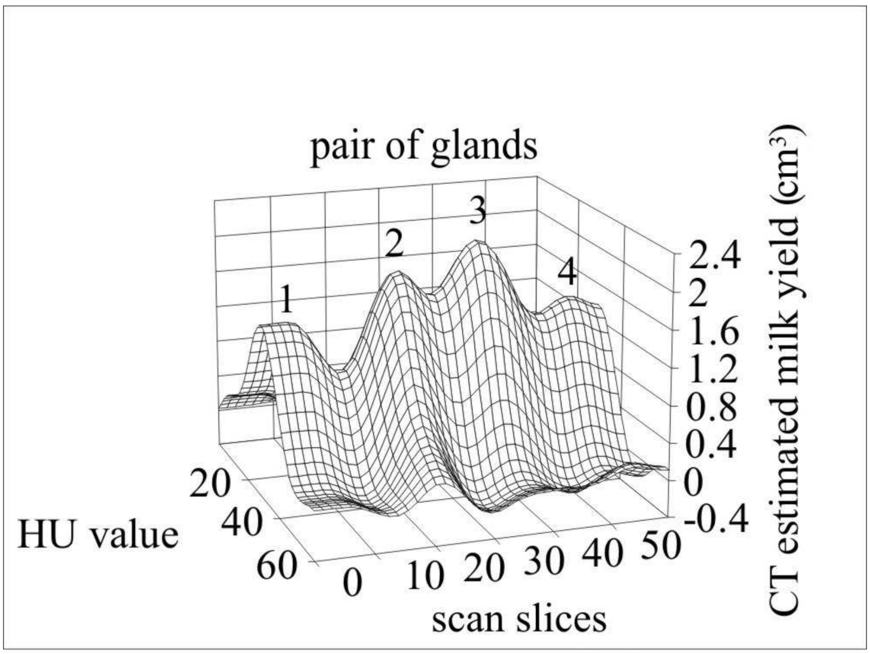

Figure 4: CT estimated milk yield in a doe at the second week of the lactation, depending on the location of the pair of glands

CONCLUSIONS

The applied in vivo CT examination method based on the mammary gland density values seems to be suitable for estimating milk yield depending on the location of the pair of glands.

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