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BODY COMPOSITION: ITS INTERRELATIONSHIPS AND ESTIMATION *IN VIVO*

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Body weight is the most widely used criterion for the evaluation of livestock response in nutritional and physiological experiments. However, changes in liveweight due to environmental treatments do not necessarily have the same chemical composition or energy value per unit increase in liveweight. A knowledge of the exact chemical composition of the live animal at any given stage would therefore not only permit a more precise evaluation of feeds, but it could conceivably be applied in the genetic improvement of livestock and would undoubtedly be a useful diagnostic tool of metabolic and physiological disorders. The prediction of body composition is of particular relevance in energy balance studies since the various other indirect calorimetric methods, or the slaughter technique, which are used for this purpose either have their limitations or require expensive and laborious equipment and techniques. It is for these reasons that so much research effort has been devoted to the study of body composition and methods of estimating it in the living animal.

The purpose of this paper is to summarize our existing knowledge of the relationship between the major chemical components of the body and to indicate how these interrelationships are applied in the evaluation of the chemical composition and energy content of living animals.

Interrelationships between the major chemical components of the body

Early investigations into the chemical composition of animal bodies showed that the higher vertebrates have a more or less constant composition which is characteristic of their age and species (von Bezold, 1857; Lawes & Gilbert, 1859, 1861). It was also commonly accepted by investigators of this period (Henneberg, 1880; von Hösslin, 1881; Pfeiffer, 1887) that the fattening process is accompanied by a reduction in the (percentage) water content of the body. Voit, a student of Leibig and a leading biologist of this period, believed that this process was partly due to the replacement of water by fat but chiefly one of deposition of water-free fat in the tissues so that with the removal of fat the organism showed a nearly normal water content. In 1919 Murray again brought attention to the fact that the composition of the non-fatty matter was practically constant and that it was not influenced by the fat content of the body but that it was somewhat dependent upon age. He also brought attention to the fact that the composition of the body was obvious if its fat content could be determined. Using the data of Lawes & Gilbert on sheep, cattle and pigs he furthermore attempted to quantitate these relationships. In a later publication Murray (1922) used data published by Haecker (1920) on cattle and Swanson (1921) on pigs to produce further evidence in support of his earlier views. Murray's findings were soon to be confirmed by those of Moulton (1923), in studies on body composition at Missouri Experimental Station. Further progress in the understanding of the basic relationship between the chemical components of the body resulted from Reid, Wellington & Dunn's (1955) observation that protein and ash form a remarkably constant fraction of the dry fat-free mass of the body. Using analytical data on 251 cattle they were able to prove that this relationship is independent of body type, diet or degree of fatness. They were therefore able to show that the chemical components of the body can be divided into a constant fraction consisting of protein and ash and a highly variable fraction consisting of water and fat.

The above-mentioned workers have been singled out since their observations form the basis of our present understanding of the relationships between the major chemical components of the body.

1. *Relationship between body fat and body water*

It is now a well established fact that the percentages of water and fat in the empty body (i.e. the whole ingesta-free body) are highly correlated, inversely. Earlier studies with limited numbers suggested that this relationship is linear but by using the data on a total of 256 cattle Reid *et al.* (1955) were able to prove that the relationship is in fact significantly curvilinear. Using the results from his his own laboratory as well as those of several other workers Reid *et al.* 1968a) has produced regression equations which allow the prediction of empty body fat from empty body water with a high degree of precision. The equations are based on the data of 714 pigs (9 breeds), 330 sheep (7 breeds) and 256 cattle (12 breeds). Despite the wide range of breeds, body types, sex (intact males, intact females and castrate males) and obvious variation in nutritional history, 97,4 to 98,2% of the variability in fat concentration of the three species is associated with variation in water concentration. In a later publication Reid (1971) included a prediction equation for horses as well although it is based only on a limited number of observations. Although the relationship between fat and water is independent of age, body condition, sex, level of feeding and breed the differences between species are significant. The prediction equations which allow the estimation of either fat or water, the one from the other, in the empty body are summarized in Table 1.

Table 1

The relationship between the concentration of empty body water and empty body fat in four animal species (Reid, 1971)

Specie	Number	Correlation Coefficient	Prediction Equation	Sy.x
Pig	714	−0,990	$\hat{Y}$ = 89,11 − 1,1095X	1,71
Horse	11	−0,966	$\hat{Y}$ = 107,71 − 1,5407X	1,31
Sheep	330	−0,991	$\hat{Y}$ = 98,31 − 1,3068X	1,03
Cattle	256	−0,987	$\hat{Y}$ = 84,29 − 1,1182X	1,03

Where Y = Per cent fat in empty body;
X = Per cent water in empty body
Sy.x = Standard error of the estimate.

2. *Composition of the fat-free empty body*

Using the same data described above, Reid (1971) examined the relationship between protein and ash in the dry fat-free empty body. The results are summarized in Table 2. When expressed in these terms the percentages of protein and ash form remarkably constant proportions. Although this relationship is slightly different for different animal species it is not affected by breeds within species or by body condition (degree of fatness). Age has a very small, but nevertheless significant, effect on the relative proportions of protein and ash (Reid *et al.,* 1955; Searle, 1970) and can be used to refine the estimates of protein and ash even further. The coefficients of variation in protein concentration for the data in Table 2 are only 2,8% in pigs, 2,5% in horses, 3,0% in sheep and 2,1% in cattle.

Table 2

The composition of the fat-free dry empty body of four animal species (Reid, 1971)

Specie	Number	Protein (%)	Standard Deviation	Ash (%)	Standard Deviation
Pig	714	83,42	±2,33	17,01	±1,69
Horse	11	79,64	±1,96	20,36	±1,93
Sheep*	221	81,09	±2,40	18,91	±2,39
Cattle	256	80,26	±1,69	19,74	±1,69

* Data for sheep on a wool-free basis.

3. *Relationship between protein and water*

Another relationship which can be used for the prediction of body composition is that existing between the total weight of water and the weight of protein in empty body. The extremely close relationship (correlation coefficiently = 0,99) between the quantities of protein and water in the empty body was noted in a study of the composition of 65 sheep of various ages and nutritional history (Van Niekerk, 1962). Panaretto & Till (1963) found that a similar relationship exists between the amounts of protein and *total* body water (including gut water) and make use of this relationship in the scheme they have proposed for predicting body composition.

Prediction of Body Composition

The highly predictable relationships between the *concentration* of fat and water in the empty body, the *concentration* of protein and ash in the fat-free dry empty body and the *amounts* of protein and water in the empty body make it possible to resolve the entire *in vivo* chemical composition of the body from any one of its major chemical constituents. The most common approach has been to estimate either empty body fat or empty body water. Attempts to measure body fat by the specific gravity method (Behnke, Feen & Welham, 1942; Rathbun & Pace, 1945) or methods based on the uptake of cyclopropane (Lesser, Blumberg & Steele, 1952) and helium (Siri, 1953) have been of only limited success.

The most fruitful approach has been that of estimating total body water. This approach relies upon measuring the degree of dilution in body water of reference substances such as deuterium (Havesy & Hofer, 1934), tritium (Pace, Kline, Schachman & Harfenist, 1947) antipyrine (Soberman, 1949) and N-acetyl 4-aminoantipyrine (Brodie, Berger, Axelrod, Dunning, Porosowska & Steele, 1951). Of these substances tritiated water appears to be the most widely accepted although it also has its limitations due mainly to the fact that tritium hydrogen exchanges with the hydrogen of compounds other than water thus leading to an over-estimation of body water. All reference substances used to date also defuse, to a varying extent, into the water of the digestive tract so that the water volume measured is that of the total body.

A third approach for estimating body composition is that based on estimates of body protein. The relationship between K^{40} and body protein (Anderson 1959) and that between urinary creatinine and body protein (van Niekerk, Reid, Bensadoun & Paladines, 1963) has been applied extensively in medical research but has not come into general use for estimating body composition in animals.

1. *Estimating body composition from body water*

Several schemes for estimating body composition from body water have been proposed. The following scheme is suggested by Reid *et al.* (1955):

(i) Estimate empty body fat or water, the one from the other, using the applicable equation (Table 1).

(ii) Compute the percentage of fat-free, dry matter in the empty body:
Fat free DM = 100 − (%fat + %water).

(iii) Compute the percentages of protein and ash by using the appropriate factors for protein and ash in the fat-free, dry empty body (Table 2).

In order to obtain the actual quantities of water, protein, fat and ash from the percentage composition as estimated above it is necessary to know the weight of the empty body. At present no direct method of estimating the weight of the ingesta is available. Empty body weight can, however, readily be estimated from the close relationship between shrunk body weight (i.e. liveweight measured after a 20 hour fast) and empty body weight (Bensadoun, van Niekerk, Paladines & Reid, 1963; Reid, Bensadoun, Paladines & van Niekerk, 1963). The following equation, applicable to sheep, is used to estimate empty body weight from shrunk body weight (Reid *et al*, 1963).

$$\hat{Y} = 19{,}56 + 1{,}173X - 20{,}909 \log X$$

Where Y = empty body weight (kg)
X = shrunk body weight (kg)

Contrary to expectation, this relationship is relatively independent of the kind of diet or level or intake. The correlation coefficient between the two variables, determined in a population of 65 sheep, was 0,995 while the standard error of the estimate and coefficient of variation amounted to only 1,47 kg and 4,2% respectively. Similar equations have been developed for cattle (Gil, Johnson, Cahill, McClure & Klosterman, 1970) and sheep (Jagusch, Norton & Walker, 1970). Numerous other workers have subsequently also found body weight and empty body weight to be highly correlated. The application of this scheme obviously also requires some estimate of *empty body water*. There is, however, at present still no suitable method of measuring empty body water (or of measuring gut water). This problem can readily be overcome by simply relating the water space of a reference substance (e.g. antipyrine or tritium) with empty body water. This method has been used with considerable success by several workers and Bensadoun *et al.* (1963) have shown that estimates of empty body water obtained in this way compare favourably with actual water content determined by analysis of the empty body.

A somewhat different method of estimating body composition which does not require estimates of either empty body water or empty body weight was proposed by Panaretto & Till (1963) and Panaretto (1968). This method depends on the relationship between *total* body water and either total body fat or *total* body protein. In each case total body water is estimated from the corrected tritiated water space (i.e. tritiated water space reduced by 3–4% to allow for over-estimation of body water as measured directly). This scheme can be summarised as follows (Panaretto & Till, 1963).

(i) Estimate total body water from tritiated water space ("Total body" as used here refers to the intact body after 48 hour fast).

(ii) Total body protein is derived from the relationship between the amount of total body water and amount of total body protein.

(iii) The amount of ash is derived from total body protein by multiplying the weight of protein content by 0,25.

(iv) Total body fat is obtained by difference after correcting for the dry matter content of the digestive tract:

Fat = Liveweight - (water + protein + ash) - 1,5 L.W.

Alternatively body composition can be determined as follows (Panaretto & Till, 1963):

(i) Express the corrected tritiated water space as a percentage of total body weight.

(ii) Total body fat (%) is estimated using the relationship between % body fat and % body water in the whole body.

(iii) The fat-free dry mass is derived by difference, after correcting for the dry matter content of the digestive tract:

Fat-free mass = 100 - (%fat + %water) – 1,5.

(iv) Protein and ash percentages are derived by partioning the % fat-free dry matter by the ratio or 4:1.

(v) The amounts of water, fat, protein and ash are calculated by multiplying the total body weight by the appropriate percentage as obtained above.

This scheme has the advantage of requiring only two measurements (total body water and shrunk body weight), both of which can readily be determined in the living animal. However, it also has disadvantages if used as proposed by the authors. The use of a constant correction factor (1,0 or 1,5 L.W.) for the dry matter content of the digestive tract will result in biased results particularly if applied at extreme weights. This is due to the fact that the contents of the digestive tract do not represent a fixed proportion of the body weight. This criticism could be overcome by developing a prediction equation which would relate the dry matter content of the digestive tract to the shrunk body weight. It should also be noted that the method they use for partitioning the ash and protein contents of dry, fat-free mass also does not allow for the effect of age on this relationship.

Searle (1970) has simplified the procedure for estimating body composition from tritiated water space even further. This is achieved by relating tritiated water space (uncorrected) with the weights of protein, fat, ash and energy in the body. Additional precision is obtained by using age and/or liveweight as further variables in his prediction equations.

2. *Body Protein as a predictor of body composition*

Although body protein is not generally used as a key substance in estimating body composition the following scheme, based on known interrelationships, can be proposed for this purpose.

(i) Estimate total body protein (from urinary creatinine or K^{40}).

(ii) Estimate empty body weight from the relationship between shrunk body weight and empty body weight.

(iii) Estimate empty body water from the relationship between weight of protein and the weight of water in the empty body.

(iv) Express empty body water as a percentage of empty body weight and estimate % fat from the relationship between % fat and % water in the empty body.

(v) Estimate body ash from the relationship between the ash and protein contents of the fat-free, dry body.

3. *Determining the gross energy value of the ruminant body*

The systematization of body composition for the purpose of employing changes in body protein and fat as an index of energy storage, i.e., as an indirect calorimetric method, requires heat of combustion values applicable to the fat and protein of the composite body as opposed to those determined for pure protein and fat preparations. Such values, obtained on the composite bodies of 172 pigs, 221 sheep and 12 cattle, have been reported on by Reid *et al.* (1968b) and are recorded in Table 3.

Table 3

Calorific values of protein and fat in the composite body of sheep and cattle (Reid et al., *1968b)*

Animal	Number of observations	Protein(kcal/g)	Fat(kcal/g)
Pigs	172	5,348	9,608
Sheep*	221	5,411	9,414
Cattle	12	5,447	9,499

*Data for sheep on a wool-free basis

4. *Body weight as a measure of body composition*

An evaluation of indirect methods of estimating body composition requires an assessment of the degree of refinement that can be gained by the indirect method over that of estimates based solely on body weight. Such a comparison was made possible in a study of various indirect methods of estimating body composition of sheep (Reid *et al,* 1963). This study revealed that shrunk body weight is a surprisingly reliable predictor of body composition. It was in fact concluded that in sheep containing less than 30% fat (or approximately 50 kg liveweight), that shrunk body weight was as effective or, in the case of energy, even more effective than the other indirect methods used. Closer examination of the analytical data on the 65 sheep used in this experiment revealed that as liveweight increased the weights of water, fat, protein and ash increase at an essentially linear rate. However the weight of each component increases at a rate significantly different from that of others. The abovementioned data, as well as those of Haecker (1920) and Wood & Groves (1964) show that in sheep, cattle and pigs, the rate of increase is most rapid for water followed by fat, protein and ash in this order. As liveweight increases a point is reached where a rather dramatic increase in rate of fat deposition occurs. In the data examined, this point coincides with a liveweight of 40 kg in sheep and 300 kg in cattle. Beyond this point, the rate of increase in water, protein and ash decrease and the relationship between body weight and its chemical components tend to become curvilinear. This point probably varies between species and breeds within species. An examination of numerous experimental results on pigs, sheep and cattle by Tulloh (1963) and by Reid and co-workers (1968a,b, 1971) have shown that an equation of the type $Y = aX^b$ (where Y = weight of component and X = body weight) gives an excellent fit to the relationship between body weight and its chemical or physically separated body components.

Contrary to what might be expected from Hammond's theory of "differential growth" the relationship between body weight and its chemical components is remarkably resistant in change by nutritional or other environmental treatments. An examination by Reid *et al.* (1968a) of the body composition of a population of 714 pigs (intact females and castrate males), ranging in age of 1 to 923 days and having been exposed to various dietary treatments revealed that 95,6 to 99,3% of the variation in the logarithms of the weights of the individual chemical components is ascribable to variability in the logarithm of body weight. Reid *et al.* (1968a) could find little evidence from this study to show that age or even breed had any significant effect on the relationships with the exception of ash which is slightly influenced by age. It is also evident that castrate males have a little more fat than intact females at weights above 70 kg but the differences were not large. Studies to date appear to cast doubt on whether any real progress (from a chemical point of view) has been made in the development of "meat type" pigs. One study has shown that apparent differences in body composition between the two types of pigs are largely due to a redistribution of fat within the other body tissues (Reid *et al.*, 1968a). The results of this study are recorded in Table 4.

Table 4

Relationships between body weight and chemical components of pigs (Reid et al. *1968a)*

Body Component	Prediction equation	R^2
Water	$\hat{Y}$ = 0,86699X – 0,05111	0,993
Fat	$\hat{Y}$ = 1,46761X – 1,35758	0,976
Protein	$\hat{Y}$ = 0,97069X – 0,80271	0,992
Ash	$\hat{Y}$ = 0,90823X – 1,41247	0,958

Where Y = $\log_{10}$ Wt(kg) of chemical component
& X = $\log_{10}$ Wt(kg) empty body wt

In sheep, body composition within breeds does not vary in a manner independent of body weight irrespective of the treatments applied, provided, the animals are maintained in a continuous positive energy balance. Burton and Reid (1969) recently studied the interaction of age, energy intake and body weight on the chemical composition of 26 Shropshire wethers. In spite of the wide range of energy intake and body weight, body composition did not vary in a manner independent of body weight. Age, used in addition to body weight as a predictant resulted in very slight improvements in the accuracy with which body composition could be predicted. These results confirmed the earlier findings of Reid *et al.* (1963) that the relationship between body weight and its chemical components remain linear until a fat content of 31% is reached. However, an equation of the type $Y = aX^b$ gave the best fit over the whole range of observations. In a study of the body composition of 221 sheep (wethers) representing 6 breeds Reid *et al.* (1968a) found significant differences between breeds in their body composition at a given weight. Sex, at least in certain breeds, also appears to modify this relationship. In one study Southdown ewes were found to contain 30 to 35% more fat and 3 to 16% less protein than rams of the same weight. In spite of this it was found that 87,6 to 95,1% of the variation in the logarithm of the weights of individual chemical components (with the exception of ash) was associated with variation in the logarithm of body weight. The results of this study are summarised in Table 5. It was also found that sheep that had been fed restricted rations for prolonged periods and then refed to reach the same final weight as the control treatment contained less fat and more water and protein than control animals of the same weight. (Reid *et al.*, 1968b).

Present evidence indicates that there is no reason to believe that the relationship between body weight and its chemical composition behaves any differently in cattle than in pigs or sheep (Reid *et al.*, 1968a). The relationship is essentially linear in young animals becoming curvilinear as they grow heavier. Different planes of nutrition do not appear to affect differentially the relationships between the amounts of body components and body weight. The effect of sex and breed on this relationship does not appear to have been generally examined in cattle.

Table 5

Relationships between the chemical components and body weight of sheep (Reid et al., 1968a)

Body Component	Prediction Equation	R^2
Water	$\hat{Y}$ = 0,73976X + 0,15466	0,951
Fat	$\hat{Y}$ = 1,98776X – 2,18382	0,876
Protein	$\hat{Y}$ = 0,80148X – 0,50283	0,949
Ash	$\hat{Y}$ = 0,59921X – 0,83870	0,626

Where: Y = $\log_{10}$ wt (kg) of chemical component
& X = $\log_{10}$ wt (kg) body weight

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