COMPLEX STUDY OF THE RABBIT'S PREGNANCY

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Abstract - The authors determined the total body composition of 4x10 female, approximately 5-month-old New Zealand White rabbits, using direct chemical analysis and computerised tomography (CT). Ten nonpregnant rabbits and three times ten pregnant rabbits on Days 7, 14 and 28 of gestation have been investigated. After receiving anaesthetics, the rabbits were positioned on their abdominal side and a cross-sectional scan was taken at each of the following positions: 1. between the last dorsal and first lumbar vertebra, 2., 3., and 4 halfway between lumbar vertebra 2 and 3, 4 and 3, 4 and 5, 6 and 7, 5. at the head of the femur, 6. at the neck of the femur. After the scanning the rabbits were overslept and the empty body, the gastrointestinal content and the pregnant uterus have been analysed. Using the obtained data one could calculate the energy and protein requirements of gestation and the efficiency of ME⇒NE transformation for pregnancy. The embrional growth proved to be allometric. The CT pictures have been assessed by reducing the pixels of ten HU (Hounsfield Unit). the records were analysed according a linear regression model. After the results of the present study, one can predict the total body composition on the basis of X-ray absorption only with a medium accuracy (r=0.47). The predictability can be improved by increasing the number of tomograms.

INTRODUCTION

The success of feeding experiments in living (intact) animals is largely dependent on the thorough knowledge of the main chemical components in the diet. To measure the relative proportion of the main chemical components during and at the end of the experiment, indirect, in vivo methods are needed. The understanding of the composition of the body is immensely important not only for feed sciences (net energy determination) but also for veterinary studies. Thus dosing anaesthetics depends on fat/whole body ratio of the animal. Body composition is influenced by both genetic and environmental factors. The concept of environment involves nutrient supply, too.

Body composition can directly be estimated by direct chemical analysis, although this does not allow for monitoring the changes in body composition. Great efforts has been made to develop methods aiming at estimating body composition in living animals. Therefore measuring density, ^{40}potassium-count, metabolic balance, neutron activation, urinal excretion of kreatinine and 3-methyl-hystidine, uptake of fat soluble gases (cyclopropane, xenon, ^{85}krypton) as well as using methods of dilution water space determination (D_2O, THO, urea) could be of practical value along with human anthropometry, radiography, photon densitometry, computerized tomography (CT), NMR, infrared interacne, ultra sound, close-up infrared spectrophotometry, total body electric conductivity (TOBEC), bioelectric impendance, analysis for very low density lipoproteins (VLDL) in plasma. None of these methods proved to be perfectly safe each having their advantages and disadvantages. Methods should be chosen for experimental purposes considering the availability of devices and equipment.

KAMPHUES (1985) found that pregnancy and lactation have a pronounced influence on body composition. He used rabbits of 3.5-4.7 kg body weight to study energy, nitrogen and mineral incorporation into the foetus, placenta, uterus and lactiferous gland. The relative weight of the 15 days old rabbits is 0.007 per cent of their mothers' body weight. This figure increases during pregnancy up to 1.198 per cent. Since it is not only the changes in foetal body weight and composition that were measured but changes in placenta, uterus and mammary gland, too. The author could therefore calculate daily nutrient and energy incorporation during the last eight days of pregnancy (Table 1). Supposing that k_c value (energy conversion efficiency during concept's building) is 0.25, the energy requirement of life is 95.6 kcal/W $^{0.75}$ and the transformation efficiency of 3 g of digestible crude protein and protein into the foetus is 60 per cent, it can be concluded that by calculating energy requirement of pregnancy food intake alone does not meet pregnant does' energy requirements thus resulting in body reserves mobilization.

Table 1 : Daily energy and nutrient accretion in the last 8 days of pregnancy (KAMPHUES, 1985)

Parameter	Foetus	Annexe	Uterus	Udder
Ash, g	1.312	0.028	0.015	0.05
Protein, g	6.815	0.294	0.097	0.751
Fat, g	2.971	-	0.023	1.248
N-f.e., g	0.737	-	0.050	0.090
GE, kcal	70.3	1.68	0.98	16.5

N-f.e.= Nitrogen-free extract

PARIGI-BINI *et al.* (1990) studied protein and energy incorporation in first time pregnant rabbit does. Comparative

slaughtering method was used in the study comparing nonpregnant control rabbits to rabbits at day 21 and 30 of pregnancy. Average body weight gain (incorporation into own body tissues) was 180 g during the first 21 d in pregnant animals plus 193 g for pregnant uterus. Body weight gain and composition of uterus were as follows: 31.1% and 85%, 24.4% and 9.3%, 36.1% and 4.7%, and 8.4 and 1% for water, crude protein, crude fat, ash, respectively. Total energy retention averaged 980 kcal per animal. During the last ten days of pregnancy catabolism dominated in does' body, however, intensive uteral and foetal growth (647±58 g) occurred mainly in form of water (81.71%) and protein (11.25%) as well as total fat (5.15%) and ash (5%). Digestible energy requirement for life is 98.5 kcal/day/W $^{0.75}$ and energy conversion for conceptus building (i.e. does' body weight gain + conceptus building) is 44.7%, therefore these latter figures were not significantly influenced by pregnancy. Efficiency of protein transformation (accrued N/digested N, %) was found 16-17% during pregnancy. These physiological changes are best explained by doubling of blood plasma glucagon level. The probable reason why energy transformation efficiency in pregnant and in non-pregnant rabbits are essentially the same is that the conceptus building within the total body weight gain is low; though energy conversion is rather poor for conceptus building, therefore within total body weight gain it represents a low figure.
PARTRIDGE *et al.* studied pregnant and non-pregnant suckling does fed high and low energy diet. Litter size were equalized to make it six after birth. Bodies of does fed high calorie concentrates contained more fat (49.6% and 37% in dry matter) at birth. Different diets fed during pregnancy had no impact on litter size, though litter weight and mortality of suckling rabbits were found considerably higher for does fed high energy concentrates. Lactation diet had no impact on milk composition in contrast to pregnancy diet: does fed high energy pregnancy concentrates produced milk with high fat content (12.3% and 10.5%, respectively). Body composition figures show (Table 2) that fat mobilization takes place during early lactation followed by fat incorporation as lactation progresses. This trend was not dependent on either of the diets offered.
Energy requirement for life for pregnant and suckling does is 108 and 113 kcal DE/W$^{0.75}$ according to FRAGA *et al.* (1989). Energy requirement for pregnancy is 6.04 kcal per losing 1 g of liveweight at birth and for milk secretion it is 2.7 kcal per 1 g of secreted milk in the form of digestible energy.
In this in vivo experiment, using computer tomography, energy and protein requirements of pregnancy were taken as a function of time. Results were supported by direct chemical analysis, too. We sought to calculate prediction functions by revealing correlations between CT examination data and the real chemical composition.

Table 2 : Weights of various body components (grams) and body composition data for does of different energy diet (PARTRIDGE *et al.* 1986)

Pregnancy diet	2966 kcal ME/kg				3396 kcal ME/kg			
Lactation diet	2638 kcal		2839 kcal		2636 kcal		2839 kcal	
Day of lactation	0	18	9	18	0	18	9	18
Live weight, kg	4.07	4.57	4.49	4.52	4.35	4.49	4.3	4.48
Empty body weight	3.58	4.09	3.98	4.03	4.08	4.02	3.92	4.11
Mammary gland	120	198	192	198	106	232	199	236
Water, %	62.6	59.7	62.2	62.0	56.2	61.6	59.6	57.5
Fat, % DM	37.0	47.5	39.5	38.8	49.6	41.7	44.3	5.21
Protein, % DM	88.3	88.4	88.3	88.1	88.6	88.8	87.0	87.5
Ash % DM	11.7	11.6	11.7	11.9	11.3	11.2	13.0	12.5
GE, kcal/g DM	6.74	7.27	7.03	6.91	7.31	7.10	7.10	7.46

DM = dry matter, GE= gross energy

MATERIAL AND METHODS

This experiment involving 4x10, approximately five months old New Zealand White rabbit does was conducted to determine body composition by direct chemical analysis and computer tomography (CT). Ten empty (non-pregnant) rabbits and thirty rabbits at 7, 14, 28 d of pregnancy (ten of each occasion) were examined at the RCT Siemens Biological Center by means of a third generation tomography, type: SOMATOM DRG (HORN, 1991).
CT-pictures were taken at six different cross sectional points. This was followed by Wendee analysis (MSZ 6830, 1977; AOAC., 1975) of rabbits, their gastrointestinal content and pregnant uteri (empty uterus and foetuses separately).
CT-scanned density figures for 3-D view were arrayed in an increasing order according to the Hounsfield scale. This means approximately 4100 individual data for each scan. These data determine the number of pixels for each of the data in the scale. Their sum equals with 65'536. The interval -200 to +200 was selected. As

described above, ten sequential figures were averaged for later calculations: 1= -199 and -190, 2= -189 and -180, etc.

Correlation between CT-data and chemical composition was determined then the results were displayed in graphic charts. The interval between -150 and +120 HU was used for regression estimation. Grouping of the ten sequential figures (6-32) and their marking remained the same as above. Six scans for each of the animals or 27 HU-values for each of the scans were displayed in a 162 column-chart where column indicated the dependent variables, plus body weight for the 163rd column. Independent variables were: protein, fat and energy. Functions were generated by MGLH stepwise methods using the function generating software programme SYSTAT, Version 5.01. Figures were made by using STATGRAF programme. (Similar legends were used: "B", "C", "D", "E", "F" for cross-sectional points; H6-H32 for group of ten sequential figures of HU-units, intervals between -150, -140, etc. and +120; "W" for body weight in grams).

RESULTS

Results of chemical investigations are summarized in Table 3 and Table 4.

Table 3 - Chemical composition of the whole doe's body (carcass+hide+conceptus+gut content)

Parameter	Control (empty)	Control on d 14	d 21	d 28 of pregnancy
Live weight,g	3255	3760	3815	3938
±	506	245	256	242
DM, %	43.02	42.93	40.77	40.94
±	3.44	2.55	5.87	4.78
CP, %	20.15	18.84	19.04	18.13
±	2.06	0.67	0.91	0.74
EE, %	19.13	20.48	18.49	18.89
±	5.29	2.88	1.68	2.20
Ash, %	3.06	2.88	3.08	2.88
±	0.41	0.27	0.31	0.44
CF, %	0.14	0.13	0.14	0.10
±	0.05	0.05	0.03	0.05
CP/Ash	6.58	6.54	6.18	6.30
Fat/Ash	6.25	7.11	6.00	6.56
GE content, MJ	40.40	46.97	44.52	46.51
Retained energy, MJ	0.0	0.824	1.202	2.189
±		0.243	0.193	0.348
Foetal energy, MJ	0.0	0.018	0.256	1.090
±		-	0.032	0.265

CP= crude protein (N*6.25), EE= ether extract, CF= crude fiber, d= day

Body fat content can accurately be calculated by using CT-results:

$$FAT, g= -418.944 + 0.28\times BH15 + 0.113\times CH9 + 0.433\times DH14 + 0.169\times W.$$

Protein content cannot be calculated by using CT-results (R^2=0.356); body weight as a new variable improves accuracy of prediction (R^2=0.797).

$$PROTEIN, g = -31.481 + 0.031\times CH28 + 0.05\times DH26 -0.022\times DH27 + 0.019\times EH26 + 0.016\times CH27 + 0.156\times W.$$

Body energy level could have been accurately (similarly to fat) calculated by CT-results (R^2=0.926):

$$ENERGY, MJ = 14.419 + 0.005\times BH10 -0.037\times BH13 + 0.043xBH14 + 0.08\times CH10 + 0.011\times DH11 + 0.01\times EH14 + 0.0011\times W.$$

Accuracy of estimation was checked by using GJERDE (1987) method. Group data of 14 d and 28 d pregnant does as well as the accuracy of estimation for the remaining three groups were selected. Energy level for the ten rabbits in Group 4 was calculated by using the function of the first three groups:

Correlation between the data obtained and the chemical analysis was 0.7558 which - considering the relatively small number of animals - seems to be fairly good. Strong correlation was found between chemical analysis and CT-data even if body composition of Group 2 animals was estimated by Group 3 and Group 4 equations:

Table 4 - Weight and chemical composition of uterus (incl. forewaters) and foetuses (incl. placenta)

Parameter\group		Control		Pregnancy d 14		d 21		d 28	
		Uterus	Foetuses	Uterus	Foetuses	Uterus	Foetuses	Uterus	Foetuses
Brut weight, g		20.0*	0.0	63.3	2.08*	134.6	58.6	130.8	233.0
	±			18.6		25.4	13.0	36.3	53.1
Dry matter, %		82.39	0.0	40.74	39.70	22.21	21.53	28.96	18.65
	±			5.90		3.72	2.27	4.32	0.75
Crude protein, %		10.09	0.0	14.92	30.09	11.68	14.88	14.32	12.00
	±			2.44		1.33	1.68	1.47	0.71
Ether extract, %		70.90	0.0	23.01	4.02	11.01	2.34	13.30	4.59
	±			6.50		2.73	0.31	3.68	0.49
Ash, %		0.89	0.0	1.49	4.84	1.09	2.62	1.28	1.99
	±			0.45		0.10	0.30	0.18	0.38
Number of foetuses		0		8.8±2.14		7.7±1.7		6.4±2.12	

* analysing a group as one sample

DISCUSSION

Table 5 - Comparison of the gross energy content of whole doe, conceptus and foetus

	Control	On d 14	d 21	d 28 of
		Pregnancy		
Whole doe's body, MJ	40.4	46.970	44.520	46.510
%	100	100	100	100
Conceptus, MJ	0.0	0.824	1.202	2.189
%	--	1.750	2.700	4.710
Foetus, MJ	0.0	0.018	0.256	1.090
%	--	0.040	0.580	2.340

Based on data obtained net energy and protein requirement for conceptus building, furthermore, based on the digestibility of diet (as fed) by using WALKER and YOUNG's method (1992) the efficiency of ME⇒NE transformation were determined. Foetal growth proved to be allometric. The last three data rows in Table 3 together with data in Table 5 show that 5 per cent of the energy level of total does body was not reached by that of the foetuses and of total conceptus even at 28 d of pregnancy. In contrast to other animal species conceptus building in rabbits is not only considerable in the last trimester but also from the second half of the pregnancy.

Data for total body composition can be correctly calculated by data of absorption factor for X-rays in the tissues. As for protein calculations it is essential to include body weight variables.

Based on data described in this study experts in rabbit nutrition should, in the future, aim at recording and transferring these data in a new data processing system with new goals as well as gathering further data on rabbits' requirements in different physiological and productivity states and on energy values. A new net energy evaluation system should therefore be developed in order to estimate feeds' energy values.

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