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# SOME COMPOSITION CHARACTERISTICS OF YOUNG MALE SOUTHDOWN SHEEP FROM LINES SELECTED

# FOR HIGH AND LOW BACKFAT DEPTH

A thesis presented in partial fulfilment of the requirements for the degree of Master of Agricultural Science in Animal Science at Massey University

> Abdullah Yousef Abdullah 1989

#### ABSTRACT

Thirty-six 6-8 month Southdown ram lambs, 18 each from the high and low-backfat selection lines established at Massey University in 1976, were used in this study to evaluate some carcass composition characteristics.

Animals were randomly allocated within lines within sire groups into three lots of 12 rams each and were housed in metabolism crates on a lucerne chaff diet (1.3 maintenance). After a 10-day adjustment period, an intravenous urea challenge was administered to the animals (120 mg/kg LW) and blood samples were collected before and after the infusion. Rams were slaughtered within 5-7 days of the urea challenge and half-carcasses were separated into soft-tissue and bone.

Differences in body composition between the selection lines were greatest for measures of fatness. They were found to a lesser extent in some other characteristics, especially those that have been reported previously to have positive or negative genetic correlations with backfat depth. Thus, carcasses from the high-backfat line, when compared at the same carcass weights had significantly greater fat depths at C. J, GR, S2 and L3, by 56.7%, 37.1%, 26.1%, 33.3% and 51%, respectively. The high-backfat line group also had significantly greater amounts of kidney fat, higher chemically analysed fat percentage in the carcass soft-tissue, larger intermuscular fat cell diameter, shorter carcasses, lighter heart and liver weights, deeper (B) and narrower (A) cross sections of M. longissimus, and slightly higher (P < 0.10) dressing-out percentages.

Moreover, at the same carcass weight, the results of the current study agree well with previous studies in showing that fatter lambs had a higher proportion of the fatter cuts (rack cuts) and a lower proportion of the leaner cuts (shoulder cuts). The high-backfat line animals also had lighter total side bone weight, and shorter lengths and smaller circumferences of the humerus, radius & ulna, femur and tibia bones in the carcasses, which agrees with the negative genetic or phenotypic correlations reported elsewhere between backfat thickness and bone weight, bone percentage or bone length in sheep. At the same total side bone weight, line effects on bone distribution in the current study were less marked than the previous work with 17-month-old rams, with significantly higher weights of bone in the rack cut, lower weights of bone in the leg cut and lighter humerus and femur bone weights for the carcasses of the high-backfat line. Shoulder cut bone weight in the present study did not differ between selection lines and the difference was in the opposite direction for the total leg bone cut compared with older rams in the previous study.

At the same carcass weight, similar total weight of four muscle in the carcasses of both lines was found, but at the same fat-free soft tissue weight in the side there are few effect on the distribution of muscle. The ratio of muscle to bone weight and muscularity are higher in the high-backfat line when adjusted to the same fat-free soft tissue and total side fat-free soft tissue weight plus bone respectively. These results are consistent with previous studies in showing that the reduction in backfat thickness have little or no effect on total muscle weight, little effect on muscle distribution and lower ratio of muscle to bone weight and lower muscularity.

Line differences in muscle fibre type, proportion and area in the M. semitendinosus were not found in the present study. This result which differs from previous which showed higher proportions of (BR) red fibres for the high-backfat line.

In general, all moisture measurements showed a slightly higher weight and percentage in the low-backfat line.

The prediction of empty body water percentage from the response to a urea challenge by measuring the rate of urea dilution in the plasma was not very successful. The best extrapolation estimates of zero-time were obtained using a simple exponential model after linear adjustments were made for increasing baseline values.

It is concluded that divergent selection for and against fatness on the basis of weightadjusted ultrasonically-measured fat depth C in the present lines has led to line differences in 14 kg carcasses such that the fat line carcasses have more fat, less bone and a similar weight of muscle. The urea dilution method as used in this study was found to be unsatisfactory for the prediction of carcass composition.

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# LIST OF ABBREVIATIONS

KKCF	= Kidney knob and chanal fat
SCF	= Subcutaneous fat .
IMF	= Intermuscular fat
IMFD	= Intermuscular fat depot
βR	= Red muscle fibres
αR	= Intermediate muscle fibres
αW	= White muscle fibres
$D_2O$	= Deuterium oxide
ТОН	= Tritiated water
LW	= Liveweight
EBWT	or EBW = Empty body weight
EBH2O	= Empty body water weight
US	= Urea space
EUCC	= Estimated urea concentration change
RRW	= Reticulo-ruminal water
Н	= High-backfat line of Southdown sheep
L	= Low-backfat line of Southdown sheep
s <sub>1</sub> , s <sub>2</sub> , s	$S_3, S_4 = Sires one, two, three and four$
S	= Standard deviation
d	= diameter
v	= volume
A	= Area
RSD	= Residual standard deviation
r	= correlation coefficient between x and y
R²	= coefficient of determination
NS	= Not significant
S	= P < 0.10
*	= P < 0.05
* *	= P < 0.01
***	= P < 0.001

#### **CHAPTER 1**

#### INTRODUCTION

In recent years, a major aim of animal scientists has been to devise methods to increase the lean content of the carcasses from meat-producing animals. The trend for consumers to prefer decreasing amounts of fat in meat is the main reason behind this aim. This changes in consumer preferences are partly because of an increased awareness of the possible health risks associated with an excessive consumption of animal fat.

Improvements in lean meat production and in the distribution of fat, muscle and bone within the carcass are considered to be desirable breeding objectives for New Zealand meatsire breeds of sheep. Changes in fatness within breeds can result from either the direct selection for or against fatness (Fennessy <u>et al</u>. 1987) or they may be achieved by selection for a genetically correlated trait (Roberts, 1979), if a suitable trait could be identified.

A breeding programme based on ultrasonic measurements was initiated at Massey University in 1976 to select for high and low backfat depth in the Southdown breed of sheep (Purchas <u>et al</u>. 1982). The objectives behind this breeding programme were, first, to evaluate changes in carcass characteristics and meat quality and, secondly, to search for correlated traits in these selection lines.

Studies conducted on Southdown sheep selected from the above breeding programme have shown significant differences in backfat depths and relatively small correlated responses such that sheep from the low-backfat line were longer and taller at the same weight (Solis Ramirez, 1988). Work by Kadim (1988) has shown a significant difference in all measures of fatness and in a number of other carcass characteristics between 15 to 17 months old Southdown lines.

In a series of several studies, physiological differences between animals from the two backfat selection lines have been reported with higher levels of plasma urea in the high backfat line being one of the consistent. These differences were observed when the rams were subjected to a short-term fast (Carter <u>et al</u>. 1989) and when they were fed above maintenance level (Bremmers <u>et al</u>. 1988). Van Maanen <u>et al</u>. 1989 has also shown by 24h sampling, fasting and refeeding that low backfat rams had lower concentrations of urea in plasma.

The difference in plasma urea concentrations established in Southdown lines was derived by using a urea challenge technique. In order to investigate these differences in more detail an experiment was designed in which ram lambs from the two lines were to be challenged with an intravenous injection of urea so that clearance rates could be observed. A urea challenge of this nature also forms the basis of a technique to measure body water using urea as a body water diluent. Therefore the opportunity was taken to use these same animals to evaluate the validity of urea as a body water diluent and to determine body composition.

Thus, the reported study in this thesis used 6- to 8-months old ram lambs from the high and low backfat Southdown selection lines.

The main objectives of the study are:-

- 1. To assess the carcass composition characteristics of rams younger than those used by Kadim (1988).
- 2. To assess adipose tissue cellularity and muscle fibre type proportions of M. semitendinosus from these rams.
- 3. To evaluate the urea dilution technique for estimating body composition in young rams.

#### **CHAPTER TWO**

#### LITERATURE REVIEW

# 2.1 INTRODUCTION

This review of literature is made up of four sections: first, evidence for genetic variability in the composition of sheep will be outlined; secondly, information on genetic variability in muscle fibre types of sheep will be reviewed; thirdly, genetic aspects of adipose tissue cellularity of sheep will be discussed; and finally, studies on the measurement of body water by dilution methods will be reviewed.

Generally, reference will only be made to work with lambs although relevant work with cattle, pigs, mice and rats is included where few references for sheep are available.

# 2.2 GENETIC VARIABILITY IN THE COMPOSITION OF SHEEP

### 2.2.1 INTRODUCTION

Berg and Butterfield (1985) described the characteristics of animal body composition of importance for the marketing of meat animals including species, liveweight, carcass weight and edible product yield. They concluded that when animals are sold on a liveweight basis, dressing-out percentage is a critical factor which must be estimated. When sale is by carcass weight, edible product (muscle and acceptable level of fat) must be estimated. Distribution of edible product (muscle and fat) is also important. Berg and Butterfield (1985) described an ideal carcass from a meat animal as one which had a high muscle:bone ratio and an optimum level of fat. Wood, (1983) stated that overall lean and fat content are the most critical commercial aspects of carcass composition, although the ratios of lean to bone and subcutaneous to intermuscular fat and the shape of cuts also vary to an important degree.

In this part of the review emphasis will be toward assessing the influences of genetic factors on the body and carcass composition of sheep. The section will be divided into four sub-sections according to some characteristics of sheep composition as follows;

- 1. dressing-out percent.
- 2. fatness
- 3. muscle distribution and muscle to bone ratio.
- 4. bone percentage and distribution.

## 2.2.2 VARIABLES INVOLVED IN SHEEP BODY AND CARCASS COMPOSITION

#### 2.2.2.1 Dressing-out percentage

Dressing-out percentage is the percentage of cold carcass weight relative to the whole live weight. Factors effecting this characteristic include breed, degree of maturity, degree of fatness, sex, liveweight, wool weights and alimentary tract contents (Kirton <u>et al</u>. 1984; Wood <u>et al</u>. 1983a). The differences in dressing-out percentage within any species of meat animal will be affected to a large extent by differences in these factors, which have often been taken into account in many comparative studies between and within breeds of sheep.

Between breeds comparisons: At the same degree of maturity, McClelland <u>et al</u>. (1976) showed highly significant differences in dressing-out percentage between Soay (47.7%), Finnish Landrace (53.3%), Southdown (53.3%) and Oxford Down (56.8%) breeds. They attributed these differences to degree of maturity at slaughter, the size of the breed, and sex. In another study Wolf <u>et al</u>. (1980) showed significant differences in dressing-out percentages in the crossbred progeny, in descending order of values, of Oldenburg, Suffolk, Oxford, Texel, Ile-de-France, Dorset Down sires out of Border Leicester X Blackface cross, and Animal Breeding Research Organisation Dam line X Blackface cross ewes at constant live weight. Previously, Fourie <u>et al</u>. (1970) had also shown that Southdowns had a significantly higher dressing-out percentage than Romney sheep when slaughtered at the same age (80 Weeks), and at maturity, while Southdown X Romney cross animals were intermediate. A similar result has been also reported by Kirton <u>et al</u>. (1981) in that Southdown X Romney lambs had higher dressing-out percentages than straight Romneys. In similar, the fatter crosses breed has also been found to have higher dressing-out percentages than the straight breed (Hawkins <u>et al</u>. 1985a and Kemp <u>et al</u>. 1981).

In contrast Meyer <u>et al</u>. (1978) found no significant differences among groups of cross lambs out of Oxford, Suffolk and Southdown sires at the same weight, although dressing-out percentages differed over slaughter groups. This result was in agreement with Fahmy <u>et al</u>. (1972) who compared Southdown lambs with Suffolk lambs at the same liveweight. In a comparison of Targhee and Suffolk X Targhee breeds Lloyd <u>et al</u>. (1981) found no significant differences in dressing-out percentage. Generally, however, it is concluded that there are often real differences in dressing-out percentage between breeds, which may be attributed to fatness, sex and nutritional interaction.

<u>Within breeds comparisons</u>: Bradford and Spurlock (1972) found a significant difference in dressing-out percentages among sire-progeny groups within the Suffolk breed at the same carcass weight with fatter groups having higher dressing-out percentages.

Recently, Kadim <u>et al</u>. (1989) showed significantly higher dressing-out percentages for Southdown rams from a high backfat selection line (49.7%) than a low backfat line (47.5%), when adjusted to the same carcass weight. This differences between the two lines appeared despite the fact that the non-carcass fat depots were significantly heavier in the high-line carcasses. The differences in dressing-out percentage were thus attributed to extra fat being located in the carcass.

<u>Heritability</u>: Sheep dressing-out percentage has been found to be moderately or highly heritable. Botkin <u>et al</u>. (1969) reported an estimate of 0.41 and Bradford and Spurlock (1972)  $0.94 \pm 0.36$ . This indicates that if this parameter was measured, either by suitable live predictors or by progeny testing, and used in selection, the trait should improve. Heritability estimates have been presented in a number of papers reviewed by Wolf and Smith (1983).

# 2.2.2.2 Fatness

As the animal grows and its carcass weight increases, the most notable change in composition is in the total amount, the percentage of and the relative partitioning and distribution of carcass fat (Berg and Butterfield, 1985). For instance, there will be a large variation in level of carcass fatness within lambs of the same sex, breed and weight from the same farm. The extent and distribution of fat therefore plays an important role in the composition of meat animal carcasses (Berg and Butterfield 1985).

**Fat-percentage:** Breed differences in the amount of fatness have been clearly demonstrated. Bowman (1967) reported that there was considerable genetic variation in fat percentage in sheep. The Suffolk X Targhee group had heavier carcass weight (P < 0.05) and more external fat than straightbred Targhees (Lloyd <u>et al</u>. 1981). The Suffolk breed was found to

be less fat than five other breeds especially when the comparisons were made at body weights exceeding 30 kg (Reid et al. 1968). The amounts of fat in the carcass of Southdown, Suffolk and Oxford breeds differed considerably in descending order when compared at the same carcass weights (Meyer et al. 1978). Fourie et al. (1970) showed that the Southdown breed was smaller and fatter (P < 0.05) than the Romney breed, although the differences became less marked as maturity was attained. On New Zealand hill country, the Romney breed was leaner than Merinos, Corriedales, Perendales, Border Leicesters and Romney crossbreds at constant carcass weight, while at constant age, the Merinos had the least fat, followed closely by the Romney, with the Perendale lambs the fattest (Kirton et al. 1974). At comparable weights, the Merino breed was found to be fatter than Border Leicester X Merino crossbred (Searle and Graham, 1972), but leaner than Dorset Horn X (Merino X Border Leicester) crossbreds (Kellaway, 1973). Wood (1982) reported that the percentage of fat at a particular weight is influenced mainly by mature size, with early maturing sire breeds producing lambs which were fatter than those sired by later maturing breeds when compared at the same carcass weight. However, Wood (1982) showed that when comparisons were made at the same age, which parallels stage of development under similar feeding conditions, breed differences disappeared. To examine the effect of differences in mature weight on the composition between breeds, McClelland et al. (1976) studied four different breeds (Soay, Finnish Landrace, Southdown and Oxford) under similar conditions. They found that Soay sheep consistently contained less fat than Finnish Landrace, when comparisons were made at a constant proportion of mature weight, and concluded that differences in mature weight are not solely responsible for differences in composition between breeds. On the basis of this conclusion, and after accounting for mature size, Wood (1982) showed that the remaining variation in total fat content between Southdown, Dorset Down, Suffolk, Texel and Oxford Down at constant body weight was probably small, and probably less than 10% of the whole variation in fatness. Lee (1984) studied carcass composition in a trial involving Scottish Blackface and Welsh Mountain breeds. They showed that the Welsh breed had higher subcutaneous fat and more (P < 0.001) kidney fat than Scottish Blackface at the same age. This difference in subcutaneous fat disappeared when comparisons were made at the same degree of maturity, but there was no change in kidney fat differences. At constant live weight, Wolf <u>et al</u>. (1980) showed a significant difference in percentage fat between crossbred progeny of Oxford, Suffolk, Texel, Ile-de-France, Oldenburg and Dorset Down breeds. Dorset Down and Ile-de-France cross lambs were fattest at constant liveweight at slaughter, while the Suffolk breed had 1.7% more fat in the carcass than the Oldenburg but neither differed significantly from the Oxford cross.

**Fat-partitioning:** Fat partitioning refers to proportion of total fat in different depots such as subcutaneous, intermuscular and kidney fat (Callow, 1948). A number of studies showing that the partitioning of fat among the depots is influenced by breed have been reviewed by Kempster (1980). Vezinhet and Prod'hon (1975) reported that differences between species in the location of different depots exist when comparisons are made at the same level of total fat.

Meyer et al. (1978) reported that the Oxford cross had less internal fat (as measured by amounts of omental and kidney fat) than the Suffolk, but relatively more external fat over the loin ('C' measurement). McClelland and Russel (1972) showed that a notably prolific sheep breed (the Finnish Landrace) had particularly large amounts of internal fat relative to total fat when compared with the purebred Scottish Blackface at the same carcass weight. Wood et al. (1980) found that Clun Forest and Colbred sheep (ewe breeds) had a higher proportion of internal fat than Suffolks or Hampshire (ram breeds) at the same weight of carcass fat. They reported that these results, together with other information available from several trials, indicated that breeds of meat-type sheep can be broadly classified into ewe breeds (noted for prolificacy and milk ability) and a ram breeds (noted for meat characteristics) with the ewe breeds having a higher proportion of internal fat. The results from sheep growing over the normal slaughter weight range (Kempster, 1980) indicated a high relative growth rate for kidney knob and channel fat (KKCF) in comparison with intermuscular fat (IMF). Also, in this case subcutaneous fat (SCF) grew at a similar rate to KKCF (the allometric growth coefficients of fat depots on carcass weight were 1.81 for SCF, 0.79 for IMF and 1.17 for KKCF). Similar results was found by Wood et al. (1980). These results, together with other information available on the development of fat depots over the growing/fattening period in sheep, are thus consistent in indicating that SCF grows relatively faster than IMF (Kempster, 1980). The results of Thompson et al. (1979a) showed no differences in the partitioning of fat between carcass fat depots (SCF and IMF depots) from the progeny of Dorset Horn and Border Leicester rams crossed with either Border Leicester X Merino cross, Corriedale or Merino ewes. However, their results did show differences in the partitioning of fat between the carcass and internal fat depots, with the progeny of Dorset Horn rams and Merino ewes having a higher proportion of total fat as internal fat than the progeny of Border Leicester rams, and Border Leicester, Merino and Corriedale ewes respectively. Similarly, at the same carcass weight, Kirton et al. (1974) found that Merino lambs had less subcutaneous fat and more perirenal fat than Perendale lambs. Jones et al. (1985) also found a significant differences in fat partitioning between subcutaneous and intermuscular fats depots at equal total fat weight between several sire breeds from the Meat

and Livestock Commission's Ram Breed Evaluation. Kempster and Cuthbertson (1977) also recorded significant differences (P < 0.05) between seven-breed type groups in fat partitioning at a constant subcutaneous fat percentage. They demonstrated that the carcasses of Welsh Mountain lambs had a significantly higher total fat percentage (and by far the highest KKCF percentage and above average IMF as a percentage of total fat percentage) and the British Longwool cross group had a lower total fat percentage (and the lowest KKCF and IMF percentages) than the other five breeds (Blackface, Suffolk, Intermediate, Southdown crosses and British Longwool) when adjusted to the same overall mean subcutaneous fat percentage. The Suffolk cross group had low KKCF percentage and the highest IMF percentage. Overall, these studies of sheep indicate differential genetic pressures on fat partitioning (Kempster, 1980). Thus, there are breed differences in the partitioning of body fat, especially between the carcass fat (subcutaneous and intermuscular) and internal fat (kidney knob and channel fat, omental fat and mesenteric fat depot) (Kempster, 1980). The conclusions from these studies were clear, but they do not necessarily reflect real genetic differences because in order to demonstrate real genetic difference, animals should be compared at the same total fatness.

Fat distribution refers to its location within the various depots such as Fat-distribution: subcutaneous, intermuscular and kidney fat (Callow, 1948). Kempster, (1980) reviewed results from the British Meat and Livestock Commission trials which involved several sire breeds and concluded that breed variation in the distribution of fat existed though the differences were small. He also drew attention to the scarcity of published information on the relative growth of fat depots in different regions of the carcass. Several authors (e.g. Hammond, 1932; Donald et al. 1970 and McClelland and Russel, 1972) have reported that breeds of sheep differ in fat distribution within various fat depots. Gaili, (1978) showed that when animals were slaughtered at 42 kg liveweight, Clun lambs yielded the least total IMF, Dorset Horn lambs the most, and Hampshire lambs an intermediate amount. Also, at an equal total intermuscular fat weight, the Clun lambs had significantly less IMF in the thoracic limb than the other two breeds, and the IMF content of the neck and thorax regions in the Hampshire was lower than that in the remaining breeds. Merino lambs had higher proportions (relative to the total side SCF and IMF weight respectively) of SCF and IMF in the loin and flank joints and a lower proportion in the thorax than the Dorset Horn (Border Leicester X Merino) when slaughtered at the same liveweight (Seebeck, 1968). Nevertheless, Kempster (1980) concluded that there was relatively little variation between breeds in fat distribution, especially when comparisons between breeds are made at equal total depot weight.

<u>Heritability and within breed comparisons</u>: Genetic selection to change lamb carcass composition may involve selection between breeds based on comparative information or within-breed selection based on some form of testing of individual rams (Wood, 1983). Although genetic differences occur in carcass composition, manipulation of these differences by genetic means depends largely on controlling the proportion of fat (Wolf and Smith, 1983).

Selection responses are affected by the accuracy of the lean measurement. Several ultrasonic techniques to estimate carcass leanness (lean weight/carcass weight) in young rams have been compared and correlations between ultrasonic measurements and carcass leanness of 0.45 have been reported in sheep (Smith <u>et al</u>. 1986).

Heritability, selection intensity and generation interval are the factors affecting selection responses in lamb carcass composition (Smith et al. 1986). Heritability estimates of carcass traits in sheep have been presented in a number of papers, and have been summarized in detail by Wolf and Smith (1983). They indicated that percentage of fat in the carcass is moderately heritable (in some reports relatively high heritability was recorded for fat measurements) with low values being reported for the heritability of the subcutaneous to intermuscular fat ratio. Also they stated that the studies of fat distribution at constant weight of total dissectible tissue showed low heritabilities for this trait. Although most heritability estimates would not have been on live-animal measurements, selection of individuals on their own performance should allow continued improvement in these characteristics provided they can be measured (Wolf and Smith, 1983). Wood (1983) reported that ultrasonics have been used to select lean animals for breeding in sheep, although less successfully than in pigs. This is partly because in ruminants there is less subcutaneous fat than in pigs, and the loose hide or fleece presents a greater problem. They concluded that changing breeds in these species (sheep and cattle) seems a much quicker route to genetically leaner carcasses, although in some situations this may not be a practical alternative. Wolf and Smith (1983) concluded that estimates of the heritability and phenotypic standard deviations of carcass traits showed that useful responses may be expected from within breed selection. Progress as often been slower than expected because it is difficult to identify differences in composition (Wood, 1983).

In most lamb studies from which genetic parameters have been derived, only one fat depot has been measured, although where more than one depot was measured, genetic correlations between individual depots are often low (Wolf <u>et al</u>. 1981) and sometimes

negative (Olson et al. 1976). These correlations suggest that direct selection for a change in one depot may not result in concomitant changes in other fat depots within the body. Fennessy et al. (1982) reported that selection of sires on the basis of backfat thickness could be an effective means of reducing carcass fat thickness in lambs. The difference between the progeny of four lean sires and that of four fat sires was significant for two fat measurements, namely the backfat (P < 0.05) thickness over the 12th rib, and S2 (P < 0.01) a fat depth in the shoulder region. Generally those lambs sired by the lean rams were leaner than average for GR and chemical fat parameters measured (but not significantly different), while those sired by fat rams were fatter than average. They concluded that the significant regression relationship between the progeny and sires indicated that selection of sires on the basis of backfat thickness measured ultrasonically could be expected to assist in reducing backfat thickness of the carcasses produced. Lean, fat and control lines of Coopworth sheep were established in 1980 at Invermay (Fennessy et al. 1987) on the basis of backfat thickness (measured ultrasonically over the 12th rib) adjusted for liveweight. Marked difference between the lines in backfat thickness were reported indicating a considerable response to selection. Consequentially an experiment was undertaken by McEwan et al. (1989) to identify possible developmental changes in body components that had resulted from this Coopworth breed selection experiment. Preliminary results for the subcutaneous fat depths and internal fat depots indicated that selection had resulted in significant changes in the carcass fat depth at the selection site when compared at the average carcass weight of all animals in the trial. They also reported that there were correlated changes in fat depths throughout the subcutaneous fat depot and also changes in the internal fat depot weights. The response was generally a greater for the high-backfat line. McEwan et al. (1989) concluded that the implication from the indirect response in internal depots was that genetic selection in these flocks was acting at least partly on common processes or controls shared between all fat depots.

Meyer <u>et al</u>. (1981/1982) selected the fattest (high) and leanest (low) rams from the Rotomahana Southdown and Suffolk flocks and the Ruakura Southdown flock based on weight-adjusted ultrasonically-measured fatness over the loin (location C) and progeny tested them over Romney ewes. The measurement of the 'C' fat depth taken over the loin showed the progeny of low-fat sires to be leaner than the progeny of high sires in both breeds over both of two years. On average, progeny of two sires had 10% less fat at 'C'. This difference was not seen, however, in GR fatness measured over the rib, suggesting that successful selection against fatness at one carcass site may not be as beneficial as hoped in reducing total carcass fatness.

At Massey University, Kadim et al. (1989) reported from Southdown rams lines selected on the basis of backfat depths (assessed ultrasonically at position 'C' over the longissimus dorsi muscle at the last rib) that selection on the basis of a single fat depth measurement resulted in significant differences in all measures of fatness, and also in several other carcass characteristics. Kadim et al. (1989) showed that although mean carcass weights for rams from the two lines were very similar, they differed significantly in fatness with the percent of carcass dissectible fat and intramuscular fat being 19.8 and 47.8 percent higher, respectively, in the high backfat, when these lines were compared for partitioning and distribution, Kadim et al. (1989) found that, at the same total side fat weight, animals from the high backfat line had more fat in the subcutaneous depot (P < 0.001,  $R^2 = 0.97$ ), more intramuscular fat in the longissimus muscle (P < 0.0001,  $R^2 = 0.67$ ), and less intermuscular fat (P < 0.001,  $R^2 = 0.90$ ), but similar weights of the internal fat depots. Differences between the two lines in the ways in which fat was distributed amongst four cuts(shoulder, rack, loin and leg) were small with a slight tendency for a greater proportion of both subcutaneous and intermuscular fat to be in the rack (P < 0.01) and loin (P < 0.05) cuts for the high backfat line.

#### 2.2.2.3 Muscle - Distribution and muscle to bone ratio

Distribution: Distribution of muscle tissue during growth and development does not differ between breeds to the same extent as fat (Kempster and Cuthbertson, 1977). Research work on muscle growth has shown only small differences between breeds in the distribution of lean in different parts of the body, especially when allowance is made for the total amount of lean and the stage of development of the animals (Lohse et al. 1971). However, there is still disagreement between researchers on the value of the differences which exist (Seebeck, 1968; Jury et al. 1977; Taylor et al. 1980). Butler-Hogg and Whelehan (1987) noted that the distribution of muscle weight in sheep did not change between breed to an extent which would have commercial implications in the first year of life. They showed that Clun and Southdown lambs had similar distributions of muscle weight at the same age despite their differences in mature size and conformation. The high-valued muscle constituted 513.8 g/kg total muscle in Clun and 514 g/kg total muscle in Southdown lambs at an age of 200 days (Butler-Hogg and Whelehan 1987). Detailed anatomical studies of the growth and development of the musculature of sheep have revealed a close relationship between muscle weight distribution and total muscle weight in the carcass (Lohse et al. 1971; Jury et al. 1977). Thus, when comparisons were made at a constant total muscle weight both Jury et al. (1977) and Seebeck (1968) found significant breed effects only on the muscles of the neck, thorax

and abdomen. Work with crossbred lambs conducted by Wolf (1982) gave a similar result to the above findings, with small but significant differences due to breed when comparisons were made at a constant weight of total lean. Also, the breed of sire did not significantly affect the proportion of total lean in the higher-priced joints, and the maximum differences between breeds for proportion of lean in each joint never exceeded 7.7 g/kg. This work examined eight standard joints of crossbred lambs slaughtered at constant liveweights of either 35 or 40 kg. The sire breeds used were Dorset Down, Ile-de-France, Oldenburg, Oxford, Suffolk and Texel (Wolf, 1982). McClelland et al. (1976) showed large differences in total muscle weight between Soay, Finnish Landrace, Southdown and Oxford Down breeds at the same degree of maturity, but most of the breed differences disappeared when expressed as a percentage of body or carcass weight. Moreover, total muscle as a percentage of body weight remained unchanged from one stage of maturity to another in the range of 40 to 76% of mature body weight, although total muscle as a percentage of carcass weight declined as maturity increased and this showed significant differences between breeds. Similar results where obtained by Taylor et al. (1980) between the same four breed based on 12 individual muscles obtained from the prime retail joints. They also obtained highly significant breed differences in the weights of individual muscles, but these differences were greatly reduced (non-significant in some muscles) when values were expressed as a percentage of total muscle weight. The combined muscles comprised 40.8, 43.4, 40.5, and 39.8% of total muscle weight respectively in the Soay, Southdown, Finnish Landrace and Oxford Down breeds. The carcass lean percentage of lambs of nine sire breeds (Border Leicester, Dorset Down, Hampshire Down, Ile-de-France, North Country Cheviot, Oxford Down, Southdown, Suffolk and Wensleydale) were reported by Croston et al. (1979) after adjustments were made to equal carcass SCF percentage (11.7%). Significant differences were recorded between sire breeds with the highest values for lambs by Ile-de-France and Wensleydale sires (56.7 and 56.5% respectively) and the lowest for lambs by Dorset Down, Oxford down and Hampshire Down sires (55.8, 55.8 and 55.6% respectively). They also reported that significant, but (from the economic point of view) relatively unimportant, breed differences were recorded in the percentage of total lean occurring in the higher-priced joints.

<u>Muscle to Bone Ratio</u>: It has been suggested by Butterfield (1974) that any variation in the percentage of muscle in a carcass is brought about primarily by change in fatness, and secondly by changes in the muscle to bone ratio. Thus, as an animal grows, its muscle:bone ratio increases to a plateau at maturity and the fat:muscle ratio also increases to maturity. In meat animals, Berg and Butterfield (1985) reported that muscle weight relative to liveweight seemed to increase predictably with weight within a genotype and considerable differences

exist between various breed types. Boccard (1981) suggests that for meat animals the muscle:bone ratio is of particular interest since it measures their meat yield at cutting. He added that in the final stages of the preparation of the animals, and from the point of view of meat production, this ratio does not depend on the growth rate and seems to be a breed characteristic.

At constant liveweight the progeny of Dorset Down, Oxford, Ile-de-France, Oldenburg and Texel sires differed significantly in percentage lean, lean/bone ratio, lean/fat ratio and eye-muscle area (Wolf <u>et al</u>. 1980). The proportion of lean in the carcass of the Texel cross lamb was 3.7 percentage points above the mean for all breeds due to high lean/bone and lean/fat ratios. Differences in percentage lean in the side were larger when compared at equal liveweights than after adjusting to an equal percentage SCF in the side, but the Texel breed produced a high percentage of lean because of a high ratio of lean to bone. Wolf <u>et al</u>. (1980) concluded that most emphasis must be placed ultimately upon the differences between breeds in the weight of total lean produced. Similar results were reported by Kempster and Cuthbertson (1977) in a survey of the carcass characteristics of the main types of British lamb when the data were adjusted to the overall mean subcutaneous fat percentage.

Anous (1989) reported that the ratio of muscle to bone weight offers an index for selection purposes of the degree of carcass muscling, as it was found to be an inherited characteristic. However, age, size, sex, nutrition and genotype were found to effect the muscle to bone ratios of sheep. Anous (1989) showed that the muscle:bone ratio in the hind limb of ram lambs of Rava, Ile-de-France, Charmois, Rouge-de-l'Ouest, Vende-e, Suffolk, Charolais, Romanov, Tarasconnais, Southdown and crossbred breeding varied, from three units for breeds with poor conformation (but of comparable fatness) to about six units for breeds with good conformation. Thus each one of the pure breeds was showed to be distinguished from the others by a specific muscle:bone ratio, so that animals of the same bone weight varied considerably in the amount of muscle and thus their muscle:bone ratios (Anous, 1989). Also, there was considerable variation in the ratios of individual muscle weights relative to the weights of the supporting bone. The coefficients of variation of muscle:bone ratios of individual muscles varied also from one muscle to another. However, the correlations between the different muscle:bone ratios showed that variability of many ratios is widely independent from those of the other ratios. Thus it seemed that a part of the variability of the muscle:bone ratios is related to the specific nature of each one. On the contrary, all the muscle:bone ratios showed a high correlation with the total muscle:bone ratio, except three of the ratios, the highest one was M. semimembranosus/femur ratio (r = 0.94). He concluded that, in the selection of meat animals, this ratio could be used with other ratios such as M. gluteobiceps/femur (r = 0.89), M. adductores/femur (r = 0.89) and M. semitendinosus/femur (r = 0.87) as indicators of the degree of relative muscling of animals.

Muscle to bone ratios was found to be higher for crosses of the Southdown breed than the Suffolk breed when the data from seven breed-type groups compared by Kempster <u>et al</u>. (1976).

<u>Heritability Estimates</u>: Smith <u>et al</u>. (1986) reported that, although selection responses are affected by the accuracy of the lean measurements, breed differences in the percentage of lean in the carcasses of sheep are not large and long-term selection within breeds will be necessary to improve this trait. Wolf (1982) suggested that there was little justification for the selection of a breed or of individuals in the hope of obtaining superior muscle weight distribution, as the gains to be made are likely to be insignificant compared with other variables.

Heritability estimates from several studies reviewed by Wolf and Smith (1983) indicated that in general percentage lean in the carcass was moderately heritable. Wolf and Smith, (1983) reported that the combination of moderate heritability estimate and phenotypic standard deviations of about 3.0 percentage units from studies reviewed indicated a potentially useful response to direct selection for percentage lean in the carcass. Heritability estimates for lean tissue distribution established by Wolf (1982) ranged from 0.07 (s.e. 0.08) to 0.65 (s.e. 0.16) for the proportion of total lean in the best-end neck and higher-priced cuts respectively. However, for most traits involving muscle, heritability estimates were only moderate. Where heritability estimates were highest, the coefficients of variation were low. Also, the dissection errors may have possibly made large contributions to the variation in the proportion of total lean in small joints, thus explaining the association between size of joint and coefficient of variation. These considerations support the conclusion that selection to change lean distribution would be possible but slow (Wolf, 1982). Taylor et al. (1980) reached a similar conclusion that carcass improvement by altering muscle distribution was unlikely to be very effective. Wood (1983) stated that selection within a breed to change carcass composition should be possible since it is moderately heritable. Kadim et al. (1989) showed that at the same side weight, high-backfat-line Southdown rams had significantly lower weights of muscle and bone but higher muscle to bone ratios (P < P

0.05). When adjusted to a constant total-side muscle weight, any line effects on individual muscle weights or muscle weights in the four cuts were small, although two of the major muscles of the proximal pelvic limb were significantly heavier (M. semimembranosus and M. biceps femoris) in the high-backfat selection line. Also, muscularity in terms of muscle weight relative to skeletal size was greater for the high-backfat selection line carcasses, when assessed either for total side muscle relative to body length, or the weights of individual muscles relative to the length of adjacent bones.

From these studies it can be concluded that:

- 1. There are real breed effects in the percentage of lean, in muscle weight distribution, muscle/bone ratio and in muscularity.
- 2. Some, but not all, of these characteristics should be considered for genetic selection.
- 3. All these characteristics should be at least measured and monitored.

#### 2.2.2.4 Bone - Percentage and Distribution

There have been few studies of bone weight distribution in sheep and present knowledge of the factors influencing it is limited. Wolf and Smith (1983) reported that evidence available until now, however, is consistent in indicating that there are small but economically unimportant effects of breed on bone distribution. They showed a significant differences between Dorset Down, Ile-de-France, Oldenburg, Oxford Down, Suffolk and Texel in the distribution of bone weight in standard joints (leg, middle-neck, shoulder and scrag) when comparisons were made at a constant weight of bone in the half carcass. Seebeck (1968) and Thompson <u>et al</u>. (1979b) both also reported on breed differences in the distribution of bone in sheep between standard joints. Thompson <u>et al</u>. (1979b) reported that the progeny of Dorset Horn and Border Leicester rams out of Merino, Corriedale and Border Leicester X Merino ewes differed significantly for bone tissue distribution between standard joints.

It has been shown that sheep breeds differ in bone percentage relative to body or carcass weight (McClelland <u>et al</u>. 1976; Wolf <u>et al</u>. 1980; Fourie <u>et al</u>. 1970). Fourie <u>et al</u>. (1970) showed that Romney carcasses, contained significantly more bone than those of Southdown and Southdown X Romney crosses when slaughtered at similar age and maturity.

McClelland et al. (1976) reported that differences between breeds in weight of bone is probably a reflection of their mature size. They found large differences (P < 0.001) in bone weight between Soay, Southdown, Finnish Landrace, and Oxford breeds when comparisons were made at the same degree of maturity with total bone weight ranging from a mean of 1.3 kg in the Soay to 4.2 kg in the Oxford Down breed. Moreover, these differences were still observed when expressed as a percentage of body or carcass weight, but in a reverse situation, the Soay having the highest percentage bone, with a mean value of 10.3 compared with 7.3, 8.8 and 9.3% for Southdown, Finnish Landrace and Oxford respectively. Similar results were obtained between six terminal sire breeds of sheep in the percentage of bone relative to carcass weight (Wolf et al. 1980). The significant differences in bone weight between the six terminal sire breeds still existed when the sire breeds were compared after adjusting the data to an equal percentage of SCF in the side with the only changes in ranking being for the Texel, Dorset Down and lle-de-France crosses (Wolf et al. 1980). Kempster and Cuthbertson (1977) also showed significant differences (P < 0.05) in bone percentage in the side with and without adjustment of the data to a constant SCF percentage for British Longwool crosses, Suffolk crosses, Intermediate, Southdown crosses and British Longwool sire breeds. The results showed that side bone weight ranging from a mean of 15.1% in the Intermediate sire breed group to 16.4% in the Suffolk crosses after adjusting the data to the equal SCF percentage. However, it has been found that lower percentages of bone in carcasses were largely the result of higher level of fatness (Berg and Butterfield, 1974).

# 2.3 GENETIC EFFECTS ON MUSCLE FIBRE CHARACTERISTICS

#### 2.3.1 INTRODUCTION

Kiessling <u>et al</u>. (1982) reported that skeletal muscles of many animals are known to vary in fibre types, numbers, sizes and colour, and that there is a marked heterogeneity of the individual fibres which compose vertebrate skeletal muscles. This heterogeneity has been confirmed biochemically as well as physiologically and is most readily demonstrated by the application of the modern histochemical methods (Kiessling <u>et al</u>. 1982).

The object of this section is to investigate the genetic variation in muscle fibre characteristics of sheep, starting with some information on the changes in muscle fibre characteristics with growth. Genetic differences between and within lines or breeds will be described with emphasis on the relevant information from sheep and from other meat animal species, but research with mice, rats and chickens will also be considered.

#### 2.3.2 CHANGES WITH GROWTH

Skeletal muscle is the predominant tissue of the mammalian body. Muscle fibres are the basic unit of skeletal muscle and comprising 75 to 90% of total muscle mass (Hegarty, 1971). Cassens and Cooper (1971) suggested that striated muscle of each mammal has its own fibre type profile which determines its physiological function during life. They concluded that the distribution of these fibre types and their relative contribution to the total muscle mass show species and individual variation. Staun (1972) noted that these fibre types will differ both within and between muscles of an individual and are subject to dynamic changes due to age, sex, physical activity and feeding level.

Animals are born with a certain number of fibres in each muscle. The prenatal origin of these fibres are myoblast cells (Swatland, 1984). Premyoblasts give rise to myoblasts which fuse to form muscle fibre precursors called myotubes. Myoblasts fuse with each other and with myotubes to form multinucleate cells, which give rise to multinucleate skeletal muscle fibres at birth. During postnatal development, new nuclei derived from satellite cells are added to the muscle fibre. During postnatal development, the fibres grow radially and longitudinally (Swatland, 1984), and with continued development there is an increase in the content of nucleic acids and in the amount of protein accumulated per unit of nucleic acid (Young, 1985). Postnatal muscle growth appears to be associated largely with increases in fibre cross-sectional area and length rather than in fibre number. Ashmore and Addis (1972) reported that all muscle growth in lambs after 140 days of gestation was the result of increases in the size of fibres present at that time.

Individual skeletal muscle fibres can be identified and evaluated using histochemical methods to indicate the activity of enzymes such as succinic dehydrogenase, adenosine triphosphatase in the alkaline range (ATPase) and phosphorylase (Ashmore and Doerr, 1971). These three enzymes are localized within mitochondria, at the 'A' band and in the space between myofibrils, respectively.

Several classification systems have been described for the identification of skeletal muscle fibre types (Stein and Padykula, 1962; Guth and Samaha, 1970; Peter <u>et al</u>. 1972). One classification that has been widely used in muscle studies of meat animals is the one described by A shmore and Doerr (1971) which distinguishes between red ( $\beta R$ ), intermediate ( $\alpha R$ ) and white ( $\alpha W$ ) fibres on the basis of ATPase and oxidative enzyme activities. Also the fibres capable of aerobic metabolism are designated as red (R) and those mainly capable of only anaerobic metabolism as white (W) (Ashmore, 1974).

#### 2.3.3 GENETIC INFLUENCE

Staun, (1972) stated that the size of an individual muscle is a function of its cell size and cell number. Therefore, variation in muscle weight will depend on the variation in one or both of these variables and any difference in extracellular material (eg. fat). The relative weights of muscles change as total muscle weight increases, with the greatest changes occur during immediate post-natal growth (Butterfield, 1963). The mechanism regulating the number of myotubes and hence the number of muscle fibres that are formed is not known (Goldspink, 1977), but it is almost certainly under genetic control. Muscle hypertrophy (double muscling) in domestic cattle is an inherited disorder of skeletal muscle growth and development, and the primary alteration appears to be an increase in the proportion of white muscle fibres (Holmes and Robinson, 1970). Genetically, in Charolais, muscle hypertrophy appears to be the results of one gene pair with incomplete dominance resulting in the heterozygote being phenotypically closer to the homozygous (muscle hypertrophy) animal (Oliver and Cartwright 1968). The double-muscled animal is, therefore, an extreme example of a difference in muscle fibre number within breeds of cattle. The following discussion examines the genetic influence on muscle fibre characteristics between and within animals that are not double-muscled.

Between Breeds: In order to increase meat production it is desirable to select for increases in fibre number, as there are physiological limits for fibre size (Staun, 1972). Also, Baldwin and Black (1979) postulated that the major determinant of ultimate organ size in mammals is the genetic control of cell number, with smaller differences resulting from the genetic control of potential cell size. To examine this postulate in sheep, Black (1983) studied the DNA content of 3-6 year old rams from several genotypes (viz: Cheviot, Dorset Horn, Corriedale and three strains of Merino) ranging in body weight from 59-90 kg. All animals were offered a good quality diet ad libitum for at least 12 weeks before slaughter to reduce the effect of previous nutrition on cell size. Results for the triceps muscle confirmed that muscle weight and cell number were correlated (r = 0.91) within and between all breeds, and that there was little overall effects of muscle weight on cell size. Nevertheless, the cells of Corriedales were consistently larger than the cells of Dorset Horns. That DNA content of a muscle may not reflect the muscle fibre number (because larger fibre have more nuclei and because muscles contain a significant proportion of connective tissue nuclei) was not considered in the conclusion. In similar work, Colebrook et al. (1988) studied the relative importance of cell number and cell size in determining the mass of 16 organs and tissues in mature rams of six different breeds (Fine Wool, Strong Wool and Camden Park Merino, Dorset Horn,

Corriedale and Cheviot) ranging in mean fleece-free empty body weight (FFEBW) from 54.6  $\pm$  0.3 kg for Camden Park Merinos to 76.7  $\pm$  1.6 kg for Strong Wool Merinos. The number of cells in an organ was expressed as g DNA and cell size was expressed as tissue weight per unit DNA. Results were interpreted to show that cell number, not cell size, was largely responsible for differences in muscle weight between mature sheep of different breeds. Cell number increased significantly with muscles weight. Vastus lateralis muscle differed between breeds in the number of cells which accounted for between-breed differences in muscle weight. Cell size was significantly related to muscle weight. However, vastus lateralis and triceps muscle differed in cell size between breeds. These differences for the triceps muscle were breed specific, whereas for the vastus lateralis muscle it was attributed to breed differences in muscle weight. Colebrook <u>et al</u>. (1988) concluded that these results largely confirmed the hypothesis of Baldwin and Black (1979) that the reason for differences in weight of most organs and tissues between sheep breeds was a difference in ultimate cell number but not cell size. "Cell number" for these studies was not, however, the same as "fibre number".

Solomon <u>et al</u>. (1981) found that the effect of breed was limited to intermediate ( $\alpha R$ ) fibres and white  $(\alpha W)$  fibres in both M. longissimus and M. semimembranosus. Suffolk X (Finnish - Southdown) lambs had more ( $\alpha W$ ) fibres (P < 0.01 and P < 0.05, respectively) and fewer ( $\alpha R$ ) fibres (P < 0.05 and P < 0.10, respectively) than the Suffolk X (Suffolk -Rambouillet) lambs, slaughtered at the same weight. Furthermore, (BR) red fibres were significantly larger in the M. semimembranosus of the Suffolk X (Finnish Landrace -Southdown) lambs than in that of the Suffolk X (Suffolk - Rambouillet). It was concluded that breed may affect the transformation from  $\alpha R$  to  $\alpha W$  fibres and this may be advantageous for improving the quantity of muscle. Differences in muscle fibre number and size have also been demonstrated between breeds of cattle (Dreyer et al. 1977) and pigs (Staun, 1963). Dreyer et al. (1977) found that Friesland cattle had a larger percentage of white muscle fibres and a lower percentage of red muscle fibres than the Afrikaner breed. Johnston et al. (1975) reported that Charolais steers had larger fibre diameters and areas than Angus steers. Johnston et al. (1981) reported that breed had no significant effect on fibre numbers or percentages of each type within the semimembranosus muscle, but the Angus steers had larger (P < 0.01)  $\alpha$ R and  $\alpha$ W diameters than the Simmental cross steers. The semitendinosus muscle from the Simmental cross steers had both a larger number and a higher percentage of  $\beta R$  fibres (P < 0.01) and fewer  $\alpha R$  fibres (P < 0.05) than Angus cattle. In contrast, breed had no significant effect on fibre diameter of the semitendinosus muscle. The biceps femoris muscle from the Simmental cross steers had a larger number (4%; P < 0.01) and a

correspondingly higher percentage of  $\beta R$  (4%; P < 0.01) and a lower percentage of  $\alpha R$  and  $\alpha W$  fibres (2%; P < 0.01) than the Angus steers. The  $\alpha R$  fibres from the Simmental cross steers were 1.4  $\mu$ m larger (P < 0.01) than those from the Angus steers. It appeared that muscle-fibre type characteristics were related to breed, sex and method of feeding. The effects of these variables on individual fibre types was not consistent between experiments, thereby indicating that additional factors may also be influencing fibre characteristics.

Within Breed: The effect of genetic selection for large body size on the physical characteristics of muscle has been reported for mice (Hanrahan et al. 1973) chickens (Aberle and Stewart, 1983) and pigs (Ezekwe and Martin, 1975). Luff and Goldspink (1967) found that mice selected for large body size had larger muscles than mice selected for smaller body size. The increased muscle weight was accompanied by increased fibre number and mean fibre diameter. They concluded that selection had a greater influence on fibre number than an fibre diameter. From a survey of inbred lines of mice Luff and Goldspink (1970) concluded that fibre number and muscle weight were independent, and that fibre number, but not fibre diameter, was genetically determined. The effects of selection for increased or decreased body weight in mice, at 5 or 10 weeks of age, on the fibre number, fibre diameter and weight of M. sternomastoideus and M. anterior tibialis muscles were studied by Hanrahan et al. (1973). They found that selection for increased body weight resulted in increased muscle weight and fibre number but fibre diameter was increased only when selection was for 5-week body weight. Similarly, Byrne et al. (1973) found increases in muscle weight, fibre number and fibre diameter in mice selected for high 5-week weight, and decreases in lines selected for low weight when compared to control lines. In another selection programme in mice based on body weight, Lax and Pisansaraki (1982) demonstrated that selection for weight at 3 weeks of age, or gain in weight between 3 to 6 weeks, resulted in increased fibre number in all muscles counted except the M. soleus. The weight of the high 10-week weight selected line from the same experiment had a significantly higher proportion of white fibres ( $\alpha W$ ) in the M. longissimus dorsi than a low 10-week weight line. The 3-week weight line did not differ from the control line in fibre type, but the 3 to 6 week gain line did have non-significant increases in the proportions of white fibres  $(\alpha W)$  in both muscles. Similar observations have been obtained using fast-growing lean pigs (Yorkshire) and the slow-growing obese pigs (Ossabaw) by Ezekwe and Martin (1975). They suggested that the greater muscle growth in the Yorkshire pigs was achieved by greater cell numbers and size. In chickens, selection for body size or growth rate resulted in increased muscle weight and myofibre number (Smith, 1963). The results from this work indicated that broiler-type birds had larger diameter muscle fibres than layer birds at 10-weeks and at

maturity. The primary difference in muscle weight between these groups could be attributed largely to muscle cell size (Smith, 1963). Aberle et al. (1979) found that broiler-type chickens had a significantly greater percentage of BR fibres and fewer  $\alpha$  fibres in the sartorius muscle. However, greater differences were found in the percentages of intermediate ( $\alpha R$ ) and white ( $\alpha W$ ) fibres. In the broiler line, approximately 28% of the  $\alpha$ fibres were of the (W) type, while in layer-type chickens only 11% were (W) fibres. They also found that the broiler-type birds had larger fibres in both fibre type classification than in the layer line. There was no evidence for preferential enlargement of one type of fibre over the other type. Similarly, Aberle and Stewart (1983) reported that broiler birds grew more rapidly than layer birds so that in comparisons at equal age, broilers had heavier body and muscle weights, larger fibre diameters and greater myofibre numbers. The percentage of intermediate ( $\alpha R$ ) type myofibres decreased and that of white ( $\alpha W$ ) type myofibres increased during growth. These changes occurred at a younger age in broiler-type birds. However, at equal body weights, broiler- and layer-type birds had similar proportions of the various myofibre types, and broilers had larger diameters and more rapid growth rates for myofibre diameters than layers. In contrast, layer-type birds had larger red (BR) myofibres than did broilers and the rate of radial growth was similar between breeds. They concluded that more rapid growth and greater muscularity of broiler-type birds are caused by more rapid myofibre hypertrophy and the presence of more myofibres.

Staun (1972) suggested that if practical selection for cell size and number could be carried out independently of body size, it was possible that genetic or physiological limits for muscle size could be extended. Selection for increased growth rate or body size has sometimes resulted in greater fat deposition rather than increases in muscle tissue (Biondini <u>et al</u>. 1968). As a result, selection for high meat yield or for muscularity would not be expected to produce the same results as selection for body weight, since the selection for high muscularity would tend to counter high fat deposition as a component of the larger body size (Ashmore, 1974). This consideration was confirmed by Stickland and Goldspink (1973) when they found no increases in fibre number with muscles of pigs during growth in response to selection.

In a study of the effects of selection for backfat depth on muscle fibre characteristics in the M. semitendinosus of Southdown rams, Kadim (1988) indicated that, at the same weight, the high fat line sheep possessed significantly higher proportions of red fibres ( $\beta R$ ), with non-significant decreases in the proportions of intermediate ( $\alpha R$ ) and white fibres ( $\alpha W$ ). Mean fibre diameters within fibre type did not differ significantly between lines. In a

similar experiment involving selection for or against fatness in two pig breeds, Staun (1963) showed a significant differences only between the two lines (P < 0.05) of the Duroc pigs in the number of fibres in M. longissimus dorsi. This work did not show any significant differences between breeds or lines of the Yorkshires in terms of the total number of muscle fibres or in fibre diameter. From this study Staun (1963) concluded that pigs selected with a thin layer of back fat possessed genes for a large area of M. Longissimus dorsi, which in turn resulted in an increase in the number of muscle fibres. This phenomenon was only apparent in the Duroc breed, probably because there had been selection within this breed from several generations. Nostvold et al. (1979) investigated muscle fibre characteristics of female and castrated male pigs from the 8th generation of a selection experiment which included a control line (C), a HP-line selected for high rate of gain and low fat thickness, the LP-line selected for the opposite traits. The HP-line M. longissimus had a significantly higher percentage of  $(\alpha R)$  intermediate fibres and a corresponding lower  $(\alpha W)$  white fibre percentage than the LP-line, but no significant line differences were observed for (BR) red frequencies a constant weight. The LP-line had smaller muscle fibre diameters than the HPline at the same weight, but the differences were significant for the  $(\alpha W)$  white fibres only.

# 2.4 GENETIC EFFECTS ON FAT CELLULARITY

## 2.4.1 INTRODUCTION

In this section, changes in fat cellularity with growth will be reviewed first and then genetic differences in fat cellularity between and within different breeds will be considered.

# 2.4.2 CHANGES WITH GROWTH

The new born farm animal has little reserve of adipose tissue (1-4%), but the carcass of the adult may contain as much as 30 to 40%% (Leat, 1976). Cleary <u>et al</u>. (1977) reported that the primordia for adipose tissue depots which are often present in the late fetal stage or in the neonate, grow rapidly postnatally in concert with overall growth of the animal. Adipose tissue increases in size and in weight by a combination of an increase in the number of cells, (i.e. hyperplastic growth) and by enlargement of the size of adipocytes (i.e. hypertrophic growth) (Cleary <u>et al</u>. 1977).

Johnson and Francendese (1985) noted that an adipose depot has the capacity to expand or to decrease in total size and weight in the adult. This changing ability reflects not only the ability of adipocytes to change markedly in size as they accumulate or lose intracellular lipid, but also reflects processes by which new adipocytes proliferate and lead to expansion of the tissue mass. Variations in the total fat mass in animals of different species, age and metabolic conditions have been shown to be the results of differences in the number and (or) size of the constituent mature adipocytes. Kirtland and Gurr (1979) reported from several studies that epididymal and perirenal adipose depots in the rat develop by an increase in fat cell size and observable numbers of fat cells up to about 14 weeks of age. From 14 weeks to at least six months of age, cell number remained virtually constant. Furthermore, adipose tissue cellularity in adult rats and mice did not appear to be permanently affected by various manipulations, including, starvation, hypothalamic lesions, gold-thioglucose treatment and exercise. They stated that similar patterns of development may apply to other adipose depots in the rat and to other species, although the precise time at which a stable cell number is reached might vary. Hood and Allen (1977) reported that the number of fat cells in the perirenal and extramuscular adipose tissue in both lean Hampshire X Yorkshire and fat Minnesota 3x1 pigs increased up to 20 weeks of age. By 24 weeks of age, hyperplasia was complete in Hormel Miniature pigs. But in another study with Large White pigs by Gurr et al. (1977) the number of observable fat cells in subcutaneous backfat increased up to about 50 weeks of age. In cattle, Hood and Allen (1973) reported that by 14 months of life hyperplasia was complete in the subcutaneous and perirenal adipose tissue from animals of the leaner Holstein breed and the fatter Hereford X Angus, but not in interfascicular adipose tissue. The results of this study suggested that interfascicular adipose tissue was a late developing depot and that hyperplasia is still an active process in this depot at 14 months of life. In sheep, Hood and Thornton (1979) found that both hyperplasia and hypertrophy of adipocytes contributed to the growth of subcutaneous adipose tissue of Dorset Horn X Merino wethers until they reached about 12 months of age. Thereafter growth of this fat depot occurred only by hypertrophy of the existing adipocytes. The general conclusion from two reviews (Allen, 1976; Hood, 1977) was that growth of extramuscular fat depots of sheep resulted primarily from hypertrophy rather than hyperplasia of adipose cells. The contribution of hyperplasia and hypertrophy of adipocytes to the growth of subcutaneous, perirenal and omental fat depots of 32 growing adult Border Leicester X Merino wethers slaughtered over the liveweight range of 29 to 56 kg were examined by Thornton et al. (1984). Subcutaneous tissue had the largest population of adipocytes, but they were the smallest, and showed the slowest rate of volume increase. Omental tissue had the lowest number of cells, but they were the largest and grew the fastest. Perirenal tissue was intermediate in terms of

cell number, size and growth rate. They concluded that differential hypertrophy of existing adipocytes could alone account for the growth of all three adipose tissue depots. In a serialslaughter experiment involving 5 pre- and 14 postnatal Romney ewes, Broad <u>et al</u>. (1980) reported that the growth of total adipose tissue and of each of the fat depots (subcutaneous, intermuscular and internal cavity) occurred mainly by an increase in the adipocyte volume, which increased over 70-fold from 120 days gestation to 5 years of age. During the same growth period, the total number of adipocytes increased about 6-fold, with 20-, 5- and 1.6fold increases in the number of adipocytes in the subcutaneous, intermuscular and internal cavity fat depots respectively. They concluded that although the mean number of adipocytes per half carcass increased over the whole of the growth-range studied, the variability was such that the increase in total adipocyte number in 5-year-old ewes compared with 6-monthold lambs did not appear to be statistically significant.

Kirtland and Gurr (1979) reported that constancy of fat cell number in the adult is not universally accepted. However, some of the evidence purporting to show fat cell formation in adult life must be viewed with scepticism on two counts, first, on methodological grounds and secondly, on interpretation. It is now believed that the adipose depot grows initially by a combination of hyperplasia and hypertrophy (Johnson and Francendese, 1985). Cell numbers reach a plateau early in life while cellular hypertrophy continues until a "maximum fat cell size" is reached. When the "maximum fat cell size" is reached, a hypothetical signal is generated that results in the recruitment of new fat cells. The addition of these newer, smaller cells to the pool of mature adipocytes results in a lowering of the mean fat cell size. Theoretically, adipocytes can again reach "maximum fat cell size" generating a new wave of recruitment. The origin of these new cells have been questioned by Johnson and Francendese (1985): "Are they truly newly generated fat cells derived from cellular proliferation in the adult phase of life; or do they arise from dormant precursors that were generated during the juvenile growth phase and are then "recruited" by lipid filling or differentiation?". They stated that the former would suggest that fat cell depot growth is essentially limitless; while the latter would suggest that the ultimate mass of the tissue is restricted to those cells formed during early proliferative stages of growth. Kirtland and Gurr (1979) reported that there is a strong tendency to assign an increase in observable fat cells to the synthesis of new fat cells. Perhaps this may be true in the young animal where adipose tissue is being laid down rapidly, but it may not be true in adult animals. The problem of distinguishing between new cell synthesis and the filling of empty fat cells is hampered by the difficulty of recognising an empty fat cell. Some workers have sought to identify these cells on morphological grounds and others by looking for enzyme markers.

Kirtland and Gurr (1979) reported that using the radio-labelled thymidine methods proved to give a direct indication of cell division and hence to give a truer reflection of the length of the hyperplastic period than just counting observable fat cells. Thus Kirtland and Gurr (1979) reported from the rat epididymal fat pad that, although the standard counting methods indicated that the number of observable fat cells increased between birth and 12 weeks of age, the thymidine method indicated that the majority of fat cell synthesis was over by five weeks of age.

## 2.4.3 GENETIC INFLUENCE ON FAT CELLULARITY CHARACTERISTICS

Over the past 20 years, there has been an appreciable decrease in body fat content of every livestock species as a result of selection. Data from swine selection for reduced fat deposition is one of the most visible example of increased leanness and decreased fatness of live market animals (Steele et al. 1974). In general, it has been demonstrated that genetic and environmental factors influence cellular mechanisms which lead to variation among animals relative to the proportions of adipose tissue in the body. Allen (1976) reported that whereas the number of adipocytes in a particular fat depot in older sheep appear to be largely unaffected by diet, the size of these cells is related to the level of feed intake. However, Van Middelkoop et al. (1977) stated that the potential to deposit triglyceride in adipose tissue must be influenced to a large extent by the number of available cells within each depot, which in turn depends basically on genotype.

Between Breed: Merkel et al. (1973) studied the mean cell number, volume and volume of adipose tissue occupied by adipocytes (specific volume) in subcutaneous adipose tissue of lambs differing in age (8, 16 and 32 weeks), sex (ewe, wether and ram) and breed (Southdown and Suffolk sired lambs), and showed no significant differences between breeds in all traits measured. Butler-Hogg and Wood (1983) reported that it is known that the difference in fat depots between beef (Hereford) and dairy (Friesian) cattle types can be attributed to the greater number of fat cells (adipocytes) in the subcutaneous depot of the Hereford. They showed that in serially slaughtered Clun (ewe type) and Southdown (ram type) lambs, the increase in KKCF weight was due largely to adipocyte hypertrophy, whereas in SCF hyperplasia was important. Butler-Hogg and Wood (1983) concluded that there is a pattern of development of fat depots common to sheep and cattle. KKCF increases through hypertrophy and SCF through hypertrophy and hyperplasia, but there was no effect of breed type in sheep comparable with that found in cattle. In a serial-slaughter experiment involving Hereford and British Friesian steers, Truscott <u>et al</u>. (1980) reported that in both breeds the perirenal depot grew almost exclusively through cell enlargement, whereas the subcutaneous depot grew principally through cell enlargement to about 17 months of age and thereafter by cell recruitment as well as cell enlargement. At equivalent ages, the Herefords were fatter and had more fat in the subcutaneous depot than Friesians which contained more fat in the intra-abdominal depot. Also, the subcutaneous depot in the Herefords contained more cells than that in the Friesians but the cells were of a similar size in both breeds. In the perirenal depot, cell size increased more rapidly with age in the Friesians resulting in larger cells in this breed at 20 months. Furthermore, the number of cells in the perirenal depot of the Friesians was greater than that in the Herefords. They concluded that differences between depots in the cellular nature of fat growth were more obvious than differences between breeds, also the results did not support the view that a particular fat cell size provides the trigger for cell recruitment in all depots.

Within Breed: Thompson et al. (1988) examined the cellular characteristics of dissected carcass (subcutaneous and intermuscular fat) and non-carcass (kidney fat, omental and mesenteric fat) fat depots at maturity in rams and ewes from flocks of Peppin Merino sheep selected for high (weight-plus) or low (weight-minus) weaning weight and from a randomlybred control flock. Selection for high or low weaning weight had no effect on adipocyte volume among five dissected fat depots in the mature animals, with the increased weight of fat in the weight-plus animals due to an increased number of adipocytes in the dissected fat depot. The kidney and omental fat depots had the largest adipocytes, followed by the mesenteric fat depot, with the smallest in the subcutaneous and intermuscular fat depot (Thompson et al. 1988). Cellularity of subcutaneous adipose tissue from strains of genetically lean and obese pigs was studied by Steele et al. (1974). High (DH) and low (DL) backfat lines and a control line (DC) of Duroc pigs were compared at either a constant age (100 days) or a constant weight (95) after 18 generations of selection. At a constant weight, average backfat depth (mm) and backfat percentage were 77, 41 and 34 and 18, 8.8 and 5.4 for the DH, DC and DL lines respectively. The results also showed that at constant weight, adipocyte volume (u3 X 104) as 241, 156 and 92 for DH, DC and DL respectively, and that total adipocyte number in the carcass (X109) was 16.5, 10.2 and 9.2 for DH, DC and DL respectively. Steele et al. (1974) concluded that these changes in fatness among lines were effected through a significant changes in adipocyte volume and apparent significant increase in adipocyte number in the high backfat line versus the low backfat line. Kadim et al. (1989) reported that sides from Southdown rams from a line selected for high backfat depths contained 18% more dissectible fat at the same side weight as a low backfat line, and they showed that the greater weights of four dissectible fat depots (subcutaneous, intermuscular, omental and kidney and pelvic fat depots) were mainly the result of larger rather than more adipocytes. The results from a number of non-ruminant studies are summarized in Table 2.1.

Reference	Genetic groups	Age compared	Characteristics of adipocytes of the fatter group
Trayhurn et al., (1979)	Ad-obese Ad-lean	6 months	Males: larger, but a similar number Females: larger and more per depot
Aubert et al., (1985)	DW db obese DW db lean	6 months	Males: larger, but a similar number Females: larger and more per depot
Eisen et al., (1978)	M16 = rapid gain at 3-6 week postweaning H6 = large bodyweight at 6 week	10 week	<ul> <li>M16 = larger and more per depot than leaner controls</li> <li>H6 = The same volume but more per depot than leaner controls</li> </ul>
Martin et al., (1979)	ICR - Albino selected for 3 to 6 week postweaning growth	6 and 15 weeks	Larger and more per depot than leaner controls
Kaplan et al., (1976)	ob/ob - obese non.ob/ob - lean	less and larger than 4 weeks	At 3 week: the same volume but more per depot At 26 week: larger and more per depot
Johanson et al., (1971)	Zucker fa/fa - obese fa/Fa & Fa/Fa - lean	compared at different ages from 3 to 37 week	Larger and more per depot with differences increasing up to week 26

 Table 2.1
 The cellular characteristics of adipose tissue from mice or rats in a sample of studies involving groups with genetically different fatness levels

#### 2.5 MEASUREMENT OF BODY WATER BY DILUTION METHODS

#### 2.5.1 INTRODUCTION

Dilution techniques were described by the Cuthbertson (1975) as involving the introduction of a known amount of tracer which becomes uniformly distributed throughout a compartment in the animal body such as body water. Body water content can be then estimated by the change in concentration of the tracer or diluent after equilibration with body water. As a result, Shebaite (1977) concluded that it is possible to predict the overall chemical composition of the animals because of the close relationship between fat and water.

The purpose of this section is to review work on the urea dilution technique as a mean of predicting live body composition in domestic animals. Advantages and disadvantages of other dilution methods will be described first and then urea dilution as a method of estimating body water will be evaluated.

# 2.5.2 DILUTION METHODS, OTHER THAN THOSE INVOLVING UREA

Two kinds of tracer have been described in the literature as reviewed by Cuthbertson (1975), Russel and Wright (1982) and Purchas (1977). The first are known as exogenous diluents and include tritiated water, deuterium oxide and urea, and the second type, known as endogenous diluents, include muscle potassium and plasma creatinine (Purchas, 1977). For these endogenous diluents, the concentration or content should be proportional to muscle weight, but for exogenous diluents a knowledge of the quantity administrated together with the concentration permits the calculation of water percent (Purchas, 1977). Endogenous diluents will not be considered further here. However, Preston and Kock (1973) noted that any exogenous tracer should possess the following properties to be acceptable as a measure of total body water:

- 1. It should not be toxic.
- 2. It must not be metabolized, selectively excreted from, or stored in, the body.
- 3. It should be easily measurable.
- 4. It must diffuse homogeneously into all the volume to be measured within a short time.

5. It should not be a substance that is not essential to the body.

According to Panaretto and Till (1963), labelled water, in the form of either tritiated water (TOH) or deuterium oxide ( $D_2O$ ), constituted a major advance in the field of dilution methods and offered a means of estimating body water with a higher precision and confidence than was possible using other markers. The use of these forms of labelled water in ruminants has been discussed in detail by Robelin (1976) who came to the following conclusions:

- 1. The two tracers (TOH or  $D_2O$ ) are not significantly different.
- Standardisation of feeding is essential for accurate determination because gut water is included in the assessment. Variation in the amount of ingesta can cause major inaccuracies in estimated composition.
- 3. Reasonable accuracy can be obtained in comparisons, but absolute measurements are not generally of sufficient accuracy for practical application.

However, of these two methods using labelled water, deuterium oxide would seem to be the more suitable, and it has the advantage that, it is more accurate than TOH and not radioactive (Robelin, 1976). However, it is expensive, and it is not particularly easily determined without sophisticated equipment (Robelin, 1976). Also it requires relatively large dose rates of between 0.5 and 1.0 g/kg liveweight (Russel and Wright, 1982). Tritiated water on the other hand is easily determined, can be infused in small volumes, but being radioactive and also requiring expensive measuring equipment, is generally considered as unsuitable for use in animals destined for human consumption (Meissner, 1976). Meissner (1976) also reported that although the tritium dilution technique is convenient and probably the most accurate method, its application for practical use with farm animals is less convenient. The main reasons are, first, the tritium molecule equilibrates relatively slowly in the fluid medium of the body, taking approximately 5 to 8 hours in sheep, compared with two hours for D<sub>2</sub>O and, secondly, standard laboratories have to be especially adapted to cope with radioactive materials. However, both methods require several hours to determine body water turnover rate which is required to estimate initial body water dilution and body composition. With either isotope, Smith and Sykes (1974) reported that total body water and hence body composition can be estimated either from a single sample (usually of blood) after equilibration has been attained or by extrapolation to the time of administration from a series of samples taken over several days.

Many other substances have been tested as agents for the measurement of total body water including antipyrene, N-acetyl-antipyrene, thiourea and sulfanilamid (Reid <u>et al</u>. 1958; Robelin, 1976). Results from work with antipyrene have generally demonstrated that results were too variable to give good estimates of total body water (Panaretto and Till, 1963). In general, these substances have received little attention from the research workers since the introduction of TOH and  $D_2O$ .

# 2.5.3 UREA DILUTION AS A METHOD OF ESTIMATING BODY WATER

Bartle <u>et al</u>. (1985) reported that urea dilution showed a level of accuracy for estimating body water in sheep comparable to that obtained for radioactive substances including tritiated water and deuterium oxide. Meissner (1976) showed the urea dilution technique to be somewhat less reliable than tritium dilution for sheep, but he suggested that due to low cost it could be useful for routine calculations of body composition. San Pietro and Rittenberg (1953) reported that urea appeared to meet all the requirements of a satisfactory body water tracer, and urea space and deuterium oxide space were similar in size when the space was defined as the volume of water with which urea equilibrates. Meissner, (1976) described the advantages of using urea dilution as following:

- 1. It is not toxic.
- 2. It is not foreign to the body and causes no physiological disturbances when adminstered in sufficiently small amounts.
- 3. Urea is not selectively stored, secreted or metabolized.
- 4. It is excreted sufficiently slowly to allow time for uniform distribution in the body.
- 5. Urea can be measured accurately and easily in either whole blood or plasma.
- 6. The urea molecule appears to equilibrate in a short time. For instance, within 30 minutes in goats, only 12 minutes in cattle and 14 to 20 minutes in sheep.

Bartle <u>et al</u>. (1985) concluded from work involving the evaluation of urea dilution as a method of estimating body composition in 42 finishing lambs, that urea dilution was a better estimator of carcass fat percentage than liveweight alone. Although fewer studies have been

conducted with sheep than with cows and pigs, the results have shown that urea space gave satisfactory prediction results for both fat and lean weights in lambs (Bartle <u>et al</u>. 1988), steers (Hammond <u>et al</u>. 1984), cows (Bartle <u>et al</u>. 1987), and pigs (Stansbury <u>et al</u>. 1985).

Kock and Preston (1979) studied the reliability and usefulness of urea space measured at varying times after urea infusion for estimating body composition in beef type steers of varying liveweight and degree of fatness. Plasma urea concentration measured 12 minutes following urea infusion proved to be the most closely correlated with rib soft tissue composition and carcass specific gravity. Overall correlations with rib water, protein and fat percentages and carcass specific gravity were 0.84, 0.73, -0.84, and 0.68 respectively. Similar results were found when the cattle were divided into groups according to age, cold carcass weight or fatness. Kock and Preston (1979) concluded from this work that urea space appeared to be a reliable and practical measure from which to estimate body composition in live cattle. Similar results were obtained by Meissner (1976) in work performed on different breeds of sheep ranging in body mass from 13 to 80 kg. He reported that urea space gave a highly satisfactory prediction of body water ( $R^2 = 0.93$ ) and a moderate prediction of body fat ( $R^2 = 0.54$ ) in lambs. Stansbury et al. (1985) showed from a study with growing-finishing swine that urea had good potential as a body water dilutent for the determination of body composition of swine. In this work, the water percentage from chemical analysis of the soft tissue of the body and that determined from urea dilution at 54, 79 and 101 kg body weight respectively was as follows : 58.2, 57.6; 52.5, 54.0; and 52.5, 51.5. The corresponding values for fat were 25.5, 26.4; 33.5, 31.5 and 33.5, 34.9 respectively. Correlations between the urea space at zero time and chemically analyzed components were -0.73, 0.76 and -0.72 for water, fat and protein respectively over all weights. Similar correlations were obtained between the change in plasma urea from prior to infusion to 15 minutes after infusion and chemical analyses. Bartle et al. (1983) showed also that the urea dilution technique provide useful estimates of body composition differences between groups of mature cows. The percentage urea space was calculated relative to either liveweight (LW) or empty body weight (EBW), and was compared with carcass fat estimated from carcass specific gravity and from the chemical composition of the 9-10-11th rib section. Coefficients of determination between percentage fat estimated from carcass specific gravity and from urea space (LW) were 0.35 and 0.17 for beef and dairy types, respectively. When urea space (EBW) values were used the coefficients of determination were 0.50 and 0.28. The coefficients of determination between actual chemical fat and that estimated from urea space (LW) were 0.36 and 0.09 and from urea space (EBW) were 0.64 and 0.41 for beef and dairy types respectively. Therefore, the close relationship between urea space and other estimates of body fat content indicated that urea space can be used to estimate body composition in live cows. Hammond et al. (1984) determined urea space in 68 mixed breed and 50 Angus steers (210 to 517 kg) and showed a linear relationship between empty body water weight (EBH2O) and urea space (r = 0.96, P < 0.001). Prediction of EBH2O weight was improved by including liveweight or empty body weight as an independent variable along with urea space in a multiple regression equation. Although, EBH2O weight was determined by direct chemical analysis, (Hammond et al. 1984), its correlation with urea space percentage of liveweight was similar to that for the data of Kock and Preston (1979) where composition was estimated by carcass specific gravity. The results of Hammond et al. (1984) were in agreement with those of Bartle et al. (1983) who found that urea dilution methods could be used to estimate body composition in mature, non-pregnant cows, and also with Meissner et al. (1980) who demonstrated that urea dilution could quantify body composition in bulls. However, Jones et al. (1982) demonstrated urea dilution to be an unreliable method for the prediction of both fat and lean weights in lambs, steers and cows. Thirty eight lambs (41.9 ± 9.7 kg (SD), 25 Holstein cows  $(624 \pm 62.8 \text{ kg})$  and 30 steers  $(466 \pm 63.2 \text{ kg})$  were evaluated for urea space estimation. All lambs and cattle were slaughtered within 2 days and half carcasses were separated into fat, lean and bone. Urea space in lambs was poorly related to the weight of half carcass lean tissue ( $R^2 = 0.10$ ) and also did not improve the level of explained variation in half carcass lean tissue weight over that explained by liveweight alone ( $R^2 = 0.73$ ). Urea space showed a closer association with half carcass lean weight in cows ( $R^2 = 0.55$ ) than in steers ( $R^2 = 0.14$ ), but again did not improve on the relationship with liveweight alone ( $R^2 = 0.60$ ). Jones et al. (1982) concluded that urea space was not very useful in these studies because the animals were either mature (cows), or still in a lean body condition (lambs and steers).

Jones <u>et al</u>. (1982) suggested some other points which should be considered in any future work using urea to predict body composition of live animals as follows:

- 1. Smaller volumes of more concentrated urea solution may be more rapidly distributed in the body water, and may substantially alter the time to equilibrate with the body fluids.
- 2. The best sampling time after urea injection showed to associated with the best prediction of composition (Kock and Preston, 1979). Urea space measured 12 min in cattle and 9-12 min in lambs following urea infusion proved to be the best estimate of body composition.

3. The effects of a fasting period should be examined (there was no fasting period applied by Jones <u>et al.</u> (1982)).

However, Bartle and Preston (1986) examined the last point above in four (450 kg) heifers and concluded that urea did not diffuse into reticulo-ruminal water (RRW) in significant amounts in fasted animals. RRW therefore, influenced urea dilution estimation of body composition only as a variable component of liveweight. Urea dilution determined EBH2O plus urine, and therefore overestimated EBH2O by the urine volume produced in the 12 min period. They concluded that to decrease the variation in liveweight due to RRW and potentially to limit urea transfer across the rumen wall, ruminants should be held off feed for 20 to 24 hours period before urea dilution procedures are performed. Bartle <u>et al</u>. (1987) suggested that the low correlations of Jones <u>et al</u>. (1982), and Meissner <u>et al</u>. (1980) may have been influenced by different sampling times or urea infusion concentrations, or by a somewhat limited range in body composition.

Rule <u>et al</u>. (1986) used the urea dilution equations developed by three separate groups of investigators (Hammond <u>et al</u>. 1984; Preston and Kock, 1973; Meissner <u>et al</u>. 1980) to compare predicted empty body water with that measured chemically in 6-12 and 18 monthold crossbred beef steers. They concluded that the overall comparison of urea space with components of the carcass and empty body indicated that urea dilution had potential for use in the prediction of body composition of beef steers. Also, the overall accuracy of prediction and stabilization of variation was improved when liveweight was included in the regression models. Without liveweight, however, urea space accounted for greater than 60% of the variation in percent body composition and mass of lipid in the carcass and empty body, and greater than 80% of the variation in mass of protein and water in the carcass and mass of EBH2O. A feature of LW inclusion was the elimination of age effects as revealed by analyses of residual plots.

Finally, Bartle <u>et al</u>. (1988) evaluated urea dilution in two experiments (n = 46 and n = 56) as an estimator of body composition in lambs and concluded from the regression equation that the relationship between urea space and percentage of empty body water in lambs was different from that reported for cattle. In the combined experiments, urea space was related to percentage of empty body water by the equation  $31.7 \pm 0.471(US)$  (empty body weight basis: r<sup>2</sup> = 0.56, P < 0.001). As a result Bartle <u>et al</u>. (1988) concluded that urea dilution was related to body composition in lambs, but considerable variation was noted. However, they claimed that the proportion of variation in body composition accounted for by

urea space was not satisfactory. They emphasised the importance of accurate sample timing and precise plasma urea-N determination and indicated that the urea space procedures may require modification for use in lambs.

## **CHAPTER THREE**

# **MATERIALS AND METHODS**

# 3.1 ANIMALS AND EXPERIMENTAL DESIGN

# 3.1.1 ANIMALS

Thirty-six Southdown ram lambs, 18 from each of the Massey University high and low backfat selection lines (Purchas <u>et al.</u> 1982), were used in this experiment. The selection programme from which the rams were drawn was initiated in 1976, and was based on selection for either increased (high-line) or decreased (low-line) weight-adjusted backfat depths as assessed by ultrasound (Purchas and Beach,1981). Further details on the Massey University backfat selection lines have been provided by Bremmers <u>et al.</u> 1988. For this experiment, two sires were equally represented in each line, and within each sire by line group (Table 3.1). Animals were randomly allocated into three "lots" of 12 rams. All the rams used in this study were born in August and September 1987 and ranged in age from 158 - 233 days at slaughter.

## 3.1.2 EXPERIMENTAL PROCEDURE

After the animals were transported from the field to the Animal Physiology Unit, they received a 10-day adjustment period in individual pens. Rams were fed once daily (at 17.00h) chaffed lucerne hay at a rate equivalent to 1.3 times maintenance. Fresh water was available ad libitum. On day 10 the rams were cannulated as described by Bremmers et al. (1988). An intravenous urea challenge was administered on day 12. Within each lot the animals were divided into two slaughter groups of 6 rams, each balanced for line. The same procedures were applied for the three lots (Table 3.1).

The two groups in each lot were slaughtered 5-7 days after the urea space measurement (Table 3.1). The following measurements were taken at and after slaughter:

- 1. Body components measurements at slaughter (Section 3.4)
- 2. Carcass measurements (Section 3.5)

- 3. Dissection measurements (Section 3.6)
- 4. Adipose tissue measurements (Section 3.7)
- 5. Muscle fibre type measurements (Section 3.8)
- 6. Chemical analysis (Section 3.9)

On the day of slaughter, the carcasses and all the non-carcass components were transported to the Massey University (meat laboratory) in separate polyethylene bags. The carcasses were kept overnight in the chiller and the non-carcass components were frozen immediately at a temperature of c. -20 °C.

Table 3.1:Layout of the experimental design.Each "lot" of 12 animals represent 6 animalsfor each line and 3 animals from each sire, and was slaughtered within 2 succeeding days.

Lot No.	Number of animals	Transfer indoors	Date of Urea challenge	Date of Slaughter
1	6	31 Jan	12 Feb	17 Feb
	6	31 Jan	12 Feb	19 Feb
2	6	21 Feb	04 Mar	09 Mar
	6	21 Feb	04 Mar	11 Mar
3	6	13 Mar	25 Mar	30 Mar
	6	13 Mar	25 Mar	31 Mar

Note: all the dates refer to the 1988 year.

# 3.2 UREA SPACE MEASUREMENT

Urea was administered intravenously as a single bolus (120 mg of urea/kg liveweight) of a solution (300 mg/ml) in sterile pyrogen-free physiological saline. It was follow by 6 ml of saline to wash out the catheter. Blood samples of approximately 8 ml were collected through the catheter at 60, 40 and 20 minutes prior to infusion and at 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 300, 360 and 420 min after the time of the urea challenge infusion. The blood samples were withdrawn into tubes containing 0.1 ml of 35% (w/v) sodium citrate as an anticoagulant, and immediately placed on ice. The blood samples were then centrifuged (20

min at 2500 g and 4°C) within 1 hour of sampling. Plasma blood was harvested and stored at c. -20°C until assayed. The auto-analyser method described by Marsh <u>et al</u>. (1965) was used to determine plasma urea concentration.

# 3.3 SLAUGHTER PROCEDURES

Six animals were slaughtered at one time (Table 3.1). All animals were slaughtered and dressed at the Animal Physiology Unit at Massey University following normal commercial procedures with a captive bolt pistol being used to stun the animals. The animals were fasted for c. 17.0 hours before slaughter.

# 3.4 BODY COMPONENTS MEASURED AT SLAUGHTER

# 3.4.1 BLOOD COLLECTION

The blood was collected in tared plastic tray (30 cm x 50 cm) by carefully holding the animal over the tray and allowing it to bleed completely after cutting the neck vessels. After weighing, triplicate samples (approximately 100 ml each) were transferred to tared aluminium containers for estimation of moisture content.

# 3.4.2 NON-CARCASS COMPONENTS MEASUREMENTS

After exanguination, animals were flayed and eviscerated according to normal dressing procedures, and always in the same order. The following non-carcass components were weighed:

- 1. Head and feet (g)
- 2. Skin (g)
- 3. Foregutfull(g)
- 4. Foregut empty (g)
- 5. All intestine full (g)
- 6. All intestine empty (g)
- 7. Heart (g)
- 8. Liver (g)

- 9. Both kidneys (g)
- 10. Spleen (g)
- 11. Lungs and Trachea (g)
- 12. Both Testes (g)
- 13. Omental fat (g)
- 14. Kidney fat (g)
- 15. Other components (g) (all remaining pieces from non-carcass components)

The weights of all parts were recorded immediately after removal from the body. The foregut and the intestine were weighed with all the ingesta included and also after being cleaned and washed under cold running water and allowed to drip. The head and feet for each animal were sealed in a plastic bag and frozen at c.-20°C after weighing. The viscera components (items 3 to 15 above) were also stored in a plastic bag at c.-20°C for moisture content determination. The dressing and visceration procedures were done over a large (80 cm x 150 cm) stainless steel tray to avoid any loss of blood and internal body fluids.

#### 3.4.3 SKIN AND WOOL SAMPLING

Three rectangular samples (10 cm x 30 cm) were cut from the pelts of the lambs after slaughter. The sampling positions were chosen on the basis of results of Wodzicka (1958) and their location on the body of the animal is outlined in Figure 3.1. The pelt samples were weighed and frozen at c. -20 °C. After thawing, wool was removed from the samples using animal clippers (Sunbeam animal clipper, Model HCAA, detachable blade). The skin percentage (SK %) in these samples was obtained by dividing the skin weight (SK Wt.) by the total weight (TS Wt.), and the wool weight (W Wt.) was determined by the difference between the total weight (TS Wt.) and the skin weight (SK Wt.)

 $SK \% = \frac{SK Wt.}{TS Wt.}$ 

W Wt. = TS Wt. - SK Wt.

The total skin weight in a pelt (TSK Wt.) was obtained by multiplying the skin percentage (SK %) by the total pelt weight (TP Wt.) and dividing by 100. The total wool weight (TW Wt.) was obtained by the difference between total pelt weight (TP Wt.) and total skin weight.

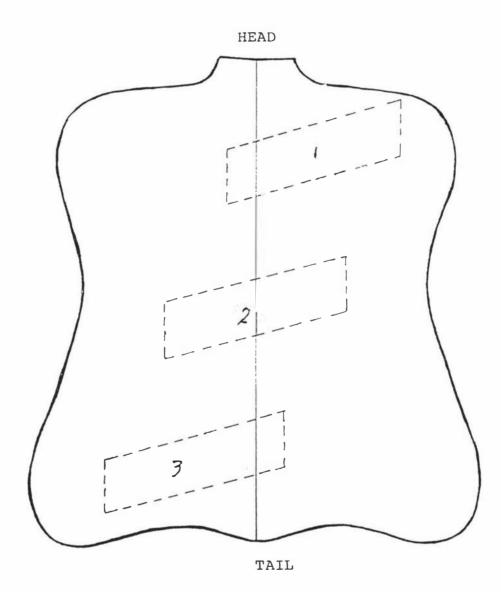


Figure 3.1 Sampling positions on the pelt.

	Measurement	Definition
1.	Body length (LB)	From the point where the gambrel is inserted through the Achilles tendon to a point just anterior to thepoint of the humerus (Moxham and Brownlie, 1976)
2.	Leg length (T)	From the distal end of the tarsals to the centre of the tuberosity of the tibia, which is visible on the ventral aspect of the hanging carcasses (Palsson, 1939).
3.	Gigot width (G)	Maximum width of the gigots, with the carcass suspended from the gambrel. The measurement was taken at right angles to the length of the carcass at a line level with the femoral trochanter (Palsson, 1939).
4.	Maximum shoulder width (WF)	Maximum width of the shoulder, measured at the level of the scapula from one lateral surface to the other, using a caliper (Palsson, 1939).
5.	Width behind shoulder (WTh)	Minimum width behind the scapula (Palsson, 1939).
6 <sup>a</sup> .	Fat thickness (C)	The depth of subcutaneous fat over B at right angles to the skin (Palsson, 1939).
7 <sup>a</sup> .	Fat thickness (J)	The depth of subcutaneous fat over the <u>M. obliquus externus</u> abdominis (Palsson, 1939).
8 <sup>a</sup>	Fat thickness (S2)	The depth of subcutaneous fat over the <u>M. latissius dorsi</u> at apoint at right angles to the mid-line bone (Kirton <u>et al</u> ., 1967).
9 <sup>a</sup> .	Tissue thickness (GR)	The depth of tissue over the surface of the rib at a point 110 mm from the mid-line (Frazer, 1976).
10 <sup>a</sup> .	<u>M. longissimus</u> with (A)	The maximum width across the surface of the M. longissimus.
11 <sup>a</sup> .	<u>M. longissimus</u> depth (B)	The maximum depth at right angles to the width measurement.
12 <sup>a</sup> .	Area of <u>M.</u> longissimus	Area of the cut surface was traced on a tracing paper and the area was determined by using a digitising tablet attached to an Hitachi personal computer (Model MB-16003[E]).
13 <sup>b</sup> .	Fat thickness (L3)	Fat thickness over the ventral edge of <u>M. gluteus medius</u> (Kirton and Johnson, 1979).

 
 Table 3.2
 Definitions of the carcass linear measurements
 (Adapted from Kadim, 1988)

<sup>a</sup> Measurements were taken on the cut surface between ribs 12 and 13 (Figure 3.4)
 <sup>b</sup> Measurements were taken on the cross-section of the leg cut (Figure 3.4)

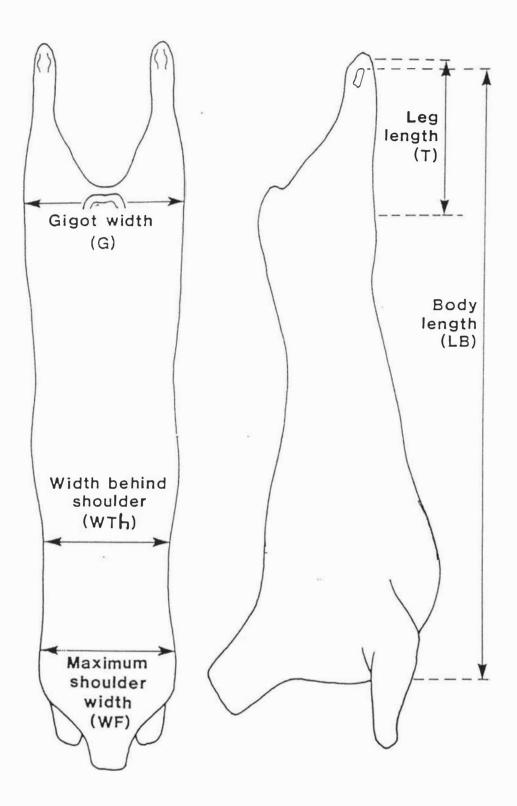


Figure 3.2 A diagram indicating where measurements were taken on the hanging carcass. (Kadim, 1988)

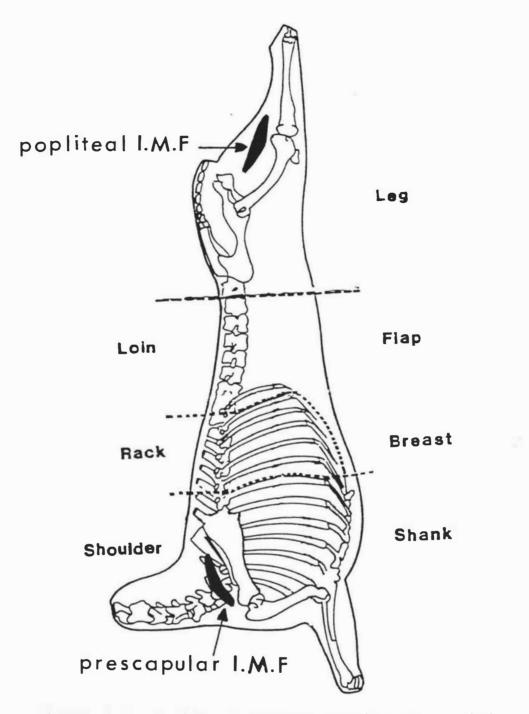


Figure 3.3 A side of carcass showing the positions of the standardised cuts using dotted lines. The locations of the two intermuscular fat depots used in this study are also shown.

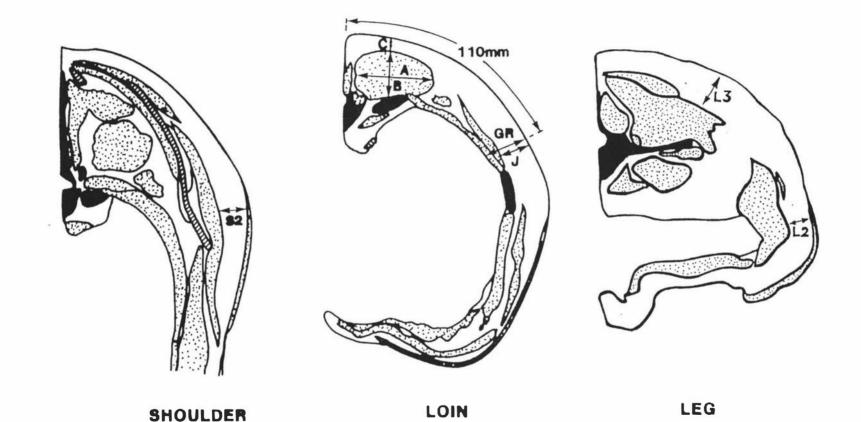


Figure 3.4 Diagrams indicating where measurements were taken on some cut surfaces of the carcases. The shoulder cut was made between ribs 7 and 8, the loin cut was between the 12th and 13th ribs, and the leg cut was between the last and second to last lumbar vertebrae.

TSK Wt. = 100 SK % x TP Wt.

TW Wt. = TP Wt. - TSK Wt.

#### 3.5 CARCASS MEASUREMENTS

Following a chilling period of approximately 24 hours at 1-3°C within a plastic bag, carcasses were weighed and the water that dripped from the carcasses was also weighed.

Linear measurements made on the whole carcass included items 1 to 6 defined in Table 3.2 and shown in Figure 3.2. After completing the above measurements, carcasses were cut into four units (shoulder, rack, loin and leg) (Figure 3.3). The shoulder was separated from the rack by first cutting with a knife along a line against the caudal edge of the 7th rib on each side, and then the vertebrae was sawn through. The rack was separated from the loin by cuts against the caudal edge of the 12th rib, the ventral edge of the costal cartilages, and through the intervertebral joint between the 12th and 13th thoracic vertebrae. The leg was separated by cutting between the last and second to last lumbar vertebrae. Linear measurements (items 7 to 12, 14 and 15 in Table 3.2) were then made on the surfaces of the cuts as illustrated in Figure 3.4. Calipers and a metal rule were used to make the measurements. Each cut from both sides of the carcasses was weighed after being split into halves. All cuts were sealed in plastic bags and frozen, at approximately -20°C for periods ranging from 3 to 60 days.

# 3.6 DISSECTION PROCEDURES AND MEASUREMENTS

Dissection commenced 2 to 3 days after the time of carcass measurements. All cuts from the left side of the carcass taken from freezer at random were thawed for about 12 hours in a cooler at 1 to 2°C, while still in plastic bags. After weighing the dissection was done as quickly as possible (using knives and scalpels) to avoid moisture losses. The cuts and dissected parts were kept covered with damp towels prior to weighing.

Cuts were dissected into the following components:

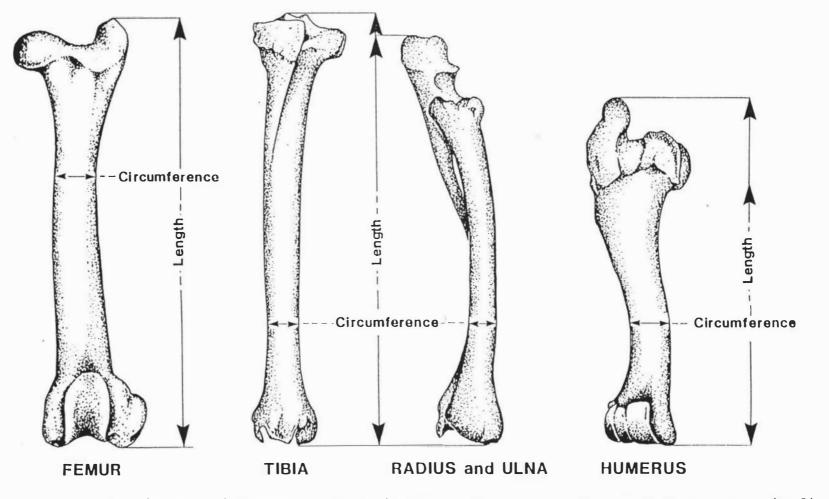


Figure 3.5 Diagrams indicating where measurements were made for four bones. (Kadim, 1988)

- 1. Soft tissue: This included all the muscle and fat.
- Individual Muscles: These included M. semitendinosus, M. semimembranosus and M. biceps femoris dissected from the leg; and M. supraspinatus, dissected from the shoulder. All muscles were weighed immediately after separation from the cuts. The dissection of individual muscles was based on the description of Fourie (1962).
- 3. <u>Bone</u>: This included the total weight of bone plus cartilage in each cut, The following individual bones were removed from the shoulder and leg cuts, scraped clean and weight, length and circumference recorded (Figure 3.5):
  - 1. Humerus and Radius-Ulna (shoulder).
  - 2. Femur and Tibia (leg).

The circumference of each bone was measured with a fine cotton string at the narrowest point on the shaft. The length was measured by calipers between the distal and proximal extremities (Figure 3.5).

- 4. <u>Intermuscular Fat Depots (IMFD)</u>: Two IMFD's were dissected from between the muscles as following:
  - I. Popliteal IMFD: This was dissected from the leg cut after removing the Biceps femoris and Semitendinosus muscles
  - II. Prescapular IMFD: This was dissected from the shoulder cut after removing the subcutaneous fat, Brachiocephalicus muscle and Omotransversarius muscle.

The respective lymph nodes (Popliteal lymph node and Prescapular or superfeicl cervical lymph node) were left embedded in the fat depots (Figure 3.3).

The dissected soft tissue and bone from left side of each carcass were frozen separately after weighing in plastic bags for subsequent determination of moisture content.

# 3.7 ADIPOSE TISSUE MEASUREMENTS

Cubes of approximately 10 to 15 mm of subcutaneous and intermuscular adipose tissues were taken from the fat above and beneath M. lliocostalis at the cranial end of the rack. After 2 to

3 months of storage in a formaldehyde solution (4%) at 4°C, the fat tissue was attached to a chuck with several drops of Tissue-Tek II O.C.T compound, and allowed to equilibrate to the Cryostat temperature (-25°C) for 30 minutes. The tissue block was then sectioned on a Cryostatic microtome (LIDSHAW CRYO TOME Model 1500) to about 90  $\mu$ m thickness for each cut. The sections were turned over on the knife with a small fine brush, and then transferred to slides, where they were mounted in a water-based mountant and covered with a coverslip (Sjostrom et al. 1971).

The diameter of two hundred randomly selected adipocytes from each slide were measured using a projection microscope (REICHERT WIEN VISOPAN) at a magnification of 125X. Care was taken to ensure that the same region was viewed only once in each slide.

The mean diameter (d) of the adipocyte and its standard deviation (S) were calculated. The mean adipocyte volume (V) was obtained using the formula of Goldrick (1967):

$$V = (\pi/6)(3S^2 + d^2) d$$

#### 3.8 MUSCLE FIBRE-TYPE MEASUREMENTS

The right M. semitendinosus from 31 animals removed from the carcass at about 20 to 30 minutes post-mortem. The muscle was cleaned of fat, weighed, placed in a plastic bag and kept on ice until the group of muscles were transported to the Histology laboratory. A longitudinal strip, up to 10 mm in length along the muscle and 5 mm in diameter was removed from each muscle, approximately 60 to 90 minutes post-mortem. The sample was wrapped in aluminium foil and frozen in 100 ml isopentane to  $-160^{\circ}$ C in 100 ml of liquid nitrogen for 10 minutes. Samples were then mounted on a cryostat chuck with Tissue-Tek II O.C.T compound, and serial sections of 10  $\mu$ m thickness were cut and mounted on clean glass slides at  $-20^{\circ}$ C in a cryostat (LIDSHAW CRYO TOME Model 1500) after the sample was allowed to equilibrate to  $-20^{\circ}$ C. All the slides were then left in a refrigerator for at least 30 minutes before staining to prevent the sections separating from the slides at later stages in the staining procedures.

The demonstration of Succinate dehydrogenase (SDH) using the Nitro Blue Tetrazolium method (Nachlas <u>et al</u>. 1957) was used to stain the sections. A cover slip was then placed over the tissue section using glycerol jelly to fix it in place. A projection microscope (REICHERT WIEN VISOPAN) (magnification of 315X) was used to measure the diameter of muscle fibres. These were selected from four muscle fibre bundles in each sample. 18 randomly selected fibres of each of the 3 muscle fibre types were measured from each bundle.

Muscle fibre area (A) was calculated from the diameter (D) using the following formula:

$$\begin{array}{c} D\\ A = \pi .(---)^2\\ 2\end{array}$$

The proportion of fibres of each of the muscle fibre types was calculated by counting all the fibres of each type in an area containing approximately 200 to 300 fibres traced on to tracing paper. This was done for four muscle fibre bundles for each animal.

# 3.9 CHEMICAL ANALYSIS

The chemical analysis was determined in the following components:

- 1. Blood sample, head and feet, viscera and skin sample for moisture determination.
- 2. The soft tissue and bone from the dissected left side cut for moisture and fat determination.
- 3. M. longissimus from the right side cuts for fat determination.

# 3.9.1 WATER DETERMINATION

Water content was calculated from triplicate samples as the difference between the fresh sample weight and the dry weight after drying at 90-100  $^{\circ}$ C (A.O.A.C.,1984).

Triplicate blood samples (100 ml each) were collected after slaughter, and within 3-4 hours post-mortem dried in an oven (90-100  $^{\circ}$ C) for 24 hours, cooled in a desiccator and then weighed. This procedure was followed since when the first block of blood samples was dried for several days until there was no appreciable water loss after 24 hours.

The moisture in the head and feet and viscera was determined approximately one week post-mortem (for each block). Similarly with soft tissues and bone, the moisture was determined approximately one week after dissection of the left side carcass was completed. All the samples were taken from the freezer at random for analysis, at the rate of 10 to 15 samples per working day, so that all the samples were removed within approximately 3 months of initial freezing.

The minced samples were prepared by initially thawing the sample at room temperature for one hour and then sawing the frozen samples into slices of 10 to 20 mm thickness. These were minced in a 11 kW WOLFKING MINCER, fitted with two die plates containing 16 and 10 mm holes respectively. After thorough mixing by hand, the mince was put through the same mincer again. After another mixing, the mince was put through a 1.1 kW HOBART MINCER with a plate-hole diameter of 6 mm. Once assured of a homogeneous product, triplicate samples (15-20 g each) were weighed out on to tared aluminium foil, and dried in an oven (90-100°C) for 24 hours.

The above procedure was followed only when the samples contained bone, such as the head and feet and the bone for one side. Otherwise the samples were minced three times only in the HOBART MINCER, for example the soft tissue and eviscerate. Approximately 100 g samples from the minced bone and soft tissues were stored in a deep freezer for later determination of fat.

After one hour thawing at room temperature, the wool was shaved and longitudinal strips (1 cm x 10 cm) were then taken from all over the three skin samples. Triplicate samples (15-20 g each) were weighed out on to tared aluminium foil and dried in an oven for 24 hours at 90 to  $100^{\circ}$ C.

#### 3.9.2 FAT DETERMINATION

The fat content were determined in the soft tissue and bone from the left side cuts and in the M. longissimus from the right side rack cut. This fat content were estimated by SOXHLET extraction of duplicate minced and freeze dried samples (10-14 g wet weight) for about 9 hours with petroleum ether (B.P. 40 to  $60^{\circ}$ C) (A.O.A.C., 1970). The minced samples used came from the separate soft tissue and bone which were prepared previously and stored, and from the M. longissimus which was dissected on the day of linear measurement and stored after trimming of subcutaneous fat, connective tissue, intermuscular fat and epimysium. All

the samples were thawed partially by microwave and then diced into very small pieces by knife. After mixing thoroughly, triplicate samples (10-14 g each) were weighed out on to tared aluminium foil to estimate the fat content.

## 3.10 STATISTICAL METHODS

Two kinds of data were available from this study as follows:

- 1. Body and carcass composition data.
- 2. Measurements associated with the urea dilution experiments.

The data were subjected to analyses of variance, and general least-squares procedures were used to examine the effects of the 3 slaughter lots, the four sires and lot x sires interactions on both sets of data after being adjusted by covariance analyses for differences in the appropriate covariate. The computations were carried out using the generalized linear models computing programme (REG) held at the Massey University computer centre (Gilmour, 1985).

# 3.10.1 BODY AND CARCASS COMPOSITION

The model used to describe body composition characteristics was:

Yijk = U + Li + Sj + (LS)ij + b(xijk) + eijk

Where:

- Yijk = is kth observation in jth sire and in the ith slaughter lot
  - U = is the overall mean
  - Li = is the effect of lot
  - Sj = is the effect of sires
- (LS)ij = is the lot X sire interaction
  - b = is the linear regression coefficient of Yijk on the covariate Xijk
  - xijk = is equal to (Xijk X-), X- being the overall mean of the covariate Xijk
  - eijk = is a random error, assumed to be normally and independently distributed with zero mean and constant variance

It is important to note that the components of the model were always fitted in the following sequences: the covariate, lot, sires and interaction. The lot X sire interaction term was seldom found to be significant (P > 0.1) and was therefore excluded from the model.

Differences between the means of the 4 sire groups were evaluated using a set of 3 orthogonal contrasts as follows:

1. Ho: -1S1 - 1S2 + 1S3 + 1S4 = 02. Ho: -1S1 + 1S2 = 0 = 03. Ho: 0 = 0 - 1S3 + 1S4 = 0

### Where:

S1 and S2 represent the 2 sire groups within the high-backfat line and:

S3 and S4 represent the 2 sire groups within the low-backfat line.

# 3.10.2 UREA DILUTION

In order to predict body water from changes in urea concentration following the administration of a urea challenge, it is necessary to obtain an accurate estimate of the increase in urea concentration that would have occurred if mixing had been instantaneous. That is, the zero-time concentration minus the baseline value.

The accuracy of various estimates of urea concentration increases were assessed from the closeness of relationships between empty body water percentage calculated from these estimates and empty body water percentage which was measured directly.

The estimation of empty body water percentage (EBH2O%) from urea dilution data was based on the following equation:

## EBH2O% = (EBH2O X 100) / EBWT

Where EBWT = empty body weight, and EBH2O = weight of empty body water (kg) = volume of empty body water (L)

= (1.998 X BWT) / (EUCC)

Where BWT = Body weight (full) on the day of challange and 1.998 = 120 / 60.06 = (Urea dose rate (mg/kg))/(Urea molecular weight)

Several methods were used to calculated EUCCs from plasma concentrations of urea before and after the challenge as outlined below. Plasma urea concentrations available for each animal included the three baseline samples before the challenge and 13 samples after the challenge.

#### 1. METHOD (A):

 The following 2-pool model was fitted using all 13 available data points (Bartle et al., 1988) using iterative procedures in the BMDP statistical package (Dixon et al., 1985). Only the second component of the model was of direct interest since it represented the phase of clearance, while the first component represented the initial mixing of the urea in body water.

$$U(t) = ae^{-bt} + ce^{-dt}$$
 .....(1)

where:

U(t) = is the plasma urea concentration at time t.

- a = is the zero-time urea concentration for pool 1 (mixing curve).
- e = is the exponential constant = 2.718 (the base of natural logarithms)

b and d = is the fractional urea decay constants.

- C = is the zero-time urea concentration for pool 2 (clearance rate).
- Average pre-challenge baseline concentrations were subtracted from c. Difficulty was experienced in fitting the model if baseline values were subtracted beforehand.

# 2. METHOD (B):

- Within each animal all values beyond the first post-challenge value that was within 2 percent of the final 420 min value were excluded from the analysis. A two percent range was chosen because it was the approximate intra-assay coefficient of variation between replicates for the urea assay.
- 2. The 2-pool model (1) was fitted to the remaining values for each animal as for method A.
- Average pre-challenge baseline concentrations were subtracted from c. Difficulty was experienced in fitting the model if baseline values were subtracted beforehand.

# 3. METHOD (C):

 Average pre-challenge baseline concentrations were subtracted from the 15 min (C15), 30 min (C30), 45 min (C45) and average of the 15, 30, and 45 min (Cav) post-challenge values for each animal.

# 4. METHOD (D):

- 1. Within each animal all values beyond the first post-challenge value that was within 2 percent of the final 420 min value were excluded from the analysis.
- 2. A simple exponential equation of the form:

$$U(t) = c e^{-dt}$$

was fitted to the baseline-adjusted data by linear regression of loge U(t) on t.

- 3. For method D1 the baseline was assumed to be constant and equal to the mean of the 3 pre-challenge samples.
- 4. For method D2 the baseline was assumed to be represented by a straight line between the mean of the 3 pre-challenge samples at time zero and the last sample to be included in the analysis for that animal as specified under 1 above.

# **CHAPTER FOUR**

# RESULTS

# 4.1 INTRODUCTION

The results for this study are given in two sections. Results concerning Body and carcass composition characteristics are presented in section 4.2 (Tables 4.1 to 4.12; Figures 4.1 to 4.3) and, those relating to urea dilution studies are outlined in section 4.3 (Tables 4.13 and 4.14; Figures 4.4 and 4.5).

#### 4.2 BODY AND CARCASS COMPOSITION CHARACTERISTICS

# 4.2.1 FINAL LIVEWEIGHT, CARCASS WEIGHT AND DRESSING-OUT PERCENTAGE

The difference between sires and lines for liveweight, carcass weight and dressing-out percentage of rams are presented in Table 4.1. Final liveweight was non-significantly higher for rams from the low-backfat line, but this line had slightly lower carcass weight. Dressing-out percent however, were higher for the high-backfat line (P < 0.10) than the low-backfat line when adjusted to a constant carcass weight. Mean slaughter liveweights for the three lots were 29.9, 30.1 and 35.4 Kg respectively and their ages were 171, 197 and 223 days respectively.

# 4.2.2 NON-CARCASS BODY COMPONENTS

Least-squares means of all non-carcass components in Table 4.1 were similarly higher for rams from the low-backfat line than the high-backfat line, when the data was adjusted to a constant carcass weight, with the exception of skin plus wool, liver, spleen and heart weight, being significantly heavier.

The skin plus wool weight was the only body component which showed significant differences between rams within a line. Table 4.1 also shows significant effects of lot on the

				res									
	Overall	High		-	Fat	2		Significance					
ltem	Mcan	1	2	3	4	r <sup>2</sup>	RSD	H vs L <sup>a</sup>	S <sub>1</sub> vs S <sub>2</sub> <sup>b</sup>	S <sub>3</sub> vs S <sub>4</sub> <sup>c</sup>	Loid	Covariate	
Ngc at slaughter (d)	197	198	200	196	194	0.92	7.1	NS	NS	NS	***		
Fasted livewcight (kg)	31.5	31.4	30.0	32.5	32.2	0.26	4.28	NS	NS	NS	*		
Empty livewcight (kg)	24.7	25.1	23.3	25.3	25.1	0.29	3.8	NS	NS	NS	**		
Gut contents (%)	20.8	19.4	22.2	21.0	20.6	0.41	4.05	NS	NS	NS	**	**	
Carcass weight (kg)	14.0	14.6	14.1	13.3	14.1	0.85	1.1	NS	NS	NS	NS	***	
Dressing-out percentage	44.2	45.2	44.4	43.2	44.1	0.70	1.89	S	NS	NS	S	***	
Blood weight (g)	1426	1448	1374	1437	1445	0.78	102	NS	NS	NS	NS	***	
Skin + wool weight (g)	2551	2330	2405	2851	2618	0.81	234	***	NS	*	NS	***	
lead and f cet weight (g)	2128	2092	2138	2173	2109	0.91	73	NS	NS	S	+	***	
Empty fore gut weight (g)	876	860	878	863	903	0.50	88.3	NS	NS	NS	NS	***	
ntestines empty weight (g)	1571	1584	1557	1546	1599	0.67	134	NS	NS	NS	NS	***	
Liver weight (g)	483	460	452	511	508	0.71	46	***	NS	NS	•	***	
Kidncy weight (g)	91	88	95	90	92	0.64	8	NS	S	NS	**	***	
Splccn weight (g)	43	42	42	44	46	0.63	4.5	S	NS	NS	NS	***	
leart weight (g)	144	143	135	150	150	0.80	10	**	NS	NS	*	***	
ungs and trachea weight (g)	390	392	358	416	393	0.25	59	NS	NS	NS	NS		
Testes weight (g)	359	368	353	364	351	-0.67	53	NS	NS	NS	**	***	

Table 4.1 Least-squares means of age at slaughter, fasted liveweight, empty liveweight, percentage gut contents, carcass weight, dressing-out percentage, and non-carcass component weights of Southdown rams within two sire groups from each of the high and low backfat lines.

<sup>a</sup> High versus Low backfat lines <sup>b</sup> Sire one versus sire two

<sup>C</sup> Sire three versus sire four

d Lot refers to the three groups of animals slaughtered at three different times. For more details refer to Table 3.1

The covariate was fasted liveweight for gut contents % and carcass weight analysis and carcass weight for all other variables

RSD = Residual standard deviation

 $r^2$  = coefficient of determination

NS = not significant; S = P < 0.10; \* = P < 0.05; \*\* = P < 0.01; \*\*\* = P < 0.001

head plus feet weight, liver weight, kidney weight, heart weight and testes weight between the groups of rams which were slaughtered at different times.

#### 4.2.3 LINEAR AND AREA MEASUREMENTS

When data was adjusted to a constant carcass weight, the carcasses from the rams of the lowbackfat line were significantly longer (by 39 mm) and had significantly longer hind legs (by 5.5 mm) than the rams from high-backfat line (Table 4.2). However, the low-backfat line had narrower shoulders (WF) (P < 0.1) and a similar width behind the shoulders (WTh).

The least-squares means for all fat depth measurements were significantly greater for rams from the high-backfat line when compared at the same constant carcass weight. On average the ram carcasses from the high-backfat line had 1.7, 2.15, 1.85, 1.75 and 3.5 mm greater depth at C, J, GR, S2 and L3 respectively than the low-backfat line. These in turn represent an increase of approximately 56.7%, 37.1%, 26.1%, 33.3% and 51% in the high-backfat line, relative to the low-backfat line.

The cross-sectional area of M. longissimus at the 12/13 rib was similar for the two lines, but the shape of this muscle was significantly deeper (B) and narrower (A) for the high-backfat line carcasses at a constant carcass weight.

Significant differences between sires within either one or both lines existed for leg length (T), fat depth C, J, GR, and S2 and also between the area and width (A) for M. longissimus (Table 4.2). Lot effects were also significant for body length (LB) and fat depth (C) (Table 4.2).

#### 4.2.4 CARCASS COMPOSITION

#### 4.2.4.1 Weights of individual cuts

Least-squares means for 4 cuts (average of both sides), together with total side soft tissue weight and total side bone weight are presented in Table 4.3. At the same carcass weight the carcasses of the high-backfat line had significantly lighter shoulder cuts, heavier rack cuts (P < 0.001) and loin and leg cuts of a similar weight to the carcasses of the low-backfat line. Also, total side bone weight was significantly lower in the high-backfat line.

			Si	res									
Item	Overall	_High Fat_		Low Fat		2		Significance					
	Mean	1	2	3	4	r <sup>2</sup>	RSD	HvsL	S <sub>1</sub> vs S <sub>2</sub>	S <sub>3</sub> vs S <sub>4</sub>	Lot	Covariate	
Body length LB (mm)	897	882	874	923	911	0.91	14	***	NS	S	**	***	
Leg length T (mm)	160	157	158	166	160	0.67	4.7	**	NS	**	NS	***	
Width behind shoulder WTh (mm)	156	155	158	154	156	0.85	5.2	NS	NS	NS	NS	***	
Maximum shoulder width WF (mm)	170	171	173	168	170	0.93	4.4	S	NS	NS	NS	***	
Gigot width G (mm)	215	215	215	216	215	0.80	4.4	NS	NS	NŚ	NS	***	
Fat depth C (mm)	2.1	2.6	3.4	1.2	1.4	0.81	0.7	***	**	NS	*	***	
Fat depth J (mm)	4.7	5.3	6.3	3.2	4.1	0.83	1.0	***	*	S	S	***	
Tissue depth GR (mm)	6.2	6.6	7.6	4.8	5.7	0.91	0.9	***	*	*	NS	***	
Fat depth S2 (mm)	4.4	4.3	6.2	3.2	3.8	0.78	1.0	***	***	NS	NS	***	
Fat depth L3 (mm)	5.1	6.4	7.3	3.3	3.4	0.87	1.1	***	S	NS	NS	***	
M. longissimus:													
Width A (mm)	54	53	52	56	53	0.61	2.0	***	NS	**	NS	***	
Depth B (mm)	25	25	26	25	25	0.85	1.1	*	NS	NS	NS	***	
Area (cm <sup>2</sup> )	10.6	10.7	10.6	11.1	10.2	0.84	0.7	NS	NS	**	NS	***	

Table 4.2 Least-squares means for carcass linear dimensions of Southdown rams within two sire groups from each of the high and low backfat lines

¶ The covariate was carcass weight

For definitions of abbreviations see Table 4.1

	Overall	High		I ou	Fat			Significance				
ltem weight (g)	Mean	1	2	3	4	r <sup>2</sup>	RSD	HvsL	S <sub>1</sub> vs S <sub>2</sub>	S <sub>3</sub> vs S <sub>4</sub>	Lot	Covariate
Left and right leg	4879	4936	4851	4885	4843	0.98	100	NS	NS	NS	NS	***
Left and right loin	2069	1983	2154	2023	2114	0.92	139	NS	**	NS	NS	***
Left and right rack	1206	1203	1277	1174	1172	0.98	39	***	***	NS	***	***
Left and right shoulder	5824	5862	5698	5892	5843	0.98	131	*	**	NS	S	***
Total side bone	987	950	927	1051	1018	0.86	54.1	***	NS	NS	**	***
Fat-free soft tissue weight	4400	4364	4260	4533	4442	0.96	165	*	NS	NS	NS	***
Weight of 4 muscles <sup>a</sup>	547	567	541	550	530	0.93	29.3	NS	S	NS	NS	***

Table 4.3 Least squares means for cut weights and dissected tissue weights of Southdown rams within two sire groups from each of the high and low backfat lines

¶ The covariate was carcass weight

<sup>a</sup> M. semitendinosus, M. semimembranosus, M. biceps femoris, M. supraspinatus

For definitions of abbreviations see Table 4.1

The shoulder cut was heavier and the loin and rack cuts were lighter in the carcasses of rams from sire number one than sire number two within the high-backfat line. Table 4.3 also shows that the rack cut weight and total side bone weight were the only two components differing significantly between lots.

# 4.2.4.2 Bone distribution and dimension

The effects of sires on bone distribution are presented in Table 4.4, with the high-backfat line carcasses having a significantly higher weight of bones in the rack (7.5%) at the same total side bone weight. However, the high-backfat line had significantly lower weight of bone (3.8%) in the leg cut. The humerus and femur bone weight were also significantly lighter by 3.9% and 6.3% respectively in the high-backfat line.

The humerus and tibia bone weight and the bone weight in the loin cut differed significantly between the ram carcasses within the sires of the low-backfat line. Also, the radius and ulna bone weights differed significantly between the ram carcasses within sires of the high-backfat line. The lot affected only the tibia bone weight.

The length and circumference of the humerus, radius and ulna, femur and tibia bones at the same carcass weight, were significantly lower in the high-backfat line (Figure 4.1). Similar results were obtained when these linear measurements were cubed to ensure that they were measured in units of the same dimension before being corrected for carcass weight (results not shown).

#### 4.2.4.3 Partitioning of fat among the depots

Table 4.5 shows the least-squares means for several carcass and non-carcass fat depots including omental and kidney fat depots, popliteal and prescapular intermuscular fat depots, and the fat percentage in M. longissimus muscle in the rack cut as an intramuscular fat depot.

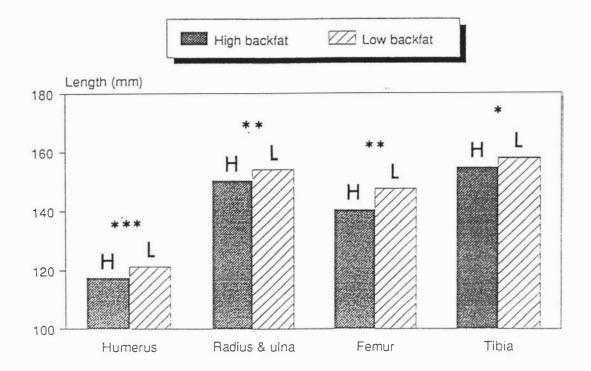
At the same carcass weight, the kidney fat depot was significantly heavier (+14.1%) in the high-backfat line, but line differences were non-significant for omental, popliteal, and prescapular and intramuscular fat depots.

The fat percentage in the soft-tissues of carcasses from the high-backfat line was 12.3% greater than the low-backfat line (Table 4.5), but the fat percentage in carcass bone did not differ between the lines.

 Table 4.4
 Least square means for bone weight distribution within total side bone of Southdown rams within two sire groups from each of the high and low backfat lines

	Overall	High		ires	v Fat				S	ignificance		
Item weight (g)	Mean	1	2	3	4	r <sup>2</sup>	RSD	H vs L	S <sub>1</sub> vs S <sub>2</sub>	S <sub>3</sub> vs S <sub>4</sub>		Covariate
Shoulder bone	466.0	469.3	462.3	470.1	462.2	0.96	14.3	NS	NS	NS	NS	***
Humerus bone	67.1	65.1	66.6	69.8	67.0	0.94	2.2	**	NS	**	NS	***
Radius and ulna bone	53.1	51.3	54.3	53.5	53.1	0.91	2.1	NS	**	NS	NS	***
Rack bone	101.3	105.0	105.1	100.9	94.2	0.73	8.5	**	NS	NS	NS	***
Loin bone	89.8	85.8	95.1	83.4	94.8	0.65	11.4	NS	NS	*	NS	***
Leg bone	328.9	321.7	323.7	332.6	337.7	0.92	13.7	*	NS	NS	NS	***
Femur bone	89.5	86.2	87.3	93.7	90.8	0.89	4.3	**	NS	NS	S	***
Tibia bone	70.0	69.5	71.8	70.8	68.0	0.89	2.9	NS	S	*	*	***

¶ The covariate was total side bone weight



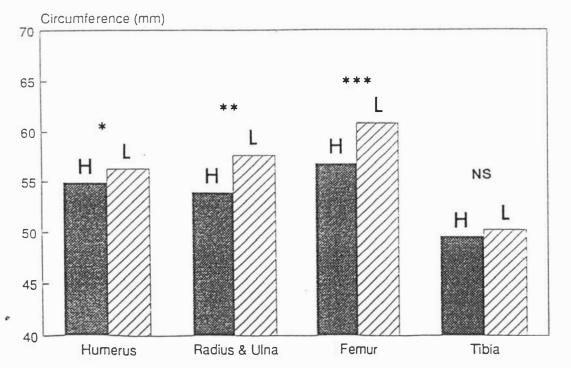


Figure 4.1 Bone dimensions adjusted to a constant Carcass weight for high (H) and low (L) backfat lines of Southdown rams.

Table 4.5 Least squares means for internal body fat depots, two intermuscular fat depots and fat percentage in M. Longissimus, soft tissue and bone of Southdown rams within two sire groups from each of the high and low backfat lines

	Overall	High		$\frac{\text{Low Fat}}{3}$					S	ignificance		
Item	Mean	1	2	3	4	r <sup>2</sup>	RSD	H vs L	S <sub>1</sub> vs S <sub>2</sub>	S <sub>3</sub> vs S <sub>4</sub>	Lot	Covariate
Omental fat weight (g)	325	304	325	291	382	0.90	48	NS	NS	***	NS	***
Kidney fat weight (g)	135	134	156	105	144	0.68	31	*	NS	**	**	***
Popliteal intermuscular fat depot weight in leg (g)	16.7	16.2	17.5	16.4	16.7	0.65	3.2	NS	NS	NS	NS	***
Prescapular intermuscular fat depot weight in shoulder (g)	68.2	68.2	72.9	60.2	71.4	0.74	10.5	NS	NS	S	NS	***
Chemical analysis:												
Fat percentage in M. longissimus (%)	3.5	3.6	3.2	4.2	2.9	0.71	0.81	NS	NS	**	S	***
Fat percentage in carcass soft tissue (%)	25.1	25.9	27.6	22.3	24.6	0.75	2.87	**	NS	S	NS	***
Fat percentage in carcass bone (%)	14.6	14.1	14.3	15.3	14.8	0.32	1.58	NS	NS	NS	*	NS

¶ The covariate was carcass weight

Omental fat depot, kidney fat depot and intramuscular fat percentage in M. longissimus differed significantly between the sires of the low-backfat line, and kidney fat depot weight differed between the slaughter lots (Table 4.5).

#### 4.2.4.4 Adipose cell diameter and volume

Rams from the high-backfat line had significantly larger fat cells (diameter and volume) for the intermuscular fat depot and a non-significantly smaller cells for the subcutaneous fat depot (Table 4.6), when the data was adjusted to a constant carcass weight. The subcutaneous fat depot had, on average, larger cell diameters and volumes than those from the intermuscular fat depot.

The results in Table 4.6 also show several significant differences between sires within lines and between lots.

## 4.2.4.5 Muscle weight and distribution

At the same carcass weight, the weights of total 4 muscles, dissected from the leg and shoulder cuts, were similar in both high and low backfat line (Table 4.3). However, the fat-free soft tissue weight in the left side which is considered to be closely corresponding to the total muscle weight in the side, showed slightly higher weight (P < 0.05) in the low backfat line carcasses.

Table 4.7 gives least-squares means for muscle weights and muscle to bone ratios after adjustment to a constant fat-free soft tissue weight. Fat-free soft tissue weight was used as a covariate because it should correspond closely to total muscle weight.

The high-backfat line carcasses had significantly heavier M. semimembranosus, M. biceps femoris but significantly lighter M. semitendinosus. The weight of M. supraspinatus did not differ significantly between the lines.

All the ratios of the muscle to bone weight were significantly higher in the highbackfat line except for the ratio of M. semitendinosus to femur weight.

Only the weight of M. semitendinosus and their ratios were significantly different between the sires of the high-backfat line and between the slaughter lots (Table 4.7).

Table 4.6 Least squares means for fat cellularity characteristics of two adipose tissue depots of Southdown rams within two sire groups from each of the high and low backfat lines

	Overall						RSD	Significance					
Item	Mean	1	2	3	4	r <sup>2</sup>	RSD	H vs L	S <sub>1</sub> vs S <sub>2</sub>	$S_3 vs S_4$	Lot	Covariate¶	
Subcutaneous fat cell	00 <i>(</i>	20.2	0.2.1	70.0	00.2	0.60			210				
diameter (µm)	82.6	79.7	83.1	79.2	88.3	0.68	7.8	NS	NS	*	*	***	
Subcutaneous fat cell volume ( $\mu m^3 \times 10^3$ )	342	304	352	303	409	0.64	101.4	NS	NS	*	**	***	
Internuscular fat cell diameter (µm)	76.6	74.6	82.7	73.4	75.7	0.70	5.5	*	**	NS	NS	***	
Internusclar fat cell volume (μm <sup>3</sup> x 10 <sup>3</sup> )	268	249	333	238	252	0.65	60.8	*	**	NS	NS	***	

¶ The covariate was carcass weight

 Table 4.7
 Least squares means for muscle weight distribution and ratios of muscles to bones adjusted to a constant total fat-free soft tissue weight of Southdown rams within two sire groups from each of the high and low backfat lines

				res								
	Overall	_High			/ Fat	2				ignificance		
Weight or ratio	Mean	1	2	3	4	r <sup>2</sup>	RSD	H vs L	S <sub>1</sub> vs S <sub>2</sub>	S <sub>3</sub> vs S <sub>4</sub>	Lot	Covariate¶
M. semitendinosus (g)	88.3	86.9	85.3	91.6	89.5	0.93	11.2	**	**	NS	***	**
M. semimembranosus (g)	203.0	217.0	203.7	196.9	194.3	0.81	18.4	*	NS	NS	NS	***
M. biceps femoris (g)	192.8	203.3	204.2	183.3	180.3	0.88	13.4	***	NS	NS	NS	***
M. supraspinatus (g)	78.3	80.4	78.5	76.6	77.6	0.81	7.3	NS	NS	NS	S	***
Muscle to bone ratios (g/g):												
Three leg muscles Femur	5.43	6.01	5.83	4.87	5.01	0.74	0.41	***	NS	NS	***	NS
<u>M. semitendinosus</u> Femur	1.00	1.04	1.05	0.95	0.97	0.97	0.08	NS	**	NS	***	***
M. semimembranosus Femur	2.27	2.57	2.39	2.03	2.09	0.58	0.23	***	NS	NS	NS	***
<u>M. biceps femoris</u> Femur	2.16	2.40	2.39	1.89	1.95	0.78	0.15	***	NS	NS	NS	***
<u>M. supraspinatus</u> Humerus	1.16	1.26	1.21	1.05	1.13	0.48	0.13	**	NS	NS	NS	***

¶ The covariate was fat-free soft tissue weight in the side

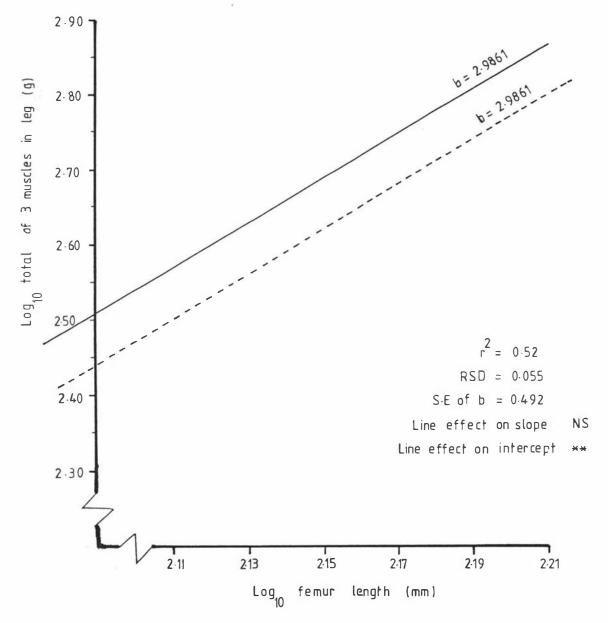


Figure 4.2 Double-log regression lines relating the weight of three leg muscles to femur length separately for high (solid line) and low-backfat (dashed line) lines of Southdown rams.

#### 4.2.4.6 Muscularity

Least-squares means for the ratios of certain individual muscle weights in leg and shoulder cuts relative to femur and humerus lengths respectively are given in Table 4.8. After adjustment to the same total side fat-free soft tissue plus bone weight. All the ratios were significantly higher in the high-backfat line than the low-backfat line except for the ratio of M. supraspinatus weight to humerus length. The latter was non-significantly higher (0.05 < P < 0.10) in the high-backfat line.

The total weight of the three muscles in the leg relative to femur length was significantly higher between the slaughter lot, also the ratio of M. semitendinosus to femur length was significantly higher between the sires of the high-backfat line and between the slaughter lot.

Double-log regression equations relating the weight of the 3-muscles to femur length have been plotted separately for the two selection lines in Figure 4.2. This Figure shows that the regression lines for the two selection lines had the same slopes, but significantly different intercepts, so that when lines were compared at the same femur length the high-backfat line animals had a greater weight of muscle.

## 4.2.4.7 Muscle fibre type - area and proportion

Table 4.9 shows the least-squares means for cross-sectional area, and proportion of the total number for the three muscle fibre types (red fibre ( $\beta R$ ), intermediate fibre ( $\alpha R$ ), and white fibre ( $\alpha W$ ) ) of M. semitendinosus. There were no significant differences between lines or between sires within lines, except between sire three and sire four for red fibre type area.

The analysis of muscle fibre characteristics was carried out within four muscle fibre bundles for each animal so that the variation between animals could be compared with variation between bundles in a nested model. This animal effect was highly significant for each characteristic (Table 4.10).

# 4.2.5 CARCASS AND NON-CARCASS EMPTY-BODY WATER

Least-squares means for the weight and percentage of water in carcass and non-carcass empty-body components are given in Tables 4.11 and 4.12, and Figure 4.3 after adjustment to the same empty body weight. 

 Table 4.8
 Least squares means for ratios of muscle weights relative to bone lengths of Southdown rams within two sire groups from each of the high and low backfat lines

Ratio of muscle weight to	Overall	High	<u>Sires</u> <u>High Fat</u> Low Fat 1 2 3 4		2	$\frac{Significance}{RSD H vs L S_1 vs S_2 S_3 vs S_4 Lot Covariate}$						
bone length (g/mm)	Mean	1	2	3	4	r <sup>2</sup>	RSD	H vs L	S <sub>1</sub> vs S <sub>2</sub>	S <sub>3</sub> vs S <sub>4</sub>	Lot	Covariate
Three muscles of the leg Femur	3.35	3.58	3.52	3.12	3.19	0.80	0.23	***	NS	NS	***	***
<u>M. semitendinosus</u> Femur	0.61	0.62	0.61	0.61	0.61	0.95	0.06	***	***	NS	***	***
<u>M. semimembranosus</u> Femur	1.40	1.53	1.45	1.30	1.33	0.74	0.13	**	NS	NS	NS	***
<u>M. biceps femoris</u> Femur	1.33	1.43	1.45	1.21	1.24	0.84	0.09	***	NS	NS	NS	***
<u>M. supraspinatus</u> Humerus	0.65	0.68	0.66	0.61	0.65	0.75	0.06	S	NS	NS	NS	***

¶ The covariate was total side fat-free soft tissue plus bone weight

	Overall	Hig	Sires h Fat	Lov	v Fat				Sic	nificance	
Item	Mean	1	2	3	4	ر2	RSD	H vs L	S <sub>1</sub> vs S <sub>2</sub>		Covariate
Average Area (µm <sup>2</sup> ):											
Red	249	236	254	271	236	0.43	43	NS	NS	*	NS
Intermediate	474	482	483	486	446	0.54	69	NS	NS	NS	NS
White	527	556	549	519	488	0.61	77	NS	NS	NS	NS
Proportion (%):										5	
Red	41.5	39.8	42.0	41.9	42.1	0.53	4.7	NS	NS	NS	NS
Intermediate	31.1	31.5	31.1	31.8	29.8	0.49	4.4	NS	NS	NS	NS
White	27.5	28.8	26.8	26.3	28.0	0.46	4.6	NS	NS	NS	NS

Table 4.9 Least squares means for muscle fibre area and proportions of the three muscle fibre types (red, intermediate and white) from M. semitendinosus of Southdown rams within two sire groups from each of the high and low backfat lines

The covariate was carcass weight

Table 4.10 Mean squares with degrees of freedom from the nested analyses of variance for muscle fibre areas and proportions of the three muscle fibre types (red, intermediate and white) from four bundles within M. semitendinosus of Southdown rams within two sire groups from each of the high and low backfat lines

	Degrees of		Area			Proportion	
Source of variation	Freedom (D.F.)	Red	Intermediate	White	Red	Intermediate	White
Between sires	3	28612	20878	67066	149.966	80.346	129.941
Between animals	26	3759	16269	27033	87.255	63.588	58.679
Error: (within animals between bundles)	93	1818	4701	5975	22.283	19.194	21.028
Significance of animal effect		**	***	***	***	***	***

	Overall	High		ires	Fat				S	ignificance		
Item	Mean	1	2	3	4	r <sup>2</sup>	RSD	H vs L	S <sub>1</sub> vs S <sub>2</sub>	and share the second	Lot	Covariate¶
Moisture weight in (g):												
Blood	1180	1204	1147	1177	1192	0.79	80.4	NS	NS	NS	NS	***
Skin	1144	1079	1062	1320	1113	0.57	190	*	NS	*	NS	***
Head and feet	1151	1120	1157	1182	1144	0.84	46	NS	S	S	S	***
Viscera	3184	3162	3120	3232	3224	0.70	233	NS	NS	NS	NS	***
Total non-carcass components	6659	6566	6487	6910	6672	0.87	307	*	NS	NS	NS	***
Percentage of water in:												
Blood	82.8	82.7	82.8	82.9	82.8	0.31	0.83	NS	NS	NS	NS	**
Skin	70.7	71.0	70.1	71.0	70.9	0.23	2.29	NS	NS	NS	NS	*
Head and feet	54.2	53.4	53.9	55.0	54.4	0.59	1.14	*	NS	NS	NS	***
Viscera	68.9	68.9	68.0	71.0	67.7	0.77	1.87	NS	NS	***	*	***
Total non-carcass components	68.0	67.9	67.3	69.2	67.6	0.72	1.24	NS	NS	*	S	***

Table 4.11 Least squares means for moisture weight and percentage of all non-carcass components of Southdown rams within two sire groups from each of the high and low backfat lines

¶ The covariate was empty body weight

			Si	ires								
	Overall	High	Fat_		Fat	2				ignificance		
Item	Mean	1	2	3	4	r <sup>2</sup>	RSD	H vs L	$S_1 vs S_2$	S <sub>3</sub> vs S <sub>4</sub>	Lot	Covariate
Moisture weight (g) in:												
Carcass bone	700	683	656	736	724	0.64	51.4	**	NS	NS	NS	***
Carcass soft tissue	6794	6936	6624	6895	6721	0.91	352	NS	S	NS	NS	***
Total carcass	7628	7748	7424	7765	7577	0.92	356	NS	S	NS	NS	***
Empty liveweight	14.3	14.3	13.9	14.7	14.2	0.95	0.46	*	S	S	NS	***
Percentage of water in:												
Carcass bone	35.7	35.7	35.4	35.6	36.0	0.51	1.7	NS	NS	NS	S	***
Carcass soft tissue	58.0	57.8	55.9	60.4	57.8	0.74	2.5	*	NS	*	NS	***
Total carcass	55.0	55.2	53.0	56.8	54.7	0.70	2.3	S	NS	S	NS	***
Empty liveweight	57.9	58.1	56.6	59.4	57.7	0.72	1.8	S	NS	S	NS	***

 Table 4.12
 Least squares means for moisture weight and percentage of water in carcass components and total empty liveweight of Southdown rams within two sire groups from each of the high and low backfat lines

¶ The covariate was empty body weight

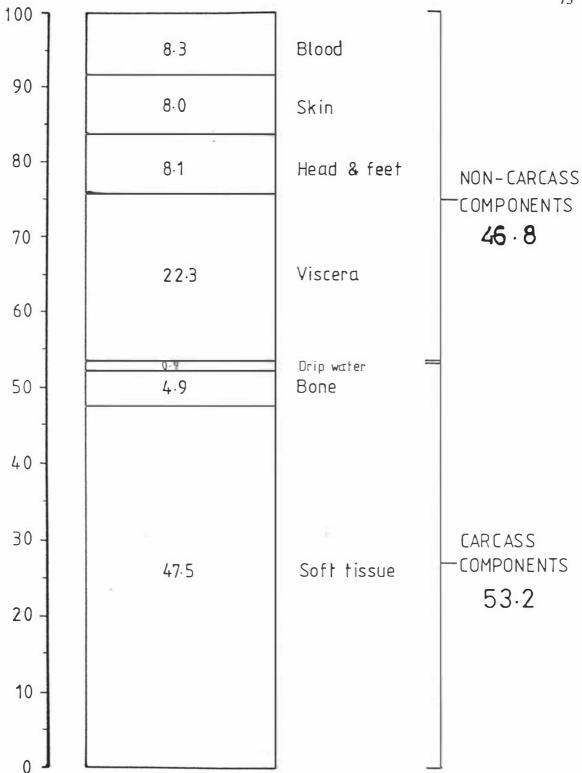


Figure 4.3 The amount of moisture in Carcass and non-carcass components expressed as percentages of the total moisture weight in the empty body for Southdown rams of both lines.

In general, all moisture measurements show a slightly higher weight and percentage in the low backfat line. The low backfat line rams had significantly higher moisture weight in skin, total non-carcass components, carcass bone and empty liveweight by 146, 264.5, 114.5 and 400 g, respectively than the rams from the high backfat line. Moreover, the low backfat line rams had also significantly higher moisture percentage in head and feet, carcass softtissue, total carcass and empty liveweight.

Some of the components had also shown signs of a significant difference between the sires of the two selection lines with non-significant differences between all the components for lot effects except for moisture percentage in viscera.

## 4.3 MEASUREMENTS OF BODY WATER BY DILUTION METHODS

Mean changes in plasma urea levels for animals of the high and low-backfat lines are plotted against time after an intravenous urea challenge in Figure 4.4. The data in this figure represent increases from the average of the 3 pre-challenge baseline samples. The only significant difference between lines was at 15 min when values were higher for the highbackfat line.

Overall means with standard errors (SE) for actual empty body water percentage, and estimated empty body water percentage as calculated by four different methods are given in Table 4.13 together with relationships between actual and estimated values.

For methods A and B (see section 3.10.2) the double exponential model was fitted iteratively using unadjusted urea levels and then the pre-challenge baseline values were subtracted from the estimated concentration at zero time. The expected urea concentration changes, (EUCCs) predicted by methods A and B included some negative values with the result that some estimated body water percentages were also negative, and as the EUCC approached zero from either the positive or negative side the estimated body water percentage approached infinity. This gave rise to exceptionally high standard errors (Table 4.13), so these methods were not considered further. Attempts to fit the double exponential equation after adjusting the data for pre-challenge baseline values were unsuccessful as the iterative procedure could not be made to converge to a meaningful stable result.

Methods C15, C30 and C45 involved the calculation of percentage moisture from the single urea concentrations at 15, 30 and 45 min respectively after subtracting the baseline

values. Method Cav involved the use of the average of the three values 15, 30 and 45 min after subtracting the baseline. The best relationships with actual empty body water percentage were for C45, C30 and Cav respectively, but these methods overestimated empty body water percentage. Although, not presented in Table 4.13, the relationship between direct and estimated empty body water percentage calculated by methods C and D gave similar results to those presented in Table 4.13 when selection line was included in the model.

Method D involved fitting a simple exponential equation to values which had been adjusted to either a constant baseline (method D1; section 3.10.2) or a sloping baseline (method D2; section 3.10.2). Use of method D2 led to a rising baseline for all animals, as shown for the group means in Figure 4.4, and gave a close estimation of the mean actual empty body water percentage (Table 4.13). However, it had a lower relationship with actual empty body water percentage than method D1, which overestimated actual empty body water percentage by 15.67 percentage points. The correlation between empty body water percentage estimated by methods D1 and D2 was 0.43. The mean number of samples that were within two percent of the final (420 min) sample was 2.14 (range 1 to 5).

Least-squares means for urea clearance rates and baseline urea concentrations (average of three pre-challenge samples) [together with actual and estimated empty body water percentages] are shown in Table 4.14 for the high- and low-backfat lines. Baseline urea values and urea clearance rates did not differ significantly between the lines, but the low-backfat line rams had a higher empty body water percentage. In addition, the empty body water percentage estimated from increases in urea concentrations at 15, 30 and the average of the three values (15, 30 and 45 minutes after the challenge), or by methods D1 and D2 were significantly higher for the low-backfat line (Table 4.14 and Figure 4.5). The empty body water percentages estimated from 15-minute post-challenge urea concentrations are plotted against empty body weight in Figure 4.6.

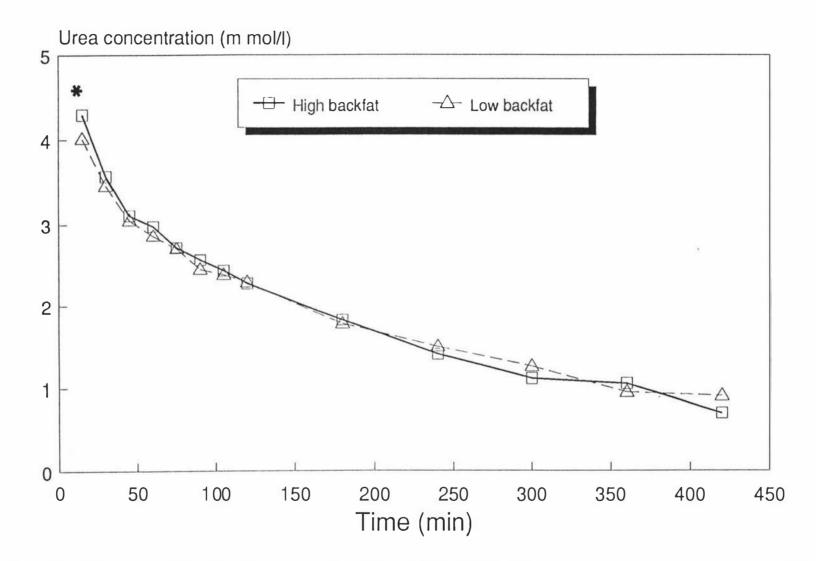


Figure 4.4 Changes in mean plasma urea concentration with time after an intravenous urea challenge for Southdown rams of high and low-backfat selection lines. Pre-challenge baseline values (mean=10.9 m mol/l) have been subtracted from all values. (\*=P<0.05 for line effect)

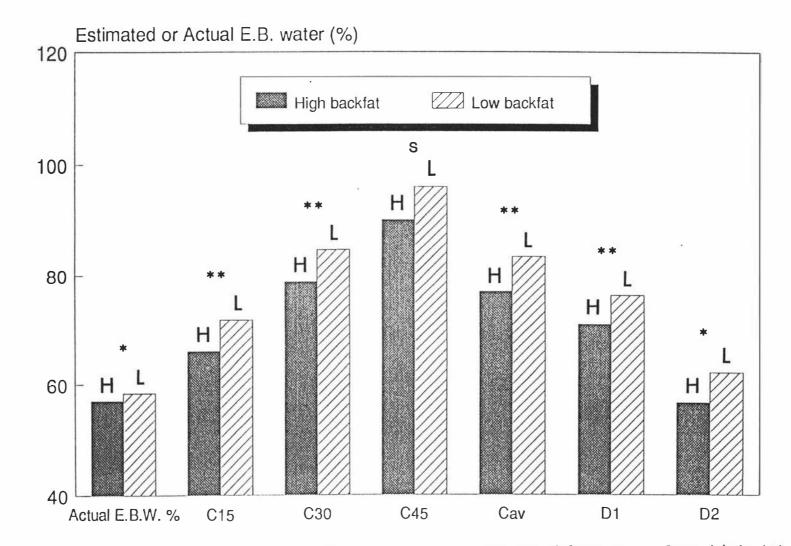


Figure 4.5 Actual and estimated empty body water percentages for Southdown rams from high (H) and low (L) backfat lines. Estimation methods (designated Cl5, C30, C45, Cav, Dl and D2) are described in the text.

Table 4.13 Means and standard errors of the actual and estimated empty body water percentages calculated by four different methods and relationships between actual and estimated values

Item	Mean	(SE)	Relationsl r <sup>2</sup>	nip with direct n RSD	neasurement Significance
Direct empty body water %	57.94	0.53			
Estimated empty body water %:					
Method (A): <sup>a</sup> (double exponential equation model) C - B.L. (13 sample)	141.1	29.7			
Method (B): (double exponential equation model) C - B.L. (2% cut of f)	58.2	31.1			
Method (C): (single urea concentration) (C15) 15 min sample - B.L.	68.99	1.26	0.09	3.07	NS
(C30) 30 min sample - B.L.	81.68	1.44	0.22	2.84	**
(C45) 45 min sample - B.L.	93.09	1.72	0.29	2.71	***
(Cav) 15, 30 and 45 min samples - B.L.	80.03	1.27	0.22	2.85	**
Method (D): (simple exponential model) (D1) adjusted to constant baseline	73.40	1.25	0.16	2.95	**
(D2) adjusted to a sloping baseline	59.30	1.33	0.10	3.06	S

 $^{\rm a}$  For definitions of all methods used refer to section 3.10 in Chapter 3.

				res								
	Overall	High	Fat_		Fat	2				ignificance		
Item <sup>a</sup>	Mean	1	2	3	4	r <sup>2</sup>	RSD	H vs L	S <sub>1</sub> vs S <sub>2</sub>	S <sub>3</sub> vs S <sub>4</sub>	Lot	Covariate
Base line (B.L.) urea												
concentration (mmol/I)	10.9	11.3	10.8	10.8	10.4	0.42	0.97	NS	NS	NS	**	S
Urea clearance rates by Method D <sub>2</sub>	0.01	05 0.00	090 0.01	03 0.01	09 0.01	16 0.12	0.004	NS	NS	NS	NS	NS
Estimated percentage empty body water:												
By Method (C):												
(single urea concentration)												
C15	69.0	66.3	64.4	72.0	73.3	0.48	5.96	***	NS	NS	NS	***
C30	81.7	80.5	77.0	84.4	84.8	0.46	6.96	**	NS	NS	NS	***
C45	93.1	89.7	90.6	94.2	97.9	0.39	8.84	S	NS	NS	NS	***
Cav	80.0	77.4	76.3	82.3	84.1	0.55	5.59	**	NS	NS	NS	***
By Method (D):												
(simple exponential model)												
D	73.4	71.9	69.6	76.3	75.9	0.45	6.09	**	NS	NS	NS	***
$D_2^{1}$	59.3	59.4	53.8	61.0	63.0	0.33	7.19	*	NS	NS	NS	*

Table 4.14 Least squares means for baseline urea concentration, urea clearance rates, and actual and estimated empty body water percentage calculated by different methods of Southdown rams within two sire groups from each of the high and low backfat lines

<sup>a</sup> For definitions of all methods used refer to section 3.10 in Chapter 3 For definitions of abbreviations see Table 4.1

¶ The covariate was empty body weight

# **CHAPTER FIVE**

# DISCUSSION

### 5.1 INTRODUCTION

The present experiment involved animals from the same high and low backfat selection lines that were assessed by Kadim <u>et al</u>. (1989), but the rams used were younger (7 mo vs 16 mo).

As the main objective was to assess the differences between the two selection lines at younger ages, the discussion will concentrate on differences between the lines in order to compare the results with the previous trial. Few differences appeared between slaughter lots or between sires within the two lines. The lot differences can generally be explained in terms of differences in age and liveweight, and the within-line sire differences may be explained in terms of differences in backfat depth. Thus, offspring of sire numbers one and three had lower backfat depths than those of sire numbers two and four in the high and low-backfat lines respectively (Table 4.2).

In addition to an assessment of line effects, this experiment was designed to further investigate differences in plasma urea levels between the two selection lines by evaluating the urea dilution technique for estimating body composition.

### 5.2 BODY AND CARCASS COMPOSITION

### 5.2.1 DRESSING-OUT PERCENTAGE

The higher dressing-out percentage for the high-backfat group was smaller than the similar result reported by Kadim <u>et al</u>. (1989) and was not significant probably because the line differences in fatness were much larger for the heavier rams of Kadim <u>et al</u>. (1989). Bradford and Spurlock (1972) reported that higher dressing-out percentages were associated with higher levels of fatness for progeny groups of Suffolk sires.

Heritability estimates for dressing-out percentage of sheep have been reported to be moderate to high (Wolf and Smith, 1983), and to be moderately positively correlated (genetically or phenotypically) with backfat depth (Bradford and Spurlock, 1972; Boylan and Seale, 1965). This suggests that the higher dressing-out percentage in the high-backfat line was a result of the increased fatness. Similar conclusions have been reached by several groups of workers using sheep differing in fatness for reasons other than genetic selection, including breed, sex, nutrition and mature weight (Kemp <u>et al.</u>, 1981; Hawkins <u>et al.</u>, 1985a; Kirton <u>et al.</u>, 1981).

## 5.2.2 NON-CARCASS COMPONENTS

Kadim <u>et al</u>. (1989) reported that Southdown sheep from the low-backfat line had heavier internal organs than the high-backfat line after adjusting for carcass weight differences. The foregut empty, intestines, liver, heart and kidneys were higher in the low-backfat line. The only significant differences obtained from the present study after adjusting for carcass weight were those showing that the liver weight and heart weight were heavier in the low-backfat line.

The results from both trials are in general agreement with work with pigs showing that heavier non-carcass components were associated with pigs selected for low levels of backfat (Tess <u>et al.</u>, 1986; Koong <u>et al.</u>, 1983).

## 5.2.3 LINEAR AND AREA MEASUREMENTS

Measures of carcass length in the present experiment agreed well with the results of Kadim et al. (1989) in showing that the low-backfat line rams had significantly longer bodies (LB) and legs (T) after adjustment for carcass weight. Purchas et al. (1982) also found that Southdown ewes selected for low-backfat had longer body lengths at constant weight than those selected for high-backfat. Other studies with sheep (Thorsteinsson and Bjornsson, 1982; Kemp and Barton, 1966; Wood et al., 1980; Bennett et al., 1982/1983) and pigs (Duckworth and Holmes, 1968) have also shown negative genetic correlations between carcass length and average backfat thickness at constant weight. Levels of fatness have also been shown to be reduced when pigs have been selected for increased carcass length (Duckworth and Holmes, 1968) and when sheep have been selected for long cannon bones (Purser, 1980). Kadim <u>et al</u>. (1989) found that the low-backfat line rams had significantly narrower carcass widths by 1.7, 4.7, and 0.79% for WF, WTh and G respectively than the high-backfat line. The biggest difference in the width behind shoulder (WTh) therefore, agree with the positive phenotypic and genetic correlations between this measurement and backfat thickness reported by Mohamed (1976) to be 0.45 for both correlation. In the present study, these widths were not significantly less for the low-backfat line. The small differences in width behind shoulder (WTh) percentage between the two lines in previous study (Kadim <u>et</u> <u>al.</u>, 1989), therefore resulted from the tendency for fat to accumulate in the WTh at larger age.

The M. longissimus measurements in the present experiment differed between the two lines in the same way as reported by Kadim <u>et al</u>. (1989) with similar cross-sectional areas of M. longissimus but with deeper (B) and less wide (A) muscles for carcasses from the highbackfat line. These results are supported by those of Buhlinger <u>et al</u>. (1978) with obese and lean pigs and by those of Kemp and Barton (1966), in which fatter and leaner grades of sheep were compared at the same weight. Other studies (Wood <u>et al</u>., 1983b; Kirton, 1982) have also shown that fatter carcasses tend to have deeper (B) and shorter (A) M. longissimus cross sections.

All fat depth measurements were significantly lower for the low-backfat line in the present study which is consistent with the results of Kadim <u>et al</u>. (1989) using similar but heavier animals and with studies with different breeds where sires have been selected on the basis of backfat thickness measured ultrasonically and the fatness of progeny has been assessed (Fennessy <u>et al</u>., 1982; Fennessy <u>et al</u>., 1987; McEwan <u>et al</u>., 1989; Meyer <u>et al</u>., 1981/1982).

### 5.2.4 WEIGHTS OF INDIVIDUAL CUTS

At the same carcass weight several studies have reported that fatter lambs will have a higher proportion of the fatter cuts (rack and loin) and a lower proportion of the leaner cuts (shoulder and leg) as reported by Kemp and Barton, (1966), Kirton <u>et al</u>. (1978), Kemp <u>et al</u>. (1981), Fourie, (1965), and Kadim <u>et al</u>. (1989). This conclusion agrees with the present results and with reported negative phenotypic and genetic correlations between backfat thickness and lean cuts percentage, and with positive genetic correlations between backfat thickness and loin and rack cuts percentage (Boylan and Seale, 1965).

### 5.2.5 WEIGHTS, DISTRIBUTION AND DIMENSIONS OF BONES

Genetic or phenotypic correlations between backfat thickness and bone weight, bone percentage or bone length in sheep have been reported to be generally negative, and somewhat higher for length than weight or percentage (Botkin <u>et al.</u>, 1971; Mohamed, 1976; Thorsteinsson and Bjornsson, 1982; Parratt <u>et al.</u>, 1987; Clarke <u>et al.</u>, 1984/1985). Thus selection for reduced backfat thickness would be expected to increase bone weights and particularly lengths as shown here and by Kadim <u>et al.</u> (1989). Similar results have been reported for pig selection lines (Rook <u>et al.</u>, 1987; Wood <u>et al.</u>, 1983b).

In the current study, line effects on bone distribution were significant, but the differences were less marked than those reported by Kadim <u>et al</u>. (1989). They found that the low-backfat line had significantly heavier shoulder bone, humerus bone weight and femur bone weight, but significantly lighter leg and rack bone. The weight of bone in the shoulder cut relative to total bone did not differ between selection lines in the present study, and the difference was in the opposite direction for the total leg bone. It is possible that the differences in bone distribution between the two studies were due to the different stages of growth of the rams.

The heavier bone in the carcasses of the low-backfat line in the shoulder and leg cuts will contribute to those cuts being heavier in the low-backfat line. A similar conclusion could be reached for the high-backfat line for the rack and loin cuts.

#### 5.2.6 FATNESS

## 5.2.6.1 Level of fatness and fat distribution

Fat is the most variable tissue in the body composition of sheep, and numerous estimates show that the tendency to lay down fat in sheep is moderately heritable (Wolf and Smith, 1983). This indicates that carcass composition of sheep can be changed by selection against fatness (Kirton and Morris, 1989), and available evidence indicates that carcass fat content or backfat thickness can be reduced by selection on live measurements (eg. ultrasonically) in the region of the last rib (Fennessy <u>et al.</u>, 1987; Bennett <u>et al.</u>, 1988; Wood <u>et al.</u>, 1983a; McEwan <u>et al.</u>, 1989; Kadim <u>et al.</u>, 1989). The results of the current study provide further evidence that significant progress by selection can be made. The average carcass weight of the rams used in the present study was 47% of that for the rams used by Kadim <u>et al</u>. (1989) so the overall level of fatness was considerably lower. This may explain why the present experiment did not show a significant difference in some of the measured fat depots found to differ between the lines by Kadim <u>et al</u>. (1989). The current experiment did show a significant decrease in all backfat thickness measurements and also in the weight of the kidney fat and in total fat percentage of the chemically analysed soft tissue in the low-backfat line when adjusted to the same carcass weight. However, in spite of the lack of differences in other fat depots, the results lead to conclusions similar to those of Kadim <u>et al</u>. (1988a). This suggests that differences between high and low-backfat lines in many aspects of fatness occur at an early age even though the subsequent gain period involves rapid increases in all measures of fatness. This conclusion is supported by the suggestion made by Wolf and Smith (1983) that breed differences in tissue growth prior to attaining 30% of mature size make an important contribution to tissue weight differences at heavier carcass weights.

Correlated responses in fat and tissue depths at other sites and in the weights of internal fat depots in the present study support the general finding that selection for backfat thickness (based on ultrasonic fat depth measurements at position C on the live animal) will result in significant changes in carcass fat depths at the selection site and also in the internal fat depot weights of sheep (McEwan <u>et al.</u>, 1989; Bennett <u>et al.</u>, 1988) and in pigs (Wood <u>et al.</u>, 1983a; Henderson <u>et al.</u>, 1981). Further support is provided by moderately high genetic correlations which have been reported between backfat thickness and other levels of fatness (Parratt <u>et al.</u>, 1987; Bennett <u>et al.</u>, 1981/1982; Botkin <u>et al.</u>, 1971; Wolf <u>et al.</u>, 1981; Clark <u>et al.</u>, 1984/1985).

The high backfat line had slightly higher (but not significantly higher) intermuscular fat depots (popliteal and prescapular) when adjusted to the same carcass weight. These fat depots were dissected to represent the whole intermuscular fat depot in the body although the level of correlation with total intermuscular fat was not known. No previous studies have used individual intermuscular fat depots. Kadim <u>et al</u>. (1989) found that the high-backfat line had significantly higher dissected intermuscular fat depot weights at the same side weight, which agrees with the high genetic correlations reported by Wolf <u>et al</u>. (1981) between subcutaneous and intermuscular fat depots in sheep. The difference between the present results and that of Kadim <u>et al</u>. (1989) may be because, the lymph nodes were not dissected from the popliteal and prescapular intermuscular fat depots. There is a clear variation in weight of these lymph nodes between animals (Yao, 1986). Alternatively, the difference in results could be due to a poor correlation between the popliteal and prescapular depots and total intermuscular fat, or to the different stages of development for animals in the two trials.

#### 5.2.6.2 The relationship between carcass water and fat

Carcass water percentage was very closely related to carcass fat percentage in the present study (r = -0.95), as it was in the studies of Kirton and Barton (1962), (r = -0.99) and Reid <u>et al.</u> (1968) (r = -0.98) for the concentrations of water and fat in the ingesta-free body.

The results of the current study, although showing non-significant differences between the lines for the water percentage in some carcass and non-carcass body components, indicated that the high-backfat line had lower overall water weight and percentage as a result of higher levels of fatness.

### 5.2.6.3 Adipose cellularity

In previous work with Southdown rams from the same lines, Kadim <u>et al</u>. (1989) showed that the greater weights of four dissectible fat depots (subcutaneous, intermuscular, omental and kidney and pelvic fat depots) in high-backfat rams were mainly the result of larger adipocytes rather than more adipocytes. Line differences in fat cell number were found only in the subcutaneous fat depot. In the present experiment although there were no differences in measures of intermuscular fat levels between the two lines (Table 4.5), the high-backfat line showed significantly larger fat cells in that depot. Line differences in subcutaneous fat cell size were not found, possibly because the subcutaneous fat depot is late developing (Leat, 1976). Also the numbers of fat cells were not measured in the present study. Cullen (1985) showed no line effects for the same two lines of Southdowns at birth either for number or size of adipocytes.

In general, the findings of Cullen (1985), Kadim <u>et al</u>. (1989) and the present study breed indicate that the increase the subcutaneous fat depot in the high-backfat line is due to increased fat cell number as this depot was found to have the same cell size between the two lines until 9 months of age. The increase in level of intermuscular fat appears to be due mainly to increased fat cell size, as this depot had the same cell number but larger fat cell sizes at 9 and 17 months of age. As the sheep used in the current experiment were older than those used by Cullen (1985) and younger than those used by Kadim <u>et al</u>. (1989) from the same lines of the Southdown breed, the subcutaneous and intermuscular fat cell size was intermediate. The subcutaneous fat depot had larger adipocytes than the intermuscular fat depot in all these studies.

## 5.2.7 MUSCLE

## 5.2.7.1 Muscle weight and distribution

In the current study total weights of four muscles from the leg and shoulder cuts were similar in the carcasses of both lines when adjusted to a constant carcass weight. This is contrary to the finding of Kadim <u>et al</u>. (1989) based on complete muscle dissection from one side, and this may be explained in term of differences in stage of growth. Kadim <u>et al</u>. (1989) showed that the low-backfat line had significantly higher total muscle weight in the carcasses, but this differences was very small and represented an increase of 5.7% in the low-backfat line relative to the high-backfat line. Thus, the results of Kadim <u>et al</u>. (1989) and Wood <u>et al</u>. (1983b) in sheep and Tess <u>et al</u>. (1986) in pigs are consistent in showing that the reductions in backfat thickness may have little effect on total muscle weight.

Line effects on muscle distribution in the leg, as determined by individual muscle weights adjusted to a constant fat-free soft tissue weight in the side, were similar to those found by Kadim <u>et al.</u> (1989). An exception was M. semitendinosus which was significantly heavier in the low-backfat line for the younger and lighter rams of this study only. However, this difference was only 5.1%. The covariate, fat-free soft tissue weight in the side, was used in the current study instead of total muscle weight because it should correlate highly with total muscle weight and because total muscle weight was not available. Selection against fatness in this study appears to have produced only small changes in muscle distribution compared with the changes in fatness that have been achieved. The results of Wood <u>et al.</u> (1983b) for two genetic lines of pigs and Butler-Hogg and Whelehan (1987) for two conformation groups of sheep are consistent with those reported here in showing that the distribution of muscle weight does not change to an extent which would have commercial implications.

## 5.2.7.2 Muscle to bone ratio

Muscle to bone ratio in the meat animal carcass is considered to be a characteristic of particular interest. Together with fat percentage, it determines the meat yield at cutting (see section 2.2.2.3) and thus should be considered for selection purposes since it is a moderately well inherited characteristic. The low-backfat line in the present study had a lower muscle to bone ratio which is similar to the result of Kadim <u>et al</u>. 1989. This may be due mainly to higher bone weight resulting from the selection against fatness.

The selection for low-backfat thickness in the Southdown breed has therefore resulted in a clear difference in muscle to bone ratio which agrees with the finding of Kirton <u>et al</u>. (1983) who showed a higher muscle to bone ratio in a fatty group (lamb carcasses selected for good muscling) compared with a leaner group (lamb carcasses selected for poor muscling). A similar result was obtained by Butler-Hogg and Whelehan (1984) when comparing fatter and leaner genotypes of sheep, and Butterfield <u>et al</u>. (1983a) when comparing a small strain of Merino (fatter) with a large strain of Merino (leaner).

## 5.2.7.3 Muscularity

The term muscularity was defined by Kempster <u>et al</u>. (1982a) as the thickness of muscle in relation to skeletal size. Muscularity in terms of muscle weight relative to the lengths of adjacent bones was found to be higher in the high-backfat line when the data was adjusted to a constant total side fat-free soft tissue plus bone weight. This result agrees with the findings of Kadim <u>et al</u>. (1989) and supports previous results with sheep (Kirton <u>et al</u>., 1983; Bass <u>et al</u>., 1984) and pigs (Wood <u>et al</u>., 1983b), in showing positive associations between fatness and muscularity.

The double-log regression equations showing the relationship of muscle weight to bone length (Figure 4.2) demonstrated clearly that the high-backfat line animals had a greater weight of 3 leg muscles at the same femur length.

Total dissectible muscle weight in the carcass was not measured in the current study. The possibility of a higher ratio of total muscle weight to body length as reported by Kadim <u>et al</u>. (1989) could not therefore be tested.

#### 5.2.7.4 Muscle fibre type and size

The higher proportion of  $(\beta R)$  red fibres in M. semitendinosus of the high-backfat line reported by Kadim (1988) was not found in the younger and lighter rams of the present study. This could be due to the different sires involved in the present study, or to differences in age or weight.

Swatland (1984) and Young (1985) reported that postnatal muscle growth appears to be associated largely with an increase in fibre cross-sectional area and length rather than in fibre number. Moreover, some muscle growth can be attributed to the enlargement of all muscle fibres and some to the conversion of small red fibres to large white fibres (Ashmore et al. 1972). An indication of the shift in the relative number or proportion of the different fibre types with increased weight and age can be obtained by comparing the results of this experiment with those reported by Kadim (1988). Relative to the rams of the current study those of Kadim (1988) were about 10 months older, had carcass weights that were about 15 kg heavier, and had proportions of red, intermediate, and white fibres that were 15.4 percentage points lower, 2.9 percentage points higher and 12.5 percentage points higher respectively. Thus, ( $\beta R$ ) red fibres become less numerous, while ( $\alpha R$ ) intermediate and ( $\alpha W$ ) white fibres become more numerous and the reduction in percentage of ( $\beta R$ ) red fibres appears to have been less in the high backfat line. This finding agrees with that of Moody <u>et al</u>. (1980) working with M. longissimus in lambs ranging in weight between 31 to 50 kg.

Overall means for the area of the different fibre types are shown in Table 4.9. In both lines, the  $\beta R$  fibres possessed the smallest size (area), the  $\alpha W$  fibres the largest, while the  $\alpha R$  fibres were intermediate in size. This trend is consistent with previous work in sheep (Kadim, 1988), pigs (Nostvold <u>et al.</u>, 1979) and cattle (Johnston <u>et al.</u>, 1975).

The present results also agree with those of Staun (1963) for pigs and Purchas <u>et al</u>. (1985) for mice, in showing no significant differences in the size of muscle fibres between genetic groups differing in fatness.

Table 4.10 shows clearly that the variation between animals accounted for most of the variation in all muscle fibre characteristics. By measuring muscle fibre characteristics in four separate muscle bundles within M. semitendinosus it was possible to show that between-bundle variation was of little significance relative to between-animal variation. Therefore, sampling from only one bundle is unlikely to lead to large errors when assessing muscle fibre characteristics.

#### 5.3 MEASUREMENTS OF BODY WATER BY DILUTION METHODS

The use of urea space measurements to estimate body composition of live animals is based on the relatively constant relationship between empty body water and other body components in live animals (Shebaite, 1977). It has given satisfactory results when used with cows (Hammond <u>et al.</u>, 1984; Preston and Kock, 1973; Bartle <u>et al.</u>, 1987).

Although there are few studies in sheep using urea space measurements for estimating body composition, Meissner (1976) found that urea space gave a highly satisfactory prediction of body water ( $r^2 = 0.93$ ) in sheep of different breeds ranging in body mass from 13 to 80 kg. He concluded that the urea method was more accurate at higher body fat contents. Bartle <u>et al</u>. (1988) found the urea space to be a moderate predictor of body water ( $r^2 = 0.56$ ) in lambs of different breeds ranging in body weight from 18.2 to 71.5 kg. Jones <u>et</u> <u>al</u>. (1982), however, showed that urea dilution was an unreliable method in lambs ranging in body weight from 32.2 to 51.6 kg and urea space was poorly related to the weight of half carcass lean tissue ( $r^2 = 0.10$ ). Jones <u>et al</u>. (1982) concluded that urea space was not very useful because the animals used were still in a lean body condition. This was considered the principal explanation for the disappointing results found.

The results of the present study, which used relatively light animals (22 to 38 kg body weight) from high and low-backfat lines of Southdowns, have led to conclusions similar to those of Jones <u>et al</u>. (1982). The results have clearly shown urea space to be an unreliable method of predicting empty body water percentage in these lambs. The extrapolation estimates that corresponded most closely with actual empty body water percentages were obtained using a simple exponential model after linear adjustments were made for increasing baseline values (method D2). Linear adjustments for increasing baseline were made because the urea level was still higher than that of the baseline at the end of sampling period (Figure 4.4). This method however, had a very low relationship with actual empty body water percentage ( $r^2 = 0.11$ ; R.S.D = 3.06). The closest relationships with actual empty body water percentage were for empty body water percent estimated from urea concentrations at C45, C30 and Cav respectively (see Table 4.13), but these methods overestimated the actual empty body water percentage.

In general, all methods used herein to estimate empty body water percentage had lower relationships with actual empty body water percentages than the above reported estimates. This may have been due to low variation in body water percentage for these lambs (Coefficient of Variation = 5.5%), which were of one breed and which had a lower body weight range compared with sheep used in the studies of Meissner (1976) and Bartle <u>et al</u>. (1988).

In beef type steers of varying liveweight and degree of fatness, Kock and Preston (1979) showed that urea space was a much poorer predictor of rib fat for lighter weight animals and those in lean body condition.

Some other important factors which could have been partly responsible for the low relationships between predicted and actual empty body water in this study are listed below:

- Urea space measurements for each animal were determined 5 7 days prior to slaughter and there may have been some changes in empty body water percentages over this period. In other studies the period between urea challenge and slaughter has varied from one day (Bartle <u>et al.</u>, 1988) to two days (Jones <u>et al.</u>, 1982).
- 2. The first sampling time after urea injection in the present study was at 15 min which may have been too late to obtain a clear indication of the mixing phase. Kock and Preston (1979) and Bartle et al. (1987) stated that the best sampling time after urea injection was 12 min in cattle and 9 12 min in lambs. Bartle et al. (1988) also showed that a 1 min error in the 12 min sampling time would result in an error of approximately 2.0% in the urea space estimate, but because urea clearance is not linear, they noted that this error will not be consistent over greater timing differences.
- 3. Inaccurate determination of body moisture, liveweight or empty body weight could have contributed in a very small way to the low relationships.
- 4. From the regression equations which related urea space to empty body water percentage in sheep and cattle, Bartle <u>et al</u>. (1988) noted from the intercepts that an equivalent quantity of urea (LW basis) caused a greater plasma urea-N increase in cattle than in lambs. This suggested that more urea (per kg LW) should be infused in lambs. The concentration of the infusion solution, however, should not exceed 20%, because an infusion solution containing greater than 22% urea causes red blood cell hemolysis in vitro (Bartle <u>et al</u>., 1988). A solution containing 10% urea (130 mg urea/kg LW) was used by Bartle <u>et al</u>. (1988). Meissner (1976) found more reliable results when 200 250 mg/kg of urea was administered than when 100 mg/kg was

administered because it raised the blood urea concentration to a great extent, thereby reducing the error between samples and giving a more reliable estimate. The amount administered in the present study was 120 mg urea/kg LW. In addition, all animals were weighed three days before urea injection to determine the actual volume of urea to be infused. This may have resulted in a different volume of urea due to the different liveweight which were calculated to provide 120 mg urea/kg liveweight. Thus, larger amounts of administered urea should be tested in sheep.

5. The gut contents was found in this study to be higher than some previously reported values in lambs (Gharaybeh <u>et al.</u>, 1969), in spite of the fasting period which was applied to the animals. This resulted presumably from the roughage diet which was given to the animals. However, the greater gut contents may not contribute to the error of estimating body composition. Bartle and Preston (1986) examined the fasting period in four heifers and concluded that urea did not diffuse into the reticulo-ruminal water (RRW) in significant amounts in fasted animals. Preston and Kock (1973) considered urea space in the ruminant to be a measure of empty body water rather than total body water. Reticulo-ruminal water therefore influences urea dilution estimation of body composition only as a variable component of liveweight.

As a method of predicting the body composition of sheep at an early age for lean meat production the urea dilution method has proved in this study to be unsatisfactory.

Baseline urea values did not differ significantly between the lines in the present study although the baseline urea concentration was slightly higher and urea clearance was slightly lower in the high-backfat line (Table 4.14). In 7-month-old ram lambs of the same lines used in this study, Bremmers <u>et al</u>. (1988) showed that rams fed 1.2 maintenance exhibited significantly greater urea concentrations than those fed 0.7 maintenance. The rams fed 1.2 maintenance had greater plasma urea in the high-backfat line, but line effects were not significant. Carter <u>et al</u>. (1989) showed higher values in the high-backfat line for baseline concentrations of plasma urea during both the fasting and refeeding periods. Similarly, Van Maanen <u>et al</u>. (1989) showed that high-backfat rams maintained significantly higher concentrations of urea in plasma than the low-backfat line throughout the 24 hours sampling, and refeeding.

Line effects on the responses to a urea challenge were minimal with no effects on clearance rates and a significantly higher plasma urea level at 15 minutes post challenge only

for the high-backfat line. Despite this, and despite the poor relationships between actual and predicted body water percentage, the empty body water percentage values predicted from several estimates of urea space were significantly higher for the low-backfat line. It is possible that the apparent differences between the lines in the way in which urea is metabolized and excreted (Van Maanen et al. 1989) together with the lower fat levels of the low line animals combined in such a way that the predicted line differences were an exaggeration of the actual differences in empty body water percentage.

## CHAPTER SIX

## CONCLUSIONS

Overall, the results of this study indicate that selection to alter backfat thickness in Southdown rams of the Massey University backfat selection lines has been successful. This supports previous work on older rams of the same lines and provides evidence that the ultrasonic method can be used in breeding programmes to select genetically lean sires.

The following conclusions may be drawn with regard to differences between the high and low-backfat lines in this study, and with regard to the differences between the results of this study and those of a previous study involving older and heavier rams.

- No clear line differences in dressing-out percentage were shown in the current results. In the older and heavier rams the higher dressing-out percentage for the high-backfat line was probably because the line differences in fatness were larger.
- 2. Differences in non-carcass components were less clear than in the previous trial although heavier heart and liver weights were found in the low-backfat line.
- 3. The low-backfat line carcasses had larger frames in this and the previous trial, with longer bodies (LB) and legs (T) and greater lengths of certain bones.
- 4. No line differences were found in carcass width measurements, whereas in older rams the low-backfat line had narrower carcass widths.
- 5. The low-backfat line had lower fat depth measurements at C, J, GR, S2 and L3 as was found in the older rams. The largest differences between lines in both studies was found for fat depths C and L3.
- 6. The low-backfat line had a higher proportion of the shoulder cut and a lower proportion of the rack cut, in the same way as the previous study.

- 7. The low-backfat line had lower weights of bone in the rack cut, higher weights of bone in the leg cut and heavier humerus and femur bone weight at the same total side bone weight. Thus, the line effect on bone distribution were less marked in the present work than the previous study and the difference in total leg bone weight was in the opposite direction.
- 8. The kidney fat depot was the only non carcass depot shown to be lower in the lowbackfat line in this study. In previous study several non carcass fat depots were lighter in the low-backfat line.
- 9. Smaller adipocytes were found only in the intermuscular fat depot of the low-backfat, while for the previous work lower adipocyte size was shown for all measured fat depots.
- 10. At the same carcass weight (14 kg) the low-backfat line rams had less fat, more bone and a similar weight of muscle, whereas the previous study found less fat and more muscle as well as bone in the low-backfat line.
- 11. The low-backfat line had lower muscularity scores in terms of muscle weights relative to skeletal size and in terms of shallower, but wider cross-sections of M. longissimus at the 12th rib. The differences between the two lines were similar and in the same direction for the heavier animals of the previous trial.
- 12. Small line effects on muscle distribution, as determined by individual muscle weights adjusted to a constant fat-free soft tissue weight in the side, were found which were similar to those found for older rams.
- 13. The muscle to bone ratio was lower in the low-backfat line carcasses for both studies.
- No line difference was shown in the proportion or area of muscle-fibre types in the M. semitendinosus, whereas the previous work found a decrease in the proportion of red (βR) muscle fibres.
- 15. The current study also showed, in general, that percentage moisture was slightly higher in the low-backfat line for carcass and non-carcass components.

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Prediction of carcass composition by using the urea dilution method as described in the current study with young rams was found to be unsatisfactory. These results are generally consistent with similar work reported for young and light sheep but not with older and heavier animals. There is a need for more fundamental work to evaluate the physiological implications of this method, as it may become a useful tool in breeding programmes if it could accurately predict carcass composition at an early age.

The need to reduce the fat content of New Zealand lamb carcasses has resulted in the recent importation of new breeds, such as the Texel, to New Zealand. However the present study, together with the previous study on the same selection lines has suggested that selection programmes which incorporate ultrasonic backfat measurements may provide a suitable sires to compete with the imported breeds. There is a need for a study to evaluate more closely the carcass composition of animals from these lines at various stages of their growth so that some of the differences between this and the previous study can be clarified and so that these animals can be compared more effectively with those of other breeds and lines.

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