

**PREDICTION OF BODY COMPOSITION IN RABBITS BY  
DEUTERIUM OXIDE DILUTION AND TOTAL BODY ELECTRICAL  
CONDUCTIVITY WITH VALIDATION BY DIRECT CHEMICAL ANALYSIS**

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**Abstract**

Twelve adult and 12 weaning New Zealand White rabbits were fed inclusively alfalfa pellets (A) or oat grain (O) and water ad libitum. After 40 d, 8 rabbits were subjected to total body electrical conductivity analysis and all rabbits were subjected to deuterium oxide dilution technique and direct chemical analysis. The best prediction of the body chemical composition was provided by combination of total body weight (TBWT) and total body electrical conductivity number (TOBEC) as linear predictors. Body energy content was predicted from body weight, and TOBEC number in these medium frame meat type rabbits which had not been fed or watered for 3-4 hours prior to scanning, by the following equation: Body energy (kcal) = 7.499\*TBWT - 51.83\*TOBEC - 8404 ( $r^2 = 0.92$ ;  $cv = 4.86$ )

**Introduction**

Most of the limited literature concerning rabbit body composition has focused on carcass composition (Hutchinson and Baker, 1949; Ouhayoun et al., 1979, 1981; Lukefahr et al., 1982; Varewyck and Bouquet, 1982; Butcher et al., 1983; Lobley et al., 1983; Ouhayoun and Cheriet, 1983; Ledin, 1984), with only a few reports of total body composition (Lanari et al., 1972; DeBlas and Galvez, 1975; Parigi-Bini et al., 1978; DeBlas et al, 1977; Fraga et al., 1978; Dehalle, 1981). All of the above mentioned studies were limited to destructive chemical analyses after slaughtering. Where non-invasive procedures have been used, the focus has been on using rabbits for validation of procedures or equipment for use in other species (Green and Dunsmore, 1978; Klish et al., 1984; Fioretto et al., 1987). These latter studies have not addressed situations spanning the range of diets and physiological status encountered in commercial and/or laboratory rabbit production.

A precise, non-invasive means of measuring the effects of diet on live animal body composition would be extremely useful for relatively inexpensive but accurate determinations of the net energy value of rabbit feeds. The first objective of this work was to contrast the values of three predictors of body energy and composition: 1. body weight alone, 2. body weight and deuterium oxide dilution space and 3. body weight and total body electrical

conductivity (TOBEC). The second objective was to provide a precise description of the range of conditions and independent variables used to assess these predictor parameters.

### Materials and Methods

**Animals.** Twelve adult (8-12 month-old) and 12 growing (9-week-old, previously weaned at 40-45 days) New Zealand White rabbits (from UC Davis Animal Science Department specific pathogen free breeding colony) were housed individually in stainless steel cages in a room with 12 h light and 12 h dark, a constant temperature of 17.5° C and 55-65% relative humidity. The animals were assigned to either oats or alfalfa at random within age groups.

This study was approved by the Animal Use and Care Administrative Advisory Committee of UC Davis and complied with United States and California laws regarding the use of experimental animals and with the United States Public Health Service Policy on Human Care and Use of Laboratory Animals.

#### Experimental design

- Group 1: Three adult males and three adult females were allowed 0.6x1.2 cm alfalfa pellets ad libitum (A).
- Group 2: Two adult males and four adult females were allowed whole oat grain ad libitum (O).
- Group 3: Four growing male rabbits and two growing female rabbits were allowed ration A ad libitum.
- Group 4: Three growing male rabbits and three growing female rabbits were allowed feed O ad libitum.

All rabbits were allowed ad libitum access to tap water. Feeding proceeded for 40 days. Feed composition is presented in Table 1.

The purpose of feeding two disparate diets was to create populations of rabbits that spanned a range of diet-induced body composition. Such a range of body compositions was needed to create equations capable of predicting body composition from non-invasive observations.

**Deuterium oxide dilution technique.** On day 36 of experimental diet consumption, each of the 24 rabbits was injected intraperitoneally (i.p.) with a single dose of approximately 1 g deuterium oxide (99.9 % pure, prepared by the Cambridge Isotope Laboratories, Cambridge, MA, USA) weighed to the nearest 0.001 gram. Blood samples (2-4 ml/animal) were drawn into heparinized tubes from the auricular artery each of four days following deuterium oxide injection. During the blood collections, the rabbits were tranquilized by 0.5 mg/kg BW subcutaneous (s.c.) Acepromazine Maleate Injection 10 mg/mlR (TechAmerica TM, Fermenta Animal Health Co. Kansas City, MO 64190, USA). Feed and water were available throughout the four-day blood collection period. Blood

deuterium oxide concentrations (D2O) were measured by rapid sublimation and infrared spectrophotometry as described by Beyers (1979).

Deuterium oxide space was determined from a single pool model (Brown and Taylor, 1986). The logarithm of rabbit blood [D2O] was regressed on time after injection. The antilog of the calculated "y" intercept of the resulting equation was taken to approximate the [D2O] which would have existed at time "0" had instantaneous mixing taken place. Deuterium oxide dilution space (DOS) in grams was calculated as the D2O dose in grams divided by [D2O]<sub>t0</sub> in ppm and multiplied by 1,000,000.

Table 1. Chemical composition and calculated nutritive value of experimental feeds\*.

Components	Alfalfa	Oats
Dry matter, %	88.85	90.36
Ash, % DM	11.25	2.55
Ether extract, % DM	3.04	6.53
Crude protein, % DM	19.62	11.47
Crude fiber, % DM	24.04	13.42
N-free extract, % DM	42.05	66.03
NDF, % DM	30.55	36.96
ADF, % DM	30.55	16.71
Cellulose, % DM	23.41	13.72
ADL, % DM	5.29	2.76
DE, kcal/kg DM	2169	3352
DCP, g/kg DM	107.9	89.5

NDF = neutral detergent fiber

ADF = acid detergent fiber

ADL = acid detergent lignin

DCP = digestible crude protein

DE = digestible energy

\* Digestibility coefficients and predictive equations are as described by Fekete and Gippert (1986).

**TOBEC analysis.** One male and one female representative rabbit was randomly selected from each of the four age-diet subclasses for the TOBEC analysis. These eight animals were transported from UC Davis to San Francisco General Hospital by automobile, scanned in an infant TOBEC unit and returned to UC Davis for slaughtering. Before the TOBEC

determination, the rabbits were sedated by 0.8 ml/kg Acepromazine Maleate (10 mg/ml)R subcutaneously, weighed and the distance from nose to tail was measured. The rabbits were positioned back first, upright, wrapped in a towel for gentle restraint, and scanned ten times through the magnetic field of the infant TOBEC instrument (modified EMM model M60, EM-SCAN, Auburn, IL, USA). A dry towel does not affect TOBEC measurement. This instrument produces a "TOBEC number", which is proportional to the subject's perturbation of the electromagnetic field produced by an oscillating electrical current in the machine's coil and the length of the animal.

These eight rabbits did not consume feed or water for 8 hours prior to slaughtering. The other sixteen rabbits, not subjected to TOBEC analysis, were slaughtered immediately after the last blood collection. The latter group did not experience any feed or water deprivation prior to slaughtering. This unplanned difference in treatment was due to the logistics of transporting the eight animals to and from the TOBEC measurement site, some 120 km from where the rabbits were housed and subsequently slaughtered.

Direct measurement of the body composition. All rabbits were euthanized by an intraperitoneal overdose of pentobarbiturate (pentobarbital sodium: Nembutal), the gut fill and urine removed, the whole body deep frozen, ground and sampled. The empty body was the sum of carcass, viscera, blood and hide (i.e. the difference between total body less gastrointestinal tract contents). The feeds and empty body were analyzed for water, ash, fat and crude protein (N x 6.25) as described by Garrett and Hinman (1969), except that ether extracts were prepared and determined with large Soxhlet apparatuses rather than Goldfish extractors. Feed cell wall analyses (ADF, lignin) were performed as described by Goering and Van Soest (1970). The heat of combustion of fat free dry matter and extracted body fat were determined separately by adiabatic bomb calorimetry. Body energy content of each animal was determined as "the sum of gram fat free dry matter multiplied by the heat content in kcal/g fat free dry matter" plus "gram fat x heat content per gram fat in question".

Prediction equations. Body energy, total body water, empty body water (total body water less gutfill water and urine), empty body fat, empty body lean (empty body mass minus fat), empty body ash and empty body protein were regressed on a) total body weight, b) total body weight and TOBEC number, and c) total body weight and deuterium oxide space. The GLM procedure from SAS statistical package (SAS 1986) was used to apply standard least squares procedures for multiple regression (including stepwise test of significance for each variable in a manner similar to that described by Snedecor and Cochran (1980)). SAS GLM procedures were used to contrast age/diet/sex subclasses and calculate least squares means and sample standard deviations for each subclass as well. Student's t-test was then used to contrast means of subclasses.

## Results

The rabbits used in this study showed a normal response to the two diets. Rabbits consuming oats in both age classes tended to eat less feed but gain more weight than rabbits consuming the alfalfa pellets (Table 2). Table 3 displays data indicating that oat-fed rabbits from both age classes had more body fat and energy than corresponding alfalfa-fed rabbits

Table 2. Least squares means and standard error of mean of body weight and feed intake.

	Adult		Growing	
	Oats	Alfalfa	Oats	Alfalfa
Number	6	6	6	6
Initial live weight, kg	2.90	2.88	1.87	1.77
±	0.22	0.17	0.09	0.14
Final live weight, kg	3.32	2.99	2.46	2.21
±	0.32	0.05	0.16	0.20
Empty body weight, kg	3.11	2.71	2.26	1.93
±	0.30	0.08	0.14	0.21
Feed intake, g DM/day	118	137	99	116
±	10	4	6	7

DM = dry matter.

( $P < 0.05$ ). In addition, growing oat-fed rabbits had more protein than the young alfalfa-fed animals ( $P < 0.05$ ). The males and females were about the same size, although the females may have had slightly more fat and energy than the males ( $P < 0.10$ ).

Table 4 indicates the range of body composition and predictive measurements used to create the predictive equations for the eight TOBEC rabbits (with feed restriction before slaughtering), which are found in Table 5, and the upper sections of the Tables 6 and 7. Predictive equations for the 16 other rabbits not subjected to TOBEC measurement and feed and drinking water restriction are presented in the center section of Table 6 and 7. Predictive equations for all 24 rabbits considered together without classification by feed restriction are presented at the foot of Tables 6 and 7.

Adding the TOBEC number to body weight (see Table 5) as a second independent predictor of body energy resulted in substantial reduction in the coefficient of variation (CV, i.e. 4.96 vs 8.99 %). Use of the TOBEC number also improved prediction of total (2.84 vs 4.73 %) and empty (3.73 vs 2.75 %) body water and fat (14.82 vs 18.67 %). Within the same group of eight rabbits (see Table 6), deuterium oxide dilution space resulted in a smaller improvement in body energy prediction (7.18 vs 8.99 %).

Among the 16 non-TOBEC-measured rabbits and among all 24 rabbits taken as a pool, deuterium oxide dilution space did not improve prediction of body energy (or any other body composition variable) over that attainable from body weight alone.

Discussion

Diet effects. Both diets (see Table 1) exceeded the apparent energy and protein requirements of the adult rabbits, permitting significant increases in body weight (see Table 2). The oats contained 11.47 % crude protein, which is less than the 12% crude protein the NRC (1977) indicates is needed for maintenance. The protein requirements of 1977 apparently include a safety factor of at least 4.5 %. NRC (1977) requirements for growth

Table 3. Least-squares means and standard deviations for chemical composition of all animals.

	Adult		Growing		Sex	
	Oats	Alfalfa	Oats	Alfalfa	Female	Male
Number	6	6	6	6	12	12
Total body weight, g	3317	2989	2460	2210	2786	2702
±	323	48	155	200	463	521
Empty body weight, g	3109	2713	2262	1931	2559	2449
±	295	74	139	206	468	528
Total body water, g	1774	1808	1493	1493	1611	1674
±	194	58	109	96	176	210
D2O space, g	2098	1968	1636	1604	1792	1863
±	169	185	73	29	319	271
Empty body water, g	1559	1524	1303	1232	1384	1425
±	169	49	93	92	157	197
Ether extract, g	796*	457*	360	173*	523	370
±	79	106	84	76	250	224
Crude protein, g	635	619	513*	441*	552	553
±	67	42	72	41	88	109
Ash, g	99	101	78	76	88	89
±	11	5	9	5	5	13
Gross energy, kcal	10970*	7684*	6173*	4058*	7924	6519
±	983	819	600	891	2682	2615
Lean mass, g	2313	2256	1902	1758	2036	2079
±	247	90	87	139	240	323

\* The difference between the two parallel groups is significant ( $p < 0.05$ ).

Table 4. Least squares means, range and standard deviations for chemical composition and predictive measurements of animals subjected to TOBEC measurement (n=8)†.

	Mean	Minimum	Maximum	SD
Total body weight, g	2747	1945	3620	660
Empty body weight, g	2490	1668	3365	666
Total body water, g	1846	1353	1986	254
TOBEC number	98.07	65.10	134.58	31.82
D20 space, g	1801	1182	2404	438
Empty body water, g	1395	1115	1732	241
Ether extract, g	425	102	919	303
Crude protein, g	569	375	729	134
Ash, g	91	67	114	18
Gross energy, kcal	7166	3031	12337	3432
Lean body mass, g	2065	1566	2589	3433

† This group included one male and one female from each age-diet subclass.

SD = standard deviation.

Table 5. Body composition prediction equations based on TOBEC number (TOBEC) and grams total body weight (TBWT) (n = 8).

Dependent Variable	Predictive Equation	r <sup>2</sup>	CV
Body total energy, kcal =	7.499*TBWT – 51.83*TOBEC – 8404	0.992	4.96
Total body water, g =	0.089*TBWT + 6.106*TOBEC + 802	0.982	2.84
Empty body water, g =	0.193*TBWT + 3.6.0*TOBEC + 512	0.982	2.75
Empty body fat, g =	0.680*TBWT – 5.134*TOBEC – 941	0.969	14.82
Empty body lean mass, g =	0.424*TBWT + 3.016*TOBEC + 604	0.964	4.13
Empty body ash, g =	0.011*TBWT + 0.284*TOBEC + 33	0.818	9.91
Empty body protein, g =	0.212*TBWT – 0.867*TOBEC + 73	0.715	14.92

Table 6. Body composition predicted from deuterium oxide space and total body weight.

Dependent Variable	Predictive Equation	r <sup>2</sup>	CV
<b>TOBEC Group (n = 8)</b>			
Body total energy, kcal =	6.534*TBWT - 2.318*DOS - 6660	0.984	7.18
Total body water, g =	0.200*TBWT + 0.278*DOS + 596	0.956	3.82
Empty body water, g =	0.265*TBWT + 0.152*DOS + 391	0.972	3.40
Empty body fat, g =	0.587*TBWT - 0.233*DOS - 767	0.959	17.02
Empty body lean mass, g =	0.473*TBWT + 0.147*DOS + 501	0.962	4.22
Empty body ash, g =	0.018*TBWT + 0.010*DOS + 24	0.802	10.32
Empty body protein, g =	0.188*TBWT - 0.027*DOS + 101	0.712	14.99
<b>AD LIBITUM GROUP (n = 16)</b>			
Body total energy, kcal =	5.264*TBWT + 0.528*DOS - 8134	0.823	14.67
Total body water, g =	0.356*TBWT - 0.067*DOS + 785	0.671	6.10
Empty body water, g =	0.343*TBWT - 0.046*DOS + 553	0.842	4.29
Empty body fat, g =	0.461*TBWT + 0.033*DOS - 869	0.709	27.99
Empty body lean mass, g =	0.542*TBWT + 0.002*DOS + 564	0.877	4.19
Empty body ash, g =	0.027*TBWT - 0.004*DOS + 21	0.726	7.67
Empty body protein, g =	0.162*TBWT + 0.049*DOS + 10	0.856	5.70
<b>ALL RABBITS (n = 24)</b>			
Body total energy, kcal =	5.49*TBWT - 0.507*DOS - 6930	0.895	12.63
Total body water, g =	0.325*TBWT + 0.063*DOS + 636	0.803	5.43
Empty body water, g =	0.326*TBWT + 0.41*DOS + 436	0.907	3.99
Empty body fat, g =	0.482*TBWT - 0.055*DOS - 777	0.815	24.72
Empty body lean mass, g =	0.529*TBWT + 0.052*DOS + 551	0.923	3.94
Empty body ash, g =	0.025*TBWT - 0.001*DOS + 21	0.745	8.30
Empty body protein, g =	0.171*TBWT + 0.007*DOS + 70	0.754	9.11

(2100 kcal DE/kg and 16 by protein with the oat diet and by energy with the alfalfa diet (see Table 1). Both probably occurred, since average daily gain of 11 grams with the alfalfa and 15 grams with the oats were suboptimal. It appears that alfalfa's 2169 kcal/kg DE (87% of the NRC recommendation) was more limiting to performance than the 11.47% (72% of the NRC value) oat protein. In addition, the oats may have been deficient in calcium for growing rabbits. Slightly confounded nutritional characteristics were not important here, since the purpose of employing these diets was not to contrast their nutritional value, but to create rabbit populations differing in body composition.



Table 7. Body composition prediction equations based on total body weight only.

Dependent Variable	Predictive Equation	r <sup>2</sup>	CV
TOBEC Group (n = 8)			
Body total energy, kcal =	5.123*TBW - 6959	0.970	8.99
Total body water, g =	0.369*TBWT + 632	0.920	4.73
Empty body water, g =	0.358*TBWT + 411	0.960	3.73
Empty body fat, g =	0.445*TBWT - 798	0.941	18.67
Empty body lean mass, g =	0.562*TBWT + 520	0.958	4.08
Empty body ash, g =	0.024*TBWT + 25	0.793	9.63
Empty body protein, g =	0.172*TBWT + 97	0.711	13.72
AD LIBITUM GROUP (n = 16)			
Body total energy, kcal =	5.425*TBWT - 7604	0.822	14.19
Total body water, g =	0.366*TBWT + 718	0.667	5.91
Empty body water, g =	0.329*TBWT + 507	0.839	4.17
Empty body fat, g =	0.471*TBWT - 835	0.709	27.00
Empty body lean mass, g =	0.543*TBWT + 565	0.877	4.04
Empty body ash, g =	0.026*TBWT + 17	0.723	7.43
Empty body protein, g =	0.177*TBWT + 58	0.845	5.69
ALL RABBITS (n = 24)			
Body total energy, kcal =	5.25*TBWT - 7194	0.894	12.41
Total body water, g =	0.355*TBWT + 668	0.800	5.35
Empty body water, g =	0.345*TBWT + 457	0.905	3.94
Empty body fat, g =	0.456*TBWT - 806	0.813	24.25
Empty body lean mass, g =	0.554*TBWT + 538	0.922	3.87
Empty body ash, g =	0.025*TBWT + 20	0.745	8.12
Empty body protein, g =	0.174*TBWT + 74	0.754	8.91

Alfalfa-fed rabbits had greater gut contents than oat-fed rabbits among both adult ( $9.7 \pm 0.8$  vs  $6.2 \pm 1.0$ ) and growing rabbits ( $12.7 \pm 2.0$  vs  $8.0 \pm 1.0$  % of TBW). Although this difference was statistically significant ( $P < 0.001$ ), the small absolute difference (relative to total body size) did not influence the empirical prediction of body composition in any significant way. Neither consideration of each diet group as a separate population nor inclusion of diet as a factor for the whole population improved prediction of body composition by TOBEC measurement or deuterium oxide dilution technique.

Relative value of TOBEC and deuterium oxide dilution. Because the TOBEC device used in this trial was located 120 km from where the rabbits were fed and slaughtered, an unintended and unavoidable interruption in feed and water intake occurred. For this reason,

the eight rabbits subjected to TOBEC measurement were considered separately from the other 16 rabbits which had feed and water available right up to slaughter. The latter, less traumatic situation is usually the more desirable, since metabolic perturbations brought on by restricted feeding can interfere with other research objectives of experiments that require body composition estimates. On the other hand, the precision of body energy, water and fat estimation (as indicated by the reductions in CV) was improved slightly ( $P < 0.01$ ) by addition of deuterium oxide dilution space to body weight in the "end-feed-restricted" (TOBEC) group, but not at all in the Ad Lib group (see Tables 6 and 7). This difference was probably due to reduction in inter-animal variation in urine bladder and gastrointestinal tract contents. Upon dissection, some ad lib fed rabbits had full urinary bladders and some did not, while TOBEC rabbits' bladders were consistently empty.

The combination of TOBEC scanning with body weighing gave a greater improvement in precision than the deuterium oxide dilution, even with end-feed-restricted rabbits (CV = 5 vs 9 %;  $r^2 = 0.992$  vs  $0.970$ ; root mean square - 353 vs 640 kcal). Unlike the results of previous research at Baylor University, the ability to predict body composition was not improved by using the square root of the product of TOBEC value and length in cm (Fiorotto et al., 1987). Narrower confidence intervals would be the likely result of including more animals in the creation of regression equations.

#### Conclusions

Body energy content can be predicted from body weight and TOBEC number in medium frame meat type (e.g. New Zealand White) rabbits which have not been fed or watered for 3-4 hours prior to scanning.

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