CHAPTER 4

THE *IN VIVO* ESTIMATION OF TOTAL BODY WATER AND THE PREDICTION OF BODY COMPOSITION IN POULTRY

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4.1 INTRODUCTION

It is important to obtain direct information on the body composition of livestock (Bailey *et al.* 1960; Reid *et al.* 1963; Farrell and Balnave 1977; van Gils *et al.* 1977). Some workers have purposed a detrimental effect of excessive body fat on egg production in laying hens (Greenberg 1976; Neil *et al.* 1977) but it is difficult to slaughter and analyse the necessary number of experimental birds for adequate statistical interpretation of results. Estimates of protein and fat gain in animals can be obtained by accurate energy balance trials (Farrell 1974a) but these estimates initially assume accurate prediction of nitrogen (N) balance, an assumption which is uncertain (Owen 1967; Davidson and Williams 1968). The comparative slaughter technique for measurement of energy retention in poultry may give similar estimates to those derived by direct calorimetry (Davidson *et al.* 1968) or by indirect respiration calorimetry (Farrell 1972), but the technique is laborious and there are several potential sources of error (cf. Farrell 1974a). Also, the slaughter technique is impractical for long-term energy metabolism studies. There is difficulty in estimating the efficiency of utilization of dietary metabolisable energy for egg synthesis, where body energy reserves may be utilized with a greater efficiency (Grimbergen 1970) than dietary ME. In addition to the factors already outlined, the accurate prediction of body composition in poultry is clearly an important consideration in nutritional experiments, particularly for detailed studies of energy metabolism.

Indirect methods for predicting body composition are justified to some extent on the improvement in accuracy which can be gained over that estimated by liveweight alone (Reid *et al.* 1963). Apparent differences in the relationship between liveweight and body components (Searle 1970a) necessitate the development of an accurate indirect method. Because of the close inverse relationship between total body water and fat, indirect methods of predicting body composition are usually based on the prediction, initially, of total body water. The isotopes of hydrogen, tritium and deuterium, which can form tritiated water or deuterium oxide in certain reactions, can be injected into animals in trace amounts to give an accurate estimation of total body water (Steele 1964). The extensive use

of tritiated water and deuterium oxide for this purpose was reviewed by Panaretto (1968), and the errors associated with the use of water containing hydrogen isotopes were discussed in detail by Siri and Evars (1962) and Nagy and Costa (1980).

The application of dilution techniques using tritiated water and deuterium oxide in the estimation of total body water in poultry were clearly demonstrated by Farrell (1974b), Farrell and Balnave (1977) and Kirchgessner *et al.* (1977). In ruminants, systematic studies have been carried out to derive prediction equations for body components based on the estimation of total body water for a range of liveweights and physiological conditions (Searle 1970a,b). No such detailed studies have been conducted on poultry. Derivation of general body composition prediction equations for poultry may be difficult given the diverse genetic selection programmes for different classes of poultry enterprise (e.g., meat production, progeny production, egg production) and the rapid and marked changes in liveweight and body composition which can be affected by dietary manipulation (see Literature Review, Section 1.5.1.3). Results from other animal studies (Searle 1970a; McManus *et al.* 1969; Farrell and Reardon 1972) indicate that these factors could present special problems in the prediction of body composition in poultry. Many other facets of body composition prediction in poultry require investigation. For example, it is generally recognised that prediction equations must be able to predict body components on a liveweight, not on an empty body weight, basis (Panaretto 1963; Little and McLean 1981). Although this presents problems in animals with fermentative digestion due to the large contribution of gut contents (Reid *et al.* 1963), it should not be difficult in poultry where gut contents contribute little to total liveweight and where rates of passage are rapid (see Sturkie 1976).

The experiments reported in this chapter formed a systematic programme to derive body composition prediction equations for poultry of different ages, liveweights, type, physiological state and prior nutritional history. The first section describes experiments which were aimed at estimating the potential errors in the use of tritiated water and deuterium oxide for the prediction of total body and at validation of the procedures employed in the use of tritiated water and deuterium oxide. The derivation of equations which could be used to estimate total body water *in vivo* are also reported. Attention is focussed on this latter aim, because any relationship between total body water estimated by either

tritiated water or deuterium oxide and the other body components relies initially on high accuracy of estimation of total body water. The second section of this chapter deals with the relationships between total body water and other body components and their prediction using either tritiated water space or deuterium oxide space.

4.2 MATERIALS AND METHODS

4.2.1 Poultry

Details of the strain and age of birds, the number slaughtered for body composition determination, the isotopes used to estimate total body water (TBW), the diets and the feeding regimens of birds prior to slaughter, are given in Table 4.1. Birds were placed in individual cages prior to estimation of isotope space and their subsequent slaughter. Birds in Groups 2, 8 and 9 were previously maintained on deep litter, and those in Group 12 in flat-deck cages with three birds per cage (see Chapter 2 for details). Relevant rearing and production parameters for the experimental treatments from which birds in Groups 2-7 and 10-11 were selected are given in Chapter 3, Experiments 1 and 2 respectively. Birds in Groups 1 and 8 had been on virus innoculation experiments approximately six months prior to slaughter and were in normal egg production. The mean (±SD) egg production (number per 100 hen d) for birds in Group 1 (N = 16) in the 26 d prior to slaughter was 83.9 (±13.9). Birds in Group 9 were obtained from a local commercial broiler breeder and had been offered a commercial diet.

4.2.2 Duration of starvation and site of isotope injection

A preliminary experiment was carried out to determine the effect of duration of starvation prior to isotope injection and the effect of site of injection on the ability of tritiated water (TOH) to estimate *in vivo* total body water (TBW). Birds in Group 1 were used for this experiment. Water was removed from all birds 2 h prior to injection with tritiated water and birds were given either an intraperitoneal or intramuscular (right leg muscle) injection of tritiated water (4.32 µc/kg W) (see Section 4.2.5 for injection procedures) after a prior starvation period of either 24 hr or 2 h. Blood samples were taken at intervals after injection and after sample preparation (Section 4.2.6) and estimation of equilibrium time (Section 4.2.3), tritiated water space (T) was calculated (Section 4.2.6) and compared with actual total body water. Birds were

TABLE 4.1: The strains of birds used; the age at slaughter, and the number of birds slaughtered; the isotopes used for estimation of total body water and the feeding regimens and diets used during the relevant period

Group code	Strain of bird+	Relevant Chapters	Age (d)	Number of birds	Isotope(s) used for TBW estimation	Feeding regimes		Diet No.**	
						Rearing	Laying	Rearing	Laying
1	Layer	Present	280	16	TOH	Limited-time restriction#	*Ad libitum*	1	2
2	Layer	3, 5	39	6	TOH	*Ad libitum*	-	1	-
3a	Layer	3, 5	70	6	TOH	*Ad libitum*	-	1	-
3b	Layer	3, 5	70	6	TOH	Limited-time restriction	-	1	-
4a	Layer	3, 5	101	6	TOH	*Ad libitum*	-	1	-
4b	Layer	3, 5	101	6	TOH	Limited-time restriction	-	1	-
5a	Layer	3, 5	162	6	TOH, D_2O	*Ad libitum*	-	1	2
5b	Layer	3, 5	162	6	TOH, D_2O	Limited-time restriction	-	1	2
5c	Layer	3, 5	162	6	TOH, D_2O	Quantitative restriction	-	1	2
6a	Layer	3, 5	218	6	TOH, D_2O	*Ad libitum*	*Ad libitum*	1	2
6b	Layer	3, 5	218	6	TOH, D_2O	Limited-time restriction	*Ad libitum*	1	2
6c	Layer	3, 5	218	6	TOH, D_2O	Quantitative restriction	*Ad libitum*	1	2
7a	Layer	3, 5	337	6	TOH, D_2O	*Ad libitum*	*Ad libitum*	1	2
7b	Layer	3, 5	337	6	TOH, D_2O	Limited-time restriction	*Ad libitum*	1	2
7c	Layer	3, 5	337	6	TOH, D_2O	Quantitative restriction	*Ad libitum*	1	2
8	Layer	Present	476	16	TOH, D_2O	Limited-time restriction#	*Ad libitum*	1	2
9	Broiler breeder	Present	476##	10	TOH, D_2O	NA*	*Ad libitum*	NA*	NA*
10a	Layer	3, 5	280	4	TOH, D_2O	*Ad libitum*	*Ad libitum*	1	2
10b	Layer	3, 5	280	4	TOH, D_2O	Limited-time restriction	*Ad libitum*	1	2
10c	Layer	3, 5	280	4	TOH, D_2O	Quantitative restriction	*Ad libitum*	1	2
11a	Layer	3, 5	476	4	TOH, D_2O	*Ad libitum*	*Ad libitum*	1	2
11b	Layer	3, 5	476	4	TOH, D_2O	Limited-time restriction	*Ad libitum*	1	2
11c	Layer	3, 5	476	4	TOH, D_2O	Quantitative restriction	*Ad libitum*	1	2
12a	Broiler breeder	-	126	6	TOH, D_2O	*Ad libitum*	-	++	-
12b	Broiler breeder	-	126	6	TOH, D_2O	Quantitative restriction	-	++	-
13	Broiler breeder	7	307	8	TOH	Quantitative restriction	Quantitative restriction	1	3

+ Strains were WLxA (Hyline) for groups 1-8, Allied Mills for Group 9, WLxNH (hyline) strain cross for groups 10 and 11, and Hyline for group 12. (For more information see Section 2.1, Chapter 2).

* NA; not available.

Approximate 15% reduction in liveweight compared to *ad libitum* fed birds at 22 weeks of age.

Age is approximate only.

** See Section 2.3, Chapter 2 for details of diets.

++ See Section 2.1, Chapter 2 for details.

slaughtered immediately after the final blood sample was taken and were macerated and analysed for body composition as described in Section 4.2.8. Results, given in Table 4.2, show that there were no significant differences between treatments in the estimation of total body water. However intraperitoneal injections into laying hens could not be recommended on a routine basis due to possible damage to reproductive organs. Standard management procedures were therefore adopted in all subsequent experiments reported in this chapter: feed and water were removed 2 h prior to isotope injection; tritiated water was injected intramuscularly into the right leg; deuterium oxide, when used, was injected intramuscularly into the left leg; immediately prior to blood sampling for determination of equilibrium isotope concentration, the bird was accurately weighed.

4.2.3 Time required for the isotopes to equilibrate with body fluids

Two experiments were conducted to determine the time required for the isotopes to equilibrate with body fluids. Birds in Group 1 were used for the first experiment. Details of the procedures used are given in Section 4.2.2. The second equilibrium experiment was carried out to investigate the time required for isotope equilibrium to occur when deuterium oxide (D_2O) and tritiated water (TOH) were injected simultaneously, and to determine if any differences were apparent in equilibrium time between layer strains and broiler breeder strains. Birds in Group 9 were deprived of feed and water for 2 h prior to intramuscular injections of tritiated water (TOH) and deuterium oxide (D_2O). Serial blood samples were taken either from the jugular or wing veins (Section 4.2.5) and isotope concentration determined (Section 4.2.6).

4.2.4 Loss of tritium in the expired water vapour

Two laying hens, without feed and water for 2 h, were injected intramuscularly with tritiated water (TOH). The birds were immediately weighed and placed in two respiration chambers described by Farrell (1972) except that the drying train was replaced with a round glass flask in an ice-filled container. Water vapour was calculated by periodic weighing of the flask after correction for the estimated addition of water vapour from the potassium hydroxide (KOH) solution used for the removal and subsequent measurement of respired carbon dioxide (CO_2). One ml aliquots of the condensed water vapour were assayed for tritium activity using normal procedures (Section 4.2.6).

TABLE 4.2 The effects of duration of starvation and isotope injection site on estimation of total body water (TBW) (Group 1)[1]

Treatment	No. birds	Duration of starvation prior to injection (h)	Site of isotope injection	W[2]	TBW[3]	TOH Space[4]	Estimation[5] (%)
a	4	24	Intraperitoneal	2033(±190)	508.5(±27.3)	583.1(±40.2)	14.6(±2.1)
b	4	2	Intraperitoneal	1975(±147)	536.9(±46.6)	608.4(±61.5)	13.3(±5.2)
c	4	24	Intramuscular	2015(±173)	503.9(±31.4)	599.3(±22.4)	11.2(±5.2)
d	4	2	Intramuscular	1899(±176)	533.9(±66.2)	602.9(±48.1)	13.7(±6.5)
Significance of differences between groups				NS+	NS	NS	NS
Overall SD				172.3	33.1	45.3	5.0

1. Details of these birds are given in Section 4.2.2 and Table 4.1
2. Liveweight (±SD) at equilibrium
3. Total body water (±SD); see text for method of determination
4. Tritiated water space (±SD); see text for method of calculation
5. Calculated as $\frac{(TOH-TBW)}{TBW} \times 100$

\+ NS is Not Significant.

4.2.5 Isotope injection and blood sampling

Disposable plastic syringes (5 ml) fitted with 21 gauge, 38 mm needles were used for both isotope injection and subsequent blood sampling, except when serial blood sampling was carried out for which 23 gauge, 38 mm needles were used. An amount of isotope was drawn into the syringe via a needle tightly fitted; the syringe with needle were then accurately weighed. This procedure was carried out immediately prior to injection, and needle caps were used to prevent isotope evaporation. Tritiated water (TOH) and/or deuterium oxide (D_2O) were injected intramuscularly into the right and/or left leg respectively. A small amount of air was left in the syringe prior to injection to facilitate complete expulsion of the isotope, and pressure was exerted on the injection point to prevent leakage from the needle channel. When the needle was withdrawn the plunger was partially retracted to draw any remaining isotope back into the syringe. Syringes and needles were wiped with a tissue and reweighed as soon as possible after injection to determine the net weight of isotope delivered to the bird. On the basis of results obtained in the equilibrium experiments (see Section 4.3.1.1) blood (3-5 ml) was sampled at approximately 3 h after isotope injection and placed in heparinised plastic vials. These were sealed and stored at $4^{\circ}C$ prior to analysis. Liveweights, determined immediately before equilibrium blood sampling, were used in subsequent calculations on body composition.

4.2.6 Sample preparation and the determination of the specific radioactivity of tritium and concentration of deuterium in the body water

Blood samples were subjected to vacuum sublimation essentially by the Thunberg technique of Vaughan and Boling (1961). The water sample obtained was used for the determination of the specific radioactivity (SR) of tritium (disintegrations per minute per ml of body water (dpm/ml)) and/or deuterium oxide concentration (g/100 g body water). For tritium determination (SR) a 1 ml water sample was accurately pipetted into a glass scintillation vial, 10 ml of scintillation liquid added and the vial capped tightly and mixed. The scintillant contained (per 1 litre): Toluene (692 ml, AR); Triton X-100 (308 ml); 2,5-Diphenyloxazole (PPO) (4.0 g) and 1,4-Bis-(5-phenyloxazol-2-yl) benzene (POPOP) (0.2 g). The use of Triton X-100 in scintillants for tritium counting was described by Turner (1969). Blanks were prepared by using 1 ml of distilled water. Samples were placed in a Packard Tri-carb Scintillation counter and,

after a period of 1-2 h for temperature stabilization ($4^{\circ}C$) and elimination of chemi-luminescence, each were counted twice for 10 minutes. Standards were prepared by diluting a weighed, 1 ml aliquot of the injection solution, in 1 l or 500 ml of distilled water. These were counted with the samples.

Counting efficiencies were determined by a quench correction equation established by progressively quenching a series of vials which contained a standard solution of known activity with increased quantities of chloroform ($CHCl_3$, AR). The standard used was N-hexadecane 1,2-T in Toluene, 0.299 µc/ml on 10/7/72 (Radiochemical Centre, Amersham, England). One ml of this standard was accurately pipetted into a 100 ml volumetric flask and diluted with scintillant, and 10 ml aliquots were pipetted into nine scintillation vials and 1 ml distilled water was added to each. These vials were first counted to establish uniformity of activity then quenched and recounted at least six times. Counting time per vial was 1 minute. Either the Channels Ratio or Automatic External Standardisation methods were used to determine the extent of quenching and to estimate the efficiency of counting. Counts obtained for water samples derived from blood, injection solution and blanks were corrected accordingly.

Deuterium oxide concentration (v/v) was measured in a twin-beam infrared spectrophotometer (Perkin Elmer Model 564). The basis of the measurement was described by Stansell and Mojica (1968) and procedures used were essentially those of Turner *et al.* (1960) and Foot and Greenhalgh (1970). Semi-permanent cells (Spectroscopic Accessory Company, England) with calcium fluoride (CaF_2) windows (Quentron Optics, Australia) separated by a 0.1 mm Telfon spacer were used. The cells were filled with distilled water and placed in the beams for at least 1 h prior to commencement of measurement to allow for temperature stabilisation. Distilled water remained in the reference beam and the other cell was sequentially removed, flushed with acetone, dried with nitrogen (N_2) gas and filled with the solution to be measured. The cell was placed in the sample beam for 3 minutes prior to commencement of the scan. After a scan was finished, the sample cell was removed and the procedure repeated with the next solution. Standards were prepared by adding known volumes of deuterium oxide (Koch-Light Laboratories, England; 99.7% (w/w)) to distilled water. The deuterium oxide (D_2O) concentration in each sample was calculated by the following:

$$D_2O \text{ concentration } \% = \frac{SA - LA}{HA - LA} \times (HC - LC) + LC \times 1.10729 \text{ (w/w)}$$

where SA = absorbance of the sample, LA = absorbance of the low standard, HA = absorbance of the high standard, LC = concentration of the low standard, HC = concentration of the high standard and 1.10729 was taken as the density of deuterium at 20°C. Absorbances for standards were determined before the commencement of sample measurements, at regular intervals during sample measurements, and at the completion of sample measurements.

4.2.7 Calculation of tritiated water space (T) and deuterium oxide space (D)

Tritiated water space (T, g) was calculated by

$$T \text{ (g)} = \frac{\text{Specific activity (dpm/g) of standard x Amount injected (g)}}{\text{Specific activity (dpm/g) of equilibrium body water sample}}$$

and dpm is disintegrations/minute.

Deuterium oxide space (D, g) was calculated in a similar manner by

$$D \text{ (g)} = \frac{\text{Amount of deuterium oxide injected (g)}}{\text{Concentration (g/100 g) of deuterium oxide in equilibrium body water sample}}$$

4.2.8 Recovery of isotopes from blood

Three experiments were carried out to determine the recovery of known quantities of isotopes added to blood prior to vacuum sublimation. Blood was obtained from pullets or laying hens which had not previously been injected with an isotope. Weighed amounts of blood were added to pre-weighed glass vials and amounts of either tritiated water or deuterium oxide were added, the exact amounts being determined by difference. The vials were capped tightly, shaken and allowed to stand at room temperature for 24 h. Moisture content of blood was determined by freeze-drying similar vials. A number of samples from each of the vials which contained added isotope were taken and subjected to vacuum sublimation using normal procedures. Isotope concentration found was compared with the theoretical concentration which was calculated on the basis of determined blood moisture content and the quantity of added isotope.

4.2.9 Slaughter of the birds and subsequent analysis

Birds were killed by cervical dislocation of the neck. For birds in Groups 2-7 and 10-11 the liver was removed for separate analysis (see

Chapter 5). Birds were placed in plastic bags which were sealed and stored at -20°C. For processing the birds were partially thawed, chopped into sections and macerated in a meat grinder twice to give a fince mince. The mixed mince was placed in plastic bags and stored at -20°C until subsequent analyses for moisture, fat, protein and ash. Procedures used to sample the mince and to determine chemical composition are given in Chapter 2.

4.2.10 Calculation of maturity indices

Two indices of maturity were calculated for all birds, an age index (M) and a liveweight index (N). For the age index (M) the age of birds (wks) was expressed as a proportion of 40 wks. The liveweight index (N) was calculated for individual birds by expressing actual liveweight as a proportion of the assumed final average liveweight for that particular strain of bird. For Groups 1-8 this was assumed to be 2117 g; for Groups 9, 12 and 13 to be 4000 g, and for Groups 10 and 11 to be 2110 g.

4.2.11 Statistical procedures

Statistical procedures used are given in detail in Chapter 2. However it is appropriate here to describe the technique used to determine the residual standard deviation (RSD) of the allometric regression models derived in this chapter. Regression models on a logarithmic ($\log_{10}$) scale are initially derived according to the following (Steel and Torrie 1960):

$$\log Y = a_1 + b \log X \quad \{(R^2)_1 \quad (RSD)_1\}$$

Transformation of the above equation back to the exponential, or allometric, regression model is carried out according to the following procedure:

$$Y = aX^b \quad \{(R^2)_2 \quad (RSD)_2\}$$

Note that the regression constant (a) in the allometric equation is the antilog of (a_1). The multiple regression coefficient in the logarithmic equation $(R^2)_1$ is approximately equal to $(R^2)_2$ in the allometric equation (ie. $(R^2)_1 \equiv (R^2)_2$). However the residual standard deviation for the logarithmic equation $(RSD)_1$ is not appropriate to the re-transformed (allometric) equation $(RSD)_2$. To estimate these values for the allometric equations the body components were predicted by the allometric regression models and then subtracted from the actual values of the body components for each individual bird. This derived value was then squared and all

values were summed. The summation of these values is an estimate of the residual sum of squares (RSS) for that regression model. This is used to obtain an estimate of $(RSD)_2$. Donnelly and Freer (1974) apparently used a similar procedure.

4.3 *THE ESTIMATION AND PREDICTION OF TOTAL BODY WATER IN POULTRY USING TRITIATED WATER AND DEUTERIUM OXIDE*

4.3.1 RESULTS

4.3.1.1 Equilibrium trials

Some typical curves obtained when tritiated water was injected either intramuscularly or intraperitoneally (Group 1) are shown in Figure 4.1. Equilibrium appeared to be attained approximately 60-100 minutes after injection of the tritiated water, and this was unaffected by site of injection. Birds in Group 9 were injected intramuscularly with tritiated water and deuterium oxide concurrently, and the results for five typical birds are shown in Figure 4.1 for the variation with time in concentration of isotope after injection of tritiated water and deuterium oxide. There were no differences in equilibrium times between tritiated water and deuterium oxide. Comparison of the equilibration curves in Figure 4.1 indicates no differences in equilibrium time between the layer strain of birds in Group 1 (mean liveweight 2.23 kg) and the broiler breeders in Group 9 (mean liveweight 3.58 kg). On the basis of these results, blood sampling to determine isotope dilution space was carried out 3 h after isotope injection, and for the two trials in which serial blood sampling occurred, the blood sample taken at approximately 3 h was used to determine isotope dilution space.

4.3.1.2 Evaporative water vapour loss of tritium

The estimated evaporative water vapour loss of tritium is given in Table 4.3. The amount of tritium found in the collected evaporative water as a percentage of the quantity of injected tritiated water for Bird 1 during the period 0-195, 195-524, 524-1457 and 0-1457 minutes after tritiated water injection were 0.2%, 0.4%, 1.1% and 1.8% respectively, and for bird 2 were 0.2%, 0.5%, 1.7% and 2.5% respectively. The heat production (HE) determined using the amounts of carbon dioxide produced and oxygen consumed (Brouwer 1965, see Chapter 7, Equation 7.4) for Birds 1 and 2 were 776.9 and 653.2 kJ/d respectively. Assuming 2.5 kJ/g as the latent heat of vaporisation of water the insensible heat production as a proportion of the total heat production (kJ/d) was 17%

FIGURE 4.1: The change with time in the concentration of tritium or deuterium oxide in the body water of poultry after injection of isotope.

A - Group 1, tritiated water injection:

Bird 47 - treatment a (■);
Bird 5 - treatment b (○);
Bird 2 - treatment c (▲);
Bird 53 - treatment d (●);

(see Table 4.2 for treatment details).

B - Group 9, tritiated water injection:

Bird 1 (△);
Bird 2 (▲);
Bird 3 (○);
Bird 8 (●);
Bird 9 (■).

C - Group 9, deuterium oxide injection:

Bird 1 (●);
Bird 2 (□);
Bird 3 (○);
Bird 8 (▲);
Bird 9 (△).

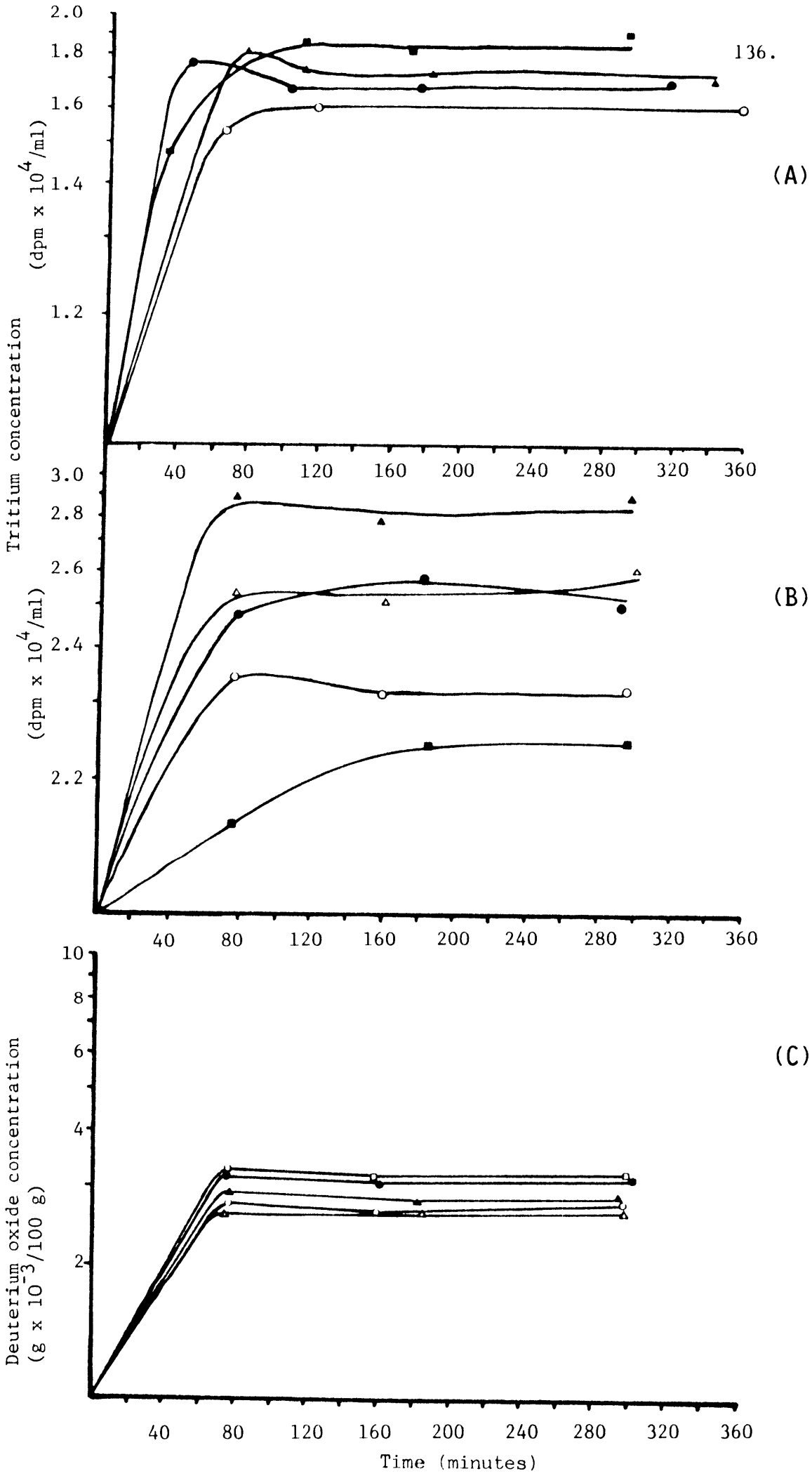

(A)
Tritium concentration (dpm x 10^4/ml)
2.0
1.8
1.6
1.4
1.2
40 80 120 160 200 240 280 320 360
(B)
(dpm x 10^4/ml)
3.0
2.8
2.6
2.4
2.2
40 80 120 160 200 240 280 320 360
(C)
Deuterium oxide concentration (g x 10^{-3}/100 g)
10
8
6
4
2
40 80 120 160 200 240 280 320 360
Time (minutes)

TABLE 4.3 Evaporative water vapour loss of tritium by two laying hens during 24 h in closed circuit respiration chambers after injection of tritiated water.

Bird No.	Liveweight (W, kg)	Amount of tritiated water injected (μc/kgW)	Time after injection (mins)	Evaporative water vapour collected[1] (g)	Measured activity[2] (dpm/ g water)	Evaporative water vapour loss of activity[3] (dpm)
1*	2147	3.625	0-195	10.11	3970	40137
			195-524	14.91	4845	72239
			524-1457	28.49	6827	194501
			0-1457	53.51		306877
2	1873	4.436	0-195	15.50	2436	37758
			195-524	19.89	5061	100663
			524-1457	35.60	9051	322216
			0-1457	70.99		460637

* Oviposition occurred during period of measurement

1. Corrected for loss of water vapour from potassium hydroxide (KOH) flask, the change in weight of which was corrected for addition of carbon dioxide (CO_2) and was assumed to be linear over 24 h.

2. Measured activity corrected for dilution by water vapour from KOH flask assuming negligible activity in water vapour from KOH flask.

3. Total estimated loss of activity from evaporative water vapour during period indicated.

for Bird 1 and 27% for Birds 1 and 2 respectively. Mean chamber temperatures were approximately 22°C.

4.3.1.3 Recovery of tritiated water and deuterium oxide from distilled water and blood

The tritium activity and deuterium oxide concentration measured after vacuum sublimation of blood and distilled water samples which contained known amounts of the isotopes are given in Tables 4.4 and 4.5 (Experiment 1), Tables 4.6 and 4.7 (Experiment 2) and in Table 4.8 (Experiment 3). Recoveries were carried out with distilled water (Experiment 1) to determine the accuracy of the procedures used, and the results indicated good recovery of the added isotopes. Vacuum sublimation of blood samples which contained known quantities of tritiated water or deuterium oxide caused apparent fractionation of the isotope to occur with a consequent reduction in the activity of tritium or concentration of deuterium oxide in the sublimated water sample relative to the calculated theoretical activity or concentration in the blood samples. The mean recovery (±SD) of added tritiated water from blood samples (N = 47) after vacuum sublimation was 93.0 (±2.6)%; the mean recovery (±SD) of added deuterium oxide from blood samples (N = 47) after vacuum sublimation was 92.4 (±5.5)%. Recoveries of tritium or deuterium oxide from blood samples were concentration-dependent. The relationship between recovery of tritium and the theoretical activity of tritium in blood was

$$Y = 101.89 - 1.85 \text{ Log } X \qquad (4.1)$$

$$N = 47; \quad R^2 = 0.138; \quad RSD = 2.4; \quad P < 0.010$$

where Y is the measured recovery of tritium from blood after vacuum sublimation, and

X is the theoretical activity of tritium in blood prior to vacuum sublimation.

The relationship between recovery of deuterium oxide and the theoretical concentration of deuterium oxide in blood was

$$Y = 84.88 - 12.20 \text{ Log } X \qquad (4.2)$$

$$N = 47; \quad R^2 = 0.377; \quad RSD = 4.4; \quad P < 0.001$$

where Y is the measured recovery of deuterium oxide from blood after vacuum sublimation, and

X is the theoretical activity of deuterium oxide in blood prior to vacuum sublimation.

TABLE 4.4 Recoveries of different quantities of tritiated water from blood and distilled water after vacuum sublimation of the sample (Experiment 1)

Medium	Theoretical activity of TOH[1] (dpm/g water)	Replicate	Activity measured[2] (dpm/g water)	Recovery[3] (%)
Blood	24761	1	23519	95.0
		2	23620	95.4
		3	23855	96.3
Blood	47626	1	43865	92.1
		2	42059	88.3
		3	43132	90.6
Blood	73550	1	67089	91.2
		2	66998	91.1
		3	69593	94.6
Distilled water	14039	1	14187	101.1
		2	14172	101.0
		3	14507	103.3
Distilled water	35506	1	34599	97.5
		2	35389	99.7
		3	35102	98.9
Distilled water	46746	1	46548	99.6
		2	46046	98.5
		3	45672	97.8

1. Calculated by:

$$\frac{\text{Activity of tritiated water added x Quantity added}}{\text{Quantity of blood in vial x Moisture content of blood}} \times 100$$

2. Activity measured using normal scintillation counting procedures (see Section 4.2.6) after prior vacuum sublimation using the Thunberg method.

3. Recovery calculated as:

$$\frac{\text{Activity measured}}{\text{Theoretical activity}} \times 100$$

TABLE 4.5 Measured recoveries of different quantities of deuterium oxide from blood and distilled water after vacuum sublimation of the sample (Experiment 1)

Medium	Theoretical concentration of D_2O (%, w/w)[1]	Replicate	Concentration of D_2O measured (%, w/w)	Recovery[2] (%)
Blood	0.1845	1	0.1896	102.8
		2	-	-
		3	-	-
Blood	0.2739	1	0.2590	94.6
		2	0.2177	79.5
		3	0.2610	95.7
Blood	0.8444	1	0.7492	88.7
		2	0.7581	89.8
		3	0.7862	93.1
Distilled water	0.1003	1	0.0984	98.1
		2	0.0994	99.1
		3	0.1008	100.5
Distilled water	0.3866	1	0.3758	97.2
		2	0.3759	97.2
		3	0.3849	99.6

1. Calculated by:

$$\frac{\text{Quantity of } D_2O \text{ added}}{\text{Quantity of blood in vial x Moisture content of blood}} \times 100$$

2. Recovery calculated as:

$$\frac{\text{Concentration measured}}{\text{Theoretical concentration}} \times 100$$

TABLE 4.6 Measured recoveries of different quantities of tritiated water (TOH) from blood after vacuum sublimation (Experiment 2)

Sample No.	Theoretical activity of TOH[1] (dpm/g blood water)	Replicate	Activity measured[2] (dpm/g blood water)	Recovery[3] (%)
1	36372	1	32912	90.5
		2	33083	91.0
2	42394	1	39850	94.0
		2	39866	94.0
3	56159	1	51891	92.4
		2	52547	93.6
4	111260	1	106417	95.7
		2	106097	95.4
5	122022	1	113656	93.1
		2	116012	95.1
6	165431	1	149051	90.1
		2	147817	89.4
7	192538	1	174475	90.6
		2	178637	92.8
8	211239	1	190914	90.4
		2	197471	93.5
9	248330	1	219036	88.2
		2	226092	91.0
10	256014	1	241291	94.3
		2	246748	96.4
11	286729	1	263862	92.0
		2	271315	94.6
12	333246	1	305298	91.6
		2	307858	92.4
13	506386	1	462430	91.3
		2	466196	92.1

Notes 1, 2 and 3, see Table 4.4.

TABLE 4.7 Measured recoveries of different quantities of deuterium oxide from blood after vacuum sublimation (Experiment 2)

Sample No.	Theoretical concentration of D_2O (%, w/w)[1]	Replicate	Concentration of D_2O measured (%, w/w)	Recovery[2] (%)
1	0.0456	1	0.0476	104.4
		2	0.0508	111.4
2	0.1232	1	0.1132	91.9
		2	0.1139	92.5
3	0.1344	1	0.1207	89.8
		2	0.1241	92.3
4	0.1728	1	0.1614	93.4
		2	0.1660	96.1
5	0.1979	1	0.1861	94.0
		2	0.1848	93.4
6	0.2108	1	-	- *
		2	0.1961	93.0
7	0.2267	1	0.2058	90.8
		2	0.2105	92.9
8	0.3008	1	0.2644	87.9
		2	0.2677	89.0
9	0.2875	1	0.2624	91.3
		2	0.2602	90.5
10	0.2842	1	0.2547	89.6
		2	0.2569	90.4
11	0.2929	1	0.2570	92.5
		2	0.2574	87.9
12	0.3212	1	0.2671	90.0
		2	0.2831	88.1
13	0.4310	1	0.3865	89.7
		2	0.3905	90.6

Notes 1 and 2, see Table 4.5.

* Sample lost due to spillage.

TABLE 4.8 Measured recoveries of different quantities of tritiated water (TOH) from blood after vacuum sublimation (Experiment 3)

Sample No.	Theoretical activity of TOH[1] (dpm/g blood water)	Replicate	Activity measured[2] (dpm/g blood water)	Recovery[3] (%)
1	5561	1	5526	99.4
		2	5428	97.6
		3	5247	94.4
		4	5438	97.8
2	11671	1	11102	95.1
		2	10914	93.5
		3	11030	94.5
		4	10167	87.1
3	51218	1	47908	93.5
		2	47243	92.2
		3	47353	92.5
		4	46805	91.4

Notes 1, 2 and 3, see Table 4.4.

4.3.1.4 Actual and estimated total body water contents

Mean liveweight (W), total body water (TBW), tritiated water space (T), deuterium oxide space (D) and an indication of the ability of tritiated water and deuterium oxide to estimate total body water are given in Table 4.9 for the individual groups of birds. Data for all the birds were combined (N = 169) and linear, multiple linear and allometric regression equations derived for the prediction of total body water (TBW, g) from the independent variables liveweight (W, g), age (A, d), tritiated water space (T, g) or deuterium oxide space (D, g) and the maturity indices M and N. These regression equations are given in Table 4.10. The relationships between total body water (TBW) and tritiated water space (T), and between total body water (TBW) and deuterium oxide space (D), are shown in Figure 4.2. The mean (±SD) total body water (TBW) of all birds (N = 169) was 1096.4 g (±424.1); inclusion of this value into equations (4) and (15) showed that tritiated water space (T) and deuterium oxide space (D) overestimated total body water by 10.4% and 8.5% respectively. The mean (±SD) total body water (TBW) for only those birds (Groups 5-12) for which tritiated water space (T) and deuterium oxide space (D) were determined concurrently (N = 115) was 1178.4 g (±333.8); inclusion of this value into equations (4) and (15) gave overestimates of 10.5% and 8.7% for tritiated water space (T, g) and deuterium oxide space (D, g) respectively. Total body water (TBW, g) was predicted more accurately from tritiated water space (T, g) rather than from deuterium oxide space (D, g) when these variables were used alone (equation 4 versus equation 15). Covariance analyses of the linear relationships between actual total body water (TBW, g) and either tritiated water space (T, g) or deuterium oxide space (D, g) (equation 4 (N = 169) versus equation 15 (N = 115), Table 4.10) showed a significant ($P < 0.05$) difference in total body water (TBW, g) after covariance adjustment to a mean isotope space but no significant difference between slopes. For those birds (N = 115) for which tritiated water space (T, g) and deuterium oxide space (D, g) were determined concurrently these effects were also evident for both the linear and allometric regression models.

The other regression equations given in Table 4.10 show that liveweight (W) alone was a good predictor of total body water (TBW) in either the linear (equation 1) or allometric form (equation 8) and that the inclusion of tritiated water space (T) in conjunction with liveweight (W) decreased the residual standard deviation (RSD) and increased the multiple

TABLE 4.9 Mean age, liveweight, total body water, tritiated water space, deuterium oxide space, and degree of accuracy of isotope dilution space in the estimation of total body water.

Group	Age (d)	N[1]	Liveweight (W)[2]		Total Body Water (TBW)		Isotope Dilution Space: Tritiated Water Space (T)		Isotope Dilution Space: Deuterium Oxide Space (D)		Estimation[3]: ET		ED	
			(g)	(SD)+	(g/kgW)	(SD)	(g/kgW)	(SD)	(g/kgW)	(SD)	(%)	(SD)	(%)	(SD)
1	280	16	1980.5	163.0	520.8	33.3	589.4	44.4	-	-	13.2	4.7	-	-
2	39	6	429.2	4.8	693.9	6.4	758.9	7.3	-	-	9.4	0.8	-	-
3a	70	6	849.5	75.0	643.1	12.3	679.2	11.1	-	-	5.6	1.4	-	-
3b	70	6	795.5	61.6	654.3	5.5	699.9	23.9	-	-	7.0	3.2	-	-
4a	101	6	1175.7	78.9	653.1	22.8	716.9	26.8	-	-	9.8	3.2	-	-
4b	101	6	1080.8	58.7	656.0	21.0	708.5	22.0	-	-	8.0	1.4	-	-
5a	162	6	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	162	6	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	162	6	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	218	6	1842.3	234.3	552.1	13.6	610.5	31.0	598.9	29.6	10.7	6.7	8.5	5.6
6b	218	6	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	218	6	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	337	6	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	337	6	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	337	6	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	476	15	2046.5	284.5	560.5	27.5	598.6	49.7	604.5	49.0	6.7	5.9	7.8	6.4
9	476	10	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	280	4	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	280	4	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	280	4	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	476	4	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	476	4	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	476	4	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	126	6	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	126	6	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	307	8	3710.6	356.7	547.6	17.1	602.8	28.6	-	-	10.2	7.2	-	-

+ Standard deviation.

1. N = Number of birds slaughtered for determination of body composition after prior estimation of isotope dilution space.

2. Measured at equilibrium blood sampling time, approximately 3 h post-injection.

3. Calculated as follows:

$$ET = \frac{T - TBW}{TBW} \times 100; \qquad ED = \frac{D - TBW}{TBW} \times 100$$

where all values used were in g/kgW.

TABLE 4.10 Linear, multiple linear and allometric regression equations established to determine the appropriate model for the prediction of total body water (TBW, g) from liveweight (W, g), age (A, d), tritiated water space (T, g) or deuterium oxide space (D, g) in poultry. (N = 169 for tritiated water space (T) and 115 for deuterium oxide space.)

Dependent Variable	Model and Independent Variables	Constants in Model							$(R^2)^1$	$(RSD)^2$	CoV_3 (%)	Equation Number
		a	b	c	d	e	f	g				
TBW(g)	a + bW	101.5	0.509						0.978	63.0	0.44	1
	a + cA	710.0		1.464					0.230	372.0	2.61	2
	a + bW + cA	111.4	0.519	-0.108					0.979	61.8	0.43	3
	a + dT	17.1			0.893				0.981	59.1	0.41	4
	a + bW + dT	46.7	0.239		0.483				0.988	45.9	0.32	5
	a + cA + dT	10.0		0.075	0.583				0.981	58.5	0.41	6
	a + bW + cA + dT	50.1	0.246	-0.027	0.475				0.988	45.9	0.32	7
	aW^b	1.560	0.866						0.981	69.6	0.49	8
	aA^c	103.8		0.423					0.471	366.5	2.59	9
	aW^bA^c	1.390	0.916	-0.048					0.984	64.4	0.45	10
	aT^d	1.045			0.980				0.988	59.3	0.42	11
	aW^bT^d	1.164	0.318		0.626				0.991	48.7	0.34	12
	aA^cT^d	1.067		0.085	0.971				0.988	-	-	13
	$aW^bA^cT^d$	1.135	0.377	-0.019	0.581				0.991	48.3	0.34	14
	a + gD	21.5						0.905	0.954	71.6	0.57	15
	a + bW + gD	51.3	0.315					0.361	0.982	44.9	0.36	16
	a + CA + gD	14.0		0.035				0.903	0.954	71.8	0.57	17
	a + bW + cA + gD	59.2	0.319	-0.035				0.357	0.982	44.9	0.36	18
	aD^g	1.265						0.956	0.946	59.7	0.48	19
	aW^bD^g	0.982	0.758					0.242	0.967	-	-	20
	aA^cD^g	1.361		0.015				0.945	0.984	99.4	0.79	21
	$aW^bA^cD^g$	1.012	0.621	-0.019				0.337	0.978	48.3	0.38	22
	a + bW + dT + eM	50.1	0.246		0.475	-7.56			0.988	45.9	0.32	23
	a + bW + dT + fN	69.1	0.268		0.446		-44.4		0.988	45.6	0.32	24
	$aW^bT^dM^e$	1.021	0.377		0.581	-0.019			0.991	48.2	0.34	25
	$aW^bT^dN^f$	0.869	0.477		0.528		-0.061		0.991	-	-	26

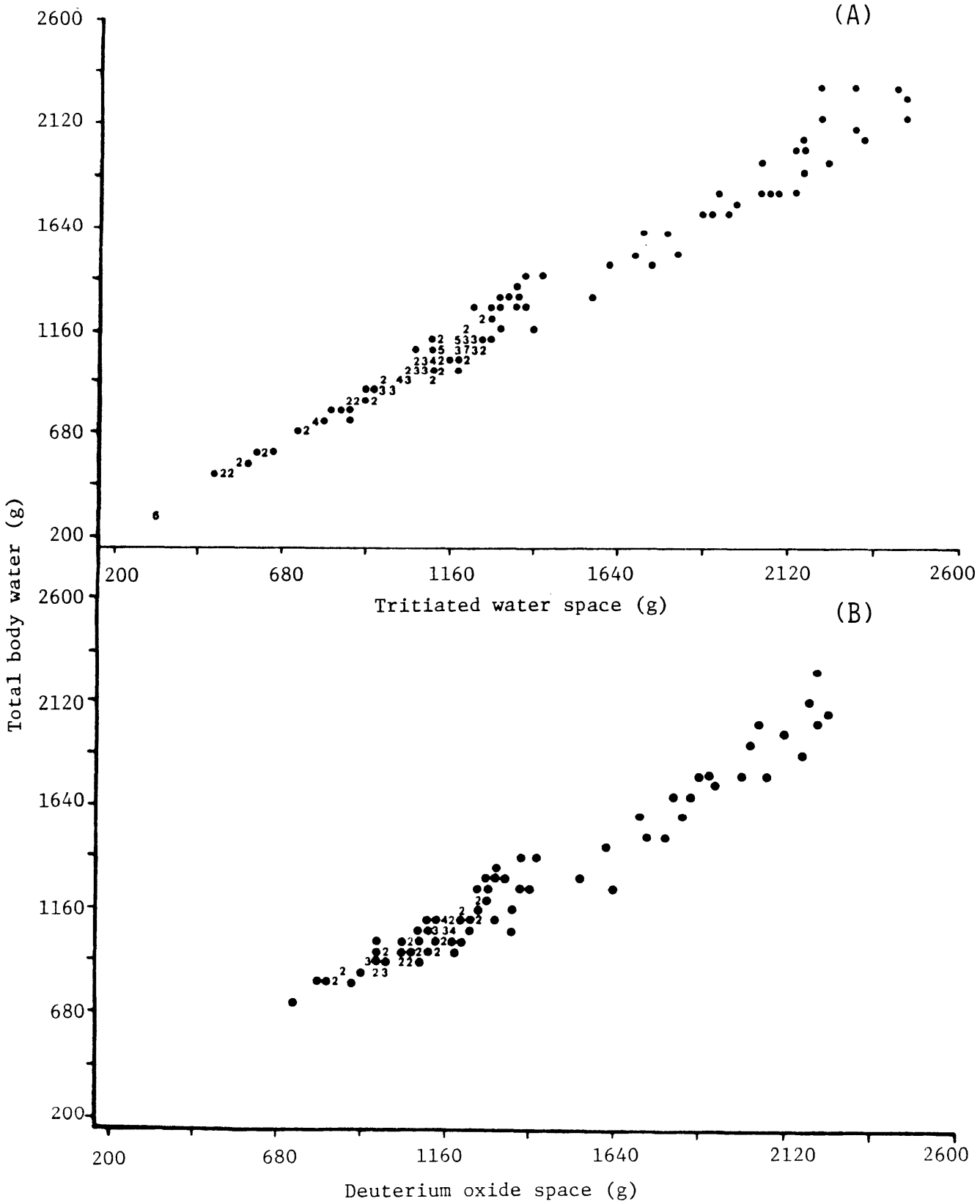
1. Coefficient of determination.

2. Residual standard deviation (RSD).

3. Coefficient of variation at mean total body water (TBW), calculated as $CoV(\%) = (RSD/\sqrt{N})$ mean TBW where N = No. of animals.

FIGURE 4.2: **A** - The relationship between total body water (TBW, g) and tritiated water space (T, g) (N = 169).

B - The relationship between total body water (TBW, g) and deuterium oxide space (D, g) (N = 115).


(A)
2600
2120
1640
1160
680
200
Total body water (g)
200
680
1160
1640
2120
2600
Tritiated water space (g)
(B)
2600
2120
1640
1160
680
200
200
680
1160
1640
2120
2600
Deuterium oxide space (g)

correlation coefficient (R^2). The inclusion of deuterium oxide space (D) with liveweight (W) decreased the RSD in the linear form (equation 16) but increased the RSD in the allometric form (equation 20). Age (A) did not increase the R^2 or decrease the RSD in any of the equations except when liveweight (W), age (A) and deuterium oxide space (D) were used together to predict total body water (TBW) (equation 22). Inclusion of either of the calculated maturity indices (M or N) did not give a further reduction in RSD compared to the inclusion of either liveweight (W) or age (A) alone. Some examples are given in Table 4.10 to illustrate this point (equations 23-26). Linear and allometric regression models for the relationships between total body water (TBW) and tritiated water space (T), and between total body water (TBW) and deuterium oxide space (D) for individual groups of birds are given in Appendix Tables A4.1, A4.2, A4.3 and A4.4. Similar relationships but with liveweight (W) included are given in Appendix Tables A4.5, A4.6, A4.7 and A4.8.

Analyses of covariance were carried out to determine differences between groups in the estimated total body water (TBW) after adjustment to a mean tritiated water space (T) and/or liveweight (W), and to test the significance of differences between groups in the regression coefficient. For the relationship given in equation 4 (Table 4.10) there were differences between groups in the adjusted total body water (TBW) ($P < 0.05$) and in regression coefficients ($P < 0.01$). There were differences between groups in the allometric relationship given in equation 11 (Table 4.10) in the adjusted total body water (TBW) ($P < 0.05$) and in the regression coefficients ($P < 0.001$). With the inclusion of liveweight (W) (equations 5 and 12) differences were increased in both the linear (equation 5) and allometric (equation 12) forms ($P < 0.001$) for both adjusted total body water (TBW) and regression coefficients. Covariance probability matrices of adjusted means for the differences between individual groups for the relationships given in equations 4, 11, 5 and 12 (see Table 4.10) are shown in Appendix Tables A4.9, A4.10, A4.11 and A4.12 respectively. The results show that birds in Groups 1 and 12 were significantly different, after covariance adjustment of total body water, for the relationships given in equations 4 and 11, from the majority of the other groups. With inclusion of liveweight in the linear form of the regression equation (equation 5) only birds in Group 1 were significantly different from the majority of the other groups (Table A4.11), but in the allometric form (equation 12) birds in Groups 1, 3 and 4 showed significant differences (Table A4.12).

TABLE 4.11 Differences between actual total body water and that predicted from individual group equations and the combined equation for the relationship TBW (g) = a + bW + dT. (See Appendix Table 4.25 for data used).

Group	Mean total body water (TBW)[1] (g) (±SD)	Total body water (g) predicted using: Group equations[2]	Combined equation[3]	Difference (%)[4] Group equations	Combined equation
1	1028.7(±72.0)	1027.6	1016.9	-0.1	-1.1
2	297.8(±3.6)	297.8	306.6	0.0	-3.0
3	533.3(±43.1)	533.4	517.2	0.0	-3.0
4	737.8(±50.1)	737.8	704.6	0.0	-4.5
5	915.6(±106.8)	915.9	908.4	0.0	-0.8
6	985.1(±99.9)	984.3	992.6	-0.1	0.8
7	1088.3(±133.0)	1088.7	1090.6	0.0	0.2
8	1140.9(±113.1)	1142.2	1123.2	0.1	-1.6
9	1935.4(±205.8)	1935.9	1936.7	0.0	0.1
10	1030.9(±71.6)	1030.4	1031.4	0.0	0.0
11	1104.3(±171.1)	1103.7	1111.9	-0.1	0.7
12	1635.7(±201.6)	1635.3	1636.9	0.0	0.1
13	2033.6(±223.0)	2031.8	2011.6	-0.1	-1.1

1. Actual total body water determined by desiccation.
2. Relevant equations are given in Appendix Table A4.5
3. Equation (5) in Table 4.10.
4. Percentage difference between precicted and actual total body water.

Analyses of covariance for similar relationships, but only for those groups in which deuterium oxide space (D) was measured, showed that for the relationship between total body water (TBW) and deuterium oxide space (D), both in the linear (equation 15) and allometric (equation 19) forms of the regression models, there were significant differences ($P < 0.001$) between groups in covariance adjusted total body water (TBW) but no significant differences in regression coefficients between groups. Covariance probability matrices for the relationships given by equations 15, 19, 16 and 20 are shown in Appendix Tables A4.13, A4.14, A4.15 and A4.16 respectively. Over all groups for both the linear and allometric regression models which described the relationship between total body water (TBW) and liveweight (W) and deuterium oxide space (D), there were significant ($P < 0.001$) differences in both adjusted total body water and regression coefficients.

The differences observed between the groups in the equations used to predict total body water (TBW) from liveweight (W) and isotope dilution space (T or D) in theory preclude the use of single equations derived from data on all birds (Table 4.10) to predict total body water (TBW) in the individual groups of birds. However the extremely good relationships derived when all birds were included, in terms of a high correlation between variables and low coefficients of variation, could indicate that the overall equations are more meaningful than equations for individual groups which have small numbers of birds. Comparisons between mean actual total body water (TBW) for the individual groups of birds and the total body water predicted from either the individual group equations (see Appendix Tables A4.1 to A4.8) or from the combined equations (given in Table 4.10) were made to determine the magnitude of differences. These values are given in Tables 4.11 and 4.12 for the multiple linear regression models for the relationship between total body water and liveweight (W) and tritiated water space (T) or deuterium oxide space (D) respectively. Comparisons between the percentage differences calculated (Tables 4.11 and 4.12) show that the individual group equations gave excellent predictions of total body water, with small divergences between actual and predicted total body water when the overall equations were used (equations 5 and 16).

To investigate the possibility that the ease and accuracy of prediction of total body water (TBW) from liveweight (W) and either tritiated water space (T) or deuterium oxide space (D) may be improved if birds

TABLE 4.12 Differences between actual total body water and that predicted from individual group equations and the combined equation for the relationship TBW(g) = a + bW + gD. (See Appendix Table A4.25 for data used.)

Group	Mean total body water (TBW)[1] (g) (±SD)	Total body water (g) predicted using: Group equations[2]	Combined equation[3]	Difference (%)[4] Group equations	Combined equation
5	915.6(±106.8)	914.7	888.0	-0.1	-3.0
6	985.1(±99.9)	985.8	1001.2	0.1	1.6
7	1088.3(±133.0)	1087.1	1092.3	-0.1	0.4
8	1140.9(±113.1)	1141.1	1139.1	0.0	-0.2
9	1935.4(±205.8)	1936.8	1916.9	0.1	-1.0
10	1030.9(±71.6)	1030.2	1038.9	-0.1	0.8
11	1104.3(±171.1)	1103.9	1127.3	-0.0	2.1
12	1635.7(±201.6)	1634.3	1627.2	-0.1	-0.5

Notes 1 and 4 see Table 4.11.

2. Relevant equations are gien in Appendix Table A4.7.

3. Equation (16) in Table 4.10.

were grouped according to some specific parameters (eg. age or liveweight) two grouping categories were devised:

(1) birds were bulked according to age and type of bird, that is, on an individual group basis, into (a) layer-type pullets, aged less than or equal to 162 d, (b) layer-type hens, aged between 162 and 280 d, (c) layer-type hens, aged between 280 and 476 d, and (d) broiler breeder strains without consideration of age. There were no pullets as defined in (a) above which had been injected with deuterium oxide.

(2) birds were bulked according to individual liveweight (W) irrespective of age, group or type of bird, into (a) liveweight less than 1500 g, (b) liveweight between 1500 to 2200 g and (c) liveweight between 2200 to the maximum liveweight recorded (approximately 4300 g). For the deuterium oxide space (D) determinations, there were only three birds with liveweights less than 1500 g, so these birds were included in the next category.

The linear, multiple linear and allometric regression models derived for the prediction of total body water (TBW) from liveweight (W) and either tritiated water space (T) or deuterium oxide space (D) for the categories of birds specified above are given in Appendix Tables A4.17 and A4.18 for tritiated water space (T) and deuterium oxide space (D) respectively. The differences between the actual total body water (TBW) and that predicted by the relationship between total body water (TBW) and liveweight (W) and either tritiated water space (T) or deuterium oxide space (D) are given in Appendix Tables A4.19 and A4.20 respectively. The observed differences between the values for total body water predicted using either the equations derived for the separate categories, or with the overall equations are small. Similar regression models are given for the liveweight categories in Appendix Tables A4.21 and A4.22 for tritiated water (T) and deuterium oxide (D) respectively. Differences obtained between actual total body water (TBW) and that predicted by the separate equations for these liveweight categories are given in Appendix Tables A4.23 and A4.24.

4.3.2 DISCUSSION

Prediction of body composition initially requires an accurate assessment of the relationships which exist between body components with special recognitionof the factors which have the potential to alter these relation-

ships in any given situation. The correlations between liveweight and the quantities of water, protein, fat and ash in the body have been shown to enable estimation of these components (Reid *et al.* 1968; Burton and Reid 1969; Searle 1970a). However the relationships between the body components irrespective of liveweight which have been identified, for example the relative constancy of the proportion of water in the fat-free body and of the proportion of protein in the fat-free dry matter (Moulton 1923; Reid *et al.* 1955; Bailey *et al.* 1960), in addition to the known influence of factors such as feeding rate and dietary differences (Searle 1970a; van Gils *et al.* 1977), make a knowledge of total body water in conjunction with liveweight essential for the accurate prediction of body fat and protein. Accuracy of prediction of total body water is therefore a very important consideration in any body composition prediction programme. Considerable attention was therefore given in the first section of this chapter to the accuracy of estimation of total body water by the water isotopes containing tritium and deuterium.

4.3.2.1 Estimation of total body water using isotope dilution techniques

There are many reports on a wide range of animal species in which water isotope dilution techniques were used to estimate total body water. Some examples from the literature, in conjunction with estimates of the accruacy of prediction and other important parameters, are given in Table 4.13. The majority of the equations given in Table 4.13 were derived by the present author from data given in the reports. The consistent overestimation of actual total body water by the isotope dilution space was observed by many authors, with the majority attributing the effect to exchange of isotope hydrogen with nonaqueous exchangeable hydrogen present in the body (see for example Nagy and Costa (1980)). However there are many potential sources of error in the technique of estimation of total body water by water isotopes. For convenience, these errors can be divided into two categories; (1) those which occur in the direct measurement of total body water and, (2) those which occur due to the use of compounds which contain isotopes of hydrogen. Potential errors in the direct measurement of total body water include inaccurate determination of liveweight, loss of carcass water during maceration of the carcass, sampling errors and weighing and desiccation errors. Any error in the determination of liveweight would affect the direct and indirect estimations of total body water to an equivalent extent where animals are slaughtered soon after equilibrium blood sampling. For serial blood sampling studies and

TABLE 4.13 The type and number of animals used, the isotope used and the amount injected, the method of determination of isotope concentration in the blood sample, and the accuracy of estimation of total body water from either tritiated water space or deuterium oxide space taken or derived from reports in the literature.

Animal	N*	Isotope Used[1]	Amount injected (per kgW)	Technique[2]	Isotopic estimation of TBW (%)	Constants in the equation: TBW(kg) = a + dT or TBW(kg) = a + gD			(R²)[3]	(RSD)[4] (kg)	CoV[5] (%)	Reference
						a	d or g					
Sheep	9	TOH	10.1 µc	A	+8.8	2.47	0.834		0.967	0.716	1.08	Panaretto (1961)
	61	TOH	22-154 µc	A	+8.8	-0.01	0.92		0.994	0.410	0.33	Searle (1970a)
	24	TOH	3.3 µc	A	+7.1	-0.765	0.970		0.981	0.344	0.38	Keenan et al. (1969)
	24	TOH	25.7 µc	B	+16.3	1.32	0.81		-	0.430	0.54	Farrell and Reardon (1972)
	11	D_2O	1 g	C	+18.0	13.84	0.682		0.727	3.17	1.82	Foot & Greenhalgh (1970)
	8	TOH	7 µc	D	+3.6	3.79	0.841		0.578	-	-	Smith & Sykes (1974)
Cattle	26	TOH	25.0 µc	B	+15.0	-	0.87		-	-	-	Carmegie & Tulloh (1968)
	12	D_2O	-	-	+9.6	37.14	0.59		0.850	10.53	1.23	Crabtree et al. (1974)
	31	TOH	1.3-1.5 µc	B	+22.5	7.96	0.78		0.968	5.88	0.75	Little & McLean (1981)
Poultry	240	TOH	17-20 µc	B	+18.0	0.03	0.82		0.910	0.05	0.35	Farrell (1974b)
	16	TOH	3-9 µc	B	+15.0	-0.057	0.917		-	-	-	Farrell & Balnave (1977)
	19	D_2O	3 g	A	-2.6	0.045	0.961		0.813	0.047	1.10	Kirchgassner et al. (1977)
Pigs	24	D_2O	1 g	C	+2.3	-0.3	0.989		-	0.89	0.38	Houseman et al. (1975)
Rodents	27	TOH	10 µc	B	-0.1*	-0.005	1.027		0.989	0.001	1.18	Holleman & Dieterich (1975)
Hamsters		TOH	8 µc	B	+2.7**	-	-		-	-	-	Kodama (1971)
Rats	32	TOH	593 824 µc	D	+12.0	-	-		-	-	-	Tissavipat et al. (1974)
	40	TOH	69-404 µc	B	+9.7	-	-		-	-	-	Gorlen et al. (1971)
Goats	13	TOH	19.1 µc	A	+5.6	0.14	0.938		0.964	0.637	1.36	Panaretto & Till (1963)
Rabbits	9	D_2O	NA	-	-1.8	-0.051	1.074		1.986	0.055	1.83	Moore (1946)

* Includes data on *Lemus trimucronatus* in which tritium dilution space underestimated total body water by 6%.
** Estimated by calculating from the % body fat and the equation given in text.
+ N = Number of animals slaughtered.

1. TOH, tritiated water; D_2O, deuterium oxide.
2. Techniques used in the experiments were: (A) Vacuum sublimation of serum;
   (B) Vacuum sublimation of blood;
   (C) Heat distillation of blood;
   (D) Plasma samples counted directly.
3. Multiple correlation coefficient.
4. Residual standard deviation.
5. Coefficient of variation at the mean total body water (TBW). Calculated as: $CoV(\%) = \frac{RSD}{\sqrt{N}} / \text{Mean TBW}$
   where N is number of animals.

NA Not available.

where total body water is estimated using the blood sample taken at equilibrium, the direct estimation of total body water would be lower than the indirect estimation due to loss of liveweight and consequent loss of body components prior to slaughter. The amount of this loss would be affected by the duration of prior starvation, especially in ruminant animals (Hecker *et al.* 1964).

Loss of carcass water during maceration may contribute to the apparent overestimation of total body water by water isotopes, but the procedures adopted in the present study, where birds were only partially thawed, maintained the macerate at temperatures considerably below room temperature. Sampling errors would also be minimized in the present study due to procedures used (see Chapter 2). Errors due to incomplete desiccation could be large if sufficient time is not allowed for drying (eg. Culebras *et al.* 1977). Also, a variable amount of muscle water may be "bound" (Ling and Negendank 1970) and therefore not measured by desiccation, but this effect is likely to be balanced by a corresponding lack of exchange with injected isotope hydrogen (Hazelwood and Nichols 1969) during the equilibration period. Loss of volatile substances or fat from the macerate during desiccation can result in an elevated apparent total body water. Under the conditions used in the present experiments, where desiccation was carried out in either a force-draught oven at 70°C for 5-6 d or by freeze-drying for 14 d (see Chapter 2), these factors would be minimized. Therefore it is not unreasonable to assume that most of the discrepancy between actual total body water and that estimated by the water isotopes in the present study, and probably in other reported studies, was due to one or more non-random errors associated with the use of the water isotopes *per se*.

Obvious errors in the use of water isotopes such as inaccurate measurement of the quantity injected and loss of isotope from the injection channel and measurement errors may occur. Failure to allow sufficient time for isotope equilibration could result in an overestimation of total body water. Carnegie and Tulloh (1968) allowed an equilibration time of only 8 h in cattle which had not previously been deprived of feed or water. Smith and Sykes (1974) found that a minimum of 8 h was required for equilibration in sheep which had been deprived of food and water for 16 h prior to injection; this may explain the large overestimation of total body water obtained with tritiated water in the study of Carnegie and Tulloh (1968). However the other large overestimations observed in published reports (see Table 4.13) (eg. Little and McLean 1981) and also

in the present study in which equilibration time was thoroughly investigated, signify that in the majority of experiments in which water isotopes were used the discrepancies observed were not due to sampling prior to complete equilibration. *In vivo* isotope fractionation effects (Pinson 1952) may cause either the selective concentration or removal of the isotope hydrogen and therefore result in non-random errors. McManus *et al.* (1969) observed variable but significantly different concentrations of tritium (SR) in the liver water and blood water of the same rabbits after a 3 h equilibration period. Selective concentration of the isotope hydrogen has also been indicated at the alveolar membrances (Siri and Evers 1962; Hatch and Mazrimas 1972; Rubsamen *et al.* 1979), which caused a subsequent reduction in the proportion of the concentration of isotope in evaporative water to that in normal body water. Siri and Evers (1962) found this effect to be particularly marked for two pigeons, a finding which prompted the present author to investigate this effect in laying hens. The measured activity (SR) of tritium in the evaporative water lost by two laying hens (Table 4.3), compared to the assumed body water activity of approximately 15000 dpm/g water, represents a very low proportion in the time period after equilibration. Results given by Till and Downes (1962) can be recalculated to show that if the quantity of insensible water lost per day reported in their experiment was at the equilibrium blood water or rumen water concentration (specific activity), then approximately 5.2% of the injected dose (rather than the figure of 3.6% given in the text) should have been lost per day. This represents a concentration of tritium (SR) in the insensible water as a proportion of the concentration of tritium (SR) in body water of 0.69 in the study of Till and Downes (1962). However these results and the results reported in the present study must be treated with caution due to the unavoidable errors associated with the procedures involved in collection of evaporative vapour loss as discussed by Nagy and Costa (1980). Nevertheless, rates of insensible water losses calculated in the present study are in good agreement with other values reported for poultry (see Dicker and Haslam 1972).

The recovery studies reported in this chapter in which known amounts of the water isotopes were added to whole blood show that the vacuum sublimation technique used to obtain a water sample from the blood contributed substantially to the discrepancies between actual and estimated total body water values found in the body composition experiments. There is the possibility of three effects in the vacuum sublimation technique which could explain the consistently poor recovery of the added water

isotope. The first of these is an isotope fractionation effect (Riley and Brooks 1964) caused by the water isotopes having a lower vapour pressure than normal water (Siri 1949; Avinur and Nir 1960). The second is incomplete desiccation of the blood sample, which would compound the first effect, and the third is exchange of isotope hydrogen with non-aqueous exchangeable hydrogen in blood. The latter effect would probably be small relative to the two former effects. Stansell and Mojica (1968) found an average 99.8% recovery of added deuterium oxide from serum samples, after vacuum sublimation of the sample, with deuterium oxide concentrations in the range 0.0150 to 0.0326% (v/v). However Graystone *et al.* (1967) found only a mean recovery of approximately 90% with added deuterium oxide from blood plasma after vacuum sublimation for a concentration range of 0.199 to 0.389% (v/v). The linear-log relationship observed between the concentration of isotope in the apparent blood water and subsequent recovery of isotope after vacuum sublimation in the present study could account for the discrepancy between the results of Stansell and Mojica (1968) and Graystone *et al.* (1967), due to the extremely small concentrations of deuterium oxide used by the former authors. Nielsen *et al.* (1971) used gas chromatography, in which serum samples were injected directly without prior treatment, and found a 100% recovery of added deuterium oxide in the concentration range of 0.0284 to 0.5821% (v/v). Direct counting techniques have been shown to give a good recovery of added tritium to plasma or urine (Foy and Schneiden 1960).

There was large variation in the quantities of isotopes injected (per kgW) between published reports (see Table 4.13). Gordon *et al.* (1971) found no significant effect of the amount of tritium injected into rats on the basis of differences between tritiated water space and actual total body water. Results on the extent of overestimation found in the present study for tritiated water determinations (N = 169) on a liveweight basis were used to derive the relationship between overestimation and the equilibrium concentration of tritium in the body water. The derived relationship was

$$Y = 7.73 + 0.000146\ X$$

$$N = 169,\quad R^2 = 0.007,\quad RSE = 5.03,\quad P>0.05\ (NS)$$

where Y is $\frac{T - TBW}{TBW} \times 100$ on a liveweight (W) basis and
X is the equilibrium concentration (SR) of tritium.

The present study therefore substantiates the lack of effect of quantity of injected isotope on the subsequent estimation of total body water for

the normal range of quantities injected.

As mentioned previously, most authors attribute an observed overestimation of total body water by the indirect method of water isotopes to exchange of isotope hydrogen with nonaqueous hydrogen. That this occurs *in vivo* is not questioned (Siri and Evers 1962; Hatch and Mazrimas 1972). Culebras and Moore (1977) calculated the maximum amount of nonaqueous exchangeable hydrogen to be approximately 5% of the total exchangeable hydrogen. The work of Sheng and Huggins (1971), in which the estimated total body water by tritium dilution was found to be 14% greater than actual total body water in growing beagles, was cited by Lewis and Phillips (1972) as illustrating the magnitude of the exchange of isotope hydrogen with nonaqueous exchangeable hydrogen. However Culebras *et al.* (1977) attributed the overestimation observed by Sheng and Huggins (1971) to the use of poor and inaccurate techniques. This was done on the basis of their experiment on rats (Culebras *et al.* 1977) in which only a 2% overestimation by tritium dilution was observed. Certainly the large overestimations obtained by the use of water isotopes reported in the literature (see Table 4.13), and those obtained in the present study, can not solely be attributed to inaccurate techniques. Culebras *et al.* (1977) concluded that isotope hydrogen exchange with nonaqueous exchangeable hydrogen was not an important factor in the explanation of the observed discrepancies. If steric conformations, solubility of proteins, accessibility of proteins and protein function are considered (T.M. Sutherland, pers. comm.), then this is a realistic conclusion. Farrell and co-workers (Farrell and Reardon 1972; Farrell and Balnave 1977) showed that only 0.2 or 0.5% of an injected dose of tritiated water was recovered in the total dry carcasses of sheep and poultry respectively. These studies (Farrell and Reardon 1972; Farrell and Balnave 1977) provide the only quantitative estimates of isotope hydrogen exchange with nonaqueous exchangeable hydrogen so far reported. On the basis of the recovery studies reported in this chapter, in conjunction with the finding that most workers used a vacuum sublimation technique to obtain a body water sample for isotope concentration determination (see Table 4.13), the present results indicate that a substantial proportion of the overestimations found in the literature can be attributed to a lack of recovery of isotope during the vacuum sublimation procedure. Some variability would probably be caused by the actual method of vacuum sublimation employed in different laboratories.

On this premise the majority of the "true" *in vivo* loss of injected

isotope can be attributed to losses which occur between injection and equilibrium in faeces and urine and by insensible water losses. The quantities of these components and therefore the extent of the loss of injected isotope could account for some of the variability associated with the estimation of total body water by isotope dilution techniques. Smith and Sykes (1974) found in their sheep that after correction for the urinary losses of tritiated water some studies overestimated total body water by only 0.8%. Rates of urine production in poultry would probably be extremely variable depending on factors such as feed intake, water intake and physiological state. Urine production rate in poultry with normal feed and water intake was estimated at 1.8 ml/kgW h^{-1} (Hester *et al.* 1940). For a 2 kg bird this could represent a urine production of approximately 11 mls during a 3 h equilibration period after isotope injection. Assuming that this urine is at the equilibrium isotope concentration then approximately 2% of the injected dose of isotope may be lost during equilibration although resorption of urine water is not considered in this calculation. Nevertheless it is apparent that some, and potentially a large amount, of the variability associated with the *in vivo* estimation of total body water by isotope dilution techniques could be explained on this basis, as found by Smith and Sykes (1974). Variability associated with site of isotope injection has also been reported (Smith and Sykes 1974) although there were no significant effects on the estimation of total body water by tritiated water due to site of isotope injection in the present study (Section 4.2.2), although Farrell (1974b) reported increased variability due to intraperitoneal rather than intramuscular isotope injection in young broiler chickens.

4.3.2.2 Equilibrium studies

The time required for equilibration of water isotopes within the body is a function of many factors. These factors, and the dynamics of water-isotope distribution have been discussed in detail elsewhere (Pinson 1952; Edelman 1952; Coleman *et al.* 1972). The two trials reported in this chapter in which serial blood samples were taken from birds injected either with tritiated water and/or deuterium oxide showed that equilibrium of the isotope hydrogen was normally complete in 60 to 100 minutes after injection. The occurrence of an extended time of equilibration for one of the birds in Group 9 (Bird 9) (Figure 4.1) after tritiated water injection illustrates the problems which may occur in sampling for the determination of equilibrium isotope concentration. The results reported

in this chapter on the time required for isotope equilibration substantiate the findings of Farrell (1974b) with young broiler chickens, and of the equilibration times reported for a range of animal species of similar liveweights (McManus *et al.* 1969; Sheng and Huggins 1971; Searle 1970a). As expected (Pinson 1952) there were no apparent differences between the gross equilibration time of tritiated water or deuterium oxide (Section 4.3.1.1).

4.3.2.3 Prediction of total body water

Despite the consistent overestimation of total body water by water isotope dilution, the technique was shown in the studies reported in this chapter to be able to give accurate prediction of total body water in a diverse range of poultry types. Analyses of the combined data for the birds used in this study showed that total body water could be predicted with a linear regression model by liveweight alone with a coefficient of variation of only 0.44% at the mean total body water of 1096.4 g. This represents an error at the mean total body water of approximately 5 g. Accuracy of prediction of total body water was improved by the inclusion with liveweight of either tritiated water space or deuterium oxide space in either the linear or allometric regression models (Table 4.10). The coefficients of variation calculated in the present study for the relationship between total body water and either tritiated water space or deuterium oxide space indicated that the accuracies obtained compare extremely favourably with those obtained in reports published previously (see Table 4.13). The larger coefficient of variation obtained for the prediction of total body water by deuterium oxide rather than tritiated water space was due to the increased variability in the estimation of total body water by deuterium oxide. In four of the eight groups of birds in which deuterium oxide and tritiated water were used concurrently the deuterium oxide space was lower than the tritiated water space, while in three of the groups the reverse was true.

For the relatively few animal studies in which deuterium oxide was used to predict total body water (Moore 1946; Foot and Greenhalgh 1970; Houseman *et al.* 1973; Crabtree *et al.* 1974; Kirchgessner *et al.* 1977), there is a tendency for a reduced overestimation, relative to tritiated water studies, or for an underestimation of total body water (see Table 4.13). The large overestimation of total body water obtained with deuterium oxide in the study by Foot and Greenhalgh (1970) was attributed by the authors to inaccurate measurement (initially) of very low deuterium

oxide concentrations. There have been no previous studies in which the estimations of total body water obtained with the concurrent use of both tritiated water and deuterium oxide have been compared with direct determination of total body water. However Stansell and Mojica (1968) found that deuterium oxide space was 0.9% lower than tritiated water space determined in the same forty-six human (male) subjects. Unlike the present study (Section 4.3.1.4), this difference was not significant. Differences in the physical properties between tritium and deuterium (Pinson 1952) arguably would result in a closer approximation of actual total body water by deuterium oxide rather than tritiated water. However the more difficult measurement procedures for deuterium probably explain the increased variability associated with its use.

Prediction equations derived from data on individual groups of birds (Section 4.3.1.4) gave improved estimates of actual total body water than when all the data were combined (Table 4.10). However the small differences obtained between the estimates with either the individual group equations or the combined equations (see Tables 4.11 and 4.12) indicate that some of the differences were due to the smaller numbers of birds associated with the derivation of the individual group equations. However covariance analyses showed that there were significant differences between groups in both the slope of the relationships and also in total body water after covariance adjustment with tritiated water space and/or liveweight. Statistically, such differences preclude the use of the overall equations derived when all data were combined. Classification of birds either on the basis of age and type or liveweight indicated that the regression models derived from the combined data gave a biased prediction of total body water in young birds and also in pullets near commencement of egg production. Further criteria for the classifications of birds undertaken in this section (Section 4.3) will be given in the next section (Section 4.4) of this chapter.

In agreement with Donnelly and Freer (1974) with sheep, total body water represented a smaller proportion of the tritiated water space in young birds. However the form of the overall allometric regression model between total body water and either tritiated water space or deuterium oxide space derived in the present study did not confirm this observation, probably due to the effect on the curve by the broiler breeders with large liveweight and total body water. In contrast to the results of Donnelly and Freer (1974), the maturity indices calculated on either an age or a mature liveweight basis did not improve the precision of the estimation

of total body water above that obtained with either age or liveweight alone.

4.4 *BODY COMPOSITION RELATIONSHIPS IN POULTRY AND THE PREDICTION OF BODY FAT, PROTEIN AND ASH IN POULTRY*

4.4.1 PREFACE

This section describes the body composition relationships found in the poultry used in the studies reported in this chapter. The previous section (Section 4.3) determined the accuracy with which either tritiated water or deuterium oxide predicted total body water *in vivo* in poultry. Liveweight is the most important variable in the prediction of body composition. Inaccuracies in the estimation of total body water can therefore only make a significant contribution to the accuracy of the prediction of the fat, protein or ash components of the body if the variation in these components is not significantly explained by liveweight alone. The prediction of body composition can be achieved by regression techniques. However it is the basic relationships which exist between the body components which determine the accuracy of such derived prediction equations. The fundamental relationship, reported for a wide range of animal species, between total body water and body fat, facilitates the estimation of body fat content and body protein content *in vivo*. This section therefore initially describes such relationships as found in the poultry used in the present study. Suitably derived body composition prediction equations are then presented. Major emphasis is placed on the use of tritiated water in the prediction of body composition because of the planned extensive use of this isotope during a major experiment with broiler breeders when in respiration chambers (see Chapter 8).

4.4.2 RESULTS

4.4.2.1 Body composition relationships in poultry

The mean (±SD) values for the fat (FW), protein (PW) and ash (ASW) components determined by carcass analyses on the birds are given for the individual groups in Table 4.14. The diversity achieved in terms of age, type of poultry and prior nutritional history resulted in a good range in each of the body components. For example, fat (FW) content varied from 51.6 g/kgW to 304.9 g/kgW, and protein (PW) content from 162.8 g/kgW to 253.8 g/kgW. The relationship between total body water (TBW, g/kgW) and

TABLE 4.14 Mean age, liveweight, body fat, protein and ash determined by carcass analyses of poultry.

Group	Age (d)	Liveweight (W)+ (g)	(SD)≠	Fat (FW) (g/kgW)	(SD)	Protein (PW) (g/kgW)	(SD)	Ash (ASW) (g/kgW	(SD)
1	280	1980.5	163.0	231.1	45.8	197.0	22.2	48.5	16.7
2	39	429.2	4.8	58.4	4.0	205.4	6.9	40.0	1.9
3a	70	849.5	61.6	96.2	21.0	217.3	8.2	43.4	5.8
3b	70	795.5	61.6	86.5	7.8	213.4	15.3	43.4	6.3
4a	101	1175.7	78.9	77.3	22.0	227.5	5.5	41.4	2.0
4b	101	1080.8	58.7	85.5	15.2	214.7	10.0	37.8	4.9
5a	162	1817.0	123.0	155.3	10.7	210.0	9.0	41.4	7.9
5b	162	1540.2	145.0	129.1	18.5	209.5	17.9	37.0	2.0
5c	162	1404.2	86.0	130.0	15.8	211.4	12.9	38.5	4.6
6a	218	1842.3	234.3	205.4	27.0	202.3	12.7	31.0	6.4
6b	218	1748.2	208.3	198.7	29.8	211.2	13.8	36.8	5.2
6c	218	1747.0	117.4	183.7	13.6	209.1	9.0	32.3	5.2
7a	337	2151.5	220.0	209.4	25.3	185.2	12.2	30.1	2.7
7b	337	1811.8	184.6	191.7	19.1	199.6	11.9	26.8	4.5
7c	337	1891.8	204.0	184.1	17.8	200.9	10.0	30.4	7.3
8	476	2046.5	284.5	185.3	33.3	189.2	14.4	40.9	6.3
9	476	3581.3	456.8	199.4	22.6	193.8	13.8	35.0	6.3
10a	280	1930.8	185.2	189.6	34.7	210.7	20.2	33.1	4.6
10b	280	1720.5	147.8	158.1	12.3	221.4	10.5	31.0	2.6
10c	280	1803.0	83.8	156.2	21.1	212.9	5.4	37.5	3.8
11a	476	1939.8	209.1	192.9	16.4	198.9	13.6	40.6	8.5
11b	476	1791.8	143.4	201.9	16.5	199.2	14.5	36.2	8.8
11c	476	2268.3	295.0	209.6	30.4	186.4	16.7	35.7	8.2
12a	126	3407.8	144.6	253.9	39.2	180.1	3.7	26.2	5.9
12b	126	2366.0	152.6	111.7	20.8	218.7	5.5	33.2	4.3
13	307	3710.6	354.7	225.0	9.8	182.0	11.7	30.1	8.0

+ Equilibration liveweight (see Table 4.9 and text (Section 4.2.5) for details).

≠ Standard deviation.

body fat (FW, g/kgW) is shown in Figure 4.3. The derived linear regression model was

$$FW(g/kgW) = 805.3 - 1.105\ TBW\ (g/kgW) \quad (27)$$
$$N = 169;\quad R^2 = 0.890;\quad RSD = 19.2;\quad P < 0.001$$

This relationship (Figure 4.3) was curvilinear ($P < 0.05$). An allometric regression model was therefore derived, but is given in the logarithmic-linear form as

$$Log\ FW(g/kgW) = 4.164 - 0.00342\ TBW(g/kgW) \quad (28)$$
$$N = 169;\quad R^2 = 0.921;\quad RSD = 0.0495;\quad P < 0.001$$

There were good relationships between body protein and body ash and liveweight. The relationship between protein (P, g) and liveweight (W, g) is shown in Figure 4.4. The linear regression model derived was

$$P(g) = 48.0 + 0.174\ W(g) \quad (29)$$
$$N = 169;\quad R^2 = 0.953;\quad RSD = 31.9;\quad P < 0.001$$

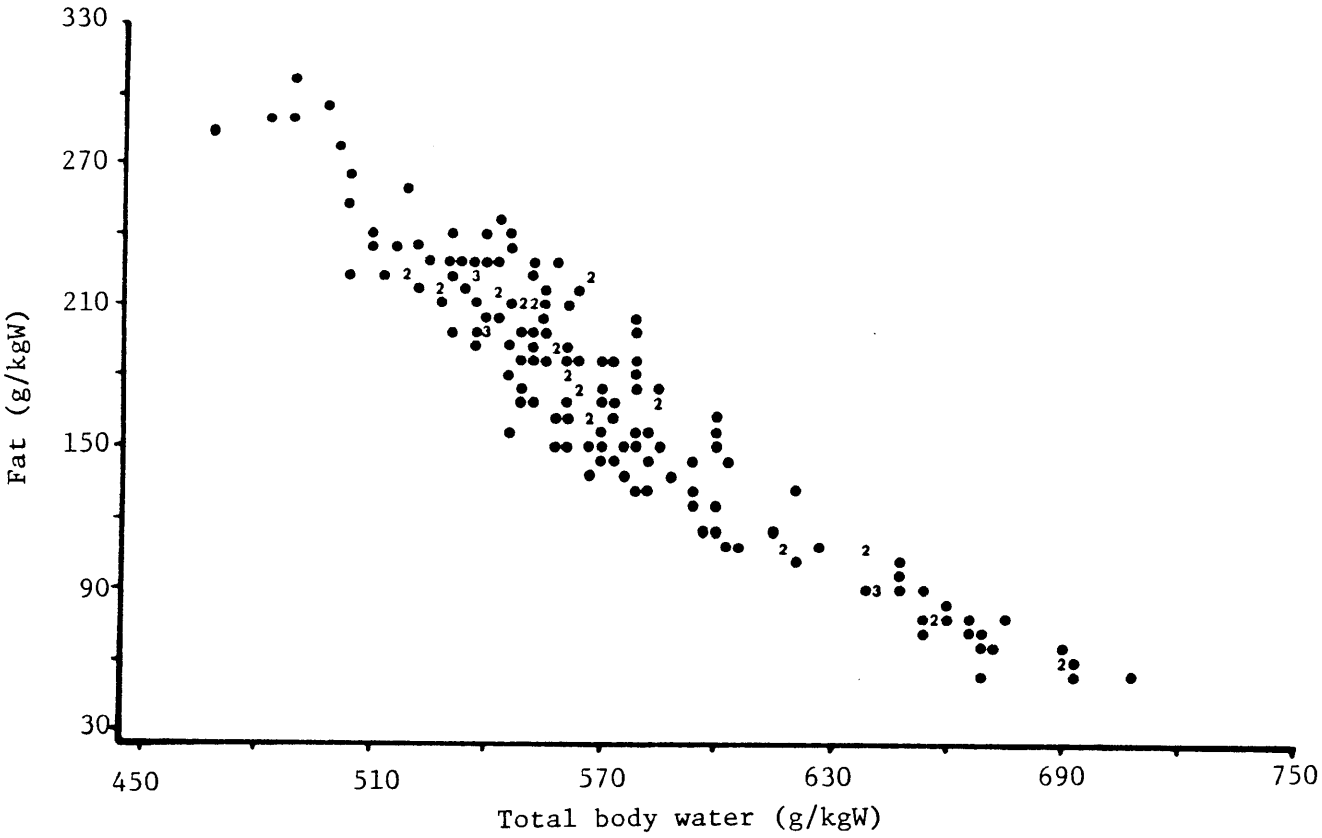
The linear regression model derived for the relationship between ash (AS, g) and liveweight (W, g) was

$$AS(g) = 16.0 + 0.028\ W(g) \quad (30)$$
$$N = 169;\quad R^2 = 0.586;\quad RSD = 19.0;\quad P < 0.001$$

The mean (±SD) values for all birds (N = 169) of total body water (TBW, g/kgW), protein (PW, g/kgW) and ash (ASW, g/kgW) were 574.6 (±49.6), 202.4 (±17.6) and 37.0 (±9.3) respectively. The coefficients of variation for these values were therefore 8.6%, 8.7% and 25.1% for total body water (TBW), protein (PW) and ash (ASW) respectively. In an attempt to reduce this variability values for the body components were recalculated on a fat-free body mass (FFM) basis, and also on a fat-free dry matter (FFDM) basis. The mean (±SD) composition of the fat-free mass (FFM) and of the fat-free dry matter (FFDM) are given in Table 4.15. The mean (±SD) values for all birds (N = 169) of water of the fat-free mass (WFFM, g/100 g), protein of the fat-free mass (PFFM, g/100 g) and ash of the fat-free mass (ASFFM, g/100 g) were 69.2 (±2.1), 24.4 (±1.6) and 4.5 (±1.1) respectively. Coefficients of variation for water (WFFM), protein (PFFM) and ash (ASFFM) components of the fat-free mass were 3.0%, 6.6% and 24.4% respectively. Therefore variability was considerably reduced for water, moderately reduced for protein but only slightly reduced for ash when these components were expressed on a fat-free body mass basis. The mean (±SD) values for all birds (N = 169) of the protein (PFFDM) and ash (ASFFDM) components of the fat-free dry matter were 79.5 g/100 g (±5.0) and

FIGURE 4.3: The relationship between body fat (F) and total body water (TBW) expressed on a liveweight basis (g/kgW).

FIGURE 4.4: The relationship between protein (P, g) and liveweight (W, g).


330
270
210
150
90
30
Fat (g/kgW)
450
510
570
630
690
750
Total body water (g/kgW)

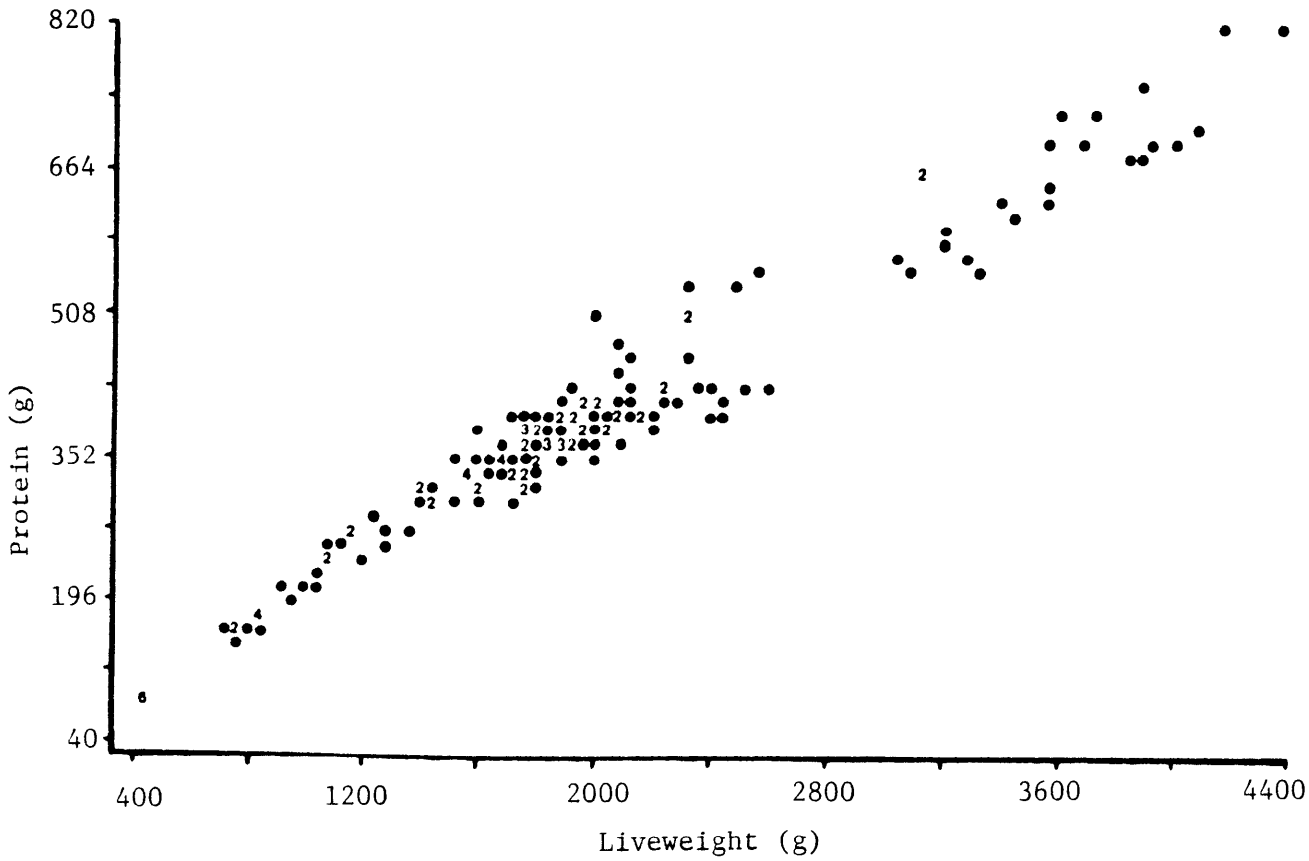

820
664
508
352
196
40
Protein (g)
400
1200
2000
2800
3600
4400
Liveweight (g)

TABLE 4.15 Mean composition of the fat-free mass and the fat-free dry matter determined by carcass analyses of poultry.

Group	Age (d)	Fat-free Mass (FFM)[1]						Fat-free dry matter (FFDM)[2]			
		Total body water		Protein[4]		Ash[5]		Protein[6]		Ash[7]	
		(g/100g)	(SD)+	(g/100g)	(SD)	(g/100g)	(SD)	(g/100g)	(SD)	(g/100g)	(SD)
1	280	67.7	1.9	25.6	2.2	6.3	2.1	79.4	5.5	19.5	6.3
2	39	73.7	0.4	21.8	0.8	4.3	0.2	82.9	2.4	16.1	0.6
3a	70	71.2	0.5	24.0	0.4	4.8	0.6	83.4	2.0	16.6	1.9
3b	70	71.6	0.5	23.3	1.6	4.8	0.7	82.3	5.2	16.7	2.3
4a	101	70.8	0.9	24.7	0.4	4.5	0.2	84.4	2.5	15.3	0.8
4b	101	71.8	1.3	23.5	1.3	4.1	0.5	83.0	1.4	14.7	2.3
5a	162	66.3	0.5	24.8	0.8	4.9	0.9	73.8	2.6	14.6	2.8
5b	162	67.8	0.4	24.1	1.8	4.3	0.3	74.5	5.8	13.2	0.7
5c	162	67.2	0.7	24.3	1.7	4.4	0.5	74.0	4.4	13.4	1.5
6a	218	69.5	1.6	25.4	1.2	3.9	0.7	83.5	2.9	12.7	1.7
6b	218	68.1	0.7	26.4	0.8	4.6	0.6	82.4	2.9	14.4	1.9
6c	218	69.1	1.6	25.6	1.3	4.0	0.6	82.9	2.6	12.8	2.3
7a	337	70.9	2.0	23.4	1.1	3.8	0.3	78.0	3.3	12.7	1.0
7b	337	69.4	1.7	24.7	1.7	3.3	0.6	80.6	2.2	10.9	2.1
7c	337	68.7	1.8	24.6	0.8	3.7	0.8	78.8	4.6	11.8	2.1
8	476	68.8	1.6	23.2	1.3	5.0	0.7	74.6	6.1	16.1	2.3
9	476	67.7	1.0	24.2	1.1	4.4	0.7	74.9	4.3	13.5	2.1
10a	280	68.3	0.3	26.0	1.4	4.1	0.5	81.9	4.1	12.8	1.4
10b	280	67.6	1.2	26.3	1.0	3.7	0.3	81.2	1.4	11.4	1.0
10c	280	68.9	0.4	25.2	0.2	4.5	0.4	81.1	1.7	14.3	1.1
11a	476	68.8	1.5	24.7	1.6	5.0	1.0	79.0	1.4	16.1	2.7
11b	476	68.0	2.1	24.9	1.4	4.5	1.0	78.1	1.9	14.0	2.6
11c	476	70.5	2.1	23.6	1.6	4.5	0.9	79.8	1.8	15.3	2.9
12a	126	70.9	0.9	24.2	0.9	3.5	0.7	83.0	3.5	12.0	2.5
12b	126	70.0	1.1	24.6	0.7	3.8	0.5	82.1	2.6	12.5	1.6
13	307	70.7	1.7	23.5	1.6	3.9	1.0	80.1	4.2	13.3	3.7

+ Standard deviation.

1. Fat-free mass (FFM) calculated by:

$$FFM(g) = W(g) - F(g)$$

where W is liveweight and
F is body fat

2. Fat-free dry matter (FFDM) calculated by:

$$FFDM(g) = W(g) - (TBW(g) + F(g))$$

where TBW is actual total body water and
W and F are as defined above.

3. Water of the fat-free mass (WFFM).

4. Protein of the fat-free mass (PFFM).

5. Ash of the fat-free mass (ASFFM).

6. Protein of the fat-free dry matter (PFFDM).

7. Ash of the fat-free dry matter (ASFFDM).

14.5 g/100 g (±3.5) respectively. Thus variation was again slightly reduced for both protein and ash. Much of the variation in the ash component can be attributed to birds in Group 1 (N = 16) for which ash determinations were not carried out directly but estimated by difference. The average values for the water and protein contents of the fat-free mass (WFFM and PFFM) and for the protein content of the fat-free dry matter (PFFDM) are within the range of values for poultry which were calculated from the literature (see Table 5.1, Chapter 5). The effects of age on the water content of the fat-free mass (WFFM, g/100 g) and on the protein (PFFDM, g/100 g) and ash (ASFFDM, g/100 g) contents of the fat-free dry matter are shown in Figures 4.5, 4.6 and 4.7 respectively. Water content of the fat-free mass (WFFM) decreased initially with increasing age but plateaued at approximately 69 g/100 g at approximately 200 d of age. Protein content of the fat-free dry matter (PFFDM, g/100 g) was constant at approximately 83 g/100 g during the first 18 weeks of age. Values for birds aged 162 d (Group 5) were markedly reduced. The pattern then showed a similar marked increase back to normal values with a consequent slow decline thereafter with age. Ash content of the fat-free dry matter (ASFFDM, g/100 g) decreased initially with age, remained relatively constant between the ages 126 d to 307 d and then increased up to 476 d of age. The probable cause of extreme deviation of the mean value of ash for birds in Group 1 has been explained previously.

The linear regression model derived for all data (N = 169) for the relationship between total body water (TBW, g) and fat-free mass (FFM, g) was

$$TBW(g) = -1.12 + 0.691\ FFM(g) \qquad (31)$$
$$N = 169;\ R^2\ 0.994;\ RSD = 32.7;\ P<0.001$$

The linear regression model for the relationship between protein (P, g) and fat-free mass (FFM, g) was

$$P(g) = 12.80 + 0.236\ FFM(g) \qquad (32)$$
$$N = 169;\ R^2 = 0.969;\ RSD = 25.8;\ P<0.001$$

The linear regression model for the relationship between ash (AS, g) and fat-free mass (FFM, g) was

$$AS(g) = 10.49 + 0.0374\ FFM(g) \qquad (33)$$
$$N = 169;\ R^2 = 0.596;\ RSD = 18.8;\ P<0.001$$

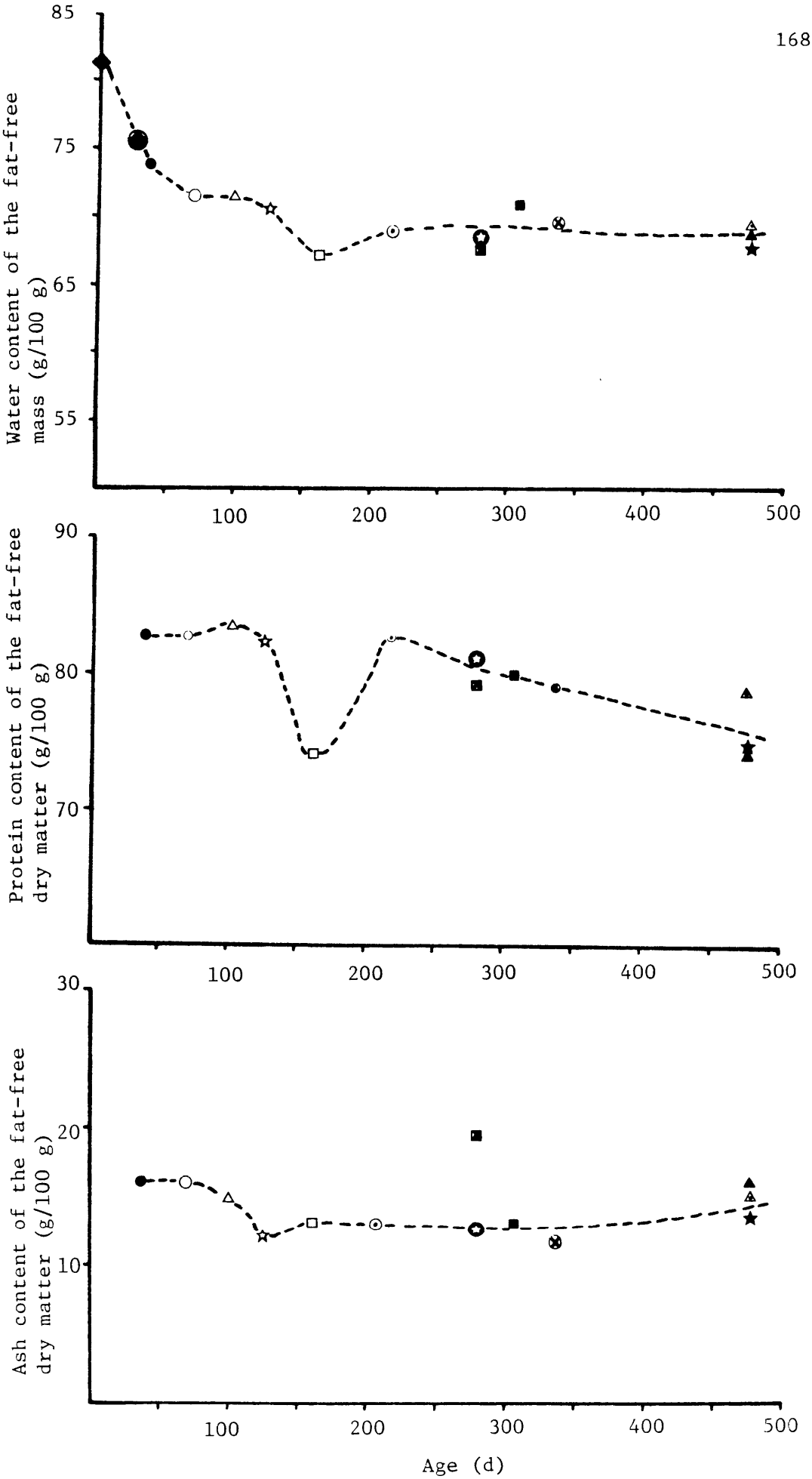
Two regression models were derived to determine the influence of age on

FIGURE 4.5: The effect of age (d) on the mean water content of the fat-free mass (g/100 g) for the groups of birds slaughtered. Symbols for each group are given below, and results recalculated from Edwards *et al.* 1973 (◆) and Edwards and Denman 1975 (◉) on young chickens (day-old and 28 d of age respectively) were included to demonstrate the probable change from hatching.

FIGURE 4.6: The effect of age (d) on the mean protein content of the fat-free dry matter (g/100 g) for the groups of birds slaughtered. Symbols for each group are given below.

FIGURE 4.7: The effect of age (d) on the mean ash content of the fat-free dry matter (g/100 g) for the groups of birds slaughtered. Symbols for each group are given below.

Groups (see Table 4.1) were: 1 (⊠); 2 (●); 3 (○); 4 (△); 5 (□); 6 (⊙); 7 (⊗); 8 (▲); 9 (★); 10 (✪); 11 (◬); 12 (☆); and 13 (■).


Water content of the fat-free mass (g/100 g)
85
75
65
55
100
200
300
400
500
Protein content of the fat-free dry matter (g/100 g)
90
80
70
100
200
300
400
500
Ash content of the fat-free dry matter (g/100 g)
30
20
10
100
200
300
400
500
Age (d)

the water content of the fat-free mass (WFFM). These are given in equations 34 and 35.

$$TBW(g) = -12.3 + (0.713 - 0.0000492\ Age)\ FFM \quad (34)$$
$$N = 169;\ R^2 = 0.995;\ RSD = 31.1;\ P < 0.001$$

$$TBW(g) = -15.39 + (0.779 - 0.0326\ Log_{10}\ Age)\ FFM \quad (35)$$
$$N = 169;\ R^2 = 0.995;\ RSD = 30.8;\ P < 0.001$$

The linear regression model for the relationship between protein (P, g) and the fat-free dry matter (FFDM, g) and age (A, d) was

$$P(g) = 5.10 + (0.827 - 0.00165\ Age)\ FFDM \quad (36)$$
$$N = 169;\ R^2 = 0.971;\ RSD = 24.9;\ P < 0.001$$

The linear regression model for the relationship between ash (AS, g) and the fat-free dry matter (FFDM, g) was

$$AS(g) = 12.98\ (0.102 + 0.000046\ Age)\ FFDM \quad (37)$$
$$N = 169;\ R^2 = 0.642;\ RSD = 17.7;\ P < 0.001$$

The effect of age was significant ($P < 0.05$) in both these relationships (equations 36 and 37).

4.4.2.2 Prediction of the protein, fat, ash and energy contents in poultry

The linear, multiple linear and allometric regression models derived for the prediction of body protein (P, g) fat (F, g), ash (AS, g) and energy (E, kJ) from all data combined are given in Appendix Tables A4.28, A4.29, A4.30 and A4.31 respectively. The most suitable regression models, chosen on the basis of the amount of variation which was accounted for by a particular model and on the coefficient of variation at the mean body component, are given in Table 4.16. With the inclusion of tritiated water space (T, g) the allometric regression models resulted in an increased correlation coefficient (R^2) but increased the residual standard deviation (RSD) for all body components (see equations 42 versus 43 for protein, equations 46 versus 47 for fat, equations 50 versus 51 for ash and equations 53 versus 54 for energy). However with the inclusion of deuterium oxide space (D, g) in similar equations, the allometric regression models decreased the residual standard deviation (RSD) (see equations 44 versus 45 for protein, 49 versus A78 for fat, 52 versus A79 for ash and 55 versus A118 for energy). The relationships between the quantities of the body components determined by analyses (protein and fat) and those predicted by equations 42 (protein) or 46 (fat) (see Table 4.16) are given

TABLE 4.16 Summary of the appropriate models* determined for the prediction of protein (P, g), fat (F, g) and ash (AS, g) from liveweight (W, g), age (A, g), tritiated water space (T, g) or deuterium oxide space (D, g) in poultry.+

Dependent Variable	Model and Independent Variables	Constants in the equation a	b	c	d	g	$(R^2)^1$	$(RSD)^2$	CoV_3 (%)	Equation Number
Protein (g)	$a + bW + dT$	29.8	0.084		0.160		0.963	28.6	0.57	42
	aW^bT^d	0.326	0.540		0.422		0.972	29.3	0.58	43
	$a + bW + gD$	47.4	0.093			0.137	0.943	26.8	0.40	44
	aW^bDg	0.679	0.447			0.421	0.928	22.7	0.34	45
Fat (g)	$a + bW + dT$	-80.2	0.604		-0.607		0.936	58.4	1.23	46
	aW^bT^d	0.0032	4.070		-2.714		0.959	79.6	1.67	47
	$aW^bT^dA^c$	0.0037	3.718	0.113	-2.446		0.962	80.3	1.69	48
	$a + bW + gD$	-109.1	0.484			-0.403	0.874	67.6	0.99	49
Ash (g)	$a + bW + dT + cA$	10.2	0.014	0.042	0.018		0.609	18.5	2.04	50
	$aW^bT^dA^c$	0.154	0.469	0.279	0.104		0.745	26.8	2.95	51
	$a + bW + gD + cA$	3.5	0.009	0.043		0.029	0.622	14.0	1.28	52
Energy (kJ)	$a + bW + dT$	-2474.0	26.01		-20.32		0.971	2117.0	0.68	53
	aW^bT^d	1.991	2.450		-1.296		0.982	2485.3	0.80	54
	$a + bW + gD$	-3207.0	21.46		-12.76		0.941	2401.0	0.56	55

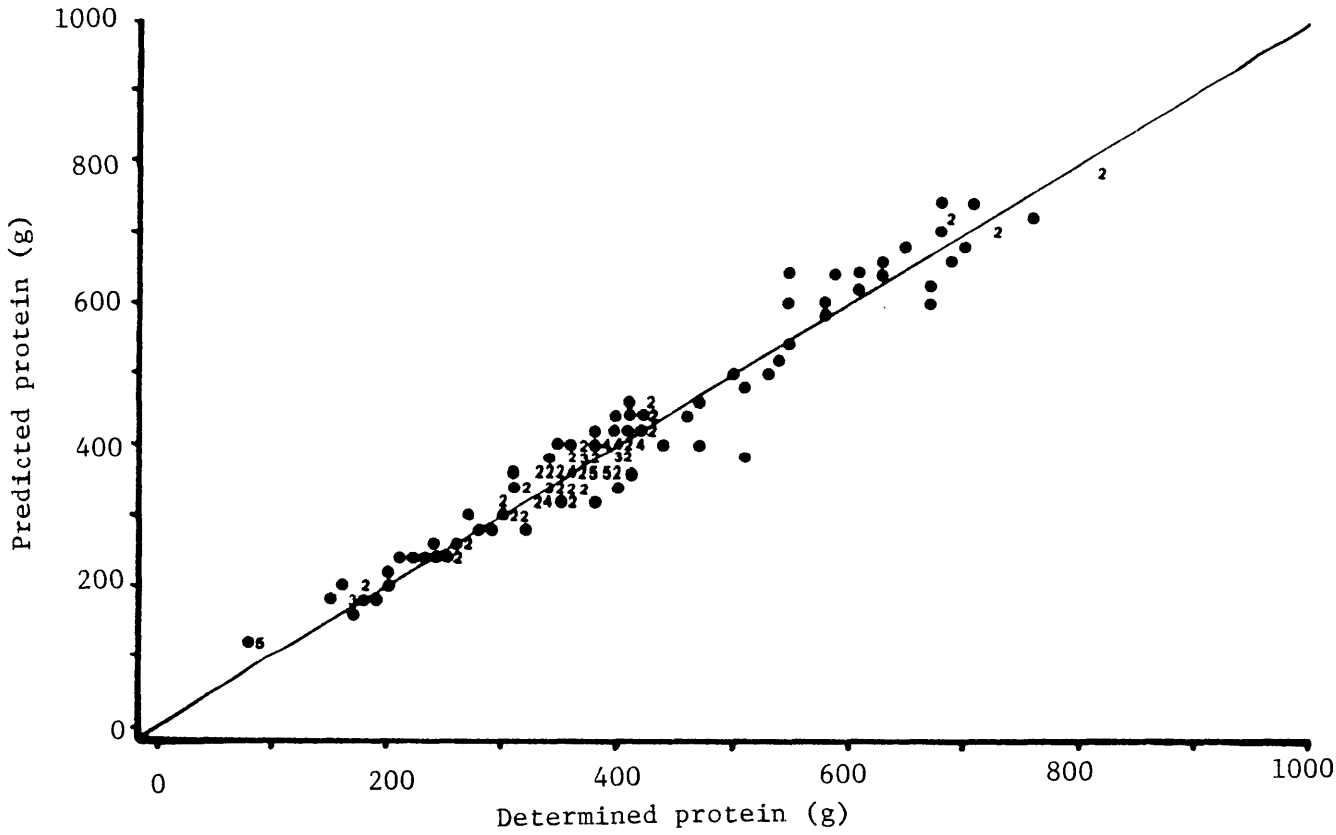
* Taken from Appendix Tables A4.28, A4.29, A4.30, A4.31.

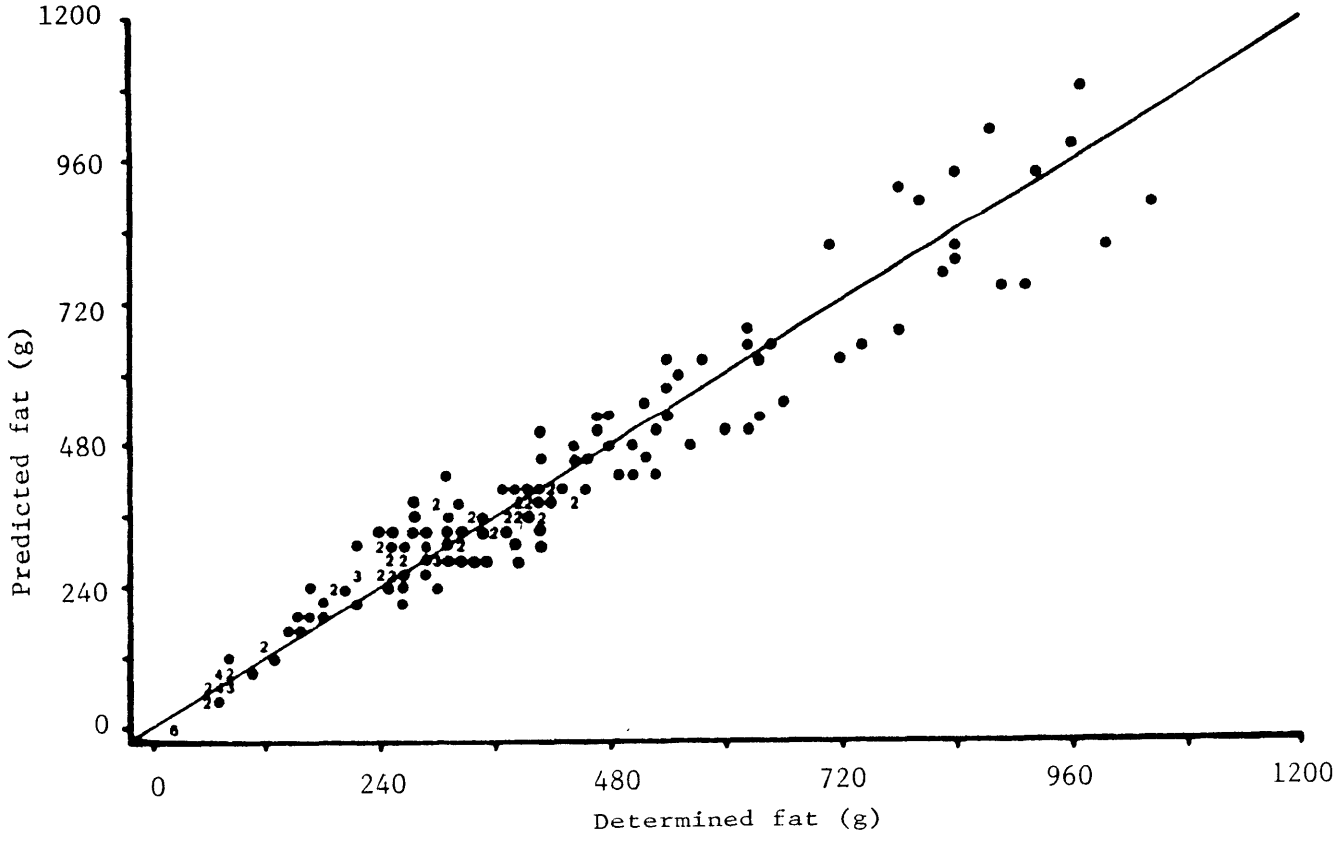
\+ N = 169 for tritiated water space (T) and 115 for deuterium oxide space (D).

Notes 1, 2 and 3, see Table 4.10.

FIGURE 4.8: The relationship between protein predicted from liveweight and tritiated water space (equation 42, Table 4.16) and protein determined by carcass analysis. The line of equality is given.

FIGURE 4.9: The relationship between fat predicted from liveweight and tritiated water space (equation 46, Table 4.16) and fat determined by carcass analysis. The line of equality is given.


Predicted protein (g)
1000
800
600
400
200
0
0
200
400
600
800
1000
Determined protein (g)


Predicted fat (g)
1200
960
720
480
240
0
0
240
480
720
960
1200
Determined fat (g)

in Figures 4.8 and 4.9 respectively. Lines of equality are included in these figures. The inclusion of age (A, d) in the regression models was significant only for the prediction of fat (F, g) and ash (AS, g). The calculated maturity indices (M and N) did not result in any improvement in precision above that obtained by the inclusion of age (A, d) or liveweight (W, g) alone so regression models with these variables (M and N) were omitted.

Multiple linear regression equations for the relationships between protein (P, g) or fat (F, g) and liveweight (W, g) and tritiated water space (T, g) for the individual groups of birds (see Table 4.1 for group details and Table 4.14 for relevant carcass composition values) are given in Tables 4.17 and 4.18 respectively. Covariance analysis of the equations between groups for the relationship between protein (P, g) and liveweight (W, g) and tritiated water space (T, g) showed no significant differences in slopes (regression coefficients) but significant ($P < 0.001$) differences in adjusted mean protein (P, g). Covariance analysis for the same independent variables but for the prediction of fat (F, g) showed that both slopes and adjusted means were significantly ($P < 0.001$) different between groups. Group differences in adjusted means for the relationships between protein (P, g) or fat (F, g) and liveweight (W, g) and tritiated water space (T, g) are given in covariance probability matrices in Tables 4.19 and 4.20 respectively.

Linear, multiple linear and allometric regression models derived after prior classification of birds on the basis of age and type and on liveweight are given in Tables 4.21 and 4.22 respectively. The percentage differences between the determined mean values for body components and those predicted from liveweight (W, g) and tritiated water space (T, g) using either category equations (Table 4.21) or combined equations (Table 4.16) are given in Table 4.23. Equations for young birds (aged less than or equal to 162 d) and birds classified as newlayers (aged 162 to 280 d) had the lowest correlation coefficients (R^2) and highest coefficients of variation (CoV%) for the prediction of body components from liveweight (W, g) and tritiated water space (T, g). Considerable deviation from the actual determined values by the use of these prediction equations was therefore evident for these two categories of birds (pullets and newlayers) (see Table 4.23).

TABLE 4.17 Multiple linear regressions of protein (P, g) on liveweight (W, g) and tritiated water space (T, g) for birds in individual groups.

Group	No. of birds	Constants in the equation: $P = a + bW + dT$					Equation Number+
		a	b	d	$(R^2)^1$	$(RSD)^2$	
1	16	-22.0	0.117	0.154	0.338	46.4	42a
2	6	-68.9	0.494	-0.169	0.263	3.7	42b
3	12	33.5	0.151	0.034	0.641	10.3	42c
4	12	-47.0	0.201	0.087	0.794	12.0	42d
5	18	11.2	0.106	0.153	0.800	20.6	42e
6	18	159.9	0.180	-0.104	0.720	17.0	42f
7	18	175.4	0.112	-0.013	0.762	15.4	42g
8	15	164.5	0.087	0.034	0.734	18.3	42h
9	10	-10.5	0.035	0.270	0.798	35.8	42i
10	12	171.2	0.042	0.125	0.542	14.9	42j
11	12	179.6	0.135	-0.052	0.673	26.6	42k
12	12	213.8	0.070	0.080	0.946	14.7	421
13	8	-159.1	0.210	0.025	0.786	48.8	42m

Notes 1 and 2, see Table 4.10.

+ Number refers to regression model given in Table 4.16.

TABLE 4.18 Multiple linear regressions of fat (F, g) on liveweight (W, g) and tritiated water space (T, g) for birds in individual groups.

Group	No. of birds	Constants in the equation: F = a + bW + dT					
		a	b	d	$(R^2)^1$	$(RSD)^2$	Equation Number+
1	16	-246.2	0.788	-0.733	0.777	57.9	46a
2	6	-36.6	0.480	-0.443	0.612	1.4	46b
3	12	-73.2	0.428	-0.359	0.638	12.3	46c
4	12	-36.1	0.537	-0.594	0.710	14.9	46d
5	18	-17.9	0.560	-0.560	0.932	15.1	46e
6	18	-149.6	0.436	-0.256	0.780	35.0	46f
7	18	-96.0	0.495	-0.406	0.904	28.0	46g
8	15	-282.7	0.448	-0.205	0.901	39.1	46h
9	10	23.0	0.514	-0.535	0.941	44.2	46i
10	12	-191.2	0.695	-0.670	0.938	21.4	46j
11	12	50.4	0.400	-0.366	0.821	42.9	46k
12	12	-404.7	0.822	-0.753	0.978	54.0	46l
13	8	58.5	0.109	0.166	0.826	36.5	46m

Notes 1 and 2, see Table 4.10.

+ Number refers to regression model given in Table 4.16.

TABLE 4.19 Covariance probability matrix of adjusted means for differences between individual groups of birds for the regression model P(g) = a + bW + dT.

Group	1	2	3	4	5	6	7	8	9	10	11	12	13
1		***	***	**	NS	NS	NS	NS	**	NS	NS	NS	NS
2			*	**	***	***	***	**	***	***	***	**	**
3				NS	***	***	*	*	***	***	*	**	*
4					*	**	NS	NS	**	***	NS	*	NS
5						NS	NS	NS	**	*	NS	NS	NS
6							*	*	*	NS	NS	NS	NS
7								NS	***	**	NS	*	NS
8									***	**	NS	*	NS
9										*	***	**	**
10											*	NS	NS
11												NS	NS
12													NS
13													

TABLE 4.20 Covariance probability matrix of adjusted means for differences between individual groups of birds for the regression model F(g) = a + bW + dT.

Group	1	2	3	4	5	6	7	8	9	10	11	12	13
1		NS	NS	*	***	*	*	***	***	***	*	NS	NS
2			*	**	***	*	*	**	**	**	NS	NS	NS
3				NS	**	NS	NS	**	**	NS	NS	NS	NS
4					NS	NS	NS	NS	*	NS	NS	NS	NS
5						**	**	NS	NS	NS	**	NS	NS
6							NS	**	**	NS	NS	NS	NS
7								*	**	NS	NS	NS	NS
8									*	NS	*	NS	NS
9										*	***	***	***
10											NS	NS	NS
11												NS	NS
12													NS
13													

TABLE 4.21 Linear, multiple linear and allometric regression equations established to predict body protein (P, g) and fat (F, g) from liveweight (W, g) and tritiated water space (T, g) for groups of birds classified on the basis of age and type into pullets (aged less than or equal to 162 d), newlayers (aged 162 to 280 d), oldlayers (aged 280 to 476 d) and broilers (broiler breeders).

Dependent Variable	Model and Indpendent Variables	Category	Constants in the equation a	b	d	$(R^2)^1$	$(RSD)^2$	Equation Number
Protein (g)	$a + bW + dT$	Pullets	-10.4	0.109	0.170	0.976	14.3	53a
		Newlayers	92.6	0.071	0.139	0.426	29.9	53b
		Oldlayers	162.4	0.100	0.017	0.706	18.7	53c
		Broilers	81.5	0.085	0.132	0.826	39.2	53d
	aW^bT^d	Pullets	0.164	0.621	0.441	0.985	-	54a
		Newlayers	1.559	0.326	0.433	0.452	-	54b
		Oldlayers	4.928	0.576	-0.003	0.718	-	54c
		Broilers	0.756	0.384	0.475	0.834	-	54d
Fat (g)	$a + bW + dT$	Pullets	-10.5	0.547	-0.635	0.975	13.5	55a
		Newlayers	-183.7	0.759	-0.755	0.818	47.3	55b
		Oldlayers	-147.6	0.404	-0.229	0.838	39.5	55c
		Broilers	-133.8	0.597	-0.569	0.852	100.1	55d
	aW^bT^d	Pullets	0.009	4.875	-3.737	0.975	-	56a
		Newlayers	0.002	3.753	-2.286	0.823	-	56b
		Oldlayers	0.007	2.045	-0.649	0.796	-	56c
		Broilers	0.0005	3.434	-1.802	0.904	-	56d
Energy (kJ)	$a + bW + dT$	Pullets	-666.8	24.3	-21.2	0.987	613.0	57a
		Newlayers	-5095.4	31.8	-26.7	0.898	1496.7	57b
		Oldlayers	-1994.8	18.5	-8.5	0.890	1494.9	57c
		Broilers	-3377.3	25.7	-19.5	0.914	3520.3	57d

Notes 1 and 2, see Table 4.10.

TABLE 4.22 Linear, multiple linear and allometric regression equations established to predict body protein (P, g) and fat (F, g) from liveweight (W, g) and tritiated water space (T, g) for groups of birds classified on the basis of liveweight.

Dependent Variable	Model and Independent Variables	Category+	Constants in the equation a	b	c	d	(R^2)[1]	(RSD)[2]	Equation Number
Protein (g)	$a + bW + dT$	1	-11.4	0.178		0.074	0.980	10.8	58a
		2	84.6	0.122		0.057	0.499	26.3	58b
		3	8.5	0.060		0.207	0.926	35.5	58c
	$a + bW + dT + cA$	1	-12.5	0.205	-0.112	0.051	0.980	10.9	59a
		2	89.0	0.121	-0.060	0.071	0.527	25.7	59b
		3	-12.6	0.046	0.057	0.233	0.929	35.1	59c
	aW^bT^d	1	0.144	0.901		-0.841	0.987	-	60a
		2	1.270	0.598		0.169	0.509	-	60b
		3	0.342	0.233		0.739	0.937	-	60c
Fat (g)	$a + bW + dT$	1	-10.8	0.542		-0.627	0.946	12.9	70a
		2	-206.8	0.677		-0.610	0.715	49.6	70b
		3	-134.2	0.587		-0.554	0.847	88.0	70c
	aW^bT^d	1	0.008	5.112		-3.983	0.960	-	80a
		2	0.001	3.582		-2.013	0.697	-	80b
		3	0.004	3.580		-2.223	0.866	-	80c
	$aW^bT^dA^c$	1	-	-	-	-	-	-	90a
		2	0.001	3.558	0.140	-2.109	0.729	-	90b
		3	0.005	3.851	-0.085	-2.492	0.876	-	90c

+ Categories were: 1. Liveweight less than or equal to 1500 g; 2. Liveweight greater than 1500 g and less than or equal to 2200 g; 3. Liveweight greater than 2200 g.

Notes 1 and 2, see Table 4.10.

TABLE 4.23 Differences between determined mean values for body components and those predicted from liveweight (W, g) and tritiated water space (T, g) after classification of birds into pullets (aged less than or equal to 162 d), newlayers (162-280 d), oldlayers (280-476 d) and broilers (broiler breeders).

Body Component[1]	Category+	Body component predicted using:		Difference (%)[4]	
		Category equations[2]	Combined equation[3]	Category equations	Combined equation
Protein	Pullets	242.7	246.9	0.08	1.81
	Newlayers	380.9	365.8	-0.10	-4.07
	Oldlayers	382.6	390.7	-0.03	2.09
	Broilers	636.4	638.9	-0.06	0.33
Fat	Pullets	128.4	144.7	0.23	12.96
	Newlayers	379.7	361.2	0.32	-4.57
	Oldlayers	382.3	392.4	-2.12	0.46
	Broilers	690.1	689.0	0.17	0.01
Energy	Pullets	10831.8	11637.1	0.43	6.97
	Newlayers	24049.8	23071.0	-0.36	-4.42
	Oldlayers	24668.6	24904.7	0.07	1.03
	Broilers	42350.5	42604.0	-0.52	0.07

+ See text for details and Appendix Table A4.26 for actual mean values of body components.

1. Determined body component.

2. Relevant equations are given in Table 4.21 (Equations 53a to d, 55a to d and 56a to d).

3. Equations 42, 46 and 53 in Table 4.16 for protein, fat and energy respectively.

4. Percentage difference between predicted and actual body component.

4.4.3 DISCUSSION

4.4.3.1 Body composition relationships in poultry

The curvilinear relationship found between body fat (g/kgW) and total body water (g/kgW) in the present study (Figure 4.3) with poultry is similar to that derived for cattle (Reid *et al.* 1955). For sheep Searle (1970a) found no evidence of curvilinearity in this relationship. Biologically the form of the relationship here can be explained as a decrease in total body water (g/kgW) without a concomitant equivalent or greater increase in body fat content (g/kgW) at high levels of total body water (g/kgW) and low levels of body fat (g/kgW), which can be expected to occur in young pullets due to high rates of protein deposition. A further contribution to non-linearity could be expected by a change in the relationship caused by maintenance of body water content at extreme rates of body fat deposition. In between these two extremes the relationship could be expected to be essentially linear. Birds were therefore grouped on the basis of age and type into categories (Section 4.3.1.4) and separate linear regression equations were derived for the relationship between body fat (g/kgW) and total body water (g/kgW). These regression equations are given in Table 4.24. Covariance analysis of these equations showed significant ($P < 0.001$) differences between slopes and adjusted mean body fat (g/kgW). Birds classified as pullets had a significantly ($P < 0.001$) lower adjusted mean body fat (g/kgW) than all the other categories. Broiler breeders had a significantly ($P < 0.05$) higher adjusted mean body fat (g/kgW) than birds classified as oldlayers.

The significant changes in slope and covariance adjusted means for the relationship between body fat (g/kgW) and total body water (g/kgW) implies *pro rata* a change in the ratio between total body water and body protein with decreasing total body water (g/kgW). As expected on the basis of Figure 4.3, the total body water to body protein ratio changed with increasing age until a relatively stable period occured. The relative rates of protein and fat deposition can be expected to have a large influence on the rate of change of this ratio. For example, van Gils *et al.* (1977) found that the water to protein ratio of the gain of young (0-40 d of age) broiler pullets was significantly affected by diet and feeding rate. Whether this finding could be attributed to differences in physiological development (Bailey *et al.* 1960) was not ascertained. Hunt (1965) found effects of breed, sex, age and diet on

TABLE 4.24 Linear regression equations for the relationship between body fat (g/kgW) and total body water (g/kgW) for groups of birds classified on the basis of age and type into pullets (aged less than 162 d), newlayers (aged 162 to 280 d), oldlayers (aged 280 to 476 d) and broilers (broiler breeders).

Category	No. of birds	Constants in the equation+ a	b	$(R^2)^1$	$(RSD)^2$	CoV_3 (%)	Equation Number
Pullets	48	561.3	-0.729	0.944	8.12	1.15	38
Newlayers	46	871.3	-1.228	0.845	16.63	1.22	39
Oldlayers	45	705.8	-0.919	0.589	17.18	1.32	40
Broilers	30	910.6	-1.277	0.868	19.92	1.82	41

+ FW (g/kgW) = a + bTBW(g/kgW)

where FW is body fat and
TBW is total body water.

Notes 1, 2 and 3. See Table 4.10.

the water to protein ratio in growing chickens (7-29 d of age). Studies on other animal species have also shown dietary-induced alterations in the relationship between protein content and liveweight (Weil and Wallace 1963; Norton *et al.* 1970). Prediction of body composition in young growing birds can therefore be expected to be extremely complex. However age can be accounted for in the derivation of prediction equations, and although such equations, on the basis of practicality, cannot account for factors such as diet and feeding rate, realistic and accurate least square estimates can nevertheless be obtained. The results of the present study indicate that the importance of variables which were shown to influence the accuracy of prediction of body composition may be large only during the growing period.

The relationships reported for different animal species in conjunction with that derived in the present study between body fat and total body water are given in Table 4.25. There is evidently a close relationship between animal species and experiments in the rate at which body water changes per unit change in body fat content, when both are expressed on a liveweight basis. The form of the relationship between body fat and water found by the various authors (Table 4.25) or by the present author, in which a change in total body water is expressed as a function of a change in body fat, is not biologically based because it is the change in body fat which would cause a corresponding change in total body water (on a liveweight basis). Rather, such regression models are statistically based because of the interest in the estimation of body fat from a knowledge of body water. Farrell (1974b) with poultry and Wood and Grooves (1963) with pigs showed that in young animals, per unit change in body fat content (liveweight basis), there is a large change in total body water content (liveweight basis). These reports substantiate the results found in the present study and those by Reid *et al.* (1955) with cattle of a curvilinear relationship between body fat and water, when both are expressed on a liveweight basis. Van Gils *et al.* (1977) showed the extremely high rates of protein deposition which can occur in young animals, with the ratio between protein and fat deposition often being negative or very small, depending on diet and feeding rate. Therefore for low rates of fat deposition, protein deposition would be high. Protein deposition occurs with substantial water deposition (van Es 1977), but would result in a net reduction in water content on a liveweight basis. There is a remarkable similarity in the regression coefficient found in the present study for birds classified as pullets (see Table 4.24) and that given

TABLE 4.25 Some examples from the literature of the constants derived for the relationship between body fat (FW, g/kgW) and total body water (TBW, g/kgW).

Animal Type	No. of animals	Constants in the equation[+] a	b	Reference
Poultry*	16	1060.0**	-1.62	Farrell and Balnave (1977)
Poultry[≠]	240	587.0	-0.72	Farrell (1974b)
Cattle	256	842.9	-1.12	Reid *et al.* (1955)
Cattle	31	809.9	-1.03	Little and McLean (1981)
Sheep	9	946.0	-1.22	Panaretto (1963)
Sheep	61	919.7	-1.18	Searle (1970a)
Hamsters	34	989.5	-1.35	Kodama (1971)
Pigs	11	686.1	-0.80	Wood and Groves (1963)
Poultry	169	805.3	-1.11	Present study

+ The equation is the linear regression model:

$$FW\,(g/kgW) = a + bTBW\,(g/kgW)$$

where FW is body fat (g/kgW) and
TBW is total body water (g/kgW)

≠ Broiler pullets at 8 weeks of age.

* Laying hens

** The regression equation given by Farrell and Balnave (1977) was in error and has been recalculated.

by Farrell (1974b) for young broiler pullets in the relationship between body fat and total body water on a liveweight basis. This similarity was apparent despite the different types of birds used, the different diets, feeding rates and growth rates between the two experiments.

There was a good relationship between body protein content and liveweight for the birds used in the present study. The coefficient of variation at the mean body protein content of 387.6 g for equation 29 was only 0.63%. As expected, there was a marked reduction in the variability of total body water, protein and ash, on a liveweight basis, when these values were expressed on a fat-free body mass basis. The mean water content of the fat-free mass of birds declined initially with increasing age; after approximately 15 weeks of age the mean value was 68.8%. Values for birds younger than those used in the present study were derived from data given by Edwards *et al.* (1973) and Edwards and Denman (1975). These values have been included in Figure 4.5 to illustrate more clearly the expected rate of decline with age in the water content of the fat-free mass in poultry. Derivation of two regression models to account for the effect of age on the water content of the fat-free mass for the birds used in the present study (equations 34 and 35) did not result in an improvement in precision of prediction of total body water from fat-free mass. The effect of age on the water content of the fat-free mass in the present study was therefore not important due to the actual ages of birds used. Results shown in Figure 4.5 for data derived from the literature (Edwards *et al.* 1973; Edwards and Denman 1975) indicate the large effect of age which could be expected, if younger birds had been used in the present study.

Protein content of the fat-free dry matter declined, and ash content increased, with increasing age. Reid *et al.* (1955) found similar trends in cattle. The decrease in the protein content of the fat-free dry matter at 162 d of age for birds in Group 5 was unexpected and is difficult to explain as there was not a simultaneous increase in the ash content of the fat-free dry matter. This was associated with incomplete dry matter recovery in these birds (92%). Omitting birds in Group 1 because ash content was not determined directly, and birds in Group 5, the mean dry matter recovery of all birds used in the present study was 97%. The low dry matter recovery for birds in Group 5 was partially caused by a low dry matter recovery for liver analysis of these birds, particularly for birds in Group 5b (limited-time feed restriction). However this particular aspect will be discussed in more detail in Chapter 5.

Neil *et al.* (1977) found a low recovery of the dry matter in the carcasses of birds slaughtered after the production of 10 eggs, with a concomitant decrease in the protein content of the fat-free dry matter. There are few possibilities which could explain the low recovery of dry matter in the birds in Group 5 (162 d of age). Increased glycogen storage can be discounted as a possible reason for the low recovery of carcass dry matter, but not for liver dry matter recovery (see Chapter 5). There was no evidence that the overestimation of total body water by isotope dilution was increased in birds in Group 5, thus indicating comparable accuracy of determination of carcass dry matter content. If fat determinations by petroleum ether extraction were in error then both the protein and ash contents of the fat-free dry matter would be decreased. Although a substantial number of repeat analyses were carried out on the carcasses of the birds in Group 5 with good repeatability, a possible explanation for the observed effect is incomplete recovery of protein. Despite excellent duplication, the reason for this is unknown.

4.4.3.2 Prediction of the protein, fat, ash and energy contents of poultry

The relationships between the body components as discussed in the previous section form the fundamental basis for the derivation of regression models which were used to predict these body components in this section. Although liveweight alone could be used to provide estimates of the various body components, the inclusion of total body water resulted in substantial improvements in the precision of the estimates for all body components except ash. Regression models which included deuterium oxide space rather than tritiated water space had lower residual standard deviations and coefficients of variation but lower correlation coefficients. This was due to the reduced numbers of birds used for determination of deuterium oxide space. As a consequence a reduced range of values were available for inclusion in the regression models. Derivation of allometric regression models which included tritiated water space increased the calculated residual standard deviation above that found for the linear or multiple linear regression models. However the residual standard deviations calculated by the method given (see Section 4.2.11) for the allometric regression models, which is apparently similar to that used by Donnelly and Freer (1974), represent only a good approximation. Coefficients of variation are nevertheless extremely small for all the major regression models.

There were major differences as determined by covariance analyses in forms of the multiple linear regression models derived for individual groups. This was expected on the basis of the complexities involved in the body composition relationships as discussed in the previous section (Section 4.4.3.1). However, rather surprisingly, there was no significant ($P > 0.20$) differences in the slopes between individual groups of birds for the relationship between protein and liveweight and tritiated water space. Consequently the equation derived for all data for the prediction of protein from liveweight and tritiated water space (equation 42) was more acceptable than the similar combined equation but for the prediction of fat (equation 46), when both were compared to the individual equations derived after prior classification of birds on the basis of age and type (see Table 4.23). The regression equations which were derived from all data combined for the prediction of protein, fat and energy (see equations 42, 46 and 53 respectively in Table 4.16) resulted in considerable bias when the predicted values were compared to the actual values for young pullets and for birds at commencement of egg production. This bias is clearly illustrated in Figure 4.9. Individual category equations after birds were classified on the basis of age and type resulted in markedly improved precision.

Farrell (1974b) and Farrell and Balnave (1977) have presented equations to predict body protein and fat in poultry from liveweight and tritiated water space. Farrell and Balnave (1977) found a coefficient of variation of 14% for the prediction of fat from these variables in sixteen laying hens. This value equates to approximately 3.5% at the mean body fat content of approximately 664 g in their study. This coefficient of variation at the mean body fat is nearly three times greater than that found in the present study (see equation 46, Table 4.16). Farrell and Balnave (1977) stated that such an equation could be used to estimate body fat subsequently in laying hens. Calculations were carried out to determine the accuracy with which the equations given by Farrell (1974b) and Farrell and Balnave (1977) could predict body fat for the birds in the present study (see Table 4.26) and it was apparent that the precision of prediction was extremely poor. This indicates the oversimplistic approach which Farrell and Balnave (1977) adopted in the probable application of the regression equations which they derived. The considerable complexities involved were not considered by these authors (Farrell and Balnave 1977). Due to the lack of sufficient data presented in the

TABLE 4.26 Determined body fat (F, g) for birds classified according to age and type and that predicted from liveweight (W, g) and tritiated water space (T, g) by two equations given in the literature.

Category+	Mean determined fat (F, g) ≠	Fat predicted* from equation in: Reference 1 (g)	Reference 2 (g)
Pullets	128.1	167.5	80.8
Newlayers	378.5	326.1	390.8
Oldlayers	390.6	350.3	439.2
Broilers	688.9	582.3	903.7

+ See text for details.

≠ Determined by carcass analysis of the birds in the present study.

* Reference 1 (Farrell 1974b):

$F(g) = -13.4 + 0.40W - 0.36T$ $N = 240$; $R^2 = 0.67$; $RSD = 35.1$

Reference 2 (Farrell and Balnave 1977):

$F(g) = -285.0 + 0.75W - 0.64T$ $N = 16$; $R^2 = 0.95$; $RSD = 93.0$

previous studies, it was not possible to determine the ability of the equations derived in the present study to predict body composition for the poultry used by these authors (Farrell 1974b; Farrell and Balnave 1977).

Little and McLean (1981) derived a linear relationship from carcass analyses in cattle between total body water plus fat and liveweight. The relationship was:

$$\{TBW + F\}(kg) = 0.77\ FLW + 0.92$$
$$N = 31;\quad RSD = 3.55;\quad R^2 = 0.997$$

where FLW is fasted liveweight (kg).

The approach which these authors (Little and McLean 1981) adopted was the initial estimation of total body water by tritiated water and the subsequent estimation of body fat, using the equation given above, by difference. Clearly, the complement of the sum of total body water and body fat is the fat-free dry matter. That is

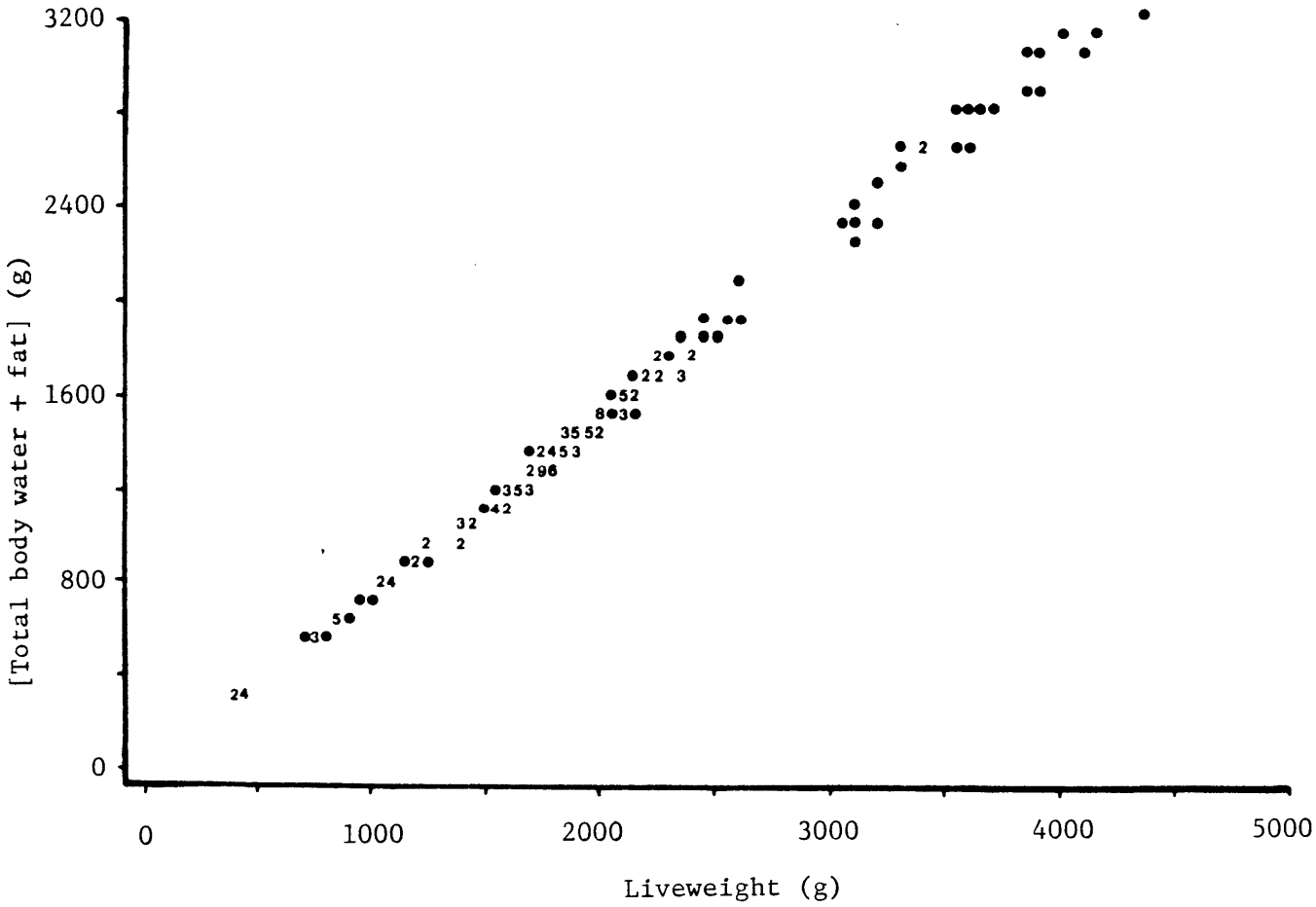
$$W(g) = TBW + F + FFDM$$

where W is liveweight, TBW is total body water (g), F is body fat (g) and FFDM is fat-free dry matter (g). To investigate if any improvement in the precision of estimation of body fat could be gained by this approach the following equation was derived from the data in the present study:

$$\{TBW + F\}(g) = -47.5 + 0.773\ W \qquad (91)$$
$$N = 169;\quad RSD = 41.7;\quad R^2 = 0.996$$

This relationship is also shown in Figure 4.10. As expected, the constant in the above regression equation is negative. For birds classified on the basis of age and type (pullets, newlayers, oldlayers, broilers) the mean total body water was predicted from the mean tritiated water space (equations A5, A6, A7 and A8 in Appendix Table A4.17). The sum of total body water and body fat was predicted by equation 91 using the mean liveweight for each of the categories, and body fat was determined by difference. The mean percentage differences between fat predicted in this manner and actual body fat were 3.4%, -0.03%, -1.22% and 0.35% for group categories of pullets, newlayers, oldlayers and broilers respectively. These values compare extremely favourably, particularly for group categories "pullets" and "newlayers", with those values for fat determined by either the individual category equations or the combined equations. This approach removes the marked bias found for predicted fat for group categories

FIGURE 4.10: The relationship between the sum of total body water and fat (g) and liveweight (g) for poulty (N = 169).


3200
2400
1600
800
0
[Total body water + fat] (g)
0
1000
2000
3000
4000
5000
Liveweight (g)

pullets and newlayers when the combined equation is used.

Summary

Poultry which varied in age, liveweight, prior nutritional history, and strain were used to derive body composition prediction equations based on liveweight and tritiated water space or deuterium oxide space. This involved the slaughter and chemical analysis of the birds after prior isotope injection and blood sampling for the determination of equilibrium isotope concentration. Initially the relationship between determined total body water and either tritiated water space or deuterium oxide space was investigated. Tritiated water space (N = 169) and deuterium oxide space (N = 115) overestimated total body water by 10.4% and 8.5% respectively. Covariance analysis showed that there was no difference in the slopes of the regression lines but a significantly higher ($P < 0.05$) total body water after adjustment to a mean isotope space for deuterium oxide space rather than tritiated water space. Major potential sources of the cause of the overestimation of total body water by isotope dilution space were identified as lack of recovery of isotope from blood samples by the vacuum sublimation technique used and loss of isotope in urine prior to equilibration. The relationships beween the body components of poultry were investigated, and suitable body composition prediction equations derived. It was not possible for general equations to be used which would give good accuracy of prediction for all birds used in the present study. Rather, individual equations for each group of birds within specific age, strain or liveweight ranges were found more appropriate.