# STUDIES ON THE BODY COMPOSITION OF BEEF COWS

IAIN A. WRIGHT

Ph.D.

University of Edinburgh

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## ABSTRACT

Suckler cow management systems generally require cows to be dependent on their body reserves of energy and protein at some stage of the production cycle. In nutritional studies with suckler cows it is therefore important that the rate of use or replenishment of body reserves be considered with contemporary nutrition. This requires the quantification of body reserves and hence a means of measuring body composition in the live animal.

The methods available for the estimation of *in vivo* body composition in animals are reviewed and a number of techniques (live weight, skeletal size, total body water as estimated by deuterium oxide dilution, blood and red cell volumes as estimated by Evans Blue dilution, ultrasonic measurement of subcutaneous fat depth and eye-muscle area, and body condition scoring) were examined using 73 non-pregnant, non-lactating cows of five genotypes (Hereford x Friesian, Blue-Grey, Galloway, Luing and British Friesian) ranging in body condition score from 0.75-4.5. Direct measurement of body composition in terms of water, fat, protein and ash were made following slaughter.

Live weight, deuterium oxide dilution, ultrasonic measurement of subcutaneous fat depth and eye-muscle area, and body condition scoring were all considered to be potentially useful predictors of body composition, but a combination of techniques offered a better prediction than did any single index. Using a combination of techniques it was possible to predict body fat and protein with residual standard deviations of 13.1 kg and 3.15 kg respectively.

Body composition changes were also examined, and it was calculated that the composition of empty body-weight change was dependent upon empty body weight, containing more fat and less

water, protein and ash at higher empty body weights.

Important breed differences were found in the partition of fat among the main adipose tissue depots, with the Friesian cows having a greater proportion of fat in the internal depots and a lower proportion in the subcutaneous depot. The implications of breed differences in fat partition are discussed in relation to *in vivo* body composition measurement.

An ancillary study was carried out into the effects of body condition on maintenance requirements and on the use of blood metabolites to measure energy status in suckler cows. This indicated that body condition affected maintenance requirements to the extent that at 500 kg live weight, maintenance requirements were 8 MJ ME/day less for each unit increase in condition score. Plasma free fatty acids were shown to be particularly useful in assessing energy status in cows, but 3-hydroxybutyrate concentrations were of little value in non-pregnant, non-lactating animals.

Finally, the conclusions of the two studies are discussed in relation to areas of study likely to prove useful in the development of efficient systems of suckled calf production.

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I hereby declare that this thesis has been composed by myself, and except where otherwise stated, the work contained herein is my own.

Iain A. Wright

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# CHAPTER 1

# INTRODUCTION

In pastorally-based systems of animal production the annual pattern of herbage production seldom, if ever, matches exactly the pattern of the animals' nutrient requirements. The growth of grass and other forages is generally seasonal, being limited at certain times of the year either by temperature or rainfall, and in some instances by both. Although most of the systems of animal management that have developed throughout the world generally attempt to ensure that the time of highest nutrient requirement coincides with the period of maximum herbage production, some conservation of fodder during the peak growing season, for use during the winter or drought periods is generally practiced. During these periods of partial or complete dependence on conserved fodder the nutrient intake of breeding livestock in particular is generally less than their requirements and they draw upon their own body tissues to meet the deficit of nutrients. At some later stage of the annual cycle, when nutrient intakes exceed requirements, the body tissues are replenished. The annual patterns of intake and requirements thus result in cyclical changes in the body composition of the animals throughout the year, the amplitude of these cycles being dependent on the relative abundance and scarcity of grazed and conserved forage and on nutrient requirements which, in turn are determined by the level of animal production demanded of the system.

Suckler cows are no exception to these generalisations. In hill and upland areas of the United Kingdom, where most of this country's beef cows are located, there can be virtually no herbage growth for up to seven months of the year. Baker (1975) has estimated that in weaned calf production systems some 85% of the total energy input is utilised by the cow, and thus there are considerable economic advantages to be gained by minimising the

amount of conserved fodder fed to suckler cows during the winter months. In all systems of suckler cow management cows store nutrients in their bodies during at least part of the grazing season, and deplete these stores in the winter months. Such systems have given, and continue to give, levels of production that are considered to be perfectly acceptable. Recent studies of the effects of undernourishment of cows in different physiological states have shown that they can withstand considerable periods of undernourishment during pregnancy (e.g. Drennan and Bath, 1976; Russel, Peart, Eadie, MacDonald and White, 1979) and in lactation (e.g. Powell, 1973; Lowman, Edwards, Somerville and Jolly, 1979; Hodgson, Peart, Russel, Whitelaw and MacDonald, 1980) without seriously impairing their production or that of their calves. Suckler cows also appear to have a considerable capacity to replenish during the grazing season the tissues and condition lost during the winter months (Hodgson *et al.*, 1980) and to undergo further cyclical changes during subsequent years.

It can however be argued that any nutritional penalty must incur some production penalty. In some instances the penalty may be so small as to make it difficult to measure; in other instances the penalty may be larger but be judged unimportant and of little consequence in relation to the savings in feed inputs; and in yet other instances it may be severe and of greater economic loss than the value of the saved fodder. Cunningham (1979) has suggested that in many environments nutrition is an important factor limiting the more efficient use of resources by cows and that a better understanding is needed of the nutritional requirements of suckler cows in different physiological states. A prerequisite of this better understanding is the quantification of the relationships

between nutrition and production penalties. Such relationships or responses are likely to be affected to a significant degree by the extent of the cow's body reserves and by the rate at which these change. It is therefore essential to be able to estimate the extent of, and changes in, cow body composition, and in particular the amount or proportion of body fat.

The estimation of cow body composition by direct measurement following slaughter is expensive in terms of animals, labour and facilities, and does not allow serial measurements to be made on the same animals during the course of an experiment. There are numerous reports in the literature of various means of estimating composition without recourse to slaughter. The majority of these however, relate to carcass composition and the yield of saleable meat rather than the composition of the whole body, and there have been few attempts to compare different approaches or techniques within one investigation.

It was against this background that an investigation was initiated to examine methods considered to be of potential use in estimating cow body composition *in vivo*. The main objectives were to assess which technique or combination of techniques were likely to have application in nutritional research on suckler cows, and to determine predictive relationships which could subsequently be applied in such research. As the study also required the direct determination of body composition following slaughter, opportunities were taken to examine the composition of the bodies of mature cows, the partition of fat among the various depots of the body, and the effect of genotype on both these areas. Techniques of estimating *in vivo* body composition are likely to be used principally in research on the effects of undernourishment on production, and for this reason

an ancillary study was also made of the possible use of concentrations of certain blood metabolites as a means of quantifying the severity of undernourishment in suckler cows.

# CHAPTER 2

# MEASUREMENT OF BODY COMPOSITION

# INTRODUCTION

## Inclusion of references

There is a considerable amount of information available in the literature on methods of estimating body composition in live animals, and a comprehensive review of the literature has not been attempted. The methods have been discussed only in relation to their suitability for use with cows. References dealing with other species have been cited only where it is necessary to explain a theory or technique, or to highlight any difficulties or advantages of a particular method. Even if a reference dealt with cattle, but did not appear to add further to the discussion it was not necessarily included.

## Definition of body composition

The composition of the animal body can be defined in a number of ways. The anatomist considers the body as comprising a number of different organs, while the butcher or worker interested in the animal body as a source of human food will regard it as comprising a number of physically separable components such as lean meat, bone and fatty tissue. To the nutritionist, who is not concerned with the immediate slaughter of the animal (as in the case of breeding stock) body composition is more meaningfully expressed in terms of chemical constituents. In this thesis the term "body composition" will be taken to mean the composition of the body in terms of its four chemical components - fat, water, protein and minerals (or ash). The quantity of carbohydrate in the body is negligible. The word "fat" is used to mean chemical fat (lipid) while "fatty tissue" is used to indicate physically separable adipose tissue.

# DIRECT MEASUREMENT OF BODY COMPOSITION

Classical information on the composition of animals has been

obtained by direct methods which involve their slaughter. In studies on the growth and development of animals the serial slaughter approach, whereby animals are slaughtered at various stages from the beginning to the end of the experiment has been adopted, to permit computation of changes in composition with time.

The main disadvantage of the direct analysis approach is that only one assessment of composition can be made on each animal. In addition, a uniform and large group of animals is required to permit precise estimates of body composition changes, by between-animal comparisons. With large animals, such as cows, experimentation relying on direct measurements of body composition can become very expensive.

For direct chemical analysis the animal body must be ground, mixed and sampled - all of which are potential sources of error (Morris and Moir, 1964). In addition the quantity of material to be handled with the bovine body is large and the bones have considerable mechanical strength, which presents problems usually requiring expensive grinding machinery.

Direct analysis must, of course, be used as the base line with which to compare all indirect techniques and has provided information upon which many indirect methods rely. As has been indicated, direct analysis is not without error and this should always be borne in mind when comparisons are made between direct and indirect methods.

## PRINCIPLES OF INDIRECT MEASUREMENT OF BODY COMPOSITION

### Relationships among body components

It is a fundamental principle that to specify completely a multi-component system, one must determine at least as many parameters as there are components. In a four component model of

an animal body it would be necessary to specify quantitatively four parameters, either the four components or three of them plus the total of the four. Fortunately it appears that there are reasonably constant relationships between some of the chemical components of the body, which greatly facilitate *in vivo* body composition measurement.

Several studies in the literature present data on the composition of the fat-free empty body of animals and show that it has almost constant composition. The data collected by Reid, Wellington and Dunn (1955) and Reid, Bensadoun, Bull, Burton, Gleeson, Han, Joo, Johnson, McManus, Paladines, Stroud, Tyrrell, Van Neikerk and Wellington (1968) is perhaps the most representative and a summary is shown in Table 2.1.

Table 2.1. Percentage composition (± standard deviation) of the fat-free, empty body of three animal species (after Reid *et al.*, 1968).

| Species | No. of animals | Water (%) | Protein (%) | Ash (%) |
|---|---|---|---|---|
| Pigs | 714 | 76.98±2.69 | 19.18±2.34 | 3.91±0.79 |
| Sheep | 221 | 74.89±1.02 | 20.35±0.84 | 4.76±0.67 |
| Cattle | 256 | 72.91±2.01 | 21.64±1.53 | 5.34±0.95 |

The data for cattle represented 12 breeds, including beef and dairy types, both male and female. The age range was from 1 to 4860 days and body fat ranged from 1.8 to 44.6% of empty body weight. Reid *et al.* (1955) had observed that the composition of the bovine fat-free body was not constant, but related to age. During the first 200 days after birth the concentration of water was shown to decline while that of protein and ash increased. From about 200 to 500 days of age the animal is approximately "chemically mature" although actual chemical maturity is not reached until about 4000 days.

The concept of chemical maturity was proposed by Moulton (1923) and is defined as the point at which the fat-free body becomes comparatively constant in composition. Moulton suggested that chemical maturity occurred at an age of about 4% of total life expectancy. Later studies on rats, rabbits, cats, pigs, mice and guinea pigs (Spray and Widdowson, 1950) supported the general concept of chemical maturity, but showed that there was variation in the age at which different components of the fat-free body approached constancy. Since then Searle (1970) has presented body composition data on sheep which support the idea of chemical maturity. In sheep ranging in age from 3 days to 18 months, no change in the composition of the fat-free body occurred after 9 months. On the other hand, Ørskov, McDonald, Grubb and Pennie (1976), using early weaned male lambs between 20 and 75 kg live weight, noticed that the water:protein and protein:ash ratios in the empty body tended to decrease with age and empty body weight.

Plane of nutrition and type of diet may affect body composition and this subject has been extensively reviewed by Pálsson (1955), Elsley, McDonald and Fowler (1964), Tulloh (1964), Fowler (1968), Seebeck (1968), Lohman (1971) and Black (1974). In general it is concluded that nutrition can have major effects on body composition when animals are compared at the same age, but these differences are substantially reduced when comparisons are made at the same weight. It is also generally agreed that most of the variation in body composition is confined to the fat content, and that variation in the fat-free body is relatively small. Evidence in the literature supports the concept that variation in the components of the fat-free body is small. A review by Lohman (1971) concludes that the biological variation in the water, nitrogen and potassium content of the fat-free

body is probably less than 2, 4 and 4% respectively, for animals older than about 4% of their life expectancy. It might be expected from the evidence of Reid *et al.* (1955), who stated that there were changes in the bovine fat-free body up until about 500 days, that the variation would be even less if only mature animals are considered.

Models for estimating body composition

Models used for indirect estimation (i.e. other than by direct measurement following slaughter) of body composition usually divide the animal into two components. If one of these can be measured, then the other can be calculated from it and live weight (or strictly empty body weight). The most widely used model is that which divides the body into fat and fat-free mass. Variations to this model have been proposed. For example, Behnke (1942) preferred to divide the body into fat and "lean body mass" which he defined as the weight of the empty body less all except the "indispensible" or essential fat, which he assumed to be 10% of the lean body mass. This figure was later revised to 2% (Behnke, Osserman and Welham, 1953) and consists of the lipids associated with cell membranes and other physiologically active lipids. The figure of 2% fat in the lean body mass is normally accepted, but Siri (1956) considered this to be the upper limit and cases of animal bodies containing less than 2% fat in the empty body have been found (Lawes and Gilbert, 1861; A.J.F. Russel, personal communication).

Keys and Brožek (1953), when considering body composition of humans, divided the human body into a standard reference body containing 14% fat and obesity tissue, while Anderson (1963) preferred a three component model consisting of adipose tissue, muscle and muscle-free mass made up of skeleton, skin, nervous system

and internal organs.

By determining one of the components of the fat-free body (by techniques to be discussed later) the composition of the fat-free body can be computed, assuming it has constant composition. The fat content can be calculated by difference from the empty body weight or by direct regression of the fat content on the component measured. The latter procedure removes the necessity to measure empty body weight.

According to Siri (1956) methods for estimating body composition by interrelating constituents will have a residual standard deviation of about 4% of live weight because of the variation in a fixed model which divides the body into two components.

In the medical field multiparameter approaches have been adopted to measure body composition, mainly because it is not possible to check indirect estimates with direct measurements of body composition. Recently there have been pleas for the adoption of a multiparameter approach to body composition measurement in the live animal. Houseman (1972) and more recently Cuthbertson (1976) have suggested that a more accurate assessment of body composition can be obtained by the simultaneous estimation of several body components than the determination of only one.

## The dilution principle

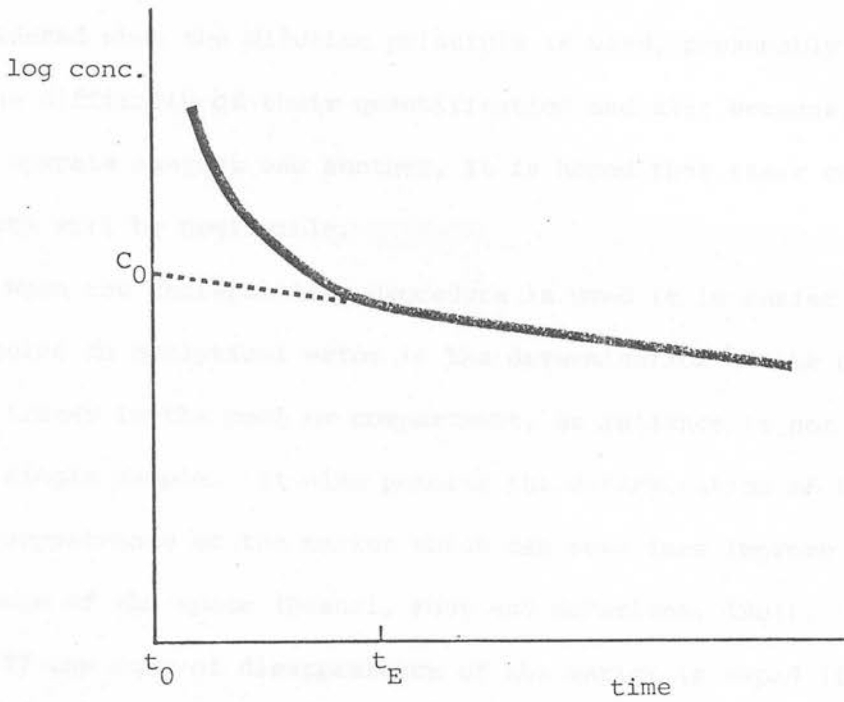
The dilution principle offers a non-destructive method of measuring the size of a body compartment. The technique involves the introduction of a known amount of a test substance, or marker, into the body compartment under consideration, and allowing the marker to mix completely and uniformly in that compartment. After mixing is complete a sample of the compartment is taken and the concentration of the marker is measured. The volume of the

compartment can be calculated from:-

$$\text{Volume} = \frac{\text{weight of marker infused}}{\text{concentration (wt/vol) of marker in compartment}}$$

The above procedure, commonly called the equilibration method, assumes that between infusion and equilibration there is no loss of the marker from the body compartment under consideration. This, however, is rarely the case. Usually the marker is lost by excretion or is metabolised slowly. If the losses were constant then the bias would in turn be constant and could be allowed for, but unfortunately this is not the case. For example, when deuterium oxide was used to estimate total body water in lambs, the loss of the marker varied from 0.09 to 4.2% per hour (Robelin, 1977). This variation can be allowed for by employing the extrapolation procedure which involves the measurement of the rate of fall in marker concentration after equilibration ($t_E$) has been reached and the extrapolation back to the time of infusion ($t_0$). This theoretical concentration ($C_0$) is the concentration that would have been achieved if distribution and mixing of the marker had been instantaneous. Most markers show a logarithmic fall off and the data are usually transformed by a logarithmic function before extrapolation. The procedure is demonstrated graphically in Figure 2.1.

The extrapolation procedure assumes that the loss of marker during the pre-equilibration phase occurs at the same rate as that after equilibration. This may not be the case as the loss is likely to be related to the concentration in only part of the body compartment. To illustrate this, consider the case of a marker for total body water estimation infused into the bloodstream. The urinary loss of the marker may be related to the blood concentration, and so the loss will be much greater during the period just after




For explanation of symbols see text

Figure 2.1. The extrapolation procedure

infusion, when the concentration in the blood passing the kidneys is at its highest, than it will be when equilibrium has been reached. This situation will lead to a slight overestimation of the space being measured.

On the other hand some losses may be lower during the pre-equilibration period, for example faecal loss, and will lead to an underestimation of the space. These two factors are not normally considered when the dilution principle is used, presumably because of the difficulty of their quantification and also because, since they operate against one another, it is hoped that their combined effects will be negligible.

When the extrapolation procedure is used it is easier to recognise an analytical error in the determination of the concentration of a tracer in the pool or compartment, as reliance is not placed on a single sample. It also permits the determination of the rate of disappearance of the marker which can sometimes improve the estimate of the space (Russel, Foot and McFarlane, 1981).

If the rate of disappearance of the marker is rapid (for example, the disappearance of Evans Blue dye from plasma) then it is important to take a sample at the exact time of equilibration (ten minutes after infusion for Evans Blue) if relying on only one equilibration sample. Taking a sample at an exact pre-determined time may be difficult, particularly with larger animals such as cattle. By using the extrapolation procedure this problem is eliminated.

The extrapolation procedure does, however, require more frequent handling, possibly over a period of days if the rate of disappearance of the marker is slow, and this may cause considerable disturbance to the animals, particularly if they are grazing, and of course the greater number of samples incurs increased analytical

costs.

Individual circumstances will determine which of the two approaches is best suited to a particular situation.

The increased sensitivity which could be obtained with the application of atomic energy, stimulated interest in the "isotope-dilution" technique because of the ease and accuracy with which a radioactive tracer could be measured. This applied particularly to the measurement of total body water, using tritiated water, but also to other body compartments such as potassium, using $^{42}K$, and thiocyanate space, using $^{35}S$-labelled thiocyanate.

## METHODS OF ESTIMATING BODY COMPOSITION IN LIVE ANIMALS

### Live weight

Live weight is the most commonly made objective measurement on live animals, and taken over a wide range, it can provide useful predictive information on body composition (Tulloh, 1963 and 1964) and probably more information than any other single measurement. In practice, however, the interest in a knowledge of composition is frequently restricted to a relatively narrow weight range, and in these circumstances the usefulness of live weight as an index of composition is reduced. Nevertheless, the importance of live weight is such that the predictive value of other indices must be judged either against live weight alone or in terms of the additional information supplied in combination with live weight.

Unfortunately live weight tends to be a less useful index of composition in ruminants than in monogastric species because of the large variations in gut fill which can constitute between 5 and 30% of live weight. At present there is no practicable means of estimating the weight of the gastro-intestinal contents, but such a technique would undoubtedly be of great value in ruminant

experimentation.

Estimation of body water in live animals

The principle of constancy of composition of the fat-free body in animals implies that if one of the components, either water, protein or ash can be measured, then the others can be calculated. The most easily measured of these components is water and, if the measurement can be made with an acceptable degree of accuracy, then, in theory at least, body fat can be calculated as the difference between the mass of the fat-free body and live weight (or strictly empty body weight) or from the inverse relationship of body water and fat. Over the years a number of tracers have been employed to estimate body water in animals using the dilution principle. The ideal tracer has to mix completely with the body water pool, and with no other body component. It must also be non-toxic and not metabolisable or at least only very slowly so. Some of the tracers used include:-

1. Urea
2. Antipyrene and its derivatives
3. Alcohol
4. Water containing isotopes of hydrogen:-
   (i) tritiated water (TOH)
   (ii) deuterium oxide ($D_2O$)

Urea

Urea is non-toxic and can be easily estimated in urine and blood. A further advantage of its use is the ease with which it can be administered orally. Endogenous urea is the end product of protein metabolism and it is assumed that the endogenous production of urea, and therefore the blood urea concentration, will remain constant during the period of total body water determination. Also, it is assumed that

diuresis, which may result from the ingestion of large quantities of urea, will not significantly affect the results. Studies on man (Steffenson, 1947; Bradbury, 1961), dogs (Painter, 1940) and pre-ruminant calves (Dalton, 1964) have shown that endogenous blood urea does in fact reach a steady state during urea space determinations.

In ruminants, however, urea is converted to microbial protein in the rumen. Houpt (1959) showed that in sheep and goats more than 52% of an ingested dose of urea was not recovered in urine nor remained in body fluids. Thus, the rapid elimination of urea as it enters the rumen effectively removes urea from body fluids and so makes it an unsuitable tracer for the estimation of total body water in ruminants.

Antipyrene and its derivatives

The analgesic and antipyretic, antipyrene (1-phenyl-2, 3-dimethyl-pyrazolone-1) has been used on several occasions in the estimation of total body water. It was observed that antipyrene (AP) rapidly and uniformly distributed through the tissues of man in close proportion to the tissue water content (Soberman, Brodie, Levy, Axelrod, Hollander and Steele, 1949), although there was a suggestion that it was slightly soluble in lipid. However Dumont (1955) observed that in pigs AP was not evenly distributed between tissues and was eliminated very rapidly from the bloodstream. This appeared to be due to preferential binding to plasma protein (Brodie, Berger, Axelrod, Dunning, Porosowska and Steele, 1951).

In some respects AP space has equated well with total body water as estimated by dessication after slaughter in rabbits (Reid, Balch and Glascock, 1958; Panaretto, 1963). With ruminants the results have been less encouraging. Garrett, Meyer and Lofgreen (1959) found

a poor association between AP dilution in sheep and cattle and specific gravity of rib cuts, but more important, AP space showed low repeatability. It appeared that the metabolism of AP was very rapid in ruminants (Reid, Balch, Head and Stroud, 1957; Garrett et al., 1959). The removal of AP from the animal body cannot be wholly attributed to its appearance in the alimentary tract (Panaretto and Reid, 1964*a)*.

It has been concluded by Garrett *et al.* (1959) that total body water as estimated by AP dilution was "too variable to have direct application in nutritional investigations with ruminants". Panaretto and Till (1963) concluded that investigations into the cause of poor results with AP would not be warranted as better methods for estimating total body water were available.

The inadequacy of AP as a marker stimulated interest in some of the derivatives of AP. Huckabee (1956) suggested the use of 4-aminoantipyrene, $^{131}$I-labelled 4-iodoantipyrene was proposed by Talso, Lahr, Spafford, Ferenzi and Jackson (1955) and N-acetyl-4-aminoantipyrene (NAAP) by Brodie *et al.* (1951). NAAP is the only one of these to be used on more than one occasion. It appeared to be negligibly bound to plasma protein, its rate of metabolism was virtually nil, it was uniformly distributed throughout tissues in proportion to their water content and seemed to be insoluble in lipid (Brodie *et al.*, 1951; Huckabee, 1956).

In cattle the NAAP space was found to be considerably lower than AP space (Reid *et al.*, 1957). NAAP appeared to diffuse only slowly into the gut water. This observation was, however, made on only four animals. The slow absorption of NAAP into the gut was further demonstrated by Reid *et al.* (1958) using rabbits. It was suggested that NAAP dilution could be used in such a way that samples taken at different intervals could give estimates

of total body water and empty body water. On the other hand Panaretto and Till (1963) found that NAAP gave a mean underestimation of empty body water of about 6% of empty body weight in sheep. The correlation coefficient between NAAP space and empty body water was only 0.89. They rejected the use of NAAP as did Panaretto and Reid (1964*a*). Panaretto and Reid (1964*b*) concluded that there was too much variability associated with the use of AP and NAAP for the estimation of gut water for the technique to have any predictive value. More recently NAAP has been used on steers to estimate total body water (Crabtree, 1976) but again the results proved too variable to be useful.

Alcohol

On one occasion total body water has been estimated in ruminants (sheep) by ethanol dilution (Budtz-Olsen, Cleeve and Oelricks, 1960). However, no comparison of ethanol space and body water as measured by dessication was made. The only justifications for using the technique were the fact that in human studies alcohol had been noted to occur in several organs at a rate which was closely correlated to their water content (Harger, Hulpieu and Lamb, 1973) and that Grüner (1957) had found close agreement between AP and ethanol spaces.

Tritiated water and deuterium oxide

Theoretically the ideal tracer for the estimation of total body water is water itself, labelled with an isotope of hydrogen, either radioactive tritiated water (TOH) or non-radioactive deuterium oxide ($D_2O$). According to Schloerb, Friis-Hansen, Edelman, Soloman and Moore (1950) they are not affected by secretory or metabolic processes. This, however, may not be strictly true for Crabtree (1976) measured $D_2O$ concentration in steers 24 hours after infusion and found that the concentration of $D_2O$ in water from various tissues

(including liver, kidney, lungs, heart, muscle, plasma and rumen contents) was the same except that from muscle tissue. The water from skeletal muscle contained 9.3% less $D_2O$ than the water from other tissues. This was attributed to exchange with labile organic hydrogen in the muscle and, it was claimed, would lead to an overestimation of total body water. Such an exchange, if it exists, would not lead to a lower $D_2O$ concentration in the muscle as compared with other tissues because as deuterium was removed from the water phase a new equilibrium between the water in muscle and other tissues would be achieved. A more likely explanation is the possibility that some of the water in skeletal muscle is bound in some way and unavailable for free exchange with deuterium. This has been shown to be the case in rat muscle; Hazelwood, Nicholas and Chamberlain (1969) found that 10% of water in rat skeletal muscle did not exchange with deuterium in 24 hours. They concluded that non-exchangeable water had less motional freedom than free water. This situation would lead to a slight underestimation of total body water.

With the development of very sensitive mass spectrometers it has been found that water in the human body (Halliday and Miller, 1977) and that of cattle (Wright, 1981) is enriched with $D_2O$ as compared with the drinking water. The reasons for such enrichment are not clear, but may be due to the differential distillation of $H_2O$ and $D_2O$ in the lungs, as expired air contains less $D_2O$ than that in body water (Halliday and Miller, 1977). Certain tissues show preferential uptake of $D_2O$, particularly the salivary glands. The saliva of four cattle contained a mean of 0.81% more $D_2O$ than the plasma (Wright, 1981). However the magnitude of such enrichment is so small as to

be of little significance in the measurement of body composition.

Dose rates. $D_2O$ is not toxic when administered in small quantities and toxicity occurs in mice only when serum $D_2O$ concentration exceeds 25% (Schloerb *et al.*, 1950). For total body water estimation in animals the $D_2O$ is usually infused intravenously as a saline solution made up to physiological concentration (9 g/l). In most cases the solution will contain over 99% $D_2O$. The dose rate used must give a concentration in the blood which will ensure accurate measurement, which in turn depends on the technique to be used for measurement. With pregnant ewes Foot and Greenhalgh (1970) used a dose of 0.65-0.87 g/kg live weight when the falling drop assay was employed, but suggested 1 g/kg live weight with infra-red spectroscopy. This latter dose rate has also been used on steers (Crabtree, Houseman and Kay, 1974) and cows (Chigaru and Topps, 1981; Trigg and Topps, 1981).

With TOH, dose rate does not seem to be important. In sheep, for example, as little as 100 µCi (Keenan, McManus and Freer, 1969) to as much as 1 mCi (Graham, 1967; Searle, 1970) has been used. These rates would be equivalent to approximately 2 and 20 µCi/kg live weight. Gordon (1970) could detect no difference in the precision of estimate of total body water among five dose rates in rats.

Equilibration time. Equilibration time refers to the time taken for the administered dose to mix completely and uniformly with all body water. It is generally accepted that in ruminants the tracers used in the estimation of total body water equilibrate less rapidly with the gut water, and the rumen water in particular, than other body compartments (Little and Morris, 1972). The rumen water equilibrates mainly by the entry of saliva into the rumen (Macfarlane, 1975).

The point at which equilibration occurs can therefore be defined as the time when the concentration in the rumen water reaches that of the other body fluids - usually represented by blood. The reported times for equilibration in cattle are shown in Table 2.2.

Table 2.2. Equilibration times for tritiated water and deuterium oxide in cattle

| Reference | Type of Animal | Tracer | Equilibration Time |
|---|---|---|---|
| Shumway, Trujillo, Bennet, Matthews and Asplund (1956) | Steers | TOH | 6 hours |
| Aschbacher, Kamal and Cragle (1965) | Jersey Cows | TOH | 3-4 hours |
| Little and Morris (1972) | Steers | TOH | 8-10 hours |
| Crabtree, Houseman and Kay (1974) | Steers | $D_2O$ | 5 hours |

Differences in equilibration times may be due to differences in live weight, health or physiological state of animals. As the rumen water equilibrates from saliva it might be expected that gut fill might affect equilibration time. However Little and Morris (1972) found fasting animals prior to infusion, which would reduce gut fill, had no effect on equilibration time. It has been suggested by Robertshaw (1981) that equilibration with water in the skin of the extremities takes longer in cold conditions because of a reduction in the blood supply to these peripheral areas, but the effect on total body water estimation is negligible.

Losses of tracer. Ideally there should be no loss of isotope from the body during the period before equilibration is reached. Such losses would lead to an overestimation of total body water.

However, in practice losses do occur and they occur in two different ways - exchange of the D and T atoms (from $D_2O$ and TOH respectively) with labile hydrogen from organic compounds and excretion of water through defaecation, urination, sweating and evaporation from the respiratory tract and skin.

When $D_2O$ is added to water the D isotopes exchange with the H atoms of the water and in effect the $D_2O$ will be present as DOH when infused into animals. This exchange of isotopes with hydrogen from water does not result in any loss. However D and T atoms will also exchange with exchangeable hydrogen atoms in organic compounds, mainly in protein, but also in other tissues such as fat and bone (Springell, 1968). Ruminal contents also represent a potential source of exchangeable hydrogen, but on a dry weight basis these represent only a very small proportion of live weight (Springell, 1968). Over-estimation of total body water as a result of exchange with hydrogen may amount to 0.5-5% (Hevesy and Jacobsen, 1940) but theoretical considerations led Springell (1968) to conclude that it is unlikely to exceed 0.2% of live weight. This low estimate was arrived at by a consideration of only the labile hydrogen in the muscle and hide of cattle. Later work on the problem suggests that there is a small, slowly exchanging labile hydrogen pool in tissues such as bone, teeth and hair, In neonatal calves this slowly exchanging pool is equivalent to 3.5% of body weight (Lewis and Phillips, 1972), but the exchange was not 99% complete until 5 days after infusion with tritiated water. During a 6-8 hour equilibration period this pool would lead to an overestimation of total body water of just under 1%. Thus the corrections of 3% of live weight used in sheep by Panaretto and Till (1963) and 2% of total body water in cattle (Aschbacher *et al.*, 1965) are probably very close to the true value.

The losses of isotopes which occur through excretion will depend on the concentration of the isotopes in the body fluids, the time interval between infusion and sampling and the ambient temperature. In sheep Till and Downes (1962) found that evaporative losses of TOH could account for up to 1.2% of the dose being lost per day at $10^{o}C$ and up to 3.6% of the dose at $36^{o}C$. The overall losses during a six hour equilibration period were found to be about 2% of the dose.

A further complicating factor is that the proportion of DOH or TOH in the water lost by evaporation is not the same as that in the body fluids. The partial vapour pressure of DOH and TOH is only about 0.93 that of $H_2O$ which means that DOH and TOH will evaporate from the surface of a liquid at only 0.93 times the rate of $H_2O$ evaporation. Furthermore, skin seems to be less permeable to TOH than $H_2O$ (Robertshaw, 1981), the specific activity of the evaporated water from skin being only 70% that of the fluid under the skin. On the other hand, skin does not appear to act as a barrier to $D_2O$ as compared to $H_2O$. These results were obtained from animals with no sweat glands and the effect of these is not known.

Theoretical considerations suggest that expired air should be lower in DOH and TOH concentrations than body fluids because of the difference in partial vapour pressure of these isotope-containing molecules and $H_2O$, and has been demonstrated experimentally (Rübsamen, Nolda and von Engelhardt, 1979; Halliday and Miller, 1977).

Any factor which will reduce the water turnover rate of the animal during equilibration will reduce losses of the marker isotope. In most studies withholding food and water from animals is reported to reduce faecal and urinary losses because of a reduction in defaecation and urination. In all but a few cases food and water

have been withheld during the equilibration period and in most cases also for a period preceding infusion.

A pre-infusion fasting period of up to 64 hours in sheep (Farrell and Reardon, 1972) has been used. To minimise disturbance to their cattle Carnegie and Tulloh (1968) did not fast their animals prior to total body water determination. Foot and Greenhalgh (1970) and Russel *et al.* (1981) did not fast their sheep either prior to or during total body water estimation for the same reason.

In addition to reducing water losses, fasting leads to a standardisation of the gastro-intestinal contents during total body water estimation. Variation in gut water could lead to serious errors when total body water is to be used for the prediction of body composition.

<u>The use of $D_2O$ and TOH to estimate total body water</u>. In most cases where isotopes of water have been used to estimate total body water in farm animals, TOH has been chosen in preference to $D_2O$. The reasons for this are probably the higher cost of $D_2O$ and the easy measurement of TOH in body fluids by liquid scintillation counting. The main disadvantage of TOH is its radioactivity. Its half life is 12.8 years and this can create problems of radioactive waste. In particular there can be problems in the disposal of the products of excretion from animals that have been dosed with radioactive isotopes, and also in the disposal of carcasses from such animals. Despite these drawbacks, TOH was widely used in nutritional and physiological investigations during the 1960's and early 1970's. With a more general awareness of the hazards of using radioactive compounds it is now perhaps less favoured.

$D_2O$ offers a non-radioactive alternative to TOH and at present

there are no restrictions as to the keeping or disposal of animals which have been given $D_2O$. For this reason, and particularly if a financial return can be achieved from the sale of such animals, $D_2O$ can in fact be cheaper to use than TOH on a routine basis.

There are relatively few reports of $D_2O$ having been used to determine total body water in farm animals. All those involving ruminants have shown an overestimation of total body water (Foot and Greenhalgh, 1970; Crabtree *et al.*, 1974; Robelin, 1977; Houseman, Robinson and Fraser, 1978) for the reasons previously discussed.

Only one report (Trigg, Domingo and Topps, 1973) found TOH to underestimate total body water by 4.6%, but this could be attributed to the method of assay used.

In cattle Carnegie and Tulloh (1968) found TOH space to overestimate total body water by 15%. This higher level of overestimation could partly be explained by the non-fasting of animals prior to TOH infusion. Values for the overestimation of total body water from TOH dilution in sheep range from 0.8% (Panaretto and Till, 1963) to 16% (Russel *et al.*, 1981). However, the interpretations of such overestimation are difficult because some authors correct TOH space for estimated losses of TOH during the equilibration period.

According to Foot (1969) the choice of a marker for the estimation of total body water in animals will depend on four main considerations:-

1. Minimal disturbance to the animal
2. The ease of accurate quantitative introduction of the marker and of obtaining a representative sample
3. The ease and accuracy of estimating the marker concentration

in the sample

4. The repeatability of the relationship between the space measured and the parameters of body composition in which one is interested

A fifth consideration is that of cost. Bearing these in mind, and considering all the factors discussed in this review, $D_2O$ appears to be the most promising tracer for the routine measurement of total body water in suckler cows.

Measurement of Blood Volume and Red Cell Volume

It is widely accepted that the oxygen consumption of mammals depends on the amount of metabolically active tissue in the body. Since adipose tissue requires relatively little oxygen, body weight and oxygen consumption may not necessarily be correlated. However, it is reasonable to expect a close correlation between the oxygen carriers (total blood volume or red cell mass) and the oxygen-consuming tissue (lean body mass) (Muldowney, 1957; Doornebal, 1968). Since the principle of *in vivo* composition measurement rests on the concept of the lean body mass having constant composition (Pace and Rathbun, 1945) it can be reasonably assumed that protein mass may be related to blood volume or red cell volume (or both).

Doornebal (1968) found close agreement between total body protein and total blood volume in pigs in the weight range 9 to 103 kg (r = 0.97). However, when body weight was included in a multiple regression equation with total body protein as the dependent variable and body weight and total blood volume as the independent variables, most of the variance could be accounted for by body weight. When the weight range was restricted, the proportion of the variance attributable to blood volume increased, but body weight

was still of major importance. It should be noted, however, that in the weight of pigs used, protein mass per unit body weight was fairly constant. In mature animals this ratio would be expected to vary more than in immature animals. It may then be postulated that the proportion of variance accounted for by blood volume will be greater in mature than in immature animals.

There are two main techniques for determining blood and red cell volume, both relying on the dilution principle. The first involves the labelling of a sample of the subject's own blood with radioactive $^{51}Cr$ (Hyde and Jones, 1962). The sample is then re-injected into the bloodstream of the subject and, after a period of 10 minutes to allow mixing, a blood sample is taken and a radioactivity count established. From the dilution factor of the radioactivity, blood volume can be determined. Red cell volume is usually measured from the application of the haematocrit value.

The major limitation of the technique is that the use of radioactivity incurrs legal and ethical problems. Regulations govern the keeping of farm animals that have been given radioactive isotopes and carcasses may not be sold for human consumption. This means that the routine use of radioactivity on large numbers of animals is both inconvenient and costly.

The second method makes use of the dye Evans Blue (T 1824) to measure plasma volume. A known quantity of dye in solution is infused into the bloodstream, where it combines with the plasma albumin. After allowing 10 minutes for equilibration to be reached, a blood sample is taken and the plasma separated by centrifugation. Dye concentration is determined colorimetrically. Alternatively, serial blood sampling can be carried out to establish a disappearance curve for the dye and the theoretical concentration at infusion time

calculated by extrapolation (Reynolds, 1953*a*). This latter procedure avoids the problems of having to sample the animal at a pre-determined time and the between animal variation that exists in disappearance rate of the dye. Red cell volume and total blood volume can be calculated from plasma volume and the haematocrit value.

Reynolds (1953*a* and *b*) first used Evans Blue to calculate plasma volume in the bovine. Since then only on two other reported occasions has blood volume in cattle been measured by this technique (Dalton and Fisher, 1961; Little and Morris, 1972). Reynolds (1953*a* and *b*) measured dye concentration directly in plasma while Little and Morris (1972) used the acetone extraction procedure described by Chinard (1951). This latter method is recommended where the plasma samples are turbid or where the absorption of blank plasma at a wavelength of 620nm is likely to vary.

Only Little and Morris (1972) have used red cell volume as determined by the Evans Blue method to predict body composition in cattle, and found it to be slightly less accurate than tritiated water space. They did not, however, combine the two in a multiple regression equation. Springell, Butterfield, Johnson and Seebeck (1968) used $^{51}Cr$ determined red cell volume, tritiated water space and body weight as predictors of total muscle weight in steers. Muscle weight was predicted with increasing precision by using these three independent variables in the following combinations.

| Variables | $r^2$ or $R^2$ |
|---|---|
| 1. Body weight alone | 0.983 |
| 2. Body weight and total body water | 0.988 |
| 3. Body weight and red cell volume | 0.991 |
| 4. Body weight, total body water and red cell volume | 0.995 |

Errors with the techniques may occur because a large proportion of the total red cells in the body may be withheld in the spleen. Turner and Hodgetts (1959) found that in sheep up to one seventh of blood volume and up to one quarter of red cells may be contained in the spleen. Exercise, excitement, age and certain drugs all affected this proportion. Intravenous administration of adrenaline has been used by workers with sheep (Turner and Hodgetts, 1959; Panaretto, 1964; Panaretto and Little, 1965) and cattle (Little and Morris, 1972) to overcome some of these problems.

Another difficulty arises from the fact that the haematocrit in large blood vessels is greater than that in small vessels. This means that the haematocrit of a blood sample taken from the jugular vein will over-estimate total blood haematocrit. In species where the red cell storage function of the spleen is unimportant the ratio of total blood haematocrit to jugular haematocrit is virtually constant and is referred to as the $F_{cells}$ ratio. In man, for example this ratio is 0.91. In animals where the spleen is important in red cell storage the $F_{cells}$ ratio is more variable, as shown by Hodgetts (1961) who reported a mean value for five sheep of 1.22 $\pm$ 0.092. Six splenectomized sheep gave a value of 0.83 $\pm$ 0.027. This illustrates the difficulty of applying the $F_{cells}$ ratio in animals in which the spleen acts as the red cell store.

<u>The use of potassium isotopes to estimate body composition</u>

The potassium (K) in animal bodies is almost completely confined to the cells and is present in almost constant concentration (Anderson, 1959). In turn, the cells are nearly all located in the lean body mass and thus a close correlation between the amount

of K in the body and the lean body mass might be expected. Two isotopes of K have been used to estimate body composition, in different ways.

Whole body counting of $^{40}K$

$^{40}K$ is a naturally occurring radioactive isotope which has a natural abundance of 0.0119% (Ward, Johnson and Tyler, 1967). Whole body counting measures the amount of this isotope by placing the animal in a whole body counter and measuring the gamma rays emitted from the body at the appropriate energy level of 1.46 MeV. The amount of radioactivity of this level emitted by an animal should be proportional to its K content and thus to its lean body mass.

The first report of the use of $^{40}K$ to measure body composition in farm animals is that of Zobrisky, Nauman, Dyer and Anderson (1959) who related $^{40}K$ in live pigs to muscle mass. The technique was then extended to other species such as sheep (Kirton, Pearson, Nelson, Anderson and Schuch , 1961; Kirton and Pearson, 1963) and cattle (Johnson and Ward, 1966; Lohman, Breidenstein, Twardock, Smith and Norton, 1966).

Results of comparisons of $^{40}K$ count and body composition have in general not been too encouraging. A major source of variation in $^{40}K$ count appears to be associated with K in the diet (Lohman and Norton, 1968; Johnson, Walters and Whiteman, 1972; Belyea, Martz, Young and Clark, 1978). Considerable amounts of radioactivity from nuclear fallout have been found in the rumen contents of cattle and a proportion of these gamma rays are counted in the $^{40}K$ channel by liquid scintillation counters. The effect of $^{40}K$ in the gut contents can be reduced to some extent by fasting animals before counting, as has been done by Frahm, Walters and McLellan (1971)

and Clark, Hedrick and Thompson (1976).

Another source of radioactivity is dirt on the coat and skin of animals. This can be reduced by ensuring that animals are thoroughly washed before counting.

The precision of counting in the early work was not great enough to allow accurate prediction of body composition in animals (Kirton *et al.*, 1961). More recent work has reduced the variation associated with counting by using an appropriate counting time (Twardock, Lohman, Smith and Breidenstein, 1966; Lohman, Coffman, Twardock, Breidenstein and Norton, 1968), improved detector positioning (Twardock *et al.*, 1966) and by careful and standard restraint of the animal (Lohman *et al.*, 1966).

When these precautions were taken $^{40}K$ count and live weight accounted for up to 94% of the variation in dissectible carcass lean muscle mass in 21 steers weighing 284-385 kg, while live weight alone accounted for 79% of the variation (Lohman *et al.*, 1966). Frahm *et al.* (1971) reported correlation coefficients between $^{40}K$ count and fat-free lean of up to 0.86 while that between live weight and fat-free lean was only 0.20. However all these animals, yearling Angus bulls, were on the same diet and were fasted for 24 hours prior to counting so that radioactivity from the gut contents was relatively constant. Clark *et al.* (1976) estimated $^{40}K$ in steers varying in live weight from 183 to 574 kg. When $^{40}K$ count and live weight were used to predict fat-free body weight a value of $R^2 = 0.97$ was obtained, but prediction from live weight alone gave a value of $r^2 = 0.95$. A similar pattern was found when other body components were predicted, i.e. the extra variation explained by $^{40}K$ count was very small. This is not surprising in view of the wide range of live weights of the steers used.

Despite the attractions of using a naturally occurring radioisotope, the great expense of a whole body counter, the considerable skill needed in its use, the preparation of the animals, the need for feed to be standardised and animals fasted before counting, as well as the doubtful accuracy of the results unless very stringent precautions are taken during counting, all make it unlikely that whole body counting of $^{40}K$ will be widely used in the routine measurement of body composition during nutritional experiments.

Measurement of total body potassium by $^{42}K$ dilution

The amount of potassium in the body can also be measured by the dilution principle. Moore (1946) first proposed the use of the radioactive isotope $^{42}K$ to measure exchangeable K in humans. The technique was further developed by Corsa, Olney, Steenburg, Ball and Moore (1950) in rabbits and human subjects. A known quantity of $^{42}K$ is infused into the subject and the equilibration concentration measured in either plasma or urine.

Comparisons of exchangeable potassium ($K_e$) with direct measurements of body composition in laboratory animals have been encouraging. Talso, Miller, Carballo and Vasquez (1960) found that 98% of body K was exchangeable and that a correlation coefficient of 0.945 existed between $K_e$ and lean solids in rats.

There are few reports of the $^{42}K$ dilution technique having been applied to farm animals, and only one case where cattle were used. Pfau (1966) found good agreement between fat-free body weight and $K_e$ in pigs, while Fuller, Houseman and Cadenhead (1971), again using pigs, found that the proportion of extractable fat in the bodies of bacon weight pigs was closely related to $K_e$ ($r = -0.92$).

In sheep, Lynch, Fries and Hiner (1965) found $K_e$ to be related to whole body water with a correlation coefficient of 0.78.

In a later experiment these same workers found $K_e$ space to be significantly affected by the diet, and in particular by the potassium content of the ration and recommended that animals be kept on low potassium diets (low roughage diets) prior to and during measurement of $K_e$ space (Lynch, Fries and Hiner, 1968).

Domingo, Trigg and Topps (1973), also working with sheep found close relationships between $K_e$ and body components. For example, empty body nitrogen could be predicted from $K_e$ with a residual standard deviation of 0.034 kg in lambs weighing 23.5-34.5 kg.

The only report of $^{42}K$ dilution having been used to estimate body composition in cattle is that of Pfau and Salem (1972). The standard error of prediction of total body water from $K_e$, expressed as a percentage of the mean, was 7.2%. This was higher than that given by tritiated water dilution (4.9%).

Despite the fact that $^{42}K$ dilution appears to hold some promise for the prediction of body composition in live animals, and its possible usefulness in some circumstances, its radioactivity (the disadvantages of which have already been discussed) make it an expensive technique for use on large numbers of farm animals, and thus it is unlikely to be used routinely in suckler cow experimentation.

Estimation of body composition from body density

The rationale for estimating body composition from density is based on the assumption that the body can be considered as a two component system, with the components being of different, but constant densities. The two components usually considered are fat and the fat-free body, although variations of this model have been used (see page 11). Whichever model is used the principles involved are the same.

If the densities of the two components are known, and the density

of the whole animal (or strictly the empty animal) can be measured, then the proportion of the two components can be calculated. Since the fat-free body has constant composition then the total composition of the animal can be calculated. An alternative approach is to relate body density (or specific gravity) directly to one of the components, usually fat, by regression analysis. With this approach a knowledge of the densities of the two components is not required.

Density is by definition mass (commonly replaced by weight) per unit volume. The major problem when applied to animals is the measurement of volume, which has been estimated by three methods:-

1. Air displacement
2. Gas dilution
3. Underwater weighing

Air displacement

Air displacement has been used mainly in human studies (Pearson, Purchas and Reineke, 1968) because co-operation of the subject is essential. The volume of the subject is calculated from pressure/ volume changes (Boyle's law) when the subject is placed in a chamber forming part of a closed system. Corrections for temperature and humidity changes are essential. Hix, Pearson and Reineke (1964) obtained good agreement (r = 0.99) between the air displacement and helium dilution methods using humans, but with pigs the correlation coefficient was only 0.59 (Hix, Pearson, Reineke, Gillett and Giacoletto, 1967). Beeston (1964) using a modified air displacement technique successfully measured the specific gravity of 14 sheep.

Gas dilution

When body volume is determined by gas dilution, the subject is placed in a chamber of known volume and an exact amount of an inert gas (usually helium) is added to the chamber. After mixing, a sample

is removed and analysed for helium concentration. The dilution of the helium is related to the volume of the chamber not occupied by the animal and hence allows animal volume to be calculated.

Siri (1956) developed the technique in which the effects of respiratory gases are fully corrected. No correction for lung volume is needed as the helium equilibrates with air in these spaces.

The helium dilution method has been used in pigs (Gnaedinger, Reineke, Pearson, Van Huss, Wessel and Montoye, 1963; Kay, 1963). A major problem is the activity of the animal which caused rapid changes in the helium concentration. Similar problems of temperature and humidity variation arise with the gas dilution technique as with the air displacement method.

Underwater weighing

Measurement of the volume of water displaced is relatively simple for eviscerated carcasses or parts of carcasses, provided care is taken to exclude trapped air, and has been used successfully for determining the density of carcasses and cuts of meat (Lofgreen and Garrett, 1954; Barton and Kirton, 1956; Garrett, 1968). Underwater weighing has been used for the determination of density of live sheep (Tallis, Moore and Gream, 1963) and pigs (Lynch and Wellington, 1963). The animals must be anaesthetised and equipped with breathing apparatus. The variable amount of air trapped in the lungs presents a problem, as does gas in the alimentary tract. The latter is a problem which is not peculiar to only underwater weighing, but occurs with all techniques of body density measurement and is particularly important in ruminants because of the large quantities of gas in the rumen and caecum. The air trapped in the fleece or hair of live animals can also present problems.

Relationship of body density to body composition

Underwater weighing was used in the classic study of Morales, Rathbun, Smith and Pace (1945) to predict body composition of guinea pigs. In general, relationships between body density and body composition in farm animals have not been very good.

Kay (1963) used a combination of water displacement and helium dilution to measure body density of pigs, but this was poorly correlated with fat content and not sufficiently accurate for predictive purposes. No significant correlations between density determined by air displacement or helium dilution and body composition of pigs could be found by Gnaedinger *et al.* (1963). However Beeston (1964) using a modified air displacement method obtained a reasonable relationship between specific gravity and the amount of fat in the bodies of 14 sheep.

The problems of measuring body volume, which arise from the difficulties of correcting for gas trapped in the lungs, coat and alimentary tract of ruminants as well as the technical problems of measuring the volume of an animal as large as a cow, preclude serious consideration of body density as a method for measuring body composition in suckler cows.

Creatinine excretion as an index of body composition

Creatinine is the end product of the metabolism of creatine and is excreted in the urine. Its use as a predictor of body composition depends on two assumptions:-

1. that the precursors (creatine and phosphocreatine) constitute a constant proportion of the lean body mass;
2. that a constant proportion of the body creatine is excreted as creatinine.

Borsook and Dubnoff (1947) stated that 98% of the creatine in

the body is found in the skeletal muscle.

The subjects for much of the earlier work on creatine and creatinine were small animals and it was commonly stated that creatine formed a constant proportion of the muscle. This seems a rather optimistic conclusion in view of the results in Table 2.3 which show considerable variation in the creatine concentration of muscle.

Table 2.3. Creatine content of muscle of small animals and man

| Reference | | Range of creatine conc. (%) |
|---|---|---|
| Myers and Fine (1913) | rabbits | 0.494 - 0.534 |
| | cats | 0.383 - 0.515 |
| Bodansky (1931) | man | 0.309 - 0.485 |
| Chanutin and Kinard (1933) | rabbits | 0.484 - 0.551 |

The amount of creatinine excreted daily has been taken to be about 2% of total creatine. Chinn (1966) obtained a value of 0.88% with a range of 0.662 to 0.958% in 29 Albino rats. He also drew attention to the fact that the creatinine coefficient (mg creatinine excreted in 24 hours per kg live weight) in his own studies and those in the previous few years (Kumar, Land and Boyne, 1959; Van Niekerk, Reid, Bensadoun and Paladines, 1963*a*) were much lower than those of the earlier workers.

The effect of diet on creatinine excretion is not clear. Dinning, Gallup and Briggs (1949) could detect no relationship between creatinine excretion and source of nitrogen in the diet of beef steers. Similarly, Albin and Clanton (1966) found that the amount of protein or energy fed to beef cattle had no effect on creatinine excretion. On the other hand, Van Niekerk, Bensadoun,

Paladines and Reid (1963*b*) obtained increased urinary output of creatinine from sheep when the protein intake was suddenly increased by a large amount, while starvation resulted in reduced urinary creatinine. There was also a carryover effect from these treatments when diets were returned to the control level and the authors attributed some of this effect to changes in lean body mass.

Despite the doubts as to the constancy of the muscle creatine content and the constancy of creatinine excretion, the relationship between lean body mass and urinary creatinine excretion has been examined on several occasions. Lofgreen and Garrett (1954) related creatinine coefficients to the amount of lean in the 9th, 10th, 11th rib cut of beef carcasses. Despite a significant correlation coefficient of 0.67, the relationship was not good enough for predictive purposes. Kumar *et al.* (1959) working with rats related urinary creatinine to body composition. Their equation for predicting fat-free mass had a coefficient of variation of only 2%, but for the females creatinine excretion did not add to information given by live weight alone. The error of prediction of fat was such as to make the method, on its own, of little value for predictive purposes.

Using identical twin and triplet steers and bulls, Rebhan and Donker (1960) found that fat steers had a lower creatinine coefficient than thin steers, but with bulls the opposite was found. No reasonable explanation could be found. Van Niekerk *et al.* (1963*a*) related creatinine excretion to body composition in sheep covering a wide range of fatness (4.86 to 46.6% of ingesta free mass). The urinary output of creatinine was highly correlated (0.97) with lean body mass. With lighter sheep, weighing less than 55 kg and containing less than 28% fat, live weight was almost as effective in predicting body protein as was urinary creatinine. From the evidence available

it is doubtful if accurate prediction of body composition could be achieved from measurements of creatinine excretion in animals over a small range of live weight or fatness.

3-methylhistidine

3-methylhistidine (3-MeH) is formed after the amino acid histidine is incorporated into the polypeptide chains which make up the myofibrillar protein in muscle. It makes up a constant proportion of muscle (Haverberg, Omstedt, Munro and Young, 1975) and the only source of free 3-MeH in the body is from the catabolism of protein, as occurs in the normal process of protein turnover. The 3-MeH is quantitatively excreted in the urine in the rat, man and adult rabbit (Harris, Milne, Lobley and Nicholas,1977). In some species, however, such as the sheep (Harris and Milne, 1980) and the pig (Harris and Milne, 1981*a*) it appears that some of the 3-MeH released during protein breakdown forms a pool of non-protein-bound 3-MeH. As this pool is several times larger than the daily urinary excretion, the excretion is not quantitatively related to muscle break-down rate. This does not appear to be the case in cattle (Harris and Milne, 1981*b*).

In those species where metabolism of the amino acid is negligible it would be expected that the total daily excretion of 3-MeH will be related to both the rate of protein breakdown and to the total amount of muscular protein in the body. Before urinary 3-MeH can be used an an index of either of these two parameters, the other must be known.

The effect of dietary protein on gross protein breakdown is not clear, but certainly a protein free diet or starvation will decrease gross protein breakdown rate in rats by about 50% compared to a "normal diet" (Millward, Garlick, James, Sender and Waterlow, 1976).

In the starved or protein deficient animal net protein breakdown will be increased because of a concomitant decrease in gross protein synthesis.

If the effect of dietary protein on breakdown of body protein could be quantified then 3-MeH excretion used in conjunction with some index of muscular protein may be of potential use as a measure of protein breakdown in the bovine body. If the range of total muscular protein is small, then perhaps 3-MeH excretion on its own might be of use as an indicator of protein breakdown. Also if the range of dietary protein intake and protein breakdown are small then 3-MeH excretion might indicate total muscular protein. As quantitative urine collection is inconvenient to use routinely on large numbers of animals, the use of circulating blood concentrations of 3-MeH as an index of protein breakdown in cattle merits investigation.

## Body condition scoring of cattle

### Development of condition scoring

Body condition was defined by Murray (1919) as "the ratio of the amount of fat to the amount of non-fatty matter in the body of the living animal", and its estimation by visual appraisal has been used for many years in livestock production. Terms such as "lean", "store", "forward store" or "fat" have been, and are still widely used to describe the condition of various classes of livestock, particularly sheep and cattle. However, despite the obvious drawbacks of using such terms and the wide discrepancies which occur in their interpretation, it was not until fairly recently that any attempt was made to standardise a system of body condition scoring. Several people (e.g. McClymont and Lambourne, 1958; Reid, 1958) considered the possible usefulness of body condition measurements in the design and interpretation of nutritional experiments, and in

1961 Jefferies devised a system of scoring body condition in sheep on a six point scale. Each point or grade was described in terms of the amount of tissue cover over the lumbar region of the spine and the use of the system for management purposes in Australia was discussed.

Everitt (1962) found considerable variation both between and within observers when sheep and cattle were condition scored. A ten point scale was used, but the points were not defined, and simply ranged from point 1 = emaciated to point 10 = very fat. Condition score was also related to objective measurements of total chemical fat and dissectable fatty tissues, and although significant relationships were obtained, they were generally poor. The conclusion was reached that condition score had little to offer and in fact could lead to erroneous conclusions.

Contrary to this, Russel, Doney and Gunn (1969) concluded that body condition scoring in sheep could "provide an acceptable and useful estimate of the proportion of fat in the animal body". They used a six point scale, based on that suggested by Jefferies (1961) where the degree of tissue cover over the lumbar area of the spine is estimated by palpation. Each of the six grades is distinctly characterised. The prediction of chemical fat in the bodies of mature Scottish Blackface ewes from condition score was better than that from live weight. It appears, however, that if animals are of very similar body size then live weight may be just as accurate a predictor of body fat (Guerra, Thwaites and Edey, 1972).

The system used for sheep by Russel *et al.* (1969) was adapted for use on cattle, and in particular suckler cows (Lowman, Scott and Sommerville, 1973). Five areas of the cow's body were assessed for fat cover:-

1. On the spinous processes of the lumbar vertebrae
2. Around the tail head
3. Over the lower rib cage
4. At the hip bones
5. At the "second thigh"

The main area of consideration was the spinous processes of the lumbar vertebrae.

After testing on a large number of suckler cows and growing/ finishing cattle the system was modified (Lowman, Scott and Sommerville, 1976) and only the first two areas were used in assessment. In particular the transverse processes of the lumbar section of the spine receive special attention. The system defines six grades and recommends that animals should be scored to the nearest half grade. It should be noted that the lowest score on the scale, grade 0, is rarely found and represents an extremely emaciated animal. With experience, and for more detailed experimental work, animals can be scored to the nearest quarter grade (B.G. Lowman, personal communicaion).

Consistency of condition scoring

Several studies have been made on the consistency with which cattle can be condition scored (Meat and Livestock Commission, 1973 and 1975; Evans, 1978) and in general the correlation coefficients of repeat scores on the same animals by the same operators have been quite high, especially after some experience with the technique, when correlation coefficients of 0.8 or higher can be expected. Repeatability between operators is also high and in one trial a correlation coefficient of 0.7 was obtained (Evans, 1978).

Relationship of condition score to live weight

The relationship between condition score and live weight can never be perfect because the former is a discrete variable and the

latter continuous. In addition the relationship will vary with the size of the animals. However, several attempts have been made to relate changes in condition score to changes in live weight in cows. The relationships found are summarised in Table 2.4

Table 2.4. Relationship of condition score change to live-weight change

| Reference | Breed | | Change in weight (kg) for 1 point change in condition score |
|---|---|---|---|
| Kilkenny (1978) | Hereford x Friesian | | 58.5 |
| | Hereford x Beef | | 57.0 |
| | Blue-Grey | | 49.0 |
| | Angus x Friesian | | 48.1 |
| | Angus x Beef | | 48.6 |
| | Welsh Black | | 49.9 |
| | Charolais cross | | 64.1 |
| | Red Breeds | | 59.7 |
| Frood and Croxton (1978)* | Friesian | Cows | 28.3 |
| | | Heifers | 16.6 |
| J.N. Peart (unpublished) | Hereford x Friesian | | 90.0 |
| | Blue-Grey | | 83.0 |

It is obvious that some disagreement exists as to the weight change associated with a change in condition score of 1 point. It seems unlikely that variation between operators could account for such wide differences between trials, and most of the variation in cow size should be removed by considering each breed separately. One factor which might explain these differences could be variation in gut fill. This might explain in particular the very low figures found by Frood and Croxton (1978). These workers recorded live weight and condition score throughout the lactations of 225 Friesian cows in commercial herds. The general trend of condition score

*includes data from pregnant cows and therefore is likely to be affected by weight increments of the conceptus.

and live weight was upwards, except for the first 2 months of lactation. However, the trial period corresponded to the drought of 1976 and was carried out over a period of diminishing grass supply. Thus live-weight increase could have been less than would normally have been expected despite the substitution of concentrates for grass.

Condition scoring and body composition

The system of condition scoring cattle is now widely recommended by the Agricultural Advisory Services in the United Kingdom as a useful aid to suckler cow management in the commercial situation, where recommendations can be given as to the required "target" condition at certain points in the production cycle of the suckler cow. As yet only one very limited attempt has been made to relate condition score to fat content of the animal (B.G. Lowman, personal communication) and in this case only the fat content of the carcass was considered, by estimation from carcass classification. No attempt has been made to relate body condition score to whole body composition and for this reason body condition scoring is of limited use at present in detailed research into the nutrition of the suckler cow.

Ultrasonic measurements

Most work using ultrasonic measurements in cattle has been aimed at predicting the commercial characteristics of the carcass. This has primarily concentrated on measuring subcutaneous fat depth and eye muscle area, although in Australia some attempt has been made to use ultrasonics to predict total muscle in the carcass (Hervé and Campbell, 1971).

As far as the author is aware no attempt has been made to relate ultrasonic measurements to the chemical composition of cattle.

In the mid-fifties, work on the use of ultrasonics on cattle

started with the estimation of subcutaneous fat depth (Temple, Stonaker, Howry, Posakony and Hazeleus, 1956). Since then most reports have been concerned with correlating ultrasonically determined subcutaneous fat depth and eye muscle area to the same measurements made on the carcass after slaughter, or with predicting the commercial value of the carcass from ultrasonic measurements made on the live animal. Some of the correlations between ultrasonic and carcass measurements are very low, but better correlations have been obtained since more sophisticated equipment has been developed.

The ultrasonic technique is based on the principle that high frequency sound waves are transmitted through the animal tissues, and when these waves reach an interface between two tissues of differing density some of the sound is reflected back. Ultrasonic equipment consists of four basic parts - a pulse generator, a transmitter-receiver probe (transducer), an amplifier and a recorder, which is often a cathode ray oscilloscope. The pulse generator transmits an electrical pulse, which the transducer coverts to an ultrasonic pulse. The transducer is placed against the animal's skin and the ultrasound transmitted to the tissues. When the ultrasonic pulse reaches an interface between two different types of tissues, for example between muscle and fat, some of the sound energy is reflected back to the transducer, which now acts as a receiver and converts the sound waves back to an electrical current. This is amplified and fed to an oscilloscope to be displayed on a screen, or on film. The distance between the echoes on the screen or film is proportional to the distance from the transducer to that interface.

There are two types of ultrasonic equipment in use at the present

time. The A-presentation displays the echoes as vertical deflections on the oscilloscope screen and is widely used in the pig industry. The B-presentation shows the echoes as spots of light, and makes it possible to obtain a two dimensional cross-sectional picture of the area being probed ultrasonically, either by using a number of transducers placed at intervals along a transect on the animal or by moving a single transducer along a transect in synchrony with the display mechanism. When a two dimensional picture is built up it becomes necessary to provide a permanent record of that picture and this is done by photographing the image on the oscilloscope screen as in the "Danscanner" or by using the photographic film as the screen itself as in the American "Scanogram".

Traditionally, ultrasonic measurements on live cattle have been carried out on the musclature and subcutaneous fat layer in the loin and back. In this region the musclature consists mainly of the *M. longissimi thoracis et lumborum* (eye muscle), which is well defined and relatively easy to measure. In addition, skeletal features in these regions are easily located, so the position of measurement can be reproduced within and between animals. When evaluating the "Scanogram" however, Tulloh, Truscott and Lang (1973) scanned cattle over the tuber coxa, over the femur and over the scapula as well as over the back and loin regions.

Table 2.5 gives the correlation coefficients between ultrasonic and carcass measurements obtained by various authors. A wide range of correlation coefficients is reported, and various reasons have been suggested for this variation. Sound frequency, trapping of air between the transducer and skin, and transducer pressure have been suggested as technical factors which affect the results (Hazel and Kline, 1959; Temple *et al.*, 1956; Wallentine, 1960).

Table 2.5. Correlation coefficients between ultrasonic and carcass measurements

| Reference | Type of Scan | Position | Fat Depth Correlation | Eye Muscle Area Correlation |
|---|---|---|---|---|
| Temple, Stonaker, Howry, Posakony and Hazeleus (1956) | A | over 13th rib | 0.39** - 0.63 | - |
| Stouffer, Wallentine, Wellington and Diekman (1961) | B | over 13th rib | 0.04 - 0.58* | 0.22* - 0.85* |
| Hedrick, Meyer, Alexander, Zobrisky and Nauman (1962) | A | between 12 & 13th ribs | 0.11 - 0.63*** | 0.58 - 0.89** |
| Hedrick, Meyer, Alexander, Lasley, Comfort, Dyer and Nauman (1962) | not stated | not stated | 0.43* - 0.80* | 0.58* - 0.92* |
| Davis, Long, Saffle, Warren and Carmon (1964) | B | between 12 & 13th ribs<br>9" posterior to 12th rib | 0.67* - 0.90** | 0.87** - 0.93**<br>0.82** |
| Levantin, Epifanov and Smirnov (1964) | not stated | over 13th rib | - | 0.92+ |
| Meyer, Moody, Hunziger, Ringkob, Alexander, Zobrisky and Hedrick (1966) | not stated | not stated | - | 0.61 - 0.92+ |
| Davis, Temple and McCormick (1966) | B | between 12 & 13th ribs | 0.64***- 0.75*** | 0.90***- 0.92*** |
| Watkins, Sherrit and Zeigler (1967) | B | between 12 & 13th ribs | 0.72** - 0.93** | 0.37* - 0.69** |
| McReynolds and Arthaud (1970*a*) | A<br>B | between 12 & 13th ribs<br>between 12 & 13th ribs | -0.19 - 0.72*<br>0.38* - 0.73* | -<br>- |
| McReynolds and Arthaud (1970*b*) | B | between 12 & 13th ribs | -0.5 - 0.61+ | -0.55 - 0.95** |
| Campbell and Hervé (1971*a*) | B | over 3rd lumbar transverse process | - | 0.94+ |
| Andersen and Ernst (1972) (cited by Andersen 1975) | B | over 1st lumbar transverse process<br>over 5th lumbar transverse process | -<br>- | 0.53***<br>0.47*** |
| Tulloh, Truscott and Lang (1973) | B | between 12 & 13th ribs<br>over 3rd lumbar transverse process | 0.93**<br>0.83** | 0.69** - 0.76**<br>0.60** - 0.62** |

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, + p not stated

Biological factors include positional variations of the animal and post-mortem changes, in particular the effects of chilling and hanging the carcass (Stouffer, Wallentine, Wellington and Diekman, 1961; Miles, Pomeroy and Harries, 1972). The latter occurs because the soft tissue of the hanging carcass moves with respect to the spine when compared with the live animal. The spine itself is also distorted and so location of the same anatomical point on both the live animal and the carcass is difficult if not impossible.

Considerable variation has also been reported between operators (Davis *et al.*, 1966; Miles *et al.*, 1972; Tulloh *et al.*, 1973). In a detailed study of operator variation Miles *et al.* (1972) found that the main reason for between operator variation was mis-identification of anatomical boundaries when interpreting Scanogram photographs. However, not all operators were experienced and operator variation appears to become less important as experience is gained (Moody, Zobrisky, Ross and Nauman, 1965). Similarly Campbell and Hervé (1971*b*) found no significant operator variation between experienced operators.

## Visual Appraisal

When beef cattle are being selected for slaughter they are usually chosen on the basis of appearance. Similarly, when they are sold alive the expected yield of meat from the carcass is assessed by visual appraisal.

Kallweit (1976) has reviewed the literature dealing with the relationship of visual assessment of the live animal and carcass evaluation. It was found that the value of the correlation coefficient decreased as the variation in the traits being examined decreased. Generally visual assessment is of little value in the prediction of body composition, especially if the variation is low.

Because of this and the diffulty of standardisation between judges, visual appraisal will not be considered further.

## External Measurements

### Linear measurements

Various attempts have been made to relate linear measurements of the live animal to the composition of its carcass, and have been reviewed by Orme (1963), Kirton (1964) and Barton (1967). In general, poor correlations have been found between the linear measurements made on an animal, or part of an animal, and the weights or proportions of saleable meat, carcass fat or other aspects of carcass composition or quality.

More recently Yadava (1970) made several linear measurements on Jersey and Holstein cows. He found the product of length of body (from the point of the shoulder to the pin bones) and height at withers (L x H) to be most closely related to live weight. After transformation to a log-log basis the regression of live weight on L x H was calculated. Condition grades were then allocated to individual cows on a 1 to 9 scale, depending on the difference between actual and predicted live weight. The allocation of grades is shown below:-

| Actual minus Predicted Weight (kg) | Condition Score |
|---|---|
| +80 or more | 9 |
| +60 to +79 | 8 |
| +40 to +59 | 7 |
| +20 to +39 | 6 |
| -19 to +19 | 5 |
| -20 to -39 | 4 |
| -40 to -59 | 3 |
| -60 to -79 | 2 |
| -80 or more | 1 |

The system was not compared with any measurement of body composition. It has the advantage that it is objective, unlike

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subjective body condition scoring, and should remove some of the variation of live weight attributable to differences in body size. It does not, however, correct for the effects of weight of gut contents. Nevertheless, because of its simplicity and cheapness it is probably worthy of further investigation.

Skinfold thickness

The skinfold appears to be a valid method of estimating subcutaneous fat depth in man (Keys and Brožek, 1953). Tulloh (1961) attempted to relate skinfold thickness, measured at the 11th rib of steers, to subcutaneous fat depth, but could find no significant relationship. Charles (1974) measured the skinfold thickness at the side of the anus (which he termed anal fold) and related it to carcass composition. Correlation coefficients were obtained between anal fold and percent carcass muscle (-0.92) and percent carcass fat (0.94). The animals used were quite fat, ranging from 17 to 44.5% fatty tissue in the carcass. Possible reasons for the better results obtained using anal fold as opposed to skinfold over the 11th rib are the thinner skin at the anal fold and the absence of the subcutaneous muscle (*M. cutaneus trunci*).

Cannon bone circumference

Kraybill, Hankins and Farnworth (1954) related, amongst other things, the circumference of the fore cannon bone (metacarpus) and overlying tissues to the proportion of bone in the carcasses of cattle. The cattle ranged in weight from 61 to 848 kg and a correlation coefficient of -0.65 was obtained. The negative correlation is a result of the decreasing proportion of bone in the body with increasing maturity. Bone, as a proportion of body weight ranged from 40 to 12.5%. The correlation coefficient for only mature cattle was not calculated.

Hidiroglou (1963) examined various cannon bone measurements, live weight and rib-eye area in cross beef cattle. Simple correlation coefficients between rib-eye area and cannon bone circumference for different breeds ranged from -0.05 to 0.31 and none reached significance.

The relationship between cannon bone circumference and total bone in the bodies of mature cows has not, as far as the writer is aware, been examined.

X-rays

Kraybill *et al.* (1954) made anthropometric measurements and took X-ray photographs of the metacarpal bone of 75 cattle, ranging in weight from 62 to 850 kg. The density of the bone, the thickness of metacarpal bone wall, measured from the X-ray plate, and the circumference of the metacarpal bone and overlying tissue, measured on the live animal, were the three measurements most closely correlated with percentage bone (R = 0.78). However, X-ray equipment is expensive and the technique needs rigid standardisation.

Lipid-soluble gases

Lipid-soluble gases have been used to estimate the body fat content of man and small animals. The technique involves measuring the uptake of a gas by the animal body, and in order that uniform distribution occurs throughout the body fat, the gas should have a high fat:water solubility ratio. Cyclopropane has been used by Lesser, Blumberg and Steele (1952) for measuring total body fat in rats. Pullar (1972), however, abandoned the use of cyclopropane to measure fat content in obese rats when he found that equilibration did not occur in 12 hours. The equilibration time in farm animals is likely to be lengthy.

Radioactive krypton ($^{85}Kr$) has been used in man to estimate body fat (Lesser and Zak, 1963). Attempts by Hytten (1969) to measure

body fat in pigs by $^{85}Kr$ dilution were not successful because of difficulties in the administration of the gas. Thus it appears from the evidence available that $^{85}Kr$ is not likely to be of use in body composition estimations in farm animals.

## PARTITION AND DISTRIBUTION OF FAT AND FATTY TISSUE

Some confusion exists in the usage of the terms "fat partition" and "fat distribution". However, recently "fat partition" has generally been taken to mean the partition of fat or fatty tissue among the various depots in the body i.e. the subcutaneous, intermuscular, intramuscular, perirenal, omental and mesenteric depots. The term "fat distribution" is usually used to describe the distribution of fat or fatty tissue within a depot e.g. the distribution of the subcutaneous fat or fatty tissue over the carcass. These definitions will be adhered to in this review.

In a recent review, Kempster (1981) concluded that breed differences in fat distribution were small, when comparisons were made at the same total fat depot weight. There is, however, more genetic variation in fat partition.

Fat partition is of considerable importance in meat production, as the partition of fat between the carcass and the non-carcass parts of the animal, as well as within the carcass, will affect its suitability for a particular market, and hence its commercial value. Differences in fat partition between breeds, and indeed within breeds, may well affect techniques for the *in vivo* prediction of body composition. This is particularly true of the assessment of total body fat from measurement of just one fat depot, such as in body condition scoring or any measurement of subcutaneous fat depth. Individual variation in fat partition may well limit the precision of prediction equations. If important differences exist between

breeds, then different prediction equations may have to be developed for different breeds (Charles and Johnson, 1976).

There are several ways of describing fat partition. Obviously the use of weights of fatty tissues in the various depots cannot be used because of differences in body weights between animals. The use of percentages overcomes this problem only if animals are compared at the same total fat weight and Kempster (1981) has suggested that in growth studies animals should be compared at similar total fat depot weight. This, of course, requires serial slaughter of animals. For breed comparisons, however, he suggested that similar subcutaneous fat weight should be used as the base-line, presumably because that is the criterion nearest to that used in the selection of commercial cattle for slaughter.

One method for describing fat partition has been proposed by Lister (1976). He suggested the use of a "Fat Partition Index" which is the quotient obtained by dividing the weight of dissectible subcutaneous fat by the sum of the weights of the intermuscular, perirenal and inguinal fat in a carcass. Truscott (1980) has criticised this index because it is not independent of stage of maturity. He proposed the use of an index based on the statistical procedure of principal component analysis, which, it is claimed, overcomes the disadvantages of Lister's Fat Partition Index.

The literature abounds with information on fat partition in the carcass of cattle because of its effect on consumer acceptability of beef and its economic significance. Less has been published on fat partition in the whole body.

It has long been known that breed differences in fat partition exist in cattle, with the earliest reference being that of Lawes and Gilbert (1859). It is widely accepted that extreme dairy breeds

of cattle deposit a higher proportion of their fat internally (in the perirenal, omental and mesenteric depots) than do the traditional beef breeds (e.g. Callow, 1948 and 1961; Barton, 1968, 1971 and 1972). However, care must be taken when considering some of the published information, for not all comparisons have been made at equal fatness.

Various hypothesis exist as to why different breeds should exhibit different patterns of fat partition. Classically it was thought that the higher proportion of subcutaneous fat in meat type animals was due to selection for "conformation" and that selection for a more "rounded" appearance had led to a shift in fat partition (Hammond, 1932; Callow, 1961), and that animals with a higher proportion of internal fat were more primitive with respect to selection for meat characteristics. Recently it has been suggested that fat partitioning within the body is indicative of a general metabolic type and efficiency of food utilisation (Lister, 1976; Allen, Beitz, Cramer and Kauffman, 1976). Truscott (1980) could, however, find no evidence of this in a study of the growth of Hereford and Friesian cattle.

Dairy cattle are known to have a particular ability to mobilise lipid in early lactation (Hart, Bines, Morant and Ridley, 1978) and to have a higher proportion of internal fat depots (Lister, 1976) which Sharfir and Wertheimer (1965) concluded are metabolically most active. Allen *et al.* (1976) suggested that a lower external to internal fat ratio may be the result of a greater turnover and mobilisation of triglycerides from the subcutaneous depot with subsequent re-deposition intra-abdominally.

In sheep also, the breeds with the low external to internal fat deposition are the prolific heavy milking breeds e.g. Finnish

Landrace (McClelland and Russel, 1972).

If there are appreciable differences in fat partition among beef cow types or breeds, this has clear implications in regard to the approach adopted by the Advisory Services in the U.K. of advocating optimum or target condition scores for beef cows at different stages of the production cycle and under different management systems. For this reason, as well as for those outlined above, fat partition in suckler cows requires examination and quantification.

CHOICE OF METHODS FOR ESTIMATING BODY COMPOSITION IN BEEF COWS

The advantages and disadvantages of various methods of indirect estimation of body composition in mature cows have been discussed. The methods were considered as to their suitability to provide information on the body composition of suckler cows during nutritional experimentation. Bearing this in mind, the techniques had to be applicable to relatively large numbers of animals on a routine basis.

For financial reasons radioactive isotopes were not considered as their use precludes the sale of carcasses from treated animals and of progeny born to, or suckling such animals.

Live weight is the most commonly made measurement in animal production experimentation, and as such had to be evaluated in terms of its ability to predict body composition. Because of the ease with which it can be measured, the other indices of body composition had to be judged against it alone or in terms of the additional information supplied in conjunction with live weight.

As explained previously, $D_2O$ appears to be the most promising tracer for the estimation of total body water. Blood volume as estimated by the Evans Blue dilution procedure was selected because

of its cheapness and its relative simplicity.

The concentration of 3-methylhistidine in the plasma was to have been measured, but through lack of time a suitable analytical technique could not be developed. However it is hoped that this technique can be pursued at a later date.

Condition scoring has obvious advantages, not least of which is its low cost. Because of this and the fact that it is now widely used on cattle in the United Kingdom and other countries, it was felt that the system should be examined in more detail to assess its predictive ability.

Although ultrasonic measurement of subcutaneous fat depth and eye-muscle area did not appear to have been used to assess whole body composition in ruminants, the results from prediction of carcass composition were sufficiently encouraging to suggest that ultrasonic techniques could have wider application.

Linear measurements used in conjunction with live weight appear to offer a cheap, simple and objective measure of body condition and merited investigation. Skinfold thickness over the ribs has only been studied on one occasion in cattle and despite the fact that this showed no significant correlation with subcutaneous fat depth, it was decided to examine the method further. Cannon bone circumference has not been examined as an index of skeletal size or proportion in mature cattle, and because of the ease with which it can be measured it was decided to include it with other external measurements.

The other methods reviewed previously were not considered further because of their doubtful value, technical problems or high cost, all of which have already been discussed.

It was hoped that the precision of estimation of body composition

could be improved by the simultaneous estimation of different parameters. For example body fat could be estimated directly by ultrasonic measurement of subcutaneous fat thickness and indirectly by $D_2O$ dilution.

Thus in summary the *in vivo* indices chosen were:-

1. Live weight
2. Total body water as estimated by $D_2O$ dilution
3. Blood and red cell volumes as estimated by Evans Blue dilution
4. Body condition score
5. Ultrasonic measurement of eye-muscle area and subcutaneous fat depth at a number of sites
6. Skeletal size, cannon bone circumference and skinfold thickness

# CHAPTER 3

## PRELIMINARY STUDIES ON THE USE OF DEUTERIUM OXIDE AND EVANS BLUE

## INTRODUCTION

Having selected the *in vivo* techniques to be examined in the main Body Composition Study, it was necessary to undertake certain preliminary investigations. The objectives of this work were, firstly to determine the time of taking the single blood sample for $D_2O$ determination for the estimation of total body water, and secondly, to study the conditions of storage and methods of determination of Evans Blue in connection with the estimation of blood and red cell volumes.

## EQUILIBRATION OF DEUTERIUM OXIDE

### Introduction

The method chosen for the prediction of total body water was the $D_2O$ dilution technique. The high cost of $D_2O$ analysis ruled out the routine use of the extrapolation method which requires several samples per cow, and it was consequently decided to adopt the equilibration approach. It was therefore important to determine the time after infusion at which equilibration had been reached.

In ruminants the rumen presents special problems in the estimation of body composition, because it contains a high proportion of the total body water. It is widely accepted that tracers used for the measurement of total body water equilibrate less rapidly with the gut water than with that in other body compartments (Little and Morris, 1972). There is, however, considerable disagreement as to the time taken for $D_2O$ to equilibrate with the body water. Table 2.2 gives the reported estimates for TOH and $D_2O$.

Fasting animals before infusion of tracer appears to have no effect on equilibration time (Little and Morris, 1972).

## Experimental

Two mature, non-pregnant, non-lactating Hereford x Friesian cows were used to establish the equilibration time for $D_2O$. The animals were fed 16 hours prior to infusion and had water available until just prior to infusion. Feed and water were withheld throughout the sampling period. A pre-infusion blood sample was taken from the jugular vein using a heparinised evacuated tube. A sample of rumen fluid was also taken before infusion and both were checked for the natural abundance of $D_2O$. Rumen sampling was carried out by inserting a flexible plastic tube (10 mm diameter) through the mouth and down the oesophagus. The end of the tube in the rumen had been drilled with several holes to avoid the problem of blockage. The tube was passed through a hard plastic pipe in the mouth to prevent the cow chewing it. A small vacuum pump was used to withdraw the rumen fluid into a glass bottle which acted as a trap.

Salinated $D_2O$ (9 g/l) was infused via a nylon catheter. The 75 cm long, 1.65 mm diameter catheter was inserted into the jugular vein towards the heart through a 12 gauge needle. Once the catheter was in position the needle was removed from the vein and skin, and a small quantity of physiological saline (approximately 5 ml) flushed down the catheter to ensure that it was in the vein and patent. The $D_2O$ was then infused from a flexible plastic bottle, of the type used for saline infusion (The Boots Company Ltd., Nottingham, U.K.) by simply attaching the bottle to the catheter and squeezing the bottle by hand. This technique allowed an infusion rate of 40 to 50 ml per minute. A dose rate of about 0.8 g of $D_2O$ per kg live weight was used, the exact weight infused being measured by weighing the bottle before and

after infusion. Immediately following infusion the catheter was flushed out with about 10 ml saline.

Blood samples and rumen samples were taken, as described previously, every 2 hours for a twelve hour period after infusion, and then at 24 hours. Blood and rumen samples were centrifuged for 20 minutes at a force of 2950 g and plasma and rumen water removed.

Analysis of $D_2O$ was carried out by mass spectrometry at the Heriot Watt University, Edinburgh.

In addition, three cows, two Hereford x Friesians and one Blue-Grey, were infused as described, but no pre-infusion blood sample was taken and no rumen sampling was carried out. A dose rate of about 0.5 g/kg live weight was used and blood samples were withdrawn at 4, 6 and 8 hours after infusion.

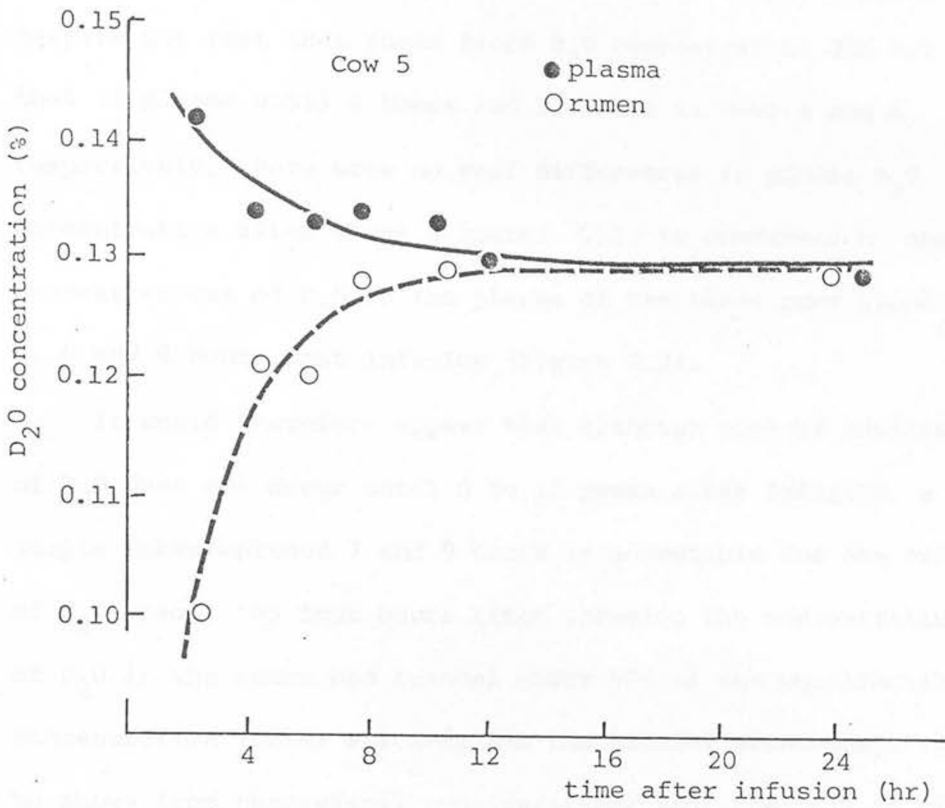
## Results and Discussion

The pre-infusion concentrations of $D_2O$ in the rumen liquor and blood plasma were, as expected, the same as the natural abundance, namely 0.015% on a molecular basis.

Figure 3.1 shows the concentration of $D_2O$ in plasma and rumen fluid of the first two cows and the parameters for curves fitted to the data are shown in Table 3.1.

Table 3.1. Relationships of concentration of $D_2O$ in water from plasma (P,%) and rumen (R, %) and time (t, hr) of the form P or R = a + $br^t$.

| Cow | Fluid | a(±s.e.) | b(±s.e.) | r (±s.e.) |
|---|---|---|---|---|
| 5 | P | 0.130(±0.0014) | 0.021(±0.0071) | 0.733(±0.1033) |
| | R | 0.129(±0.0018) | -0.081(±0.0263) | 0.622(±0.0871) |
| 3 | P | 0.134(±0.0004) | 0.271(±0.2326) | 0.292(±0.1096) |
| | R | 0.134(±0.0055) | -0.032(±0.0144) | 0.798(±0.1451) |



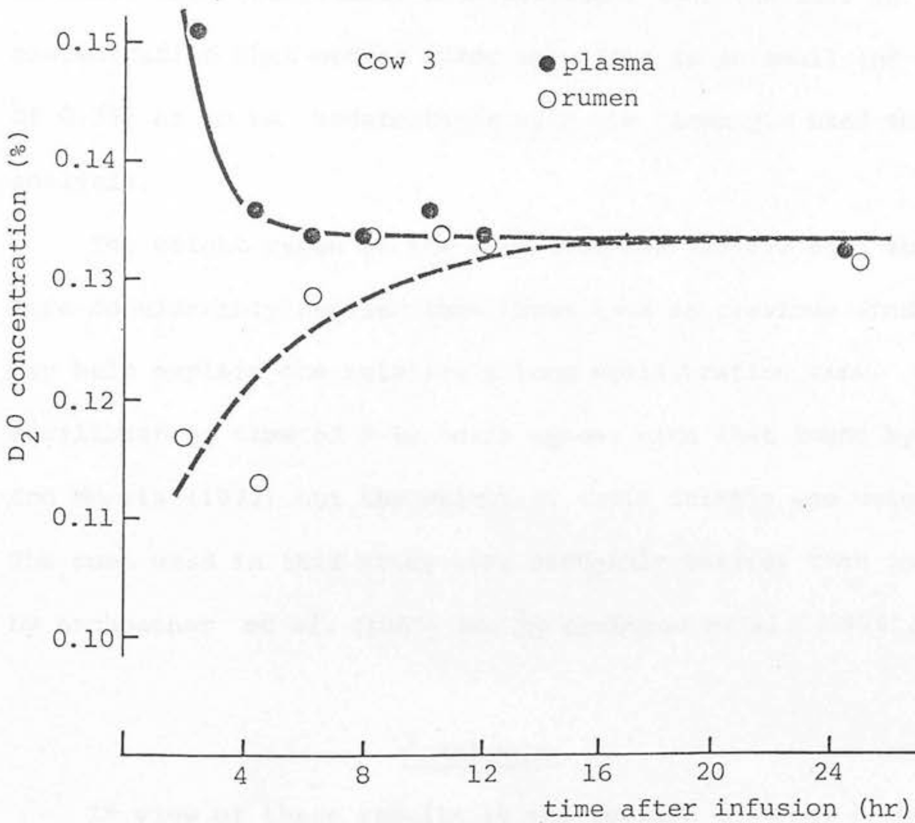




Figure 3.1. Concentration of $D_2O$ in rumen and plasma following $D_2O$ infusion

No attempt was made to force the curves to a common asymptote. Despite the fact that rumen fluid $D_2O$ concentration did not reach that of plasma until 8 hours and 12 hours in Cows 3 and 5 respectively, there were no real differences in plasma $D_2O$ concentration after about 4 hours. This is confirmed by the concentrations of $D_2O$ in the plasma of the three cows blood sampled 4, 6 and 8 hours post infusion (Figure 3.2).

It would therefore appear that although time of equilibration of $D_2O$ does not occur until 8 to 12 hours after infusion, a blood sample taken between 7 and 8 hours is acceptable for the calculation of $D_2O$ space. By four hours after infusion the concentration of $D_2O$ in the rumen had reached about 90% of the equilibration concentration (after allowing for the natural abundance). It can be shown from theoretical considerations that the fall in plasma concentration that occurs after this time is so small (of the order of 0.3%) as to be undetectable with the technique used for analysis.

The weight range of the cows used was 435-672 kg. These animals were considerably heavier than those used in previous studies, which may help explain the relatively long equilibration time. An equilibration time of 8-12 hours agrees with that found by Little and Morris (1972) but the weight of their animals was unspecified. The cows used in this study were certainly heavier than those used by Aschbacher *et al.* (1965) and by Crabtree *et al.* (1974).

## Conclusion

In view of these results it was decided that all blood samples collected for the calculation of $D_2O$ space would be taken between 7 and 8 hours after infusion.

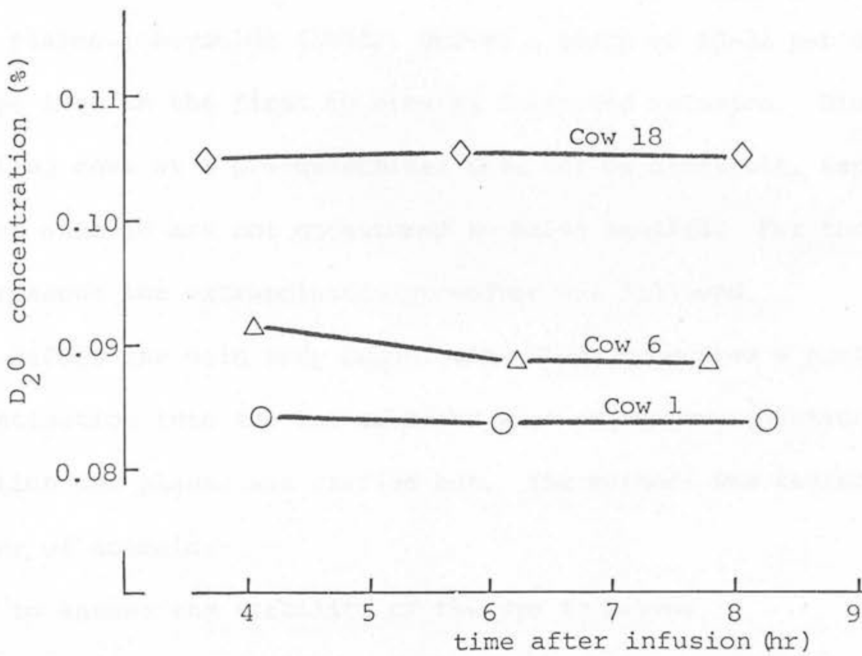



Figure 3.2. Concentration of $D_2O$ in plasma following $D_2O$ infusion

# THE USE OF EVANS BLUE TO MEASURE BLOOD VOLUME

## Introduction

One of the parameters to be considered was blood volume. This was to be estimated from measurements of haematocrit and plasma volume as determined by the Evans Blue dilution method (Reynolds, 1953*a*). Plasma volume can be estimated by either the extrapolation procedure or from a single sample collected 10 minutes after injection. The latter approach does not, however, take into consideration the individual differences in the rate of disappearance of the dye from plasma. Reynolds (1953*a*) quotes a range of 10-22 per cent of dye lost in the first 60 minutes following infusion. Blood sampling cows at a pre-determined time can be difficult, especially if the animals are not accustomed to being handled. For these two reasons the extrapolation procedure was followed.

Before the main Body Composition Study commenced a preliminary investigation into the use of Evans Blue in aqueous solution, saline solution and plasma was carried out. The method was tested on a number of animals:-

(a) to assess the stability of the dye in plasma,

(b) to determine whether the dye concentration could be measured directly in plasma, or whether the dye should first be removed by extraction with acetone (Chinard, 1951).

## Behaviour of Evans Blue in aqueous solution and in plasma under two storage conditions

Before the Evans Blue dilution method of measuring plasma volume could be used as a routine procedure the stability of the dye in plasma and in the carrier solvent had to be examined. It was decided initially to use water as the solvent as it was felt that salination of the carrier to physiological concentrations

was unnecessary because of the small volume needed in relation to the total plasma volume of a cow. It was also important to establish whether or not analysis for Evans Blue in plasma had to be carried out immediately or whether some time could be allowed to elapse.

A stock solution of aqueous Evans Blue (10 g/l) was prepared and stored overnight on the laboratory bench. The following morning a cow was blood sampled from the jugular vein using heparinised evacuated tubes (3 x 20 ml). Twenty ml of Evans Blue solution were then infused into the opposite jugular vein, using the same technique as for the $D_2O$ infusion, the exact quantity being determined by weighing the syringe before and after infusion. Time of infusion was noted and the animal was blood sampled from the same vein from which the first samples were withdrawn, ten minutes after infusion ($t_{10}$) to allow time for mixing of the dye and plasma. All blood samples were centrifuged at a force of 2950 g for 45 minutes and the plasma removed.

The blank and the dyed plasma were divided into five aliquots, one being used immediately for analysis, two being frozen and two stored on the laboratory bench. Samples of the stock solution were also stored frozen and on the laboratory bench. All samples were stored in polystyrene vials. The absorbance of blank plasma, of a standard solution of plasma containing 10 mg/l Evans Blue, and of the $t_{10}$ sample were measured on a Unicam SP 1800 ultraviolet spectrophotometer at a wavelength of 620 nm (Reynolds 1953*a*) using water as a blank.

Two days later and then again after 7 days the analyses were repeated using blanks and standards prepared from stock solution and blank plasma that had been stored under the same conditions and for the same

time as the $t_{10}$ plasma samples. The absorbance of the stock solution (diluted $1:10^4$) at 620 nm was also measured after 0, 2 and 7 days of storage under the two conditions.

The absorbance of the stock aqueous solution (diluted by a factor of $10^4$) is given in Figure 3.3, which shows a slight decrease in concentration of Evans Blue after storage for one week, particularly when kept on the bench.

Figure 3.4 shows the concentration of Evans Blue in plasma collected at $t_{10}$ and kept under the two storage conditions. The concentration was determined by comparison with standards made up on the day of measurement and also from stock solution kept under the same conditions and for the same period of time as the plasma. Figure 3.4 suggests that when stored on the bench, Evans Blue in plasma is very unstable in comparison to the aqueous solution, but when kept frozen this is not so. Reference to Figure 3.3 suggests that even when frozen the concentration of dye in aqueous solution decreases, but from Figure 3.4 it is seen that the relative concentrations of dye in aqueous solution and plasma remain constant when both are frozen. On the other hand the disappearance of Evans Blue in plasma is accelerated as compared with that in aqueous solution when stored on the bench, and indeed after 1 week the concentration had dropped to zero.

When Evans Blue is infused as an aqueous solution and when there is likely to be a delay in the determination of dye concentration in plasma, then plasma samples and preferably the stock solution should be frozen.

Disappearance of Evans Blue in plasma and in saline

In view of the differences in behaviour of Evans Blue in plasma and aqueous solution, a comparison of Evans Blue in plasma and in

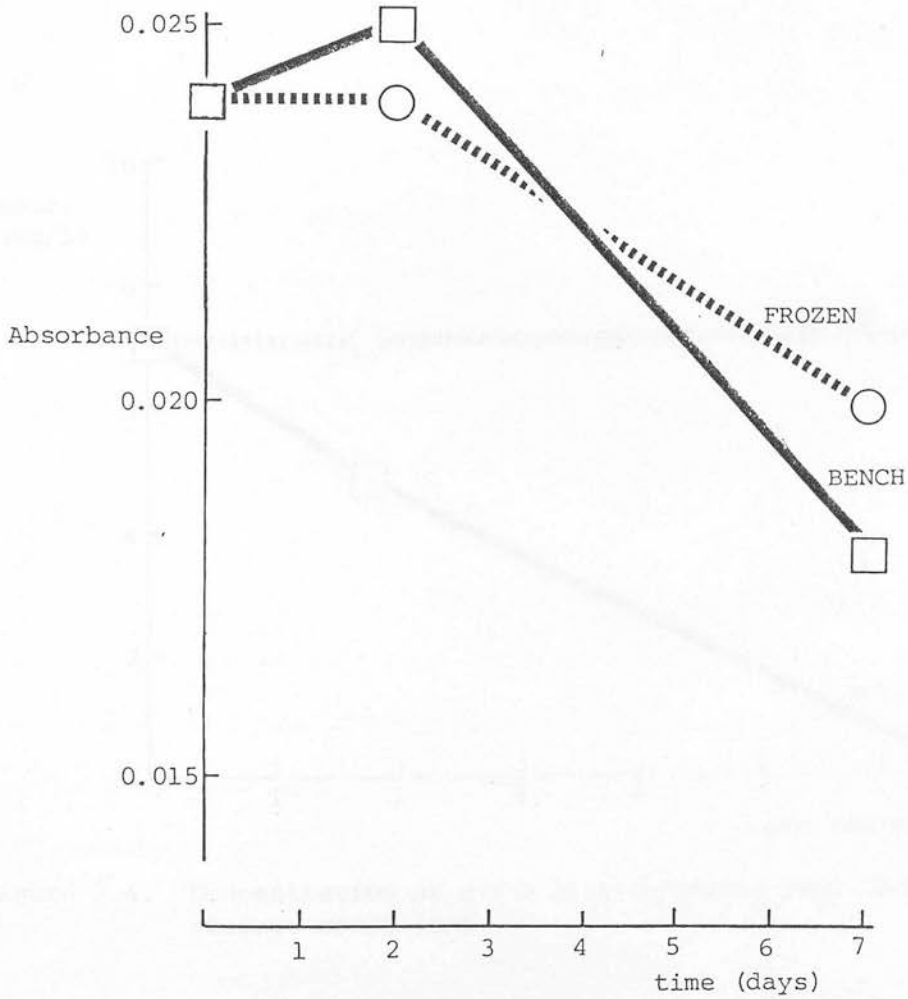



Figure 3.3. Absorbance of aqueous Evans Blue solution kept under two storage conditions

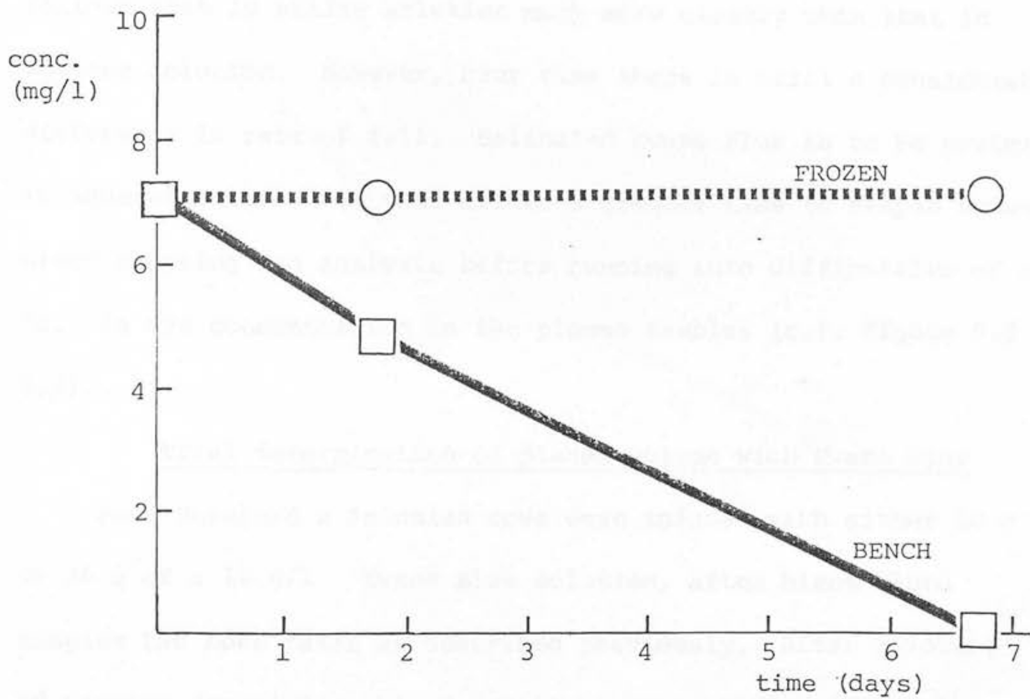



Figure 3.4. Concentration of Evans Blue in plasma kept under two storage conditions

physiological saline was carried out to determine whether the behaviour of the dye in saline followed that of plasma.

Solutions of Evans Blue (10 mg/l) were prepared in physiological saline (9 g/l) and in bovine plasma. These were stored in polystyrene vials on the laboratory bench and their absorbances measured at 620 nm at 0, 1, 3 and 6 days after preparation. The absorbance of the dye in plasma was compared with that in saline and of blank plasma, all stored under the same conditions. The results are shown in Figure 3.5. The fall in absorbance in plasma follows that in saline solution much more closely than that in aqueous solution. However, over time there is still a considerable difference in rate of fall. Salinated Evans Blue is to be preferred to aqueous solution as this allows a greater time to elapse between blood sampling and analysis before running into difficulties of a fall in dye concentration in the plasma samples (c.f. Figure 3.3 and 3.5).

Trial determination of plasma volume with Evans Blue

Four Hereford x Friesian cows were infused with either 20 g or 30 g of a 10 g/l Evans Blue solution, after blank blood samples had been taken as described previously. After allowing 10 minutes for mixing, blood sampling was carried out serially, every 10 minutes for 1 hour in the case of the two cows infused with 30 g of solution and every 5 minutes for 30 minutes in the case of those infused with 20 g. Dye concentration was determined from absorbances at 620 nm by comparison with standard solutions, and time-concentration graphs constructed.

The concentration of dye over time is given in Figure 3.6. Instead of the expected logarithmic fall in dye concentration with time the results followed no distinctive pattern. The most likely

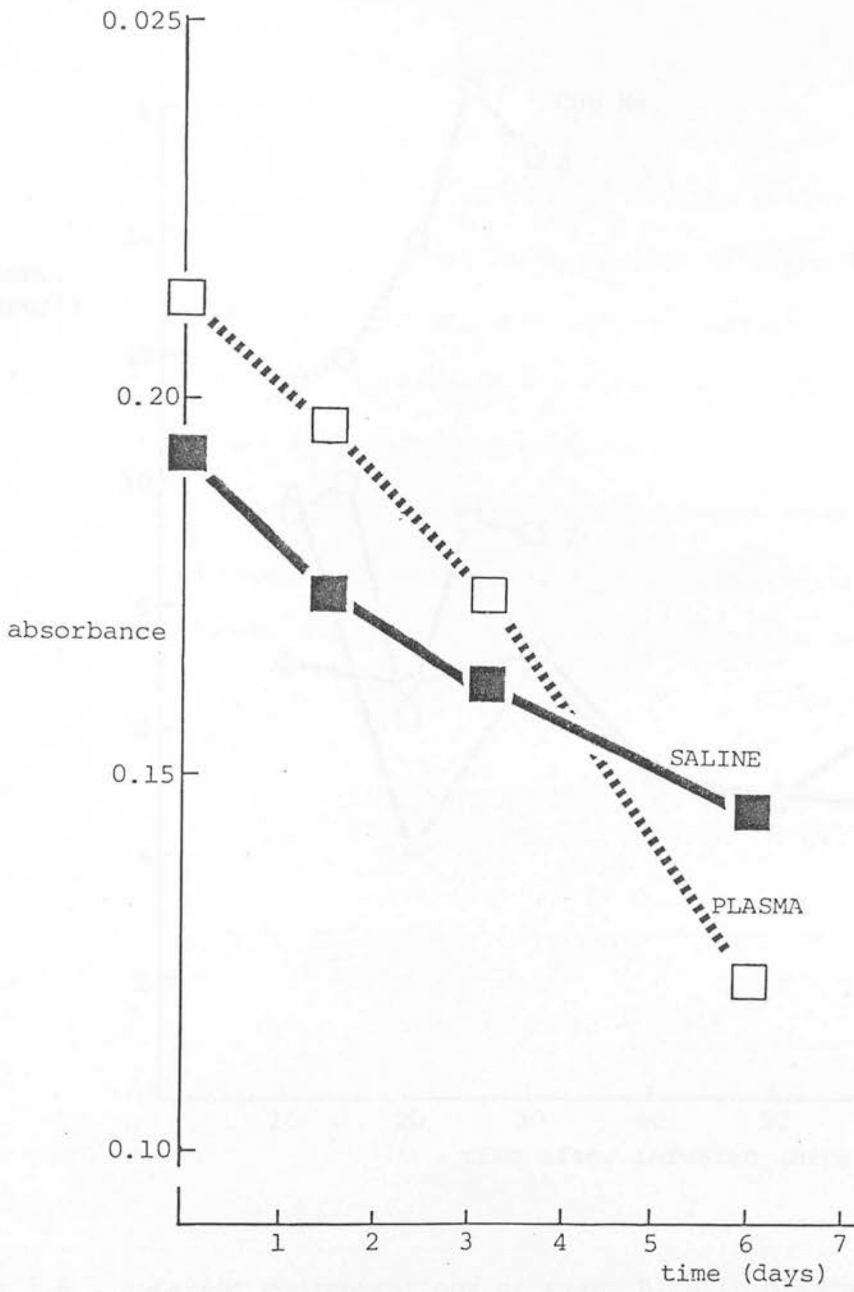



Figure 3.5. Absorbance of Evans Blue solution in plasma and saline stored on the bench

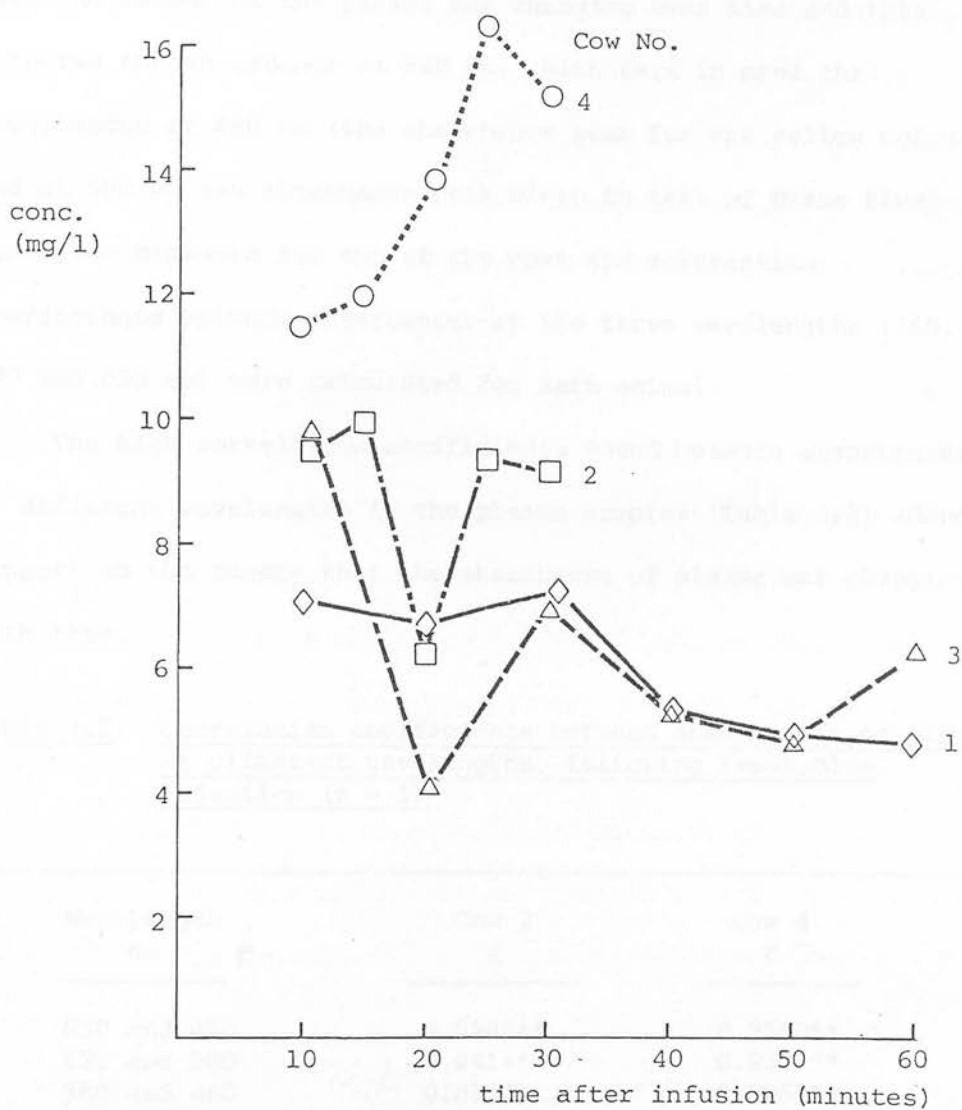



Figure 3.6. Apparent concentrations of Evans Blue in plasma following infusion

reason for this was that the absorbance of the plasma itself varied with time and that subtraction of the absorbance of the pre-infusion blank plasma was not effectively correcting for the "background" absorption of the plasma in subsequent blood samples, i.e. the colour of the plasma was changing over time and this affected the absorbance at 620 nm. With this in mind the absorbances at 460 nm (the absorbance peak for the yellow colour) and at 580 nm (an absorbance peak close to that of Evans Blue) were also measured for two of the cows and correlation coefficients between absorbances at the three wavelengths (460, 580 and 620 nm) were calculated for each animal.

The high correlation coefficients found between absorbances at different wavelengths in the plasma samples (Table 3.2) added support to the theory that the absorbance of plasma was changing with time.

Table 3.2. Correlation coefficients between absorbances of plasma at different wavelengths, following Evans Blue injection (n = 5)

| Wavelength nm | Cow 2 r | Cow 4 r |
|---|---|---|
| 620 and 460 | 0.9288** | 0.9580** |
| 620 and 580 | 0.8414* | 0.9377** |
| 580 and 460 | 0.8730* | 0.9860** |

* P <0.05 ** P <0.01

Within-cow differences in plasma absorbance

In view of the results from the previous trial it was decided to investigate the absorbance of blank plasma over time. A "simulated" Evans Blue trial was carried out. Four cows were used, two Blue-Grey and two Hereford x Friesian. Despite the fact that

Reynolds (1953a) found no evidence of lipaemia in Guernsey cows during plasma volume determinations using the Evans Blue technique, it was considered that changes in colour or turbidity could cause variation in absorbance. As it is possible that lipaemia could be related to stress, two "excitable" and two "docile" cows were used. The procedure was exactly as described for the previous trial, but 20 ml of physiological saline were infused instead of dye. The cows were serially blood sampled and the absorbance of plasma was measured at 460, 580 and 620 nm. In addition the turbidity of the plasma was measured in a nephelometer.

The absorbances at 460 nm were too high to be read on the spectrophotometer and so could not be measured.

The absorbances and turbidities for the plasma samples collected at different times are given in Figure 3.7. It is clear that the absorbance of blank plasma at 620 nm does vary over time. This would explain the poor results obtained when Evans Blue concentration was measured directly in plasma. However, the reason for this is not clear as no significant correlation coefficients were obtained between absorbance and turbidity. If lipaemia or a change in plasma protein concentration was the cause then a relationship between absorbance and turbidity would be expected.

It is concluded that the direct measurement of Evans Blue concentration in plasma samples is not advisable as the absorbance of blank plasma at a wavelength of 620 nm varies with time. This is in conflict with Reynolds (1953a) who concluded that there was no need to extract the dye from plasma as lipaemia did not interfere significantly with the transmission of light through dyed serum samples. The fact that Reynolds used serum and in the present study plasma was used seems unlikely to account for the differences.

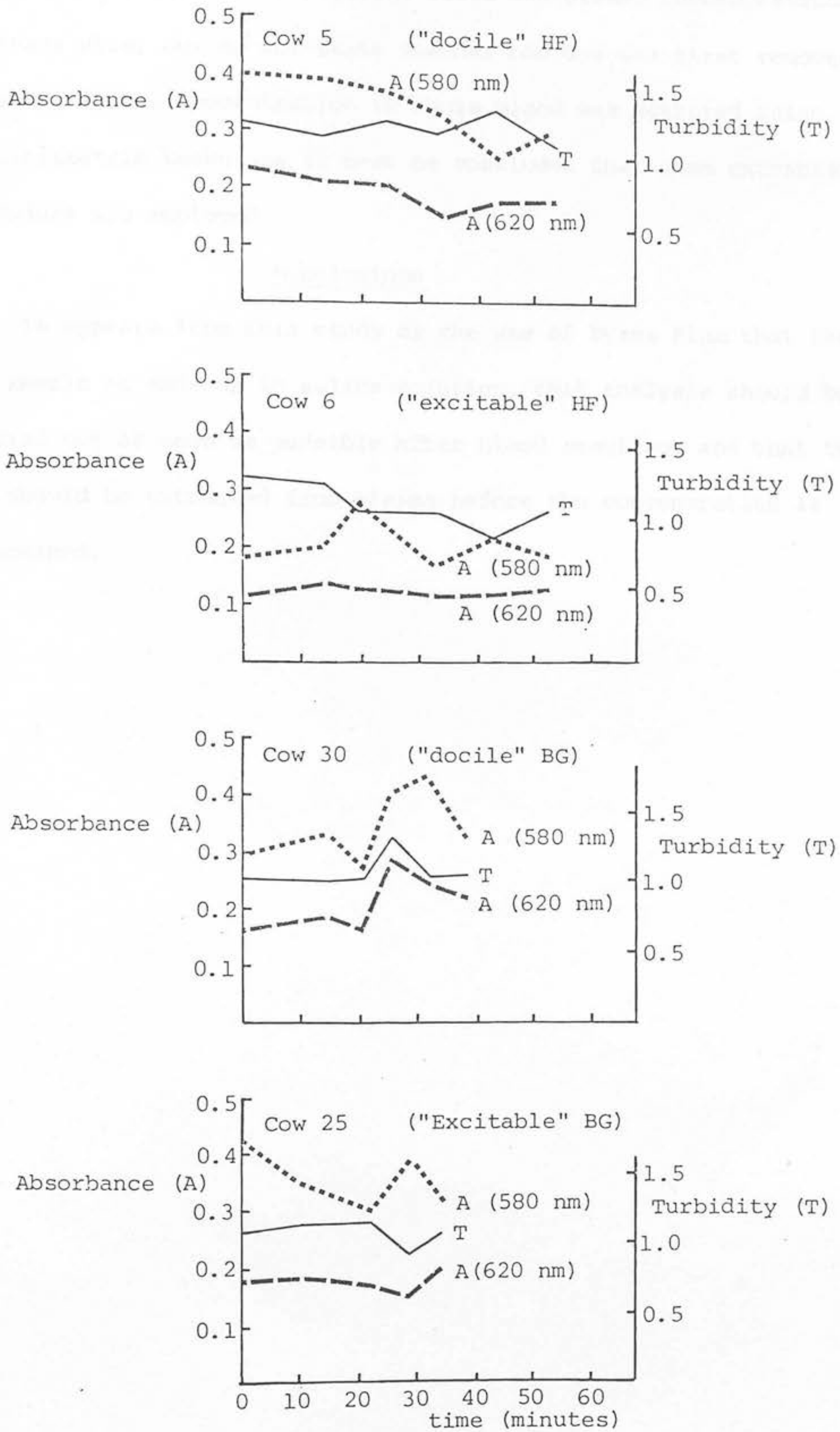



Figure 3.7. Absorbances and turbidities of plasma samples

Dalton and Fisher (1961) measured blood and plasma concentrations of Evans Blue, but do not state whether the dye was first removed. However, as the concentration in whole blood was measured using a colorimetric technique it must be concluded that some extraction procedure was employed.

## Conclusions

It appears from this study of the use of Evans Blue that the dye should be made up in saline solution, that analysis should be carried out as soon as possible after blood sampling, and that the dye should be extracted from plasma before the concentration is determined.

# CHAPTER 4

## EXPERIMENTAL PROCEDURE FOR MEASURING BODY COMPOSITION

ANIMALS AND MANAGEMENT

Initially it was intended to use 16 non-pregnant, non-lactating cows of each of the five genotypes:-

1. Hereford x Friesian (HF)

2. Blue-Grey (White Shorthorn x Galloway) (BG)

3. British Friesian (F)

4. Galloway (G)

5. Luing (L)

The British Friesians were included as a comparison of a dairy breed with the suckler cow types, and were all pure British Friesian with no Holstein Friesian blood.

One G cow died and was not replaced and six cows were found to be in calf at slaughter. The data from the pregnant cows were discarded, and so the results refer to a total of 73 cows made up as follows:-

| Breed | Number |
|---|---|
| HF | 14 |
| BG | 14 |
| F | 15 |
| G | 15 |
| L | 15 |
| Total | 73 |

All cows were mature animals, and had had at least one calf. There was a considerable range of ages, from 4 to 16 years old, although the exact age of some cows was not known.

To maximise genetic variation within a breed, and to make the cows as representative as possible of the different genotypes, they were selected from the following numbers of herds:-

| Breed | No. of Source of Herds |
|---|---|
| HF | 2 |
| BG | 4 |
| F | 6 |
| G | 5 |
| L | 3 |

All cows had been culled from their original herds, for a variety of reasons, such as failure to breed, mastitis, bad feet, and in the case of the Friesian cows, poor production.

## Body Condition of Cows

Ideally the cows of each breed were to have ranged from condition scores 1 to $4\frac{1}{2}$ (see Appendix I for definition of condition scores) with two cows of each genotype at each half grade interval. This was sought by individual feeding. However, difficulty was experienced in achieving the higher grades with the Friesians and, for reasons of time, with the Luings. The fattest cows of the F and L breeds were at condition score $3\frac{1}{2}$ and 3 respectively. The distribution of condition scores is shown in Figure 4.1.

## Housing

The cows were housed in the Metabolism Unit at HFRO's House o' Muir Research Station. Each cow was tied in a cubicle with individual feeding facilities. If the number of cows on hand exceeded the number of places available in the Metabolism Unit, then extra cows were kept on straw, in a cattle court with individual feeding using Calan-Broadbent electronic feeding gates. All cows were housed in the Metabolism Unit for at least two weeks prior to slaughter.

## Feeding

Cows were fed once per day a diet, designated Complete Diet AA6, designed to be of constant energy and protein content between years. The diet consisted of a pelleted mixture of chopped straw,

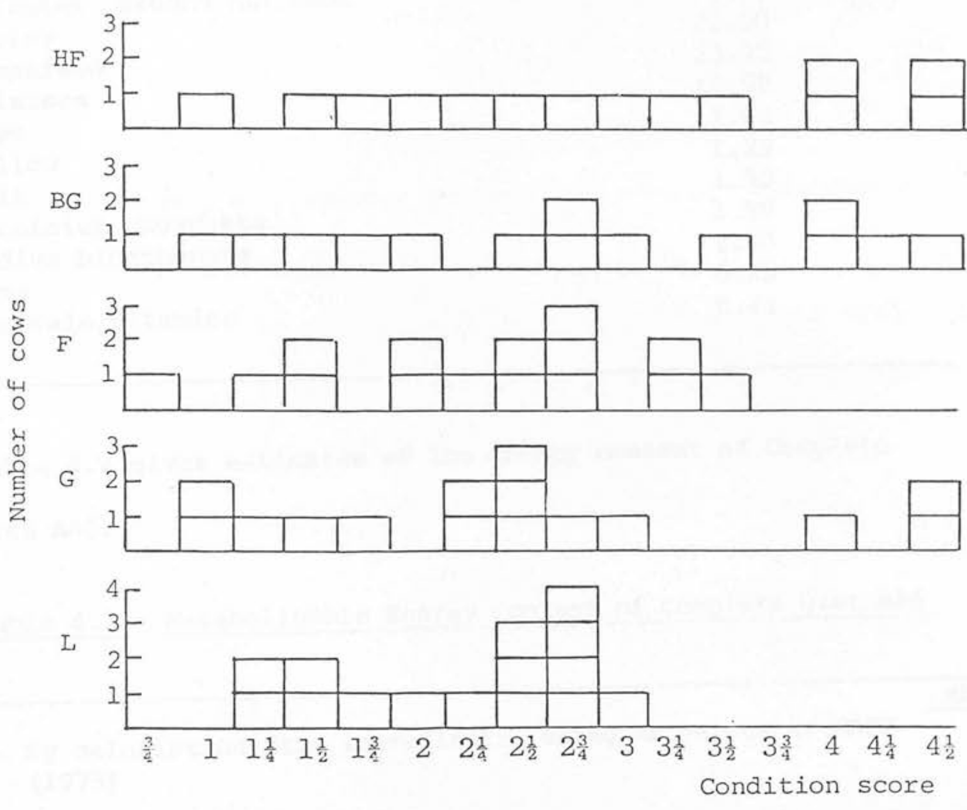



Figure 4.1. Distribution of condition score at slaughter

concentrates and molasses, and the exact composition is given in Table 4.1.

Table 4.1. Composition of Complete Diet AA6

| Component | % (fresh wt. basis) |
|---|---|
| Barley straw | 30.00 |
| Extruded ground nut meal | 7.00 |
| Barley | 22.00 |
| Wheatfeed | 23.25 |
| Molasses | 10.00 |
| Urea | 1.00 |
| Tallow | 1.25 |
| Salt | 1.50 |
| Dicalcium phosphate | 1.50 |
| Sodium bicarbonate | 2.00 |
| Lime | 0.25 |
| Minerals/Vitamins | 0.25 |

Table 4.2 gives estimates of the energy content of Complete Diet AA6.

Table 4.2. Metabolisable Energy Content of Complete Diet AA6

| | ME (MJ/kg DM) |
|---|---|
| 1. By calculation from ingredients, using ME values of MAFF (1975) | 9.70 |
| 2. From *in vitro* OMD, using ME = 0.15 DOMD (MAFF, 1975) | 9.84 |
| 3. From calorimetric measurements in sheep (Wainman, Smith and Dewey, 1975) | 9.89 |

The digestible crude protein was 11.0% on a dry matter basis, calculated from the values of the Ministry of Agriculture, Fisheries and Food (1975).

When a cow was brought in to the experiment it was allocated a "target" condition score and was subsequently fed to achieve that target. Cows which had to be reduced in condition were fed

half their estimated maintenance requirements (MAFF, 1975) while those to be fattened were fed near to their maximum dry matter intake, which was invariably close to twice maintenance requirements. If a cow reached the "target" condition score before it was possible to slaughter it, then it was fed a maintenance ration. On these diets all cows received at least their minimum requirements for digestible crude protein (Agricultural Research Council, 1965). No ration changes took place within 2 weeks of slaughter.

## LIVE ANIMAL MEASUREMENTS

Each week, on a Tuesday morning live animal measurements were made on either one or two cows. Two days later, on a Thursday morning the cows were slaughtered. These cows were fed approximately 24 hours prior to the live animal measurements being made, and were not fed again until after taking the blood samples for estimation of deuterium oxide space.

### Live weight

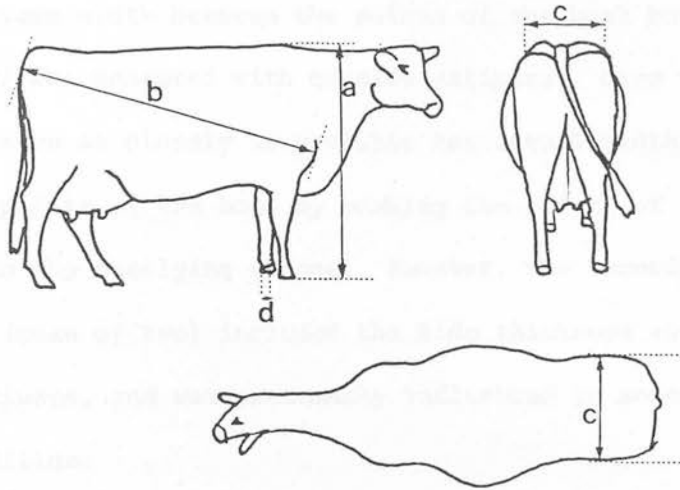
The cows were weighed in a cattle weigh crush, immediately prior to the other measurements being made.

### Skeletal size

The various measurements made of skeletal size are shown in Figure 4.2. All measurements were made when the cow was standing in a "normal" position.

#### Height at withers

The vertical distance from the ground to the highest point of the shoulder was measured using a measuring stick. To overcome the problem of any slope on the floor the height was measured twice at each side and the mean of the four measurements taken.




a  height at withers

b  length - shoulder to pin bones

c  width at hook bones

d  cannon bone circumference

Figure 4.2. Measurements of skeletal size

Length

The length from the point of the shoulder (found by palpation) to the end of the pin bones (*tuber ischii)* was measured by measuring stick, twice on each side. The four measurements were meaned.

Width at hook bones

The maximum width between the points of the hook bones (*tuber coxae)* was measured with outside calipers. Care was taken to measure as closely as possible the actual width between the points of the bone by pushing the points of the calipers into the overlying tissue. However, the recorded measurement (mean of two) included the hide thickness and some underlying tissue, and was presumably influenced to some extent by body condition.

Cannon bone circumference

The circumference of the left fore cannon bone (metacarpus) and overlying tissues was measured at its narrowest point, using a tape. The mean of two measurements was taken.

Skinfold thickness

The skinfold thickness measurement was taken on the right side over the 13th rib at a point 30 cm from the midline of the back. Skinfold callipers were used and the mean of four measurements taken.

Ultrasonic Measurements

All ultrasonic measurements were made using a Scanogram Model 722 (Ithaco Inc., Ithaca, N.Y., U.S.A.), using a TR-01 transducer, which operated at 2000kHz, and a "Type B" guide and cam stack. The instrument was set at all times to produce photographs at a scale of 2:1 (half actual size).

The machine was operated according to the manufacturer's instructions, with the exception that when scanning very thin cows the transducer was adjusted to protrude about 15 mm from its carrying block, instead of the usual 4-5 mm.

All scanning and interpretation of the photographs was carried out by one operator (the author).

Scan Sites

All scans were carried out on the right side of the animal and all sites were clipped immediately prior to scanning. Their positions are shown in Figure 4.3.

12-13th ribs - A transverse section, ventro-dorsal between ribs 12 and 13 over the position of the *M. longissimi thoracis et lumborum* (eye muscle) and spine. The scans were made upwards to allow the guide rail to fit the curvature of the cow's back. Two scans were made in this position.

3rd lumbar vertebra - A transverse section dorso-ventral over the eye muscle and spine at the position of the transverse process of the 3rd lumbar vertebra. Two scans were made.

Scapula - A section across the scapula at right angles to its spine at a point midway between the dorsal surface of the proximal extremity of the humerus and the dorsal border of the cartilage of the scapula. The scan was made either forward or backward depending on which gave the best fit with the guide tail. One scan was made in this position.

Femur - A horizontal section at right angles to the posterior surface of the leg, crossing the femur at a point midway between the hip and stifle joint. One scan was made either forward or backward.

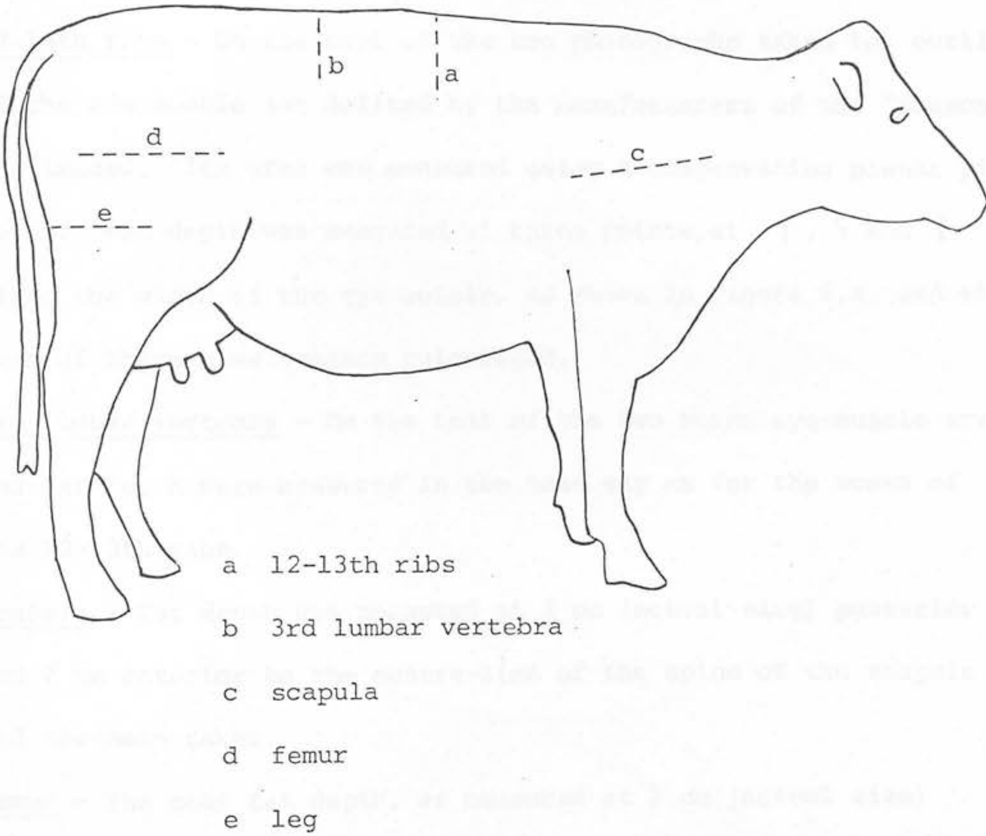



Figure 4.3. Positions of ultrasonic scans

Leg - A horizontal section on the "round" of the hind leg at a point midway between the pin bones and the hock. One scan was made, forward, starting from the centre line at the back of the leg.

Measurements on ultrasonic scans

All scans were traced on to transparent plastic film and the appropriate measurements made.

12-13th ribs - On the best of the two photographs taken the outline of the eye muscle (as defined by the manufacturers of the "Scanogram") was traced. Its area was measured using a compensating planar planimeter. Fat depth was measured at three points, at $\frac{1}{4}$, $\frac{1}{2}$ and $\frac{3}{4}$ along the width of the eye muscle, as shown in Figure 4.4, and the mean of these measurements calculated.

3rd lumbar vertebra - On the best of the two scans eye-muscle area and fat depth were measured in the same way as for the scans of the 12-13th ribs.

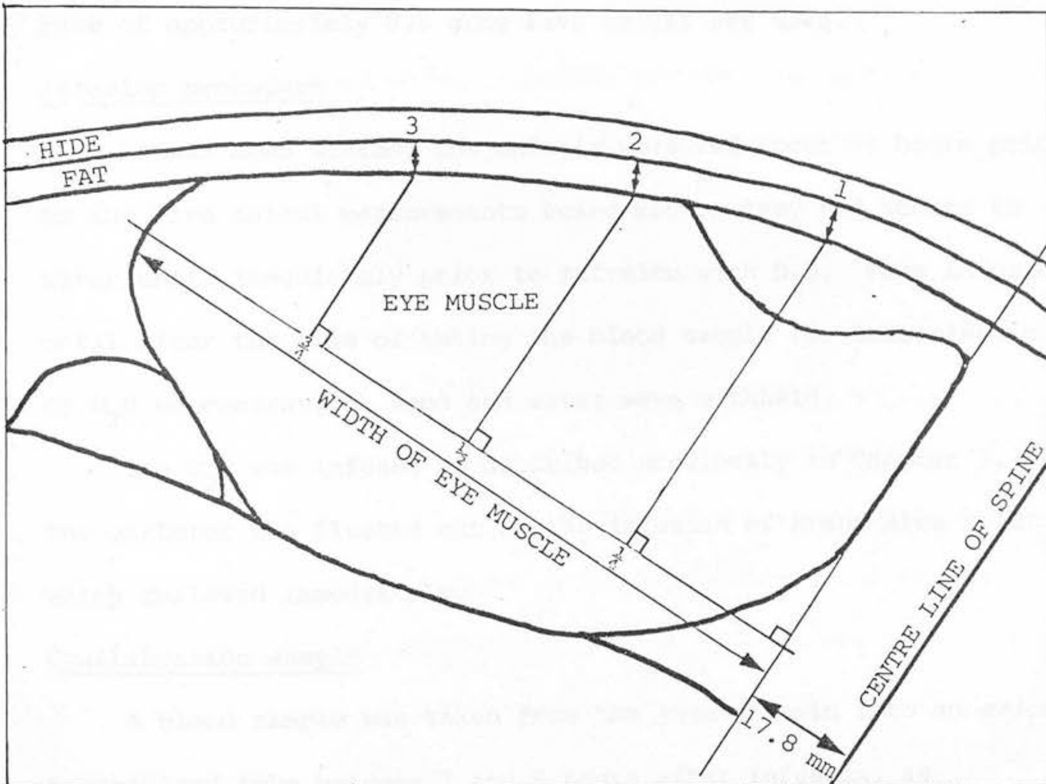
Scapula - Fat depth was measured at 2 cm (actual size) posterior and 2 cm anterior to the centre-line of the spine of the scapula, and the mean taken.

Femur - The mean fat depth, as measured at 2 cm (actual size) posterior and 2 cm anterior to the point on the hide which lay nearest the femur, was used.

Leg - Fat depth was measured at two points, 8 and 12 cm anterior to the posterior centre-line of the leg, and the mean taken.

## $D_2O$ space

Total body water was estimated by measurement of deuterium oxide ($D_2O$) space. Because of the high cost of $D_2O$ analysis the equilibration procedure was used, rather than the extrapolation method which requires analysis of a greater number of samples.




FAT DEPTH IS TAKEN AS MEAN OF MEASUREMENTS 1, 2 and 3

Figure 4.4. Measurement of ultrasonic fat depth over the eye muscle

Form of $D_2O$ infused

The deuterium oxide was supplied by the U.K. Atomic Energy Authority, Winfrith, Dorset and was 99.8% pure. Before infusion it was salinated to a concentration of 9 g/l with sodium chloride.

Dose

To allow a reproducability of 1% in the analysis of $D_2O$ it was necessary to obtain a concentration of $D_2O$ in the body water of about four times the natural abundance. Thus a dose rate of approximately 0.5 g/kg live weight was used.

Infusion procedure

As has been stated, the animals were fed about 24 hours prior to the live animal measurements being made. They had access to water until immediately prior to infusion with $D_2O$. From infusion until after the time of taking the blood sample for determination of $D_2O$ concentration, food and water were withheld.

The $D_2O$ was infused as described previously in Chapter 3, and the catheter was flushed out by the infusion of Evans Blue solution which followed immediately.

Equilibration sample

A blood sample was taken from the jugular vein into an evacuated heparinised tube between 7 and 8 hours after infusion, as indicated by the results of the preliminary study (Chapter 3). The blood was centrifuged for 20 minutes at a force of 2950 g and the plasma removed and stored at -20°C in polystyrene vials.

Analysis of $D_2O$

$D_2O$ analysis was carried out by mass spectrometery at Heriot Watt University, Edinburgh.

Calculation of $D_2O$ space

$D_2O$ space was calculated from the following equation:-

$$D_2O \text{ space (kg)} = \frac{\text{Weight } D_2O \text{ infused (kg)}}{\left(\frac{D_2O}{H_2O} - 0.00015\right) \times \frac{20}{18}}$$

where $\frac{D_2O}{H_2O}$ is the molecular ratio of $D_2O:H_2O$ in the body water at equilibration.

Blood/Plasma volume

Blood, red cell and plasma volumes were estimated using the extrapolation procedure with the dye Evans Blue (T 1824) and the haematocrit (packed cell volume).

Prior to infusion a blood sample was taken to provide blank plasma for the preparation of standards for the Evans Blue analysis.

Infusion

Approximately 30 ml of a filtered 10 g/l solution of Evans Blue made up in physiological saline was infused over a 10 second period from a syringe via the same catheter as used for $D_2O$ infusion. The time of infusion was noted as the time when half of the solution had been infused. Immediately afterwards the catheter was flushed with about 20 ml of physiological saline. The exact quantity of Evans Blue solution infused was determined by weighing the syringe before and after infusion.

Blood sampling

After allowing 10 minutes for the Evans Blue to mix with the bloodstream, 5 blood samples were taken from the opposite jugular vein at approximately 5-minute intervals, the exact times being noted.

Haematocrit

After thorough mixing of the blood, 3 graduated Wintrobe tubes were filled with blood and centrifuged for 45 minutes at 2950 g.

The ratio of red blood cell volume to total blood volume was read off the tubes directly and the mean of the three taken.

The haematocrit of the last 21 cows to be slaughtered was also measured, in duplicate, using a microhaematocrit centrifuge (Gelman Hawksley Ltd., Lancing, Sussex, U.K.) at a force of 12,000 g for 6 minutes. The relationship of the haematocrit from the Wintrobe (W, %) tubes and the microhaematocrit (M, %) was established from a regression equation, and this used to correct the Wintrobe haematocrit. The equation was as follows:-

$$M = 0.75(\pm 0.057)W + 5.76(\pm 2.689)$$

$$(n = 21, r^2 = 0.898, \text{residual s.d.} = 1.33)$$

Analysis of Evans Blue

The blood samples were centrifuged at 2950 g for 45 minutes (twenty minutes would have been sufficient, but the haematocrit was determined at the same time). The plasma was removed and the concentration of Evans Blue estimated by a modification of the method of Chinard (1951).

All analysis was carried out in duplicate. Standard solutions and the unknown samples were prepared in test tubes as described in Table 4.3.

Table 4.3. Preparation of standards and unknowns for Evans Blue analysis

All quantities in ml unless otherwise stated

| | Blank Plasma | Unknown Plasma | Evans Blue 0.1g/l soln. | Saline 9g/l soln. | Conc. of Evans Blue (mg/l) |
|---|---|---|---|---|---|
| Standard 1 | 2.0 | 0.0 | 0.0 | 0.4 | 0.000 |
| Standard 2 | 2.0 | 0.0 | 0.2 | 0.2 | 8.333 |
| Standard 3 | 2.0 | 0.0 | 0.4 | 0.0 | 16.666 |
| Unknowns | 0.0 | 2.0 | 0.0 | 0.4 | unknown |

The samples were mixed and immediately following the addition of 6 ml of acetone the test tubes were capped. After vigorous shaking for at least 10 seconds the tubes were centrifuged at 2000 g for 10 minutes to remove the precipitated protein. The supernatant containing the dye in solution was aspirated and the absorbance measured immediately at a wavelength of 620 nm in either a Unicam SP1800 or SP6-500 Ultraviolet Spectrophotometer. The concentration of Evans Blue in the post-infusion samples was calculated using the regression of concentration on absorbance derived from the standards.

Calculation of blood compartment volumes

The concentration (mg/l) of Evans Blue in the plasma was regressed on time after infusions (minutes) and the intercept of the regression taken as representing the theoretical concentration at the time of infusion (see page13 ). This concentration was used in the calculation of plasma volume. Strictly, the log of the dye concentration should have been used in the regression, but because of the short sampling time (30 minutes) there was no statistical advantage in doing this.

Plasma volume was calculated as:-

$$\text{Plasma Volume (l)} = \frac{\text{Weight of dye infused (mg)}}{\text{Concentration in plasma (mg/l)}}$$

Blood volume and red cell volume were calculated from plasma volume and the haematocrit measurement.

$$\text{Blood Volume (l)} = \frac{\text{Plasma volume (l)}}{1 - \text{Haematocrit*}}$$

$$\text{Red Cell Volume (l)} = \frac{\text{Haematocrit x plasma volume}}{1 - \text{Haematocrit*}}$$

*Haematocrit expressed as a proportion of 1.

Body condition score

The cows were condition scored to the nearest $\frac{1}{4}$ score using the system of Lowman *et al.* (1976) (see Appendix I). All condition scoring was all carried out by the author.

## SLAUGHTER AND POST-SLAUGHTER TREATMENT

### Slaughter procedure

In the afternoon of the day before slaughter the cows were transported to a commercial abattoir. In lairage they had access to water, but no food. They were slaughtered early the next morning, just under 48 hours after the live animal measurements had been made.

The animals were killed by captive bolt and then bled by severing the jugular vein. The blood was collected and a sample taken in a heparinised container for subsequent analysis. Because the animals were lying on the floor at the time of bleeding and not hanging, the efficiency of bleeding was low and the quantitative collection of blood was difficult.

The carcass was dressed and split according to normal commercial practice, with the exception that the kidneys and perirenal fat were removed and the left side of the udder left attached to the carcass to facilitate the removal of the thin flank joint in a standard manner (see below). All non-carcass components were collected and brought back to the Carcass Evaluation Unit, Bush Estate. The carcass was weighed warm and then left at the slaughterhouse for approximately 24 hours before being brought back to the Carcass Evaluation Unit.

### Treatment of non-carcass components

The fatty tissue surrounding the alimentary tract was removed with associated ligaments and connective tissue (omental and mesenteric

fatty tissue) and the perirenal fatty tissue was separated from the kidneys. The weight of gut contents was obtained by weighing the gut full and then empty. After mixing and taking a sample for dry matter determination the gut contents were discarded. The non-carcass tissues were weighed according to the following classifications:-

1. Blood
2. Omental and mesenteric fatty tissue
3. Perirenal fatty tissue
4. Remainder - head
   pluck
   liver
   gall bladder
   empty alimentary tract
   empty urinogenital tract
   tail
   feet
   hide
   kidneys
   right side of udder (the left side was added at a later stage)

After weighing, all components were stored at -20°C in polythene bags with the exception of the blood, which was discarded.

Because of the inefficiency of bleeding and the difficulty of quantitative collection, the weight of blood obtained was increased by half.

## Dissection of carcass

Approximately 24 hours after slaughter the two sides of the carcass were weighed, and the left side quartered between ribs 10 and 11 and brought back to the Carcass Evaluation Unit to be stored at 4°C for 4 days. Four days after slaughter the left side was dissected. It was weighed before dissection and then cut into wholesale joints to ease handling. The side, apart from the thin flank joint, was dissected into:

1. Subcutaneous fatty tissue
2. Bone
3. Muscle and associated fatty tissue

All bones were scraped free of adhering tissue. The periostium was removed where this came away easily but, because of the age of the animals, this was firmly attached to the bone in most cases. The *ligamentum nuchae* was included with the bone. All tissues were weighed following dissection.

Eye Muscle

The part of the *M. longissimi thoracis et lumborum* (eye muscle) between ribs 6 and 10 was removed completely free of adhering intermuscular fatty tissue and weighed. This was done so that an estimate of intramuscular fat could be obtained by chemical analysis.

Thin Flank

In an attempt to assess the amount of intermuscular fat, the thin flank joint was dissected more fully. It was removed according to the method of Kempster, Cook and Smith (1980), and then weighed and dissected into:-

1. Subcutaneous fatty tissue
2. Intermuscular fatty tissue
3. Thick connective tissue sheath
4. Muscle
5. Bone

All dissected tissues were weighed.

## Mincing of tissues

Tissues were either minced fresh or frozen according to the following scheme:

Carcass components - subcutaneous fatty tissue
muscle and associated fatty tissue
bone
thin flank subcutaneous fatty tissue
thin flank intermuscular fatty tissue
and thick connective tissue sheath
thin flank muscle
thin flank bone
part of eye-muscle

Non-carcass components - perirenal fatty tissue
omental and mesenteric fatty tissue
remainder

The subcutaneous fatty tissue, soft tissues from the thin flank, perirenal fatty tissue, omental and mesenteric fatty tissue, and part of eye muscle were each cut up and minced separately, firstly through an 8 mm plate, then mixed and minced again through a 3 mm plate. After mixing, a sample of approximately 1 kg was taken. The muscle samples were analysed within 24 hours, but the fatty tissue samples were stored at -20°C to await analysis.

The muscle and associated fatty tissue was cut up and stored for approximately 24 hours at 4°C before being weighed again and minced twice through a 3 mm plate with a thorough mixing between mincings. Throughout the second mincing nine samples of approximately 0.5 kg were taken and bulked in threes to form 3 samples of 1.5 kg. These were analysed within 24 hours.

The remainders were removed from the -20°C store about 24 hours prior to processing and so were still partly frozen during processing. They were weighed and ground in a prebreaker (Henry Balfour & Co. Ltd., Leven, Fife, Scotland) which rendered the material into particles ranging in size from a few mm to 3 cm across. The material was then mixed and minced twice through a Wolfking mincer, fitted with a 10 mm plate and a 5 mm plate, with mixing taking place between mincings. Three samples of approximately 1 kg were made up by taking small quantities of minced material throughout the second mincing.

The bones from the carcass were ground in the prebreaker while frozen. The material was mixed and about 3 kg removed and minced once in the Wolfking mincer. A sample of about 1 kg was taken.

The bones from the thin flank were partially ground in the

Wolfking mincer, but because of the small quantity (300-400 g) there was not enough to be forced through the plates of the mincer. The semi-ground material was then freeze-dried and milled through a 2 mm sieve.

## ANALYSIS OF TISSUES

The samples from all components were analysed for water, fat, ash and protein. These components were:

| | |
|---|---|
| Non carcass | - blood<br>perirenal fatty tissue<br>omental and mesenteric fatty tissue<br>remainder (3 samples) |
| Carcass (excluding thin flank) | - muscle and associated fatty tissue (3 samples)<br>subcutaneous fatty tissue<br>bone<br>part of eye muscle |
| Thin Flank | - muscle<br>intermuscular fatty tissue<br>subcutaneous fatty tissue<br>bone |

Except for the thin flank bones and the blood, all tissues were analysed similarly.

### Water

Accurately weighed, triplicate samples (each of approximately 10 g) of minced tissue were dried to constant weight, in aluminium cups, at 105$^{\circ}$C. Moisture content was calculated from the weight loss.

### Fat

The dried material from the two moisture determinations that agreed most closely was then crushed in the aluminium cups which were subsequently perforated and folded and Soxhlet-extracted with 60-80$^{\circ}$C petroleum ether for 8 hours. Fat contents were estimated by weight differences of Soxhlet flasks after evaporation of the

petroleum ether.

Ash

Accurately weighed duplicate samples (each of approximately 10 g) of minced tissue were ashed in a muffle furnace at 600°C to constant weight.

Protein

The nitrogen contents of the various minced samples were to be determined by a macro Kjeldahl procedure and crude protein calculated as 6.25 x nitrogen. However the sums of the percentages of water, fat, ash and protein were for many samples, markedly different from 100%. Repeat analysis for a number of samples suggested that the repeatability of the nitrogen analysis was poor, and so the percentage of protein in the samples was calculated as:-

% protein = 100 - (% water + % fat + % ash)

The protein fraction is in fact the fat-free organic matter, but is referred to as protein throughout this thesis.

Thin flank bones

The moisture content of the thin flank bones was determined by freeze-drying. Analyses of fat and ash were carried out on the dried, milled material, and protein calculated by difference.

Blood

The blood samples taken from the cows of each breed with the highest and lowest condition score were analysed. The moisture contents were estimated by freeze drying and the dried material was analysed for fat and ash, and protein calculated by difference. Table 4.4 gives the composition of these samples.

Table 4.4. Composition of cow blood

| Component | Composition (%, ± se) |
|---|---|
| Water | 79.92 (± 0.335) |
| Fat | 0.04 (± 0.006) |
| Ash | 0.57 (± 0.058) |
| Protein | 19.47 (± 0.337) |

As indicated by the standard errors the variation in composition is very small and probably most of this is due to analytical variation. It was therefore decided to use the figures of Table 4.4 for the composition of the blood of all the cows.

### Gut contents

Samples of gut contents of approximately 100 g were freeze dried to constant weight and the water content calculated from the weight loss.

### Calculation of whole body composition

The body composition of each cow was calculated. The quantities of water, fat, protein and ash in each of the tissues listed on page 99 were calculated from the weights of the tissues and the results of the chemical analysis.

All weight losses between slaughter and analysis were assumed to be water and compositional data for each tissue were adjusted accordingly. The weight loss from the carcass between slaughter and dissection, and during dissection was assumed to be water from muscle.

Dissection data on the left side of the carcass were adjusted for weight differences between the two sides. As there was no method

of quantifying the composition of the weight difference it was assumed that the total weight difference between the two sides was made up of differences of the physically separable components in the same proportion as in the left side.

The change in empty body weight and composition between the time of the live animal measurements and slaughter was assumed to be negligible. The difference in live weight, as measured two days prior to slaughter, and the sum of the weights of all the tissues after slaughter (including gut contents) was assumed to represent a loss of weight of gut contents. The water content of the gut contents was assumed to be the same at the time of the live animal measurements and at the time of slaughter.

Summation of the quantities of the separate chemical components in the tissues of the empty body gave the composition of the empty body. Total body water was calculated by the addition of the water in the gut contents to the empty body water. All data therefore refer to the cows at the time of the live animal measurements, i.e. two days prior to slaughter.

## STATISTICAL ANALYSES

### Computing

All statistical analyses were carried out using the statistical computer program Genstat, Version 4.03 (Rothamsted Experimental Station, 1977) as implemented by the Edinburgh Regional Computing Centre on an ICL 2980 computer.

### Testing for breed differences in regression equations

Most analyses were carried out using standard regression techniques. When a regression equation was being developed separate equations were initially calculated for each of the five breeds.

These equations were then tested individually against each other for differences ($p < 0.05$) in regression coefficients (or partial regression coefficients in the case of multiple regression) For pairs of breeds which showed no difference in regression coefficients, a common slope and separate breed intercepts were fitted to the data for the two breeds, and the intercepts tested for difference ($P < 0.05$). This procedure for testing for breed differences in both slope and intercept was considered more sensitive than a simple comparison of the residual mean squares for the combined regression for all breeds against that for the separate regressions fitted for each breed.

As will be demonstrated later the upper limit of most of the data for the L cows was lower than that for the other breeds. For this reason if significant differences were found between regression equations for the L breed and other breeds the analysis was repeated but with the range of data of the second breed restricted to that of the L cows.

Presentation of regression equations

Where the regression coefficients of any two breeds differed significantly the equations for each breed are presented together with the within breed $r^2$ (or $R^2$) and residual standard deviation (s.d.). In some cases the $r^2$ (or $R^2$) and residual s.d. are also given "pooled within breed". These are calculated from the total sums of squares of the dependent variable, the sums of the residual degrees of freedom and sums of squares of the five separate breed equations. If no breeds differed in regression coefficient, but at least one breed pair showed a statistically significant difference in intercept when a common slope was fitted to the data for these two breeds, then the regression

equation is presented with a common slope, but separate intercepts for each of the five breeds. In this case the $r^2$ (or $R^2$) and residual s.d. are given for the data "pooled with separate intercepts". These were calculated when a single regression model with a pseudovariate for breed was fitted. Finally, if there were no breed differences in either coefficients or intercepts, the data for all breeds were combined and an overall regression equation calculated and presented with its appropriate $r^2$ (or $R^2$) and residual s.d. These are referred to as pooled for all breeds.

Where breed differences were demonstrated these are indicated by listing the abbreviations for each breed and underscoring with a solid line those breeds which did not differ significantly in regression coefficients. Within each group of breeds which did not differ in regression coefficient, breeds showing no significant difference in intercept when a common slope was fitted to the data for each breed pair are underscored with a broken line. Thus differences in intercept between two breeds refer to those intercepts calculated when a common slope was fitted to the data for both breeds. All differences indicated are significant at the 5% level at least. The following will serve as an example:-

```
HF      G      BG      L      F
_________________
---------
        --------

        ___________________________
        ---------------------------
```

The configuration above indicates that the regression coefficient for the HF cows differed significantly from that of the L and F cows and that when a common slope was fitted for each breed pair that did not differ significantly in slope, the HF and BG breeds showed differences in intercept.

# CHAPTER 5

# BODY WEIGHT, SIZE AND COMPOSITION

## FORM OF PRESENTATION OF RESULTS

In this and subsequent chapters only summaries of the data are presented. Details of all weights, composition and measurements made on the live animalsare given in full in the tables of Appendix II. The numbers of animals of each breed were as follows:-

| | |
|---|---|
| HF | 14 |
| BG | 14 |
| F | 15 |
| G | 15 |
| L | 15 |
| Total | 73 |

Where relationships are presented (e.g. regression equations relating different body components or equations for the prediction of body composition) the number of animals used in the derivation of such relationships is given. In some cases these are lower than the numbers indicated above because some data values were missing and these are indicated in the tables of Appendix II.

## RESULTS

### Chemical composition of the body

A summary of the weights, condition scores and the composition of the cows is given in Table 5.1. The range in all variables is considerable and it can be seen that the manipulation of body condition score was successful in extending the range of live weight and fatness. The component with the largest range relative to the mean value is fat. It is of interest to note that, despite the fact that the highest condition score for the F cows was 3.5, the range of fatness is similar to the other breeds. The reason for this will be discussed later in Chapter 7. The ranges for all variables for the L cows were

Table 5.1. Summary of weights, condition scores and composition of cows at slaughter. (Values are means and ranges).

| Breed | n | Live weight (kg) | Empty body weight (kg) | Condition score | Components of empty body (% of empty body) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Water | Fat | Ash | Protein |
| HF | 14 | 542 | 458 | 2.89 | 55.6 | 22.2 | 4.8 | 17.6 |
| | | 350-710 | 283-623 | 1.00-4.50 | 45.2-63.6 | 9.3-36.6 | 3.6-7.0 | 14.9-20.9 |
| BG | 14 | 512 | 437 | 2.77 | 52.5 | 26.4 | 4.3 | 16.9 |
| | | 370-720 | 320-639 | 1.00-4.50 | 41.1-61.5 | 13.9-43.0 | 3.3-5.6 | 12.6-19.5 |
| F | 15 | 560 | 476 | 2.30 | 54.4 | 23.8 | 4.6 | 17.2 |
| | | 417-720 | 326-636 | 0.75-3.50 | 43.7-68.3 | 4.7-37.6 | 3.3-5.9 | 14.0-21.1 |
| G | 15 | 471 | 394 | 2.48 | 56.2 | 21.9 | 4.4 | 17.6 |
| | | 365-630 | 261-564 | 1.00-4.50 | 44.4-68.0 | 6.6-37.6 | 3.4-6.1 | 14.2-21.3 |
| L | 15 | 434 | 365 | 2.19 | 57.0 | 20.4 | 4.6 | 18.1 |
| | | 320-565 | 265-489 | 1.25-3.00 | 46.8-68.1 | 5.5-31.1 | 4.0-6.2 | 16.6-20.5 |

smaller because no cows of this breed were slaughtered at a condition score above 3.

## Relationships among body components

### Composition of the fat-free body

The percentage composition of the fat-free body was calculated for each cow and mean values are presented in Table 5.2.

Table 5.2. Percentage composition of the fat-free body

| | Water | Protein | Ash |
|---|---|---|---|
| Mean | 71.47 | 22.67 | 5.85 |
| Standard deviation | 0.901 | 0.672 | 0.564 |
| Coefficient of variation (%) | 1.26 | 2.96 | 9.64 |

To check whether the composition of the fat-free body was affected by the level of fatness, the percentages of water (W), protein (P) and ash (A) in the fat-free body were regressed in turn on the percentage of fat (F) in the empty body weight. The relationship between W and F was not significant ($P > 0.05$) but those between P and F and A and F were, the correlation coefficients being 0.328 and 0.299 respectively. These latter two regression equations, relating P and A to F were:-

$$P = 22.10\,(\pm 0.208) + 0.024(\pm 0.0085)F$$

$$(n = 73,\ r^2 = 0.108^{***},\ \text{residual s.d.} = 0.642)$$

$$A = 6.28(\pm 0.175) - 0.018(\pm 0.0072)F$$

$$(n = 73,\ r^2 = 0.089^{**},\ \text{residual s.d.} = 0.542)$$

### Relationship of body fat and body water

Body fat (F) and empty body water (W), both expressed as a percentage of empty body weight, were highly negatively correlated,

giving the following equation, which is shown in Figure 5.1:-

$$F = 98.29(\pm 1.012) - 1.36(\pm 0.018)W$$

$$(n = 73,\ r^2 = 0.987^{***},\ \text{residual s.d.} = 1.003)$$

There were no differences between the breeds and no evidence of curvilinearity in the relationship.

Relationship of live weight to condition score

The regressions of live weight on condition score are given in Table 5.3. The regression coefficients give a change in live weight associated with one unit change in condition score of 61, 97, 104, 106 and 110 kg for the G, BG, HF, L and F breeds respectively. However, only the G cows differed significantly from the other breeds.

Relationship of empty body weight to live weight

The relationship between empty body weight (BW, kg) and live weight (LW, kg) was established by regression to be:-

$$LW = -43.1(\pm 8.08) + 0.931(\pm 0.0157)LW$$

$$(n = 73,\ r^2 = 0.980,\ \text{residual s.d.} = 14.5)$$

No differences between breeds were apparent. The regression coefficient of 0.931 means that for each kg change in live weight, the corresponding change in empty body weight is 0.931 kg.

Body size

The product of height, length and width was chosen as a measure of body size, and a summary for each breed is shown in Table 5.4.

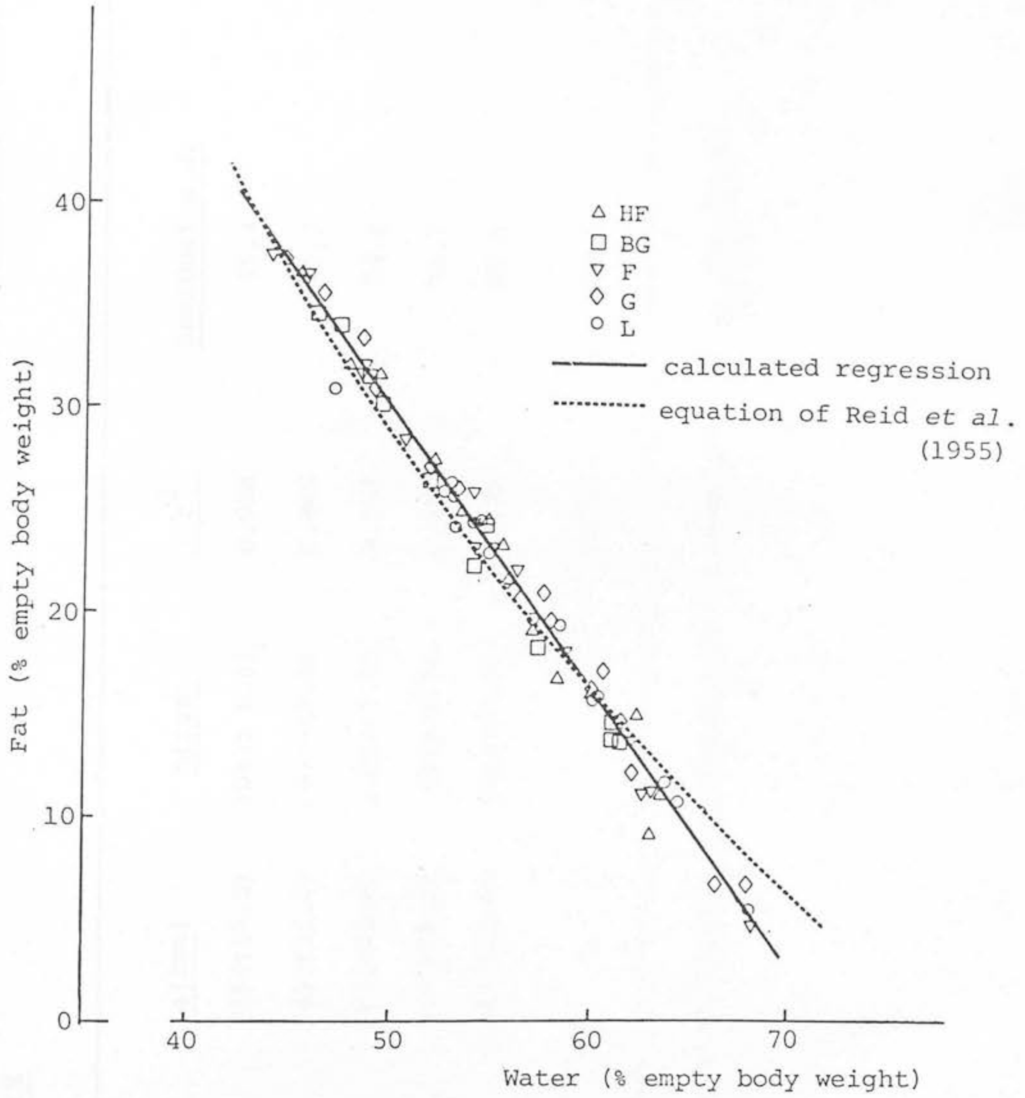



Figure 5.1. Relationship between proportions of fat and water in the empty body

Table 5.3. Regression of live weight (LW, kg) on body condition score (CS) using the model $LW = a + bCS$

| Breed | n | a(±se) | b(±se) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| HF | 14 | 239(±19.5) | 104(± 6.3) | 0.958 | 25.4 |
| BG | 14 | 243(±32.4) | 97(±10.8) | 0.869 | 45.3 |
| F | 15 | 305(±42.6) | 110(±17.5) | 0.753 | 52.2 |
| G | 15 | 319(±26.3) | 61(± 9.7) | 0.753 | 41.7 |
| L | 15 | 200(±40.6) | 106(±17.9) | 0.731 | 41.8 |
| Pooled within breed | 73 | - | - | 0.867 | 42.4 |

Differences in coefficients and intercepts (see page 103 for explanation) G L BG F HF

Table 5.4. Summary of body size

| Breed | n | Body size ($m^3$) Mean | Range |
|---|---|---|---|
| HF | 14 | 1.063 | 0.908 - 1.301 |
| BG | 14 | 1.017 | 0.799 - 1.188 |
| F | 15 | 1.140 | 0.975 - 1.294 |
| G | 14 | 0.909 | 0.827 - 1.044 |
| L | 15 | 0.923 | 0.821 - 1.065 |

Body size, as defined above, was not independent of condition, the partial correlation coefficient between body size and the proportion of fat in the empty body (independent of breed) being 0.393. The regression of body size on fatness is shown in Table 5.5.

Table 5.5. Regression of body size (S, $m^3$) on percentage of fat in the empty body (F) using the model $S = a + bF$

| Breed | n | a(±se) | b(±se) | $r^2$ † | residual s.d. † |
|---|---|---|---|---|---|
| HF | 14 | 0.954(±0.0294) | $4.87\times10^{-3}$ ($\pm9.89\times10^{-4}$) | 0.660 | 0.073 |
| BG | 14 | 0.888(±0.0325) | | | |
| F | 15 | 1.023(±0.0301) | | | |
| G | 14 | 0.802(±0.0291) | | | |
| L | 15 | 0.824(±0.0276) | | | |

† $r^2$ and residual s.d. are pooled with separate breed intercepts.

Differences in intercepts (see page 103 for explanation) F HF BG L G -----

The values of the regression constants in Table 5.5 indicate the relative sizes of the five breeds, being ranked G, L, BG, HF and F

in increasing order of size.

## DISCUSSION

### Relationships among body components

#### Composition of the fat-free body

The composition of the fat-free body (Table 5.2) agrees very closely with the figures presented by Reid *et al.* (1968) shown in Table 2.1. The coefficients of variation (CV) for the components of the fat-free body are lower in this study than those of Reid *et al.* (1968) as shown:-

| | Coefficients of Variation | |
|---|---|---|
| | Reid *et al.* (1968) | This experiment |
| Water | 2.75 | 1.26 |
| Protein | 7.07 | 2.96 |
| Ash | 17.79 | 9.64 |

This is perhaps not surprising, since the animals in the present study were all mature and the analysis was carried out in one laboratory, whereas the data of Reid *et al.* (1968) were derived from animals ranging in age from 1 to 4860 days, and it was shown that age affected the composition of the fat-free body. In addition, the data were derived from a wide range of sources and thus the analyses were carried out at many times and locations.

Lohman (1971) in his review of biological variation in body composition came to the conclusion that the CV of water in the fat-free body was probably less than 2% for animals that had completed more than 4% of their life expectancy. The figure of 1.26% above is certainly less than 2% and includes technical variation, so that the true biological variation in the water content of the fat-free bodies of mature cows is probably less than 1.26%.

Level of fatness appears to affect the composition of the fat-free body in that as cows increase in fatness there is a tendency for the proportions of protein and ash to increase and decrease respectively. However, the correlation coefficients between protein and ash (as a percentage of the fat-free body) and fatness (fat as a percentage of empty body weight), 0.328 and 0.299 respectively, are not particularly high.

Despite the slight variations in the composition of the fat-free body and the changes associated with fatness, as indicated by the above analyses, the general concept of constancy of composition of the fat-free body is upheld, as the deviations are relatively small.

Relationship of body fat and body water

Reid *et al.* (1955) found a slightly curvilinear relationship between fat and water contents of the empty bodies (expressed as percentages of empty body weight) of 256 cattle. No such curvilinearity could be demonstrated in this study, but the numbers of animals were smaller and all were mature. Nevertheless, the agreement between the curvilinear equation of Reid *et al.* (1955) and the linear regression equation derived from the present study is good, as indicated in Figure 5.1, and demonstrates the validity of predicting the proportion of fat in the body from the proportion of water, at least in the type of animals used in this experiment.

Change in live weight per unit change in condition score

The ranking of the live-weight change associated with one unit change in condition score is similar to the ranking for body size, apart from the L breed. For this breed the live-weight change seems to be larger than the body size warrants but the standard errors of these live-weight changes are relatively high (Table 5.3).

The coefficients in Table 5.3 are very similar to those of J.N. Peart (unpublished) who recorded values of 90 and 83 kg for HF and BG cows respectively (Table 2.4). Other values in Table 2.4 are all lower than this. However, if one considers that the condition score scale effectively extends from 1 to 5 (since scores below 1 should rarely be encountered in healthy animals) then the range is 4 units. The live weights of the four breeds which showed no significant differences in regression coefficient in Table 5.2 extended from 320 to 720 kg (Table 5.1), a range of 400 kg, indicating that if the condition score scale is to cover the range of all possible live weights, then each unit change must be associated with about 100 kg live-weight change. The coefficients for the HF, BG, F and L breeds are similar to this. The figures that are lower than this in Table 2.4 clearly indicate that the scale used by these operators cannot cover the range of possible live weights, and that there is a need for the standardisation of scales and scores.

Relationship of empty body weight to live weight

ARC (1980) suggested that for animals on mixed diets live weight (LW, kg) could be related to empty body weight (BW, kg) by:-

$$LW = 1.09BW + 15$$

Thus empty body weight can be estimated from live weight as:-

$$BW = 0.917LW - 14$$

From this study the coefficient equivalent to that above is 0.931(± 0.0157), and the two do not differ significantly. For the range in live weight 400-700 kg the equation from this study predicts empty body weight to be some 20 kg less than the equations from ARC (1980). However the ARC (1980) predictions probably lie within the confidence interval for predictions from the equation derived in this study, since the residual s.d. was 14.5 kg.

Body size

Any measurement of body size should ideally be independent of body condition. However in practice this is difficult to achieve, because when taking linear measurements on the bodies of cows the measurements are invariably affected by the amount of tissue cover on the selected reference points. Nevertheless the index of size chosen, namely the product of height, length and width, was effective in ranking the breeds in the order of size that was consistent with visual assessment.

# CHAPTER 6

# EMPTY BODY-WEIGHT CHANGE

## RESULTS

The relationships between the four chemical components (water, fat, ash and protein) of the empty bodies, and empty body weight allowed estimates of the composition of empty body-weight change to be made, and the energy content of empty body weight change to be calculated using standard calorific values for body fat and protein.

For each breed the weights of empty body water, fat, ash and protein were regressed, in turn, on empty body weight. Each relationship was tested for curvilinearity by fitting a quadratic model ($y = a + b_1x + b_2x^2$). The quadratic terms were not significant ($P > 0.05$), but tended to be negative in the case of water and protein, and positive in the case of fat. Ash showed no such tendency, being positive for three breeds and negative for the other two. It was considered that the inclusion of an index of body size in the models might reduce the residual variation and allow the quadratic terms to attain statistical significance. The product of height, length and width was accordingly used as an index of body size and included as a covariate. In only one model, that for body water in the L cows was the quadratic term significant, but the appropriate statistical tests indicated that the data for all five breeds could be pooled, using common partial regression coefficients and separate intercepts. These equations are shown in Table 6.1. The partial regression coefficients for body size proved to be significant ($P < 0.05$) in all four models, and the quadratic terms were significant for all equations, except that involving ash. Even although this parameter was not significant it was retained in the model, as it was thought likely that the non-significance was simply a reflection of the greater residual variation in this model (see $r^2$ values in Table 6.1).

Table 6.1. Regressions relating empty body water (W, kg), fat (F, kg), ash (A, kg) and protein (P, kg) to empty body weight (BW, kg) and body size (S, $m^3$) using the models W, F, A or P = $a + b_1BW + b_2BW^2 + b_3S$.

| Body Component | n | a(±se) | | $b_1$(±se) | $b_2$(±se) | $b_3$(±se) | $r^2$ ‡ | Residual s.d.‡ |
|---|---|---|---|---|---|---|---|---|
| W | 72 | 3.7(±41.95) | (HF) | 0.54(±0.132) | $-3.3x10^{-4}$ ($\pm 1.51x10^{-4}$) | 66(±29.0) | 0.873 | 13.4 |
| | | -13.5(±41.11) | (BG) | | | | | |
| | | -2.7(±43.42) | (F) | | | | | |
| | | -4.3(±39.47) | (G) | | | | | |
| | | -8.3(±39.85) | (L) | | | | | |
| F | 72 | -1.7(±50.6) | (HF) | 0.22(±0.159) | $4.9x10^{-4}$ ($\pm 1.82x10^{-4}$) | -93(±35.0) | 0.938 | 16.2 |
| | | 21.9(±49.6) | (BG) | | | | | |
| | | 6.8(±52.4) | (F) | | | | | |
| | | 11.4(±47.6) | (G) | | | | | |
| | | 15.3(±48.1) | (L) | | | | | |
| A | 72 | 0.7(± 4.70) | (HF) | 0.03(±0.014) | $-2x10^{-5}$ ($\pm 1.6x10^{-5}$) | 11(± 3.2) | 0.773 | 1.5 |
| | | -1.7(± 4.60) | (BG) | | | | | |
| | | -0.4(± 4.86) | (F) | | | | | |
| | | -1.5(± 4.42) | (G) | | | | | |
| | | -1.5(± 4.46) | (L) | | | | | |
| P | 72 | -1.8(± 9.38) | (HF) | 0.21(±0.029) | $-1.4x10^{-4}$ ($\pm 3.4x10^{-5}$) | 16(± 6.5) | 0.930 | 3.0 |
| | | -6.4(± 9.18) | (BG) | | | | | |
| | | -3.4(± 9.70) | (F) | | | | | |
| | | -5.2(± 8.82) | (G) | | | | | |
| | | -5.2(± 8.91) | (L) | | | | | |

‡ $R^2$ and residual s.d. values are for pooled data with separate intercepts.

Table 6.2. Regression of body size ($S$, $m^3$) on empty body weight ($BW$, kg) using the model $S = a + b_1 BW + b_2 BW^2$.

| Breed | n | a(±se) | $b_1$(±se) | $b_2$(±se) | $r^2$ † | Residual s.d. † |
|---|---|---|---|---|---|---|
| HF | 14 | 1.051(±0.1232) | $-6.730 \times 10^{-4}$ ($\pm 5.594 \times 10^{-4}$) | $1.45 \times 10^{-6}$ ($\pm 6.188 \times 10^{-7}$) | 0.793 | $5.732 \times 10^{-2}$ |
| BG | 14 | 1.017(±0.1223) | | | | |
| F | 15 | 1.117(±0.1236) | | | | |
| G | 14 | 0.938(±0.1222) | | | | |
| L | 15 | 0.968(±0.1209) | | | | |

† $r^2$ and residual s.d. values are for pooled data with separate intercepts.

Table 6.3. Equations relating empty body water (W, kg), fat (F, kg), ash (A, kg) and protein (P, kg) to empty body weight (BW, kg), using the formula W, F, A, or P = $a + b_1\ BW + b_2\ BW^2$, calculated from the equations in Tables 6.1 and 6.2.

| Body component | a | | $b_1$ | $b_2$ |
|---|---|---|---|---|
| W | 72.7 | (HF) | 0.509 | -0.000236 |
| | 53.2 | (BG) | | |
| | 70.6 | (F) | | |
| | 57.2 | (G) | | |
| | 55.3 | (L) | | |
| F | -99.4 | (HF) | 0.283 | 0.000358 |
| | -72.6 | (BG) | | |
| | -97.0 | (F) | | |
| | -75.7 | (G) | | |
| | -74.6 | (L) | | |
| A | 12.3 | (HF) | 0.023 | -0.000007 |
| | 9.5 | (BG) | | |
| | 11.9 | (F) | | |
| | 8.8 | (G) | | |
| | 9.2 | (L) | | |
| P | 14.9 | (HF) | 0.195 | -0.000118 |
| | 9.8 | (BG) | | |
| | 14.3 | (F) | | |
| | 9.7 | (G) | | |
| | 10.2 | (L) | | |

Had body size been independent of empty body weight (i.e. not correlated) then the change in any one of the components could have been calculated as the derivative of the equation with respect to empty body weight. However, not unexpectedly, body size and weight were correlated and could be related by the regression in Table 6.2.

If body size in the equations of Table 6.1 is expressed in terms of empty body weight (Table 6.2) then the four body components can be related directly to empty body weight, and these relationships are given in Table 6.3. The derivatives (i.e. slopes) of these equations in Table 6.3 correspond to the compositional changes associated with 1 kg change in empty body weight, and since for any component the equations for all breeds are parallel they are independent of breed. The equations relating these compositional changes to empty body weight are given in Table 6.4.

Table 6.4. Relationships of change in empty body water (W, kg), fat (F, kg), ash (A, kg) and protein (P, kg) to empty body weight (BW, kg) of the form W, F, A or P = a + bBW.

| Body component | a | b |
|---|---|---|
| W | 0.509 | -0.000472 |
| F | 0.283 | 0.000716 |
| A | 0.023 | -0.000014 |
| P | 0.195 | -0.000236 |

Table 6.5 shows the composition of empty body-weight change calculated from the relationships in Table 6.4. The calorific values of the changes are also given, having been calculated using standard calorific values for body fat and

protein of 39.3 and 23.7 MJ/kg respectively (Webster, 1977).

Table 6.5. Composition and energy value of 1 kg empty body weight change at different empty body weights

| Empty body weight (kg) | Water (kg) | Fat (kg) | Ash (kg) | Protein (kg) | Energy (MJ) |
|---|---|---|---|---|---|
| 300 | 0.367 | 0.497 | 0.018 | 0.124 | 22.5 |
| 400 | 0.320 | 0.569 | 0.017 | 0.100 | 24.7 |
| 500 | 0.273 | 0.641 | 0.016 | 0.077 | 27.0 |
| 600 | 0.225 | 0.712 | 0.014 | 0.053 | 29.2 |

Within the range of the data the energy content of empty body weight change (E, MJ/kg) can be related directly to empty body weight (BW, kg) by the relationship:-

$$E = 15.77 + 0.0224\ BW$$

## DISCUSSION

Despite the fact that the underlying trends of the relationships relating body components were curvilinear, the considerable computations that had to be undergone to establish that such curvilinearity existed, highlight one of the problems of trying to estimate a within-animal parameter from measurements made on different animals. The variation between animals, combined with the errors associated with measurement, may often obscure the true relationship. It is probable that with larger numbers of animals the curvilinearity of the relationships between body components and empty body weight could have been shown to be statistically significant, without recourse to the use of body size to reduce the variation between animals. However, the number of animals considered here (72, as no body measurements were taken on one cow) cannot be considered as small, and indeed is considerably

larger than those employed in many slaughter experiments.

There is little published information with which to compare the data on empty body-weight change. The only other data available from which to calculate such information is that of Ellenberger, Newlander and Jones (1950), who slaughtered a total of 132 bovines, ranging from foetuses to mature cows. Some of these data were used by ARC (1980), who analysed the data for "22 Holsteins, 12 of which were pregnant". However Ellenberger *et al.* (1950) report data on the composition of only 19 mature Holstein cows. ARC (1980) appears to have related the quantity of body fat and protein to empty body weight by fitting allometric models ($y = ax^b$) to calculate the composition and hence calorific value of empty body-weight change, as shown in Table 6.6.

Table 6.6. Fat, protein and energy content of 1 kg of empty body-weight change (ARC, 1980).

| Empty body weight (kg) | Fat (kg) | Protein (kg) | Energy (MJ) |
|---|---|---|---|
| 300 | 0.299 | 0.163 | 15.6 |
| 400 | 0.431 | 0.157 | 20.6 |
| 500 | 0.573 | 0.152 | 26.1 |

A re-analysis of the data of the 19 Holstein of Ellenberger *et al.* (1950) appears to show little justification for fitting allometric models. Linear, quadratic and allometric equations were calculated for the relationships between body fat and empty body weight and between body protein and empty body weight. In the quadratic models the quadratic parameter was positive

in the case of body fat and negative in the case of protein, but neither proved to be significant ($P > 0.05$), which is not surprising since there were only 19 animals and the quadratic terms could not be shown to be significant for the same relationships in the present study with a larger number of animals. The linear and allometric relationships relating fat (F, kg) to empty body weight (BW, kg) were:-

$$F = -82.9(\pm 25.90) + 0.398(\pm 0.0709)BW \qquad (r^2 = 0.650)$$

$$\log_e F = -9.25(\pm 2.845) + 2.25(\pm 0.484)\log_e BW \quad (r^2 = 0.560)$$

These relationships are shown in Figure 6.1.

The equivalent equations relating body protein (P, kg) to empty body weight (BW, kg) were:-

$$P = 8.53(\pm 5.405) + 0.160(\pm 0.0148)BW \qquad (r^2 = 0.873)$$

$$\log_e P = -1.09(\pm 0.485) + 0.90(\pm 0.083)\log_e BW \quad (r^2 = 0.874)$$

As indicated by the $r^2$ values there is no statistical advantage in fitting the allometric models, and in fact the linear model fits the body fat data better than the allometric one. The fitting of an allometric model implies that the function passes through the origin. The data, as shown in Figure 6.1, do not suggest that this is justified. Indeed biological considerations suggest that there is a lower limit to the amount of fat present in the bodies of mature animals, and certainly there is no biological justification for forcing the function through the origin since empty body weight can never reach zero in mature cows. Thus, although allometry is useful in describing growth and development processes in certain circumstances, it appears to be inappropriate to describe the type of data shown in Figure 6.1. The positive exponent (and therefore increasing derivative) of the allometric function of Figure 6.1 indicates that the relative growth rate of fat is greater than the

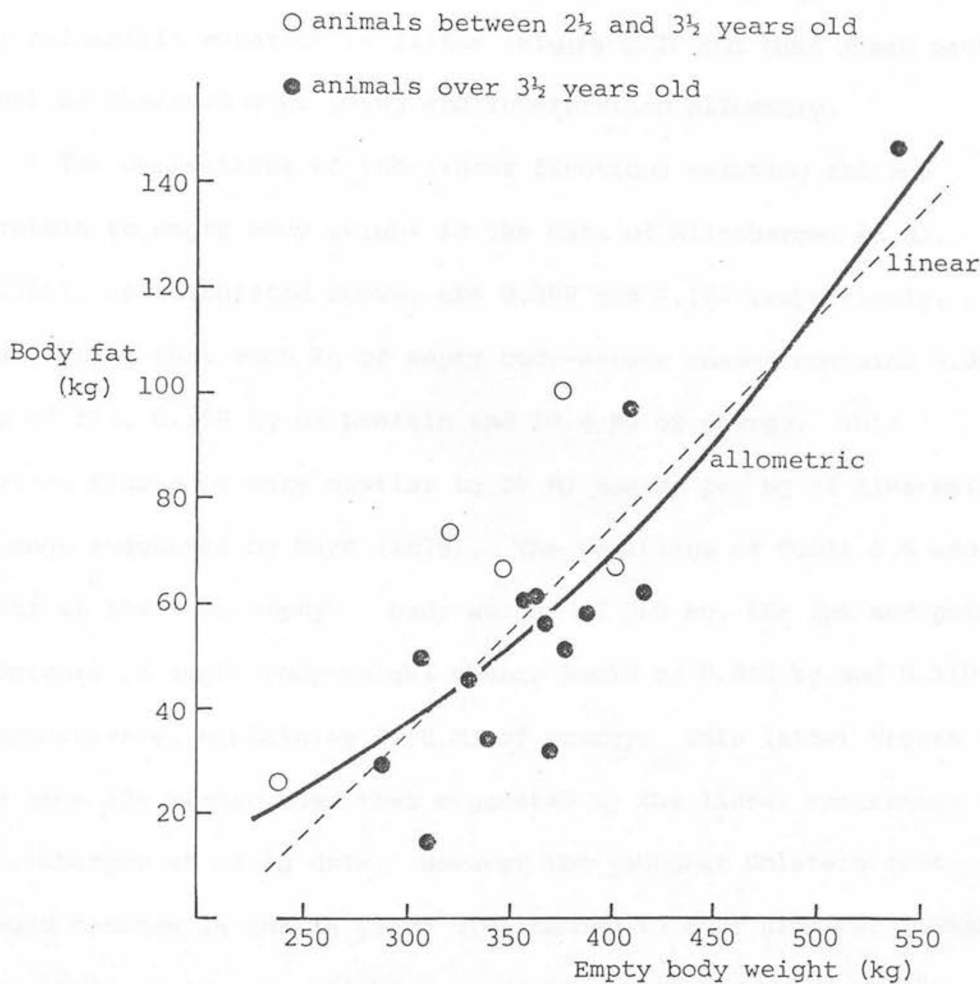



Figure 6.1. Relationship between body fat and empty body weight - data of Ellenberger *et al*.

relative growth rate of empty body weight. This is undoubtedly the case, but it is simply a reflection of the linear function having a positive slope and negative intercept, and is not necessarily indicative of a curvilinear relationship. Indeed a function with a decreasing derivative (i.e. decreasing slope) and a large negative intercept can give a positive exponent if an allometric equation is fitted (Figure 6.2) and thus great caution must be observed when using and interpreting allometry.

The derivatives of the linear functions relating fat and protein to empty body weight in the data of Ellenberger *et al.* (1950), as calculated above, are 0.398 and 0.160 respectively, indicating that each kg of empty body-weight change contains 0.398 kg of fat, 0.160 kg of protein and 19.4 MJ of energy. This latter figure is very similar to 20 MJ energy per kg of live-weight change suggested by MAFF (1975). The equations of Table 6.4 would indicate that at the mean empty body weight of 360 kg, the fat and protein contents of empty body-weight change would be 0.540 kg and 0.110 kg respectively, containing 23.8 MJ of energy. This latter figure is some 22% higher than that suggested by the linear regression from Ellenberger *et al.'s* data. However the youngest Holstein cows (aged between 2½ and 3½ years old) tended to have high fat contents for their empty body weights, suggesting that they were smaller and probably had not reached their mature size. If these are excluded, the regression coefficients for the regression of fat and protein weights on empty body weight become 0.469 and 0.147 respectively, giving an energy content of empty body-weight change of 21.9 MJ at a mean empty body weight of 369 kg. These figures are closer to those predicted by the equations of Table 6.4, which are 0.554 kg of fat, 0.107 kg of protein and 24.3 MJ of energy. The discrepancy

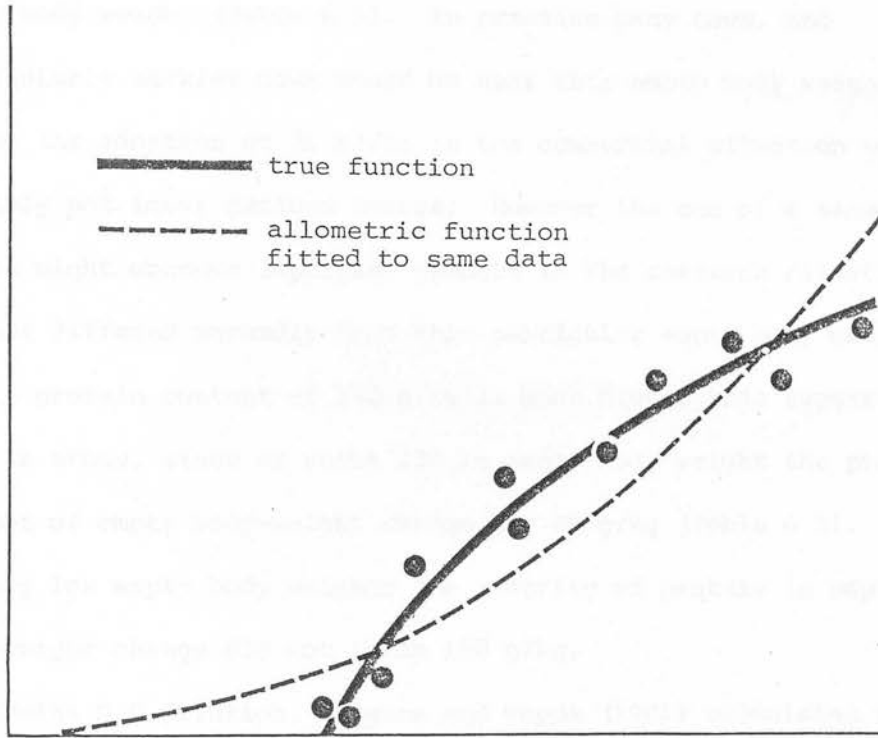



Figure 6.2.

Allometric function fitted to data where true function has negative exponent

in energy content then becomes 11%.

ARC (1980) suggest that until more information becomes available the energy value of empty body-weight change in adult cattle should be assumed to be 26 MJ/kg, and for protein they suggest a value of 150 g/kg. The former figure of 26 MJ/kg agrees well with that predicted by the data from this study for cows of about 450 kg empty body weight (Table 6.5). In practice many cows, and particularly suckler cows would be near this empty body weight, and so the adoption of 26 MJ/kg in the commercial situation would probably not incur serious errors. However the use of a single figure might obscure important effects in the research situation, if cows differed markedly from this particular empty body weight.

A protein content of 150 g/kg is much higher than suggested by this study, since at about 450 kg empty body weight the protein content of empty body-weight change was 88 g/kg (Table 6.5). Even at very low empty body weights the quantity of protein in empty body-weight change did not reach 150 g/kg.

Using $D_2O$ dilution, Chigara and Topps (1981) calculated that each kg of live-weight loss in beef cows during mid-lactation contained 615 g fat, 280 g water and 83 g protein, figures which agree remarkably well with those in Table 6.5 for cows of about 500 kg empty body weight. Their figures for the composition of live-weight gain were similar. However, the effects of physiological state is not clear. Certainly in sheep in early lactation the composition of empty body weight change can be very variable. Indeed Cowan, Robinson, McDonald and Smart (1980) recorded instances of animals losing fat, but gaining water, resulting in the energy content of empty body weight change apparently ranging from -8 to 50 MJ/kg. It would not be unreasonable to assume that similar

changes might occur in cattle. The effect of pregnancy is similarly unknown. Russel, Gunn and Doney (1968) recorded losses of empty body weight containing 0.305 kg of fat and 0.213 kg of protein per kg in early and mid-pregnancy in Blackface sheep, while in late pregnancy empty body-weight losses contained 0.706 kg and 0.076 kg of fat and protein per kg respectively (assuming 81% protein in the fat-free dry matter). It is therefore suggested that the values for composition and energy content of empty body-weight change presented in this chapter may not be appropriate to animals in physiological states other than those from which the data were derived.

# CHAPTER 7

# FAT PARTITION

INTRODUCTION

The objective of this part of the study is to describe in which parts of the body mature cows store fat, and how the storage of fat in various tissues is influenced by both the total amount of fat stored and by genotype. All results refer to the partition of chemical fat (lipid) in various depots in the body; the partition of dissectable fatty tissue has not been considered. It is, however, likely that the partition of fatty tissue will show the same general trends.

The fat in six different tissues was considered, that in the omental and mesenteric fatty tissue (OM), perirenal fatty tissue (P), subcutaneous fatty tissue (S), muscle and associated fatty tissue (MAF), carcass bone (B) and remainder (R). The exact definitions of these tissues are given in Chapter 4. It was intended to predict the quantities of intermuscular and intramuscular fat in the MAF by two methods. The first of these was to predict the quantity of carcass intermuscular fatty tissue from the thin flank intermuscular fatty tissue from prediction equations developed by the Meat and Livestock Commission (A.J. Kemptster, personal communication) and assume that the carcass intermuscular fatty tissue had the same composition as the thin flank intermuscular fatty tissue. The intramuscular fat could then be calculated as the difference between the fat in the total MAF and that in the intermuscular fatty tissue. The second approach was to assume that the part of the *M. longisimmi thoracis et lumborum* removed was representative of the muscle mass as a whole and had the same composition. If this was valid it should have been possible to calculate the quantities of muscle and intermuscular fat in the MAF from a knowledge of its mass and

composition.

Unfortunately the two methods did not agree particularly well, and in some cases absurd values were obtained, including negative percentages and masses. It was concluded that some of the assumptions that it had been necessary to make were not valid. The Meat and Livestock Commission prediction equations relating carcass intermuscular fatty tissue to the intermuscular fatty tissue in the thin flank were derived from data from young commercial beef animals, covering a smaller range of fatness than the mature cows of this experiment, and the composition of the intermuscular fatty tissue may not be constant throughout the carcass (Callow, 1962). Similarly the proportion of intramuscular fat may vary between muscles, and even within muscles (Callow, 1962; Cook, Bray and Weckel, 1964). Because of these difficulties, the attempt to quantify the amounts of intermuscular and intramuscular fat was abandoned, and the fat in the MAF was treated as one depot.

## RESULTS

A summary of the quantities of fat in each of the various tissues is given in Table 7.1. Table A3 in Appendix II gives details for each cow.

When describing fat partition the percentage of fat in the empty body was chosen as the base line with which to make breed comparisons. By doing so, basic differences in body size were removed. The quantity of fat in each depot was expressed as a percentage of the total fat, and this was regressed on the percentage of fat in the empty body. A regression with a positive slope indicates that as the proportion of fat in the empty body increases, (i.e. as animals get fatter) then the depot under consideration forms an increasing proportion of the total fat.

Table 7.1 Summary of the quantities (kg) of fat in various depots (Values are means and ranges).

| Breed | n | Empty body weight (kg) | OM | P | S | MAF | B | R | Total |
|---|---|---|---|---|---|---|---|---|---|
| HF | 14 | 458 | 13.3 | 5.6 | 22.8 | 40.5 | 9.0 | 18.3 | 109.4 |
| | | 283-623 | 1.7-45.2 | 0.6-15.2 | 0.7-48.0 | 11.0-78.9 | 5.9-13.5 | 5.2-34.2 | 26.3-228.2 |
| BG | 14 | 437 | 16.8 | 7.0 | 26.4 | 47.6 | 8.9 | 17.0 | 123.6 |
| | | 320-639 | 3.2-46.1 | 1.4-14.9 | 3.8-80.6 | 18.0-91.0 | 6.3-12.1 | 7.5-33.4 | 45.3-274.1 |
| F | 15 | 476 | 23.0 | 8.0 | 19.1 | 44.9 | 10.0 | 17.3 | 122.4 |
| | | 326-636 | 0.2-56.5 | 0.2-18.9 | 0.1-46.9 | 3.9-86.1 | 5.9-15.4 | 4.9-34.4 | 15.3-233.5 |
| G | 15 | 394 | 13.2 | 4.7 | 16.4 | 36.2 | 8.2 | 14.7 | 93.3 |
| | | 261-564 | 0.6-36.8 | 0.1-13.1 | 0.0-52.4 | 4.5-75.9 | 5.4-11.4 | 3.9-26.8 | 17.3-211.9 |
| L | 15 | 365 | 8.4 | 3.7 | 12.9 | 30.2 | 9.1 | 13.8 | 78.1 |
| | | 265-489 | 0.3-20.0 | 0.1-9.5 | 0.0-26.2 | 3.7-49.6 | 5.6-12.0 | 4.9-23.0 | 14.6-126.5 |

Similarly a negative slope indicates a decreasing proportion of the total fat, while a regression coefficient of zero for any depot would indicate that it contained a constant proportion of the total fat irrespective of fatness.

The relationships of depot fat (as a percentage of total fat) to total fat (as a percentage of empty body weight) are shown in Figures 7.1 to 7.6. The calculated relationships are given in Table 7.2. These fitted lines were chosen simply on the basis of how well they described the data. No attempt was made to force the sum of the six equations to equal the percentage of fat in the empty body.

The OM, P and S fats all show positive slopes, indicating that these depots form an increasing proportion of the fat as cows fatten, although above about 25-30% fat in the empty body the P fat tended to plateau. There are also significant breed differences in these depots, with the F cows having a higher proportion of fat in the internal depots and a lower proportion in the subcutaneous depot than the other breeds. The HF cows had the highest proportion of fat in the S depot, while the BG, G and L breeds were similar for all depots. It appears from Figure 7.3 that breed differences in the proportions of fat in the S depot do not become apparent until the fat constitutes about 15% of the empty body.

There were no breed differences in the relationships involving MAF, B and R. The B and R fat made up a smaller proportion of fat in the fatter cows, while the proportion of MAF fat increased rapidly initially and thereafter decreased slowly. It is not possible to be conclusive as to where the

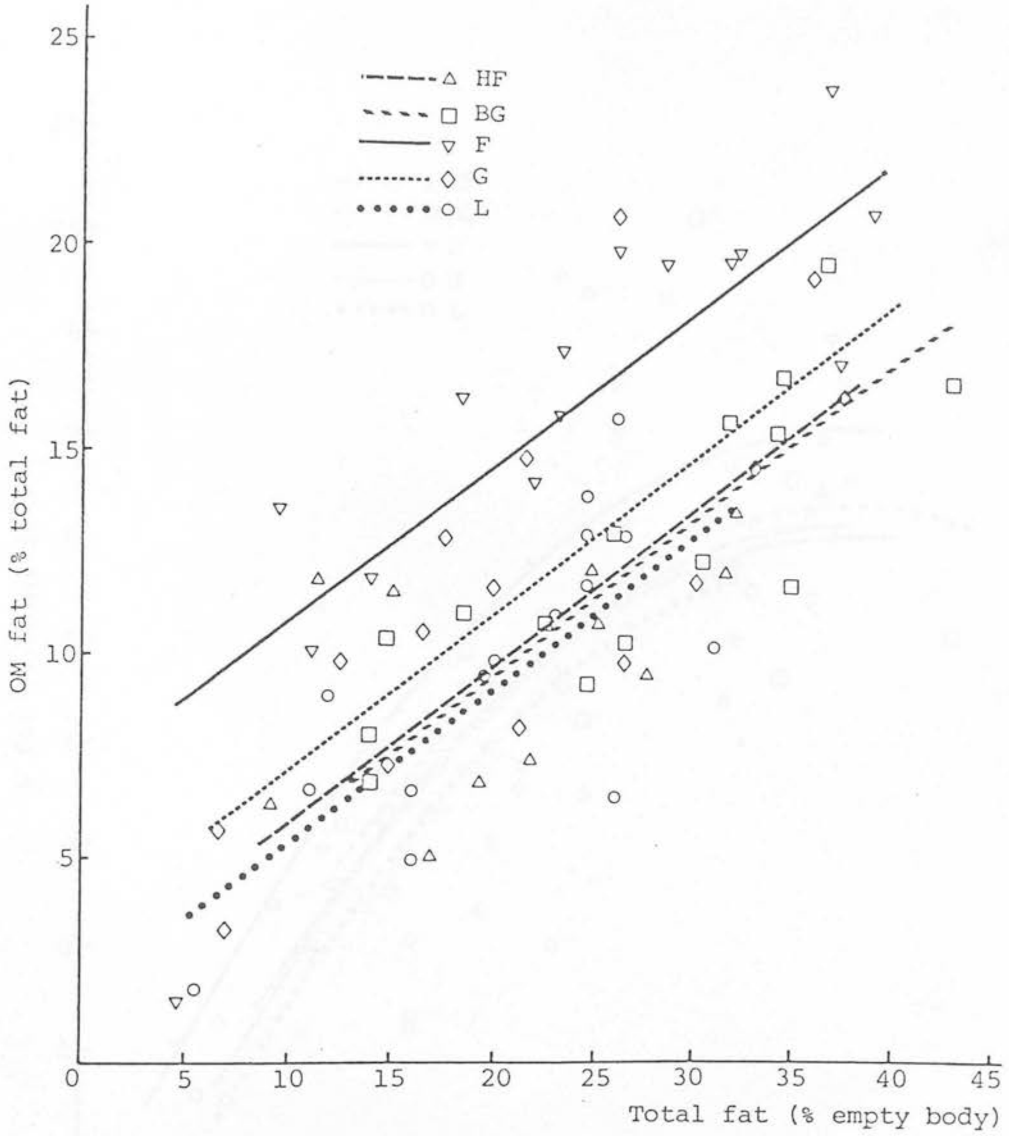



Figure 7.1. Relationship between OM fat (% total fat) and total fat (% empty body)

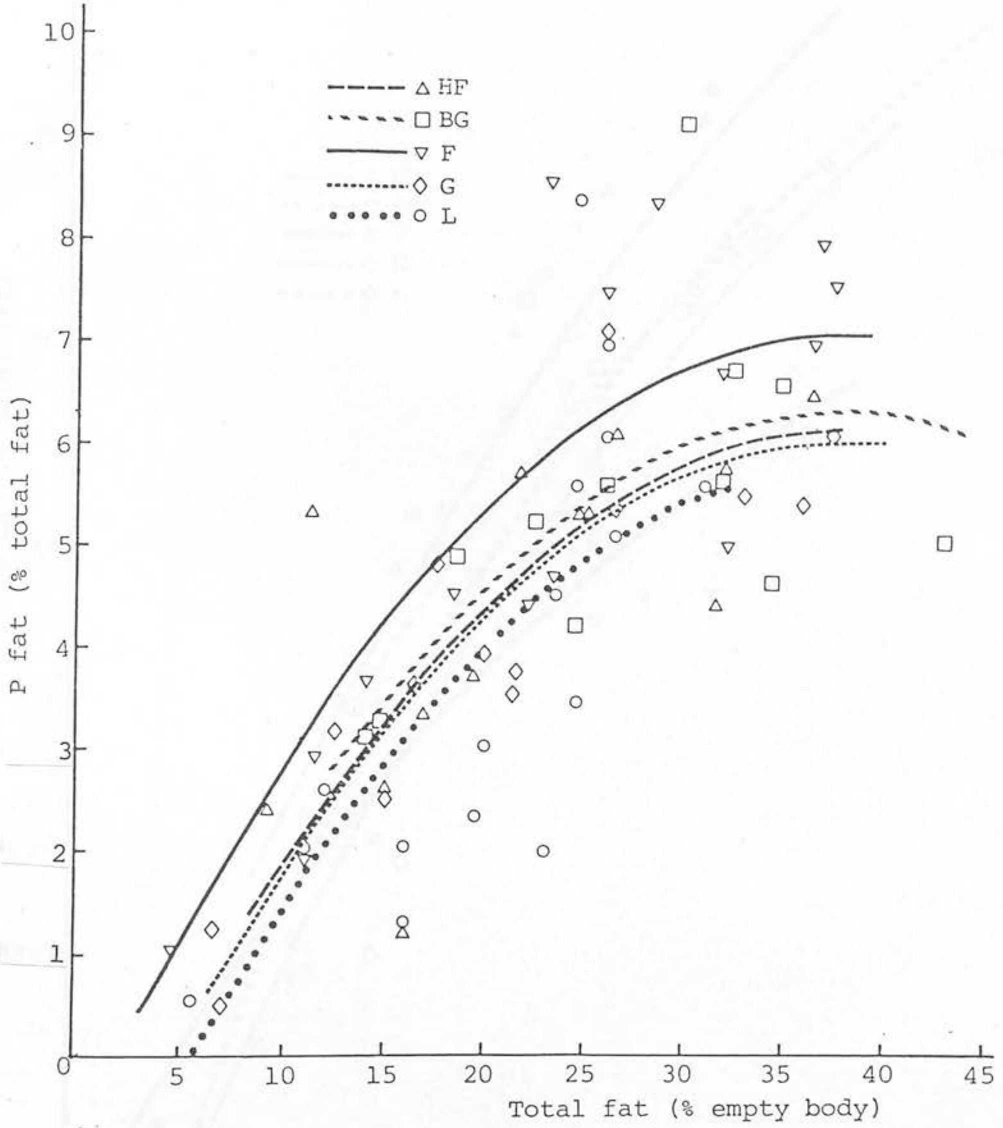



Figure 7.2. Relationship between P fat (% total fat) and total fat (% empty body)

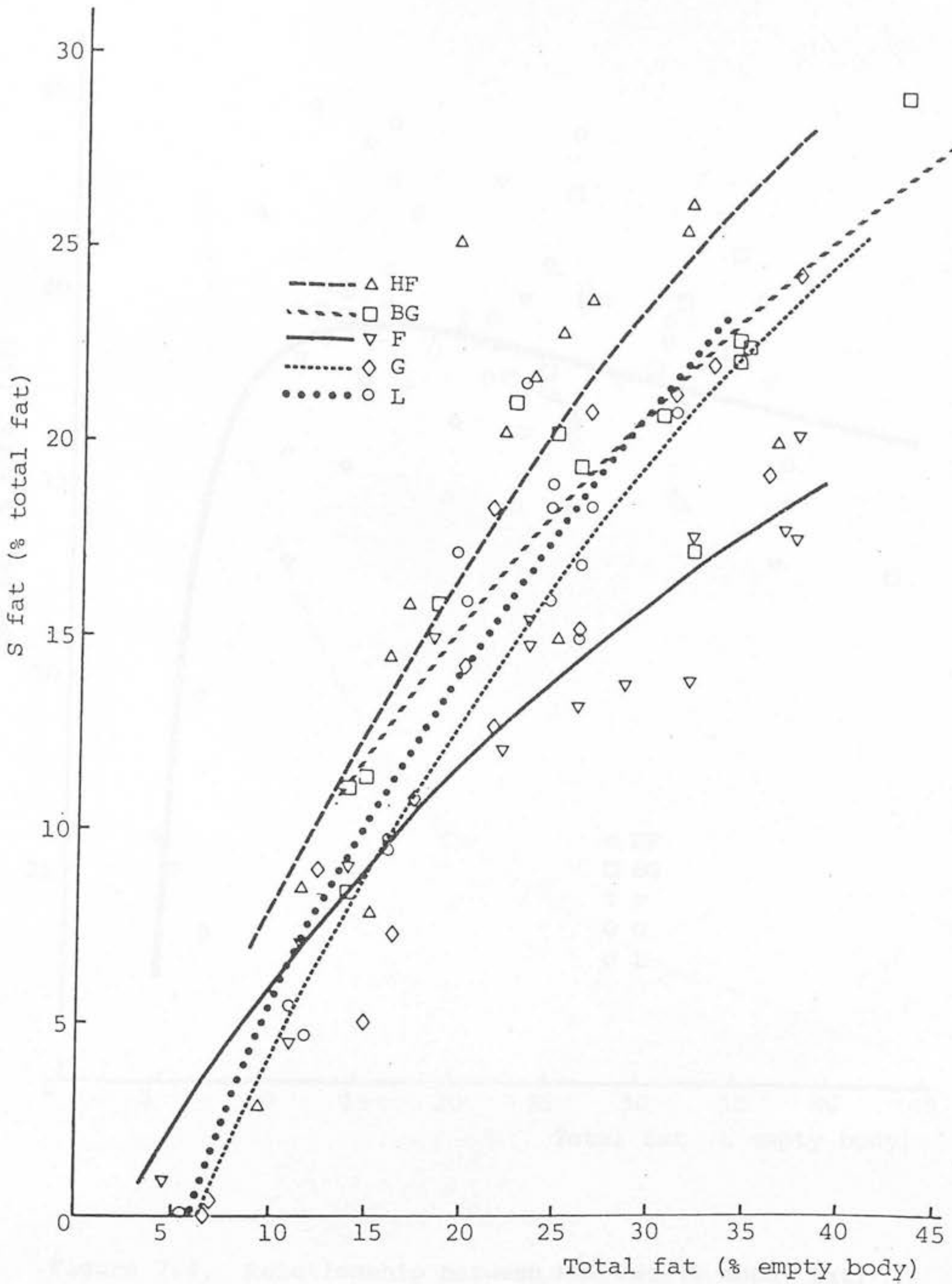



Figure 7.3. Relationships between S fat (% total fat) and total fat (% empty body)

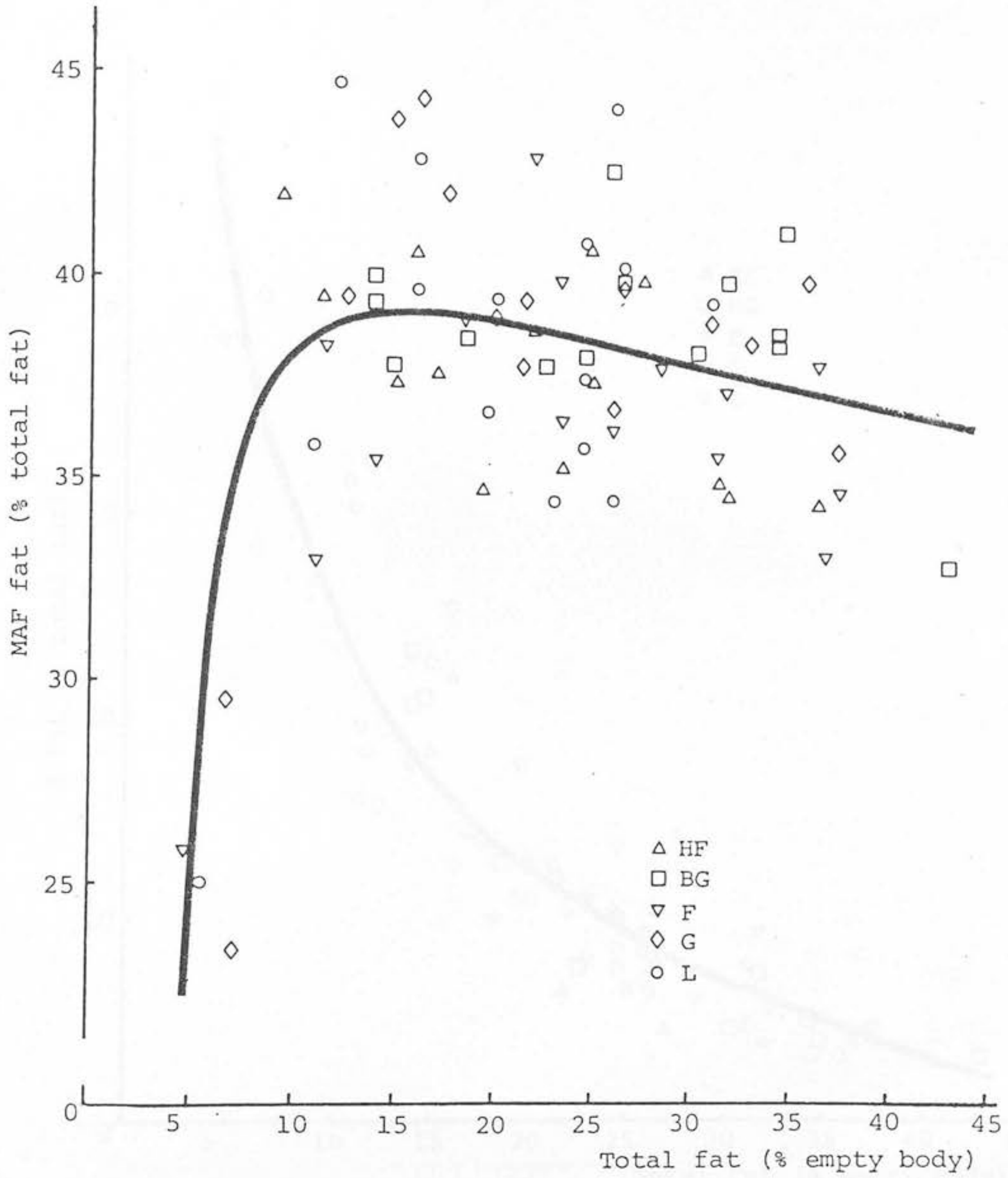



Figure 7.4. Relationship between MAF fat (% total fat) and total fat (% empty body)

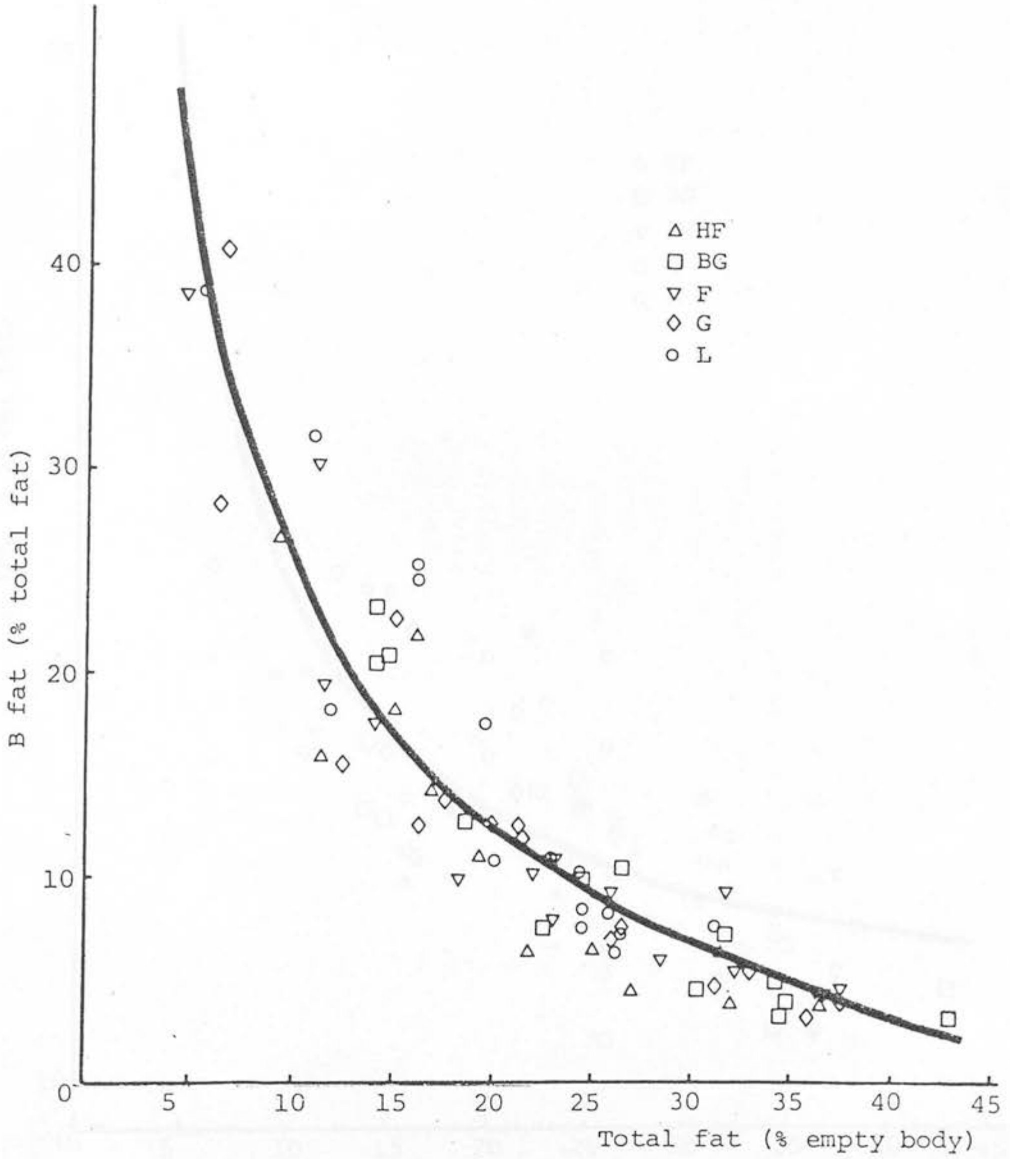



Figure 7.5. Relationship between B fat (% total fat) and total fat (% empty body)

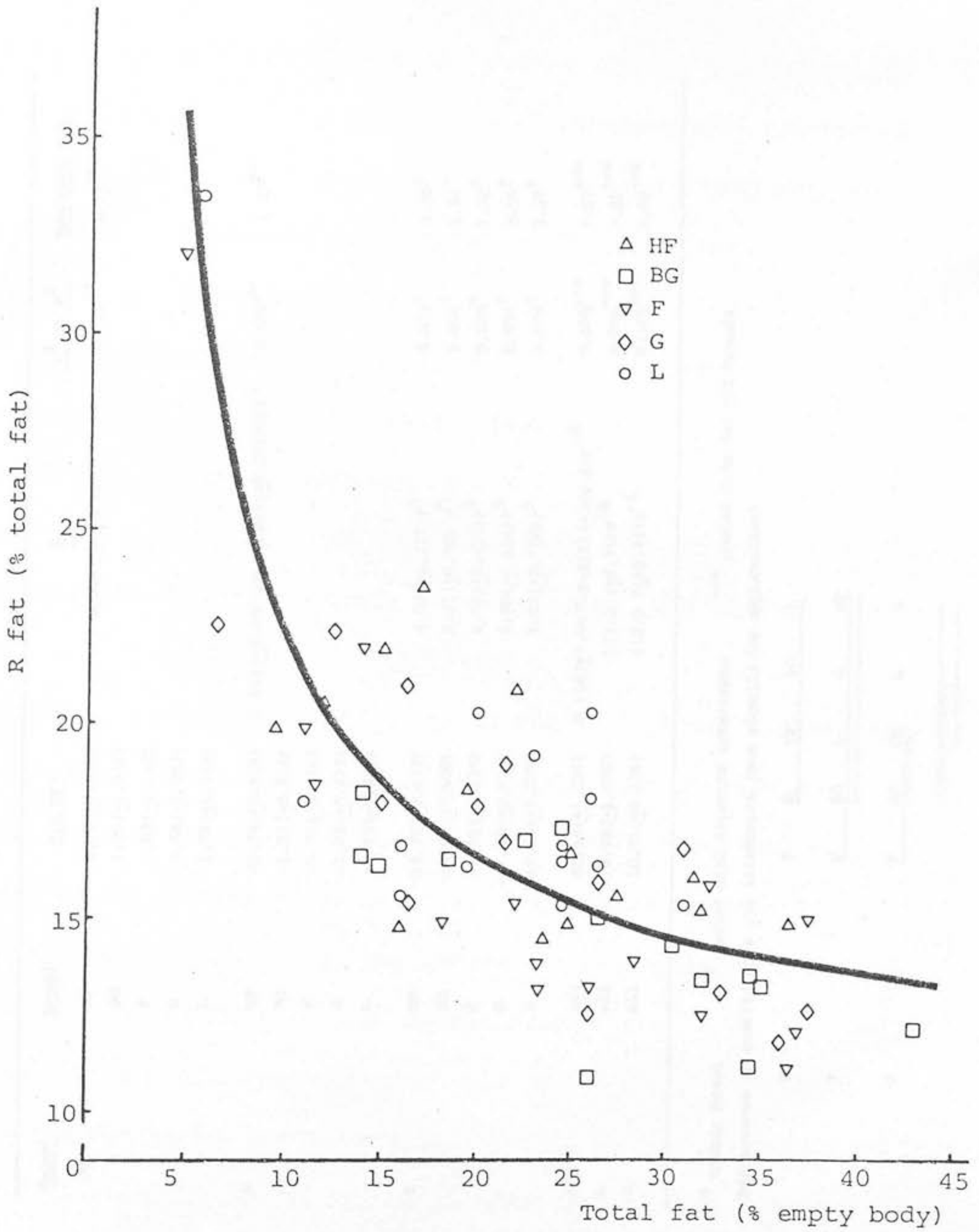



Figure 7.6. Relationship of R fat (% total fat) to total fat (% empty body)

Table 7.2. Relationships of depot fat (D, % total fat) to total fat (F, % empty body weight) of the form D = a + Z.

| Depot | Breed | a(± se) | Z | $r^2$ or $R^2$ | Residual s.d. |
|---|---|---|---|---|---|
| OM | HF | 2.19 (±1.110) | 0.384 (±0.0374)F | $0.700^{++}$ | $2.75^{++}$ |
| | BG | 1.99 (±1.231) | | | |
| | F | 7.09 (±1.140) | | | |
| | G | 3.48 (±1.083) | | | |
| | L | 1.72 (±1.043) | | | |
| P | HF | -1.74 (±0.919) | 0.418 (±0.0787)F-0.0055 (±0.00169)$F^2$ | $0.658^{++}$ | $1.25^{++}$ |
| | BG | -1.52 (±0.914) | | | |
| | F | -0.78 (±0.872) | | | |
| | G | -1.83 (±0.872) | | | |
| | L | -2.14 (±0.917) | | | |
| S | HF | -14.01 (±6.473) | 6.90 (±1.373)$F^{1/2}$ | $0.678^{+}$ | $4.38^{+}$ |
| | BG | -10.64 (±3.600) | 5.81 (±0.701)$F^{1/2}$ | $0.851^{+}$ | $2.26^{+}$ |
| | F | -7.78 (±2.134) | 4.37 (±0.437)$F^{1/2}$ | $0.885^{+}$ | $1.92^{+}$ |
| | G | -17.39 (±2.875) | 6.80 (±0.614)$F^{1/2}$ | $0.904^{+}$ | $2.56^{+}$ |
| | L | -17.13 (±3.279) | 7.02 (±0.726)$F^{1/2}$ | $0.878^{+}$ | $2.37^{+}$ |
| MAF | all | 41.48 (±1.224) | 0.114 (±0.0475)F-2165 (±304.6)$F^{-3}$ | $0.570^{+++}$ | $3.07^{+++}$ |
| B | all | -18.26 (±1.496) | 137.3 (±6.39)$F^{-1/2}$ | $0.866^{+++}$ | $3.33^{+++}$ |
| R | all | 10.71 (±0.676) | 118.9 (±10.46)$F^{-1}$ | $0.645^{+++}$ | $3.08^{+++}$ |

$^{+}$ within breed $^{++}$ pooled with separate intercepts $^{+++}$ pooled data for all breeds

Differences in coefficients and intercepts (see page 103 for explanation)

OM F G HF BG L

P F BG L G HF

S F BG HF L G

change takes place because of the lack of data at the very low end of the fatness range, but from inspection of the data (Figure 7.4) it would appear to be between 10 and 15% fat in the empty body.

Although it was not possible to estimate the total quantity of intramuscular fat, the percentage of intramuscular fat in the section of the *M. longissimi thoracis et lumborum* that was removed was related to the percentage of fat in the empty body as shown in Figure 7.7 and the regressions in Table 7.3.

There was a considerable range in the percentage of fat in the bone, from 12.3 to 34.7% with a mean of 23.0%. There was no relationship between percentage fat in bone and percentage fat in empty body ($r = 0$) although the bone of all five animals that had less than 10% fat in the empty body contained less than 20% fat.

DISCUSSION

The partition of chemical fat in the whole bodies of animals has received little attention in the past. Most studies on fat partition have concentrated on the partition of dissectible fatty tissue in the carcass, because of its importance in carcass and meat quality. However, the general trends would be expected to be similar for both fat and fatty tissue.

In all but the very thinnest cows (< 10% fat in the empty body) the fat in the MAF constituted the major depot, containing some 35-45% of the fat in the whole body (Figure 7.4). Even at 5% fat in the empty body it was still a major depot containing some 25% of the total fat. The OM depot contained from 5-10% of the fat in the thinnest cows, increasing to about 20% in the fattest, while the S depot contained almost no fat in the thinnest cows and 20-30% of the fat in the fattest animals. The P depot

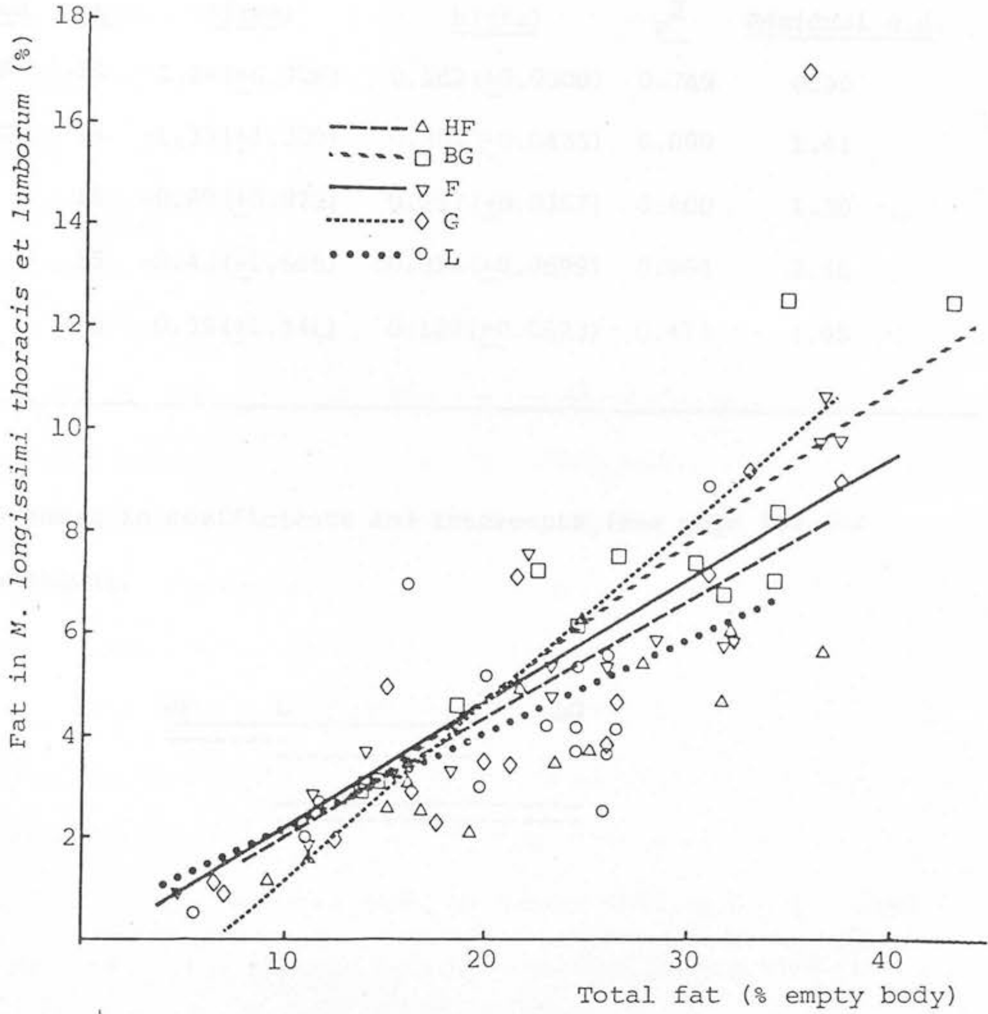



Figure 7.7. Relationship between percentage fat in *M. longissimi thoracis et lumborum* and percentage fat in the empty body

Table 7.3. Regression of percentage fat in *M. longissimi thoracis et lumborum* (M) on the percentage of fat in the empty body (F) using the model $M = a + bF$.

| Breed | n | a(±se) | b(±se) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| HF | 14 | -0.24 (±0.726) | 0.182 (±0.0308) | 0.745 | 0.90 |
| BG | 14 | -1.33 (±1.209) | 0.309 (±0.0435) | 0.808 | 1.41 |
| F | 15 | -0.60 (±0.922) | 0.257 (±0.0357) | 0.800 | 1.38 |
| G | 15 | -2.43 (±1.665) | 0.354 (±0.0699) | 0.664 | 2.56 |
| L | 15 | 0.35 (±1.341) | 0.189 (±0.0623) | 0.415 | 1.65 |

Differences in coefficients and intercepts (see page 103 for explanation).

```
HF   L    F    G    BG
--------
     -------------
     ------------------
```

appeared to be the smallest adipose tissue depot, containing between 1 and 9% of the total fat.

As the level of fatness increased the proportions of fat stored in the OM, P and S depots all increased. The increase in the OM depot appeared to be linear, but that for the S and P depots increased at a decreasing rate and in fact above 25-30% fat in the empty body the P fat appeared to reach a plateau, indicating that it was increasing at the same rate as the fat as a whole.

The fat in the MAF appears to form a decreasing proportion of the total fat as cows with above about 10-15% fat in the empty body become fatter, but the change is relatively small. Below 10-15% fat in the empty body the change in proportion is very rapid, and in the opposite direction. This would appear to be due to the intermuscular fat, since no evidence of a change appears in the percentage fat in muscle (Figure 7.7). It is possible that as fat animals become thinner they draw upon their intermuscular fat at a rate that is slightly lower than the rate at which they mobilise fat as a whole, until such time as the MAF fat constitutes some 40-45% of the total, when they are then forced to mobilise fat from the intermuscular depot at a rate that is greater than the fat as a whole, perhaps because the other depots have been almost exhausted. It is difficult, however, to find a biological explanation to support such a hypothesis.

The decreasing proportion of the total fat stored in the bone and remainder is a reflection of the increased amounts of fat stored in the main adipose tissue depots. The proportion of fat in bone seems to be little affected by level of fatness, although there is some indication that very thin animals (less

than 10% fat in the empty body) had a lower bone fat content. Russel, Doney and Gunn (1971) noted that underfed Blackface ewes with less than 15% fat in the empty body mobilised fat from the skeleton. If this occurs in cattle it would appear to be at lower levels of fatness.

As far as the author is aware no comparable study had been carried out on the partition of chemical fat in the whole bodies of mature cattle. Russel *et al.* (1971) studied fat partition in mature ewes and their results show exactly the same pattern of change for all depots as those in this study, when they are expressed in the same way. The only differences were that sheep with above about 20% fat in the empty body had a higher proportion of fat in the S depot and a lower proportion in the MAF than the cattle of this study.

The higher proportion of fat in the internal depots of the F cows and the lower proportion of subcutaneous fat is in keeping with the findings of many other studies (e.g. Callow, 1948 and 1961; Barton, 1968, 1971 and 1972; Truscott, 1980).

The proportion of fat in the OM and P depots for the HF cows was very similar to that of the three beef breeds but the HF cows had the highest proportion of S fat. However, below about 15% fat in the empty body breed differences in the S depot are greatly reduced. This may also be the case with the P and OM depots, but could not be shown with the data from this study.

Since the HF cows were crossbred animals, it seems reasonable to suggest that the pattern of fat partition of these cows would be intermediate between that of the two parent breeds i.e. Hereford and Friesian. If this is so, then it appears likely that pure Hereford cows would have a lower proportion of internal fat (OM and

P) and a higher proportion of S fat than any of the breeds examined in this investigation. This would be in keeping with the general concept that breeds which have been strongly selected for meat characteristics have a higher proportion of S fat and a lower proportion of internal fat (Hammond, 1932; Callow, 1961).

# CHAPTER 8

# PREDICTION OF BODY COMPOSITION

# RESULTS

## Prediction of total body water

Although theoretically it is possible to predict body composition from empty body weight and empty body water, in practice neither of these can be measured in the live animal. The prediction equations presented are therefore based on live weight and total body water. Total body water is the sum of the water in the empty body, the alimentary tract and the bladder. A summary of live weight has already been given in Table 5.1 and a summary of the data for total body water and $D_2O$ space is given in Table 8.1. Full data for each cow are given in Appendix II.

Table 8.1. Summary of total body water and $D_2O$ space

| Breed | Total body water (kg) | | $D_2O$ space (kg) | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| HF | 322 | 237-380 | 336 | 259-423 |
| BG | 289 | 227-369 | 298 | 228-361 |
| F | 325 | 266-377 | 327 | 261-372 |
| G | 284 | 240-337 | 298 | 276-337 |
| L | 265 | 208-328 | 277 | 212-344 |
| Overall | 297 | 208-380 | 307 | 212-423 |

### Live weight

The relationship of total body water to live weight is given in Table 8.2.

### $D_2O$ space

The mean values for total body water and $D_2O$ space were 297 and 307 kg respectively, resulting in $D_2O$ space overestimating total body water by a mean of 3.37%. The relationship between total body water (W, kg) and $D_2O$ space (DS, kg) was:-

Table 8.2. Regression of total body water (W, kg) on live weight (LW, kg) using the model $W = a + b_1LW + b_2LW^2$

| Breed | n | a(±s.e.) | $b_1$(±s.e.) | $b_2$(±s.e.) | $R^2$ | Residual s.d. |
|---|---|---|---|---|---|---|
| HF | 14 | 47.0(±47.45) | 0.725(±0.1848) | -0.00038(±0.000175) | - | - |
| BG | 14 | 23.5(±47.29) | | | - | - |
| F | 15 | 43.0(±47.99) | | | - | - |
| G | 15 | 29.6(±47.88) | | | - | - |
| L | 15 | 24.6(±46.87) | | | - | - |
| Pooled with separate intercepts | 73 | - | | | 0.850 | 17.4 |

Differences in intercepts (see page 103 for explanation) HF F G L BG

$$W = 7.5\,(\pm 16.93) + 0.942\,(\pm 0.0547)DS$$

$$(n = 72,\ r^2 = 0.809,\ \text{residual s.d.} = 19.0)$$

Total body water (W, kg) was related to $D_2O$ space (DS, kg) and live weight (LW, kg) by the following equation:-

$$W = 35.7\,(\pm 13.42) + 0.537\,(\pm 0.0694)DS + 0.190\,(\pm 0.0262)LW$$

$$(n = 72,\ R^2 = 0.892,\ \text{residual s.d.} = 14.4)$$

## Prediction of body fat

Table 8.3 gives a summary of the various measurements used in the prediction of body fat. Values for each cow are presented in the tables of Appendix II. Summaries of live weight, condition score (Table 5.1), body size (Table 5.4), body fat (Table 7.1) and $D_2O$ space (Table 8.1) have already been given.

### Prediction of fat weights and percentages

When the quantity of fat in the bodies of animals is predicted by indirect methods, prediction equations can be developed to estimate either the proportion of fat in the live weight (usually expressed as a percentage) or the absolute weight of fat. In either case live weight may or may not be included as a covariate in a multiple regression equation. All prediction equations in this Chapter express fat in absolute terms for two reasons. Firstly, the variation explained by this approach was invariably either the same as or greater than that when the proportion of fat was used as the dependent variable. An example of this is given in Table 8.4 where $r^2$ or $R^2$ values for the prediction of body fat from live weight and condition score are given.

Secondly, if fat is predicted as an absolute quantity it is possible to develop prediction equations that are independent of live weight. Thus the equations from this study which do not include live weight as an independent variable could be used,

Table 8.3. Summary of some measurements used in the prediction of body fat (values are means and ranges).

| Breed | Skinfold thickness (mm) | Ultrasonic fat depth (mm) | | | | |
|---|---|---|---|---|---|---|
| | | 12-13th ribs | 3rd lumbar | Scapula | Femur | Leg |
| HF | 8.1 | 8.2 | 11.1 | 4.5 | 4.6 | 4.9 |
| | 6.5-9.7 | 0.0-24.0 | 1.0-31.0 | 0.0-11.8 | 0.0-17.0 | 0.0-12.5 |
| BG | 6.7 | 11.3 | 12.7 | 3.8 | 3.7 | 2.3 |
| | 5.5-8.6 | 2.0-29.7 | 2.5-29.0 | 0.0-8.0 | 1.0-9.5 | 0.0-5.5 |
| F | 6.3 | 6.3 | 7.3 | 2.2 | 2.3 | 1.4 |
| | 4.9-8.6 | 0.0-13.9 | 0.0-19.2 | 0.0-4.7 | 0.0-6.2 | 0.0-5.0 |
| G | 6.6 | 8.0 | 8.5 | 3.2 | 2.3 | 1.9 |
| | 5.2-8.5 | 0.0-27.5 | 0.0-24.6 | 0.0-14.0 | 0.0-9.5 | 0.0-4.0 |
| L | 7.5 | 4.7 | 7.6 | 2.9 | 2.5 | 1.0 |
| | 6.0-9.9 | 0.0-11.0 | 0.0-18.0 | 0.0-6.5 | 0.0-6.5 | 0.0-2.0 |

Table 8.4. $R^2$ and $r^2$ values for predicting fat as absolute (abs) quantities or as a percentage (%) of live weight, from live weight (LW) and condition score (CS).

| Independent Variables | Breed | | | | | | | | | | Pooled within breed | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HF | | BG | | F | | G | | L | | | |
| | abs | % | abs | % | abs | % | abs | % | abs | % | abs | % |
| LW | 0.94 | 0.92 | 0.94 | 0.83 | 0.91 | 0.81 | 0.84 | 0.71 | 0.79 | 0.57 | 0.91 | 0.79 |
| CS | 0.93 | 0.93 | 0.89 | 0.91 | 0.87 | 0.91 | 0.92 | 0.90 | 0.80 | 0.67 | 0.90 | 0.90 |
| CS and LW | 0.95 | 0.94† | 0.96 | 0.91† | 0.95 | 0.94† | 0.95 | 0.91† | 0.86 | 0.67† | 0.95 | 0.90† |

† only one of the partial regression coefficients is significant.

albeit with caution, on pregnant animals and on animals subject to rapid changes in live weight due to variation in gut fill. If a meaningful live weight can be determined, all predicted fat weights can, of course, be converted to a percentage if so required.

Live weight

Live weight was used as the base with which to compare all other methods of *in vivo* prediction of body fat. Equations for the regression of body fat on live weight are presented in Table 8.5.

Condition score

Figure 8.1 shows the relationship between body fat and condition score; the corresponding regressions are presented in Table 8.6. The multiple regression of fat weight on condition score and live weight is given in Table 8.7. In these multiple regression equations neither of the partial regression coefficients for the HF breed was significant, nor was the partial regression coefficient for condition score for the BG cows. All other coefficients were significant, and it would therefore appear that condition score can improve upon the prediction of body fat afforded by live weight alone.

Ultrasonic subcutaneous fat depth

In relating body fat to the mean ultrasonic fat depths measured at each of the different sites, various models were investigated, as the relationship proved to be non-linear. The models tested were:-

1. $y = a + b_1x + b_2x^2$
2. $y = a + b(x + 1)^{-1}$
3. $y = a + b\log_e(x + 1)$
4. $y = a + bx^{\frac{1}{2}}$

Table 8.5. Regression of body fat (F, kg) on live weight (LW, kg) using the model $F = a + bLW$.

| Breed | n | a(±s.e.) | b(±s.e.) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| HF | 14 | -157 (±19.6) | 0.493 (±0.0354) | 0.942 | 15.2 |
| BG | 14 | -166 (±20.8) | 0.566 (±0.0396) | 0.944 | 17.2 |
| F | 15 | -256 (±33.3) | 0.676 (±0.0585) | 0.911 | 22.2 |
| G | 15 | -217 (±38.0) | 0.658 (±0.0796) | 0.840 | 24.1 |
| L | 15 | -102 (±25.6) | 0.415 (±0.058) | 0.797 | 16.9 |
| Pooled within breed | 73 | - | - | 0.912 | 19.5 |

Differences in coefficients and intercepts (see page 103 for explanation)

L HF BG G F

_____ (L–HF)
_____ (HF–G)
- - - - (BG–G)
_____ (BG–F)
- - - - (BG–G)

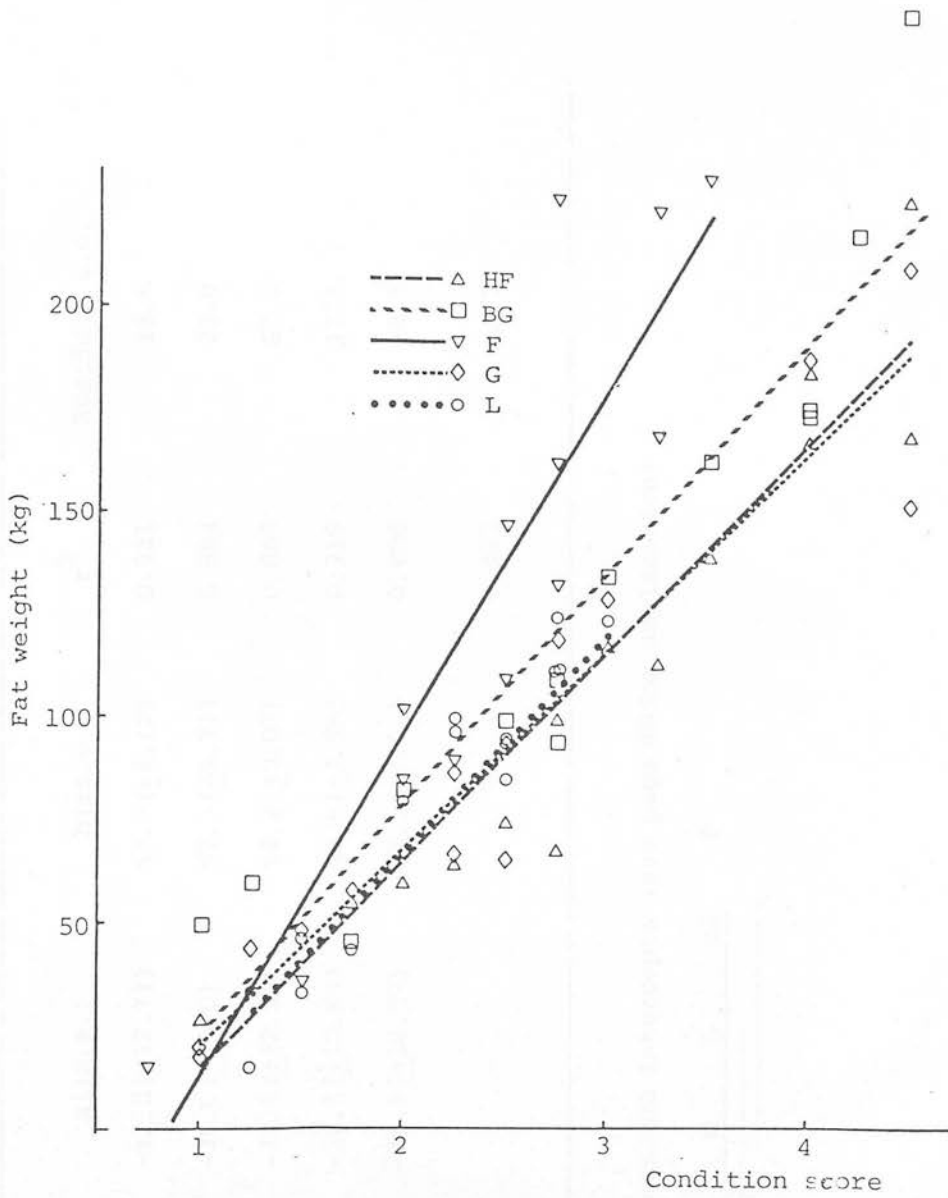



Figure 8.1. Relationship between fat weight and condition score

Table 8.6. Regression of body fat (F, kg) on condition score (CS) using the model F = a + bCS.

| Breed | n | a(±s.e.) | b(±s.e.) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| HF | 14 | -42.0(±12.71) | 52.3(±4.12) | 0.931 | 16.6 |
| BG | 14 | -35.1(±17.0) | 57.3(±5.71) | 0.894 | 23.8 |
| F | 15 | -71.1(±22.00) | 84.2(±9.07) | 0.869 | 27.0 |
| G | 15 | -27.1(±10.84) | 48.6(±3.99) | 0.919 | 17.1 |
| L | 15 | -35.7(±16.07) | 52.1(±7.09) | 0.806 | 16.5 |
| Pooled within breed | 73 | - | - | 0.902 | 20.7 |

Differences in coefficients and intercepts (see page 103 for explanation)

HF G L BG F
----------------- (HF–L)
----------------- (G–BG)

Table 8.7. Regression of body fat (F, kg) on condition score (CS) and live weight (LW, kg) using the model $F = a + b_1CS + b_2LW$.

| Breed | n | a(±s.e.) | $b_1$(±s.e.) | $b_2$(±s.e.) | $R^2$ | Residual s.d. |
|---|---|---|---|---|---|---|
| HF | 14 | -117(±42.8) | 19.3(±18.38) | 0.316(±0.1722) | 0.947 | 15.2 |
| BG | 14 | -133(±27.4) | 18.2(±10.60) | 0.403(±0.1019) | 0.956 | 16.0 |
| F | 14 | -198(±29.9) | 37.9(±11.14) | 0.418(±0.0874) | 0.955 | 16.4 |
| G | 15 | -105(±31.6) | 33.5(±6.69) | 0.246(±0.0949) | 0.948 | 14.3 |
| L | 15 | - 79(±23.7) | 29.0(±11.9) | 0.216(±0.0955) | 0.864 | 14.4 |
| Pooled within breed | 73 | - | - | - | 0.950 | 15.3 |

Differences in coefficients and intercepts (see page 103 for explanation)

L BG G HF F
(L, BG, G underlined by a common line; G, HF underlined by a common line)

The term x + 1 was used in models 2 and 3 because some of the fat depths recorded were 0 and these functions are not defined at 0. Of the four models, model 4 fitted the data best and was subsequently used in all prediction equations involving ultrasonic fat depth.

The regressions of fat weight on the ultrasonic fat depths at the 12-13th ribs site and at the 3rd lumbar site are given in Tables 8.8 and 8.9 respectively. In addition the relationship of fat weight to ultrasonic fat depth at 12-13th ribs is shown graphically in Figure 8.2. There were no significant breed differences in the regressions of fat weight on the fat depth measured ultrasonically at the scapula, femur or leg sites and these regressions are given in Table 8.10.

The numbers of cows scanned on these latter three sites is considerably lower than the total number of cows. Only 63, 51 and 35 cows were scanned on the scapula, femur and leg sites respectively. These numbers reflect the ease with which scans can be made on these sites without incurring danger to the operator. Taking scans of the hind leg in particular was difficult because of the problem of cows kicking, and without efficient restraining apparatus was impossible on many animals.

The $r^2$ values and residual s.d. of the regression of fat weight on the square root of the fat depths for the five different sites (taken from Tables 8.8-8.10) were:-

| Site | $r^2$ | Residual s.d. |
|---|---|---|
| 12-13th rib | 0.804+++ | 29.3+++ |
| 3rd lumbar | 0.689++ | 35.8++ |
| Scapula | 0.466+ | 43.5+ |
| Femur | 0.498+ | 43.1+ |
| Leg | 0.439+ | 40.7+ |

+ pooled ++ pooled with separate intercept +++ pooled within breed

Table 8.8. Regression of fat weight (F, kg) on ultrasonic fat depth at 12-13th ribs (D, mm) using the model $F = a + bD^{\frac{1}{2}}$.

| Breed | n | a(±s.e.) | b(±s.e.) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| HF | 14 | 9.8(±21.72) | 38.6(±7.58) | 0.684 | 35.5 |
| BG | 14 | -29.5(±21.52) | 49.1(±6.40) | 0.831 | 30.1 |
| F | 15 | -11.0(±22.90) | 58.2(±9.11) | 0.759 | 36.5 |
| G | 14 | - 1.0(±11.09) | 38.8(±3.93) | 0.890 | 20.8 |
| L | 15 | 9.7(±12.29) | 34.6(±5.69) | 0.740 | 19.1 |
| Pooled within breed | 73 | - | - | 0.804 | 29.3 |

Differences in coefficients and intercepts (see page 103 for explanation)

L G BG HF F

Table 8.9. Regression of fat weight (F, kg) on ultrasonic fat depth at 3rd lumbar site (D, mm) using the model $F = a + bD^{\frac{1}{2}}$.

| Breed | n | a(±s.e.) | b(± s.e.) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| HF | 14 | - 4.6(±13.86) | 37.0(±3.26) | - | - |
| BG | 14 | - 0.4(±14.51) | | - | - |
| F | 15 | 33.6(±12.10) | | - | - |
| G | 14 | 0.6(±12.59) | | - | - |
| L | 15 | -13.3(±12.25) | | - | - |
| Pooled with separate intercepts | 72 | - | | 0.689 | 35.8 |

Differences in intercepts (see page 103 for explanation) G BG HF L F (G, BG, HF and L joined by one dashed underline; F not underlined)

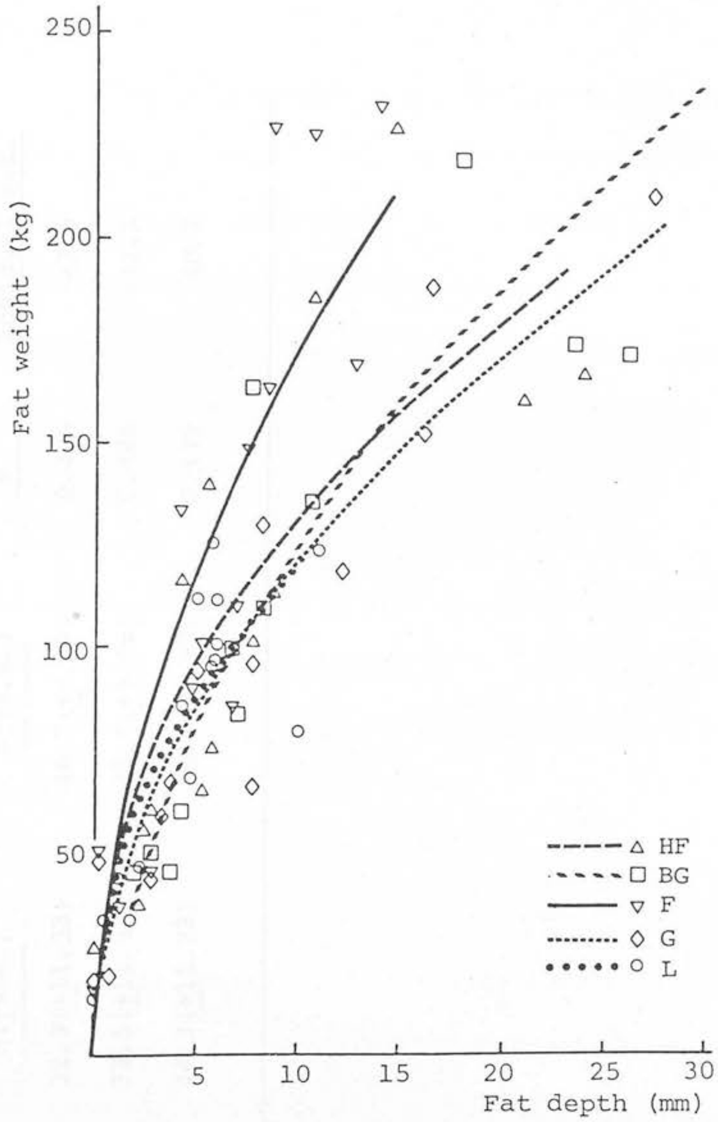



Figure 8.2. Relationship between fat weight and ultrasonic fat depth at 12-13th ribs

Table 8.10. Regressions of fat weight (F, kg) on ultrasonic fat depth (mm) measured at the scapula (S), femur (Fm) and leg (L) using the model $F = a + bx^{\frac{1}{2}}$.

| x | n | a($\pm$s.e.) | b($\pm$s.e.) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| S | 63 | 32.3($\pm$11.53) | 46.7($\pm$6.39) | 0.466 | 43.5 |
| Fm | 51 | 32.6($\pm$11.67) | 45.7($\pm$6.56) | 0.498 | 43.1 |
| L | 35 | 44.3($\pm$11.23) | 35.4($\pm$6.97) | 0.439 | 40.7 |

It thus appears that the best single site for the prediction of body fat is the 12-13th ribs site. In addition, it was not possible to reduce significantly the residual s.d. of that site by the inclusion of any of the other ultrasonic measurements of fat depth in multiple regression equations. Because of this the 3rd lumbar, scapula, femur and leg sites will not be considered further.

Table 8.11 gives the parameters in the multiple regression of fat weight on the ultrasonic fat depth at the 12-13th ribs and live weight. The partial regression coefficients for fat depth were not significant in the case of the HF and F breeds, but were for the other three breeds, and it therefore appears that fat depth, as measured at the 12-13th ribs improves upon the prediction afforded by live weight alone.

Skinfold

The regression of body fat on the skinfold measurement taken over the 13th rib was not significant ($r^2 = 0.054$, $P > 0.05$) indicating that it was of no value in the prediction of body composition.

$D_2O$ space

$D_2O$ space cannot be used on its own to predict body fat, it can be used only in conjunction with live weight. When separate regressions, relating body fat to $D_2O$ space and live weight, were fitted to the data for each breed, the partial regression coefficient for $D_2O$ space was significant in all cases except for the F cows. However, there were no breed differences in these partial regression coefficients, and the data were pooled and separate intercepts fitted for each breed (Table 8.12).

Table 8.11. Regression of fat weight (F, kg) on ultrasonic fat depth at 12-13th ribs (D, mm) and live weight (LW, kg) using the model $F = a + b_1 D^{\frac{1}{2}} + b_2 LW$.

| Breed | n | a(±s.e.) | $b_1$(±s.e.) | $b_2$(±s.e.) | $R^2$ | Residual s.d. |
|---|---|---|---|---|---|---|
| HF | 14 | -152(+24.9) | 2.6(+6.12) | 0.469(+0.666) | 0.943 | 15.8 |
| BG | 14 | -140(+18.6) | 16.2(+5.52) | 0.417(+0.0597) | 0.969 | 13.5 |
| F | 15 | -217(+42.9) | 14.0(+10.23) | 0.549(+0.1085) | 0.923 | 21.5 |
| G | 14 | -104(+39.4) | 24.4(+6.26) | 0.293(+0.1094) | 0.934 | 16.9 |
| L | 15 | - 74(+19.3) | 18.7(+4.89) | 0.265(+0.0564) | 0.908 | 11.8 |
| Pooled within breed | 72 | - | - | - | 0.944 | 16.3 |

Differences in coefficients and intercepts (see page 103 for explanation)

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HF    L    BG    G    F
      --------
      ______________
      --------------
           ______________
           --------------
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Table 8.12. Regression of body fat (F, kg) on deuterium oxide space (DS, kg) and live weight (LW, kg) using the model $F = a + b_1 DS + b_2 LW$.

| Breed | n | a ($\pm$s.e.) | $b_1$ ($\pm$s.e.) | $b_2$ ($\pm$s.e.) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|---|
| HF | 13 | -91 ($\pm$20.3) | -0.557 ($\pm$0.0882) | 0.715 ($\pm$0.0315) | - | - |
| BG | 14 | -77 ($\pm$17.8) | | | - | - |
| F | 15 | -95 ($\pm$19.5) | | | - | - |
| G | 15 | -78 ($\pm$18.2) | | | - | - |
| L | 15 | -78 ($\pm$17.0) | | | - | - |
| Pooled with separate intercepts | 72 | - | | | 0.933 | 16.7 |

Differences in intercepts (see page 103 for explanation) L G BG HF F

Linear measurements

Measurements of body size were used in several ways to estimate body composition. One approach was similar to that used by Yadava (1970). The body size of the cows was estimated by the product of height, length and width and the "average" relationship between live weight (LW, kg) and body size (S, $m^3$) established by regression. This relationship was as follows:-

$$LW = -169(\pm 75.1) + 665(\pm 73.8)S$$

$$(n = 72,\ r^2 = 0.537,\ \text{residual s.d.} = 75)$$

There were no breed differences in this equation.

A condition index was then constructed, being the difference between actual live weight and live weight as predicted by the above equation. Thus, a cow with a positive condition index would be above the average weight for body size, and one with a negative condition index would be below the average weight for body size. The regression of fat weight on condition index is given in Table 8.13. The relationship of fat weight (F, kg) to condition index (CI, kg) and live weight (kg) from pooling the data for all breeds was:-

$$F = -124(\pm 17.4) + 0.139(\pm 0.0500)CI + 0.475(\pm 0.0340)LW$$

$$(n = 72,\ R^2 = 0.866,\ \text{residual s.d.} = 23.0)$$

There were differences between some of the breeds in this relationship, but if the breeds were considered separately the partial regression coefficients for condition index proved, in all cases, to be non-significant ($P > 0.05$), indicating that the variation in body size within a breed was too small for the condition index to be of value in improving upon live weight alone when predicting body fat within a single breed.

Table 8.13. Regression of body fat (F, kg) on condition index (CI, kg) using the model $F = a + bCI$.

| Breed | n | a(±s.e.) | b(±s.e.) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| HF | 14 | 107 (±9.7) | 0.672 (±0.0604) | - | - |
| BG | 14 | 120 (±9.7) | | - | - |
| F | 15 | 142 (±9.5) | | - | - |
| G | 14 | 70 (±9.9) | | - | - |
| L | 15 | 86 (±9.4) | | - | - |
| Pooled with separate intercepts | 72 | - | | 0.680 | 36.2 |

Differences in intercepts (see page 103 for explanation)

```
F     BG     HF     L     G
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                    ------
```

The second approach was to predict fat weight (F, kg) from both live weight (LW, kg) and body size (S, $m^3$) in a multiple regression equation. This equation was as follows:-

$$F = -101(\pm 23.9) - 92(\pm 33.3)S + 0.596(\pm 0.0367)LW$$

$$(n = 72,\ R^2 = 0.866,\ \text{residual s.d.} = 23.0)$$

The $R^2$ value and residual s.d. are identical to that obtained using the condition index. Again when the breeds were considered separately the partial regression coefficients for body size were not significant ($P > 0.05$).

Prediction of body fat from more than one parameter

The prediction of body fat from a combination of methods is considered firstly without live weight and secondly with live weight.

(i) Without live weight

Three indices were available for predicting body fat without the use of live weight, namely condition score, ultrasonic fat depth (as measured between the 12 and 13th ribs) and body size. Body fat was regressed in turn, in multiple regression equations, on each combination of two indices and also on the combination of all three and the residual mean square of each regression (pooled within breed where appropriate) tested against those of the simpler models for significant reductions.

Only two multiple regressions gave a significantly lower residual mean square. These were the regressions of body fat on condition score and body size and on ultrasonic fat depth at the 12-13th ribs site and body size. There was no advantage in using both condition score and ultrasonic fat depth in the same prediction equation.

No breed differences were apparent in the regression of body fat (F, kg) on ultrasonic fat depth at the 12-13th ribs (D, mm) and

body size (S, $m^3$) which was:-

$$F = -151(\pm 26.7) + 36.8(\pm 2.72)D^{\frac{1}{2}} + 164(\pm 28.0)S$$

$$(n = 72, R^2 = 0.823, \text{residual s.d.} = 26.4)$$

There were breed differences in the regressions of body fat on condition score and body size, which are shown in Table 8.14. All partial regression coefficients except that for body size for the HF breed were significant ($P < 0.05$).

(ii) With live weight

Multiple regression equations for predicting body fat from live weight and two, three or four other indices were calculated. Only two of these equations significantly reduced the residual mean square below that given by a simpler regression. These were the regressions of body fat on ultrasonic fat depth at 12-13th ribs, $D_2O$ space and live weight and on condition score, $D_2O$ space and live weight. There were significant breed differences in both these regressions and they are given in Tables 8.15 and 8.16 respectively. Not all the partial regression coefficients were significant in these separate breed regressions, although the residual s.d.'s pooled within breed were significantly lower than those for the simpler models. In the regressions of Table 8.15 the partial regression coefficients for fat depth for the HF, F and G cows were not significant ($P > 0.05$) nor were those for $D_2O$ space for the BG, F and G cows. In Table 8.16 the partial regression coefficient for condition score was not significant for the HF cows nor were those for $D_2O$ space in the equations with the F and G cows.

Comparison of different indices for predicting body fat

The $r^2$ (or $R^2$) values and the residual s.d. for the regression equations for predicting body fat are given in Table 8.17. In general

Table 8.14. Regression of body fat (F, kg) on condition score (CS) and body size (S, $m^3$) using the model $F = a + b_1CS + b_2S$

| Breed | n | a (±s.e.) | $b_1$ (± s.e.) | $b_2$ (±s.e.) | $R^2$ | Residual s.d. |
|---|---|---|---|---|---|---|
| HF | 14 | -100 (±67.0) | 48.7 (±5.82) | 64 (±72.9) | 0.936 | 16.7 |
| BG | 14 | -196 (±57.7) | 50.3 (±5.12) | 178 (±61.9) | 0.939 | 18.8 |
| F | 15 | -283 (±63.8) | 72.1 (±7.56) | 210 (±61.2) | 0.934 | 19.9 |
| G | 14 | -172 (±66.6) | 45.6 (±3.87) | 167 (±76.4) | 0.945 | 15.4 |
| L | 15 | -151 (±54.9) | 40.4 (±8.23) | 152 (±70.1) | 0.861 | 14.6 |
| Pooled within breed | 72 | - | - | - | 0.938 | 17.2 |

Differences in coefficients and intercepts (see page 103 for explanation)

```
HF   L   G   BG   F
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     --------------
```

Table 8.15. Regression of body fat (F, kg) on ultrasonic fat depth at 12-13th ribs (D, mm), deuterium oxide space (DS, kg) and live weight (LW, kg) using the model $F = a + b_1 D^{\frac{1}{2}} + b_2 DS + b_3 LW$

| Breed | n | a (±s.e.) | $b_1$ (±s.e.) | $b_2$ (±s.e.) | $b_3$ (±s.e.) | $R^2$ | Residual s.d. |
|---|---|---|---|---|---|---|---|
| HF | 13 | -88 (±28.8) | -1.5 (±4.91) | -0.452 (±0.1609) | 0.654 (±0.0837) | 0.970 | 12.2 |
| BG | 14 | -91 (±31.2) | 12.5 (±5.35) | -0.322 (±0.1720) | 0.531 (±0.0811) | 0.977 | 12.2 |
| F | 15 | -130 (±57.0) | 15.2 (±9.09) | -0.473 (±0.2296) | 0.665 (±0.1113) | 0.945 | 19.0 |
| G | 14 | -31 (±95.8) | 18.5 (±9.44) | -0.379 (±0.4498) | 0.408 (±0.1756) | 0.938 | 17.1 |
| L | 15 | -43 (±14.7) | 16.1 (±3.26) | -0.389 (±0.0943) | 0.455 (±0.0589) | 0.964 | 7.7 |
| Pooled within breed | 71 | - | - | - | - | 0.961 | 14.3 |

Differences in coefficients and intercepts (see page 103 for explanation)

HF G BG L F

Table 8.16. Regression of body fat (F, kg) on condition score (CS), deuterium oxide space (DS, kg) and live weight (LW, kg) using the model $F = a + b_1CS + b_2DS + b_3LW$

| Breed | n | a(±s.e.) | $b_1$(±s.e.) | $b_2$(±s.e.) | $b_3$(±s.e.) | $R^2$ | Residual s.d. |
|---|---|---|---|---|---|---|---|
| HF | 13 | -88(±37.5) | 0.7 (±16.63) | -0.441(±0.1673) | 0.643(±0.1814) | 0.970 | 12.2 |
| BG | 14 | -52(±34.9) | 18.3(±8.16) | -0.473(±0.1619) | 0.519(±0.0879) | 0.976 | 12.3 |
| F | 15 | -145(±40.8) | 34.3(±10.45) | -0.330(±0.1859) | 0.530(±0.1025) | 0.965 | 15.1 |
| G | 15 | -47(±80.2) | 28.0(±9.61) | -0.289(±0.3616) | 0.334(±0.1453) | 0.951 | 14.5 |
| L | 15 | -45(±20.7) | 24.0(±9.10) | -0.420(±0.1316) | 0.433(±0.0988) | 0.929 | 10.8 |
| Pooled within breed | 72 | - | - | - | - | 0.967 | 13.1 |

Differences in coefficients and intercepts (see page 103 for explanation)

```
HF      G      L      BG      F
--------------
       -------------
```

Table 8.17. Precision of prediction of body fat from different indices

| Indices[‡] | $r^2$ or $R^2$ | Residual s.d. | Residual s.d. as % of mean |
|---|---|---|---|
| LW | $0.912^{+}$ | $19.5^{+}$ | 18.6 |
| D | $0.804^{+}$ | $29.3^{+}$ | 27.9 |
| CS | $0.902^{+}$ | $20.7^{+}$ | 19.7 |
| CI | $0.680^{++}$ | $36.2^{++}$ | 34.4 |
| D and LW | $0.944^{+}$ | $16.3^{+}$ | 15.5 |
| CS and LW | $0.950^{+}$ | $15.3^{+}$ | 14.5 |
| CI and LW | $0.866^{+++}$ | $23.0^{+++}$ | 21.9 |
| S and LW | $0.866^{+++}$ | $23.0^{+++}$ | 21.9 |
| DS and LW | $0.933^{++}$ | $16.7^{++}$ | 15.9 |
| CS and S | $0.938^{+}$ | $17.2^{+}$ | 16.4 |
| D and S | $0.823^{+++}$ | $26.4^{+++}$ | 25.2 |
| D, DS and LW | $0.961^{+}$ | $14.3^{+}$ | 13.6 |
| CS, DS and LW | $0.967^{+}$ | $13.1^{+}$ | 12.5 |

‡ LW = live weight

D = ultrasonic fat depth at 12-13th ribs site

CS = condition score

CI = condition index

S = body size

DS = $D_2O$ space

$^{+}$ pooled within breed $^{++}$ pooled with separate intercepts $^{+++}$ pooled data for all breeds

the degree of precision of prediction increases with the number of indices used to predict body fat.

Prediction of body protein

A summary of the measurements used in the prediction of body protein is given in Table 8.18, while full data for each cow are given in Appendix II. Summaries of the data for live weight (Table 5.1) and $D_2O$ space (Table 8.1) have already been given.

Live weight

The regression of body protein on live weight is given in Table 8.19.

$D_2O$ space

The relationship of body protein (P, kg) to $D_2O$ space (DS, kg) was:-

$$P = 5.9(\pm 5.35) + 0.217(\pm 0.0173)DS$$

$$(n = 72,\ r^2 = 0.694,\ \text{residual s.d.} = 6.01)$$

When $D_2O$ space was included with live weight in a multiple regression equation, then both partial correlation coefficients were significant and this regression is shown in Table 8.20.

Evans Blue blood volume and red cell volume

The regressions of body protein on Evans Blue blood volume and red cell volume are given in Tables 8.21 and 8.22 respectively. The use of both blood volume and red cell volume in a multiple prediction equation did not significantly improve upon the prediction afforded by either alone. Also, there was no advantage in including either in a multiple regression equation with live weight.

Ultrasonic eye-muscle area

Tables 8.23 and 8.24 give the regressions of body protein on ultrasonically determined eye-muscle area at the 12-13th ribs and 3rd lumbar sites respectively. When both estimates of eye-

Table 8.18. Summary of some measurements used in the prediction of body protein (Values are means and ranges)

| Breed | Body protein (kg) | Evans Blue dilution | | Ultrasonic eye-muscle area ($cm^2$) | |
|---|---|---|---|---|---|
| | | Blood volume (l) | Red cell volume (l) | 12-13th ribs | 3rd lumbar |
| HF | 78.8<br>59.1-92.7 | 33.7<br>23.9-46.0 | 13.1<br>9.3-19.9 | 60.1<br>26.8-89.6 | 59.6<br>23.1-98.4 |
| BG | 71.6<br>60.8-93.4 | 31.0<br>21.9-40.7 | 13.3<br>9.2-18.7 | 57.9<br>26.4-104.5 | 53.4<br>33.9-87.1 |
| F | 79.7<br>66.4-91.3 | 37.8<br>27.3-48.9 | 15.2<br>12.0-23.8 | 60.0<br>28.6-104.7 | 59.4<br>32.5-99.9 |
| G | 67.9<br>55.4-82.2 | 30.1<br>24.4-35.8 | 13.1<br>9.7-19.6 | 59.2<br>20.6-107.3 | 49.8<br>26.7-85.8 |
| L | 65.5<br>52.1-82.5 | 28.7<br>20.4-36.9 | 12.9<br>9.3-19.1 | 51.6<br>27.0-82.0 | 45.0<br>23.6-80.4 |

Table 8.19. Regression of body protein (P, kg) on live weight (LW, kg) using the model $P = a + b_1 LW + b_2 LW^2$

| Breed | n | a (±s.e.) | $b_1$ (±s.e.) | $b_2$ (±s.e.) | $R^2$ | Residual s.d. |
|---|---|---|---|---|---|---|
| HF | 14 | 4.4 (±8.76) | 0.197 (±0.0341) | $-1.07 \times 10^{-4}$ (±$3.23 \times 10^{-5}$) | - | - |
| BG | 14 | -0.2 (±8.73) | | | - | - |
| F | 15 | 3.6 (±8.86) | | | - | - |
| G | 15 | -0.8 (±8.84) | | | - | - |
| L | 15 | 0.5 (±8.66) | | | - | - |
| Pooled with separate intercepts | 73 | - | | | 0.918 | 3.21 |

Differences in intercepts (see page 103 for explanation)

```
HF------F     L     BG     G
        -------
              ---------------
```

Table 8.20. Regression of body protein (P, kg) on $D_2O$ space (DS, kg) and live weight (LW, kg) using the model $P = a + b_1DS + b_2LW + b_3LW^2$

| Breed | n | a(±s.e.) | $b_1$(±s.e.) | $b_2$(±s.e.) | $b_3$(±s.e.) | $R^2$ | Residual s.d. |
|---|---|---|---|---|---|---|---|
| HF | 13 | 0.4(±8.81) | 0.034(±0.0169) | 0.179(±0.0351) | $-9.82\times10^{-5}$ ($\pm3.228\times10^{-5}$) | - | - |
| BG | 14 | -3.3(±8.71) | | | | - | - |
| F | 15 | -0.1(±8.88) | | | | - | - |
| G | 15 | -4.3(±8.85) | | | | - | - |
| L | 15 | -2.7(±8.64) | | | | - | - |
| Pooled with separate intercepts | 72 | - | | | | 0.923 | 3.15 |

Differences in intercepts (see page 103 for explanation) G BG L HF F

(G – BG – L underlined; BG – L – HF underlined; L – HF – F underlined)

Table 8.21. Regression of body protein (P, kg) on Evans Blue blood volume (BV, l) using the model $P = a + bBV$

| Breed | n | a(±s.e.) | b(±s.e.) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| HF | 13 | 50.5(±6.34) | 0.881(±0.1768) | - | - |
| BG | 14 | 44.3(±5.86) | | - | - |
| F | 14 | 46.7(±6.99) | | - | - |
| G | 13 | 42.3(±5.75) | | - | - |
| L | 13 | 39.8(±5.53) | | - | - |
| Pooled with separate intercepts | 67 | - | | 0.513 | 7.83 |

Differences in intercepts (see page 103 for explanation) HF F BG L G

(dashed lines underneath join HF–F; F–BG–L; BG–L–G)

Table 8.22. Regression of body protein (P, kg) on Evans Blue red cell volume (RV, l) using the model P = a + bRV

| Breed | n | a (±s.e.) | b (±s.e.) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| HF | 13 | 59.5 (±4.61) | 1.59 (±0.312) | - | - |
| BG | 14 | 50.5 (±4.63) | | - | - |
| F | 14 | 55.7 (±5.18) | | - | - |
| G | 13 | 48.0 (±4.62) | | - | - |
| L | 13 | 44.7 (±4.56) | | - | - |
| Pooled with separate intercepts | 67 | - | | 0.519 | 7.78 |

Differences in intercepts (see page 103 for explanation) HF F BG G L (HF–F underlined together; F–BG–G–L underlined together)

Table 8.23. Regression of body protein (P, kg) on ultrasonic eye-muscle area at 12-13th ribs site ($A$, $cm^2$) using the model $P = a + bA$

| Breed | n | a(±s.e.) | b(±s.e.) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| HF | 14 | 57.5(±2.45) | 0.354(±0.0323) | - | - |
| BG | 14 | 51.2(±2.39) | | - | - |
| F | 15 | 58.5(±2.42) | | - | - |
| G | 14 | 46.8(±2.43) | | - | - |
| L | 15 | 47.2(±2.21) | | - | - |
| Pooled with separate intercepts | 72 | | | 0.748 | 5.61 |

Differences in intercepts (see page 103 for explanation) F HF BG L G

Table 8.24. Regression of body protein (P, kg) on ultrasonic eye-muscle area at 3rd lumbar site (A, $cm^2$) using the model $P = a + bA$

| Breed | n | a(±s.e.) | b(± s.e.) | $r^2$ | Residual s.d. |
|---|---|---|---|---|---|
| HF | 14 | 56.4 (±3.25) | 0.376 (±0.0458) | - | - |
| BG | 14 | 51.6 (±3.02) | | - | - |
| F | 15 | 57.6 (±3.21) | | - | - |
| G | 14 | 49.0 (±2.89) | | - | - |
| L | 15 | 48.6 (±2.68) | | - | - |
| Pooled with separate intercepts | 72 | - | | 0.649 | 6.63 |

Differences in intercepts (see page 103 for explanation) F HF BG G L

muscle area were included in a multiple regression equation the precision of prediction was significantly better than when only one was used, and this multiple regression is given in Table 8.25 When included with live weight in a multiple regression equation neither improved upon the prediction given by live weight alone.

Prediction of body protein from more than one parameter

Multiple regression equations were constructed and tested in the same way as those for body fat. There was no advantage in including any index other than $D_2O$ space in a multiple regression equation with live weight. Any combination of two of the three indices, Evans Blue red cell volume, $D_2O$ space and ultrasonic eye-muscle area at 12-13th ribs significantly increased upon the precision of prediction of body protein given by only one index. There was no advantage in using all three together. In some cases the use of blood volume and eye-muscle area at the 3rd lumbar site in multiple regression equations also proved useful, but the $R^2$ values were lower than those given by red cell volume and 12-13th ribs eye-muscle area, and so these regressions are not presented.

The regression of body protein (P, kg) on Evans Blue red cell volume (RV, l) and $D_2O$ space (DS, kg) was:-

$$P = 3.7(\pm 5.03) + 0.515(\pm 0.2422)RV + 0.203(\pm 0.0183)DS$$

$$(n = 66,\ R^2 = 0.756,\ \text{residual s.d.} = 5.45)$$

The regression of body protein on red cell volume and 12-13th ribs eye-muscle area is given in Table 8.26, while Table 8.27 gives the regression of body protein on $D_2O$ space and 12-13th ribs eye-muscle area.

Comparison of indices for predicting body protein

The $r^2$ (or $R^2$) values and the residual s.d. of the various prediction equations for predicting body protein are given in Table 8.28.

Table 8.25. Regression of body protein (P, kg) on ultrasonic eye-muscle area at 12-13th ribs ($A_R$, $cm^2$) and 3rd lumbar ($A_L$, $cm^2$) sites using the model $P = a + b_1A_R + b_2A_L$

| Breed | n | a(±s.e.) | $b_1$ (±s.e.) | $b_2$ (±s.e.) | $R^2$ | Residual s.d. |
|---|---|---|---|---|---|---|
| HF | 14 | 54.6 (±2.69) | 0.272 (±0.0472) | 0.131 (±0.0567) | - | - |
| BG | 14 | 48.9 (±2.52) | | | - | - |
| F | 15 | 55.7 (±2.65) | | | - | - |
| G | 14 | 45.1 (±2.46) | | | - | - |
| L | 15 | 45.6 (±2.26) | | | - | - |
| Pooled with separate intercepts | 72 | - | | | 0.767 | 5.43 |

Differences in intercepts (see page 103 for explanation) F - - - HF BG - - - L - - - G

(Dashed lines join F–HF, BG–L, and L–G.)

Table 8.26. Regression of body protein (P, kg) on Evans Blue red cell volume (RV, l) and ultrasonic eye-muscle area at 12-13th ribs site (A, $cm^2$) using the model $P = a + b_1RV + b_2A$

| Breed | n | a (±s.e.) | $b_1$ (±s.e.) | $b_2$ (±s.e.) | $R^2$ | Residual s.d. |
|---|---|---|---|---|---|---|
| HF | 13 | 50.1 (±3.23) | 0.868 (±0.2258) | 0.300 (±0.0339) | - | - |
| BG | 14 | 42.7 (±3.20) | | | - | - |
| F | 14 | 48.8 (±3.53) | | | - | - |
| G | 12 | 38.0 (±3.31) | | | - | - |
| L | 13 | 38.9 (±3.10) | | | - | - |
| Pooled with separate intercepts | 66 | - | | | 0.796 | 5.15 |

Differences in intercepts (see page 103 for explanation) HF F BG L G

Table 8.27. Regression of body protein (P, kg) on $D_2O$ space (DS, kg) and ultrasonic eye-muscle area at 12-13th ribs site (A, $cm^2$) using the model $P = a + b_1DS + b_2A$

| Breed | n | a (±s.e.) | $b_1$ (±s.e.) | $b_2$ (±s.e.) | $R^2$ | Residual s.d. |
|---|---|---|---|---|---|---|
| HF | 13 | 22.0 (±4.87) | 0.127 (±0.0161) | 0.237 (±0.0274) | - | - |
| BG | 14 | 20.0 (±4.31) | | | - | - |
| F | 15 | 23.8 (±4.73) | | | - | - |
| G | 14 | 15.7 (±4.31) | | | - | - |
| L | 15 | 18.0 (±4.03) | | | - | - |
| Pooled with separate intercepts | 71 | - | | | 0.878 | 4.04 |

Differences in intercepts (see page 103 for explanation) F HF BG L G

(dashed lines under the breeds: F–HF; HF–BG–L; L–G)

Table 8.28. Precision of prediction of body protein from different indices

| Indices[ǂ] | $r^2$ or $R^2$ | Residual s.d. | Residual s.d. as % of mean |
|---|---|---|---|
| LW | $0.918^{+}$ | $3.21^{+}$ | 4.42 |
| DS | $0.694^{++}$ | $6.01^{++}$ | 8.27 |
| BV | $0.513^{+}$ | $7.83^{+}$ | 10.78 |
| RV | $0.519^{+}$ | $7.78^{+}$ | 10.71 |
| $A_R$ | $0.748^{+}$ | $5.61^{+}$ | 7.72 |
| $A_L$ | $0.649^{+}$ | $6.63^{+}$ | 9.13 |
| DS and LW | $0.923^{+}$ | $3.15^{+}$ | 4.34 |
| $A_R$ and AL | $0.767^{+}$ | $5.43^{+}$ | 7.47 |
| RV and DS | $0.756^{++}$ | $5.45^{++}$ | 7.50 |
| RV and $A_R$ | $0.796^{+}$ | $5.15^{+}$ | 7.08 |
| DS and $A_R$ | $0.878^{+}$ | $4.04^{+}$ | 5.56 |

ǂ LW = live weight

DS = $D_2O$ space

BV = Evans Blue blood volume

RV = Evans Blue red cell volume

$A_R$ = Ultrasonic eye-muscle area at 12-13th ribs site

$A_L$ = Ultrasonic eye-muscle area at 3rd lumbar site

+ pooled data with separate intercepts for each breed

++ pooled data for all breeds

Prediction of weight of bone from cannon bone circumference

A summary of carcass bone weight and cannon bone circumference is given in Table 8.29.

Table 8.29. Summary of weight of carcass bone and cannon bone circumference

| Breed | Carcass bone (kg) | | Cannon bone circumference (cm) | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| HF | 42.2 | 34.9 - 48.5 | 19.6 | 18.8 - 21.4 |
| BG | 36.8 | 33.8 - 40.4 | 18.5 | 17.6 - 19.4 |
| F | 45.5 | 39.1 - 54.1 | 18.5 | 16.6 - 20.2 |
| G | 36.5 | 30.7 - 46.8 | 18.4 | 17.1 - 20.0 |
| L | 36.2 | 31.2 - 46.0 | 18.6 | 17.4 - 20.2 |
| Overall | 39.4 | 30.7 - 54.1 | 18.7 | 16.6 - 21.4 |

When the weight of bone in the carcass was related to cannon bone circumference the regression was only significant for the L breed ($n = 10$, $r^2 = 0.810$). The regressions for the other four breeds were all non-significant ($r^2 < 0.188$). When the data for all breeds were pooled the regression relating carcass bone (B, kg) to cannon bone circumference (C, cm) was significant:-

$$B = -4.7\ (\pm 11.9) + 2.37(\pm 0.634)C$$

$$(n = 64,\ r^2 = 0.184,\ \text{residual s.d.} = 4.73)$$

DISCUSSION

Prediction of total body water

Live weight

The relationship of total body water to live weight is of the

same form as that relating empty body water to empty body weight (Table 6.3), increasing with live weight, but at a decreasing rate. In both relationships there were no significant breed differences in slope, and the intercepts form two groups, with the HF and F cows being higher than the BG, G and L cows.

$D_2O$ space

The overestimation of total body water by $D_2O$ space of 3.37% agrees very well with that found by Foot and Greenhalgh (1970) in pregnant ewes, when $D_2O$ space was 2.98% greater than total body water. In this study food and water were withheld between infusion and the collection of the blood sample for determination of $D_2O$ concentration, while in the experiment of Foot and Greenhalgh (1970) the ewes had continuous access to food and water. In steers Crabtree *et al.* (1974) found $D_2O$ space was 9.6% greater than total body water, and TOH space has been found to overestimate total body water by as much as 20% (Little and McLean, 1980) although in the latter case it is not reported whether food and water were withheld.

It is probable that by starving the cows and withholding water for a time prior to infusion of $D_2O$ as well as during the equilibration period, the level of overestimation of total body water could have been reduced because of a decrease in water turnover and a consequent decrease in loss of $D_2O$. However, in circumstances where $D_2O$ dilution is likely to prove useful in the estimation of body composition in suckler cows, doing so would lead to unacceptable interruption in the animals' feeding activity and possibly lactational performance.

The precision with which $D_2O$ space predicts total body water is perhaps more important than the magnitude of any discrepancy between total body water and $D_2O$ space. The residual s.d. of the regression of total body water on $D_2O$ space, expressed as a percentage of the mean total body water, is 6.4% which is slightly higher than

values calculated from other studies e.g. 3.9% in sheep (Foot and Greenhalgh, 1970) and 4.2% in steers (Crabtree *et al.*, 1974).

In view of the results of Russel *et al.* (1981) is it possible that the degree of precision of prediction of total body water could have been increased by using the extrapolation procedure and also by considering water turnover rate. This however necessitates the collection of more blood samples and incurs increased analytical costs.

The residual s.d. was reduced to 14.4 kg (4.8 % of the mean) when live weight and $D_2O$ space were used together to predict total body water.

Prediction of body fat

From the point of view of suckler cow nutrition, fat is perhaps the most important body component , since it is the major store of energy in the body.

Live weight

The single most effective index for measuring body fat was, not unexpectedly, live weight. It must be remembered, however, that the range of live weight had been deliberately extended, and so the $r^2$ values relating body fat to live weight were higher than might be expected with a reduced range of live weight. Indeed this was so for the regression equation relating body fat to live weight in the L breed (Table 8.3) which had a smaller range of live weight than the other breeds.

It was shown earlier in Chapter 6 that the underlying relationship between body fat and empty body weight was curvilinear, and it would be reasonable to assume that the relationship between body fat and live weight should show the same trend. However, since there appeared to be breed differences in the relationship of body

fat to live weight, each breed had to be considered separately and with the numbers involved, it was not possible to show any significant curvilinearity in the relationships.

Condition score

The breed differences in the relationships between fat weight and condition score reflect the differences between breeds in fat partition. However, because of the different sizes of the breeds, the ranking of the breeds in the relationship of body fat to condition score is not the same as that indicated in Figure 7.3 where the proportion of fat in the subcutaneous depot was related to the proportion of fat in the empty body. If, however, fat as a percentage of the live weight is related to condition score, as in Figure 8.3, then a similar pattern to that in Figure 7.3 emerges. At low condition scores all breeds were similar in the proportion of fat, but at higher conditions scores the F cows were fatter because they had a lower proportion of their total fat in the subcutaneous depot. Similarly, the HF cows were the least fat at higher condition scores because they had the greatest proportion of subcutaneous fat. In both Figures the BG, G and L cows occupy intermediate positions.

It was speculated in Chapter 7 that pure Hereford cows might have a greater proportion of their total fat in the subcutaneous depot than any of the breeds studied in this experiment. If this is the case then at higher condition scoresthey might be less fat than any of the five breeds studied here. This could have important implications if condition scoring is used as the basis on which to make management decisions with regard to suckler cow feeding, especially if recommendations are made as to the "target" condition score that should be achieved at particular times of the year.

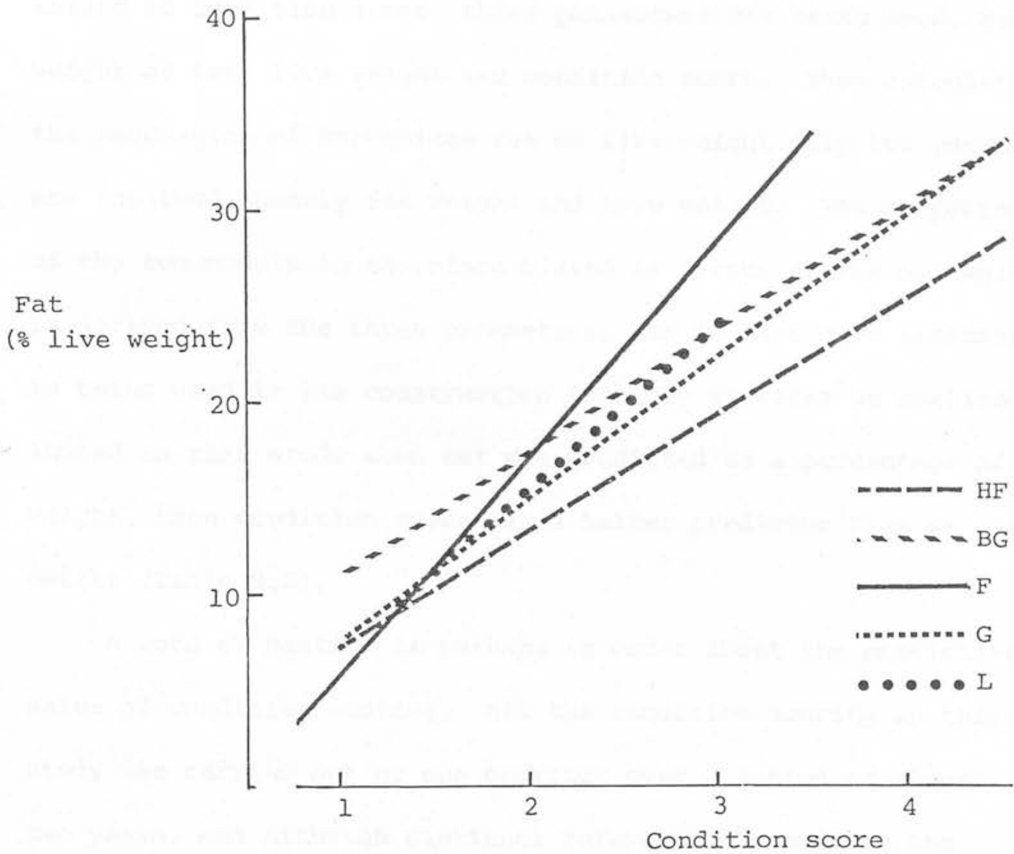



Figure 8.3. Relationship between percentage fat in live weight and condition score

Clearly this area needs further examination.

The prediction of body fat from condition score appears to be almost as good as that from live weight. Russel *et al.* (1969) found that condition score predicted the percentage of fat in the live weight of sheep better than did live weight. However, when calculating the regression relating percentage of fat in live weight to condition score, three parameters are being used, namely weight of fat, live weight and condition score. When calculating the regression of percentage fat on live weight only two parameters are involved, namely fat weight and live weight. The comparison of the two models is therefore biased in favour of the one which is derived from the three parameters, for in fact more information is being used in its construction than may at first be realised. Indeed in this study when fat was predicted as a percentage of live weight, then condition score was a better predictor than was live weight (Table 8.2).

A word of caution is perhaps in order about the predictive value of condition scoring. All the condition scoring in this study was carried out by one operator over a period of almost two years, and although continual reference was made to the definition of each point on the condition score scale, and periodically the standards were checked with one of the authors of the system described by Lowman *et al.* (1976), it is conceivable that the scoring was inconsistent over the two year period.

It is also known that, as with any subjective assessment, there are differences between operators in condition scoring, and if these are taken into consideration then the real predictive value of condition scoring is probably less than that indicated

by the results of this study. Between-operator differences are perhaps not so important if a number of people condition score each animal, as suggested by Evans (1978). If one is more interested in condition score change than absolute condition, e.g. in an experiment designed to assess the effect of two levels of nutrition on change in condition, then between-operator differences are probably less important. However, if absolute levels of condition are of interest then between-operator differences could become very important, especially if there is perhaps a minimum (or maximum) body condition score necessary to achieve required levels of performance.

Nevertheless, condition scoring offers a very cheap, non-destructive method of assessing the amount of fat in the bodies of cows with a degree of precision that is perfectly acceptable for use in commercial situations and for many research purposes, provided that the breed differences which result from the differences in fat partition are appreciated.

Ultrasonic subcutaneous fat depth

For prediction of total body fat from ultrasonic fat depth the 12-13th ribs site was the best of the five sites examined, and there appeared to be no advantage in using any of the other positions. The predictive value of fat depth (residual s.d. = 29.3 kg) appears to be less than that offered by condition scoring (residual s.d. = 20.7 kg). However, the ultrasonic measurement of fat depth is a more objective measure than condition scoring and differences between experienced operators and interpreters of ultrasonic photographs would appear to be small (Moody *et al.*, 1965; Tulloh *et al.*, 1973).

The main reason for misinterpretation of photographs is misidentification of tissue boundaries. This problem is less important in leaner animals, since there is not the problem of having to identify two layers of subcutaneous fatty tissue, and so interpretation of Scanogram photographs taken on suckler cows, which are in general not as fat as young commercial beef animals ready for slaughter, should be more consistent.

The problem of two separate layers of fatty tissue may be part of the reason for the poorer predictive value of the 3rd lumbar site, since at this position the two layers were apparent on thinner cows before they could be seen at the 12-13th ribs site, and so misidentification of boundaries or confusion with multiple reflections may have led to misinterpretation of the photographs. The layer of subcutaneous fatty tissue on the other three sites appeared to be rather variable and disjointed, particularly on thin cows, which probably explains their poor predictive value.

The breed differences in the regressions of body fat on fat depth can be explained by differences in fat partition in the same way as the breed differences in condition scoring.

It therefore appears that, provided breed differences are taken into account, ultrasonic measurement of fat depth between the 12th and 13th ribs offers an objective measure of the amount of fat present in the bodies of cows, particularly when used in conjunction with live weight.

Skinfold

The results of this study confirm those of Tulloh (1961); that skinfold as measured over the 13th rib is of no value in the prediction of body fat. The reason for this is probably that

the *M. cutaneus trunci* prevents the subcutaneous fatty tissue being included in the thickness measurement.

$D_2O$ space

To predict body fat from $D_2O$ space, live weight must also be known. The level of prediction afforded by $D_2O$ space and live weight (residual s.d. = 16.7 kg) seems to be acceptable. The residual s.d. as a percentage of the mean (15.9%) is similar to that found by Crabtree *et al.* (1974) in steers (14.0%) and Houseman *et al.* (1978) in ewes at 90 days of lactation (14.9%). It is, however, higher than that found by Foot and Greenhalgh (1970) (4.2%) in pregnant sheep and by Cowan *et al.* (1979) in ewes at 12 days of lactation (6.5%). These latter two studies used, however, only 7 and 4 sheep respectively.

It is possible that the use of the extrapolation procedure would have improved the relationship between $D_2O$ space and total body water as explained on page 191 , thus giving an improved prediction of body fat.

Body size

When the data for all breeds were pooled, body size in conjunction with live weight offered an improvement over the use of live weight on its own. However, within breeds it proved to be of no value. In situations where live weight is confounded by gut-fill changes or by the products of conception, body size can be used in conjunction with either condition score or ultrasonic fat depth to improve upon the prediction afforded by one of these techniques alone.

The objectively determined condition index proved to be less useful than subjective body condition score and would appear to have little to offer in the way of predictive ability.

Multiple prediction equations

The attempt to improve upon the precision of prediction of body fat by a combination of techniques proved to be successful. The lowest residual s.d.'s were obtained when body fat was predicted from live weight, $D_2O$ space and ultrasonic fat depth (residual s.d. = 14.3 kg) or live weight, $D_2O$ space and condition score (residual s.d. = 13.1 kg). The combination of a direct measure (condition score or ultrasonic fat depth) with an indirect measure ($D_2O$ space and live weight) thus appears to be a valid means of increasing the precision of prediction of body composition as has been suggested by Houseman (1972) and Russel and Wright (1981).

## Prediction of body protein

Live weight

The curvilinear relationship between body protein and live weight (i.e. body protein increasing with live weight, but at a decreasing rate) is very similar to that relating body protein to empty body weight in Table 6.3. The best single measurement for predicting body protein was live weight.

$D_2O$ space

There was a relatively close correlation between $D_2O$ space and body protein, giving a residual s.d. of 6.01 kg when body protein was predicted from $D_2O$ space. $D_2O$ space was the only measurement that was of any value in a multiple regression equation with live weight, and in fact the combination of these two measurements offered the best prediction of body protein of any of the prediction equations examined.

Evans Blue blood volume and red cell volume

Since quantitative collection of blood at slaughter was not

possible there was no useful purpose to be served by relating the indirect measures of blood volume to the volume of blood collected at slaughter. The mean blood volume was 64.8 ml/kg live weight with a standard deviation of 11.72. This value will be a slight overestimate since no correction was made for trapped plasma when the haematocrit was measured. There is, however, general agreement with the volumes reported in other studies with cattle e.g. Reynolds (1953*b*) (57.4 ml/kg live weight) and Dalton and Fisher (1961) (63 ml/kg live weight).

Blood volume and red cell volume as estimated by Evans Blue dilution were less useful predictors of body protein than the other indices. Red cell volume was marginally better than blood volume.

With steers Little and Morris (1972) found Evans Blue red cell volume to be a less useful predictor of body water than TOH space, although they concluded that red cell volume could provide useful information on body composition. There was no advantage in combining $D_2O$ space and either red cell volume or blood volume in a multiple regression equation to predict body protein. Crabtree (1976) similarly found no advantage in combining these two measurements to predict total body water in steers.

The major problem associated with the measurement of blood volume and red cell volume is the variation in the haematocrit due to the release or red blood cells from the spleen. No attempt was made to standardise this by the administration of adrenaline as it was considered that if the technique was to be of value in the routine measurement of body composition in suckler cows it would have to be so without the use of the hormone.

It is doubtful whether alone red cell volume or blood volume would be of use in the determination of body protein in suckler cows.

Ultrasonic measurement of eye-muscle area

To date most work on the ultrasonic measurement of eye-muscle area has been concerned with either the correlation between direct and indirect measurement of eye-muscle area or with the prediction of carcass composition. However, there appears to be a reasonably close relationship between body protein and ultrasonic eye-muscle area, particularly at the 12-13th ribs site. Indeed, apart from live weight, eye-muscle area measurement at this site gave the best prediction of body protein from a single measurement. The use of both sites gave a small improvement over the use of only one site.

Prediction of body protein from more than one index

When $D_2O$ space, Evans Blue red cell volume and ultrasonic eye-muscle area at 12-13th ribs were used in multiple regressions in any combination of two indices, the level of prediction of body protein was improved compared to that given when only one was used. The best prediction of body protein without the use of live weight was given by a combination of $D_2O$ space and ultrasonic eye-muscle area at 12-13th ribs, when the residual s.d. was 4.04 kg.

Prediction of carcass bone from cannon bone circumference

Even although there was a significant correlation between carcass bone and cannon bone circumference it would appear that the relationship is of little value for predictive purposes.

The use of prediction equations to estimate body composition in suckler cows

Various prediction equations have been presented for the prediction of different body components, but no indication has been given as to whether they are likely to prove useful in the context

of nutritional investigationswith suckler cows. It is essential that, before deciding upon the use of a prediction equation, the equation be examined to see if it is satisfactory for the intended purpose.

The body component likely to be of most interest in nutritional research with suckler cows is body fat, and it is possible to calculate the minimum difference in fat content of two groups of cows required to show significant differences when a prediction equation is used. As an example, consider the prediction equation for body fat for BG cows, based on condition score, $D_2O$ space and live weight which gave a residual s.d. of 12.3 kg (Table 8.16). To ease calculation consider a group of cows, four in number, which have values for condition score, $D_2O$ space and live weight which give the same mean values as the mean values of the data used in the derivation of the prediction equation. The predicted mean body fat is 124 kg. The number of observations used in the calculation of the regression equation was 14. In this case the s.e. of the mean value for the body fat of the four cows, based on the prediction from the mean of the four values of condition score, $D_2O$ space and live weight can be calculated from the formula:-

$$\text{s.e.} = \text{residual s.d.} \sqrt{\frac{1}{m} + \frac{1}{n}}$$

where m = no. of observations making up mean of group to be predicted, and n = no. of observations used in calculation of prediction equation. The s.e. of the mean body fat of the group of cows then becomes 7.0 kg. It should be noted that the above formula only applies at the mean values of the independent variables used in the construction of the prediction equation and that the s.e. of the predicted mean increases as the independent variables move from the mean values. However if

the s.e. of the predicted mean is 7.0 kg the least significant difference (LSD) can be calculated from:-

LSD = t x (s.d. of predicted mean)

where t = t for n - 4

The 95% LSD for the example given is then 15.6kg. This means that to show significant differences between two groups of four cows the differences in fat weight would have to be over 15.6 kg. This would imply a difference in live weight of almost 28 kg (assuming 560 g of fat in each kg of live weight difference). If twelve cows are used per group the LSD (95%) becomes 11 kg or about 20 kg live weight. These are, of course, minimum figures, since the further the observed values are from the mean values of the independent variables, then the greater the LSD. Nevertheless, it can be seen that the prediction equation is likely to be of value in predicting body composition in suckler cows since the difference between maximum and minimum live weight throughout the year can be almost 100 kg (e.g. Hodgson *et al.* , 1980).

If a prediction equation is to be used, it should be developed from within the population to which it is to be applied and under identical conditions. This means that if in an experiment, body composition is to be predicted, then a number of the animals should be slaughtered and a prediction equation derived, or at least an existing equation validated. However, this may not be possible in practice, especially with suckler cows, because of the expense and practical difficulties involved in direct measurement of body composition. The equations presented in this Chapter may thus be of value to predict body composition during the course of an experiment. However, physiological state may influence some of the relationships, especially those involving live weight, $D_2O$ space

and possibly Evans Blue blood and red cell volumes. It is likely, although not inconceivable, that the relationships predicting body composition from ultrasonic measurements, condition score and body size would be less influenced by physiological state and it may be possible to apply these, with caution, to cows that are either pregnant or lactating.

# CHAPTER 9

## MAINTENANCE REQUIREMENTS OF SUCKLER COWS AND BLOOD METABOLITES AS INDICES OF ENERGY STATUS

FOREWORD

The experiment described in this chapter was designed and carried out by Dr A.J.F. Russel. However all subsequent statistical analyses and conclusions drawn from the results are the work of the author.

## INTRODUCTION

Economic considerations often require that animals be fed less than their requirements. When this is so, as in the case of the winter feeding of suckler cows, the difference between the optimum economic feeding level (determined by the relative values of inputs and outputs) and the level at which production begins to decline sharply may be particularly small. In such cases it is important to be able to quantify with precision the optimum level of feeding. However, the level of feeding is not the sole criterion to be considered - the animals' requirements must also be taken into account. In fact the net effects of nutrient intake and requirements i.e. nutrient status, may well be more important than absolute level of intake.

The assessment of nutrient status in suckler cows presents serious difficulties in both the research and commercial situations. The difficulties of measuring feed intake in group-fed or grazing animals, coupled with the uncertainty of the exact nutritional requirements of cows in certain physiological states, particularly pregnancy, or in certain environments, e.g. outwintered, is such that meaningful measurements of the adequacy (or inadequacy) of nutrition cannot readily be made.

Although changes in live weight and body condition may reflect nutritional status to some extent, they do so in only the long term and are not effective in assessing short term nutritional inadequacy. The measurement of the circulating levels of certain blood metabolites is one possible way of assessing nutrient status, particularly energy status.

### Catabolism of fat in underfed ruminants

During periods of undernourishment, fat from adipose tissue is

catabolised. This results in the release of free fatty acids into the bloodstream, and these are subsequently metabolised to help meet the animal's demand for energy. Annison (1960) and Reid and Hinks (1962*a*, 1962*b*) showed that the concentration of fatty acids in the bloodstream is related to the rate of fat catabolism with maximum levels of 1500 to 2500 μmol/l occurring in starved sheep. Further increases in the rate of fat catabolism do not result in higher concentrations, probably because of an increase in the turnover rate of free fatty acids in the bloodstream.

In the liver, free fatty acids are oxidised via $\beta$-oxidation to acetyl CoA. The normal pathway for the further oxidation of acetyl CoA via the TCA cycle involves the use of oxaloacetate. However, oxaloacetate is also required in gluconeogenesis from lactate, pyruvate, propionate and those amino acids which, on degradation, yield pyruvate (Krebs, 1965). Thus, in ruminants which have a high demand for glucose, a large proportion of glucose must be formed from gluconeogenesis which reduces the supply of oxaloacetate for the entry of acetyl CoA into the TCA cycle; this results in the formation of acetoacetate and 3-hydroxybutyrate (ketone bodies) (Figure 9.1).

Ketone bodies in moderate quantities are not harmful and in fact are readily utilised by many tissues in respiration, in the absence of glucose.

Plasma concentration of 3-hydroxybutyrate is closely related to production (Leng, 1965).

The approach of using plasma concentrations of free fatty acids and ketone bodies as estimates of nutritional status has been adopted in sheep by Reid and Hinks (1962*b*), Russel, Doney and Reid (1967) and Russel and Doney (1969) and has been successfully used to

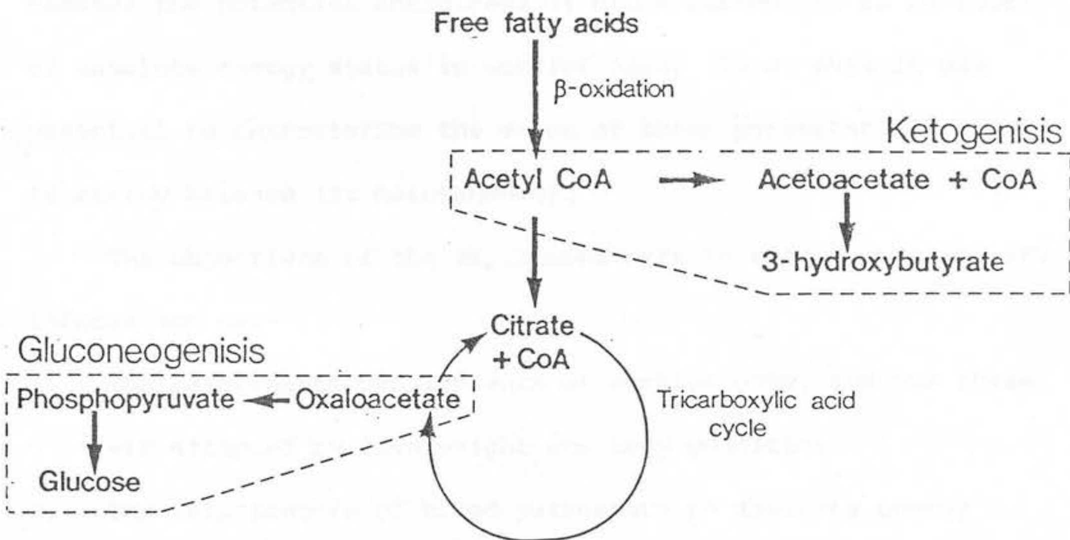



Figure 9.1. The relationship between ketogenisis and gluconeogenisis

maintain sheep at predetermined levels of undernutrition (Russel, 1978).

A close relationship has been demonstrated between energy intake and plasma 3-hydroxybutyrate concentration in suckler cows during late pregnancy (Russel *et al.* 1979). This provided a reasonable index of relative nutritional status, but because of the uncertainty of bovine foetal energy requirements it could not be used to measure absolute energy status.

### Objectives of Experiment

The experiment described in this chapter was designed to examine the potential usefulness of blood parameters as an index of absolute energy status in suckler cows. To do this it was essential to characterise the value of these parameters in cows in energy balance (at maintenance).

The objectives of the experiment were to provide preliminary information on:-

1. The maintenance requirements of suckler cows, and how these are affected by live weight and body condition.
2. The relationship of blood parameters to absolute energy status.

To characterise maintenance requirements, non-pregnant, non-lactating animals were used to avoid the problems of growth of the products of conception and of lactation.

## MATERIALS AND METHODS

### Animals

Twenty-nine HF and 27 BG non-pregnant, non-lactating cows were used.

### Pre-experimental Treatment

A range of body condition was created within each genotype.

Forty-eight cows (24 HF and 24 BG) were divided within genotype into two equal groups according to body condition score. The "lean" group was housed and fed individually to reduce body condition. The "fat" group grazed pasture such that they maintained or increased body condition. In addition another five HF and three BG cows were included at the start of the experiment.

## Experimental Design

Cows were fed one of six levels of energy intake, approximately 50, 60, 70, 80, 95 or 120% of anticipated maintenance requirements (MAFF, 1975). Initially 48 cows (24 HF and 24 BG) were allocated to treatments by restricted randomisation, taking account of genotype, condition score and live weight. The additional eight cows (five HF and three BG) were allocated at random within genotype to the sub-maintenance levels of feeding.

## Procedure

All cows were tied in cubicles and fed individually. The experiment lasted 42 days, after a two week preliminary period to allow for changes in gut fill.

All cows were weighed, condition scored and blood sampled by jugular venepuncture twice weekly before feeding. The blood was centrifuged and the plasma removed and frozen to await analysis.

## Feeding

The cows were fed once per day a diet of chopped poor-medium quality hay. The analysis is shown in Table 9.1.

Table 9.1. Hay analysis

| | |
|---|---|
| Dry matter (%) | 90.40 |
| Organic matter (% in dry matter) | 94.74 |
| Organic matter digestibility (%)+ | 56.75 |
| ME (MJ/fresh)++ | 7.29 |

+ from *in vitro* analysis
++ based on ME (dry basis) = 0.15 x DOMD% (MAFF, 1975)

The estimated maintenance requirements of each cow were calculated at the beginning of the experiment from the equation:-

Maintenance = 0.55 MJ $ME/kg^{0.73}$ (MAFF, 1975)

Cows were fed individually in relation to their estimated maintenance requirements according to allocation to treatment.

Digestibility

To check that the different levels of feeding did not affect the digestibility of the hay, 12 cows (two HF and two BG from each of the 50, 80 and 120% feeding levels) were dosed with $Cr_2O_3$-impregnated paper twice per day for 12 days commencing on day 32, and faeces samples were collected and bulked for each cow for the last five days of $Cr_2O_3$ dosing.

In addition, faeces samples were collected from all cows on days 18 and 38.

Analytical

Hay

As has been indicated, the *in vitro* organic matter digestibility of the hay was determined. The hay was also analysed for cell wall constituents by the method of Van Soest and Wine (1967).

Faeces

The faeces from the cows dosed with $Cr_2O_3$ were analysed for $Cr_2O_3$ by a modification of the method of Williams, David and Iismaa

(1962), and the faeces collected from all cows on the two occasions were analysed for cell wall constituents by the same method as was the hay.

Plasma

Plasma was analysed for different metabolites by the methods indicated, or by modifications of those methods:-

1. Free (non-esterified) fatty acids - Patterson (1963)
2. 3-hydroxybutyrate - Zivin and Snarr (1973)
3. Glucose - Gutteridge and Wright (1968)
4. Urea - Marsh, Fingerhut and Miller (1965)
5. Total protein - Collier (1970)
6. Albumin - " "

Plasma globulin was calculated as the difference between total protein and albumin.

Statistical Analysis

All statistical analysis was carried out by the statistical program Genstat Version 4.01 (Rothamsted Experimental Station, 1977) using standard regression techniques.

RESULTS

Digestibility

The results of both the $Cr_2O_3$ dosing and the analysis of cell wall constituents showed no effect of level of feeding on digestibility of the feed consumed.

Liveweight and condition score changes

The regression of live weight and of condition score on time, computed separately for each cow, indicated that these relationships were linear. Estimates of rates of daily live-weight change were provided by the regression coefficients of live weight on time, and of initial live weights and condition scores by the two sets of

regression constants.

The mean live-weight changes for each treatment group are given in Table 9.2.

Table 9.2. Daily live-weight changes

| Mean energy intake (MJ ME/day) | Live-weight change (kg/day) |
|---|---|
| 23.2 | -1.029 |
| 27.1 | -0.919 |
| 32.9 | -0.597 |
| 37.0 | -0.462 |
| 43.4 | -0.369 |
| 55.7 | 0.112 |

Individual daily live-weight changes per kg live weight (LWC, g/kg) were regressed on energy intake per kg live weight ($E_{LW}$, MJ ME/kg) and condition score (CS). Both partial regression coefficients proved to be significant ($P < 0.05$). There was no difference between the two breeds in the relationship which is given below:-

$$LWC = -5.5(\pm 0.36) + 37.5(\pm 2.96)E_{LW} + 0.6(\pm 0.12)CS \quad \text{(Equation 9.1)}$$

$$(n = 56,\ R^2 = 0.771,\ \text{residual s.d.} = 0.47)$$

The use of metabolic live weight ($kg^{0.75}$) did not improve the relationship.

Relationship of live weight and condition score

The regression of initial live weight (LW, kg) on initial condition score (CS) was:-

$$LW = 281(\pm 19.2) + 84.9(\pm 9.14)CS \quad \text{(Equation 9.2)}$$

$$(n = 56,\ r^2 = 0.615,\ \text{residual s.d.} = 34.0)$$

There was no significant difference between the two breeds.

Estimation of maintenance requirements

From Equation 9.1 it is possible to calculate the energy intake associated with zero live-weight change and therefore for maintenance (assuming zero live-weight change corresponds to maintenance). From Equation 9.1:-

$$LWC = -5.5 + 37.5E_{LW} + 0.6\ CS$$

If LWC = 0, then

$$E_{LW} = 0.147 - 0.016\ CS$$

Since $E_{LW}$ is energy intake per kg live weight, actual energy intake for maintenance (M, MJ ME/day) will be related to both live weight (LW) and condition score (CS):-

$$M = 0.147LW - 0.016CS.LW \qquad \text{(Equation 9.3)}$$

Equation 9.4 gives the maintenance requirement of a 500 kg cow.

$$M = 73.5 - 8.0\ CS \qquad \text{(Equation 9.4)}$$

Table 9.3 gives maintenance requirements calculated from Equation 9.3, and for comparison those given by ARC (1980).

Table 9.3. Maintenance requirements (MJ ME/day)

| | Condition score | | | ARC |
|---|---|---|---|---|
| Live weight | 1 | 2 | 3 | (1980) |
| 350 | 45.8 | 40.2 | 34.6 | 40.3 |
| 400 | 52.4 | 46.0 | 39.6 | 44.2 |
| 450 | 58.9 | 51.7 | 44.5 | 47.9 |
| 500 | 65.5 | 57.5 | 49.5 | 51.5 |
| 550 | 72.0 | 63.2 | 54.4 | 55.0 |
| 600 | 78.6 | 69.0 | 59.4 | 58.4 |

Efficiency of utilisation of catabolised tissue

The reciprocal of the partial regression coefficient for energy intake in Equation 9.1 is 0.0267 MJ/g or 26.7 MJ/kg. This latter figure represents the dietary energy equivalent contributed by 1 kg of live-weight loss. If the efficiency of utilisation of dietary energy for maintenance is assumed to be 0.67 (ARC, 1980) then the energy content of 1 kg of live-weight loss is 17.9 MJ, or 19.2 MJ/kg empty body-weight loss (using the equation relating empty body weight to live weight on page 109). The mean live weight of the cows used was 455 kg, or 380 kg empty body weight. From the results in Chapter 6 the energy value of empty body-weight change would have been 24.3 MJ/kg. Thus the efficiency of utilisation of body tissue for maintenance was 79%.

Blood metabolites

The concentrations of the various blood metabolites in individual cows did not show any trends over the six week period, and mean values were therefore calculated. Maintenance requirements for each cow were calculated using Equation 9.3 ($M_1$) and also using the values recommended by ARC (1980) ($M_2$). The treatment mean values for the various metabolites were regressed on the following indices of energy status:-

1. Energy intake (E)
2. Energy intake per kg live weight (E/kg)
3. Energy intake per $kg^{0.75}$ ($E/kg^{0.75}$)
4. Energy intake minus $M_1$ ($ED_1$)
5. Energy intake minus $M_2$ ($ED_2$)

The data for free fatty acid concentration were transformed to a natural logarithm basis before regression.

Plasma concentrations of total protein, albumin and urea were not significantly affected by treatment. Significant breed differences were found in the concentrations of glucose and urea. The mean values of these latter two are given in Table 9.4.

Table 9.4. Mean plasma concentrations of urea and glucose

| | Hereford x Friesian | Blue Grey | s.e. of difference |
|---|---|---|---|
| Urea (mmol/l) | 3.71 | 3.06 | 0.139*** |
| Glucose (mmol/l) | 3.21 | 3.49 | 0.041*** |

Table 9.5 shows $r^2$ (or $R^2$) values for the relationships of the group means of those blood parameters that were significantly affected by treatment, to the various measures of energy status.

Table 9.5. $R^2$ and $r^2$ values for relationships of group means of blood metabolites to energy status

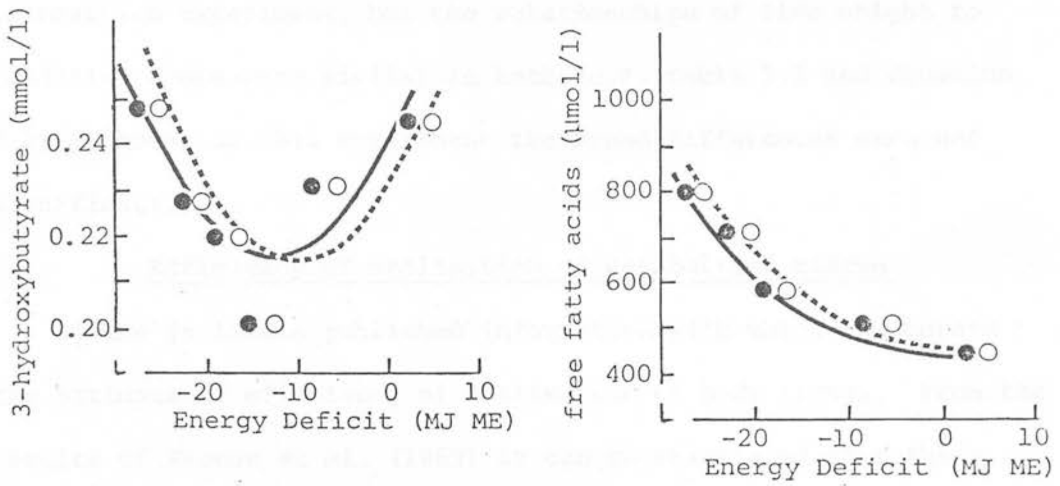
| | E | E/kg | $E/kg^{0.75}$ | $ED_1$ | $ED_2$ |
|---|---|---|---|---|---|
| 3-hydroxybutyrate+ | 0.709 | 0.777* | 0.759 | 0.724 | 0.749 |
| free-fatty acids ($\log_e$) + | 0.994*** | 0.979** | 0.984** | 0.975** | 0.976** |
| glucose | 0.721* | 0.681* | 0.692* | 0.673* | 0.682* |
| globulin+ | 0.732 | 0.798* | 0.781* | 0.751 | 0.775 |

\+ indicates quadratic relationship

The regression equations relating 3-hydroxybutyrate, free fatty acids, glucose and globulin to $ED_1$ and $ED_2$ are shown in Table 9.6 and are illustrated graphically in Figure 9.2.

Table 9.6. Regressions of group means of blood metabolites on energy status of the form $y = a + b_1 x + b_2 x^2$.

| y | x | a ($\pm$ s.e.) | $b_1$ ($\pm$ s.e.) | $b_2$ ($\pm$ s.e.) |
|---|---|---|---|---|
| 3-hydroxybutyrate (mmol/l) | $ED_1$ | 0.239 ($\pm$0.0092) | 0.0038 ($\pm$0.00141) | 0.00015 ($\pm$0.000054) |
| | $ED_2$ | 0.230 ($\pm$0.0073) | 0.0031 ($\pm$0.00109) | 0.00015 ($\pm$0.000049) |
| $\log_e$ free fatty acids (μmol/l) | $ED_1$ | 6.10 ($\pm$0.035) | -0.0014 ($\pm$0.00547) | 0.00072 ($\pm$0.000209) |
| | $ED_2$ | 6.11 ($\pm$0.029) | -0.0060 ($\pm$0.00436) | 0.00064 ($\pm$0.000197) |
| glucose (mmol/l) | $ED_1$ | 3.43 ($\pm$0.033) | 0.0053 ($\pm$0.00184) | - |
| | $ED_2$ | 3.41 ($\pm$0.028) | 0.0052 ($\pm$0.00177) | - |
| globulin (mmol/l) | $ED_1$ | 41.8 ($\pm$0.41) | -0.189 ($\pm$0.0636) | -0.0072 ($\pm$0.00224) |
| | $ED_2$ | 42.2 ($\pm$0.33) | -0.152 ($\pm$0.0491) | -0.0070 ($\pm$0.00222) |




● Energy deficit calculated from Equation 9.3

○ Energy deficit calculated from ARC (1980)

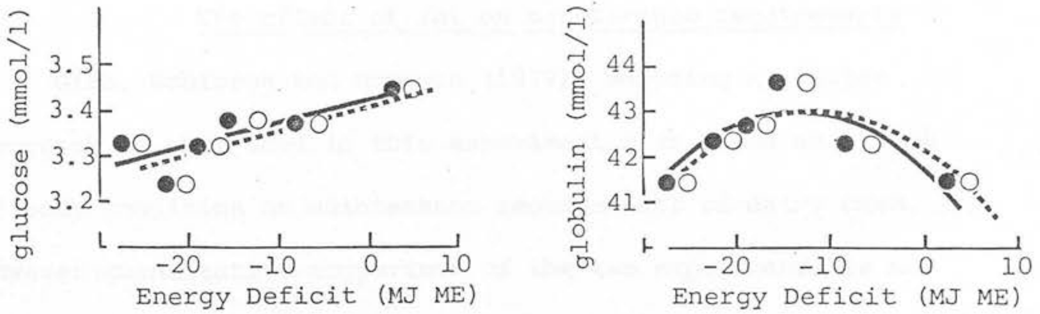



Figure 9.2.
Relationships between plasma 3-hydroxybutyrate, free fatty acids, glucose and globulin, and energy deficit.

## DISCUSSION

### Relationship of live weight to condition score

The ranges of condition score and live weight were less in this experiment than for the HF and BG cows in the Body Composition experiment, but the relationships of live weight to condition score were similar in both (c.f. Table 5.3 and Equation 9.2) although in this experiment the breed differences were not significant.

### Efficiency of utilisation of catabolised tissue

There is little published information with which to compare the estimate of efficiency of utilisation of body tissue. From the results of Keenan *et al*. (1969) it can be calculated that the efficiency of use of catabolised tissue in sheep was 55%, which is considerably lower than the present estimate. However it must be remembered that there may be considerable errors in the various factors used in the computation of the efficiency in this experiment.

### The effect of fat on maintenance requirements

Gibb, Robinson and McGowan (1977), adopting a similar approach to that used in this experiment also noted an effect of body condition on maintenance requirements of dairy cows. However quantitative comparison of the two experiments is not possible because different condition scoring systems were used.

Classically, maintenance requirements of livestock are estimated from calorimetric measurements of fasting metabolism. Graham (1967) made serial measurements of fasting heat production of adult sheep as they lost live weight and body condition, but found no significant effect of fatness (as estimated by TOH dilution). Fasting heat production varied with live weight$^{0.73}$,

but heat production per $kg^{0.73}$ did "tend to be highest when sheep were thin". However, fasting heat production varied directly with estimates of fat-free body weight.

There is a close relationship between heat production and total protein synthesis ($r^2 = 0.90$) in rats (Webster, Lobley, Reeds and Pullar, 1978). The correlation between heat production and body protein ($r^2 = 0.72$) was much greater than that between heat production and body weight ($r^2 = 0.59$). Webster (1980) also showed that most protein synthesis takes place in the fat-free body, and particularly in the gut and liver.

Furthermore, it seems likely from theoretical considerations that the turnover of fat is relatively low, at least under conditions of continuous growth, and the energetic efficiency of fat synthesis and deposition is high (Webster, 1980).

In view of the above information it is not unreasonable to postulate that the maintenance requirement of the fat-free body (where most protein synthesis takes place) is higher than that of fat. This could explain why, when compared at constant live weight, the maintenance requirements of fatter cows in this experiment are lower (Table 9.3).

## The effect of changing live weight and condition score on maintenance requirements

Each individual cow will have its own live weight-condition score relationship, depending on basic body size (ignoring changes due to pregnancy and gut fill). However, if Equation 9.2 is considered to be the "average" relationship between live weight and condition score for HF and BG cows

it is possible to calculate from Equations 9.2 and 9.3, the "average" maintenance requirements for cows as they change in live weight and consequently in condition score. Re-arranging Equation 9.2 gives:-

$$CS = 0.0118LW - 3.310$$

Substituting this expression for CS in Equation 9.3:-

$$M = 0.147LW - 0.016LW\,(0.0118LW - 3.310)$$

$$= 0.200LW - 0.00019LW^2 \qquad \text{(Equation 9.4)}$$

Figure 9.3 compares Equation 9.4 with the estimates of ARC (1980).

It is interesting to note that the curvilinear relationship between maintenance requirements and live weight is of a similar nature to the relationships between body protein and empty body weight that were calculated in Table 6.3, i.e. they both increase at a decreasing rate, adding support to the theory that maintenance requirements are closely related to body protein.

## Blood metabolites

### Urea and glucose

No reasonable explanation can be found for the unexpected breed differences noted in the plasma concentrations of urea and glucose, and further confirmation of this finding is required before indulging in speculation as to its biological significance.

As expected, the concentration of glucose in the plasma increased with increasing energy status (Figure 9.2), although the increase was small. Plasma glucose concentration is subject to very strict homeostatic control (Lindsay, 1978), and does not constitute a very satisfactory index of energy status.

### Globulin

No plausible explanation can be found for the curvilinear

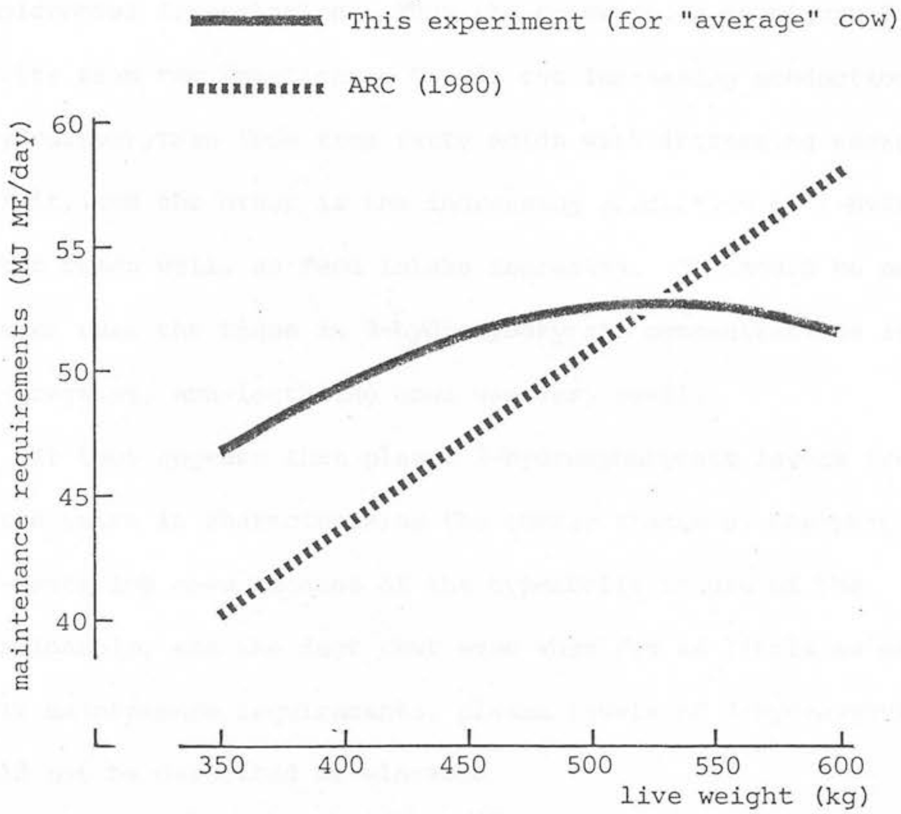



Figure 9.3. Estimates of maintenance requirements

relationship of plasma globulin levels and energy status (Figure 9.2).

3-hydroxybutyrate and free fatty acids

The curvilinear relationship between energy status and 3-hydroxybutyrate levels (Figure 9.2) is considered to reflect the two sources of 3-hydroxybutyrate in the ruminant. 3-hydroxybutyrate is produced from acetyl CoA following the breakdown of fatty acids, and also in the epithelial cells of the rumen wall from the products of microbial fermentation. Thus the curve shown in Figure 9.2 results from two functions. One is the increasing production of 3-hydroxybutyrate from free fatty acids with increasing energy deficit, and the other is the increasing production of 3-hydroxybutyrate by the rumen wall, as feed intake increases. It should be noted however that the range in 3-hydroxybutyrate concentrations in these non-pregnant, non-lactating cows was very small.

It thus appears that plasma 3-hydroxybutyrate levels are of little value in characterising the energy status of non-pregnant, non-lactating cows because of the hyperbolic nature of the relationship, and the fact that even when fed as little as half their maintenance requirements, plasma levels of 3-hydroxybutyrate could not be described as elevated.

Plasma free fatty acid concentrations are closely correlated with energy status, reflecting increasing fat mobilisation with increasing energy deficit, and are clearly of value in characterising energy status in non-pregnant, non-lactating suckler cows. A concentration of about 450 µmol/l appears to characterise maintenance, being similar to that found by Russel and Doney (1969) in sheep.

It is of interest to compare the plasma 3-hydroxybutyrate and free fatty acid levels of the cows in this experiment with those

in the experiment of Russel *et al.* (1979). In that experiment cows were fed at different levels of energy intake during late pregnancy. Figure 9.4 shows the 3-hydroxybutyrate levels recorded by Russel *et al.* (1979), 3 weeks pre-partum, compared to those in the present study. The data for the energy intake of the pregnant animals have been converted to an energy status basis (energy intake minus energy requirement) using the energy requirements of ARC (1980), such that a cow of 500 kg maternal body weight has a total metabolisable energy requirement of 82.5 MJ/day three weeks pre-partum. The plasma free fatty acid concentrations from the same late pregnancy experiment (A.J.F. Russel, unpublished) are compared with those of the present study in Figure 9.5. Again the energy intake data have been converted to an energy status basis. Despite there being a significant difference between years 1 and 2 in the data from the pregnancy experiment it is clear that free fatty acid levels are of similar magnitude in both pregnant and non-pregnant cows at a comparable energy status.

The difference in plasma 3-hydroxybutyrate levels between the pregnant and non-pregnant cows can be explained by the difference between the two groups in the demand for glucose. The ruminant foetus is unable to utilise the main products of carbohydrate digestion from the maternal rumen (volatile fatty acids) because these cannot cross the placenta. The foetus must have a sufficient supply of glucose, which can cross the placental barrier. This creates a high demand for glucose in pregnant ruminants, particularly in late pregnancy when the foetus is growing quickly. Thus, even at the same energy deficit, pregnant cows would be expected to have a much higher

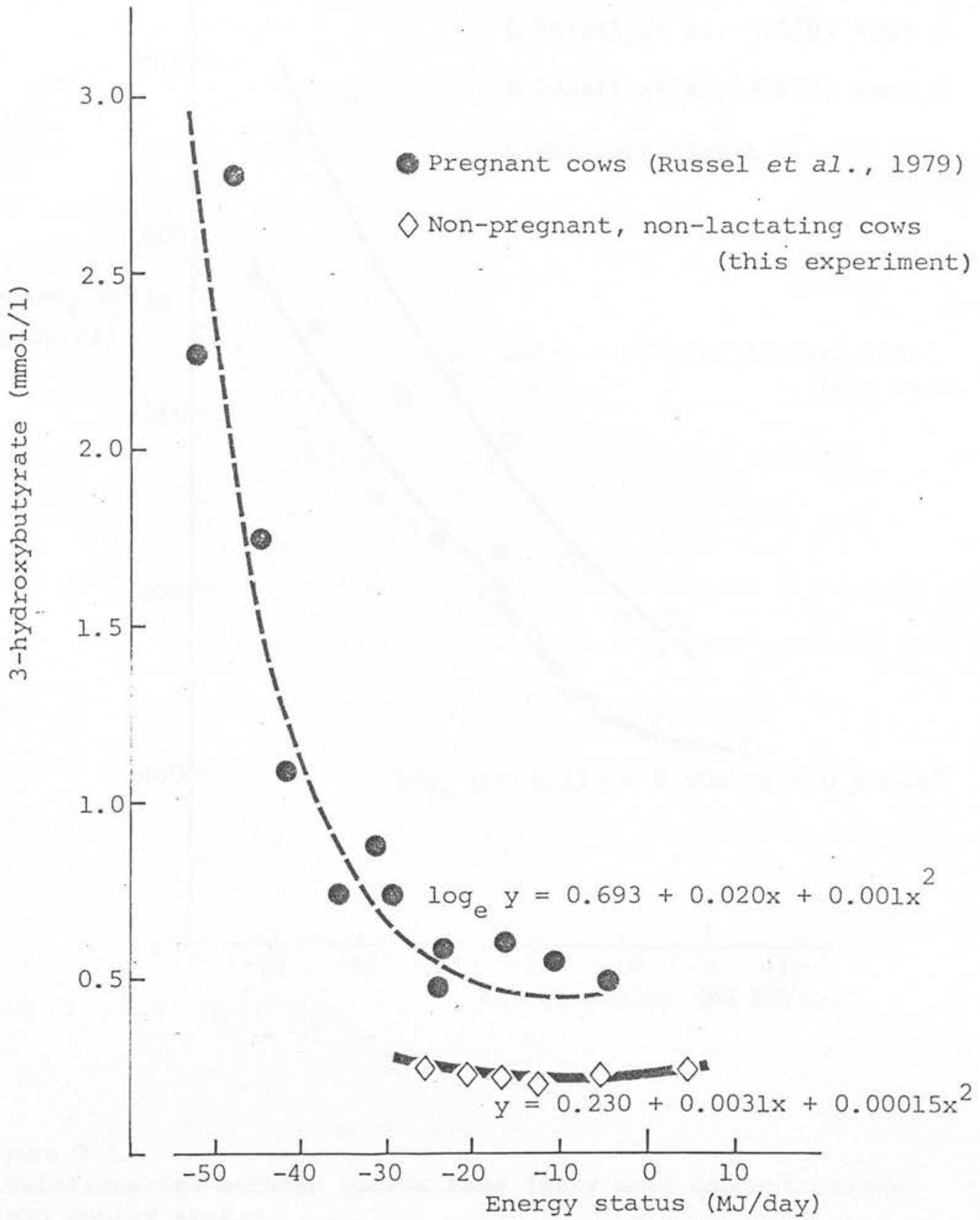



Figure 9.4.

Relationships between plasma 3-hydroxybutyrate concentrations and energy status

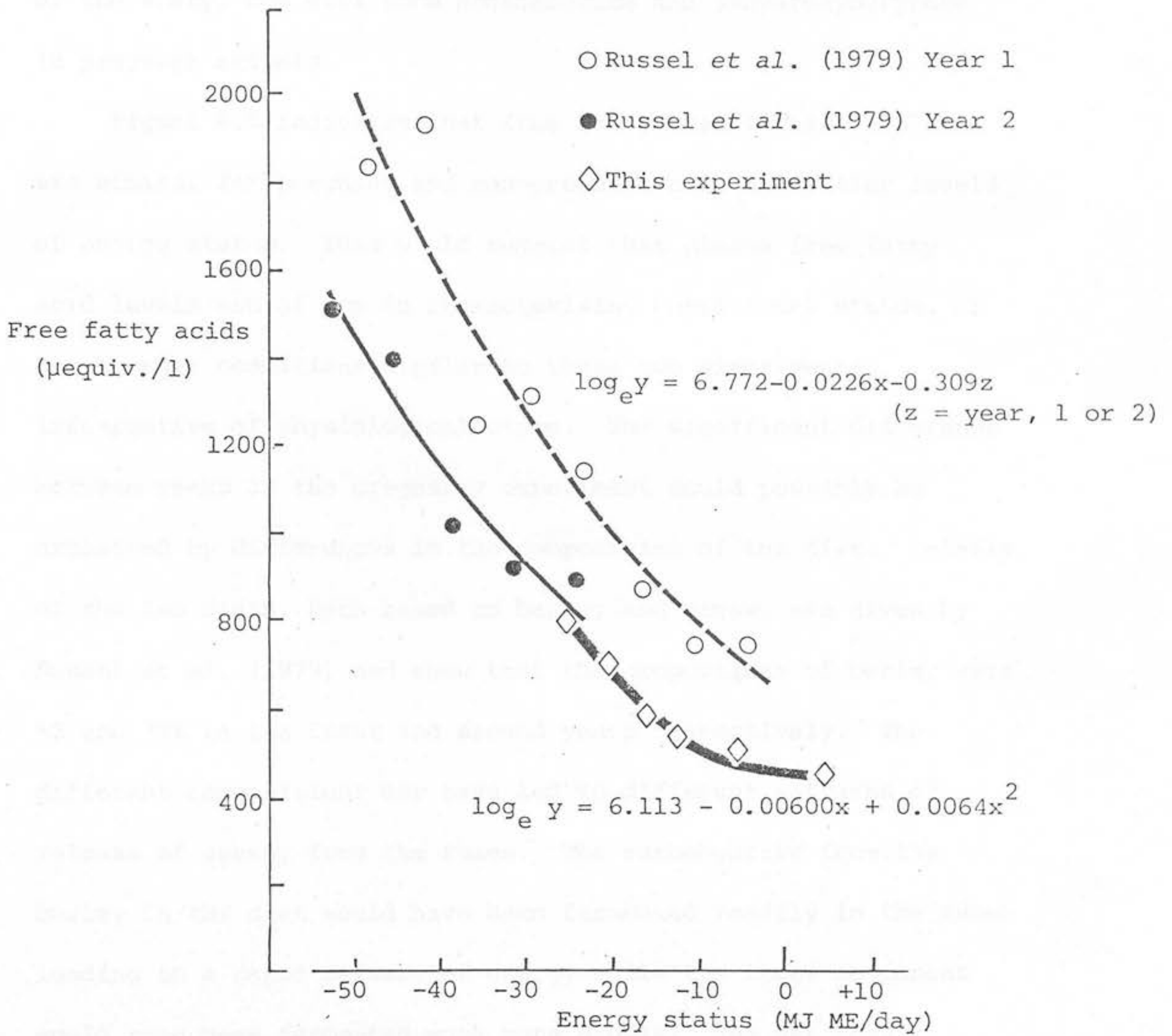



Figure 9.5.
Relationships between plasma free fatty acid concentrations and energy status.

demand for glucose. This demand would have to be met by gluconeogenesis from propionate and a key intermediate metabolite in gluconeogenesis is oxaloacetate for the oxidation of acetyl CoA produced from the $\beta$-oxidation of fatty acids. Thus more of the acetyl CoA will form acetoacetate and 3-hydroxybutyrate in pregnant animals.

Figure 9.5 indicates that free fatty acid levels in plasma are similar for pregnant and non-pregnant cows at similar levels of energy status. This would suggest that plasma free fatty acid levels are of use in characterising nutritional status, at least under conditions similar to these two experiments, irrespective of physiological state. The significant difference between years in the pregnancy experiment could possibly be explained by differences in the composition of the diet. Details of the two diets, both based on barley and straw, are given by Russel *et al*. (1979) and show that the proportions of barley were 52 and 37% in the first and second years respectively. The different compositions may have led to different patterns of release of energy from the rumen. The carbohydrate from the barley in the diet would have been fermented readily in the rumen leading to a rapid release of energy while the straw component would have been fermented much more slowly. The net result may have been a rather low level of "background" release of energy (supplied by the straw) supplemented by pulsatory releases of energy, after feeding, from the barley. In the first year the barley:straw ratio was higher and so the "background" release of energy from straw may well have been lower, and the peaks of energy release from barley, higher. The diurnal fluctuation in short term energy status may then have been different in the two

years, even at the same energy status in terms of MJ ME/day. As Figure 9.6 suggests, the energy status at the time of blood sampling (before feeding) may have been different with the two diets.

The effect of diet type on plasma levels of free fatty acids seems to warrant further investigation, and until such information is available care should be exercised in the use of plasma free fatty acids to characterise nutritional status in suckler cows. However, within one experiment, when animals are fed the same diet, plasma free fatty acids appear to offer a practicable method of estimating nutritional status in differing physiological states. The major limitations to their use are that they are very sensitive to stress and that variation occurs within animals and between samplings. This can be overcome to some extent by the use of large numbers of animals, and/or more frequent sampling.

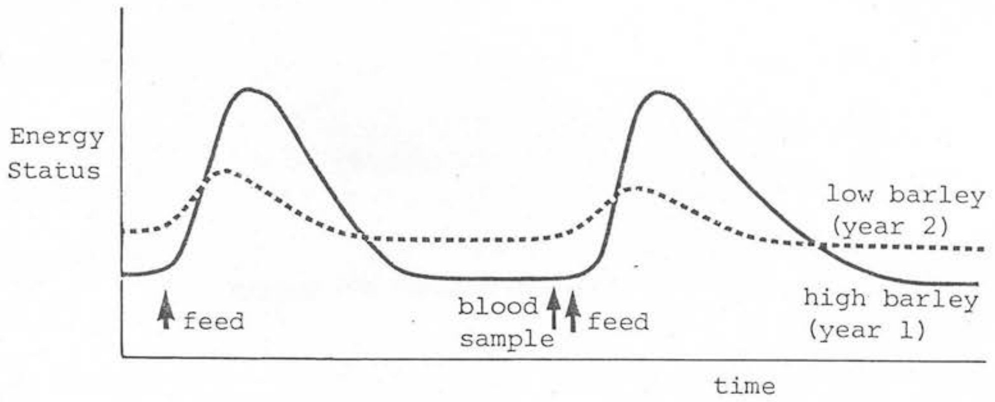



Figure 9.6.
Stylised representation of possible diurnal variation in energy status of cows in experiment of Russel *et al.* (1979)

# CHAPTER 10

# TOPICS FOR FUTURE STUDIES

## INTRODUCTION

While the studies reported in this thesis have attempted to answer several questions related to the body composition of suckler cows, many remain unanswered and several more have been posed. It is relevant to outline these as possible areas for further study.

## AREAS OF FURTHER STUDY

### The effects of direction of live-weight change

In the Body Composition experiment most of the cows slaughtered at the lower end of the condition score scale had previously been fed to reduce condition, while those at the upper end of the scale had deliberately been fattened. It would have been useful to have had animals approaching each "target" condition score from both above and below in order to investigate possible interactions with direction of change of live weight and condition. However time did not permit such an approach, and so the results presented have considered only static effects.

Information was presented on the composition of empty body-weight change, but it is not known whether the composition of empty body-weight gain is the same as that of empty body weight loss in mature animals. It would seem reasonable to suggest that, in the long term, gain and loss must be of the same composition, otherwise animals which undergo frequent cyclical changes in weight would progressively be changing their composition at any given empty body weight, and would in effect become compositionally unbalanced. It is known, however, that in the short term the composition of empty body-weight gain may be different from loss e.g. as in the case of sheep in which weight gain contained more water and protein than weight loss (Keenan *et al.*, 1969) and it is conceivable that animals may change in composition while maintaining body weight.

While energy status normally determines whether an animal will gain or lose weight it is possible that protein nutrition may affect body composition. R.D. Baker (personal communication) found, for example, that the weight gains of cattle on an all silage diet contained more fat and less protein than did those of cattle supplemented with concentrates, possibly because the high non-protein nitrogen content of the silage resulted in protein being limiting and the excess energy being stored as fat. Lindsay and Davies (1981) noted that the dietary concentration of protein affected the composition of a rib joint in steers. There is no information available in the literature as to the effect of diet on the weight gains of mature cattle. The effect of protein in the diet on the composition of weight loss is similarly unknown. In lactating sheep it appears that the level of dietary protein can affect the magnitude of weight loss (Robinson, 1978), but the effect on the composition of that loss is not known. If protein supply affects the composition of body-weight loss it is also possible that the nutrient demand may have a similar effect.

The breed differences in the partition of fat between the different adipose tissue depots are important in the estimation of *in vivo* body composition from techniques such as body condition scoring and ultrasonic measurement of subcutaneous fat depth. If direction of change of empty body weight influences fat partition, this could also have important effects on the use of these techniques. In pregnant sheep catabolising body fat it has been noted that initially the fat from the subcutaneous depot appears to be depleted at a greater rate than that from other depots, whereas in the later stages the omental and mesenteric depot is utilised to a greater extent (Russel *et al.*, 1968). However, it is not clear in which order the different

depots are replenished when the animals subsequently deposit fat, or even if they return to the same state with regard to fat partition.

The effects of physiological state

It was indicated in Chapter 6 that the data for the composition and calorific value of empty body weight change may not be appropriate to cows that are pregnant or lactating. Since suckler cows should normally be either pregnant or lactating, or both, it is important that information appropriate to these physiological states be obtained. Similarly, the prediction equations presented in Chapter 8, particularly those involving live weight and $D_2O$ space, may not be applicable to pregnant or lactating cows. Prediction of body composition in nutritional experimentation is likely to prove most useful in late pregnancy and during lactation, and prediction equations require to be developed for these particular conditions.

Breed differences in fat partition

It was speculated in Chapter 7 that pure Hereford cows might have a greater proportion of their fat in the subcutaneous depot than any of the breeds examined in the Body Composition study. Because of the possible implications of this with regard to the estimation of body composition from techniques which assess only the subcutaneous depot, fat partition in pure beef breeds requires examination.

Estimation of gut fill

No attempt was made to estimate the amount of gut fill in the live animals in either of the experiments described in previous Chapters, as it was considered that no method was sufficiently reliable. However, a method of accurately assessing gut fill, and hence of estimating empty body weight, would undoubtedly constitute a major advance in the field of animal production from

ruminants. As well as being a more reliable indicator of animal performance than live weight, the use of empty body weight would improve upon the precision afforded by the techniques of *in vivo* body composition measurement which in practice have to be based on live weight, but which theoretically should be based on empty body weight.

Maintenance requirements

The effect of body condition and composition on the maintenance requirements of cattle is clearly one that would merit further investigation. Differences between maintenance requirements of cows, as given by ARC (1980) and those presented in Chapter 9 can be explained in terms of body composition. Similarly differences noted by ARC (1980) in maintenance requirements between sexes, whereby bulls have a higher maintenance requirement than heifers may also be attributable to differences in body composition; at the same empty body weight bulls have a greater weight of body protein than heifers. However, the hypothesis that maintenance requirements are closely related to the quantity of protein in the bodies of animals remains to be tested experimentally. This is important not only in relation to maintenance requirements *per se*, but also because estimates of requirements for productive processes frequently include a maintenance component in their computation.

Blood metabolites

The differences between the HF and BG cows in the plasma concentrations of glucose and urea could not be explained, nor could the curvilinear relationship between plasma globulin and energy status, and further work is required to provide an understanding of their physiological basis and importance. Plasma urea did not appear to be related to feed intake and a simple

index of nitrogen status in suckler cows would prove useful in assessing the adequacy of nitrogen supply.

If plasma free fatty acids and 3-hydroxybutyrate are to be used in the assessment of energy status in suckler cows it will be necessary to establish quantitatively how the pattern of feed intake and composition of the diet affects these parameters.

## CONCLUSION

If more efficient systems of suckled calf production are to be developed these must make optimum use of the ability of cows to undergo cyclical changes in body composition throughout the annual production cycle. Advances in the production and utilisation of pasture during the grazing season are likely to lead to a greater difference between the levels of summer and winter nutrition and hence place even greater importance upon the utilisation and replenishment of body reserves of nutrients, particularly energy.

It is clearly important to gain a better understanding of the contribution that the use of body tissue can make to production and of the requirements for the replenishment of such tissue. Information presented in earlier sections of this thesis, which enable estimates of changes of body composition to be made on the live animal, is likely to be of use in research programmes designed to provide this understanding and to answer at least some of the questions posed.

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# APPENDIX I

# BODY CONDITION SCORING SYSTEM

Body condition scores (Lowman *et al.*, 1976)

Score 0

The animal is emaciated with the spinous processes, hip bones, tail head and ribs projecting prominently. No fatty tissue can be detected and the neural spines and transverse processes feel very sharp.

Score 1

The individual spinous processes are still fairly sharp to the touch and there is no fat around the tail head. The hip bones, tail head and ribs are still prominent but appear less obvious.

Score 2

The spinous processes can be identified individually when touched, but feel rounded rather than sharp. There is some tissue cover around the tail head, over the hip bones and the flank. Individual ribs are no longer visually obvious.

Score 3

The spinous processes can only be felt with firm pressure. The areas on either side of the tail head now have a degree of fat cover which can easily be felt.

Score 4

Fat cover around the tail head is evident as slight "rounds", soft to the touch. The spinous processes cannot be felt even with firm pressure and folds of fat are beginning to develop over the ribs and thighs of the animal.

Score 5

The bone structure is no longer noticeable and the animal presents a "blocky" appearance. The tail head and hip bones are

almost completely buried in fatty tissue, and folds of fat are apparent over the ribs and thighs. The spinous processes are completely covered by fat and the animal's mobility is impaired by the large amount of fat carried.

APPENDIX II

DATA TABLES

Table A1. Breed, condition score, live weight and empty body weight

| Cow | Breed | Condition score | Live weight (kg) | Empty body weight (kg) |
|---|---|---|---|---|
| 1 | HF | 4.50 | 672 | 581 |
| 2 | HF | 4.00 | 693 | 588 |
| 3 | HF | 2.25 | 435 | 384 |
| 4 | HF | 4.50 | 710 | 623 |
| 5 | HF | 2.50 | 467 | 394 |
| 6 | HF | 2.75 | 545 | 465 |
| 7 | HF | 3.25 | 582 | 484 |
| 8 | HF | 3.50 | 635 | 554 |
| 9 | HF | 1.00 | 350 | 283 |
| 10 | HF | 4.00 | 640 | 522 |
| 11 | HF | 1.50 | 400 | 326 |
| 12 | HF | 1.75 | 412 | 345 |
| 14 | HF | 2.00 | 470 | 391 |
| 15 | HF | 3.00 | 577 | 474 |
| 17 | BG | 1.00 | 390 | 335 |
| 18 | BG | 3.00 | 535 | 450 |
| 19 | BG | 4.00 | 590 | 498 |
| 20 | BG | 1.50 | 400 | 327 |
| 21 | BG | 2.50 | 485 | 405 |
| 22 | BG | 2.75 | 405 | 354 |
| 23 | BG | 2.75 | 495 | 423 |
| 24 | BG | 4.25 | 716 | 639 |
| 25 | BG | 4.50 | 720 | 637 |
| 26 | BG | 4.00 | 610 | 516 |
| 27 | BG | 3.50 | 615 | 517 |
| 30 | BG | 2.00 | 445 | 371 |
| 31 | BG | 1.25 | 370 | 320 |
| 32 | BG | 1.75 | 400 | 324 |
| 33 | F | 1.50 | 417 | 329 |
| 34 | F | 0.75 | 435 | 326 |
| 35 | F | 2.00 | 530 | 460 |
| 36 | F | 2.50 | 556 | 471 |
| 37 | F | 2.25 | 453 | 386 |
| 38 | F | 2.75 | 553 | 468 |
| 40 | F | 1.50 | 455 | 360 |
| 41 | F | 3.50 | 710 | 636 |
| 42 | F | 3.25 | 720 | 620 |
| 43 | F | 2.50 | 650 | 569 |
| 44 | F | 2.75 | 615 | 514 |
| 45 | F | 2.75 | 675 | 607 |
| 46 | F | 1.25 | 464 | 399 |
| 47 | F | 3.25 | 605 | 524 |
| 48 | F | 2.00 | 558 | 466 |

(cont'd)

Table A1. (cont'd)

| Cow | Breed | Condition score | Live weight (kg) | Empty body weight (kg) |
|---|---|---|---|---|
| 49 | G | 1.50 | 385 | 321 |
| 50 | G | 2.50 | 435 | 360 |
| 51 | G | 2.50 | 535 | 437 |
| 52 | G | 2.75 | 513 | 457 |
| 53 | G | 1.00 | 365 | 276 |
| 54 | G | 1.00 | 370 | 261 |
| 55 | G | 2.50 | 390 | 333 |
| 56 | G | 4.00 | 595 | 527 |
| 57 | G | 4.50 | 545 | 461 |
| 58 | G | 1.75 | 446 | 355 |
| 59 | G | 2.25 | 490 | 386 |
| 60 | G | 1.25 | 427 | 350 |
| 61 | G | 3.00 | 470 | 417 |
| 62 | G | 4.50 | 630 | 564 |
| 64 | G | 2.25 | 476 | 411 |
| 65 | L | 2.50 | 480 | 390 |
| 66 | L | 1.25 | 348 | 283 |
| 67 | L | 2.00 | 412 | 346 |
| 68 | L | 2.75 | 430 | 349 |
| 69 | L | 1.50 | 363 | 288 |
| 70 | L | 1.50 | 362 | 302 |
| 71 | L | 1.25 | 320 | 265 |
| 72 | L | 1.75 | 325 | 274 |
| 73 | L | 3.00 | 463 | 397 |
| 74 | L | 2.75 | 530 | 457 |
| 75 | L | 2.50 | 525 | 430 |
| 77 | L | 2.75 | 490 | 428 |
| 78 | L | 2.25 | 432 | 381 |
| 79 | L | 2.25 | 462 | 394 |
| 80 | L | 2.75 | 565 | 489 |

Table A2. Weight (kg) of chemical components of bodies of cows

| Cow | Total body water+ | Empty body water | Body fat | Body ash | Body protein |
|---|---|---|---|---|---|
| 1 | 378.1 | 302.4 | 160.9 | 25.2 | 92.1 |
| 2 | 379.7 | 289.4 | 186.2 | 23.8 | 89.1 |
| 3 | 269.9 | 224.5 | 64.8 | 20.8 | 74.7 |
| 4 | 353.2 | 281.6 | 228.2 | 22.7 | 92.7 |
| 5 | 289.6 | 224.8 | 75.8 | 22.1 | 73.6 |
| 6 | 329.0 | 260.2 | 100.9 | 20.9 | 82.9 |
| 7 | 356.2 | 269.2 | 113.5 | 21.7 | 82.1 |
| 8 | 366.6 | 296.7 | 139.4 | 25.1 | 92.7 |
| 9 | 237.2 | 178.4 | 26.3 | 19.9 | 59.1 |
| 10 | 352.2 | 250.2 | 167.7 | 23.9 | 80.7 |
| 11 | 271.3 | 207.3 | 36.8 | 17.1 | 64.9 |
| 12 | 266.4 | 207.2 | 55.5 | 17.5 | 64.9 |
| 14 | 311.7 | 243.8 | 58.9 | 18.3 | 72.3 |
| 15 | 348.1 | 259.5 | 117.4 | 17.4 | 80.9 |
| 17 | 252.0 | 204.7 | 49.4 | 18.0 | 62.9 |
| 18 | 293.3 | 222.1 | 136.4 | 20.3 | 71.8 |
| 19 | 310.2 | 230.2 | 173.4 | 18.2 | 77.3 |
| 20 | 263.8 | 201.0 | 45.6 | 18.2 | 62.2 |
| 21 | 291.3 | 221.6 | 99.7 | 15.3 | 69.6 |
| 22 | 227.5 | 183.5 | 94.0 | 13.8 | 62.8 |
| 23 | 287.3 | 225.3 | 109.7 | 18.1 | 70.8 |
| 24 | 368.9 | 303.6 | 220.0 | 21.6 | 93.4 |
| 25 | 333.4 | 261.8 | 274.1 | 20.8 | 80.5 |
| 26 | 322.6 | 244.3 | 176.8 | 16.9 | 77.4 |
| 27 | 335.9 | 252.5 | 164.0 | 19.4 | 80.9 |
| 30 | 264.4 | 200.8 | 83.5 | 17.6 | 69.3 |
| 31 | 226.5 | 183.4 | 59.2 | 16.9 | 60.8 |
| 32 | 270.5 | 198.2 | 45.3 | 17.2 | 63.3 |
| 33 | 284.6 | 206.0 | 36.8 | 19.5 | 67.0 |
| 34 | 316.6 | 222.6 | 15.3 | 18.9 | 68.9 |
| 35 | 316.4 | 258.6 | 101.8 | 21.0 | 81.2 |
| 36 | 330.2 | 259.3 | 109.9 | 21.6 | 80.8 |
| 37 | 266.0 | 208.9 | 89.8 | 20.3 | 66.4 |
| 38 | 307.5 | 237.0 | 133.5 | 22.0 | 76.4 |
| 40 | 305.5 | 223.2 | 50.1 | 17.6 | 68.7 |
| 41 | 354.4 | 290.8 | 233.5 | 21.2 | 88.9 |
| 42 | 368.2 | 282.4 | 226.2 | 24.0 | 89.1 |
| 43 | 377.1 | 307.6 | 148.4 | 21.8 | 91.3 |
| 44 | 338.4 | 247.8 | 163.1 | 19.5 | 83.9 |
| 45 | 323.7 | 265.1 | 228.3 | 23.3 | 89.5 |
| 46 | 308.0 | 252.1 | 45.7 | 21.1 | 79.5 |
| 47 | 324.2 | 254.3 | 169.1 | 19.8 | 80.9 |
| 48 | 353.1 | 273.5 | 85.1 | 23.8 | 83.5 |

(cont'd)

Table A2. (cont'd)

| Cow | Total body water+ | Empty body water | Body fat | Body ash | Body protein |
|---|---|---|---|---|---|
| 49 | 253.5 | 197.5 | 47.8 | 14.4 | 62.3 |
| 50 | 254.5 | 189.8 | 95.7 | 13.9 | 62.3 |
| 51 | 336.7 | 251.6 | 94.3 | 17.3 | 73.8 |
| 52 | 291.2 | 243.2 | 119.6 | 19.4 | 77.3 |
| 53 | 263.3 | 187.6 | 19.2 | 14.9 | 55.4 |
| 54 | 270.9 | 173.1 | 17.3 | 15.9 | 55.6 |
| 55 | 240.5 | 192.9 | 66.0 | 13.8 | 60.6 |
| 56 | 303.0 | 244.1 | 188.9 | 18.1 | 74.7 |
| 57 | 292.3 | 218.3 | 153.0 | 17.5 | 71.0 |
| 58 | 292.1 | 213.0 | 58.1 | 16.5 | 66.8 |
| 59 | 325.7 | 234.2 | 67.2 | 17.4 | 69.6 |
| 60 | 283.5 | 217.2 | 43.6 | 18.9 | 69.2 |
| 61 | 254.9 | 204.8 | 130.0 | 14.9 | 67.8 |
| 62 | 307.8 | 250.4 | 211.9 | 19.5 | 82.2 |
| 64 | 286.1 | 229.7 | 87.3 | 23.0 | 70.7 |
| 65 | 286.8 | 210.4 | 95.7 | 17.6 | 66.4 |
| 66 | 236.5 | 180.4 | 33.3 | 14.8 | 56.3 |
| 67 | 246.2 | 189.6 | 79.6 | 14.7 | 62.1 |
| 68 | 274.6 | 203.7 | 68.3 | 14.7 | 62.0 |
| 69 | 239.7 | 173.9 | 46.3 | 13.7 | 54.5 |
| 70 | 246.4 | 194.5 | 33.0 | 14.0 | 61.9 |
| 71 | 228.2 | 180.5 | 14.6 | 16.5 | 53.9 |
| 72 | 208.2 | 164.5 | 43.8 | 14.1 | 52.1 |
| 73 | 242.8 | 186.0 | 123.7 | 18.7 | 68.8 |
| 74 | 307.5 | 243.0 | 111.6 | 18.3 | 82.5 |
| 75 | 327.4 | 245.3 | 85.9 | 20.4 | 78.4 |
| 77 | 280.1 | 226.9 | 111.8 | 18.2 | 71.3 |
| 78 | 245.4 | 201.1 | 101.2 | 15.2 | 64.1 |
| 79 | 273.7 | 215.2 | 96.8 | 16.9 | 66.9 |
| 80 | 327.5 | 262.0 | 126.5 | 20.2 | 81.3 |

+ includes water in alimentary tract and bladder

Table A3. Weight of fat (kg) in various depots

| Cow | Omental and mesenteric | Perirenal | Subcutaneous | Muscle and associated fatty tissue | Bone | Remainder | Total |
|---|---|---|---|---|---|---|---|
| 1 | 15.4 | 9.9 | 38.6 | 64.5 | 7.3 | 25.2 | 160.9 |
| 2 | 22.7 | 8.3 | 48.0 | 65.4 | 11.8 | 30.0 | 186.2 |
| 3 | 3.3 | 2.2 | 10.4 | 24.4 | 9.2 | 15.3 | 64.8 |
| 4 | 45.2 | 15.2 | 46.3 | 78.9 | 8.5 | 34.2 | 228.2 |
| 5 | 5.2 | 2.8 | 19.1 | 26.4 | 8.4 | 13.9 | 75.8 |
| 6 | 7.8 | 5.8 | 20.5 | 39.1 | 6.5 | 21.2 | 100.9 |
| 7 | 17.9 | 5.2 | 24.7 | 40.2 | 9.0 | 16.5 | 113.5 |
| 8 | 15.0 | 7.4 | 32.1 | 52.3 | 9.2 | 23.3 | 139.4 |
| 9 | 1.7 | 0.6 | 0.7 | 11.1 | 7.0 | 5.2 | 26.3 |
| 10 | 23.1 | 9.7 | 44.5 | 58.2 | 6.6 | 25.6 | 167.7 |
| 11 | 4.4 | 2.0 | 3.1 | 14.6 | 5.9 | 6.9 | 36.8 |
| 12 | 3.6 | 0.7 | 8.1 | 22.6 | 12.3 | 8.2 | 55.5 |
| 14 | 6.9 | 1.5 | 4.7 | 22.0 | 10.8 | 12.9 | 58.9 |
| 15 | 14.6 | 6.3 | 17.8 | 47.9 | 13.5 | 17.4 | 117.4 |
| 17 | 5.2 | 1.6 | 5.4 | 18.7 | 10.4 | 8.1 | 49.4 |
| 18 | 17.0 | 12.6 | 28.8 | 52.2 | 6.3 | 19.5 | 136.4 |
| 19 | 20.4 | 11.6 | 39.7 | 71.7 | 7.0 | 23.0 | 173.4 |
| 20 | 3.2 | 1.6 | 3.8 | 18.0 | 10.7 | 8.3 | 45.6 |
| 21 | 9.3 | 4.3 | 20.5 | 38.3 | 10.1 | 17.3 | 99.7 |
| 22 | 9.8 | 4.9 | 17.3 | 37.7 | 10.0 | 14.3 | 94.0 |
| 23 | 14.4 | 6.2 | 21.6 | 46.9 | 8.8 | 11.8 | 109.7 |
| 24 | 37.5 | 14.9 | 50.7 | 85.3 | 7.4 | 24.1 | 220.0 |
| 25 | 46.1 | 14.0 | 80.6 | 91.0 | 9.0 | 33.4 | 274.1 |
| 26 | 27.6 | 8.3 | 39.8 | 68.2 | 8.9 | 24.1 | 176.8 |
| 27 | 26.0 | 9.4 | 28.8 | 65.8 | 12.1 | 22.1 | 164.0 |
| 30 | 9.0 | 4.4 | 17.7 | 31.7 | 6.5 | 14.2 | 83.5 |
| 31 | 6.6 | 2.9 | 9.4 | 22.9 | 7.6 | 9.9 | 59.2 |
| 32 | 3.7 | 1.4 | 5.0 | 18.2 | 9.3 | 7.5 | 45.3 |
| 33 | 3.7 | 0.7 | 1.7 | 12.2 | 11.2 | 7.3 | 36.8 |
| 34 | 0.2 | 0.2 | 0.1 | 3.9 | 5.9 | 4.9 | 15.3 |
| 35 | 14.6 | 4.5 | 12.4 | 44.0 | 10.5 | 15.7 | 101.8 |
| 36 | 19.4 | 9.5 | 17.1 | 40.1 | 8.7 | 15.0 | 109.9 |
| 37 | 14.3 | 4.2 | 13.5 | 36.0 | 10.0 | 11.9 | 89.8 |
| 38 | 26.4 | 11.3 | 18.6 | 50.7 | 7.9 | 18.6 | 133.5 |
| 40 | 6.0 | 1.8 | 4.6 | 17.8 | 8.8 | 11.0 | 50.1 |
| 41 | 56.5 | 18.9 | 42.2 | 77.6 | 10.2 | 28.1 | 233.5 |
| 42 | 47.9 | 16.0 | 40.5 | 86.1 | 10.8 | 25.0 | 226.2 |
| 43 | 29.8 | 11.2 | 19.7 | 54.0 | 13.9 | 19.7 | 148.4 |
| 44 | 32.4 | 11.1 | 22.9 | 60.9 | 15.4 | 20.4 | 163.1 |
| 45 | 39.6 | 17.4 | 46.9 | 79.5 | 10.5 | 34.4 | 228.3 |
| 46 | 6.2 | 1.3 | 3.2 | 17.5 | 9.0 | 8.4 | 45.7 |
| 47 | 34.0 | 8.6 | 30.2 | 60.4 | 9.2 | 26.8 | 169.1 |
| 48 | 13.9 | 3.9 | 12.8 | 33.3 | 8.5 | 12.7 | 85.1 |

(cont'd)

Table A3. (cont'd)

| Cow | Omental and mesenteric | Perirenal | Subcutaneous | Muscle and associated fatty tissue | Bone | Remainder | Total |
|---|---|---|---|---|---|---|---|
| 49 | 3.5 | 1.2 | 2.4 | 21.1 | 10.9 | 8.6 | 47.8 |
| 50 | 9.4 | 5.2 | 20.2 | 38.3 | 7.4 | 15.2 | 95.7 |
| 51 | 14.1 | 3.5 | 12.0 | 37.3 | 11.4 | 16.0 | 94.3 |
| 52 | 25.1 | 8.6 | 18.4 | 44.1 | 8.4 | 15.0 | 119.6 |
| 53 | 0.6 | 0.1 | 0.1 | 4.5 | 5.4 | 8.5 | 19.2 |
| 54 | 1.0 | 0.2 | 0.0 | 5.1 | 7.1 | 3.9 | 17.3 |
| 55 | 7.7 | 2.6 | 9.5 | 25.9 | 8.4 | 11.9 | 66.0 |
| 56 | 36.8 | 10.3 | 36.9 | 75.8 | 6.2 | 22.9 | 188.9 |
| 57 | 22.6 | 8.5 | 34.3 | 59.0 | 8.5 | 20.2 | 153.0 |
| 58 | 6.2 | 2.1 | 4.3 | 25.9 | 7.4 | 12.2 | 58.1 |
| 59 | 8.7 | 3.3 | 7.2 | 28.4 | 9.3 | 10.4 | 67.2 |
| 60 | 4.3 | 1.4 | 4.0 | 17.3 | 6.8 | 9.8 | 43.6 |
| 61 | 15.5 | 7.3 | 28.2 | 50.9 | 6.3 | 21.9 | 130.0 |
| 62 | 34.9 | 13.1 | 52.5 | 75.9 | 8.6 | 26.8 | 211.9 |
| 64 | 7.2 | 3.1 | 16.1 | 33.1 | 11.0 | 16.6 | 87.3 |
| 65 | 11.2 | 3.3 | 17.8 | 39.3 | 8.2 | 15.7 | 95.7 |
| 66 | 3.0 | 0.9 | 1.6 | 14.9 | 6.1 | 6.9 | 33.3 |
| 67 | 8.9 | 1.6 | 17.3 | 27.6 | 8.8 | 15.4 | 79.6 |
| 68 | 6.6 | 1.6 | 11.8 | 25.1 | 12.0 | 11.2 | 68.3 |
| 69 | 3.1 | 1.0 | 4.5 | 18.4 | 11.5 | 7.9 | 46.3 |
| 70 | 2.2 | 0.7 | 1.8 | 11.8 | 10.5 | 6.0 | 33.0 |
| 71 | 0.3 | 0.1 | 0.0 | 3.7 | 5.6 | 4.9 | 14.6 |
| 72 | 2.2 | 0.6 | 4.2 | 18.9 | 11.2 | 6.8 | 43.8 |
| 73 | 12.7 | 7.0 | 26.2 | 49.1 | 9.6 | 19.1 | 123.7 |
| 74 | 15.7 | 9.5 | 18.0 | 40.0 | 11.4 | 17.1 | 111.6 |
| 75 | 8.5 | 2.6 | 13.8 | 34.0 | 9.4 | 17.5 | 85.9 |
| 77 | 7.4 | 7.8 | 17.0 | 49.6 | 7.1 | 22.8 | 111.8 |
| 78 | 12.3 | 5.2 | 18.8 | 40.9 | 7.4 | 16.6 | 101.2 |
| 79 | 12.6 | 5.5 | 18.6 | 36.4 | 7.4 | 16.3 | 96.8 |
| 80 | 20.0 | 7.7 | 21.5 | 43.9 | 10.4 | 23.0 | 126.5 |

Table A4. Red cell volume, blood volume and $D_2O$ space

| Cow | Red cell volume (l) | Blood volume (l) | $D_2O$ space (kg) |
|---|---|---|---|
| 1 | 19.9 | 45.9 | 387 |
| 2 | 16.4 | 46.0 | 392 |
| 3 | 10.3 | 29.4 | 292 |
| 4 | 16.4 | 36.6 | 353 |
| 5 | 9.7 | 23.9 | 297 |
| 6 | 11.8 | 29.0 | 350 |
| 7 | 12.2 | 30.5 | 340 |
| 8 | 17.5 | 40.6 | 423 |
| 9 | * | * | 259 |
| 10 | 13.3 | 32.9 | 353 |
| 11 | 9.3 | 28.4 | 305 |
| 12 | 9.8 | 25.8 | 286 |
| 14 | 10.4 | 33.4 | * |
| 15 | 13.0 | 35.6 | 332 |
| 17 | 12.3 | 31.2 | 276 |
| 18 | 18.7 | 40.7 | 290 |
| 19 | 14.2 | 31.1 | 335 |
| 20 | 10.4 | 29.7 | 292 |
| 21 | 17.9 | 33.2 | 316 |
| 22 | 10.6 | 26.1 | 265 |
| 23 | 12.8 | 31.9 | 322 |
| 24 | 14.8 | 38.0 | 361 |
| 25 | 16.4 | 35.1 | 316 |
| 26 | 13.1 | 30.4 | 308 |
| 27 | 15.1 | 31.8 | 337 |
| 30 | 11.0 | 25.8 | 262 |
| 31 | 9.2 | 21.9 | 228 |
| 32 | 9.5 | 27.3 | 263 |
| 33 | 12.2 | 36.6 | 325 |
| 34 | 14.1 | 40.4 | 284 |
| 35 | 13.4 | 36.7 | 293 |
| 36 | 15.3 | 41.0 | 335 |
| 37 | 14.3 | 27.3 | 261 |
| 38 | * | * | 296 |
| 40 | 15.8 | 45.0 | 300 |
| 41 | 12.4 | 31.0 | 367 |
| 42 | 23.8 | 48.9 | 354 |
| 43 | 19.3 | 45.8 | 372 |
| 44 | 17.4 | 41.8 | 371 |
| 45 | 12.0 | 34.2 | 344 |
| 46 | 17.1 | 39.1 | 310 |
| 47 | 12.2 | 31.4 | 351 |
| 48 | 13.9 | 29.4 | 350 |

(cont'd)

Table A4. (cont'd)

| Cow | Red cell volume (l) | Blood volume (l) | $D_2O$ space (kg) |
|---|---|---|---|
| 49 | 10.2 | 28.7 | 299 |
| 50 | 12.6 | 31.2 | 277 |
| 51 | 14.7 | 31.4 | 337 |
| 52 | 16.5 | 34.1 | 302 |
| 53 | 9.7 | 27.2 | 308 |
| 54 | * | * | 306 |
| 55 | 12.2 | 27.1 | 279 |
| 56 | 13.8 | 29.1 | 307 |
| 57 | 19.6 | 35.8 | 284 |
| 58 | 15.3 | 34.9 | 296 |
| 59 | 14.1 | 31.6 | 302 |
| 60 | * | * | 289 |
| 61 | 10.3 | 24.4 | 276 |
| 62 | 11.4 | 29.7 | 315 |
| 64 | 9.7 | 26.3 | 302 |
| 65 | * | * | 331 |
| 66 | 10.2 | 22.1 | 228 |
| 67 | 19.1 | 34.7 | 265 |
| 68 | 14.4 | 31.1 | 308 |
| 69 | 12.0 | 28.1 | 273 |
| 70 | 11.1 | 26.4 | 266 |
| 71 | 10.5 | 26.0 | 222 |
| 72 | 9.3 | 20.8 | 212 |
| 73 | * | * | 256 |
| 74 | 18.1 | 36.9 | 303 |
| 75 | 13.4 | 32.4 | 344 |
| 77 | 13.6 | 29.1 | 286 |
| 78 | 9.6 | 20.4 | 252 |
| 79 | 14.0 | 32.0 | 276 |
| 80 | 12.0 | 33.7 | 330 |

*indicates missing value

Table A5. Ultrasonic measurements

| Cow | Fat depths (mm) | | | | | Eye-muscle area ($cm^2$) | |
|---|---|---|---|---|---|---|---|
| | 12-13th rib | 3rd lumbar | scapula | femur | leg | 12-13th rib | 3rd lumbar |
| 1 | 21.0 | 18.3 | 11.8 | 3.0 | 6.5 | 81.0 | 68.2 |
| 2 | 10.8 | 15.7 | 6.8 | 5.0 | 7.8 | 62.7 | 98.4 |
| 3 | 5.2 | 10.3 | 4.0 | 2.8 | 8.0 | 44.5 | 23.1 |
| 4 | 14.7 | 19.5 | 5.2 | 17.0 | 4.0 | 89.6 | 62.6 |
| 5 | 5.7 | 6.2 | * | 4.5 | 4.0 | 59.0 | 51.1 |
| 6 | 7.8 | 15.5 | 6.0 | 2.8 | 8.0 | 42.3 | 66.8 |
| 7 | 8.7 | 9.3 | 3.0 | * | * | 72.7 | 67.5 |
| 8 | 5.5 | 13.0 | 5.8 | 1.8 | 12.5 | 80.0 | 69.6 |
| 9 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 26.8 | 31.6 |
| 10 | 24.0 | 31.0 | 6.0 | 8.0 | * | 61.4 | 63.6 |
| 11 | 2.2 | 2.7 | 2.0 | * | * | 43.7 | 56.9 |
| 12 | 2.3 | 2.8 | 1.0 | 2.0 | 1.2 | 38.1 | 42.1 |
| 14 | 2.8 | 2.7 | * | 0.8 | 1.0 | 52.1 | 75.6 |
| 15 | 4.2 | 7.0 | 2.0 | 7.5 | 0.5 | 88.0 | 57.9 |
| 17 | 2.7 | 2.5 | 0.0 | * | * | 26.4 | 33.9 |
| 18 | 10.7 | 20.2 | 3.8 | 3.2 | * | 59.9 | 52.5 |
| 19 | 26.3 | 29.0 | 5.5 | 9.5 | 5.5 | 77.8 | 75.2 |
| 20 | 2.0 | 2.5 | 1.0 | 1.0 | 0.8 | 29.6 | 39.6 |
| 21 | 6.8 | 13.3 | 4.0 | 2.8 | * | 41.2 | 42.1 |
| 22 | 7.8 | 8.8 | 4.2 | 1.5 | 0.0 | 44.8 | 37.4 |
| 23 | 8.3 | 14.3 | 6.8 | 1.5 | 3.0 | 40.9 | 35.9 |
| 24 | 18.0 | 24.7 | 8.0 | * | * | 104.5 | 85.0 |
| 25 | 29.7 | 19.2 | * | 5.0 | * | 76.8 | 87.1 |
| 26 | 23.5 | 15.8 | 5.5 | 4.8 | * | 82.8 | 68.0 |
| 27 | 7.7 | 11.5 | 3.8 | 4.0 | * | 82.8 | 63.0 |
| 30 | 7.0 | 8.0 | 2.5 | 4.0 | * | 61.0 | 44.9 |
| 31 | 4.2 | 3.5 | 1.0 | * | * | 41.8 | 44.0 |
| 32 | 3.8 | 4.0 | * | * | * | 40.5 | 40.0 |
| 33 | 1.2 | 0.2 | 0.5 | 0.0 | 0.0 | 28.6 | 49.6 |
| 34 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 40.4 | 32.5 |
| 35 | 5.2 | 5.5 | 1.8 | 1.0 | 0.5 | 49.3 | 58.2 |
| 36 | 7.0 | 8.5 | 2.0 | 1.8 | 0.0 | 49.8 | 58.7 |
| 37 | 4.8 | 7.3 | 2.5 | 5.8 | 2.0 | 52.1 | 53.5 |
| 38 | 4.3 | 10.3 | 0.0 | 2.5 | * | 60.1 | 45.7 |
| 40 | 0.3 | 1.0 | 1.5 | 2.0 | 0.0 | 30.6 | 36.1 |
| 41 | 13.9 | 11.3 | 4.7 | * | * | 78.5 | 72.7 |
| 42 | 10.8 | 11.4 | 2.5 | * | * | 104.7 | 99.9 |
| 43 | 7.5 | 6.5 | 2.5 | 3.0 | 5.0 | 56.3 | 54.7 |
| 44 | 8.5 | 19.2 | 3.0 | 0.5 | 2.8 | 68.1 | 67.7 |
| 45 | 8.8 | 10.3 | 4.0 | 6.2 | * | 89.0 | 71.6 |
| 46 | 2.8 | 0.3 | 2.8 | * | * | 49.3 | 44.5 |
| 47 | 12.8 | 12.0 | 4.2 | 5.2 | * | 76.8 | 85.7 |
| 48 | 6.8 | 6.0 | 0.8 | 0.0 | 2.0 | 65.7 | 60.5 |

(cont'd)

Table A5. (cont'd)

| Cow | Fat depths (mm) | | | | | Eye-muscle area ($cm^2$) | |
|---|---|---|---|---|---|---|---|
| | 12-13th rib | 3rd lumbar | scapula | femur | leg | 12-13th rib | 3rd lumbar |
| 49 | 0.3 | 0.5 | 0.0 | * | * | 33.2 | 49.9 |
| 50 | 7.7 | 16.0 | 2.0 | 2.2 | 3.0 | 45.2 | 39.7 |
| 51 | 5.0 | 14.0 | 4.0 | * | * | 68.7 | 45.5 |
| 52 | 12.2 | 8.8 | 2.2 | 9.5 | * | 63.2 | 41.6 |
| 53 | 0.7 | 0.5 | 0.0 | 0.0 | * | 43.1 | 28.3 |
| 54 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20.6 | 26.7 |
| 55 | 7.7 | 9.7 | 6.0 | 0.5 | 2.0 | 46.1 | 40.8 |
| 56 | 16.5 | 20.7 | * | * | * | 107.3 | 85.8 |
| 57 | 16.2 | 9.5 | 4.5 | 3.8 | 4.0 | 74.9 | 72.7 |
| 58 | 3.2 | 2.5 | 1.2 | 0.0 | 0.5 | 54.6 | 43.6 |
| 59 | 3.7 | 3.2 | * | * | * | 51.4 | 49.4 |
| 60 | 2.7 | 1.5 | 1.2 | * | * | 46.3 | 32.0 |
| 61 | 8.3 | 7.7 | 2.7 | * | * | 70.4 | 65.4 |
| 62 | 27.5 | 24.7 | 14.0 | * | * | 103.6 | 76.6 |
| 64 | * | * | * | * | * | * | * |
| 65 | 5.7 | 9.3 | 1.8 | 3.0 | 1.8 | 55.7 | 34.1 |
| 66 | 0.5 | 0.0 | 0.0 | 0.8 | 1.0 | 63.1 | 39.0 |
| 67 | 10.0 | 17.5 | 3.8 | 2.2 | 1.5 | 39.4 | 45.4 |
| 68 | 4.7 | 4.7 | 2.0 | 4.0 | * | 41.2 | 40.3 |
| 69 | 2.3 | 6.7 | 2.0 | 1.2 | 2.0 | 35.6 | 39.1 |
| 70 | 1.7 | 1.5 | 0.0 | 1.0 | 0.0 | 37.9 | 56.9 |
| 71 | 0.0 | 0.0 | * | 0.0 | 0.0 | 27.0 | 24.0 |
| 72 | 1.2 | 7.0 | * | 2.5 | * | 28.8 | 23.6 |
| 73 | 11.0 | 18.0 | 6.2 | * | * | 63.6 | 43.9 |
| 74 | 6.0 | 7.7 | * | * | * | 65.3 | 80.4 |
| 75 | 4.3 | 3.8 | 3.0 | 3.5 | * | 58.2 | 49.7 |
| 77 | 5.0 | 6.3 | 3.5 | 6.5 | * | 53.9 | 59.7 |
| 78 | 6.0 | 6.5 | 3.5 | * | * | 48.2 | 44.9 |
| 79 | 5.8 | 14.7 | 6.5 | * | * | 74.8 | 37.0 |
| 80 | 5.7 | 10.8 | 2.2 | * | * | 82.1 | 56.3 |

* indicates missing value

Table A6. Linear measurements

| Cow | Height to withers (cm) | Length pin bone to shoulder (cm) | Width of hook bones (cm) | Skinfold thickness (mm) | Cannon bone circumference (cm) |
|---|---|---|---|---|---|
| 1 | 125.0 | 157.7 | 58.5 | 9.7 | 21.4 |
| 2 | 126.0 | 172.4 | 59.9 | 8.9 | 19.6 |
| 3 | 124.2 | 150.6 | 56.2 | 8.8 | 19.8 |
| 4 | 130.3 | 164.7 | 52.1 | 7.6 | 18.8 |
| 5 | 123.2 | 151.5 | 55.9 | 8.1 | 19.6 |
| 6 | 124.6 | 161.2 | 52.7 | 9.1 | 19.5 |
| 7 | 123.5 | 154.7 | 54.0 | * | 18.9 |
| 8 | 117.8 | 152.7 | 58.6 | * | 20.6 |
| 9 | 124.9 | 153.8 | 53.5 | 7.4 | 19.8 |
| 10 | 122.2 | 155.2 | 56.2 | 7.5 | 19.2 |
| 11 | 126.0 | 146.4 | 49.2 | 6.5 | 19.0 |
| 12 | 121.2 | 154.5 | 52.1 | 8.0 | 19.2 |
| 14 | 124.6 | 154.0 | 53.8 | 7.0 | 19.6 |
| 15 | 124.6 | 160.2 | 53.3 | * | 19.3 |
| 17 | 125.4 | 156.0 | 53.8 | 6.1 | 19.4 |
| 18 | 116.9 | 160.3 | 53.0 | 7.9 | 19.0 |
| 19 | 119.5 | 150.6 | 56.2 | * | 18.4 |
| 20 | 121.2 | 150.6 | 53.2 | 7.2 | 18.1 |
| 21 | 125.6 | 153.5 | 55.2 | * | 17.8 |
| 22 | 114.2 | 142.0 | 49.3 | 6.5 | 18.4 |
| 23 | 125.2 | 150.0 | 52.3 | 6.8 | 18.9 |
| 24 | 126.5 | 162.7 | 57.7 | * | 19.0 |
| 25 | 127.6 | 154.5 | 59.4 | 6.2 | 17.9 |
| 26 | 124.2 | 150.6 | 58.4 | 5.5 | 18.6 |
| 27 | 118.9 | 155.5 | 53.4 | 6.4 | 18.6 |
| 30 | 118.5 | 152.8 | 53.6 | 8.6 | * |
| 31 | 121.9 | 151.5 | 52.2 | 5.5 | 17.6 |
| 32 | 124.5 | 152.5 | 52.2 | 6.9 | 18.3 |
| 33 | 124.6 | 156.0 | 54.3 | 5.9 | 16.6 |
| 34 | 134.9 | 155.5 | 54.9 | 4.9 | 17.9 |
| 35 | 127.8 | 159.0 | 57.7 | 6.4 | 18.2 |
| 36 | 127.4 | 161.9 | 52.9 | 5.8 | 17.9 |
| 37 | 125.6 | 154.2 | 53.5 | * | 17.3 |
| 38 | 127.9 | 158.0 | 55.6 | * | 18.2 |
| 40 | 127.1 | 146.9 | 52.2 | 5.6 | 18.6 |
| 41 | 134.1 | 163.1 | 56.2 | * | 18.0 |
| 42 | 136.5 | 157.6 | 59.1 | 6.2 | 20.2 |
| 43 | 136.7 | 162.6 | 58.2 | 7.0 | 20.0 |
| 44 | 131.8 | 150.6 | 52.2 | 6.6 | 19.7 |
| 45 | 138.8 | 153.8 | 60.6 | 8.6 | 18.6 |
| 46 | 126.7 | 152.8 | 56.4 | 5.6 | 19.8 |
| 47 | 129.4 | 154.3 | 57.4 | * | 17.8 |
| 48 | 130.9 | 153.2 | 56.6 | 6.8 | 19.3 |

(cont'd)

Table A6. (cont'd)

| Cow | Height to withers (cm) | Length pin bone to shoulder (cm) | Width of hook bones (cm) | Skinfold thickness (mm) | Cannon bone circumference (cm) |
|---|---|---|---|---|---|
| 49 | 116.4 | 152.5 | 49.6 | 6.2 | 20.0 |
| 50 | 115.8 | 147.6 | 50.1 | 6.2 | 19.3 |
| 51 | 113.7 | 145.5 | 50.5 | 6.6 | 19.3 |
| 52 | 115.8 | 156.2 | 53.1 | 6.2 | 17.6 |
| 53 | 117.2 | 151.4 | 52.8 | 5.9 | 18.0 |
| 54 | 114.7 | 147.9 | 50.7 | 5.5 | 18.0 |
| 55 | 116.6 | 145.1 | 48.9 | 6.2 | 17.1 |
| 56 | 117.9 | 150.8 | 53.6 | 8.5 | 18.5 |
| 57 | 117.3 | 149.5 | 52.6 | 5.2 | 18.7 |
| 58 | 116.7 | 148.8 | 53.0 | 5.2 | 18.6 |
| 59 | 116.8 | 150.8 | 49.8 | * | * |
| 60 | 116.4 | 154.3 | 54.0 | 8.2 | 18.0 |
| 61 | 117.1 | 145.2 | 51.9 | 8.5 | 17.7 |
| 62 | 121.1 | 160.5 | 53.7 | 7.5 | * |
| 64 | 123.3 | 151.4 | * | * | * |
| 65 | 121.2 | 145.3 | 52.2 | 6.6 | 19.1 |
| 66 | 117.4 | 142.2 | 51.8 | 7.2 | 17.4 |
| 67 | 118.2 | 148.0 | 52.6 | 6.8 | 17.6 |
| 68 | 117.2 | 141.7 | 51.5 | * | * |
| 69 | 119.4 | 143.7 | 51.3 | * | 17.6 |
| 70 | 118.3 | 141.9 | 49.8 | 8.2 | 18.8 |
| 71 | 122.3 | 141.4 | 51.9 | * | 17.7 |
| 72 | 117.0 | 141.8 | 49.5 | 6.0 | * |
| 73 | 119.4 | 152.2 | 53.6 | * | 18.7 |
| 74 | 119.4 | 152.1 | 55.2 | 8.0 | * |
| 75 | 121.1 | 152.0 | 53.8 | 9.9 | 20.1 |
| 77 | 124.0 | 154.1 | 52.4 | 8.2 | 18.3 |
| 78 | 124.3 | 148.6 | 52.1 | * | * |
| 79 | 115.8 | 146.4 | 50.2 | 6.7 | * |
| 80 | 120.2 | 159.0 | 55.7 | * | 20.2 |

* indicates missing value