

IN VIVO ESTIMATION OF BODY FAT CONTENT OF NEW-BORN RABBITS USING THE TOBEC METHOD

MILISITS G., GYARMATI T., SZENDRŐ ZS.

Pannon Agricultural University, Faculty of Animal Science, H-7401 KAPOSVÁR, P.O. Box 16, Hungary

ABSTRACT: An EM-SCAN SA-2 type Small Animal Body Composition Analyser (TOBEC) was used to determine the total conductivity index (E value) of 50 new-born rabbits. After the TOBEC measurements the animals were slaughtered and their bodies were homogenised by grinding twice; fat content was then determined by a chemical procedure. A weak correlation ($r=-0.09$) was obtained between E value and body fat percentage. Due to the high correlation between E value and body weight ($r=0.90$), estimated fat content was corrected for this

trait [CF(%) = $11.7 - 0.1127 \times (1.61 \times (BW - BW_i) + E_i)$], resulting in a marked improvement in the correlation ($r=0.51$) between predicted and measured fat content. In spite of this improvement, the medium correlation makes the TOBEC method unsuitable for the accurate prediction of body fat content of new-born rabbits, although it can be used for the identification of animals with extremely high or low fat content.

RESUME : Estimation *in vivo* du gras corporel chez les lapereaux nouveaux nés par la méthode TOBEC

Un appareil "TOBEC" (analyseur de composition corporelle d'après la conductivité électrique) de type EM-SCAN SA-2 a été utilisé pour déterminer l'index de conductivité totale (valeur E) chez 50 lapereaux nouveaux nés. Après l'évaluation TOBEC les animaux ont été abattus et les carcasses ont subi un double broyage pour homogénéisation. Le contenu lipidique total du corps a été déterminé par l'analyse chimique. La corrélation entre la valeur E et le pourcentage de lipides est faible (r

= -0.09). Compte tenu de la forte corrélation entre le poids vif et la valeur E ($r = +0.90$), la teneur en lipides a été corrigée en prenant en compte ce caractère [Lip Corr (%) = $11,7 - 0,1127 \times (1,61 \times (BW - BW_i) + E_i)$], améliorant notablement la corrélation ($r = +0.51$) entre le contenu lipidique prévu et celui mesuré. En dépit de cette amélioration, la corrélation médiocre rend la méthode TOBEC impropre pour des évaluations précises du contenu lipidique des lapereaux nouveaux nés, bien qu'elle puisse être utilisée pour l'identification d'animaux au contenu lipidique très élevé ou très faible.

INTRODUCTION

It is known that exact information on body composition is very important in the determination of chemical maturity, nutritional requirements and body condition of animals. For this reason several experiments have been carried out to determine body composition at different developmental stages, mostly using experimental slaughter and chemical analysis as invasive methods (DE BLAS and GÁLVEZ, 1975; FRAGA *et al.*, 1978; PARIGI-BINI *et al.*, 1986, 1991). To avoid the slaughter of genetically valuable animals different types of non-invasive methods have been developed (FEKETE, 1992), which allow fairly good estimation of the body composition of living animals.

Two of these methods, computer tomography (CT) and magnetic resonance imaging (MRI), were used by our research team for the determination of body composition (especially of fat content) of young rabbits and does (MILISITS *et al.*, 1996; ROMVÁRI *et al.*, 1996; KÖVÉR *et al.*, 1998). Another non-invasive method, the TOBEC (Total Body Electrical Conductivity), gave close correlations ($r=0.88-0.99$) between the E value and lean mass or the weight of total body water of small birds and mammals (CUNNINGHAM *et al.*, 1986; FIOROTTO *et al.*, 1987; FEKETE and BROWN, 1993; STAUDINGER *et al.*, 1995), but only medium accuracy ($r=0.59$) in the prediction of total body fat percentage (FEKETE *et al.*, 1995). The high correlation between E value and live weight allowed us to achieve fairly good prediction of the weight of different body parts (especially for the lean body mass), but did not provide useful information on body fat percentage.

Therefore, the objective of this study was to clarify the relationship between E value and body fat percentage and to develop prediction equations for the *in vivo* determination of the total body fat content of new-born rabbits.

MATERIAL AND METHOD

Fifty new-born Pannon White rabbits with an average live weight of 54.9 ± 5.8 g were used. The rabbits originated from 8 multiparous does kept in individual cages (800x500mm) in a closed building and fed *ad libitum* with a commercial pelleted diet (DE 10.30 MJ/kg, crude protein 17.5%, crude fat 3.6%, crude fibre 12.4%) during pregnancy. The parturition was induced by an I. M. injection of 3 IU oxytocin on the 31st day of pregnancy. The new-born pups were weighed immediately after birth (before the first suckling) and measured by TOBEC at the same time.

The TOBEC measurements were carried out with an EM-SCAN SA-2 type Small Animal Body Composition Analyser (EM-SCAN Inc., Springfield, Illinois, USA), which allows a rapid, non-invasive measurement of the total conductivity index (E value) of small animals. This method is useful for detecting energy absorption in the presence of a radio-frequency electromagnetic field, which is created when a 10 MHz frequency is passed through a copper wire wound around a Plexiglas tube. In this system more energy is absorbed by conductive materials such as normally hydrated lean tissue than by resistant materials such as body fat (FUNK, 1991). The net energy absorption detected by TOBEC is compared to the chemical determination of body composition as reference. With this method the fat-free body

mass could be measured directly and the fat content calculated from it.

Because this method requires immobility of the animals during the measuring procedure, the pups were given a lethal dose of anaesthetic by I. M. injection of Rometar (SPOFA) and Calypsovet (RICHTER GEDEON; 0.2 mg xylazine and 2 mg ketamine per pup).

The position of the animal in the measurement chamber is also critical. In repeated examination the animal has to lie in exactly the same place. In our studies the E value of pups was determined three times and the mean was used for the calculation of prediction equations. Intra-animal variability (CV) of the E value was lower than 1% for each animal.

The reading of the E value is proportional to the square of the cross sectional area of the lean tissue. A short, broad animal will give a greater signal than a long, thin animal of the same weight, lean mass, and volume. For this reason, it is very important to stretch the animals to their greatest length (without harming them) during the measurement (EM-SCAN MODEL SA-2 OPERATION MANUAL, 1991).

After the TOBEC measurement (approximately 10 minutes after the anaesthetic overdose was administered) the whole body of each rabbit was cut into pieces and homogenised by grinding it twice. From the homogenates, 28g samples were taken and transferred into a 40 mm tube and used both for TOBEC measurement and for chemical analysis. The Soxhlet method was used for the determination of fat content.

For the in vivo estimation of the body fat content of the pups prediction equations were developed by multiple linear regression using the SPSS statistics software package (SPSS 7.5 for WINDOWS, 1996):

$$\begin{aligned} \text{Eq. 1} \quad CF_{(\%)ij} &= \mu + E_i + e_{ij} \\ \text{Eq. 2} \quad CF_{(g)ij} &= \mu + E_i + e_{ij} \\ \text{Eq. 3} \quad E_{ij} &= \mu + BW_i + e_{ij} \\ \text{Eq. 4} \quad CF_{(\%)jk} &= \mu + E_i + b(BW - BW_j) + e_{ijk} \\ \text{Eq. 5} \quad E_{ij} &= \mu + BL_i + e_{ij} \\ \text{Eq. 6} \quad CF_{(\%)jk} &= \mu + E_{iBW} + E_{jBL} + e_{ijk} \\ \text{Eq. 7} \quad CF_{(g)jk} &= \mu + E_{iBW} + E_{jBL} + e_{ijk} \\ \text{Eq. 8} \quad CF_{(\%)ij} &= \mu + E_{iS} + e_{ij} \end{aligned}$$

where

- $CF_{(\%)ij}$ = Crude fat (%),
- $CF_{(g)ij}$ = Crude fat (g),
- E_{ij} = E value,
- μ = Overall mean,
- BW = Mean body weight (g),
- BW_i = Body weight of the i^{th} pup (g),
- BL_i = Body length of the i^{th} pup (mm),
- E_{iBW} = Corrected E value for the i^{th} pup by body weight,
- E_{jBL} = Corrected E value for the j^{th} pup by body length,
- E_{iS} = E value for the homogenised sample from the i^{th} pup,
- e_{ijk} = Random error.

Based on the estimated fat content, the 5 most fatty and the 5 leanest rabbits were chosen from the population and the differences in their chemically determined fat content

Table 1 : Basic data for the new-born rabbits.

	Average	S.D.	Min.	Max.
Weight (g)	54.9	5.8	44	65
Length (mm)	97.8	4.2	89	107
E value	64.8	10.3	46	86
Fat (%)	4.4	1.0	2.6	7.3
Fat (g)	2.4	0.6	1.3	4.4

were tested with one-way ANOVA (LSD test), also using the SPSS software package.

RESULTS AND DISCUSSION

The basic data for the new-born rabbits are shown in Table 1.

As the first step of the evaluation the correlation between E value and chemically determined fat content was examined. It was found that the E value in itself was not sufficient to estimate the body fat percentage of new-born rabbits due to the low ($r=0.09$) correlation obtained (Eq. 1):

$$\text{Eq. 1 : } CF_{(\%)i} = 5.0 - 0.0089 \times E_i \\ (R^2=0.01, p>0.05)$$

It is known that the weight of the animal exerts a major effect on the E value. Therefore, a slightly better ($r=0.25$) correlation was obtained when the total lipid mass was predicted instead of the fat percentage (Eq. 2):

$$\text{Eq. 2 : } CF_{(g)i} = 1.4 + 0.0155 \times E_i \\ (R^2=0.06, p<0.1)$$

To improve the prediction of fat percentage, first the

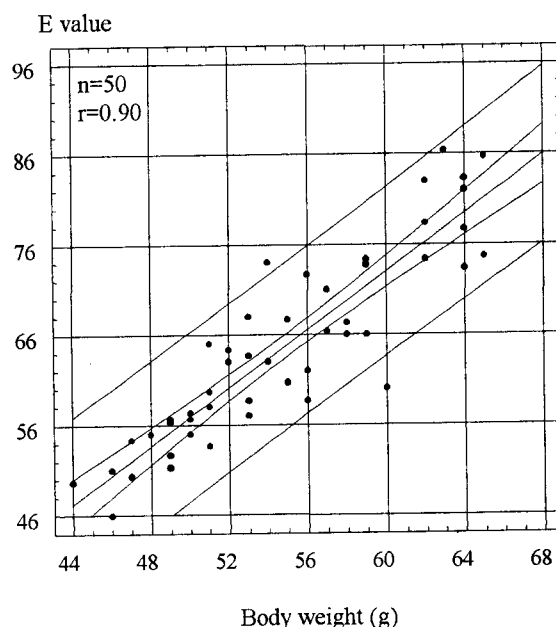


Fig. 1 : Relationship between E value and body weight

correlation between E value and live weight had to be clarified and a 0.90 correlation was found. In the weight range examined the linear regression model (Fig. 1) appeared to be the best way to represent the relationship (Eq. 3):

$$\text{Eq. 3 : } E_i = -23.6 + 1.61 \times BW_i \\ (R^2=0.81, p<0.001)$$

In view of the high correlation between E value and live weight a correction based on body weight seemed to be necessary for predicting the body fat content more precisely. The correction in Eq. 4 eliminated most of the effect of live weight on E value, resulting in much higher accuracy of prediction of fat content:

$$\text{Eq. 4 : } CF_{(%)i} = 11.7 - 0.1127 \times (1.61 \times (BW - BW_i) + E_i) \\ (R^2=0.26, p<0.001)$$

It is known that the TOBEC measurements are also affected by the length of the animal. The correlation between the E values measured and the length of the rabbits (from nose to end of legs in this case) was 0.60 in the present experiment (Eq. 5):

$$\text{Eq. 5 : } E_i = -80.2 + 1.48 \times BL_i \\ (R^2=0.36, p<0.001)$$

The correction for body length was performed in the same way as for live weight, but due to the high correlation ($r=0.78$) between the E values corrected for body weight and the E values corrected for body length, the latter had no significant effect on the R^2 value of the prediction equation (Eq. 6):

$$\text{Eq. 6 : } CF_{(%)i} = 12.8 - 0.1666 \times E_{iBW} + 0.0367 \times E_{iBL} \\ (p<0.001) \quad (p<0.001) \quad (p>0.05) \\ (R^2=0.30)$$

A slightly better result was obtained when the weight of the fat was predicted (Eq. 7):

$$\text{Eq. 7 : } CF_{(g)i} = 8.2 - 0.1472 \times E_{iBW} + 0.0581 \times E_{iBL} \\ (p<0.001) \quad (p<0.001) \quad (p<0.001) \\ (R^2=0.45)$$

These results are in agreement with the previous results of FEKETE *et al.* (1995), who obtained an $r=0.59$ correlation between the predicted and chemically determined fat content of dwarf and normal rabbits. It appears, therefore, that the use of the TOBEC method results in only medium accuracy in predicting total body fat content alien to the prediction of lean mass and total body

Table 2 : Differences in live weight, E-value and fat content between fatty and lean rabbits

Parameters	Fatty (n=5)		Lean (n=5)		Level of significance
	average	S. D.	average	S. D.	
Live weight (g)	60.8	3.7	56.6	5.5	NS
E value	67	7.4	75	9.2	NS
Corrected E value (by weight on whole body)	57	3.0	73	1.8	***
Predicted fat content (%)	5.4	0.4	3.5	0.2	***
Chemically analysed fat content (%)	5.5	1.1	3.9	0.4	*

* $p<0.05$, *** $p<0.001$

water (CUNNINGHAM *et al.*, 1986; FIOROTTO *et al.*, 1987; FEKETE and BROWN, 1993; STAUDINGER *et al.*, 1995). This is also accounted for by the medium correlation ($r=-0.56$) between the original E values for the homogenates and the chemically determined fat content (Eq. 8):

$$\text{Eq. 8 : } CF_{(%)i} = 11.7 - 0.1701 \times E_{is} \\ (R^2=0.31, p<0.001)$$

In the latter case, in the absence of the effect of weight and length no corrections were needed.

Comparison of fat predicted on the basis of the whole body with fat predicted based on the homogenate produced a 0.39 correlation.

The medium accuracy observed in this experiment indicates that the TOBEC method is unsuitable for the exact prediction of the body fat content of new-born rabbits, but it can be used for the selection of the most fatty and leanest rabbits from a given population. To confirm this, a significant difference was observed between the chemically analysed fat content of the 5 most and 5 least fatty rabbits chosen by predicted fat content from the population examined (Table 2).

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