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The prediction of body composition in poultry by estimation in vivo of total body water with tritiated water and deuterium oxide

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1. Birds (*n* 169) which varied in age, live weight, nutritional history, physiological state and genotype were slaughtered and analysed for total body water. Before slaughter, birds were injected with the water isotopes tritiated water (TOH) or deuterium oxide (D_2O), or both, to determine TOH space or D_2O space, or both, as estimates of total body water in vivo.

2. At the mean total body water of all birds determined by desiccation, of 1096·4 (SD 424·1) g, TOH space and D_2O space overestimated total body water by 10·4 and 8·5% respectively. The difference between the isotopes was significant ($P < 0·05$).

3. Based on recovery of isotope it was postulated that the main reason for the observed overestimation of total body water in vivo was incomplete recovery of isotope due to the vacuum sublimation technique. The mean recovery (%) of added isotope to whole blood after vacuum sublimation was 93·0 (SD 2·6) and 92·4 (SD 5·5) of the theoretical concentrations of TOH and D_2O respectively.

4. Nevertheless, accurate prediction of total body water was obtained from regression equations which included live weight and isotope-dilution space. Values required logarithmic (base 10) transformation before derivation of linear and multiple linear regression equations, and the precision of prediction was determined by the residual standard deviation (RSD).

5. Total body water could be predicted with nearly equal accuracy from live weight or isotope-dilution space (RSD 0·025 and 0·020 respectively). Prediction of carcass protein was more accurate from live weight (RSD 0·033) than from TOH space (RSD 0·036), and inclusion of both variables resulted in only a marginal decrease in RSD to 0·031.

6. The prediction of carcass fat and energy was markedly improved by the inclusion of isotope-dilution space in conjunction with live weight compared with live weight alone.

7. The relations show the developmental nature of body composition of domestic fowl given diets adequate in nutrients. The prediction equations demonstrate the precision possible for studies in which estimates of body composition in poultry are required without slaughter.

It is often important to predict body composition of poultry, particularly when composition is required in serial determinations and in association with other physiological and production measurements. It is usually expensive and time-consuming to slaughter and to determine the chemical composition of the large number of birds needed to measure small changes in composition and for statistical interpretation of the results. Changes in the body composition of the same bird with time may be desirable, and indirect methods for estimating these changes are therefore justified. Indirect methods are usually more accurate than using changes in live weight to predict changes in chemical composition of livestock (Reid *et al.* 1963) since there are sometimes differences in the relations between live weight and chemical components (Searle, 1970; van Gils *et al.* 1977).

Numerous studies have been carried out with a range of animal species on the application of isotope dilution techniques, usually with the water isotopes tritiated water (TOH) or deuterium oxide (D_2O) (for reviews, see Panaretto, 1968; Nagy & Costa, 1980). Isotope dilution is used to estimate total body water, and because this varies inversely with body fat content on a live weight basis, the latter can usually be predicted from the former with acceptable accuracy, for example sheep (Panaretto, 1963), cattle (Little & McLean, 1981),

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pigs (Houseman *et al.* 1973), goats (Panaretto & Till, 1963), poultry (Farrell, 1974; Kirchgessner *et al.* 1977).

In an experimental programme which included a study on the effects of quantitative feed restriction of poultry during rearing on body composition both during the restriction period and subsequently during egg production, serial slaughter and carcass analysis were required. This study provided the opportunity to examine the use of isotope dilution to estimate total body water and to derive suitable prediction equations of body composition in poultry.

TOH and D_2O were used often concurrently in the studies to be reported. D_2O is a stable isotope and its use in laying hens would not contaminate eggs. At the commencement of our work the use of D_2O had not been reported for poultry and was limited to two main studies on pigs (Houseman *et al.* 1973) and pregnant sheep (Foot & Greenhalgh, 1970).

EXPERIMENTAL

Birds and their management

The types of birds used, their age, number slaughtered and the isotope(s) injected before slaughter are given in Table 1. Group 1 birds had been reared from 42 to 154 d of age on a limited-time feed restriction programme (allowed *ad lib.* feed consumption for 24 h in every 72 h) and had been allowed *ad lib.* feed intake thereafter to slaughter. Mean rate of egg production in the 26 d before slaughter was 83·9 (SD 13·9) eggs/100 hen days. Details of birds in groups 2–7 inclusive and in groups 10 and 11 were given by Johnson *et al.* (1984). In that report the birds in groups 2–7 were derived from Expt 1 and those in groups 10 and 11 from Expt 2. Birds in group 8 were managed similarly to those in group 1, and were used for the initial study of the concurrent use of TOH and D_2O. Heavy hybrid birds (groups 9, 12 and 13) were from experiments in which feed restriction strategies were examined (R. J. Johnson and D. J. Farrell, unpublished results). Rearing management varied, both in terms of housing (deep-litter or cage) and feeding (*ad lib.*, limited-time or quantitative feed restriction) but these variables were not considered important in the context of the present paper. All birds were allowed free access to feed and water during the laying period, except for birds in group 13 where quantitative feed restriction regimens were used during the laying period (see Johnson & Farrell, 1982).

Birds received least-cost diets formulated to meet their nutrient requirements (see Johnson *et al.* 1984). Illumination was natural during rearing and was kept constant (16 h/d) during the laying period.

Isotope injection and blood sampling

Disposable plastic syringes (5 ml) fitted with 21 gauge, 38 mm needles were usually used for injection of isotopes and subsequent sampling of the blood. Syringes were weighed before and after isotope injection and the amount injected determined by difference. Blood (3–5 ml) was sampled usually from the jugular vein, but on occasions from the wing vein. Samples were placed in heparinized plastic screw-cap vials and stored at 4° before analysis.

Recovery of water from blood samples and measurement of tritium and deuterium

Water was recovered from blood by vacuum sublimation (Vaughan & Boling, 1961). For the determination of the specific radioactivity (SR) of tritium a 1 ml water sample was pipetted into a glass scintillation vial and 10 ml scintillation liquid added and thoroughly mixed. The scintillant contained (/l) 692 ml toluene, 308 ml Triton X-100, 4 g 2,5-diphenyloxazole (PPO), 0·2 g 1,4-bis-(5-phenyloxazol-2-yl) benzene (POPOP). Blanks were

Table 1. *The age and number of birds on different dietary regimens which were slaughtered to determine body composition after estimation of total body water with tritiated water (TOH) or deuterium oxide (D_2O), or both*

Group*	No. of birds slaughtered	Age (d) at slaughter	Dietary regimen†		Isotope(s) used to measure total body water
			Rearing	Laying	
1	16	280	LTR	F	TOH
2	6	39	F	—	TOH
3a	6	70	F	—	TOH
3b	6	70	LTR	—	TOH
4a	6	101	F	—	TOH
4b	6	101	LTR	—	TOH
5a	6	162	F	—	TOH, D_2O
5b	6	162	LTR	—	TOH, D_2O
5c	6	162	QR	F	TOH, D_2O
6a	6	218	F	F	TOH, D_2O
6b	6	218	LTR	F	TOH, D_2O
6c	6	218	QR	F	TOH, D_2O
7a	6	337	F	F	TOH, D_2O
7b	6	337	LTR	F	TOH, D_2O
7c	6	337	QR	F	TOH, D_2O
8	16	476	LTR	F	TOH, D_2O
9	10	476	—‡	F	TOH, D_2O
10a	4	280	F	F	TOH, D_2O
10b	4	280	LTR	F	TOH, D_2O
10c	4	280	QR	F	TOH, D_2O
11a	4	476	F	F	TOH, D_2O
11b	4	476	LTR	F	TOH, D_2O
11c	4	476	QR	F	TOH, D_2O
12a	6	126	F	—	TOH, D_2O
12b	6	126	QR	—	TOH, D_2O
13	8	307	QR	QR	TOH

* Groups 1–8 were White Leghorn × Australorp hybrid birds; groups 9, 12 and 13 were broiler breeders (heavy hybrids); groups 10 and 11 were White Leghorn × New Hampshire hybrid birds. For details see p. 110.

† All diets were formulated to meet known nutrient requirements (Agricultural Research Council, 1975), but feed intake was either *ad lib.* (F) or restricted by limited-time (LTR) or quantitative (QR) methods to control live weight to approximately 60–70% of F birds at end of rearing (154 of age).

‡ Not known.

prepared with distilled water. Samples prepared in this manner were counted (Packard Tri-carb Scintillation Counter) for a 10 min period. Standards were prepared by diluting a weighed portion of the injection solution (3–5 μCi/g) in distilled water. Counting efficiencies were determined by a quench correction equation based on standard techniques, and counts obtained for water samples derived from blood, blanks and injection solution were corrected accordingly.

D_2O concentration (v/v) was measured in a twin-beam infra-red spectrophotometer (Perkin Elmer Model 564). The basis of the measurement was described by Stansell & Mojica (1968) and the procedures were essentially those of Turner *et al.* (1960) and Foot & Greenhalgh (1970) using calcium fluoride windows in semi-permanent cells. Standards were prepared by the addition of known volumes of D_2O (997 g/kg; Koch-Light Laboratories) to distilled water and concentration (g/kg) calculated according to

procedures given by Foot & Greenhalgh (1970), on the assumption that the density of deuterium was 1·1072 g/ml at 20°.

TOH space and D_2O space were calculated according to standard isotope dilution procedures.

Duration of starvation, site of injection and equilibration time

A preliminary study was carried out to determine the effects of duration of starvation before injection of TOH and site of injection on the accuracy of estimation of total body water. Sixteen birds (group 1 in Table 1) were divided into four equal groups, two of which received intraperitoneal TOH injections while the other two received intramuscular (right leg muscle) TOH injections (4·44 μCi/kg live weight). For each site of injection one group of birds was deprived of food for 24 h and the other group for 2 h before TOH injections. Drinking water was removed from all birds 2 h before TOH injection. Birds were slaughtered 6 h after injection and carcass analyses were carried out as described later (see p. 113). Serial blood samples were taken at approximately 0·5, 1·0, 2·0, 3·0 and 5·0 h after injection to determine equilibration time of the isotopes. Results are not presented but showed that equilibrium was complete by approximately 1·5 h after injection. To avoid any possibility of errors due to equilibration it was decided on a standard basis in future studies to sample blood 3 h after injection. There were no significant differences between groups in the estimation of TOH although intraperitoneal injections increased the variability. Additionally there was some concern that intraperitoneal injections may damage the reproductive organs of laying hens. Starvation for a 24 h period tended to decrease total body water (g/kg live weight) but did not influence the accuracy of estimation of total body water by TOH.

Based on these preliminary observations the following standard procedure was used in all subsequent determinations (groups 2–13 in Table 1): (a) water and food were removed 2 h before intramuscular injection of isotope; (b) TOH (4–10 μCi/kg live weight) or D_2O (1–2 g/kg live weight), or both, were injected into the right and left legs respectively; (c) blood was sampled 3 h after isotope injection, at which time live weight was recorded. To evaluate the unlikely possibility that the equilibration time of D_2O was different from that of TOH, or that the equilibration time for heavy-hybrid layers was slower than that for the medium-hybrid birds used in preliminary studies, serial blood samples were taken from birds in group 9 after concurrent injections of TOH and D_2O. This study confirmed that a 3 h delay after injection was the most appropriate time interval for sampling of blood from birds up to a live weight of approximately 4 kg, and showed no gross differences in equilibration time between TOH and D_2O.

Recovery of isotope from blood

Initial studies on poultry here and previously (for example, see Farrell, 1974) confirmed those on other animal species (for example, see Panaretto, 1963; Little & McLean, 1981) that water isotopes overestimated actual total body water determined by desiccation. On the assumption that this implied loss of the injected isotope, a series of experiments were carried out in which the recovery of known quantities of isotopes added to whole blood before vacuum sublimation was determined. Blood was obtained from pullets or laying hens which had not previously been injected with either TOH or D_2O. Weighed amounts of blood were added to glass vials and known amounts of either TOH or D_2O were added. The exact quantities of isotope added were determined by difference in weight. The vials were capped, shaken and allowed to stand at room temperature (approximately 20°) for 24 h. Moisture content of blood was determined by freeze-drying. Isotope concentration found after vacuum sublimation was compared with the theoretical concentrations

calculated on the basis of blood moisture content determined by freeze-drying and the quantity of added isotope.

Slaughter and carcass analysis procedures

All birds were killed by cervical dislocation then placed in plastic bags which were sealed and stored at −20° until analysed. Whole birds were chopped into sections in the frozen state and macerated in a meat grinder twice to give a fine mince. The usual procedure for chemical analyses was to take immediately a 5 g sample of the minced carcass for crude protein (nitrogen × 6·25) determination by a Kjeldahl technique. The remaining carcass mince was re-frozen (−20°) and samples were taken by using a core sampler attached to an electric drill. Duplicate samples (20–30 g) for each bird were placed in dried and pre-weighed cellulose extraction thimbles and were either oven-dried (OD) in a force-draught oven at 70° for 4–5 d or freeze-dried (FD) for 14 d to determine dry matter (DM) content. Three studies were carried out to determine any differences in DM content due to the method of drying. Carcass samples from birds in groups 8, 10 and 12 (Table 1) were used for this purpose. Results (g DM/kg) were as follows: group 8 (*n* 16) 441 (SD 29) for OD, 447 (SD 37) for FD; group 10 (*n* 12) 435 (SD 19) for OD, 442 (SD 30) for FD; group 12 (*n* 18) 421 (SD 44) for OD, 433 (SD 52) for FD. There was a small but non-significant ($P = 0{\cdot}30$) decrease in DM due to drying samples by OD compared with the FD technique. No distinction is therefore made between DM determined by the two methods in the present report.

After DM determination the extraction thimbles containing the dried carcass samples were immediately placed in a Soxhlet apparatus and extracted with solvent (light petroleum, b.p. 40–60°) for 24 h; then dried (100° for 24 h) before re-weighing. Loss of weight was considered to be light petroleum extractives, referred to here as fat. For ash determinations 5 g samples of carcass mince were placed in tared porcelain crucibles, dried at 80° for 24 h and combusted at 600° for 4 h in a muffle furnace. Energy content of the carcass was calculated from the quantities of protein and fat using the values 23·8 and 39·7 kJ/g respectively (Zaniecka, 1969).

Statistical analyses

Regression and covariance analyses were carried out using standard procedures (Steel & Torrie, 1960). Regression analyses were first carried out on 'raw' values and the assumptions that the errors were independent with zero mean and a constant variance, and distributed normally were tested by examination of the residuals as described by Draper & Smith (1981).

RESULTS

Recovery of isotopes from blood in vitro

Typical results are given in Table 2. The mean recoveries (%) of added TOH and D_2O from whole blood samples (*n* 47 for each isotope) after vacuum sublimation were 93·0 (SD 2·6) and 92·4 (SD 5·5) respectively. Studies using distilled water gave 100% recovery of added isotopes. The significant ($P < 0{\cdot}01$) relations between percentage recovery and theoretical concentration of isotope in the blood was for TOH:

$$Y = 101{\cdot}9 - 1{\cdot}85 \log X, \quad n\ 47, \quad R^2\ 0{\cdot}138, \quad \text{RSD}\ 2{\cdot}4,$$

and for D_2O:

$$Y = 84{\cdot}88 - 12{\cdot}20 \log X, \quad n\ 47, \quad R^2\ 0{\cdot}377, \quad \text{RSD}\ 4{\cdot}4$$

where Y is the measured amount of isotope (disintegrations/min per g for TOH and g/kg for

Table 2. *Measured recoveries of tritium using vacuum sublimation after different quantities of tritiated water were added to whole blood of birds*

Calculated activity (disintegrations/min per g blood water)	Replicate	Measured activity (disintegrations/min per g blood water)*	Recovery (%)†
5561	1	5526	99·4
	2	5428	97·6
	3	5247	94·4
	4	5438	97·8
11671	1	11102	95·1
	2	10914	93·5
	3	11030	94·5
	4	10167	87·1
51218	1	47908	93·5
	2	47243	92·2
	3	47353	92·5
	4	46805	91·4

* Activity of recovered water after vacuum sublimation of blood. For details, see p. 112.

† Recovery calculated by: $\frac{\text{activity measured (disintegrations/min per g)}}{\text{theoretical activity (disintegrations/min per g)}} \times 100.$

D_2O) in the water after vacuum sublimation of the blood expressed as a percentage of the theoretical activity, and X is the theoretical activity of isotope in blood water before vacuum sublimation.

Estimation of total body water by TOH and D_2O

Mean live weight, total body water, TOH space and D_2O space of birds in each of the groups are given in Table 3. Observations on one bird (group 8) were discarded due to loss of the collected blood sample. For all birds combined (n 169), TOH space overestimated total body water by 10·2 (range 4·8–16·1)%. For birds injected with both TOH and D_2O (n 115) concurrently, TOH overestimated total body water by 10·6% and D_2O by 9·2%. Covariance analysis of the linear relations between total body water and either TOH or D_2O space showed that at the same mean total body water, TOH space was significantly ($P < 0{\cdot}05$) greater than D_2O space.

Prediction of body composition

Determined values of body fat, protein and ash for each of the groups of birds are given in Table 4.

There was a linear ($P < 0{\cdot}05$) relation between body fat (g/kg live weight) and total body water (g/kg live weight), which was:

$$Y = 805 - 1{\cdot}11\ X, \quad n\ 169, \quad r\ 0{\cdot}94, \quad \text{RSD}\ 19{\cdot}2,$$

where Y is body fat (g/kg live weight) and X is total body water (g/kg live weight).

Analyses (see p. 113) showed that all values required logarithmic (base 10) transformation before the derivation of prediction equations. For all relations tested, it was found that residuals were not distributed normally, but increased as means increased. Logarithmic transformation of variables was found to correct these problems. Accordingly, log transformed linear and multiple linear regression models for the prediction of total body

Table 3. *Live weight (W) and total body water (TBW) of birds slaughtered, and tritiated water (TOH) space and deuterium oxide (D_2O) space and their estimation of TBW determined before slaughter using isotope-dilution techniques (ET and ED respectively)*

(Mean values and standard deviations for individual observations)

					Isotope dilution space				Estimation (%)‡			
	W (g)†		TBW (g/kg W)		TOH (g/kg W)		D_2O(g/kg W)		ET		ED	
Group*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1980·5	163·0	520·8	33·3	589·4	44·4	—		13·2	4·7	—	
2	429·2	4·8	693·9	6·4	758·9	7·3	—		9·4	0·8	—	
3a	849·5	75·0	643·1	12·3	679·2	11·1	—		5·6	1·4	—	
3b	795·5	61.6	654·3	5·5	699·9	23·9	—		7·0	3·2	—	
4a	1175·7	78·9	653·1	22·8	716·9	26·8	—		9·8	3·2	—	
4b	1080·8	58·7	656·0	21·0	708·5	22·0	—		8·0	1·4	—	
5a	1817·0	123·0	560·2	5·7	613·2	8·0	577·3	11·8	9·5	1·4	3·1	1·9
5b	1540·2	145·0	589·9	14·1	652·4	36·6	599·6	30·1	10·6	4·4	1·6	4·4
5c	1404·2	86·3	584·3	15·7	640·7	21·0	590·6	29·6	9·7	1·6	1.1	3.6
6a	1842·3	234·3	552·1	13·6	610·5	31·0	598·6	29·6	10·7	6·7	8·5	5·6
6b	1743·2	208·3	545·0	19·7	593·7	20·6	593·4	25·8	8·9	2·2	8·9	2·9
6c	1757·0	117·4	564·1	21·3	615·4	26·2	628·6	24·8	9·1	1·9	11·5	5·1
7a	2151·5	220·0	553·2	26·6	588·9	11·7	593·4	17·4	6·6	4·2	7·5	3·6
7b	1811·8	184·6	560·7	23·6	617·9	19·2	605·9	17·1	10·3	2·9	8·2	4·3
7c	1891·8	204·0	560·5	13·8	639·7	17·8	621·2	39·5	14·2	7·7	10·9	7·1
8	2046·5	284·5	560·5	27·5	598·6	49·0	604·5	49·0	6·7	5·9	7·8	6·4
9	3581·3	456·8	541·9	18·9	601·4	39·2	573·9	35·3	10·9	4·5	5·9	4·2
10a	1930·8	185·2	553·6	23·2	602·5	30·2	608·3	37·4	8·8	1·8	9·8	3·5
10b	1720·5	147·8	569·3	8·4	642·5	12·6	651·5	14·7	12·9	1·1	14·4	2·3
10c	1803·0	83·8	581·1	13·8	639·8	21·2	642·0	20·3	10·1	3·0	10·5	3·4
11a	1939·8	209·1	555·5	15·6	623·8	53·1	679·9	52·7	12·2	6·9	22·4	9·8
11b	1791·8	143·4	542·8	8·8	632·8	12·1	614·3	15·9	16·6	3·0	13·2	2·8
11c	2268·3	295·0	556·7	21·6	582·2	43·9	569·7	36·7	4·8	9·9	2·5	8·8
12a	3407·8	144·6	528·7	30·4	600·9	48·5	592·7	42·5	16·1	0·6	12·1	3·5
12b	2366·0	152·6	621·7	20·5	712·8	22·2	708·7	20·4	12·0	2·5	14·1	3·8
13	3710·6	356·7	547·6	17·1	602·8	28·6	—	—	10·2	7·2	—	—

* For details of age, nos. of birds and dietary regimen at slaughter see Table 1.

† Measured 3 h after isotope injection when equilibrium blood samples were taken.

‡ Calculated as follows: $ET = \frac{TOH - TBW}{TBW} \times 100$, $ED = \frac{D_2O - TBW}{TBW} \times 100$, where all values used were in g/kg W.

Table 4. *Body composition determined after slaughter by chemical analyses of different groups of poultry*

(Mean values and standard deviations)

	Fat (g/kg W)		Protein (g/kg W)		Ash (g/kg W)	
Group*	Mean	SD	Mean	SD	Mean	SD
1	231·1	45·8	197·0	22·2	nd	nd
2	58·4	4·0	205·4	6·9	40·0	1·9
3a	96·2	21·0	217·3	8·2	43·4	5·8
3b	86·5	7·8	213·4	15·3	43·4	6·3
4a	77·3	22·0	227·5	5·5	41·4	2·0
4b	85·5	15·2	214·7	10·0	37·8	4·9
5a	155·3	10·7	210·0	9·0	41·4	7·9
5b	129·1	18·5	209·5	17·9	37·0	2·0
5c	130·0	15·8	211·4	12·9	38·5	4·6
6a	205·4	27·0	202·3	12·7	31·0	6·4
6b	198·7	29·8	211·2	13·8	36·8	5·2
6c	183·7	13·6	209·1	9·0	32·3	5·2
7a	209·4	25·3	185·2	12·2	30·1	2·7
7b	191·7	19·1	199·6	11·9	26·8	4·5
7c	184·1	17·8	200·9	10·0	30·4	7·3
8	185·3	33·3	189·2	14·4	40·9	6·3
9	199·4	22·6	193·8	13·8	35·0	6·3
10a	189·6	34·7	210·7	20·2	33·1	4·6
10b	158·1	12·3	221·4	10·5	31·0	2·6
10c	156·2	21·1	212·9	5·4	37·5	3·8
11a	192·9	16·4	198·9	13·6	40·6	8·5
11b	201·9	16·5	199·2	14·5	36·2	8·8
11c	209·6	30·4	186·4	16·7	35·7	8·2
12a	253·9	39·2	180·1	3·7	26·2	5·9
12b	111·7	20·8	218·7	5·5	33·2	4·3
13	225·0	9·8	182·0	11·7	30·1	8·0

W, live weight; nd, not determined.
* For details, see Table 1.

water, fat, protein and energy for all birds (*n* 169) in which TOH space was determined are given in Table 5. Similar equations are given in Table 6 for those birds (*n* 115) for which D_2O space was determined concurrently with TOH space. Table 6 therefore allows direct comparisons to be made concerning the relative merits of using either TOH or D_2O to predict body composition.

There were good relations between the major chemical components and live weight. Inclusions of either TOH or D_2O space increased the accuracy of prediction of all components, particularly body fat. However inclusion of either TOH or D_2O space in conjunction with live weight for the prediction of body protein resulted in a relatively small improvement in the precision of prediction (see eqns (9) and (11) in Table 5 and eqns (22), (23) and (24) in Table 6). The prediction of body fat was markedly improved when either TOH or D_2O space was included with live weight in the regression model (for example, see eqns (4) and (8) in Table 5).

Table 5. *Log-transformed linear and multiple linear regression models for the prediction of total body water (TBW), fat, protein and energy in poultry* (n *169*) *using tritiated water (TOH) space and live weight (W)*
(Regression coefficients and standard errors)

Dependent variable	Model and independent variables	*a*	SE	*b*	SE	*c*	SE	R^2	RSD	Equation no.
Log TBW (g)	$a+b$ log W	0·193	0·030	0·866	0·009			0·982	0·025	1
	$a+c$ log TOH	0·019	0·026			0·986	0·008	0·988	0·020	2
	$a+b$ log W $+c$ log TOH	0·065	0·023	0·318	0·041	0·626	0·046	0·990	0·017	3
Log fat (g)	$a+b$ log W	−3·051	0·131	1·693	0·040			0·913	0·106	4
	$a+c$ log TBW	−3·097	0·195			1·845	0·065	0·829	0·149	5
	$a+b$ log W $+c$ log TBW	−2·305	0·066	5·047	0·132	−3·872	0·151	0·982	0·048	6
	$a+c$ log TOH	−3·091	0·196			1·818	0·064	0·828	0·150	7
	$a+b$ log W $+c$ log TOH	−2·500	0·099	4·070	0·175	−2·714	0·198	0·958	0·073	8
Log protein (g)	$a+b$ log W	−0·401	0·041	0·909	0·013			0·968	0·033	9
	$a+c$ log TOH	−0·565	0·047			1·023	0·015	0·964	0·036	10
	$a+b$ log W $+c$ log TOH	−0·487	0·042	0·540	0·075	0·422	0·085	0·972	0·031	11
Log energy (kJ)	$a+b$ log W	0·034	0·064	1·315	0·020			0·964	0·052	12
	$a+c$ log TOH	0·058	0·113			1·432	0·037	0·899	0·087	13
	$a+b$ log W $+c$ log TOH	0·298	0·049	2·450	0·087	−1·297	0·098	0·982	0·036	14

R^2, coefficient of determination; RSD, residual standard deviation.

Table 6. *Log-transformed linear and multiple linear regression models for the prediction of total body water (TBW), fat, protein, ash and energy in poultry* (n *116*) *using tritiated water (TOH) space, deuterium oxide (D_2O) and live weight (W)*

Dependent variable	Model and independent variables	*a*	SE	*b*	SE	*c*	SE	R^2	RSD	Equation no.
Log TBW (g)	$a+c$ log D_2O	0·112	0·066			0·952	0·021	0·947	0·025	15
	$a+c$ log TOH	0·016	0·055			0·981	0·018	0·964	0·021	16
	$a+b$ log W $+c$ log D_2O	−0·020	0·044	0·605	0·048	0·348	0·050	0·978	0·016	17
	$a+b$ log W $+c$ log TOH	−0·049	0·039	0·495	0·045	0·474	0·047	0·982	0·014	18
Log fat (g)	$a+b$ log W	−2·108	0·238	1·410	0·072			0·805	0·089	19
	$a+b$ log W $+c$ log D_2O	−2·054	0·210	2·685	0·227	−1·382	0·236	0·826	0·078	20
	$a+b$ log W $+c$ log TOH	−1·916	0·174	3·383	0·202	−2·167	0·214	0·882	0·064	21
Log protein (g)	$a+b$ log W	−0·156	0·081	0·836	0·025			0·912	0·030	22
	$a+b$ log W $+c$ log D_2O	−0·173	0·073	0·425	0·079	0·446	0·082	0·929	0·027	23
	$a+b$ log W $+c$ log TOH	−0·203	0·071	0·350	0·083	0·534	0·088	0·933	0·026	24
Log energy (kJ)	$a+b$ log W	0·458	0·120	1·187	0·036			0·904	0·045	25
	$a+b$ log W $+c$ log D_2O	0·481	0·110	1·750	0·119	−0·609	0·124	0·922	0·041	26
	$a+b$ log W $+c$ log TOH	0·548	0·093	2·118	0·108	−1·023	0·115	0·945	0·034	27

R^2, coefficient of determination; RSD, residual standard deviation.

DISCUSSION

The significant relations between live weight and the quantities of water, protein, fat and ash in the body enable their estimation from live weight (Reid *et al.* 1968; Burton & Reid, 1969; Searle, 1970). However the existence of interrelations between the body components which are independent of live weight (Moulton, 1923; Reid *et al.* 1955; Bailey *et al.* 1960), in addition to the influence of diet (van Gils *et al.* 1977), make the measurement of total live weight essential for accurate prediction of body composition. Isotope dilution can estimate total body water in vivo which can be used to predict the remaining body components with equations previously established by slaughter and carcass analysis. The accuracy with which isotopes can estimate total body water is therefore an important determinant of the ultimate precision of body-composition prediction.

In the present study isotope dilution space was greater than total body water determined by desiccation of carcass material irrespective of the type of isotope used (tritium or deuterium). This finding is similar to results found in other animal species, some of which are summarized in Table 7. The magnitude of the overestimation using TOH was less than that found in broiler chickens (Farrell, 1974) and medium-hybrid laying hens (Farrell & Balnave, 1977), but given the many potential sources of error in the technique it is not surprising that differences occur between and within studies. These errors can be divided into two categories, (1) those which occur in the direct measurement of total body water, (2) those which occur due to the use of isotopes. Potential errors in the direct measurement of total body water include loss of carcass water during mincing, sampling errors and weighing and desiccation errors. Procedures adopted in the present study essentially eliminated these sources of error, and the extent of 'bound' muscle water (Ling & Negendank, 1970), not measured by desiccation, is likely to be balanced by a corresponding lack of exchange of isotope hydrogen during equilibration (Hazelwood & Nichols, 1969). The majority of the discrepancy between actual total body water and isotope dilution space in the present and previous studies (see Table 7) can be attributed to non-random errors associated with the use of isotopes *per se*.

Two sources of error associated directly with the use of H isotopes are insufficient equilibration time after injection, and isotope fractionation effects in vivo (Pinson, 1952). As in the present study, time of equilibration was sufficiently well investigated in most other studies so that this source of error can be discounted. Isotope fractionation in vivo has been observed between liver water and blood water in rabbits (McManus *et al.* 1969) and at the alveolar membrane in pigeons and rats (Siri & Evers, 1962; Hatch & Mazrimas, 1972; Rubsamen *et al.* 1979). This latter aspect was tested (R. J. Johnson and D. J. Farrell, unpublished results) by using respiration chambers (Farrell, 1972) in which expired moisture was collected and radioactivity determined after TOH injection into two laying hens. As with the studies previously reported, it was found that expired moisture was not at equilibrium with the total body water, being lower in radioactivity than body water.

Most workers who have investigated the estimation of total body water by isotope dilution have concluded that overestimation of total body water was due to exchange of isotope H with non-aqueous H. However, calculations by Culebras & Moore (1977) showed that for rats the maximum amount of non-aqueous exchangeable H was approximately 5% of the total exchangeable H, and Farrell and others using sheep (Farrell & Reardon, 1972) and laying hens (Farrell & Balnave, 1977) found by combustion that only 0·2 and 0·5% of an injected dose of TOH was recovered in the total dry carcass material of sheep and poultry respectively. Culebras *et al.* (1977) concluded that the overestimation of total body water by isotope dilution usually observed was due to the use of poor and inaccurate techniques.

Table 7. *Examples from the literature on the use of tritiated water (TOH) or deuterium oxide (D_2O) to estimate total body water (TBW) in a range of animal species*

Source	Species	No. of animals	Isotope	Amount injected (/kg W)	Technique*	Isotope estimation of TBW (%)	TBW = $a+b$ (T or D)				
							a	b	R^2	RSD	CV
Panaretto (1963)	Sheep	9	TOH	10 μCi	A	+ 8·8	2·47	0·83	0·96	0·82	3·24
Searle (1970)	Sheep	61	TOH	22–154 μCi	A	+ 8·8	−0·01	0·92	0·98	0·41	2·58
Farrell & Reardon (1972)	Sheep	24	TOH	25 μCi	B	+16·3	1·32	0·81	—	0·48	2·65
Foot & Greenhalgh (1970)	Sheep	13	D_2O	1 g	C	+18·0	13·84	0·68	0·72	3·17	6·56
Crabtree *et al.* (1974)	Cattle	12	D_2O	—†	—†	+ 9·6	87·14	0·59	0·85	10·53	4·26
Little & McLean (1981)	Cattle	31	TOH	1–2 μCi	B	+22·5	7·96	0·78	0·96	5·88	4·18
Farrell (1974)	Poultry	240	TOH	17–20 μCi	B	+18·0	0·03	0·82	0·90	0·05	5·42
Farrell & Balnave (1977)	Poultry	16	TOH	3–9 μCi	B	+15·0	−0·06	0·92	—	—	—
Kirchgessner *et al.* (1977)	Poultry	19	D_2O	3 g	A	− 2·6	0·05	0·98	0·81	0·05	4·79
Houseman *et al.* (1973)	Pigs	24	D_2O	1 g	C	+ 2·3	−0·05	0·99	—	0·89	1·86
Panaretto & Till (1963)	Goats	13	TOH	19 μCi	A	+ 5·6	14	0·94	0·96	0·64	4·90

* Technique of determination of isotope concentration in blood samples was by vacuum sublimation of serum (A), vacuum sublimation of whole blood (B) or heat distillation of blood (C).

† Not given.

T, tritiated water space; D, deuterium oxide space; W, live weight; R^2, coefficient of determination; RSD, residual standard deviation; CV, coefficient of variation ((RSD/mean TBW) × 100).

The vacuum sublimation technique used to obtain a representative water sample from blood was identified as the major factor contributing to the overestimation of total body water in the present study. In addition, the observed relation between the concentration of isotope in the blood water and subsequent recovery of isotope after vacuum sublimation would account for some of the variability associated with the use of the isotopes. Such a relation could explain some of the discrepancies in the literature. For example, Stansell & Mojica (1968) found on average a 99·8 % recovery of added D_2O from serum samples after vacuum sublimation of the sample with D_2O concentrations ranging from 0·150 to 0·326 ml/l. However, Graystone *et al.* (1967) found a mean recovery of 90 % of added D_2O from blood plasma after vacuum sublimation with a range in concentration of 1·99 to 3·89 ml/l. Nielsen *et al.* (1971) used gas–liquid chromatography, in which serum samples were injected directly without previous treatment, and found a 100 % recovery of added D_2O. Similarly, direct counting was shown to give nearly complete recovery of added tritium from plasma and urine (Foy & Schnieden, 1960).

It was not possible in the present study to consider in more detail the reasons for the lack of recovery of added isotope, but the effect may be due to isotope fractionation (Riley & Brooks, 1964) caused by the water isotopes having a lower vapour pressure than normal water (Siri, 1949; Avinur & Nir, 1960). The majority of published reports on the use of TOH and D_2O to estimate total body water in animals used a vacuum sublimation technique to recover water from blood (see Table 7). Direct measurement of the SR of tritium in plasma samples was found (Smith & Sykes, 1974; Culebras *et al.* 1977) to give a marked reduction in the observed overestimation of total body water by TOH space (1–4 %).

We conclude therefore that incomplete recovery of isotope in vitro accounted for 70–80 % of the observed overestimation in vivo in the present study, and this may be the reason for the consistent overestimation of total body water by isotope dilution found in previous studies in which vacuum sublimation was used to recover a water sample from blood (see Table 7). The remaining 20–30 % is probably due to a combination of factors, some of which have already been discussed. Urinary loss of injected isotope could account for the majority of the remainder (Smith & Sykes, 1974).

There has been no previous study in which the estimation of total body water concurrently with TOH and D_2O has been compared with direct determination of total body water. However, as in the present study, Stansell & Mojica (1968) found that D_2O space was lower than TOH space in forty-six human subjects. Differences in the physical properties of tritium and deuterium (Pinson, 1952) could arguably result in a closer approximation of total body water by D_2O rather than TOH. The estimation of total body water by D_2O was more variable than with TOH in the present study (Table 3). Mean overestimation of total body water by D_2O space ranged from 1·1 to 22·4 %, while those for TOH space ranged from 4·8 to 16·1 % (see Table 3). In a total of nineteen groups of birds in which TOH and D_2O were used concurrently, the overestimation of total body water was less in ten groups when D_2O rather than TOH was used and was greater in eight groups. These findings were evident in the lower precision of prediction of total body water by D_2O rather than TOH (see Table 4). Kirchgessner *et al.* (1977) found that D_2O space underestimated total body water by 2·5 (SD 4·5) % in broiler chickens of 1600 g average live weight. An obvious area contributing to increased variability when D_2O is used is the analysis procedure which is more laborious and time consuming than TOH analysis and which requires regular standardization during sample analyses.

Both Farrell (1974) and Farrell & Balnave (1977) investigated the prediction of body composition in poultry, using broiler chickens and laying hens respectively. Farrell (1974) did not find a good relation between body fat and total body water on a live weight basis

(r 0·72), but there was only a small range in the total body water and body fat of the chickens slaughtered; means of total body water and body fat (g/kg live weight) were 625 (SD 2) and 128 (SD 2) respectively. This degree of variation can be compared with that of the present study (see Table 4), in which the range of body fat was from 52 to 305 g/kg live weight. Farrell & Balnave (1977) investigated the estimation of body fat in vivo in sixteen laying hens aged between 220 and 640 d; body fat content ranged between 90 and 390 g/kg live weight, somewhat similar to the range of that constituent in the present study. The RSD for the prediction of fat from live weight and TOH space in the study of Farrell & Balnave (1977) was 93 g, which for sixteen observations and a mean body fat of 664 g represents a coefficient of variation (CV) of 14%. This value is comparable with a CV of about 16% found in the present study for the prediction of body fat from TOH space and live weight.

The prediction equations given in the present paper are based on poultry which had received standard diets formulated with conventional ingredients to meet the known nutrient requirements. Diet is one of the most important factors which can influence the basic relations between the chemical constituents of the animal body (Hunt, 1965; van Gils *et al.* 1977). Variation in the basic relations will diminish the accuracy of the prediction of body composition. van Gils *et al* (1977) found high rates of protein deposition in young broiler chickens, where the ratio, protein: fat deposition was often very large or negative, but dependent on diet and feeding rate. However, the observed effects of diet were achieved with large variation in protein content, ranging from 250 to 700 g/kg. The results of Hunt (1965) represent a more meaningful appraisal of the possible effects of dietary protein content on the basic relations of the chemical constituents of the body, in this case body N:water ratio. Protein content of the diets used was either 220 or 150 g/kg, but the effects of these diets on body N:water ratio differed between the two experiments carried out.

The effect of feeding rate has clear implications if it alters the basic relations in a manner not related to its effect on live weight *per se*. Farrell & Reardon (1972) concluded that different prediction equations should be applied to well-nourished or undernourished sheep. This was after a prolonged period of undernutrition (> 1 year) for the undernourished sheep, in which body fat content was severely depleted, and was due, at least in part, to a greater amount of water in the rumen-reticulum of the undernourished sheep. In the present study, feed restriction was imposed only during the growing phase (about 6–22 weeks of age), and was of a severity sufficient to reduce live weight by approximately 20% at 22 weeks of age relative to birds fed *ad lib.* (Johnson *et al.* 1984). There were no permanent effects of this level of feed restriction after all birds were allowed to feed *ad lib.* at 22 weeks of age. As with the results of Burton & Reid (1969) on sheep subjected to varied levels of energy intake before slaughter, undernutrition in the present studies did not affect body composition independent of its effect on live weight (see Johnson *et al.* 1985).

The relations derived in the present study are predominantly based on medium-hybrid (layer) domestic fowl although some groups of mature heavy-hybrid (broiler) poultry were included to extend the range of measurements. The applicability of these relations to predict body composition of young, rapidly-growing meat-type (broiler) poultry would be limited primarily because these types of poultry were not included in the analysis at the lower region of live weight. However, Farrell (1974) has previously shown that isotope dilution can be used in the prediction of body composition in young broiler chickens which were given diets of a large range of nutrient densities.

The present study has clearly demonstrated the applicability of isotope dilution techniques in the estimation of body composition in vivo in poultry. The nature of the

relation between the major carcass constituents in poultry has been determined across a broad spectrum of ages, genotypes and live weights for the first time. Clearly the prediction equations based on isotope dilution space will be influenced by the degree of overestimation of TBW, and, as previously discussed, this could be affected by the method of TOH recovery from the biological fluid. Relatively small-scale validation experiments would be required before the direct use of the prediction equations, and this may or may not limit their usefulness depending on the circumstances.

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