# AN INVESTIGATION INTO GROWTH IN JERSEY, HOLSTEIN AND HEREFORD HEIFERS ON KIKUYU PASTURE*(Pennisetum clandestinum)* USING N-ALKANES TO ESTIMATE INTAKE AND RUMINAL OUTFLOW RATE.

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## ABSTRACT

The experiment was designed to investigate why dairy heifers perform poorly on kikuyu pasture compared to a beef breed like the Hereford. The main assumption was that the lower growth rate in dairy breeds was due to low intake as a result of slower passage of digesta out of the reticulorumen. Eight animals in each breed (Jersey, Hereford and Holstein) were grazed continuously on 6.25 hectares of kikuyu. The Jerseys had to be dropped from the second part of the experiment due to health problems. The experiment was conducted from December to April and was split into two trials with half the animals in each breed on a maize supplement during the last eight weeks of the experiment. During the first trial (T1), average daily gain was 1.18, 0.54 and 0.2kg per daily in Herefords, Holsteins and Jerseys respectively. Herefords grew significantly faster than both the Holsteins and the Jerseys ($p<0.01$). The Holsteins also grew significantly faster than the Jerseys ($p<0.05$). In trial 2 (T2), average daily gains were 0.732 and 0.561 in Herefords and Holsteins respectively. Supplemented Herefords had and average daily gain of 0.797 compared to 0.668kg in non-supplemented animals. Supplemented Holsteins had an average daily gain of 0.497 compared to the 0.624 in non-supplemented animals. There were no differences in growth in T2 ($p>0.05$). N-alkane estimated intake in T1 using the C32/C33 alkane pair was 117.7, 92.7 and 97.6g/kg$LW^{0.75}$ in Herefords, Holsteins and Jerseys respectively. The Herefords had significantly higher intake compared to both the Holsteins and the Jerseys ($p<0.05$). In T2, the Herefords had a significantly higher intake ($p<0.05$) of 104.4 compared to 98.0g/kg $LW^{0.75}$ in the Holsteins. In T1, n-alkane estimated digestibility was 59.8, 56.8 and 51.4% in Herefords, Holsteins and Jerseys respectively. The Herefords had significantly higher apparent digestibility compared to the Jerseys ($p<0.05$). In T2, the Herefords had a digestibility of 61.6 compared to the Holsteins at 60.5%. The difference was not significant ($p>0.05$). Estimated outflow rate (k1) using $Cr_2O_3$ was 0.056, 0.062, 0.061 and 0.056/h in supplemented Herefords, non-supplemented Herefords, supplemented Holsteins and non-supplemented Holsteins respectively when data was fitted into a multi-compartment model. When the data was fitted into a two-compartment model, estimated k1 values were 0.069, 0.063, 0.090, and 0.061/h also respectively. When data obtained from using alkane coated hay was used, k1 values obtained by a graphical procedure were 0.035, 0.042, 0.038 and 0.042/h also respectively. Neither breed nor supplement had a significant effect on outflow rate ($p>0.05$).

## TABLE OF ABBREVIATIONS.

| | |
|---|---|
| **ADF** | Acid detergent fibre |
| **ADG** | Average daily gain |
| **CF** | Crude fibre |
| **CP** | Crude protein |
| **CPS** | Critcal particle size |
| **CV** | Coefficient of variation. |
| **DCP** | Digestible crude protein |
| **DM** | Dry matter |
| **DMI** | Dry matter intake |
| **DOM** | Digestible organic matter |
| **GC** | Gas chromatography |
| **GIT** | Gastro-intestinal tract |
| **LW** | Live weight |
| **ME** | Metabolisable energy |
| **MFW** | Mobile feed wagon |
| **MRT** | Mean retention time |
| **NDF** | Neutral detergent fibre |
| **NFE** | Nitrogen free extracts |
| **ROO** | Reticulo-omasal orifice |
| **RR** | Reticulorumen |
| **SE** | Standard error |
| **UDP** | Undegradable dietary protein |

# CHAPTER 1
# INTRODUCTION

## 1.1. Introduction.

Exotic dairy breeds are dominant in commercial milk production in most tropical regions. Restrictions on their productivity include such diverse factors as climate, disease and inadequate nutrition and management, with nutrition probably the most limiting factor.

Nutritionists generally recommend that the solution to the nutritional limitations in milk production in developing countries lies in the production of adequate and high quality forage crops. Tropical pastures however tend to be of low nutritive value compared to temperate species due to partly genetic and partly environmental effects (Minson, 1990). As a result, animal performance is generally lower on tropical relative to temperate grasses.

Evidence of relatively poor utilisation of kikuyu pasture in Holstein heifers has been apparent in recent trials by Allwood (1994) and Horne (1996). Performance seems to be only enhanced at the added cost of supplements, while in the other breeds, the same supplementary regimes result in less dramatic changes in growth (Horne, 1996). Given the need to meet high growth standards for dairy heifers to facilitate a younger age at first calving at a minimum of feed costs, it is necessary to investigate possible factors responsible for the poor performance. These factors include intake, or with respect to efficiency of growth, either differences in protein accretion or in maintenance requirements (Rohr and Daniecke, 1978). Garrett (1971) observed significantly higher intake in Holstein steers compared to Herefords. In one trial, the Herefords also had higher energy retention than Holsteins, indicating a higher efficiency of gain. The Herefords tended to have lower maintenance requirements and higher protein retention. When the Holstein was compared to other beef breeds, there was a similar relationship with the Charolais and the Limousin (Beranger, 1978) and the Simmental (Daniecke and Rohr, 1978; Rohr and Daniecke, 1978). The latter however did not find significant differences in maintenance requirements. Langholz (1978) concluded that breed differences in body weight gain were mostly a result of differences in the capacity for protein retention. In comparison with an earlier maturing breed like the Hereford, Holsteins would not be expected to have lower intake. It must be noted also that

observations in most of the literature cited above are based on high planes of nutrition where differences in the composition of gain are more distinct. On pasture alone, where there is much lower fat accretion, differences in gain are thus likely to be restricted mainly to factors affecting either intake and or digestion in the gastro-intestinal-tract (GIT).

It is unlikely however that differences in the rumen environment with regards to physiological factors affecting digestive capacity can result in marked differences in performance. Such breed differences have been found to be small and usually insignificant (Moran and Vercoe, 1972; Frisch and Vercoe, 1977). It is likely however that variation in intake will largely explain the observed differences in comparison with the Hereford. This was confirmed by Horne (1996) who found significantly lower intake in Holsteins.

On a forage diet, where intake is likely to be limited mainly physically through gut capacity, differences in digesta movement within the reticulorumen (RR) may significantly affect nutrient flow due to a direct effect on dry matter intake (DMI). In comparisons between Brahman crosses and purebred Hereford steers, Kennedy (1982) found more extensive digestion in the rumens of the crossbreeds resulting from longer retention time. Since this was a restricted diet, differences in weight gain could thus have derived from either extensive digestion in the rumen or from differences in the efficiency of synthesis of body proteins. Lower rate of passage in this case could be a natural adaptation to a diet both poor in quality and limited in supply. On an *ad lib* diet however, differences in retention time suggested above, could result in significantly less total dry matter intake and thus lower growth rates. Smuts *et al.* (1995) observed differences in retention time in the rumen in sheep which were highly correlated with both intake and wool production. The lower intake apparent in Holsteins could therefore be due to restricted flow of digesta through the rumen. Holsteins have been selected for high milk production on high quality diets. It may be that there has been an inadvertent selection against roughage intake.

This study seeks to verify differences in performance between the breeds which are apparently largely a result of differences in intake and investigates ruminal outflow rate as a possible major limitation to intake in the Holsteins.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1. Some aspects of growth in dairy heifers.

Growth can be defined as an increase in size and change in body composition (Pomeroy, 1978). In growing dairy heifers, relatively rapid growth is desirable to achieve conception at an early age. Swanson (1967) described optimal growth as that regime which will develop their full milking potential at the desired age with the minimum of expense.

Changes in body composition associated with rate of gain may however have important implications in the milk production ability during lactation due to variations in the physiological development associated with the plane of nutrition. Underfeeding and early conception resulted in low milk yield and calving problems as a result of physiological and anatomical underdevelopment (Little and Kay, 1979). Research also shows that prepubertal heifers fed high levels of energetically dense diets have reduced subsequent milk yield compared to heifers grown at no more than 700g per day (Swanson and Spann, 1954; Little and Kay, 1979; Sorensen, 1989). It appears that the rapid growth results in relatively higher fat deposition in the udder tissues at the expense of milk secreting tissue (Swanson, 1960; Stelwagen and Grieve, 1990). Mammary glands of heifers grown at less than 0.74kg per day had 39% more weight and 68% more milk secreting tissue than those of heifers grown at 1kg per day at 11 months (Stelwagen and Grieve, 1990). Amir and Kali (1974) cited by Little and Kay (1979) suggested an optimum feeding regime to produce 450 to 700g/day gain in small and large breeds respectively.

### 2.1.1. Maturation and changes in body composition.

While the composition of the fat free body is relatively constant (Wright and Russel, 1984), fat content is dependent partly on the maturity status, and, to a large extent, on the nutritional plane of the animal (Butterfield, 1988). Also, the tendency for fat tissue to mature relatively late (Butterfield, 1988) means that as the animal grows older, the proportion of fat in body weight gain increases. Research also shows that genetic mature size has a marked influence on the rate of maturation of the animal (Beranger, 1978; Taylor, 1985; Butterfield, 1988). A large mature

body weight imposes a late maturing pattern resulting in low body fat content when compared on an age or weight constant basis with smaller breeds (Beranger, 1978). Thus, although growth in the whole body, growth of individual tissues and organs, and change in energy requirements and growth rate tend to follow a similar sigmoid shape in different animals, parameters of the curve are largely determined by the mature body weight of the animal.

### 2.1.2. Estimating body composition.

Body composition is an important quality attribute in meat science and in nutritional research, since energy utilisation is also affected by the nature of tissue deposition (ARC, 1980). A wide range of indirect *in vivo* methods of estimating body composition have shown relatively high levels of accuracy compared to conventional methods although they are restricted to specialised research due to the high costs under normal production or experimental situations (Lister, 1984). Wright and Russel (1984), working on mature cows, used body condition score, body weight, skeletal size, ultrasonic fat measurements and total water space as predictors of body composition. Their results showed that body fat and protein had high correlations with body weight, condition score and total water space. Multiple regression using combinations of these indices showed even better accuracy of prediction.

In dairy farming, condition scoring is widely used to alter feeding regimes both during growth and during lactation. In research situations where precise data on body composition may not be necessary, condition scoring is the most practical and often used indicator of body condition because of the ease of obtaining data. Body condition is defined in this case as the ratio of the amount of fat to the amount of nonfatty matter in the body of the animal (Wright and Russel, 1983). Given that correlation between different operators can be as high as 0.7, and between repeat scores on the same animal as high as 0.8 (Wright and Russel, 1984), condition scoring is a good method of assessing fatness in animals.

In comparative growth studies however, comparison among breeds which may differ in the distribution of fat reserves does not allow for direct comparison on the same scoring standard (Callow, 1961). Milk breeds for example tend to deposit more fat in internal depots while beef breeds tend to deposit more subcutaneous fat (Williams, 1978). Williams (1978) observed more

subcutaneous and less channel fat in Herefords compared to Holsteins. Variation in the distribution of subcutaneous fat in different anatomical regions may also arise as animals fatten (Williams, 1978). The subjective nature of condition scoring and the fact that predictive equations are population specific also means that where precise data on tissue composition is required, condition scoring is not ideal.

### 2.1.3. Factors affecting rate and efficiency of gain.

An extensive review on growth aspects was done by Horne (1996) in which it was evident that differences in rates of maturation between animals of different breeds and between individual body tissues resulted in relative changes in tissue composition as the animal grows which indirectly affect both rate and efficiency of gain.

Energy requirements can be partitioned into requirements for maintenance and production (ARC, 1980). The energy available for growth thus depends on the difference between intake and maintenance requirements. Also, since efficiency of utilisation for maintenance and growth are different (ARC, 1980), level of intake influences both growth rate and efficiency of gain. The degree of maturity has a marked influence on the energy demand and utilisation of energy within an animal since changes in growth, intake of metabolisable energy (ME) and heat production occur in an animal as it grows to maturity. As the animal matures, the difference in ME intake and heat production falls as the energy demand for growth falls (Webster, 1989). This induces a decline in efficiency of utilisation of metabolisable energy due to the resultant relatively higher maintenance costs.

Differences between individual animals in the composition of gain also have an influence on the efficiency of utilisation of energy for growth (Van Es, 1978). Fat deposition, unlike protein, is not accompanied by water deposition (Van Es, 1978). The energetic efficiency of protein deposition is however lower than that of fat deposition (Beranger, 1978) such that while efficiency in terms of live weight gain increases with increase in proportion of protein in achieved gains, in terms of energy, animals with high protein growth potential are less efficient than in animals that fatten early. An increase in the energy cost of live weight gain thus occurs as the animal matures and the proportion of fat increases. The combined effect of relatively higher maintenance costs

and a higher proportion of body fat results in an overall decline in the efficiency of utilisation of metabolisable energy, per unit of weight gain.

When comparisons between animals of different breed types are standardised to correct for the differences in rates of maturation and body size to give the same 'metabolic age' (Taylor, 1965), the curve for these relationships can be superimposed. If standardisation in this manner is assumed to be close to the ideal, any observed differences can be confidently described as genetic. Such correction however only serves to reduce and not eliminate the influence of either maturity or metabolic size (Beranger, 1978). It means however that at a given metabolic age, the ratio of intake to fasting metabolism is near constant among breeds (Frisch and Vercoe, 1977), and any differential depression of intake among different breeds will affect their relative growth rates.

## 2.2. Nutritive value of kikuyu.

### 2.2.1. Definition and prediction of nutritive value.

Nutritive value in forages is a rather complex concept to describe quantitatively. It can perhaps only be adequately discussed with respect to the likely impact of nutritive attributes of the available herbage on both intake and the extraction of nutrients. Variation in intake, arising from either plant or animal factors, accounts for most of the variation in performance of grazing animals (Crampton *et al.* 1960). Dry matter intake accounted for up to 70% of the variation in performance, the remainder mainly accruing from differences in forage digestibility. Based on this observation, Crampton *et al.* (1960) suggested a nutritive value index which combines both intake and digestibility.

Ulyatt (1970) also described the value of a forage as a function of both intake and "nutritive value". Nutritive value was in this case defined as the net energy per unit of intake, which could be affected by factors including the chemical and physical composition, digestibility, rate of digestion and site of digestion. He pointed out however that many of these factors are also affected by intake such that intake and nutritive value cannot be independent entities. The inclusion of the net energy concept and of factors providing a more dynamic description of nutritive value was an improvement on the definition by Crampton *et al.* (1960) given the more precise nature of the metabolisable energy (ARC, 1980) and net energy systems for animal nutrition (NRC, 1987).

An essential requirement in applied nutrition, and often not readily apparent due to the wide application of predictive methods of forage assessment is a quick but accurate estimate of the 'quality' and potential intake of the diet. Most intake prediction equations are centred on the relationship between intake and the digestibility of the feed (Poppi, 1996). Prediction of digestibility of forages has in turn been based on their relationship with nutritional entities such as crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin content or on *in vitro* estimates of digestibility (Moore and Mott, 1973).

Given that the relationship between intake and digestibility does not necessarily apply across a

range of diets and various plant-animal interactive factors such as level of intake and associative effects of feed mixtures(Minson, 1990), and that in tropical forages, the relationship between the nutritional entities described above and digestibility *in vivo* seems to be more variable (Duble *et al.* 1971), the use of predictive equations is limited. On kikuyu, this was demonstrated by Dugmore and du Toit (1988), who observed a poor relationship between chemical fractions and digestible organic matter(DOM). Such observations reflect the limitations of empirical methods for predicting quality in tropical forages since they are population specific (Pienaar *et al.* 1993). Dugmore and du Toit (1988) went on to suggest that predicting DOM of kikuyu using commonly used laboratory analyses is not possible and could have unexpected results.

Structural differences in terms of the cell wall constituents among species are the likely cause of a departure from expected relationships (Moore and Mott, 1973). Lignification, for example tends to have varying effects depending on plant species (Van Soest, 1994). Often, forage antiquality components such as alkaloids, fungal endophytes or tannins may be much more important than the conventional indicators of quality (Hart and Hoveland, 1988).

More dynamic models for the prediction of forage quality need to be developed. These are models that include the dynamic nature of rate constants for fermentation and passage out of the rumen, rumen composition and rumen fill (Pienaar, 1994).

### 2.2.2. Yield in kikuyu pastures.

Yield on kikuyu pastures in KwaZulu Natal varies widely with yields of 5-8 tonnes per hectare under low fertilisation (60-150kgN/HA) up to 16 tonnes under heavy fertilisation rates (275-375kgN/HA) (Dugmore, 1995a). Heard and Wiseman (1973) cited production levels of up to 18t/Ha.

### 2.2.3. Nutrient composition.

For effective fermentation in the rumen, microbes require at least 1.5% nitrogen and 65-70% digestible energy (Kennedy *et al.* 1986). Efficiency of microbial protein synthesis in the rumen also depends on nutrients such as phosphorus, sulphur, branched chain fatty acids and possibly

vitamins (Owens and Goetsh, 1986).

Van Ryssen *et al.* (1976) cited digestible organic matter values in kikuyu from the literature ranging from 47 to 63.2%. In the same review, they also cited crude protein values ranging from 14 to 30% in kikuyu. Van Ryssen *et al.* (1976) also obtained ME values ranging from 9.5MJ/kg in December to 9.8MJ/kg in March. The large variation in the nutrient composition is a reflection of the effects of factors such as season and stage at which the grass is cut.

**Table 2.1.** Nutrient concentrations (g/kgDM) of well managed kikuyu and perennial ryegrass pluck samples.

| Nutrient | Kikuyu | Ryegrass |
|---|---|---|
| Crude protein (N*6.25) | 207.5[a] | 252.3[b] |
| Total amino acid (g/kg CP) | 713.0 | 918.0 |
| NPN (g/kg CP) | 221.0 | 245.0 |
| Nitrate | 0.26 | 0.46 |
| DOM | 733.7[a] | 842.1[b] |
| ADF | 230.9[a] | 177.4[b] |
| NDF | 602.5[a] | 395.0[b] |
| Ca | 3.05[a] | 5.92[b] |
| P | 3.08[a] | 3.33[b] |
| K | 30.72 | 34.37 |
| Na | 0.15[a] | 3.67[b] |
| Mg | 2.24 | 2.38 |
| Total oxalic acid | 6.80[a] | 1.20[b] |
| Soluble oxalic acid | 1.10 | <1.00 |
| Water soluble carbohydrate | 19.3[a] | 91.0[b] |
| Starch | 34.4[a] | 66.0[b] |

Source (Reeves *et al.* 1996).

[ab]Means within rows followed by different letters are significantly different (p=0.05)

More recently, Reeves *et al.* (1996) compared the nutrient content of kikuyu with that of rye grass (Table 2.1). Crude protein, non-protein nitrogen, water soluble carbohydrate, starch, Ca and Na were significantly higher in ryegrass than in kikuyu. Acid detergent fibre, neutral detergent fibre and total oxalates were higher in kikuyu. The soluble carbohydrate to protein ratio was also higher in ryegrass. Ryegrass had 92% of nitrogen as amino acids compared to 71% in kikuyu.

The nutrient composition of kikuyu from a widely cited manual of nutritive value of South African feeds by Bredon *et al.* (1987) is shown in Table 2.2.

**TABLE 2.2** Chemical composition, digestible protein and energy and TDN of kikuyu pasture (Bredon *et al.* 1987).

| SEASON | CP (%) | CF (%) | NFE (%) | DCP (%) | DOM (%) | TDN (%) | ME MJ/kg | Ca g/kg | P g/kg |
|---|---|---|---|---|---|---|---|---|---|
| Kikuyu | | | | | | | | | |
| Summer | 18.0 | 29.0 | 37.5 | 12.2 | 58.7 | 61 | 9.15 | 3.2 | 3.5 |
| Autumn | 15.0 | 30.0 | 40.4 | 9.7 | 55.9 | 58 | 8.70 | 2.2 | 3.5 |
| Spring | 11.5 | 32.0 | 42.1 | 6.8 | 54.9 | 57 | 8.55 | 1.8 | 3.0 |
| *Rye grass | 17.8 | 27.1 | 44.3 | 13 | 59.3 | 61 | 9.15 | 3.5 | 2.2 |

*Average well fertilised perrenial rye grass.

### 2.2.4. Animal performance on kikuyu.

Yearling dairy heifers can achieve growth rates of 0.5-0.55kg per day in the KwaZulu-Natal mistbelt of South Africa (Dugmore, 1995b). Allwood (1994) also observed growth rate of 0.52kg/day in Holstein heifers with significant response to maize meal supplement above 0.4kg/day of supplement.

In terms of milk production, a 550 kg Holstein cow on kikuyu summer pasture can produce from 12l on low quality kikuyu to 14-16l of milk in well managed pastures, with yields dropping to 6-8l

without supplementation in Autumn (Dugmore, 1995b). Dugmore and du Toit (1988) however observed that despite nutrient levels in kikuyu being equivalent to the chemical composition of rye grass, animal performance on kikuyu was low. Milk production at Cedara was considerably less than expected. Much research has focused on the supply and utilisation of the major nutrients, protein and energy.

### 2.2.5. Possible factors affecting intake on kikuyu.

#### 2.2.5.1. Protein supply from kikuyu.

Loosli *et al.* (1949) first reported that microbes in the rumen were able to synthesise essential amino acids. Subsequent research later focused on the nitrogen supply in the rumen (Webb and Bergman, 1991). It became increasingly evident that at higher levels of production, the quality of amino acids reaching the duodenum was important (Charmers *et al.* 1954; Egan, 1965; Little and Mitchell, 1967). More recently, research efforts have focused on protein escape from the rumen and ways of manipulating protein metabolism in the rumen (Van Soest, 1994).

Reeves *et al.* (1996) argued that although the crude protein content of well fertilised kikuyu pastures may exceed recommended levels, the high non-protein nitrogen levels suggests crude protein values (N*6.25) may overestimate the value of the protein to the animal. Unlike cows in lactation, growing animals on good quality forage are however able to satisfy their protein requirements from microbial protein alone (Van Soest, 1994). This has been confirmed on kikuyu pasture by Allwood (1994) who found no significant effects when undegradable dietary protein (UDP) was supplemented in yearling dairy heifers. Protein supply to the rumen is therefore not likely to be limiting.

#### 2.2.5.2. Protein and energy balance.

The imbalance in nitrogen and energy has often been cited as the main reason for poor utilisation of nitrogen and the resultant lower than expected performance (Dugmore and du Toit, 1988; Pienaar, 1994). Tainton *et al.* (1982) demonstrated that increasing levels of nitrogen fertiliser reduced the growth of steers. In high protein forages, energy deficiency may result in escape of

ammonia from the rumen and subsequent excretion as urea (Van Soest, 1994). Demand for energy is high in the rumen since all nitrogen pools pass through a breakdown and resynthesis process through the ammonia molecule (Walker, 1970). When forage crude protein exceeds 15% DM, a loss of feed nitrogen can be expected (Walker, 1970). Egan and Ulyatt, (1980) estimated up to 69% of dietary urea was excreted in the urine of sheep on rye grass. This implies the value of the forage based on apparent digestibility is overestimated, and any anticipated productivity based on the commonly used proximate analysis will not be obtained. Minimising the loss of such high proportions of dietary nitrogen is necessary given the high cost of nitrogen.

Heavily fertilised pastures contain high proportions of non-protein nitrogen (Van Soest, 1994). This soluble nitrogen is rapidly degraded to ammonia in the rumen. Such readily available nitrogen constituted up to 30.8% of nitrogen in leaf material from kikuyu (Pienaar, 1994). Reeves *et al.* (1996) observed values of 22.1% and 24.5% as a proportion of total CP as NPN in pluck samples of kikuyu and rye grass respectively. In *in vitro* studies, Satter and Slyter (1974) observed optimal microbial protein synthesis at 50mg $NH_3$-N per litre of rumen fluid. Above this level, loss of nitrogen as urea occurs. Although this report is widely quoted in literature, the variation in other such estimates points to the interaction with other dietary factors. Pienaar (1994) suggested that ruminal ammonia concentration alone could not be directly used to determine ammonia flux to the liver since the effect of production, and uptake of ammonia could not be separated.

Evidence of a negative relationship between either intake (rumen fill) or growth and the level of nitrogen in kikuyu leads to the conclusion that high nitrogen levels may restrict intake on kikuyu pasture (Pienaar, 1994). Voluntary intake is affected when dietary urea exceeds 1.5% (Wilson *et al.* 1975). A calculated urea equivalence of soluble nitrogen in kikuyu at one site of 1.64%, and apparent high rumen pH levels, led Pienaar *et al.* (1993b) to suggest that the urea load was responsible for the lower than expected intake. Such effects may, however, be moderated by the observation that grazing steers select herbage containing around an optimum of 14%CP (Dugmore and du Toit, 1988; Dugmore, *et al.* 1991). Fistulated animals also apparently selected a diet higher in crude fibre than cut samples, a result of selection for more mature herbage. They suggested lower levels of fertilisation although that would require investigating the optimum between nitrogen content and inevitable loss of yield. Alternatively, they suggested grazing

pastures at a later stage of maturity than currently recommended so as to maintain both yield and an optimum crude protein level.

Energy supplements can also be used to balance the energy deficit. Although primary growth in the more nutritious temperate species may contain 150-300gCP/kgDM with apparent digestibility as high as 77-86% (McRae and Ulyatt, 1974) such species also contain readily fermentable carbohydrates (Egan and Ulyatt, 1980; Beever *et al.* 1986). Readily fermentable carbohydrate is available in small quantities in tropical forage (Reeves *et al.* 1996) and hence energy easily becomes limiting since the rapidly fermentable carbohydrates constitute the only form of energy which can be available at rates necessary for the high demand for energy in ruminal protein synthesis (Van Soest, 1994). Reeves *et al.* (1996) observed that starch and water soluble carbohydrate constituted 3.44% and 1.93% of dry matter in kikuyu (Table 2.1). Ryegrass had 6.6% and 9.1% of starch and water soluble carbohydrate respectively. In kikuyu, Reeves *et al.* (1996) observed that the water soluble carbohydrates tended to rise during the day as photosynthesis proceeds . They suggested that utilisation of kikuyu pasture may be improved by grazing animals during specific periods of the day.

Supplementary sugar or starch in animals on high nitrogen forage reduced the ammonia level in the rumen, indicating more rapid assimilation of ammonia nitrogen into microbial protein (Van Soest, 1994). This confirms that a shortage of ATP for microbial protein synthesis is the main limiting factor on a fresh forage diet. Van Ryssen *et al.* (1976) supplemented lambs on kikuyu pasture with molasses. Although they could not attribute the response in gains entirely to the supplement due to possible confounding with stocking rate effects, there was a positive response.

Other energy supplements, such as cereal grain, may not show dramatic changes in rumen ammonia level, possibly due to their own contribution to the rumen ammonia pool (Van Soest, 1994). Similarly, the effect of protein supplement was similar to that of maize meal, probably because the high protein supplement was high in energy and hence what was realised was essentially an effect of energy rather than protein (Allwood, 1994).

### 2.2.5.3. Other plant attributes.

Although C4 biochemistry allows for more efficient photosynthesis in tropical species such that they tend to accumulate a large herbage mass compared to temperate species, the quality of the forage is much less compared to temperate species due to structural and to biochemical adaptations associated with the C4 biochemistry (Van Soest, 1994).

C4 plants have a lower ratio of mesophyll to vascular tissue (Akin, 1986). Mesophyll in C3 plants is thin walled, and unlignified with large intercellular spaces allowing for access of microbes and hence better digestibility (Hanna *et al.* 1973). The parenchyma bundle sheath of C4 plants is more rigid, thick walled and lignified and hence slowly and partially degraded in the rumen (Hanna *et al.* 1973; Akin, 1982; Akin, 1986). Tropical pasture thus tends to have higher fibre than temperate species. The chloroplasts in C4 plants tend to concentrate within the bundle sheaths rather than in the mesophyll when compared to temperate grasses (Akin and Burdock, 1977). The otherwise readily available carbohydrate is thus effectively protected from microbial action.

Pienaar *et al.* (1993a) also observed that oxalates and nitrates were higher in young plants. Although oxalate levels exceeded those considered toxic to the animal, rapid metabolism in the rumen and the fact that only insoluble oxalate was observed could have minimised toxic effects. Selection by animals for low oxalate was also apparent. Nitrate changes suggested that a period of about two weeks of active growth following nitrogen application was necessary in order to minimise the risk of nitrate poisoning. Reeves *et al.* (1996) observed that only 3.71% (Table 2.1.) of NPN in kikuyu consisted of nitrate, which is below the level considered harmful to cattle. At levels above 23% CP however, the nitrate levels rose sharply, which could affect the rate of digestion in the rumen.

## 2.3. The control of intake and passage of digesta through the GIT of ruminants

### 2.3.1. Metabolic control of intake.

Evidence of sensitivity to chemical properties of digesta in the gut and to concentrations of products of digestion in the liver point to a metabolic control of intake (Forbes, 1995). Monogastric animals, which generally consume diets of high nutrient density, tend to eat to a set energy status (Forbes, 1995). Conrad *et al.* (1964) suggested that in ruminants, metabolic control of intake takes place in forages above 67% digestible energy.

The homeostatic mechanisms for intake are thought to be regulated through mechanisms related to energy metabolism and linked to the central nervous system, consisting of an intake facilitatory (hunger) and inhibitory (satiety) centre (Bines *et al.* 1969). In non-ruminants, the main dietary product involved in the chemostatic control is glucose, which effects short term regulation like size and frequency of a meal (Forbes, 1995). In ruminants, the active substances are mainly volatile fatty acids absorbed after fermentation in the rumen (Forbes, 1995). In the long term, control of intake appears to be linked to the release of fatty acids from the main energy depots (Forbes, 1995).

These mechanisms do not operate independently, and are also linked to other homeostatic centres. The interaction between temperature and intake(Forbes, 1995) suggests that the control of intake is linked to temperature regulation centres. The regulatory thresholds will also depend on the physiological state of the animal, since nutrient demand also depends on physiological states such as pregnancy, lactation and realimentation (Forbes, 1995).

### 2.3.2. Physical regulation of intake.

The ruminant animal possesses a 'static capacity' through controlled outflow of digesta in the RR, compared to hindgut fermenters which rely on a faster rate of throughput. This provides for the slow microbial fermentation processes by increasing residence time in the RR, but at the same time restricting feed intake due to physical limitations of gut capacity (Waldo, 1986). The RR is

the site of most of the digestive processes in ruminants on a forage diet. When digestibility falls to about 66% the rate of flow of digesta out of the rumen is restricted (Conrad *et al.* 1964). Important factors in the emptying of the RR are the digestible energy concentration of the feed (Blaxter *et al.* 1961), fermentation and flow kinetics (Crampton, 1960). The positive relationship between these parameters and voluntary intake is evidence of the physical limitation to intake (Forbes, 1995). Intake is physically regulated through mechano-receptors localised mainly in the epithelia of the RR (Leek, 1986), which are mostly responsible in regulating size and frequency of meals (Forbes, 1995)

The possible roles of rumen fill, digestion kinetics, and microbial protein synthesis in the control of intake on kikuyu pasture were investigated by Pienaar *et al.* (1993b). The rates of passage and fermentation did not show any significant relationship with intake. Efficiency of microbial protein synthesis was also high at 43.2 gN/kgOM.

#### 2.3.2.1. Parameters of rumen clearance.

Digesta clearance from the RR occurs through digestion , absorption of digestion products and passage of both digestible and indigestible fractions through the reticul-omasal orifice (ROO) (Waldo *et al.* 1972). Disappearance from the RR can thus be explained in terms of rate constants for digestion and passage out of the RR. The two rate constants also interact since ruminal digestion is a function of both the rate of fermentation and residence time in the rumen (Dhanoa *et al.* 1985). Also, faster outflow from the rumen increases the efficiency of microbial protein synthesis and the proportion of undegraded protein escaping fermentation in the rumen resulting in a more balanced plane of amino acids and a higher supply of microbial protein to the animal (Smuts *et al.* 1995). Factors that can affect the rate constants include the physical and chemical nature of the diet, the level of intake (Warner, 1981) and the physiological state of the animal (Faichney, 1986).

In high fibre diets, due to the restrictive effect of physical fill, voluntary intake is effectively influenced by factors affecting rate of breakdown and passage of particles out of the rumen (Johns, 1965; Thorton and Minson, 1972; Thorton and Minson, 1973). Ulyatt (1970) pointed out that there is genetic variation in animals in their ability to utilise forage.

The variation in digesta flow though the RR could account for a large part of this variation in performance in grazing animals (Burns *et al.* 1991).

### 2.3.2.2. The concept of critical particle size (CPS).

Hungate (1966) suggested that the low passage rate of digesta in the rumen of cattle fed low quality roughage could be attributed to a large pool of large particles with low probability of exit from the RR. Numerous reports agree on the concept that digesta particles must be reduced to a critical particle size (CPS) before they can exit the RR (Poppi and Norton, 1980). Lechner-doll *et al.* (1991), in a review, suggested that the CPS for passage through the RR lies in the range 1-2mm for sheep and cattle. In practice, especially for modelling purposes, particles have been classified into large (greater than 1mm) and small particle (smaller than 1mm) (Pond *et al.* 1988). Below this critical size, the probability of a particle passing through the RR increases exponentially as particles become smaller (Poppi and Norton, 1980). The preferential passage of small particles is physiologically logical since they are the most extensively digested (Deswysen and Ellis, 1988; Kaske and Engelhardt, 1990).

There seems to be controversy as to whether the CPS remains the same in different animals, diets and levels of intake. Changes in the CPS could be linked to changes in retention time in the rumen. Bae *et al.* (1983) found that faecal particle size did not differ with size in cattle ranging from 261 to 861kg. Also, neither level of intake, physical form, nor the digestibility of the diet significantly affected the CPS (Ulyatt *et al.* 1986; Shaver *et al.* 1988). However, Deswysen *et al.* (1987), Van Soest *et al.* (1986) and Okine and Mathison (1991) found evidence of a relatively high proportion of large particles in the duodenum and faeces at high intake. Although the concept of particle size in linear dimensions is most widely used to describe the type of particle passing through the ROO, most of the variation in mean retention time (MRT) derives from particle density (Des Bordes and Welch, 1984; Kaske and Engelhardt, 1990). Des Bordes and Welch (1984) suggested an optimum functional specific gravity of 1.2. Kaske and Englehardt (1990) observed maximum passage in particles within the range of 1.38 to 1.5g/ml. The normal range of density of particles in the rumen is 0.8-1.5g/ml (Lechner-doll *et al.* 1991).

Factors affecting the functional density of a particle include its size, structure, shape, microbial population and fluid or gas inside it (Welch, 1986). Physical breakdown during mastication increases the functional density of particles since the larger the particle size, the lower the functional density of a particle due to reduced surface area to volume ratio. The density of particles also increases partly as a result of pockets of fermentation gases being expelled during comminution (Welch, 1986).

### 2.3.2.3. Mechanisms of selective retention of particles in the RR.

Although there is general agreement in the mechanisms involved in the selective retention of particles in the RR, such mechanisms are not clearly defined (Ulyatt *et al.* 1986). Balch and Campling (1962) suggested that discrimination occurred before the omasum. The presence of higher proportions of larger particles in the reticulum compared to distal organs is indicative of differential passage through the ROO and or intermittent return of large particles (Sutherland, 1988; Shaver *et al.* 1988; Okine and Mathison, 1991). The fact that the ROO is up to ten times larger than the CPS (Macbride *et al.* 1983) however means that the size of the ROO is not the limiting factor to digesta passage. Particles greater than the critical size are also present in the faeces, which could be due to either end-on delivery across the ROO (Kaske and Engelhardt, 1990) or the somewhat random nature of passage once particles are in proximity to the ROO.

The type of digesta in the RR and RR movements seem to be the likely sorting mechanisms allowing for the retention of large particles for further rumination and digestion (Ulyatt *et al.* 1986). Desbordes and Welch, (1984) suggested that small dense particles descend to the ventral section of the rumen, with occlusion and entrapment in a raft of stratified large particles in the dorsal sac slowing or completely obstructing large particles. Once in the ventral rumen particles are subsequently carried cranially in the ventral digesta flow with the lower dry matter pool in the reticulum allowing for further settling out of denser particles (Ulyatt *et al.* 1986). Supportive evidence showed digesta in the ventral section of the RR had higher proportions of small particles compared to the dorsal section (Evans *et al.* 1973; Shaver *et al.* 1988; Okine and Mathison, 1991; Sutherland 1988). The concept of a raft forming is widely supported in literature and even positive effects of fibre in concentrate diets are thought to partially accrue from the formation of this fibrous raft and stimulation of RR activity for sorting of particles since paradoxically, ground

material resulted in an increase in faecal particle size (Van Soest, 1994).

The role of the reticulum in the selective retention process is also not fully understood. Differential propulsion of particles seems to result from the biphasic motility pattern of the reticulum (Van Soest, 1994). The first of the observed biphasic contractions of the reticulum occurs when the ROO is closed and large particles in the dorsal section are thrown back to the cranial rumen (Ehrlein, 1980, cited by Ulyatt *at al.* 1986). The second contraction is very strong and virtually obliterates the reticular lumen (Wyburn, 1980, cited by Ulyatt *et al.* (1986). This occurs when the ROO is open and the high pressure built up forces particles to the omasum. These reticular contractions are also coordinated with rumination (Van Soest, 1994), enabling large particles to be regurgitated for remastication. In cattle, the proportion of large particles in the regurgitated bolus is larger than mixed RR contents (Ulyatt *et al.* 1986), suggesting origins either in the dorsal rumen, the cranial sac or the reticulum. The very rapid selection of large particles regurgitated could also occur in the pharynx, mouth or cheeks (Ulyatt *et al.* 1986).

Waghorn *et al.* (1986) speculated that particles do not necessarily selectively reach the reticulum. When the reticulum is relaxed, reticular cells of the mucosal honeycomb are open and the ridges separating them are low (Hoffman, 1973). Small dense particles settle and are trapped in these grooves while large particles floating in reticular fluid are thrown back during the first contraction. As the reticulum goes into the second contraction, digesta is forced towards the reticula groove and ROO (Hoffman, 1973) since the ROO is open. Small particles trapped in the reticular grooves thus find their way through the ROO.

#### 2.3.2.4. Processes of particle size reduction.

Since both particle size and density depend on the reduction in size of ingested material, residence time in the RR depends largely on the rate of comminution of large particles to smaller fractions for further digestion or passage through the RR (Waldo *et al.* 1972). Forages are degraded by ingestive and rumination mastication, detrition by digestive movements and microbial action (Pond *et al.* 1987). Ingestive and rumination mastication are however the most important processes in comminution of particles (Chai *et al.* 1984; Pond *et al.* 1984; Ulyatt *et al.* 1986; Welch, 1986).

Although it is generally believed that the physical rate of breakdown of large to small particles during eating or rumination limits outflow (Ulyatt *et al.* 1986), some reports suggest that there is a larger increase in dry matter intake with increase in passage of small particles than with an increase in breakdown of large particles (Poppi *et al.* 1981; Woodford and Murphy, 1988), implying that despite the crucial role of physical breakdown of particles, passage out of the rumen is rate limiting. However, Reid *et al.* (1979), cited by Kennedy (1985), observed that quantities of dry matter undergoing rumination exceeded both intake and daily passage out of the rumen, suggesting that rumination is rate limiting. This is confirmed by increases in passage in ground or pelleted diets with associated reduced ruminating chews (Kennedy, 1985). The two are not completely independent however since saliva secreted during rumination is a major contributor to rumen fluid, and hence may increase small particle flow (Woodford and Murphy, 1988).

#### 2.3.2.5. Efficiency of comminution of particles.

More time is spent chewing during rumination than during eating, with the chewing slower and more deliberate (Ulyatt *et al.* 1986). The latter also suggested that chewing during rumination is more efficient than during eating. Animals that chew more efficiently should be able to eat more of high fibre diets. Consistent differences in chewing efficiency have been observed (Ulyatt *et al.* 1986). Deswysen *et al.* (1988) argued that such variation suggests genetic differences in mastication ability. Efficiency of chewing could thus be an important factor in explaining breed differences in intake. Significant differences in outflow rate from the rumen in sheep also suggested inherent differences in aspects related to passage of material out of the rumen (Smuts *et al.* 1995).

Animal factors that could be responsible for the variation in particle comminution include such factors as size, age, anatomy of jaws and teeth, frequency of chewing and time spent chewing (Ulyatt *et al.* 1986). Deswysen *et al.* (1987) suggested that variation between animals in outflow rate could also result from differences in RR anatomy and the strength of contractions facilitating particle movement.

Frequency of chewing is relatively constant within species (Ulyatt *et al.* 1986) and relatively unaffected by the quality of the diet. Hooper and Welch (1983) and Nelson (1988), found that

sheep with a more rapid chewing rate broke down feed particles more thoroughly and yielded digesta with higher *in vitro* fermentation. Sheep also had a higher frequency of chewing during eating than cattle (Gill *et al.* 1966) which could be responsible for the apparent more chewing effectiveness in sheep than in cattle (Ulyatt *et al.* 1986).

An important parameter in assessing rumination is the time spent ruminating (Ulyatt *et al.* 1986). Animals have been observed to ruminate for up to a limit of about ten hours per day (Bae *et al.* 1983; Ulyatt *et al.* 1986). Animals able to ruminate more efficiently during this relatively constant period can thus consume more forage and be more productive. Luginbuhl *et al.* (1989) and Okine and Mathison, (1991) observed that at high intake, ruminating time per kilo of dry matter intake is low. Thus, for a given feed, high intake could be a reflection of better efficiency of chewing.

Stockman (1979), cited by Ulyatt *et al.* (1986) found significant correlations between mandible shape and diet in African Bovidae, and Ndiema (1980), cited by Ulyatt *et al.* (1986) observed similar relationships between jaw musculature and diet in intermediate feeders. There is little if any information on breed differences in anatomical structures related to comminution of particles in cattle. Welch *et al.* (1970) observed significant breed effects on comminution in dairy cows. In contrast, Bae *et al.* (1983) reported no apparent breed differences in chewing efficiency in Jersey, Holstein and Aryshire mature cows. Body size seemed to be the most important variable affecting chewing efficiency, with rumination, eating and total chewing time per kilogram of cell wall decreasing significantly with body mass (Bae *et al.* 1983; Hooper and Welch, 1983).

Differences in the ruminative process in animals can also explain part of the between animal variation in chewing efficiency. Variations in bolus sizes and selective regurgitation of large particles may cause differences in efficiency of rumination. Gill *et al.* (1966) observed differences in bolus sizes between animals. In dry diets fed to cattle however (Kennedy, unpublished cited by Ulyatt *et al.* 1986) reported relatively low variation in dry matter in the material actually retained for mastication after regurgitation.

Stage of plant maturity, plant species and physical form also affect the ease of comminution of particles (Ulyatt *et al.* 1986). Resistance to chewing of a roughage can be expressed as total chewing time per unit of roughage intake (Kennedy, 1985). This "roughage index" reflects

potential rate of particle breakdown, and hence relates indirectly to intake. The principal factor limiting intake in forages is usually fibre due to its influence on the breakdown of large to small particles, and also slow microbial fermentation rate (Johns, 1965). Fibrous diets require more chews per kilo and tend to be chewed less efficiently (Nelson, 1988). Tropical forages tend too have a relatively long MRT (Thorton and Minson, 1972; Woodford and Murphy, (1988) due to the high fibre content (Akin, 1986). The structural characteristics responsible for the higher fibre content in C4 plants have been described in section 2.3.5.3.

## 2.4. The use of n-Alkanes in measuring digesta kinetics and intake in grazing animals.

### 2.4.1. Limitations and opportunities in understanding the nutrition of the grazing animal.

Practical on farm decisions on animal management with respect to maintaining an adequate feed flow require accurate methods of estimating nutrient supply from the basal diet. While understanding the nutritional requirements of the animal has improved markedly in recent years (Beever, 1993; Baldwin, 1995), the lack of accurate methods for determination of forage quality, intake and utilisation in general is often cited as a major limitation in understanding the nutrition of grazing animals (Beever, 1993).

Substantial progress in the understanding of ruminal processes and the advent of more advanced computer technology offers the opportunity for developing more dynamic models that allow for more accurate predictive equations of the performance of the grazing animal (Beever, 1993). There is need however for precise quantitative data on intake and digesta kinetics.

The potential use of long chain plant alkanes, which are relatively indigestible, as markers when investigating intake, botanical composition of consumed herbage and digestive function in grazing animals has aroused interest since the first documented literature in the application of alkanes in intake studies in the mid to late eighties (Mayes and Lamb, 1984; Mayes *et al.* 1986a; 1986b; 1986c; 1988). Plant alkanes are found in the cuticular waxes of forage plants. These are mostly in the range of 25 to 35 carbon atoms ( Mayes *et al.* 1986a). Against a background of failure of conventional marker substances to meet necessary requirements in measuring intake and digesta kinetics (Faichney, 1986), the alkane method has been a subject of major interest.

### 2.4.2. Applicability of common methods used in estimating intake.

Pienaar (1994) argued that it is unlikely that a high degree of accuracy in estimating intake in grazing animals can be obtained given the complexity of the associated factors. However, various methods of estimating intake have been widely applied, but for most, applicability is limited to specific situations.

### 2.4.2.1. Methods based on pasture characteristics.

Measurements based on pasture changes due to grazing involve sampling of herbage at the start and end of a grazing period, with the difference being herbage utilised by the animals (Mannetje, 1978). The latter suggested that although such estimates may be confounded with plant growth, this error can be ignored within short grazing intervals, or corrected from daily growth estimates obtained from rest period measurements.

On relatively long grazing periods however, growth cannot be ignored nor conditions assumed the same during the grazing and rest periods. Enclosures can however be used and utilisation over short periods determined by the same principle (Mannetje, 1978). Although extensive research has been focused on developing appropriate sampling and computation procedures to account for many of the sources of variation in the estimate of utilisation in this method, there is no consistent precision under different pasture and management conditions. Highly variable results have been obtained and the methods seem applicable only on intensively utilised homogeneous pastures (Linehan, 1952). Pasture based estimates also only provide group intake measures and given the variation in nutritional needs, such data are difficult to interpret and apply (Hancock, 1952), and are more relevant to studying pasture characteristics.

### 2.4.2.2. Animal based techniques.

Penning and Hooper (1985), attempted to estimate intake by measuring short term changes in animal weight. They obtained intake estimates similar to those with $Cr_2O_3$, and pointed out the advantage of the short time within which intake estimates can be obtained. They suggested that this may be the only method ideally suited to situations where pasture conditions change rapidly. There are however several limitations to the use of this method. Harnesses for faeces and urine collection may affect animal movement. Since weight changes are measured over short periods, there is still need for applying cumbersome methods used in measuring grazing time. Also, the need for accurate estimation of evaporative effects makes the method subject to weather conditions, added to the difficulty in determination of dry matter of grazed herbage (Penning and Hooper, 1985).

The reverse use of feeding standards can also be used to obtain an estimate of feed intake (Corbett, 1978). This means there is need to have precise knowledge of the actual quality of herbage being grazed and a precise measurement of animal performance (Minson *et al.* 1976). The method inevitably ignores the limitations in methods used in establishing feeding standards discussed earlier. Differences in calculated values may be confounded with factors that cause unexpected deviation in nutrient extraction or utilisation of a particular forage. Effective nutrient intake, extraction or utilisation can also be affected by factors that have proved difficult to integrate as variables in predictive models (Beever, 1993).

An alternative method is based on the observation that number and size of bites is strongly correlated with daily intake compared to other parameters of ingestive behaviour (Allden and Whittaker, 1970; Stobbs, 1973; Chacon *et al.* 1976; Forbes, 1988; Forbes and Hodgson, 1985). The problem arises in obtaining accurate estimates of the parameters involved (Jamieson and Hodgson, 1979; Meijs, 1981). Estimates of bite size have been obtained using animals fitted with oesophageal fistula (Stobbs, 1973). Number of bites in a normal day is however a highly variable parameter and depends on numerous animal and plant factors. Stobbs (1973) estimated that number of bites vary from 12000 to 36000 on a normal grazing day. Direct counting from visual observation is tiring and often not practical, such that estimates are usually obtained indirectly from extrapolation of short time measurements (Minson *et al.* 1976), or the use of vibracorders (Coleman *et al.* 1989). Coleman *et al.* (1989), noted that estimates of intake using the bite method were usually higher than those obtained using indicator methods. They suggested that the number of bites per day as estimated from short intervals of grazing assumed continuous grazing within each short grazing period which tended to reflect maximal rate of grazing. Measurement with vibracorders also tended to show the same direction of error due to false recordings resulting from aspects like social interactions (Coleman *et al.* 1989).

### 2.4.2.3. Marker dilution techniques.

Most intake data in grazing trials is obtained from estimating faecal output and then dividing by an estimate of the coefficient of indigestibility (Dove and Mayes, 1991). This technique is more applicable in research focused on animal or plant animal interactive parameters since it gives a measure of between animal variability (Dove and Mayes, 1991).

In grazing animals, total faecal collection involves labourious work and the use of faeces collection bags can affect the behaviour of the animal (Dove and Mayes, 1991) especially in low density pastures where the animal has to move over long distances (Van Soest, 1994). Alternative methods involve the estimation of faecal output using externally administered markers ( Hankock, 1952; Chacon *et al.* 1976). The limitation is the use of *in-vitro* estimates of indigestibility in computing intake. Such estimates overlook the dynamic nature of rumen kinetics (Beever, 1993). They are based on regressions of *in vivo* digestibility determined mostly on sheep data and often applied to cattle (Beever, 1993). Similarly, *in vivo* estimates of digestibility with external markers have the same limitation since they are inevitably performed only at predetermined and narrow ranges of intake (Blaxter *et al.* 1956).

The opportunity for concomitantly estimating the digestibility coefficient arises when a marker naturally occurring in the forage is used concurrently (Fahey and Jung, 1983; Egan and Doyle, 1984; Faichney, 1986; Mayes *et al.* 1986a; Dove *et al.* 1989a; Laredo *et al.* 1991).

#### 2.4.2.4. Limitations in conventional marker substances.

The limitations of the conventional marker techniques are probably best evaluated by reference to the requirements of an ideal marker for each specific purpose. These were reviewed extensively by Faichney (1975) and Van Soest *et al.* (1986). An ideal marker is one which does not interfere with normal digesta kinetics and also allows for the validity of the computation of digesta kinetics based on proportionality of the marker concentration at the input and output points (Faichney, 1975; Fahey and Jung, 1983). It should not be metabolised, absorbed or contaminated with endogenous secretions. In rate of passage studies, a marker should move through the tract in a manner that approximates the movement of the target component (Van Soest, 1994; Faichney, 1975; Fahey and Jung, 1983). The marker must be firmly associated with the particulate digesta phase and not transfer to the fluid phase so that it passes out at the same rate as that component. The close association should at the same time not affect the physical and biochemical characteristics of digesta (Van Soest *et al.* 1986). Thus, particulate markers are generally more problematic in terms of meeting the requirements of an ideal marker (Van Soest *et al.* 1986).

In digestibility measurements, lower than expected digestibility apparent with most particulate markers could also be a result of interference with microbial attachment to the particles (Van Soest *et al.* 1986).

Insoluble identifiable materials added to feed such as plastic or rubber, or stained particles have been used as markers (Van Soest *et al.* 1986). The stained particle technique has the main disadvantage in that it is impossible to quantify (Van Soest *et al.* 1986), while the plastic or rubber particles may not behave in a manner identical to digesta particles.

Heavy metal compounds and complexes have also been used as particulate markers. The coordinated complex of Ruthenium (Ruthenium phenathroline) has a high affinity for particulate matter and has been used extensively in nutrition research, although it has the tendency to migrate to the fluid phase (Faichney, 1986). Chromium oxide complexes of plant cell wall and protein are also insoluble and stable in the rumen (Van Soest *et al.* 1986). Although widely used in intake studies, chromium oxide tends to affect the digestibility of feed. At 8 to 10%, chromium oxide will reduce digestibility to zero (Van Soest *et al.* 1986). At high concentration, changes in the specific gravity in digesta particles may also alter the processes of particle passage out of the RR (Van Soest *et al.* 1986).

Rare earth metals like ruthenium, dysprosium and ytterbium form weaker ligands than chromium oxide (Van Soest *et al.* 1986). Most studies however indicate limited absorption of rare earth metal compounds used as markers (Faichney, 1975). Hunt *et al.* (1984) found ytterbium determined estimates of faecal output similar to total faecal collection. However, Krysil *et al.* (1988) suggested that ytterbium as a marker tends to underestimate intake. Rare earth metals also tend to have wide reactivity in the rumen, especially with those compounds with free carboxyl groups such as oxalates (Van Soest *et al.* 1986). The tendency of rare earth metals to migrate limits their use in measuring particle breakdown (Faichney, 1986).

Potentially, the most ideal compounds or entities are those that form part of the feed particles (internal markers). The use of an internal marker provides an *in vivo* digestibility coefficient for an individual animal, thus accounting for differences in digestibility arising from animal or animal-plant interactive factors such as rate of breakdown of particles (Waldo *et al.* 1972), level of intake

(Blaxter *et al.* 1956), age (Dove and Mayes, 1991) or associative effects apparent with concentrate supplements (Merhrez *et al.* 1983).

The inert nature of lignin in the rumen makes it attractive as a particulate marker. Attempts have been made to use lignin in digestibility and intake studies (Fahey and Jung, 1983; Egan and Doyle, 1984). Lignin is partially digestible in the GIT although this is partly attributed the crudeness of analytical techniques (Fahey and Jung, 1983). Egan and Doyle (1984) observed that up to 10% of lignin was apparently digested in the GIT of hay fed sheep. Similar digestibilities were also obtained by Egan *et al.* (1975). The greatest apparent digestion of lignin appears to occur in the rumen, although modification of lignin has also been reported in the lower gut (Fahey and Jung, 1983). Due to this modification, faecal and dietary lignin are different in chemical structure (Fahey and Jung 1983). Gaillard and Richards (1975) cited by Fahey and Jung (1983) also observed the formation of a soluble lignin-carbohydrate complex in the rumen. Later, Nelson and Richards (1978) also cited by Fahey and Jung (1983) observed that this complex precipitated in the abomasum. Fahey and Jung (1983) noted the implications of such a reaction on the interpretation of compartmental digesta kinetics when lignin is used as marker. Thus, solubilisation in the rumen may facilitate more rapid passage out of the rumen. It is not known if markers like rare earth metals that are adsorbed on particulate matter are adsorbed to lignin associated molecules or whether or not they pass into solution with the lignin-carbohydrate complex (Fahey and Jung, 1983). Allison and Orsbourn (1970) found chemicals of non dietary origin in the faeces that analysed quantitatively as lignin in sheep fed sainfoin. The presence of such non-dietary quantities may cause under estimation of digestibility.

Various other plant entities have been tried as internal marker substances. They include $^{14}C$ plant material (Van Soest *et al.* 1986), plant pigments (Greenhalgh and Corbett, 1960), silica and acid insoluble ash (Egan and Doyle, 1984; Van Soest, 1994), pepsin insoluble nitrogen (Hunt *et al.* 1984) ADF and NDF (Hunt *et al.* 1984; Lippke *et al.* 1986 and Krysil *et al.* 1988). Isotopic labling with $^{14}C$ is limited due to measurement difficulties (Van Soest *et al.* 1986.). The use of plant pigments, as is the case with lignin, is also affected by their modification in the GIT (Greenhaugh and Corbett, 1960). Soil contamination limits the use of silica and acid insoluble ash to clean animals (Van Soest, 1994). Egan and Doyle (1984) observed high recovery rates of acid insoluble ash, although there was considerable variation between different animals. Hunt *et al.*

(1984) found that pepsin insoluble nitrogen and acid insoluble ash tended to over-estimate digestibility. Indigestible ADF gave values similar to total collection. Krysil *et al.* (1988) used residues from *in vitro* fermentation subjected to acid or neutral detergent. Recovery of ADF and NDF was low, which was in agreement with the low recovery of ADF obtained by Hunt *et al.* (1984) and Lippke *et al.* (1986). Krysil *et al.* (1988) concluded that the use of such markers was questionable.

### 2.4.3. The use of alkanes in estimating intake.

Alkanes as markers in studies on digestive function offer several advantages over most of the markers discussed above. They are relatively easily and accurately analysed (Mayes and Lamb, 1984). The use of the double alkane method (Mayes *et al.* 1986a) solves the major limitation of faecal recovery, and also allows for the use of a simple faecal alkane ratio that is less variable than the absolute concentrations.

#### 2.4.3.1. Assumptions in calculation of intake and digestibility.

The formulae used here are those derived for the double alkane technique of (Mayes *et al.* 1986a).

$$D = 1 - \frac{H_i}{F_i} \quad \text{.....1}$$

$$FO = \frac{(D_j + I \times H_j)}{F_j} \quad \text{.....2}$$

$$I = \frac{\frac{F_i}{F_j} \times D_j}{H_i - \frac{F_i}{Fj} \times H_j} \quad \text{.....3}$$

The main assumption in equations (1) and (2) is that the marker alkanes are completely recoverable (Laredo *et al.* 1991). However, alkanes are also not fully recovered in the faeces (Table 2.3). Neither the site of absorption nor the nature of their metabolism has been fully investigated. A study by Mayes *et al.* (1988) and Kafilzadeh and Parker (1990) indicated that absorption occurs in the small intestines (Table 2.4.). It appears rumen microbes are not able to metabolise plant alkanes. Also, there should be no confounding with gastric secretions since animal excretions are also minimal (Dove and Mayes, 1991).

**Table 2.3.** Faecal recovery of plant and synthetic C32 alkanes in rye grass.

| Alkane | C27 | C29 | C31 | C32 | C33 | C35 | Source |
|---|---|---|---|---|---|---|---|
| Recovery | 0.45 | 0.608 | 0.744 | 0.817 | 0.816 | 0.934 | Mayes *et al.* (1986b). |
| | - | - | 0.594 | 0.770 | 0.870 | - | Mayes *et al.* (1986c). |
| | 0.447 | 0.722 | 0.831 | - | 0.909 | 0.975 | Mayes and Lamb (1984). |
| | 0.594 | 0.697 | 0.779 | 0.859 | 0.839 | 0.953 | Mayes *et al.* (1988). |
| | 0.713 | 0.745 | 0.854 | 0.889 | 0.891 | 0.931 | Mayes *et al.* (1986a). |

**Table 2.4.** Recovery of dosed and natural alkanes.

| ALKANE. | C31 | C32 | C33 | C35 | C36 | Source |
|---|---|---|---|---|---|---|
| Duodeneum | 1.21 | 1.02 | 1.04 | 1.05 | 1.05 | Kafilzadeh and Parker (1990). |
| Ileum | 0.94 | 0.98 | 0.92 | 0.90 | 0.97 | |
| Faeces | 0.78 | 0.92 | 0.85 | 0.85 | 0.97 | |
| Duodenum | 0.965 | 0.821 | 0.988 | 1.013 | 0.841 | Mayes *et al.* (1988). |
| Ileum | 0.815 | 0.819 | 0.875 | 0.977 | 0.876 | |
| Faeces | 0.779 | 0.859 | 0.839 | 0.953 | 0.922 | |

* Values relative to $Cr_2O_3$.

In rats, studies with $^{14}C$ showed that 16-18% of dietary alkane carbon that disappears in the GIT end up as respiratory $CO_2$ (Kolattukudy and Hankin, 1966). Most of the labled $^{14}C$ ended up as

part of fatty acid moieties of liver phospholipids.

The fate of absorbed alkanes in ruminants has not been established (Dove and Mayes, 1991), and further investigation of the sites of disappearance and likely influences of alkane synthesis or secretion into the GIT may be necessary (Dove and Mayes, 1991). Also, although no quantitative evidence exists, alkenes may be saturated in the RR (Dove and Mayes, 1991), thus contributing to the dietary alkane pool.

According to the equations 1 and 2 , disappearance of alkane in the GIT leads to an underestimation of digestibility due to an overestimation of faecal output. Table 2.3 shows that recovery improves with alkane chain length, and the differences in recovery between adjacent alkanes becomes smaller with chain length. Dove and Mayes (1991) also observed that recovery of synthetic even chain alkanes was slightly higher than expected from its chain length. Although it is not readily apparent why this is so, the phenomenon reduces the differences in recovery between adjacent alkanes, thus justifying the use of a combination with the odd chain alkane one carbon more than the dosed alkane. It can be deduced from equation (3) which is a derivation based on equation (1) and (2) that given that adjacent pairs of odd and even chain alkane have similar faecal recovery, errors in the intake calculation tend to cancel out (Dove and Mayes 1991), since in the faeces, only the ratio of the external to the internal marker is critical. The implications of the tendency for the dosed alkane to have slightly higher faecal recovery than expected on possible differences in the behaviour of the alkane pair in the GIT needs further investigation (Dove and Mayes, 1991). The choice of the alkane pair is based on the concentration of the plant alkane, often meaning that a compromise has to be made with the length of the alkane chain. A minimum alkane concentration of greater than 50mg/ml is desirable (Casson *et al.* 1990). On rye grass, Mayes *et al.* (1986b) and Mayes *et al.* (1986c) suggested the use of plant C33 alkane and a dosed C32 in estimating intake. C33 was the best comprise on chain length and herbage concentrations.

Although available cattle data shows somewhat more variability, recovery rates of the commonly used C32 and C33 are very similar (Dove and Mayes, 1991). Equation (3) can also be used to demonstrate that any error arising from a difference in the recovery of the two alkanes is smaller than that caused by an equivalent deviation in an *in vitro* estimated digestibility.

In calculating digestibility, equation (1) requires that information be available on the faecal recovery of the alkane. Alkane faecal recovery especially in the shorter chain alkanes can be highly variable under different experimental conditions (Dove and Mayes, 1991) such that obtaining a correction factor for determining digestibility becomes a problem. Mayes and Lamb (1984) and Dove and Mayes (1991) suggested that the high and consistent recovery of C35 makes it ideal for such digestibility determinations. Estimates of faecal recovery of C35 based on penned animals thus be used as corrections for trials under grazing conditions (Dove and Mayes, 1991).

#### 2.4.3.2. Alkane content in tropical grass species.

The alkane content of grasses varies with species, plant component, age and season (Laredo *et al.* 1991). The later observed that while the concentration of C33 was sufficiently high in some species, low concentration in others may necessitate the use of shorter chain alkanes. Generally, alkane concentration tended to decrease with age of leaf. Even-chain alkane concentration was much lower compared to odd-chain alkanes. The alkane concentration was also generally lower in stem, compared to leaf fractions. Changes in plant maturity and proportions of morphological fractions are likely to induce cyclic changes in the alkane concentration of grazed material under intermittent grazing systems (Laredo *et al.* 1991).

Not much literature is available on the alkane profile in kikuyu. Marais (1995a) observed that C33 had the highest concentrations in both leaf and stem fractions. Horne (1996) also observed Higher levels of C33 compared to C31 and C35.

#### 2.4.3.3. Accuracy in estimating intake using alkanes.

##### 2.4.3.3.1. Herbage sampling.

Equations 1, 2 and 3 also require that the quantity of internal and external alkane be measured precisely. However, given the selective behaviour of grazing animals (Vulich *et al.* 1993), variation in plant alkane concentration complicates obtaining representative samples of the material actually ingested by the animal. Sampling has been achieved through the use of animals fitted with an oesophageal fistula, by mechanical clipping or by hand plucking (Vulich *et al.*

1993). Vulich *et al.* (1993) observed significant variation in alkane concentration among weeks and among days within weeks, indicating the need for sampling throughout the experimental period. There were no significant differences in the alkane concentrations of clipped, plucked and extrusa samples, and also no differences in variability within the methods. Their combined results indicated that the coefficient of variation was in the order of 8%, 14%, and 9% for the C31, C32 and C33 respectively. It was also evident in this trial that the size of sample had a marked effect on the variability of the alkanes.

Vulich *et al.* (1993) suggested that the small between animal variation in extrusa alkane concentration meant that a single animal was adequate for sampling purposes, provided samples were collected more than once daily. They concluded that simple harvesting procedures such as hand plucking or clipping could be used to obtain representative herbage samples, although in principle, extrusa samples would be ideal since the fistulated animal exhibits similar foraging behaviour to the experimental animal, provided they have normal social interaction with the rest of the experimental animals. However, under less uniform pasture conditions, it is unlikely that hand plucking will provide representative samples (Dove and Mayes, 1991). There will probably be also a requirement for more than just one fistulated animal where animals are to be used.

Dove and Mayes (1991) argued that even with fistulated animals, emphasis should be placed on obtaining sufficient numbers of samples, more samples than would be necessary for *in vitro* digestibility since it is possible for animals to select a diet of similar digestibility while containing different species mixtures and hence alkane concentrations. Vulich *et al.* (1993) also showed that although they are positively correlated, sampling variation in plant C33 caused a larger bias in the estimate of intake compared to variation in plant C32. Any bias arising from sampling errors for herbage alkanes is however especially relevant in experiments comparing intake in different pasture conditions, comparative experiments on the same pasture would not be markedly affected (Vulich *et al.* 1993).

#### 2.4.3.3.2. Dosing and sampling schedules.

Unlike with other conventional markers, it is not the absolute alkane concentration, but rather the ratio of the dosed and internal alkane whose diurnal variation will have important contribution to

the variation in the intake estimate (Dove and Mayes, 1991). It is possible to have temporal variation in faecal concentrations of each alkane, with their ratio remaining relatively constant (Dove and Mayes, 1991). Apart from the need to determine accurately the herbage alkane concentration, it is important that variation in this ratio is minimised. Diurnal variations in faecal marker concentration results from dosing and faeces collection schedules, marker behaviour, intake, digestibility, and pasture and climate induced changes in foraging behaviour (Raymont and Minson, 1955; Blaxter *et al.* 1956). Variation tends to be more marked with feeds of higher passage rate (Blaxter *et al.* 1956). A study , also by Blaxter *et al.* (1956) showed there was no difference between passage of a meal fed once versus twice daily.

Faecal dosed alkane concentration reaches a steady state after 5-6 days of dosing constant amounts of alkane (Mayes *et al.* 1986a) A regular, constant and precise dosing schedule should be maintained in order to achieve this equilibrium. Most intake studies with other conventional markers obtain satisfactory results with a twice daily dosing schedule (Langlands *et al.* 1963; Meijs, 1981). With alkanes, Mayes *et al.* (1986a) did not find significant diurnal variation in the ratio of the faecal concentration of the odd chain and synthetic even chain alkane with a once or twice daily dosing schedule respectively. Dillon and Stakellum (1988) cited by Doves and Mayes (1991) found greater diurnal variation of this ratio with once daily compared to twice daily dosing. Dove *et al.* (1989b) concluded twice daily dosing and faecal sampling was more satisfactory. Variation in the internal alkane concentrations remained relatively constant throughout the day, suggesting that variation in the ratio arose from the dosed alkane. This could be due to the association of the internal and external marker with the particulate and fluid phases respectively (Mayes *et al.* 1988 cited by Dove and Mayes, 1991). Centrifuging resulted in 95% of the natural alkane compared 60-70% of the dosed alkane precipitating, suggesting that the natural alkane was more associated with particulate matter (Mayes *et al.* 1988).

The method of administration of the marker is important in ensuring that the right quantities of marker are administered and since it may affect the manner in which the marker distributes in digesta and hence its pattern of excretion. Differences in marker excretion patterns can also arise from the nature of the material on which the marker is mounted particularly where it is not reasonably representative of the forage consumed by the experimental animal (Pond *et al.* 1984).

Dove and Mayes (1986a) calculated a coefficient of variation of 2-5% in the alkane content of shredded paper pellets, while Dove *et al.* (1988a) obtained variation of 1-2% in gelatine capsules of alkane on cellulose powder. Vulich *et al.* (1991) filled gelatine capsules with cellulose powder coated with alkanes in a rotary evaporator. Marais (1995b), in a similar procedure, coated milled hay with alkane and administered it as a suspension in 0.4% Ketrol jelly with a dosing gun.

Faeces collection should be undertaken over the last six or more days of dosing (Mayes *et al.* 1986a). Vulich and Hanrahan (1995) investigated different methods of faecal sampling. They concluded that although the highest level of precision can only be achieved using sampling methods based on obtaining representative samples of total faecal out, simple methods such as rectal grab sampling could be used without much compromise on the precision of the intake estimates. Precision could then be improved by more frequent sampling.

### 2.4.3.2. Comparison with known estimates and conventional marker estimates.

**Table 2.5.** Accuracy of the alkane method in estimating intake.

| Experiment Type. | Known Intake | Known-estimated. | Deviation. (%) | Source |
|---|---|---|---|---|
| Lambs on Fresh herbage. | 579g/day | 0 | 0 | Mayes *et al.* (1986a). |
| Lambs on milk + Fresh herbage. | 112-273g/day | 0.04kg/day | - | Mayes *et al.* (1986b). |
| Mature beef cows on Fresh Herbage. | 4kg/day | 0.07kg/day | -1.7 | Mayes *et al.* (1986c). |
| Dairy Cows on Fresh Herbage. | 14.18kg/day | 0.09kg/day | -0.6 | Dillon and Stakelum (1989). |
| Dairy Cows on Fresh Herbage. | 13.27kg/day | 0.10kg/day | -0.8 | Stakelum and Dillon (1990). |

A review by Dove and Mayes (1991), showed the largest deviation from known intake of only 1.7% (Table 2.5.).

Dove *et al.* (1989a) grazed sheep at two stocking rates and supplemented one group with concentrate. A comparison of C32/C33 alkane markers with $Cr_2O_3$/*in vitro* estimates of intake showed that at the high stocking rate, alkane estimate was significantly higher than that of chromium oxide but was lower at the low stocking rate, and that the point at which the estimates were equal was the level at which *in vitro* estimates were calibrated, implying that deviation was due to errors associated with the *in vitro* based estimate of digestibility used in the $Cr_2O_3$ method.

### 2.4.4. Marker techniques in estimating rate of passage in the rumen.

Rate of passage can be estimated by feeding a marker with the food and subsequently measuring the marker concentration either in the faeces or in a specific segment of the GIT. Estimating rate of passage is more difficult with particulate matter than with the fluid phase (Faichney, 1975). The problem arises partly from the tendency of conventional markers to migrate and from the conflicting need for the marker to closely associate with the target component which often entails alterations in its physical and chemical behaviour and thus affects flow parameters. The different methods of estimating rate of passage are widely reviewed by Faichney (1975) and Warner (1981).

#### 2.4.4.1. The graphical procedure of estimating outflow rate constants.

Fractional outflow rate in compartments of the GIT can be determined graphically through a curve peeling procedure in which the slopes (ie. rate constants) of the ascending and declining phases of the curve of the faecal marker excretion curve can be estimated after a single dose of marker (Grovum and Williams, 1973). This effectively solves the need for cannulation of test animals in order to obtain direct compartmental analysis.

Compartmental flow is assumed to follow the first order relationship thus:

$$C_t = C_o e^{-kt} \quad \text{.....4}$$

where $C_t$ and $C_0$ are the faecal concentrations at time t and o respectively, and k is the rate

constant. Logarithmic transformation gives the linear function:

$$\ln C_t = \ln C_o - kt \quad \text{.....5}$$

from which the slope can be determined by least squares in a linear regression model. Extrapolation of the least squares function of the declining phase allows for the calculation of residuals by subtracting the observed values from the antilog of the extrapolated function, such that a second rate constant can also be determined in the same way, representing the fractional outflow in compartments distal to the RR (Grovum and Williams, 1973).

### 1.4.4.2. Modelling digesta flow in the GIT.

Outflow parameters in the GIT have also been estimated by modelling digesta flow. This has been facilitated by rapid calculations using computer programmes that can iteratively fit non-linear least squares mathematical functions on the faecal excretion curve. As with the graphical procedure, the tendency is to describe the whole excretion curve as dominated by two mixing compartments (Grovum and Williams, 1973) (equation 6). Dhanoa *et al.* (1985) developed a multi-compartmental model (equation 7) which seemed to fit better than the two compartment model.

Models:

$$y = Ae^{-k1(t-r)} - Ae^{-k2(t-r)} \quad \text{.....6}$$

where y and A are adjusted marker concentrations in faecal dry matter, $k_1$ and $k_2$ are rate constants, r is a calculated time for first appearance of marker in faeces and t is the sample time after the pulse dose.

$$\ln\frac{dX_N}{dt} = \ln A - k1t - (N-2)e^{-k2-k1)t} \quad \text{.....7}$$

where $X_N$ represents the amount of unit marker in the compartment concerned at time t, and $k_1$ and $k_2$ are rate constants.

It is possible therefore to estimate k1 and k2. There is some controversy however, on the suggestion that the declining phase of the curve represents passage out of the RR (Pond *et al.* 1988). Although Blaxter *et al.* (1956) argued that one of the rate constants definitely described processes in the rumen, there was no proof to that effect. The other constant was not identified. Observation that there was selective passage out of the rumen led Hungate (1966) to suggest that the compartments were inside the rumen, the liquid-small particle phase and a large particle pool. This was supported by Waldo and Smith (1972). Grovum and Williams (1973) argued that the faster rate constant (k2) related to the caecum/proximal colon and the slower rate constant to events in the RR. It can be seen from equation 4 and 5 that it is the same model by Blaxter *et al.* (1956) but assigned different biological meaning to the rate constants.

Faichney and Boston (1983) also suggested that retention time in the rumen may not always be longer than in distal compartments and suggested the abomasum should not be completely ignored. Further, there also are limitations arising from inadequacies in the mathematical approaches used (Faichney, 1975; Dhanoa *et al.* 1985; Pond *et al.* 1988). The curve does not always fit a simple exponential function (Faichney 1975; Danhoa *et al.* 1985). Differential passage of material out of the RR (Lechner-doll *et al.* 1991) means that a minimum of two, that is, large and small particles needs to be distinguished, resulting in deviation from a simple first order curve.

A comparison of predicted and observed estimates however seemed to confirm the biological relevance of outflow parameters (Grovum and Williams, 1977). The estimated parameters however also necessarily relate only to the specific marker used (Faichney, 1975).

### 2.4.4.3. Use of alkanes in estimating rate of passage.

The internal alkanes should be ideal for measuring flow of particulate matter (Dove and Mayes, 1991). Their use in measuring rate of passage of particulate matter is not yet well documented (Mayes *et al.* 1986a; Dove *et al.* 1991; Laredo *et al.* 1991). The major problem could be achieving a pulse dose of natural marker. Odira (1988) cited by Dove and Mayes (1991) used $^{14}C$ labled natural alkane in comparison of flow characteristics with Yb and Cr-mordant markers.

Based on his observations, Dove and Mayes (1991) suggested there was potential for use of the labled alkane method in measuring digesta flow.

Externally administered alkanes however tend to associate with both the particulate and liquid phases (Mayes *et al.* 1988). When a low proportion of external alkane is coated on the solid support, more of the alkane might be associated with the solid than the liquid phase (Marais 1995c), allowing one to get relative rates of particulate matter flow. Such an assumption probably needs to be verified with concomitant administration of a different marker.

While results obtained using these methods should be interpreted with caution, they are useful in evaluating relative differences between animals (Warner, 1981).

# CHAPTER 3.

# GROWTH IN HEIFERS GRAZING ON KIKUYU PASTURE.

## 3.1. Introduction.

An investigation was carried out to determine changes in body weight, condition score and height at withers in Holstein, Hereford and Jersey heifers on kikuyu pasture.

## 3.2. Materials and methods.

### 3.2.1. Experimental design.

The experiment was conducted at Cedara, which lies in the KwaZulu-Natal mistbelt of South Africa, 1076m above sea level. Holstein and Hereford heifers from the Cedara herd were run together with Jersey heifers obtained from a different commercial herd. The heifers were grazed at an initial stocking rate of about four heifers per hectare. There were eight animals of each breed. The experiment effectively ran from December to April. All animals had been on kikuyu pasture from the beginning of the season. During the first eight weeks (T1), the animals were fed on pasture alone with mineral supplement. They were later divided into two equal groups for the remainder of the study (T2), one group on kikuyu plus supplement and the other on kikuyu alone. Animals were grazed on a continuous grazing programme. Four of the Cedara Holstein heifers were replaced in T2 with heifers from an adjacent farm because they were considered significantly older than the other Holsteins. The Jerseys were dropped from T2 due to health problems.

### 3.2.2. Animals.

An attempt was made to use heifers within a narrow range of degree of maturity. Mean body weights and ages at the start of T1 and T2 are shown in Table 3.1. and 3.2. respectively. There was no significant difference in age at the start of both trials ($p>0.05$). Weight differences were also not different between the Herefords and the Holsteins at the start of T1 ($p>0.05$), both of which were however significantly heavier than the Jerseys ($p<0.05$). The Herefords were significantly heavier than the Holsteins($p<0.05$) at the start of T2 due to the introduction of new

animals. The availability of animals was a major constraint in having the most ideal ages and weights.

**Table 3.1.** Mean body weights (kg) at the start of experimental periods.

| BREED | T1 | | T2 | | | |
|---|---|---|---|---|---|---|
| | INITIAL | | INITIAL | | | |
| | | | S | | NS | |
| | WEIGHT | SE | WEIGHT | SE | WEIGHT | SE |
| Jerseys | 222.2[a] | 9.4 | - | - | - | - |
| Herefords | 275.9[b] | 7.9 | 340.0[a] | 12 | 342.5[a] | 12 |
| Holsteins | 290.0[b] | 14.0 | 312.5[a] | 27 | 313.8[a] | 22 |

[ab] Means within each trial period (T1,T2) with different superscripts are significantly different ($p<0.05$).

**Table 3.2.** Animal ages (Days) at the start of T1 and T2.

| Animal No. | T1 Age (days) | T2 Age (days) |
|---|---|---|
| **Jerseys** | | |
| **Mean** | **426.0[a]** | |
| **SE** | **7.8** | |
| **Herefords** | | |
| **Mean** | **467.0[a]** | **523.0[a]** |
| **SE** | **6.2** | **6.2** |
| **Holsteins** | | |
| **Mean** | **447.0[a]** | **467.0[a]** |
| **SE** | **20.7** | **9.4** |

[ab]Means within each column with different superscripts are significantly different($p<0.05$).

### 3.2.3. Animal health.

Animals were dosed for internal parasites at the beginning of the trial, and were dipped frequently. All necessary inoculations had been administered prior to the trial. Despite the frequent dipping, the Jerseys had problems with babesiosis. Eventually, two Jerseys were lost during the adaptation period in T2, and the rest were subsequently withdrawn from the trial.

### 3.2.4. Feeds.

#### 3.2.4.1. Pastures.

Animals were grazed on 6.25 hectares of kikuyu pasture. The pasture was not uniform however, with about half of the total area under a poor stand covered with some weeds. The previous season had been very bad and it was decided to use a rather conservative stocking rate from the start. Available herbage was estimated using a disc metre. The disc metre is based on the principle of the relationship between both depth and density of the canopy with yield. It consists of a flat circular disc sliding on a light calibrated bar. The disc metre is dropped at random points in the field while reading the height in centimetres on the vertical bar. This allows numerous measurements to be taken within a short time. Three hundred disc metre readings were taken weekly during T1 and fortnightly for the remainder of the trial. The disc metre was calibrated once during the last week of January. A total of thirty six points were used in the calibration. Herbage was cut to about 1cm height using sheep shears and dried in an oven at $70^0$ C for 48 hours. During calibration, the heights recorded at each point were regressed on the yield (DM) of the area under the disc metre to obtain a linear relationship between yield and height. The yield was then estimated from the regression equation and adjusted to per hectare basis. Herbage samples for quality analysis were obtained by hand plucking in January and in March/April during the intake assessment periods(Chapter 4).

#### 3.2.4.2. Supplements.

The animals were given a mineral lick throughout the experiment. Allwood (1994) showed that feeding with UDP rich protein sources had a similar effect on animal growth to feeding a maize meal supplement. In T2, animals were therefore offered maize meal at the rate of 1kg per animal.

### 3.2.4.3. The Mobile Feed Wagon (MFW).

Feed was dispensed using an automatic individual feeding system fitted on a mobile feed wagon. The use of the mobile feed wagon is described in detail by Allwood (1994). The system comprises a cattle code system that allows one to feed animals two types of concentrate on an individual basis. A code on a transponder attached to the neck of the animal activates an interrogator fitted at the base of the feed trough. The interrogator relays the code to a central computer which activates a motor fitted to an auger at the base of the feed bin. One auger revolution dispenses a predetermined amount of feed into the feed trough. The system is fitted with eight feed bins (two on each feed trough) each with a separate auger. Since only maize meal was used, all feed bins were filled with maize meal. Wetting due to rain necessitated frequent servicing and calibration of each auger.

The system was programmed to dispense one kilogram feed per animal in each 24 hour cycle starting at 0930 hours, which was divided into two twelve hour subcycles. Each animal could thus eat up to a limit of 0.5kg of concentrate every 12 hours. The system automatically zeroes for the new cycle at the preset time hence daily intakes have to be recorded just before the start of a new cycle. It also records cumulative intakes for individual animals.

The cattle feeding system is powered by a 24V supply from two 12V heavy duty batteries. Solar panels fitted to the top of the MFW recharge the batteries during the day. However, the solar panels could not adequately recharge the batteries and a second set of batteries had to be constanly recharged on a heavy duty battery charger. The season was particularly wet such that due to frequent periods of cloud cover, batteries had to be changed sometimes as frequently as every 24 hours. Occasionally, the charger had problems resulting in disruption of concentrate feeding over short periods.

### 3.2.5. Measurements.

Animals were weighed weekly at 0830 hours. Condition scores and height at withers were measured at the same time. Condition scoring was done using a dairy condition scale (Mulvaney, 1977). Most of the time, there were two people involved in the scoring. Height at withers was obtained using a sliding horizontal bar fitted to a calibrated vertical bar which allowed the quick reading of data, by dropping it on the animal once it attained the correct posture.

Animals were restrained using a head clamp.

### 3.2.6. Statistical analysis.

Average daily gain (ADG) was estimated using regression analysis in Minitab. In T1, the estimated ADG was fitted into a one way model using Minitab. T2 data were fitted into a general linear model. More complex analyses on height and condition score were performed using Genstat. Changes in body weight, condition score and height at withers were estimated by regressing each of these variable on time. T-tests were used to separate differences between means.

## 3.3. Results.

### 3.3.1. Quality, availability of pasture and concentrate intake.

#### 3.3.1.1. Herbage availability.

Disc metre readings over the experimental period and calibration points are shown in Appendix 1a and 1b respectively. Although initially the pasture had been divided into two sections, the pooled calibration equation, which accounted for 58.9% (Appendix 1c) of the variation in the model was used to estimate overall herbage availability. Pasture yields estimated from the regression equations are shown in Table 3.3. The standard error of the estimated available herbage was determined from the formula given by Rayner (1967), cited by Horne (1996) shown in Appendix 1d. There was a marked increase in herbage available after the removal of the Jerseys when stocking rate fell from 3.84 animals per hectare to 2.76 animals per hectare.

**Table 3.3.** Herbage availability in the pasture (kg/ha) over the experimental period..

| Date | 20/12 | 27/12 | 3/1 | 10/1 | 17/1 | 8/2 | 21/2 | 7/3 | 27/3 |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 1581.0 | 1605.0 | 1660.0 | 1495.0 | 1545.0 | 2565.0 | 2077.0 | 2768.0 | 2659.0 |
| SE | 155.8 | 153.8 | 153.8 | 166.3 | 153.8 | 280.8 | 254.8 | 437.1 | 407.6 |

### 3.3.1.2. Herbage and concentrate quality.

The composition of pooled hand-plucked samples of herbage taken during the last weeks of both phases of the trial are shown in Table 3.4.

**Table 3.4.** Chemical composition of grazed kikuyu (100% DM).

| | CP (%) | ADF (%) | NDF (%) | Ca (g/kg) | P (g/kg) |
|---|---|---|---|---|---|
| Kikuyu | | | | | |
| January | 17.86 | 33.23 | 66.72 | 2.39 | 3.69 |
| March/April | 17.61 | 30.85 | 63.33 | 2.54 | 3.42 |
| Maize Meal | 10.13 | 3.77 | 11.67 | 0.11 | 2.50 |

### 3.3.1.3. Concentrate intake.

The mean concentrate intake in supplemented animals are shown in Table 3.5.

**Table 3.5.** Concentrate intake.

| Animal No. | Daily intake (g/day) |
|---|---|
| HEREFORDS | |
| Mean | 539.5[a] |
| SD | 194 |
| HOLSTEINS | |
| Mean | 267[b] |
| SD | 106.8 |

[a]Means with different superscripts are significantly different ($p<0.01$).

While the Herefords learnt to use the MFW by the third week, the Holsteins took time to learn and did not fully utilise the machine up to the end of the trial. They had significantly lower ($P<0.01$) concentrate

intake than the Herefords (Appendix 2.)

### 3.3.2. GROWTH.

#### 3.3.2.1. Average daily gain (ADG).

Individual body weights in T1 and T2 are shown in Appendix 3a and 3b. Average daily gains are presented in Table 3.6. Analysis of variance on ADG/100kg live weight is shown in Appendix 4a and 4b. In T1, analysis of variance on ADG per 100kg live weight showed significant breed differences ($p<0.05$). T-tests showed Herefords grew significantly faster than both the Holsteins and the Jerseys ($p<0.05$). The difference between the Holstein and the Jersey was also significant at ($p< 0.05$). In T2 however, neither breed, diet nor their interaction was significant ($p>0.05$). Since there was no significant concentrate effect, a comparison between all animals in each breed (Appendix 5.) showed that the Herefords had a significant drop in ADG in T2 ($p<0.01$) while there was no significant change in ADG in Holsteins ($p>0.05$).

**Table 3.6.** Individual ADG in T1 and T2.

| Animal No. | T1 ADG (kg/day | T2 ADG (kg/day) | |
|---|---|---|---|
| | | Supp | Non-Supp |
| Jerseys | 0.200 | | |
| Herefords | 1.180 | 0.797 | 0.668 |
| Holsteins | 0.540 | 0.497 | 0.624 |

#### 3.3.2.2. Change in height at withers.

Weekly height measurements for individual animals in T1 and T2 are shown in Appendix 6a and 6b. Analyses of variance are presented in Appendix 7a and 7b. A linear regression model was fitted to estimate weekly changes in height. In T1 change in height was not significant in all breeds ($p>0.05$). In

T2, a test of model suitability showed that a linear model was not appropriate for the data set. The apparent measurement error which resulted in unexpected fluctuations in height may have obscured any change in height over the relatively short experimental period. Tests of between-week differences within each breed and dietary level showed no significant($p>0.05$) changes over the trial period.

#### 3.3.2.3. Change in condition score.

Weekly condition score measurements in T1 and T2 are shown in Appendix 8a and 8b. Analyses of variance are shown in Appendix 9a and 9b. In T1, a linear regression model was also fitted on mean breed condition score over time to estimate weekly change. In T1, change in condition score in Herefords was significant ($p<0.05$). In T2, a graphical plot suggested change in condititon score was not linear, and a test of model suitability showed that the linear regression model was not suitable for the data. Tests of between-week differences within each breed or dietary level also showed no significant($p>0.05$) changes over the trial period.

## 3.4. Discussion.

### 3.4.1. The quantity and quality of feeds offered to the animals.

#### 3.4.1.1. Available herbage.

The two main factors influencing intake are quality and quantity available. Quantity is usually first limiting (NRC, 1987). Abundant forage also allows for selectivity in grazing. Intake in cattle and sheep on continuous grazing, tended to be maximal at about 2.25t per hectare available herbage (Rayburn, 1986), cited by (NRC, 1987). The available herbage in this trial was an average 2 tonnes per hectare, which should have allowed for a high degree of selectivity in grazing especially during the second stage of the experiment. The increase in available herbage with time means that grass growth was greater than utilisation.

than utilisation.

### 3.4.1.2. Herbage quality

In chapter 2, reference was made to the negative effect of high levels of nitrogen in kikuyu on intake. It was also noted that animals tended to select mature herbage of around 14%CP which in turn tended to have more fibre than the total herbage on offer. The quality of the total canopy was not analysed in this experiment, and the quality of the herbage obtained would largely reflect the composition of grazed material, assuming that the sampling procedure was accurate.

The CP content in pluck samples was similar to values of around 18% obtained by Horne (1996). Both ADF and NDF were also similar to those obtained by Horne (1996) of 30.85 to 33.23% and 55.7% respectively. The overall quality of the diet was not likely to have had significant limitations in growth.

### 3.4.1.3. Concentrate intake.

Based on the proportion of concentrate intake to that which was offered, the Herefords made 54% use of the MFW compared to only 27% in Holsteins. These results are similar to those obtained by Allwood (1994), in which the Holsteins ate about 23% of available concentrate. He argued that the low usage of the MFW was due to individual animal differences in learning behaviour, and external influences such as placement of the wagon, size of paddock and the quality of the available herbage. It was evident in his trial that animals reduced MFW usage on entry into a new paddock. The major limitation in this trial was the placement of the machine in a large continuous grazing paddock, and the relatively low stocking rate. Part of the problem could also have been bullying by the Herefords. A major limitation also was the power problem which continually disrupted the feeding, and may thus have contributed to the lack of interest.

### 3.4.2. Growth in Heifers.

The Holsteins had a slower growth pattern compared to the Herefords. The poor performance of the Jerseys should be viewed with caution given the apparent problems in adapting to the conditions at Cedara. The lower ADG in Herefords in T2 compared to T1 was probably mainly a result of a maturity

effect. Review of literature in Chapter 2 showed that fatness in cattle results in depressed intake, and that as the composition of gain increasingly constitutes fat, ADG is lower since, unlike protein accretion, although fat excretion is energetically more efficient, in terms of live weight gain, the efficiency is lower. The decline in intake could also be partly due to internal fat deposition with possible negative effects on physical fill (NRC 1987). The introduction of younger Holsteins in T2 effectively reduced the mean age in Holsteins, thus increasing the disparity in degree of maturity.

The faster change in condition score in the Herefords in T1 corresponded to the rapid change in weight. The lack of change in condition score in Holsteins could be a result of a slow rate of maturing, and could also be partly due to the lower rate of body mass gain. In Chapter 2, reference was also made to the fact that there are distinct breed differences in the distribution of fat in the various fat depots, and possible differences in the distribution of subcutaneous fat in various anatomic regions. Accordingly, conclusions about fat assessments between beef and dairy breeds using condition scores must be drawn with caution. The nonlinear condition score response in T2 could have resulted from a systematic underestimation of condition scores towards the end of the trial, especially in Herefords, a likely consequence of the subjective nature of condition scoring. Wright and Russel (1984), working on mature cows and using a similar scale, observed a unit change in condition score corresponding to 104kg and 110 kg body weight gain in Hereford-Holstein crosses and Holsteins respectively. In this trial, the fact that the animals were younger means that a unit change in condition score would probably take a more marked change in body weight. The range in condition score during the course of the trial used in the linear regression was therefore also limited.

The relatively low ADG in the Holsteins seems to confirm the results by Horne (1996). The pattern of growth in Holsteins was also very similar to that observed by Allwood (1994), who obtained ADG of 0.52kg in non supplemented Holsteins. Review of literature showed that there were no marked differences in maintenance requirements between the two breeds, and that any differences in efficiency of body weight gain were only likely to be a result of the differences in the composition of gain. Horne (1996) observed that herbage intake was in fact lower in Holsteins compared to Herefords. The suggestion was therefore that given evidence of significant differences in the flow of digesta in the RR which correlated well with wool production in sheep (Smuts *et al.* 1995), the Holsteins could be inherently incapacitated in effectively utilising the forage due to selection under conditions that do not favour optimum use of forage.

In this trial failure to effectively utilise the MFW makes it difficult to separate the effect of supplement on each breed. The Herefords however were expected to respond to supplement. An increase in herbage intake is to be expected especially when an energy supplement is offered to animals grazing on well fertilised, high nitrogen kikuyu pastures. This is mainly due to changes in the energy supply within the rumen which allows microbes to efficiently utilise the high levels of ruminal nitrogen and thus reduce the urea load generally believed to be responsible for the lower than expected intake (Pienaar, 1994). Allwood (1994) suggested ADG starts being affected at levels of energy supplement of 0.4kg/day. It must be noted however that such responses will largely depend on the relative difference in quality between the supplement and the forage. Although the Herefords had higher supplement intake than this, there was no significant supplement effect on ADG. Horne (1996) also suggested that the lack of response to supplement in Herefords compared to Holsteins could be indicative of relatively lower herbage intake in Holsteins, such that higher levels of gutfill in Herefords resulted in a substitution effect, whilst intake in the Holsteins was additive. Substitution would normally however only have been expected at rates exceeding about 25% of total intake (Obara *et al.*, 1991).

# CHAPTER 4.

# THE USE OF N-ALKANES TO ESTIMATE INTAKE IN ANIMALS GRAZING ON KIKUYU.

## 4.1. Introduction.

The experiment was conducted to investigate intake and digestibility in Holstein, Hereford and Jersey heifers grazing on kikuyu pasture.

## 4.2. Materials and methods.

The alkane method described in chapter 2 was used in estimating intake and digestibility. Marais (1995a) found that C33 had the highest concentration in leaf and stem fractions of kikuyu pasture. In a previous trial by Horne (1996), C33 also had a higher concentration compared to both C31 and C35. C32 was therefore used as the external alkane in this experiment. The experimental design and dietary treatments are as described in Chapter 3. Animals were dosed with C32 over a one week adaptation and a seven day experimental period at the end of T1 and a six day period in T2. Animals were grazed on a continuous basis, since Horne (1996) suggested the higher in intake his trial could have resulted partly from inaccuracies in the estimation of alkane content in grazed material due to changes in morphological composition of herbage under a rotational grazing system.

### 4.2.1. Alkane preparation and administration.

C32 was coated at 10% on milled hay in a rotary evaporator using 60-80BP petroleum ether. The hay had been milled through a 1mm screen and sieved through a 0.5mm screen, retaining all material caught on a 0,25mm screen underneath. After coating, the hay was again passed through a 1mm sieve to break up lumped particles, and mixed thoroughly. The coated alkane was prepared in bulk for both T1 and T2. Samples of the coated grass were retained for alkane analysis.

A suspension of the coated grass in a 0.4% solution of xanthan gum in water was dosed using a

dosing gun to achieve a calculated 0.5g of alkane per animal. The xanthan gum retained particles evenly in the suspension. The suspension was later diluted to facilitate smooth flow of particles such that animals had to be given two doses each. The suspension was prepared and left overnight to allow gas bubbles to come to the surface and was stirred gently before dosing. The quantity of suspension administered at each dosing was estimated by weighing the dosing gun output before dosing. Dosing was done once a day at 0830 hours.

### 4.2.2. Herbage sampling.

Samples were obtained daily by hand plucking over the trial periods. Sampling started two days before the start of faecal collection in order to obtain material representative of that to be eventually collected in the faeces. Samples were taken while following the animals as closely as possible to obtain material representative of herbage consumed. Sampling was also done in the morning which coincided with the period of maximal intake. Samples were dried at $70^0$C for 24 hours, and milled through a 0.5mm screen.

### 4.2.3. Faeces collection and preparation.

Grab faecal samples were collected at 0830 hours over the last seven days in T1 and over 6 days in T2. Animal number H18 skipped a dose and was subsequently dropped from the experiment. The faeces was immediately frozen and later dried in an oven at 84°C before milling through a 0.5mm screen.

### 4.2.4. Analysis of herbage and faecal samples.

Vulich and Hanrahan (1990), cited by Dove and Mayes (1991), suggested that the increase in precision obtained from duplicate analysis did not justify the increase in number of samples. Thus, only one sample was prepared for analysis.

Extraction of alkanes was done using a method developed at the Cedara laboratory (Marais, 1995b). Hexatriacontane (C36) was used as internal standard. A 0.4g sample of the internal standard was made up to 200g with undecane (C11). A 0.2g sample of the solution

was then weighed into a 50ml glass-stoppered tube to which 1g or 1.5g of the faecal or feed samples, respectively, were added. Forty millilitres of petroleum ether (80-100$^0$CBP) were added and the suspension heated in a water bath at 70$^0$C for two hours with occasional shaking. The supernatant was then decanted into a 50ml beaker and allowed to evaporate. The extract was dissolved in about 3ml of warm petroleum ether (60-80$^0$CBP) and the solution passed through a column of silica gel (60 microns) into a test tube. A further 3ml of warm petroleum ether (60-80$^0$CBP) was used to rinse the beaker and was passed through the same column into a different test tube. The solution was evaporated, and the second test tube washed into the first with 3ml warm petroleum ether (60-80$^0$CBP) and again evaporated to dryness. The alkanes were then dissolved in 0.7ml of warm hexane in a screw cap vial. One microgram of the solution was injected into a Varian 3600 GC, fitted with a capillary column (megabore, 15m, 53mmID, 1.0 micron 100% dimethyl polysiloxane). Column temperature was set at 240$^0$C for 2.5 minutes rising to 288$^0$C at 3$^0$C per minute and to a final temperature of 298$^0$C.

### 4.2.5. Analysis of coated grass.

A 0.5g sample of coated grass was weighed into a glass stoppered tube with 0.05g of internal standard dissolved in 10ml of undecane. The suspension was then heated in a water bath at 70$^0$C with occasional shaking. An aliquot (0.3ml) was passed into a test tube through a silica gel column (60 microns) and evaporated to dryness. The alkanes were then dissolved in 0.3ml of hexane for the GC analysis.

### 4.2.6. Statistical analysis.

Analysis of variance was performed using Genstat. T -tests were used to separate differences between means.

## 4. 3. Results.

### 4.3.1. Intake of the external alkane.

Data used to estimate the variation in alkane administered to the animal is shown in Appendix 10a, 10b and 11 and summarised in Table 4.1.

**Table 4.1.** Quantity of C32 administered per dose in T1 and T2.

| | C32 ( .g/kg) | Gun output (g) | | C32 intake (mg/day/animal) | |
|---|---|---|---|---|---|
| | | T1 | T2 | T1 | T2 |
| N | 10 | | | 10 | 10 |
| MEAN | 90.8 | 101.8 | 101.5 | 493.0 | 492.0 |
| SE | 1.50 | | | 9.00 | 8.00 |
| CV (%) | 1.65 | 0.09 | 0.07 | 1.72 | 1.72 |

### 4.3.2. Alkane concentration in herbage samples.

Table 4.2 and Appendix 12 show the alkane concentration in pluck samples in TI and T2.

**Table 4.2.** Mean concentrations of n-alkanes in herbage samples (mg/kg).

| T1 | | | T2 | | |
|---|---|---|---|---|---|
| | Mean | SE | | Mean | SE |
| C31 | 103 | 4.0 | C31 | 96 | 6.0 |
| C32 | 8 | 0.4 | C32 | 13 | 2.0 |
| C33 | 215 | 5.0 | C33 | 200 | 14.0 |
| C35 | 211 | 7.0 | C35 | 205 | 16.0 |

### 4.3.3. Alkane estimated intake.

The faecal (Appendix 13 a and 13b), dosed (Table 4.1) and herbage (Table 4.2) alkane concentrations were fitted into equation 3 in chapter 2. Comparisons were done on a metabolic body weight basis. Estimated intake and analyses of variance are presented in Appendix 14a, 14b and 15a, 15b respectively, and summarised in Table 4.3.

**Table 4.3**. Mean intake estimates (g/kgLW$^{0.75}$/day).

| | T1 | | T2 | |
|---|---|---|---|---|
| Breed | Intake | SE | Intake | SE |
| JERSEY | | | | |
| C31 | 98.5$^{a}$ | 5.7 | | |
| C33 | 97.6$^{a}$ | 3.0 | | |
| C35 | 98.4$^{a}$ | 4.3 | | |
| HEREFORD | | | | |
| C31 | 119.4$^{a}$ | 4.6 | 104.3$^{a}$ | 3.0 |
| C33 | 117.7$_{b}$ | 2.9 | 104.4$^{a}$ | 2.3 |
| C35 | 113.8$^{b}$ | 3.1 | 113.3$^{b}$ | 3.0 |
| HOLSTEIN | | | | |
| C31 | 92.7$^{a}$ | 2.7 | 93.6$^{c}$ | 2.9 |
| C33 | 92.7$^{a}$ | 2.7 | 98.0$^{c}$ | 2.2 |
| C35 | 93.5$^{a}$ | 2.5 | 108.6$^{d}$ | 2.3 |

$^{ab}$Means within each treatment with different superscripts are significantly different ($p<0.05$)

In T1, the estimated intake decreased based on the odd chain alkane used in the order C31< C33< C35. There was no significant difference($p>0.05$). The reverse occurred in T2, with C5 estimates significantly higher($p<0.05$) than the C31 and C32 estimates. Herefords had significantly higher intake than both the Holsteins and Jerseys ($p<0.01$). The difference between the Holsteins and the Jerseys was not significant ($p>0.05$). In T2, the Herefords had higher intake

than the Holsteins ($p<0.01$). A comparison of intake between T1 and T2 (Appendix 16) showed no significant differences in the Holsteins ($p>0.05$), with a significant drop in intake in the Herefords($p<0.05$)

### 4.3.4. Estimated digestibility.

Dove and Mayes (1991) suggested a factor of 0.95 to correct for the incomplete recovery of C35 in estimating digestibility. This factor was used in estimating digestibility. Table 4.4 shows the estimated digestibility of kikuyu using equation 2. Individual and daily digestibilities and analyses of variance are presented in Appendix 17a, 17b and 18a, 18b respectively. T-tests in T1 showed the Jerseys had a significantly lower digestibility than the Herefords ($p<0.01$) In T2, however there were no significant differences between breeds in digestibility ($p>0.05$).

**Table 4.4**. Estimated digestibility using C35.

| | T2 | | T2 | |
|---|---|---|---|---|
| Breed | Digestibility (%) | SE | Digestibility (%) | SE |
| J | $51.4^{b}$ | 2.1 | - | |
| H | $59.8^{a}$ | 1.3 | $61.6^{a}$ | 1.6 |
| HF | $56.8^{ab}$ | 1.5 | $60.5^{a}$ | 2.3 |

$^{ab}$Means within each treatment with different superscripts are significantly different ($p<0.05$)

than the Holsteins ($p<0.01$). A comparison of intake between T1 and T2 (Appendix 16) showed no significant differences within each breed ($p>0.05$).

### 4.3.4. Estimated digestibility.

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**Table 4.4**. Estimated digestibility using C35.

| | T2 | | T2 | |
|---|---|---|---|---|
| Breed | Digestibility (%) | SE | Digestibility (%) | SE |
| J | 51.4[b] | 2.1 | - | |
| H | 59.8[a] | 1.3 | 61.6[a] | 1.6 |
| HF | 56.8[ab] | 1.5 | 60.5[a] | 2.3 |

[ab]Means within each treatment with different superscripts are significantly different ($p<0.05$)

## 4.7. Discussion.

### 4.7.1. Intake and digestibility of kikuyu.

The estimated digestibilities are similar to those obtained by Horne (1995). The lower apparent digestibility in Jerseys compared to Herefords in TI was not expected. Given possible differences in the concentrations of alkanes in different plant morphological components (Laredo *et al.* 1991), differences in the selectivity of herbage between the two breeds may result in differences in the estimated digestibility coefficients since the plucked samples were assumed to be representative of material ingested by animals in all breeds. There is not much information on changes in the relative proportions of alkanes in different plant morphological components in kikuyu. Such differences have not been demonstrated. The observed differences may however be a result of experimental error.

The variation in estimated intake within breeds in this trial was relatively lower than in a previous trial by Horne (1996). In the latter study, it was suggested that intake could have been over-estimated due to difficulty in obtaining representative grazed samples in a rotational grazing system, given the morphological differences in alkane content. It was suggested also that the relatively lower external alkane dose could have reduced the precision of alkane analysis. The continuous grazing regime and the higher external alkane dosage seem to have overcome these limitations in this trial.

The faecal samples in this trial were dried at a temperature higher than normally recommended for alkanes. The effects of oven drying have not been fully investigated. Normally, freeze drying is recommended since alkane recovery tends to fall at temperatures above $70^0$C (Dove and Mayes, 1991). It is not known wether this is due to loss of alkanes or failure to effectively extract them.

Vulich et al. (1993) suggested a procedure such as the hand plucking method used in this trial could provide representative grazed herbage. Half the grazing area in this trial was not uniform, and was infested with weeds, and probably required a more rigorous sampling procedure. The inconsistency in the ranking of the alkane estimated intakes between T1 and T2 in this trial, which

(1981), working on steers on kikuyu obtained values of 107.61g/kgL.$W^{0.75}$. Preston (1972) concluded intake in beef cattle was 95g/kgL.$W^{0.75}$ with a 95% confidence interval of 88 to 100g/kgL.$W.^{0.75}$.

Intake will however depend to a large extent on various animal and pasture characteristics. The animals in the trial by Horne (1996) were under a higher stocking rate than in this trial, and had lower herbage availability than in this trial. Although Horne (1996) attempted to estimate the degree of maturity of the test animals, which he calculated at 47% and 49% in Herefords and Holsteins respectively, it is difficult to estimate the degree of maturity of animals in this trial since they came from different herds. However, given that both the Herefords and Holsteins used in T1 came from the same herd as the animals used in the trial by Horne (1996), and assuming the same estimated mature masses of 500kg and 550kg for the Herefords and Holsteins respectively (Horne, 1996), animals in this trial were also more mature at about 54% and 52.7% degree of maturity respectively at the start of the trial.

The relatively lower intake in the Herefords in T2 compared to T1 may be due to the maturity effect discussed in chapter 3, which corresponds to the decline in ADG observed in chapter 3. Relative intake tends to decline at a condition score of 5 on a scale of 1-9, corresponding in this experiment to a condition score of 3. Intake of Holsteins over the trial periods remained relatively unchanged and consistent with the pattern obtained in ADG, reflecting partly the slower maturing pattern of the Holsteins.

The lower intake in the Jerseys and the Holsteins in T1 is the most likely explanation for the lower ADG apparent during this period. The Herefords had a 25% and a 7% higher intake compared to Holsteins in T1 and T2 respectively. This is lower than the 55% observed by Horne (1995). Again, the low performance of the Jerseys must be viewed with caution given the adaptation problems.

There is no readily apparent reason for the lower intake in the Holsteins. Genetic variance in intake in cattle has been suggested in the literature. In a range of experiments, heritability of intake varied from 43 to 76% (NRC, 1987), with a mean of 62%. It is thus possible to induce relative differences in intake through selection, as suggested by Horne (1996).

# CHAPTER 5.

# PASSAGE OF DIGESTA IN THE RUMEN OF HEIFERS GRAZING ON KIKUYU PASTURE USING CHROMIUM OXIDE AND DOTRIACONTANE(C32).

## 5.1. *Introduction.*

During the second part of the trial (T2), an experiment was conducted to measure the flow of digesta in the rumen, while at the same time testing the possibility of using a synthetic alkane coated on grass as a marker

## 5.2. Material and methods.

Three animals from each treatment combination (Chapter 3) were used in this study. Two markers $Cr_2O_3$, and C32 were administered at the same time. In n effort to minimise alkane migration to the fluid phase, the alkane was coated at a rate of 5% compared to the 10% used in the intake estimates. The markers were administered two weeks before the start of the intake studies in T2 to allow the alkane to completely clear out of the gastro-intestinal tract so as not to influence the intake study. The animals grazed on the same pasture for most of the day but were moved to a paddock closer to the handling facilities at night during the sampling period to enable easier sampling during the night.

### 5.2.1. Marker administration.

Animals were dosed at 1100 hours with a capsule containing 10g chromium oxide. At the same time, 21g of C32-coated and coarsely milled hay was also administered to the animal, giving an alkane intake of 1g per animal. The hay had been milled through a 1mm screen and coarse material caught on a 2mm sieve was coated with alkane in a rotary evaporator using 60-80BP petroleum ether. The coated grass (moulded into a small ball with water and bagasse) and the $Cr_2O_3$ capsule were placed by hand as far back as possible into the mouth of the animal to avoid any spitting.

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### 5.2.1. Dosage of marker.

Animals were dosed at 1100 hours with a capsule containing 10g chromium oxide. At the same time, 21g of C32-coated and coarsely milled hay was also administered to the animal, giving an alkane intake of 1g per animal. The hay had been milled through a 1mm screen and coarse material caught on a 2mm sieve was coated with alkane in a rotary evaporator using 60-80BP petroleum ether. The coated grass (moulded into a small ball with water and bagasse) and the $Cr_2O_3$ capsule were placed by hand as far back as possible into the mouth of the animal to avoid any spitting.

### 5.2.2. Sampling schedule.

Samples were obtained by grab sampling from the rectum. The sampling times are shown in Appendix 19 and Appendix 22 for chromium oxide and C32 respectively. An attempt was made at each sampling to avoid faeces that could have been stored in the rectum by moving the hand well off the caudal end. Animals that defecated just before sampling had to be allowed some time before a sufficient quantity of sample could be obtained. Such animals were sampled again at the end of sampling period, if a sufficient sample could be obtained, otherwise no sample was taken for that period.

### 5.2.2. Laboratory analysis.

The samples were heated to dryness at 84$^0$C and milled through a 0.5mm screen. The alkane was analysed by the same method described in chapter 4. The same samples were also analysed for $Cr_2O_3$.

### 5.2.3. Mathematical and statistical analysis.

A computer programme (SAS) was used to fit the models by Blaxter *et al.* (1956) and Dhanoa *et al.* (1985)(chapter 2) on faecal excretion data. The model of Blaxter *et al.* (1956) was analysed using the interpretation of Grovum and Williams (1973) in which k2 represented outflow in compartments distal to the rumen, and k1 represented outflow within the RR. A graphical procedure by Grovum and Williams (1973) described in section 2.4.3.5. was also used to estimate outflow parameters for the C32 data due to apparent inconsistencies in the non linear iterative procedures. A general linear model was fitted on k1 values to investigate treatment effects on k1.

## 5.3. Results.

### 5.3.1. Analysis of $Cr_2O_3$ using the nonlinear methods.

Faecal marker concentrations and sampling times for $Cr_2O_3$ are presented in Appendix 19. The parameter estimates are presented in Appendix 20. Analysis of variance is presented in Appendix 21. The mean estimated rate constants for k1 are also shown in Table 5.1a. k1G and k1D, denote rate constants determined by the methods of Blaxter *et al.*, (1986), Dhanoa *et al.*, (1985) respectively.

Convergence was met in all the data sets with both models when the $Cr_2O_3$ data was fitted. The intercept of the regression of k1G on k1D was not significantly different from zero and the slope not different from 1($p>0.01$). Rate constants form the nonlinear least squares procedure were therefore used for assessing treatment effects. Both breed and dietary effects were not significant ($P>0.05$)

### 5.3.2. Analysis of C32 data using nonlinear procedures and the graphical procedure of Grovum and Williams (1973).

Faecal C32 concentrations and sampling times are shown in Appendix 22. A summary of nonlinear model parameters is also presented in Appendix 23. The alkane data had limited data points, and both non-linear procedures tended to give unrealistic parameter estimates and non-repeatable parameter estimates on individual heifer data with changes in initial parameters set. There was no significant correlation between k1D and k1G (Appendix 24). Only the graphical procedure was subsequently used for testing treatment effects on k1 (Appendix 25). Regression was performed on breed and treatment combination means of the natural log of C32 (LN) concentration. The mean k1 values are shown in Table 5.1b.

Analysis of variance on k1 also showed that neither breed, diet nor their interaction was significant ($p>0.05$).

**Table 5.1a**. k1 for $Cr_2O_3$ data using nonlinear iterative procedures.

| Parameter. Treatment. | k1D | SE | k1G | SE |
|---|---|---|---|---|
| Herefords Supp | 0.056[a] | 0.0045 | 0.069[a] | 0.0098. |
| Herefords No-Nupp | 0.062[a] | 0.0044 | 0.063[a] | 0.0083 |
| Holsteins Supp | 0.061[a] | 0.0067 | 0.090[a] | 0.0250 |
| Holsteins No Supp | 0.056[a] | 0.0029 | 0.061[a] | 0.0076 |

[a] Means within each column with different superscripts are significantly different at ($p<0.05$)

**Table 5.1b**. k1 values using the graphical procedure on alkane data.

| Parameter/ Treatment. | k1 | SE |
|---|---|---|
| Herefords Supp | 0.025[a] | 0.008 |
| Herefords No Supp | 0.035[a] | 0.008 |
| Holsteins Supp. | 0.042[a] | 0.008 |
| Holsteins No Supp | 0.038[a] | 0.008 |

[a] Means within each column with different superscripts are significantly different at ($p<0.05$)

**5.4. Discussion.**

Judged by the criteria suggested by Dhanoa *et al.*, (1985), the models for faecal marker excretion using $Cr_2O_3$ seemed to fit most of the data sets relatively well. The criteria include no systematic under or overestimation of parts of the curve, biologically acceptable estimates, and convergence to a repeatable solution for different initial parameter estimates. The main problem seemed to be insufficient points in the ascending phase which seemed to require a more frequent sampling schedule than the one undertaken. The model by Blaxter *et al.*, (1956) tended to give higher estimates of both k1 and k2 compared to the multi-compartmental of Dhanoa *et al.*, (1985). The alkane data gave values of k1 lower than the chromium data. Chromium mordants are conventionally used in estimating out flow (Uden et al., 1982). The chromium oxide tends to complex with soluble compounds such that by administering it in capsules, part of the chromium forms soluble compounds in the rumen, or may simply escape along with the fluid phase, thus giving the lower estimates of k1. The low alkane concentration in the faeces in the descending phase probably contributed to failure to detect C32 in some of the samples selected. Due to the apparently low alkane dosage, the variation in the naturally occurring C32 could also have been of sufficient magnitude to interfere with estimates of the dosed alkane. It is probably necessary to dose the animals with higher quantities of the C32. The lower k1 values obtained with C32 mean that probably relatively more of the alkane was attached to particulate matter compared to the chromium oxide. The material that the alkane had been coated on was milled coarsely, but it is unlikely the particle sizes milled through a 0.5mm screen, although greater than 2mm because of the 2mm sieve used, could have significantly influenced the rate of passage of hay versus fresh herbage particles. More work is necessary to investigate the migration of the coated alkane. The apparent lack of fit of the nonlinear iterative procedures could have been due to limited data points. Generally however, the pattern of alkane excretion shows potential use of the procedure in measuring relative differences in outflow, provided there is no need for a large number of samples to reduce the variation.

The lack of supplement effect on k1 especially in Herefords which had a relatively higher concentrate intake is rather surprising. In the Holsteins, this could be explained by the low level of supplement intake. It is possible that even in the Herefords, the level was still not high enough to markedly change rumen fermentation kinetics, which in any case may not have been limiting on a good quality kikuyu pasture.

Variation in retention time in the RR, can be as high as up to 24% (Warner 1981). Such variation could explain differences in productivity in animals on the same diet. Smuts *et al.*, (1995) found significant correlations between DMI and outflow from the RR in sheep which could have accounted for the apparent differences in wool growth. The lack of a breed effect in this trial seems to invalidate the argument that differences in the flow of digest in the RR could be responsible for lower intake in the Holsteins.

## SUMMARY AND CONCLUSION.

The lower ADG and intake in Holsteins compared to Herefords obtained in this trial are consistent with the results obtained by Horne (1996). Review of literature however showed that most comparative experiments between Holsteins and a range of beef breeds show that the Holsteins generally have higher intake, although with lower efficiency of gain .

Results obtained in this trial suggest that the rate of passage is not a limiting factor on intake in Holsteins. The nutrient composition of kikuyu from this trial and from literature cited in chapter 2. Shows that there are no marked deficiencies nor toxicity that could have differential effects on intake. The excess nitrogen which has been shown to affect on well fertilised pastures has not been shown to have differential effects on intake among different breeds.

The limitations on intake in Holsteins are thus likely to be inherent in the animal. While rate of passage in the rumen is an important factor in determining feed intake, the apparent disparity in intake is large enough to suggest there are factors severely restricting intake. It is possible that the gut capacity in Holstein heifers is limiting, and it may be necessary to estimate either rumen volume or gut weight. Differences in gut capacity are however difficult to explain given that selection for high milk yield should indirectly select for increased intake capacity. If there are such limitations in gut capacity, differences in foraging behaviour would also be expected, with more frequent but shorter grazing periods. There is no evidence of such differences in grazing behaviour between Holsteins and Herefords.

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# APPENDICES.

## Appendix 1a. Disc metre readings and available herbage over the trial period.

| Date | 20/12 | | | | | | 27/12 | | | | | | 3/1 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 6 | 5 | 1 | 5 | 4 | 7 | 3 | 4 | 1 | 1 | 3 | 12 | 4 | 3 | 1 | 3 | 9 |
| | 2 | 5 | 6 | 0 | 3 | 5 | 4 | 3 | 7 | 1 | 2 | 9 | 4 | 6 | 14 | 2 | 2 | 4 |
| | 5 | 5 | 8 | 4 | 2 | 6 | 14 | 6 | 7 | 2 | 8 | 3 | 9 | 11 | 6 | 3 | 2 | 3 |
| | 1 | 4 | 9 | 1 | 3 | 6 | 5 | 5 | 12 | 3 | 7 | 1 | 11 | 9 | 2 | 3 | 2 | 4 |
| | 2 | 7 | 5 | 9 | 1 | 3 | 6 | 12 | 5 | 1 | 2 | 7 | 3 | 10 | 2 | 5 | 1 | 6 |
| | 2 | 5 | 10 | 3 | 3 | 6 | 7 | 8 | 9 | 3 | 2 | 3 | 4 | 17 | 1 | 9 | 12 | 7 |
| | 5 | 2 | 6 | 1 | 2 | 7 | 8 | 7 | 4 | 8 | 10 | 3 | 2 | 9 | 1 | 2 | 6 | 5 |
| | 2 | 2 | 8 | 4 | 3 | 2 | 4 | 9 | 5 | 5 | 3 | 3 | 8 | 7 | 5 | 2 | 2 | 3 |
| | 6 | 7 | 11 | 4 | 7 | 4 | 4 | 8 | 8 | 3 | 5 | 2 | 4 | 11 | 3 | 2 | 2 | 5 |
| | 7 | 5 | 7 | 6 | 4 | 3 | 6 | 9 | 7 | 3 | 5 | 4 | 7 | 5 | 8 | 2 | 0 | 9 |
| | 5 | 4 | 7 | 3 | 3 | 5 | 5 | 8 | 2 | 1 | 8 | 4 | 8 | 9 | 5 | 1 | 3 | 7 |
| | 7 | 5 | 9 | 4 | 1 | 5 | 9 | 4 | 9 | 3 | 3 | 9 | 6 | 7 | 10 | 3 | 3 | 4 |
| | 9 | 4 | 2 | 3 | 8 | 6 | 4 | 2 | 10 | 4 | 3 | 7 | 6 | 7 | 7 | 1 | 4 | 3 |
| | 8 | 13 | 8 | 3 | 4 | 6 | 8 | 7 | 11 | 3 | 1 | 3 | 4 | 4 | 7 | 3 | 2 | 5 |
| | 8 | 4 | 5 | 2 | 5 | 3 | 4 | 8 | 1 | 2 | 3 | 3 | 7 | 6 | 10 | 5 | 3 | 4 |
| | 7 | 3 | 5 | 3 | 4 | 2 | 3 | 8 | 12 | 3 | 3 | 4 | 6 | 2 | 7 | 1 | 2 | 2 |
| | 6 | 8 | 5 | 3 | 2 | 3 | 8 | 2 | 14 | 9 | 2 | 1 | 3 | 9 | 10 | 1 | 8 | 6 |
| | 7 | 6 | 3 | 3 | 1 | 1 | 5 | 4 | 8 | 4 | 2 | 5 | 3 | 11 | 6 | 3 | 4 | 4 |
| | 2 | 13 | 3 | 3 | 1 | 3 | 4 | 5 | 4 | 4 | 7 | 8 | 2 | 8 | 3 | 1 | 7 | 8 |
| | 5 | 3 | 4 | 4 | 2 | 4 | 3 | 6 | 4 | 2 | 2 | 6 | 1 | 8 | 8 | 0 | 7 | 4 |
| | 4 | 10 | 2 | 4 | 2 | 4 | 7 | 3 | 3 | 2 | 3 | 14 | 4 | 5 | 10 | 3 | 9 | 2 |
| | 5 | 1 | 4 | 2 | 6 | 3 | 14 | 13 | 5 | 3 | 4 | 9 | 4 | 6 | 7 | 4 | 2 | 3 |
| | 7 | 6 | 6 | 2 | 3 | 5 | 6 | 7 | 3 | 0 | 4 | 7 | 6 | 7 | 6 | 2 | 6 | 2 |
| | 2 | 5 | 3 | 2 | 2 | 6 | 0 | 3 | 3 | 4 | 0 | 6 | 5 | 8 | 4 | 7 | 11 | 3 |
| | 5 | 8 | 3 | 2 | 7 | 5 | 3 | 5 | 22 | 2 | 1 | 4 | 5 | 6 | 6 | 4 | 3 | 6 |
| | 14 | 2 | 10 | 2 | 3 | 3 | 3 | 9 | 10 | 0 | 4 | 3 | 5 | 17 | 1 | 3 | 7 | 2 |
| | 3 | 7 | 7 | 4 | 3 | 11 | 5 | 10 | 6 | 6 | 4 | 8 | 3 | 7 | 3 | 1 | 8 | 3 |
| | 7 | 4 | 12 | 2 | 5 | 3 | 5 | 5 | 8 | 2 | 1 | 5 | 4 | 15 | 4 | 1 | 7 | 2 |
| | 3 | 3 | 9 | 2 | 4 | 3 | 2 | 9 | 13 | 3 | 2 | 4 | 4 | 7 | 2 | 2 | 2 | 6 |
| | 3 | 7 | 12 | 2 | 5 | 4 | 3 | 11 | 4 | 2 | 3 | 7 | 8 | 5 | 5 | 6 | 7 | 6 |
| | 3 | 4 | 6 | 3 | 7 | 7 | 5 | 11 | 13 | 0 | 7 | 3 | 3 | 10 | 1 | 3 | 2 | 6 |
| | 5 | 3 | 11 | 2 | 4 | 4 | 14 | 6 | 5 | 1 | 9 | 6 | 5 | 7 | 3 | 4 | 7 | 3 |
| | 5 | 5 | 8 | 2 | 3 | 3 | 3 | 9 | 2 | 1 | 3 | 5 | 3 | 11 | 3 | 2 | 7 | 4 |
| | 3 | 3 | 6 | 2 | 3 | 7 | 3 | 5 | 2 | 1 | 4 | 6 | 7 | 7 | 6 | 1 | 8 | 4 |
| | 8 | 6 | 3 | 3 | 5 | 0 | 4 | 3 | 2 | 1 | 2 | 6 | 4 | 8 | 2 | 0 | 10 | 2 |
| | 9 | 3 | 1 | 2 | 4 | 5 | 2 | 5 | 1 | 3 | 2 | 4 | 6 | 6 | 4 | 4 | 1 | 4 |
| | 7 | 6 | 1 | 2 | 2 | 4 | 4 | 6 | 2 | 5 | 5 | 8 | 10 | 5 | 3 | 7 | 2 | 3 |
| | 8 | 12 | 3 | 3 | 4 | 9 | 3 | 1 | 3 | 3 | 6 | 7 | 7 | 9 | 4 | 2 | 1 | 6 |
| | 8 | 7 | 5 | 2 | 11 | 8 | 1 | 3 | 7 | 6 | 11 | 5 | 7 | 2 | 5 | 1 | 2 | 9 |
| | 10 | 6 | 5 | 3 | 3 | 5 | 3 | 7 | 6 | 5 | 8 | 8 | 6 | 7 | 11 | 3 | 5 | 10 |
| | 8 | 10 | 7 | 2 | 3 | 4 | 7 | 7 | 2 | 6 | 7 | 1 | 8 | 6 | 11 | 6 | 9 | 8 |
| | 5 | 6 | 4 | 5 | 3 | 6 | 2 | 6 | 3 | 3 | 3 | 1 | 5 | 0 | 9 | 2 | 5 | 3 |
| | 6 | 11 | 4 | 1 | 2 | 3 | 7 | 4 | 4 | 5 | 2 | 3 | 7 | 3 | 7 | 2 | 6 | 1 |
| | 7 | 19 | 11 | 2 | 3 | 2 | 5 | 4 | 4 | 2 | 4 | 6 | 8 | 8 | 4 | 0 | 14 | 7 |
| | 10 | 3 | 8 | 0 | 5 | 4 | 7 | 1 | 6 | 4 | 5 | 5 | 13 | 3 | 8 | 3 | 15 | 12 |
| | 11 | 6 | 2 | 0 | 2 | 3 | 13 | 1 | 5 | 5 | 4 | 3 | 6 | 1 | 0 | 1 | 6 | 2 |
| | 1 | 8 | 4 | 3 | 6 | 8 | 8 | 2 | 3 | 2 | 3 | 8 | 5 | 5 | 7 | 2 | 10 | 6 |
| | 0 | 8 | 1 | 3 | 3 | 4 | 7 | 5 | 1 | 3 | 2 | 3 | 6 | 2 | 7 | 2 | 7 | 5 |
| | 1 | 8 | 2 | 3 | 5 | 9 | 7 | 13 | 8 | 2 | 3 | 3 | 11 | 5 | 7 | 2 | 6 | 6 |
| | 12 | 4 | 6 | 3 | 5 | 3 | 7 | 10 | 4 | 4 | 4 | 16 | 14 | 2 | 3 | 2 | 6 | 9 |
| Mean(cm) | 4.8 | | | | | | 5.0 | | | | | | 5.2 | | | | | |
| DM (kg/ha) | 1580.9 | | | | | | 1605.7 | | | | | | 1660.6 | | | | | |
| SE | 155.8 | | | | | | 153.8 | | | | | | 153.8 | | | | | |

## Appendix 1a(Cont). Disc metre readings and available herbage over the trial period.

| Date | 10/1 | | | | | | 17/1 | | | | | | 8/2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8 | 4 | 6 | 0 | 3 | 2 | 6 | 8 | 9 | 0 | 3 | 3 | 7 | 10 | 12 | 5 | 5 |
| | 4 | 6 | 6 | 1 | | 1 | 6 | 7 | 12 | 2 | 3 | 4 | 8 | 12 | 13 | 3 | 7 |
| | 8 | 7 | 3 | 3 | 2 | 5 | 12 | 6 | 2 | 1 | 9 | 2 | 8 | 7 | 7 | 0 | 9 |
| | 3 | 7 | 6 | 2 | 1 | 2 | 3 | 4 | 13 | 7 | 2 | 3 | 4 | 8 | 5 | 3 | 3 |
| | 9 | 7 | 2 | 0 | 0 | 6 | 4 | 4 | 13 | 8 | 2 | 2 | 13 | 12 | 4 | 9 | 7 |
| | 5 | 7 | 2 | 2 | 2 | 3 | 7 | 6 | 7 | 13 | 5 | 7 | 14 | 5 | 3 | 14 | 5 |
| | 5 | 8 | 5 | 6 | 1 | 4 | 3 | 3 | 5 | 4 | 2 | 9 | 7 | 11 | 5 | 19 | 9 |
| | 7 | 8 | 4 | 3 | 2 | 2 | 5 | 2 | 5 | 6 | 3 | 8 | 8 | 9 | 5 | 15 | 6 |
| | 6 | 8 | 6 | 3 | 1 | 2 | 10 | 4 | 8 | 7 | 4 | 6 | 12 | 7 | 4 | 7 | 3 |
| | 10 | 4 | 4 | 5 | 3 | 1 | 9 | 4 | 4 | 5 | 1 | 5 | 10 | 5 | 6 | 7 | 5 |
| | 2 | 4 | 6 | 0 | 2 | 2 | 4 | 7 | 5 | 8 | 4 | 4 | 11 | 3 | 5 | 5 | 3 |
| | 5 | 4 | 5 | 0 | 1 | 4 | 8 | 9 | 2 | 11 | 10 | 8 | 18 | 6 | 9 | 9 | 7 |
| | 7 | 7 | 5 | 3 | 4 | 7 | 9 | 4 | 4 | 3 | 2 | 3 | 17 | 8 | 7 | 7 | 9 |
| | 12 | 6 | 7 | 3 | 8 | 4 | 1 | 5 | 4 | 5 | 7 | 8 | 8 | 6 | 3 | 9 | 7 |
| | 6 | 3 | 3 | 4 | 2 | 4 | 4 | 8 | 2 | 8 | 7 | 10 | 12 | 3 | 7 | 11 | 4 |
| | 8 | 3 | 3 | 5 | 3 | 2 | 3 | 6 | 6 | 3 | 3 | 3 | 17 | 7 | 9 | 8 | 8 |
| | 4 | 5 | 15 | 4 | 4 | 5 | 6 | 6 | 6 | 7 | 5 | 4 | 13 | 4 | 4 | 11 | 6 |
| | 3 | 6 | 10 | 2 | 7 | 8 | 3 | 3 | 2 | 7 | 5 | 4 | 9 | 10 | 7 | 5 | 5 |
| | 9 | 4 | 8 | 2 | 2 | 10 | 1 | 7 | 6 | 4 | 7 | 2 | 14 | 9 | 10 | 5 | 17 |
| | 6 | 8 | 6 | 7 | 0 | 7 | 2 | 4 | 2 | 4 | 4 | 7 | 13 | 6 | 8 | 4 | 9 |
| | 2 | 9 | 13 | 1 | 3 | 7 | 7 | 4 | 7 | 3 | 3 | 6 | 8 | 5 | 20 | 11 | 7 |
| | 2 | 6 | 7 | 2 | 6 | 3 | 2 | 10 | 9 | 4 | 3 | 5 | 11 | 6 | 9 | 13 | 7 |
| | 2 | 6 | 4 | 3 | 7 | 1 | 3 | 3 | 14 | 3 | 6 | 5 | 4 | 7 | 12 | 7 | 4 |
| | 3 | 5 | 0 | 3 | 4 | 3 | 2 | 4 | 14 | 4 | 2 | 5 | 5 | 15 | 11 | 6 | 4 |
| | 1 | 6 | 4 | 3 | 2 | 3 | 3 | 9 | 3 | 4 | 2 | 5 | 12 | 6 | 7 | 7 | 3 |
| | 4 | 3 | 2 | 2 | 4 | 2 | 6 | 10 | 7 | 0 | 2 | 6 | 13 | 8 | 8 | 5 | 3 |
| | 4 | 3 | 5 | 3 | 2 | 2 | 9 | 9 | 10 | 4 | 4 | 4 | 6 | 12 | 6 | 9 | 3 |
| | 4 | 3 | 7 | 3 | 1 | 3 | 5 | 3 | 14 | 7 | 4 | 2 | 10 | 5 | 2 | 7 | 4 |
| | 2 | 7 | 8 | 2 | 1 | 8 | 2 | 2 | 13 | 2 | 4 | 2 | 10 | 9 | 5 | 6 | 8 |
| | 3 | 10 | 6 | 2 | 4 | 2 | 8 | 4 | 4 | 4 | 5 | 4 | 6 | 5 | 3 | 8 | 5 |
| | 4 | 5 | 2 | 2 | 2 | 6 | 4 | 4 | 4 | 6 | 2 | 2 | 5 | 11 | 6 | 5 | 5 |
| | 4 | 4 | 3 | 2 | 2 | 6 | 4 | 13 | 5 | 4 | 4 | 3 | 13 | 8 | 10 | 9 | 11 |
| | 5 | 4 | 8 | 2 | 1 | 4 | 5 | 5 | 6 | 3 | 1 | 2 | 13 | 8 | 9 | 9 | 4 |
| | 4 | 6 | 7 | 2 | 1 | 3 | 11 | 2 | 2 | 5 | 6 | 1 | 11 | 16 | 19 | 14 | 4 |
| | 6 | 6 | 5 | 2 | 1 | 2 | 5 | 4 | 5 | 1 | 7 | 9 | 18 | 9 | 11 | 9 | 4 |
| | 2 | 7 | 6 | 2 | 5 | 2 | 6 | 9 | 4 | 3 | 6 | 4 | 16 | 14 | 13 | 12 | 12 |
| | 3 | 5 | 6 | 4 | 2 | 3 | 6 | 4 | 2 | 3 | 4 | 4 | 29 | 9 | 9 | 5 | 14 |
| | 1 | 6 | 8 | 2 | 1 | 2 | 7 | 14 | 2 | 3 | 3 | 5 | 25 | 5 | 6 | 9 | 6 |
| | 3 | 9 | 8 | 2 | 3 | 4 | 8 | 4 | 6 | 4 | 5 | 2 | 18 | 11 | 4 | 7 | 11 |
| | 6 | 6 | 2 | 1 | 2 | 6 | 7 | 8 | 6 | 7 | 1 | 3 | 18 | 17 | 12 | 9 | 14 |
| | 9 | 7 | 9 | 1 | 4 | 6 | 7 | 4 | 3 | 0 | 7 | 5 | 24 | 9 | 7 | 5 | 5 |
| | 10 | 6 | 9 | 2 | 3 | 1 | 7 | 2 | 6 | 1 | 6 | 3 | 19 | 6 | 9 | 3 | 4 |
| | 9 | 5 | 8 | 5 | 9 | 4 | 10 | 2 | 3 | 5 | 2 | 6 | 5 | 10 | 5 | 3 | 11 |
| | 3 | 8 | 2 | 3 | 5 | 6 | 3 | 4 | 7 | 3 | 2 | 6 | 10 | 8 | 3 | 4 | 9 |
| | 1 | 8 | 4 | 3 | 4 | 5 | 7 | 5 | 10 | 0 | 4 | 7 | 20 | 11 | 11 | 5 | 6 |
| | 5 | 7 | 6 | 2 | 2 | 6 | 3 | 5 | 4 | 1 | 8 | 4 | 11 | 9 | 14 | 10 | 2 |
| | 7 | 6 | 3 | 1 | 2 | 3 | 7 | 8 | 3 | 1 | 8 | 6 | 12 | 9 | 8 | 5 | 5 |
| | 4 | 9 | 2 | 1 | 5 | 3 | 6 | 10 | 5 | 2 | 7 | 3 | 8 | 13 | 7 | 3 | 3 |
| | 6 | 7 | 4 | 3 | 2 | 3 | 4 | 7 | 5 | 1 | 6 | 2 | 15 | 12 | 5 | 7 | 4 |
| | 10 | 4 | 2 | 1 | 2 | 2 | 5 | 1 | * | 2 | 2 | 8 | 8 | 6 | 14 | 5 | 4 |
| Mean | 4.32 | | | | | | 5.0 | | | | | | 8.1 | | | | |
| DM (kg/ha) | 1495.9 | | | | | | 1545.4 | | | | | | 2565 | | | | |
| SE | 166.3 | | | | | | 153.8 | | | | | | 280.8 | | | | |

## Appendix 1a(Cont). Disc metre readings and estimated available herbage over the trial period.

| Date | 21/2 | | | | | | 7/3 | | | | | | 27/3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 10 | 6 | 2 | 5 | 7 | 7 | 14 | 11 | 7 | 3 | 11 | 5 | 15 | 3 | 5 | 7 | 14 |
| | 8 | 1 | 2 | 3 | 4 | 8 | 11 | 16 | 9 | 9 | 4 | 22 | 12 | 13 | 7 | 7 | 7 | 4 |
| | 14 | 7 | 5 | 4 | 7 | 7 | 10 | 11 | 9 | 7 | 4 | 3 | 15 | 12 | 6 | 5 | 4 | 22 |
| | 9 | 11 | 8 | 10 | 3 | 10 | 7 | 10 | 5 | 12 | 8 | 7 | 12 | 12 | 7 | 4 | 6 | 3 |
| | 16 | 8 | 6 | 9 | 8 | 5 | 13 | 11 | 10 | 9 | 9 | 18 | 20 | 8 | 10 | 21 | 2 | 13 |
| | 15 | 14 | 5 | 16 | 9 | 5 | 12 | 12 | 11 | 17 | 12 | 15 | 22 | 17 | 18 | 12 | 6 | 8 |
| | 5 | 6 | 9 | 14 | 8 | 8 | 15 | 14 | 5 | 17 | 4 | 1 | 16 | 9 | 5 | 30 | 4 | 13 |
| | 12 | 6 | 12 | 11 | 3 | 8 | 23 | 7 | 9 | 19 | 10 | 4 | 8 | 11 | 9 | 15 | 3 | 7 |
| | 12 | 3 | 3 | 9 | 4 | 14 | 14 | 8 | 7 | 18 | 5 | 12 | 7 | 12 | 7 | 20 | 11 | 15 |
| | 11 | 6 | 7 | 4 | 4 | 7 | 7 | 31 | 8 | 17 | 5 | 8 | 6 | 9 | 7 | 2 | 2 | 4 |
| | 10 | 12 | 3 | 20 | 5 | 7 | 7 | 15 | 3 | 18 | 4 | 10 | 7 | 9 | 4 | 7 | 5 | 12 |
| | 13 | 4 | 6 | 15 | 8 | 7 | 9 | 9 | 7 | 7 | 5 | 4 | 10 | 18 | 10 | 7 | 5 | 19 |
| | 8 | 5 | 9 | 7 | 8 | 4 | 4 | 14 | 11 | 7 | 7 | 7 | 6 | 15 | 7 | 9 | 5 | 26 |
| | 9 | 11 | 7 | 5 | 13 | 10 | 10 | 14 | 8 | 22 | 8 | 10 | 2 | 11 | 9 | 27 | 7 | 4 |
| | 18 | 4 | 14 | 8 | 7 | 11 | 7 | 13 | 7 | 7 | 6 | 8 | 14 | 8 | 7 | 7 | 9 | 3 |
| | 6 | 13 | 8 | 12 | 3 | 9 | 12 | 16 | 5 | 19 | 7 | 6 | 4 | 11 | 7 | 7 | 7 | 2 |
| | 8 | 10 | 9 | 12 | 2 | 5 | 12 | 9 | 11 | 15 | 9 | 7 | 9 | 7 | 5 | 6 | 6 | 16 |
| | 16 | 8 | 10 | 8 | 3 | 8 | 16 | 7 | 17 | 15 | 7 | 5 | 18 | 9 | 5 | 10 | 5 | 6 |
| | 3 | 7 | 10 | 8 | 3 | 4 | 5 | 11 | 15 | 9 | 22 | 6 | 11 | 7 | 7 | 13 | 10 | 8 |
| | 9 | 6 | 12 | 10 | 5 | 8 | 4 | 7 | 9 | 7 | 18 | 4 | 7 | 12 | 12 | 18 | 6 | 8 |
| | 7 | 6 | 6 | 6 | 4 | 4 | 10 | 18 | 3 | 4 | 7 | 8 | 24 | 7 | 5 | 11 | 11 | 9 |
| | 7 | 8 | 4 | 5 | 13 | 5 | 13 | 7 | 14 | 12 | 3 | 16 | 7 | 12 | 7 | 3 | 8 | 8 |
| | 2 | 11 | 8 | 4 | 3 | 9 | 12 | 8 | 24 | 7 | 7 | 12 | 11 | 16 | 6 | 6 | 9 | 15 |
| | 11 | 12 | 2 | 2 | 5 | 8 | 12 | 9 | 7 | 8 | 3 | 18 | 18 | 14 | 6 | 6 | 7 | 9 |
| | 8 | 9 | 9 | 3 | 5 | 5 | 9 | 14 | 9 | 10 | 16 | 7 | 15 | 7 | 7 | 2 | 7 | 2 |
| | 10 | 9 | 8 | 2 | 4 | 18 | 12 | 6 | 3 | 7 | 21 | 6 | 12 | 7 | 7 | 11 | 8 | 11 |
| | 5 | 13 | 4 | 2 | 5 | 9 | 11 | 5 | 7 | 4 | 13 | 9 | 11 | 9 | 17 | 12 | 9 | 7 |
| | 8 | 5 | 10 | 4 | 4 | 5 | 13 | 18 | 5 | 4 | 10 | 7 | 13 | 14 | 11 | 4 | 24 | 3 |
| | 5 | 2 | 6 | 3 | 4 | 7 | 7 | 20 | 14 | 5 | 6 | 9 | 12 | 15 | 16 | 4 | 7 | 4 |
| | 6 | 14 | 2 | 3 | 4 | 7 | 8 | 7 | 13 | 13 | 5 | 9 | 7 | 11 | 7 | 2 | 10 | 7 |
| | 12 | 8 | 6 | 1 | 5 | 8 | 11 | 11 | 11 | 4 | 13 | 13 | 10 | 8 | 11 | 3 | 7 | 8 |
| | 9 | 6 | 9 | 2 | 6 | 4 | 7 | 18 | 6 | 3 | 12 | 19 | 14 | 10 | 2 | 7 | 6 | 7 |
| | 6 | 4 | 12 | 2 | 5 | 6 | 12 | 9 | 6 | 12 | 3 | 14 | 14 | 12 | 10 | 7 | 4 | 6 |
| | 15 | 10 | 13 | 4 | 0 | 4 | 17 | 4 | 14 | 9 | 8 | 5 | 10 | 7 | 6 | 16 | 16 | 5 |
| | 8 | 3 | 6 | 2 | 6 | 4 | 8 | 11 | 7 | 4 | 12 | 23 | 18 | 14 | 17 | 8 | 13 | 3 |
| | 8 | 3 | 3 | 5 | 4 | 4 | 11 | 11 | 4 | 3 | 9 | 6 | 17 | 10 | 19 | 7 | 11 | 8 |
| | 7 | 6 | 3 | 15 | 3 | 20 | 13 | 6 | 7 | 19 | 9 | 20 | 18 | 11 | 14 | 4 | 10 | 2 |
| | 12 | 11 | 8 | 2 | 2 | 10 | 13 | 7 | 12 | 5 | 14 | 5 | 10 | 7 | 4 | 7 | 9 | 9 |
| | 4 | 10 | 8 | 2 | 8 | 5 | 2 | 11 | 3 | 4 | 7 | 5 | 12 | 12 | 7 | 5 | 9 | 11 |
| | 4 | 11 | 5 | 9 | 3 | 11 | 11 | 5 | 7 | 7 | 7 | 14 | 11 | 10 | 5 | 8 | 12 | 6 |
| | 7 | 11 | 6 | 3 | 7 | 14 | 16 | 13 | 17 | 15 | 13 | 9 | 7 | 9 | 3 | 5 | 9 | 5 |
| | 3 | 9 | 5 | 4 | 7 | 19 | 15 | 11 | 19 | 3 | 14 | 3 | 9 | 2 | 8 | 5 | 12 | 11 |
| | 11 | 7 | 2 | 4 | 7 | 19 | 9 | 15 | 17 | 7 | 7 | 10 | 11 | 7 | 7 | 8 | 12 | 15 |
| | 8 | 9 | 4 | 6 | 6 | 6 | 8 | 8 | 7 | 3 | 7 | 10 | 12 | 3 | 14 | 4 | 7 | 8 |
| | 12 | 5 | 1 | 3 | 12 | 6 | 9 | 14 | 12 | 3 | 6 | 7 | 8 | 8 | 8 | 6 | 15 | 7 |
| | 8 | 5 | 7 | 3 | 9 | 3 | 12 | 9 | 11 | 6 | 7 | 6 | 7 | 9 | 5 | 5 | 15 | 20 |
| | 8 | 5 | 14 | 4 | 4 | 8 | 10 | 11 | 13 | 3 | 6 | 9 | 4 | 8 | 9 | 6 | 9 | 11 |
| | 7 | 4 | 0 | 4 | 1 | 6 | 7 | 5 | 8 | 4 | 7 | 6 | 14 | 6 | 13 | 9 | 23 | 8 |
| | 11 | 7 | 2 | 9 | 4 | 5 | 5 | 5 | 14 | 11 | 24 | 7 | 9 | 9 | 6 | 3 | 10 | 9 |
| | 4 | 5 | 6 | 3 | 3 | 4 | 10 | 8 | 4 | 17 | 13 | 9 | 9 | 4 | 7 | 7 | 27 | 18 |
| Mean | 7.7 | | | | | | 10.3 | | | | | | 9.9 | | | | | |
| DM kg/ha | 2076.6 | | | | | | 2768.7 | | | | | | 2659 | | | | | |
| SE | 199.9 | | | | | | 437.1 | | | | | | 406 | | | | | |

## Appendix 1b. Disc metre calibration.

| Sample | Metre Reading | DM |
|---|---|---|
| 1 | 3 | 36 |
| 5 | 10 | 84 |
| 6 | 12 | 122 |
| 8 | 13 | 90 |
| 11 | 6 | 64 |
| 13 | 12 | 70 |
| 14 | 2 | 39 |
| 16 | 11 | 59 |
| 17 | 7 | 58 |
| 21 | 2 | 61 |
| 31 | 4 | 58 |
| 34 | 6 | 42 |
| 46 | 8 | 58 |
| 50 | 3 | 42 |
| 51 | 6 | 56 |
| 58 | 7 | 78 |
| 59 | 6 | 47 |
| 3 | 9 | 69 |
| 7 | 5 | 90 |
| 12 | 4 | 58 |
| 18 | 3 | 30 |
| 20 | 1 | 24 |
| 23 | 2 | 21 |
| 27 | 3 | 40 |
| 28 | 4 | 41 |
| 38 | 3 | 28 |
| 36 | 3 | 64 |
| 39 | 3 | 22 |
| 40 | 8 | 52 |
| 41 | 5 | 47 |
| 43 | 3 | 51 |
| 48 | 1 | 32 |
| 49 | 5 | 59 |
| 52 | 4 | 46 |
| 53 | 1 | 24 |
| 54 | 1 | 27 |
| N=36 | 5.13 | |

## Appendix 1c. Regression equations for Disc metre calibration.

| SOUTH | | NORTH | |
|---|---|---|---|
| Regression Output: | | Regression Output: | |
| Constant | 31.95 | Constant | 23.01 |
| Std Err of Y Est | 15.22 | Std Err of Y Est | 14.20 |
| R Squared | 0.45 | R Squared | 0.47 |
| No. of Observations | 17 | No. of Observations | 19 |
| Degrees of Freedom | 15 | Degrees of Freedom | 17 |
| X Coefficient (s) | 4.44 | X Coefficient (s) | 5.7 9 |
| Std Err of Coef. | 1.06 | Std Err of Coef. | 1.51 |

OVERALL

| Regression Output: | |
|---|---|
| Constant | 26.61 |
| Std Err of Y Est | 14.42 |
| R Squared | 0.59 |
| No. of Observations | 36 |
| Degrees of Freedom | 34 |
| X Coefficient (s) | 5.03 |
| Std Err of Coef. | 0.72 |

**Appendix 1d. Calculation of the standard error of the herbage estimates.**

The standard error of the available herbage was estimated using the formula by Rayner (1967):

$$SE = RSD\sqrt{\frac{1}{m}+\frac{1}{n}+\frac{(x-X)^2}{\Sigma(X-X)^2}}$$

where m is the number of disc metre measurements (300) n is the number of calibration points in the regression equations (36), x is the mean disc metre height of estimation points, X is the mean disc height of calibration points (5.13) and RSD is the SE of the Y estimate in the regression equation (14.42).

**Appendix 2. One-Way Analysis of Variance for concentrate intake.**

Analysis of Variance Table

| Source | DF | SS | MS | F | p |
|---|---|---|---|---|---|
| Breed | 1 | 144196 | 144196 | 5.88 | 0.052 |
| Error | 6 | 147249 | 24541 | | |
| Total | 7 | 291445 | | | |

| Level | N | Mean | StDev |
|---|---|---|---|
| 1 | 4 | 535.5 | 194.2 |
| 2 | 4 | 267.0 | 106.7 |

Pooled StDev = 156.7

Two Sample T-Test and Confidence Interval

Two-sample T for Holsteins vs Herefords

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| Holsteins | 4 | 267 | 107 | 53 |
| Herefords | 4 | 536 | 194 | 97 |

T-Test mu Holstein = mu Hford (vs not =): T= -2.42 P=0.072 DF= 4

Conclusion.

The significance of the t-ratio means that the Herefords had higher concentrate intake than the Holsteins.

## Appendix 3a. Body weight measurements(kg) during T1.

JERSEYS

| Animal No. | INTEREST | INTRO | DOLPHIN | ISMAY | ILVA | DAMASK | DONGA |
|---|---|---|---|---|---|---|---|
| Date | | | | | | | |
| 13/12 | 260 | 236 | 200 | 210 | 225 | 210 | 202 |
| 20/12 | 260 | 235 | 215 | 207 | 232 | 218 | 204 |
| 27/12 | 254 | 235 | 210 | 200 | 229 | 210 | 200 |
| 3/1 | 260 | 232 | 209 | 199 | 231 | 211 | 201 |
| 10/1 | 266 | 240 | 209 | 207 | 237 | 215 | 206 |
| 25/1 | 270 | 240 | 219 | 215 | 230 | 192 | 215 |

HEREFORDS

| Animal No. | H69 | H18 | H62 | HNM | H12 | H24 | H9 | H63 |
|---|---|---|---|---|---|---|---|---|
| 13/12 | 272 | 276 | 251 | 270 | 252 | 300 | 270 | 316 |
| 20/12 | 276 | 282 | 251 | 275 | 256 | 312 | 275 | 321 |
| 27/12 | 281 | 291 | 260 | 279 | 260 | 315 | 281 | 330 |
| 3/1 | 294 | 301 | 268 | 292 | 265 | 326 | 285 | 332 |
| 10/1 | 305 | 310 | 275 | 306 | 279 | 340 | 300 | 345 |
| 25/1 | 325 | 320 | 300 | 325 | 295 | 350 | 320 | 365 |

HOLSTEINS

| Animal No. | F29 | F33 | F34 | F28 | F26 | F30 | F31 | F32 |
|---|---|---|---|---|---|---|---|---|
| Date | | | | | | | | |
| 13/12 | 335 | 245 | 240 | 325 | 335 | 280 | 270 | 290 |
| 20/12 | 330 | 240 | 238 | 341 | 335 | 289 | 274 | 290 |
| 27/12 | 335 | 244 | 245 | 351 | 335 | 295 | 282 | 290 |
| 3/1 | 342 | 235 | 250 | 355 | 344 | 293 | 282 | 287 |
| 10/1 | 350 | 244 | 250 | 367 | 350 | 310 | 290 | 296 |
| 25/1 | 360 | 255 | 245 | 370 | 365 | 310 | 290 | 300 |

## Appendix 3b. Body weight measurements(kg) during T1.

HEREFORDS

| Animal No. | H69 | H18 | H62 | HNM | H12 | H24 | H9 | H63 |
|---|---|---|---|---|---|---|---|---|
| Date | | | | | | | | |
| 21/2 | 330 | 335 | 315 | 340 | 320 | 375 | 340 | 375 |
| 28/2 | 335 | 343 | 325 | 350 | 329 | 385 | 347 | 395 |
| 6/3 | 335 | 350 | 330 | 355 | 327 | 392 | 345 | 405 |
| 14/3 | 345 | 351 | 335 | 360 | 337 | 400 | 356 | 410 |
| 27/3 | 344 | 365 | 340 | 368 | 343 | 415 | 362 | 408 |
| 3/4 | 347 | 370 | 345 | 375 | 350 | 420 | 367 | 422 |

HOLSTEINS

| Animal No. | F58 | F33 | F34 | F54 | F61 | F53 | F31 | F32 |
|---|---|---|---|---|---|---|---|---|
| Date | | | | | | | | |
| 21/2 | 310 | 255 | 265 | 370 | 320 | 385 | 300 | 300 |
| 28/2 | 325 | 262 | 280 | 386 | 343 | 405 | 310 | 310 |
| 6/3 | 320 | 275 | 285 | 385 | 330 | 395 | 315 | 310 |
| 14/3 | 320 | 275 | 293 | 400 | 342 | 405 | 317 | 320 |
| 27/3 | 320 | 275 | 295 | 395 | 340 | 405 | 323 | 325 |
| 3/4 | 329 | 285 | 305 | 400 | 345 | 420 | 325 | 330 |

**Appendix 4a. One-Way Analysis of Variance for T1 weight change (kg/100kgLW/day).**

Analysis of Variance.

| Source | DF | SS | MS | F | p |
|---|---|---|---|---|---|
| BREED | 2 | 0.2381 | 0.1190 | 11.89 | 0.000 |
| Error | 19 | 0.1902 | 0.0100 | | |
| Total | 21 | 0.4283 | | | |

| Level | N | Mean | StDev |
|---|---|---|---|
| 1 | 6 | 0.0901 | 0.0385 |
| 2 | 8 | 0.3406 | 0.1445 |
| 3 | 8 | 0.1661 | 0.0724 |

The significance of the F-Value means there were breed differences in growth.

**T-test for differences between means (assuming equal variance).**

**Jerseys vs Herefords**

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| Jerseys | 6 | 0.0901 | 0.0385 | 0.016 |
| Hfords | 8 | 0.341 | 0.144 | 0.051 |

T-Test mu Jerseys = mu Hfords (vs not =): T= -4.10 P=0.0015 DF= 12

Critical $t_{(12)} = 3.055$, therefore the Herefords had higher ADG compared to the Jerseys.

**Jerseys vs Holsteins.**

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| Jerseys | 6 | 0.0901 | 0.0385 | 0.016 |
| Holstein | 8 | 0.1661 | 0.0724 | 0.026 |

T-Test mu Jerseys = mu Holstein (vs not =): T= -2.32 P=0.039 DF= 12

Conclusion.

Critical $t_{(12)} = 2.2179$, therefore there was no difference ingrowth between the Jerseys and the Holsteins.

**Herefords vs Holsteins.**

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| Hfords | 8 | 0.341 | 0.144 | 0.051 |
| Holstein | 8 | 0.1661 | 0.0724 | 0.026 |

T-Test mu Hfords = mu Holstein (vs not =): T= 3.05 P=0.0086 DF= 14

Conclusion.

Critical $t_{(14)} = 2.997$ therefore the Herefords had higher ADG compared to the Holsteins.

**Appendix 4b. General Linear Model for weight change in T2 using MINITAB.**

```
Factor Levels    Values
DIET         2     1     2
BREED        2     1     2

Analysis of Variance.

Source                DF     Seq SS     Adj SS     Adj MS       F      P
      DIET             1   0.000922   0.000624   0.000624    0.29  0.601
      BREED            1   0.000549   0.000724   0.000724    0.34  0.573
DIET2*BREED            1   0.002127   0.002127   0.002127    0.99  0.341
      Error           11   0.023612   0.023612   0.002147
      Total           14   0.027209

Treatment Means.

DIET2           Mean     Stdev
    1           0.1800   0.01638
    2           0.1930   0.01769
BREED
    1           0.1935   0.01638
    2           0.1795   0.01769
DIET2*BREED
    1      1    0.1750   0.02317
    1      2    0.1850   0.02317
    2      1    0.2120   0.02317
    2      2    0.1740   0.02675
```

Lack of significants of the F-values means than neither breed, diet nor the interaction was significant.

## Appendix 5. Test for differences in ADG in T1 and T2.

Herefords:

$$t_{(30)} = \frac{0.341 - 0.194}{\sqrt{0.051^2 + 0.00579^2}}$$

Conclusion.

The calculated t value 2.86 is significant ($p<0.01$) ie, there was a higher ADG in T2 compared to T1.

Holsteins

$$t_{(30)} = \frac{.1795.1661}{\sqrt{0.026^2 + .0.0625^2}}$$

Conclusion.

The calculated t value of 0.198 is not significant, ie, there was no change in ADG in Holsteins in T2 compared to T1.

## Appendix 6a. Height measurements(cm) during T1.

| JERSEYS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Animal No. | INTEREST | INTRO | DOLPHIN | ISMAY | ILVA | DAMASK | DONGA | |
| Date | | | | | | | | |
| 13/12 | 104 | 104 | 105 | 105 | 108 | 107 | 105 | |
| 20/12 | 112 | 109 | 106 | 105 | 111 | 104 | 110 | |
| 27/12 | 118 | 113 | 111 | 113 | 110 | 106 | 117 | |
| 3/1 | 109 | 110 | 109 | 102 | 109 | 110 | 108 | |
| 10/1 | 109 | 110 | 111 | 108 | 108 | 110 | 108 | |
| 17/1 | 106 | 109 | 112 | 109 | 112 | 110 | 107 | |
| 25/1 | 110 | 110 | 107 | 106 | 109 | 111 | 108 | |
| HEREFORDS | H69 | H18 | H62 | HNM | H12 | H24 | H9 | H63 |
| 13/12 | 103 | 105 | 105 | 106 | 106 | 109 | 102 | 107 |
| 20/12 | 109 | 109 | 102 | 109 | 107 | 113 | 111 | 111 |
| 27/12 | 111 | 118 | 115 | 113 | 112 | 110 | 109 | 106 |
| 3/1 | 108 | 107 | 104 | 108 | 105 | 109 | 108 | 111 |
| 10/1 | 110 | 106 | 104 | 108 | 106 | 110 | 110 | 113 |
| 17/1 | 110 | 108 | 105 | 109 | 106 | 110 | 109 | 109 |
| 25/1 | 111 | 108 | 109 | 110 | 109 | 114 | 113 | 113 |
| HOLSTEINS | F29 | F33 | F34 | F28 | F26 | F30 | F31 | F32 |
| 13/12 | 114 | 109 | 110 | 126 | 123 | 118 | 113 | 112 |
| 20/12 | 120 | 111 | 113 | 127 | 122 | 112 | 120 | 117 |
| 27/12 | 117 | 118 | 115 | 127 | 121 | 119 | 110 | 117 |
| 3/1 | 122 | 112 | 112 | 127 | 122 | 121 | 113 | 115 |
| 10/1 | 122 | 114 | 111 | 128 | 121 | 120 | 114 | 110 |
| 17/1 | 122 | 113 | 109 | 128 | 128 | 119 | 119 | 115 |
| 25/1 | 122 | 110 | 111 | 128 | 122 | 122 | 117 | 117 |

## Appendix 6b. Height measurements(cm) during T2.

| HEREFORDS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Animal No. | H69 | H18 | H62 | HNM | H12 | H24 | H9 | H63 |
| Date | | | | | | | | |
| 21/2 | 111 | 110 | 109 | 113 | 110 | 114 | 113 | 115 |
| 28/2 | 112 | 111 | 110 | 114 | 109 | 114 | 111 | 115 |
| 6/3 | 111 | 114 | 112 | 111 | 110 | 114 | 114 | 114 |
| 14/3 | 114 | 112 | 111 | 110 | 109 | 115 | 113 | 111 |
| 27/3 | 110 | 111 | 108 | 110 | 108 | 114 | 111 | 113 |
| 3/4 | 110 | 109 | 111 | 111 | 111 | 114 | 114 | 115 |
| HOLSTEINS | F58 | F33 | F34 | F54 | F61 | F53 | F31 | F32 |
| 21/2 | 124 | 114 | 114 | 129 | 118 | 134 | 116 | 115 |
| 28/2 | 128 | 112 | 112 | 127 | 118 | 133 | 114 | 119 |
| 6/3 | 128 | 114 | 111 | 128 | 120 | 131 | 118 | 118 |
| 14/3 | 128 | 117 | 114 | 125 | 122 | 128 | 114 | 119 |
| 27/3 | 127 | 112 | 113 | 125 | 120 | 132 | 114 | 116 |
| 3/4 | 126 | 113 | 114 | 128 | 122 | 133 | 117 | 118 |

**Appendix 7a. Regression analysis for change in height in T1. (Genstat 1995).**

***** Regression Analysis for Jerseys *****

Response variate: Height
Fitted terms : Constant + week + Animal + week.Animal

*** Summary of analysis ***

| | d.f. | s.s. | m.s. | v.r. |
|---|---|---|---|---|
| Regression | 11 | 61.1 | 5.56 | 0.46 |
| Residual | 30 | 364.0 | 12.13 | |
| Total | 41 | 425.1 | 10.37 | |
| Change | -5 | -16.3 | 3.27 | 0.27 |

Residual variance exceeds variance of Y variate
Standard error of observations is estimated to be 3.48

*** Estimates of regression coefficients ***

| | estimate | s.e. | t (30) | t pr. |
|---|---|---|---|---|
| Constant | 110.04 | 2.37 | 46.36 | <.001 |
| weekm | -0.107 | 0.658 | -0.16 | 0.872 |
| Animal 2 | -2.36 | 3.36 | -0.70 | 0.488 |
| Animal 3 | -3.25 | 3.36 | -0.97 | 0.341 |
| Animal 4 | -3.82 | 3.36 | -1.14 | 0.264 |
| Animal 5 | -0.79 | 3.36 | -0.23 | 0.817 |
| Animal 6 | -0.39 | 3.36 | -0.12 | 0.908 |
| week.Animal 2 | 0.643 | 0.931 | 0.69 | 0.495 |
| week.Animal 3 | 0.750 | 0.931 | 0.81 | 0.427 |
| week.Animal 4 | 0.321 | 0.931 | 0.35 | 0.732 |
| week.Animal 5 | 0.214 | 0.931 | 0.23 | 0.820 |
| week.Animal 6 | -0.107 | 0.931 | -0.12 | 0.909 |

**Appendix 7a. (Cont). Regression analysis for change in height in T1. (Genstat 1995).**

***** Regression Analysis for Herefords *****

Response variate: Height
Fitted terms : Constant + week + Animal + week.Animal
*** Summary of analysis ***

| | d.f. | s.s. | m.s. | v.r. |
|---|---|---|---|---|
| Regression | 15 | 173.5 | 11.569 | 1.16 |
| Residual | 40 | 399.9 | 9.997 | |
| Total | 55 | 573.4 | 10.426 | |
| Change | -7 | -37.2 | 5.321 | 0.53 |

Percentage variance accounted for 4.1
Standard error of observations is estimated to be 3.16

*** Estimates of regression coefficients ***

| | estimate | s.e. | t (40) | t pr. |
|---|---|---|---|---|
| Constant | 107.75 | 2.15 | 50.01 | <.001 |
| week | 0.750 | 0.598 | 1.26 | 0.217 |
| Animal 7 | -1.57 | 3.05 | -0.52 | 0.609 |
| Animal 8 | 1.50 | 3.05 | 0.49 | 0.625 |
| Animal 9 | -2.21 | 3.05 | -0.73 | 0.472 |
| Animal 10 | 0.50 | 3.05 | 0.16 | 0.870 |
| Animal 11 | -0.57 | 3.05 | -0.19 | 0.852 |
| Animal 12 | 2.00 | 3.05 | 0.66 | 0.515 |
| Animal 13 | -2.11 | 3.05 | -0.69 | 0.493 |
| week.Animal 7 | 0.143 | 0.845 | 0.17 | 0.867 |
| week.Animal 8 | -0.929 | 0.845 | -1.10 | 0.278 |
| week.Animal 9 | -0.500 | 0.845 | -0.59 | 0.557 |
| week.Animal 10 | -0.500 | 0.845 | -0.59 | 0.557 |
| week.Animal 11 | -0.714 | 0.845 | -0.85 | 0.403 |
| week.Animal 12 | -0.429 | 0.845 | -0.51 | 0.615 |
| week.Animal 13 | 0.321 | 0.845 | 0.38 | 0.706 |

**Appendix 7a (Cont.) Regression analysis for change in height in T1. (Genstat 1995).**

```
***** Regression Analysis for Holsteins *****

Response variate: Height
Fitted terms    : Constant + week + Animal + week.Animal

*** Summary of analysis ***
             d.f.         s.s.         m.s.      v.r.
Regression     15       1505.5      100.365     14.17
Residual       40        283.4        7.084
Total          55       1788.8       32.524

Change         -7        -45.3        6.477      0.91

Percentage variance accounted for 78.2
Standard error of observations is estimated to be 2.66

*** Estimates of regression coefficients ***
                            estimate         s.e.     t (40)  t pr.
Constant                      114.29         1.81      63.02  <.001
week                           0.143        0.503       0.28  0.778
Animal 15                       2.04         2.56       0.79  0.432
Animal 16                      -2.18         2.56      -0.85  0.401
Animal 17                      -1.75         2.56      -0.68  0.499
Animal 18                      12.04         2.56       4.69  <.001
Animal 19                       7.46         2.56       2.91  0.006
Animal 20                       1.54         2.56       0.60  0.553
Animal 21                      -0.64         2.56      -0.25  0.803

week.Animal 15                  1.036        0.711       1.46  0.153
week.Animal 16                 -0.036        0.711      -0.05  0.960
week.Animal 17                 -0.464        0.711      -0.65  0.518
week.Animal 18                  0.179        0.711       0.25  0.803
week.Animal 19                  0.179        0.711       0.25  0.803
week.Animal 20                  0.821        0.711       1.15  0.255
week.Animal 21                  0.357        0.711       0.50  0.618
```

Conclusion:

The lack of significance of the terms 'week' shows that there were no significant changes in height over the experimental period.

**Appendix 7b. Test of model suitability for height in T2 using Genstat.**

***** Analysis of variance *****

Variate: Height

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Animals.Breed.Ration stratum | | | | | |
| Breed | 1 | 1980.167 | 1980.167 | 10.25 | 0.008 |
| Ration | 1 | 22.042 | 22.042 | 0.11 | 0.741 |
| Breed.Ration | 1 | 100.042 | 100.042 | 0.52 | 0.485 |
| Residual | 12 | 2317.417 | 193.118 | 77.72 | |
| Animals.Breed.Ration.Weeks stratum | | | | | |
| Weeks | 5 | 30.708 | 6.142 | 2.47 | 0.042 |
| Lin | 1 | 0.572 | 0.572 | 0.23 | 0.633 |
| Quad | 1 | 2.051 | 2.051 | 0.83 | 0.367 |
| Cub | 1 | 9.832 | 9.832 | 3.96 | 0.051 |
| Deviations | 2 | 18.254 | 9.127 | 3.67 | 0.031 |
| Breed.Weeks | 5 | 10.458 | 2.092 | 0.84 | 0.525 |
| Breed.Lin | 1 | 3.665 | 3.665 | 1.47 | 0.229 |
| Breed.Quad | 1 | 0.779 | 0.779 | 0.31 | 0.578 |
| Breed.Cub | 1 | 0.848 | 0.848 | 0.34 | 0.561 |
| Deviations | 2 | 5.166 | 2.583 | 1.04 | 0.360 |
| Ration.Weeks | 5 | 11.583 | 2.317 | 0.93 | 0.467 |
| Ration.Lin | 1 | 0.001 | 0.001 | 0.00 | 0.984 |
| Ration.Quad | 1 | 9.270 | 9.270 | 3.73 | 0.058 |
| Ration.Cub | 1 | 0.326 | 0.326 | 0.13 | 0.718 |
| Deviations | 2 | 1.986 | 0.993 | 0.40 | 0.672 |
| Breed.Ration.Weeks | 5 | 3.833 | 0.767 | 0.31 | 0.906 |
| Breed.Ration.Lin | 1 | 2.799 | 2.799 | 1.13 | 0.293 |
| Breed.Ration.Quad | 1 | 0.036 | 0.036 | 0.01 | 0.904 |
| Deviations | 3 | 0.998 | 0.333 | 0.13 | 0.940 |
| Residual | 60 | 149.083 | 2.485 | | |
| Total | 95 | 4625.333 | | | |

## Appendix 7b(Cont). Test of model suitability for height in T2 using Genstat.

```
***** Tables of means *****

    Breed  Hereford Friesland
           111.79    120.87
   Ration       Nil supplement
           115.85     116.81

    Weeks     0.00     1.00     2.00     3.00     5.00     6.00
           116.19   116.19   116.75   117.00   115.25   116.62

    Breed   Ration        Nil supplement
 Hereford               112.33     111.25
Friesland               119.37     122.37

    Breed    Weeks     0.00     1.00     2.00     3.00     5.00     6.00
 Hereford            111.88   112.00   112.50   111.88   110.63   111.88
Friesland            120.50   120.37   121.00   122.12   119.87   121.37

    Ration    Weeks     0.00     1.00     2.00     3.00     5.00     6.00
       Nil           116.00   116.00   115.87   116.00   114.75   116.50
supplement           116.37   116.37   117.62   118.00   115.75   116.75

                      Weeks
    Breed     Ration     0.00     1.00     2.00     3.00     5.00     6.00
 Hereford        Nil   113.00   113.00   112.50   112.00   111.00   112.50
           supplement  110.75   111.00   112.50   111.75   110.25   111.25
Friesland        Nil   119.00   119.00   119.25   120.00   118.50   120.50
           supplement  122.00   121.75   122.75   124.25   121.25   122.25

*** Standard errors of differences of means ***

Table                Breed      Ration       Weeks       Breed
                                                         Ration
rep.                    48          48          16          24
d.f.                    12          12          60          12
s.e.d.               2.837       2.837       0.557       4.012

Table                Breed      Ration       Breed
                     Weeks       Weeks       Ration
                                             Weeks
rep.                    8           8           4
s.e.d.               2.926       2.926       4.139
d.f.                13.58       13.58       13.58

Except when comparing means with the same level(s) of
 Breed               0.788
 d.f.                   60
 Ration                      0.788
 d.f.                           60
 Breed.Ration                            1.115
 d.f.                                       60
```

Conclusion:

The non significance of the term 'linear' means the linear model is not suitable for comparative assessment of change in height in this data. T- Tests for differences between means(within breed comparison) using the formula:

$$t = \frac{d}{SE(difference)}$$

showed no significant differences between means($P>0.05$),i.e., there was no significant change in condition in either breed.

## Appendix 8a. Condition score measurements during T1.

| JERSEYS | | | | | | | |
|---|---|---|---|---|---|---|---|
| Animal No. | INTEREST | INTRO | DOLPHIN | ISMAY | ILVA | DAMASK | DONGA |
| Date | | | | | | | |
| 13/12 | 2.75 | 2.00 | 2.00 | 2.00 | 2.00 | 1.75 | 2.00 |
| 20/12 | 2.75 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| 27/12 | 2.50 | 2.00 | 2.00 | 2.00 | 2.00 | 1.75 | 2.00 |
| 3/1 | 2.75 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| 10/1 | 2.50 | 2.50 | 2.25 | 2.00 | 2.00 | 2.00 | 2.00 |
| 17/1 | 2.75 | 2.25 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| 25/1 | 2.75 | 2.50 | 2.00 | 2.00 | 2.00 | 1.75 | 2.00 |

| HEREFORDS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Animal No. | H69 | H18 | H62 | HNM | H12 | H24 | H9 | H63 |
| 13/12 | 2.75 | 3.00 | 3.00 | 2.50 | 2.00 | 2.50 | 2.75 | 2.50 |
| 20/12 | 2.75 | 2.75 | 2.75 | 2.50 | 2.00 | 2.00 | 2.25 | 2.50 |
| 27/12 | 2.50 | 2.75 | 2.50 | 2.75 | 2.00 | 2.50 | 2.50 | 2.75 |
| 3/1 | 2.50 | 2.75 | 2.50 | 3.00 | 2.50 | 2.75 | 2.50 | 2.75 |
| 10/1 | 2.75 | 2.75 | 2.75 | 3.00 | 2.75 | 2.75 | 2.75 | 2.75 |
| 17/1 | 2.75 | 2.75 | 2.75 | 3.00 | 2.75 | 3.00 | 2.75 | 3.00 |
| 25/1 | 2.75 | 2.75 | 2.50 | 3.00 | 2.75 | 3.00 | 3.00 | 3.00 |

| HOLSTEINS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Animal No. | F29 | F33 | F34 | F28 | F26 | F30 | F31 | F32 |
| 13/12 | 2.25 | 2.00 | 2.50 | 2.00 | 2.50 | 2.00 | 2.25 | 2.00 |
| 20/12 | 2.25 | 2.00 | 2.50 | 2.00 | 2.75 | 2.50 | 2.50 | 2.25 |
| 27/12 | 2.50 | 1.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| 3/1 | 2.50 | 1.75 | 2.75 | 2.50 | 2.50 | 2.25 | 2.50 | 2.50 |
| 10/1 | 2.75 | 2.00 | 2.50 | 2.50 | 2.75 | 2.75 | 2.50 | 2.50 |
| 17/1 | 2.75 | 2.00 | 2.50 | 2.50 | 2.75 | 2.50 | 2.50 | 2.25 |
| 25/1 | 2.75 | 2.00 | 2.50 | 2.75 | 2.75 | 2.75 | 2.50 | 2.50 |

## Appendix 8b. Condition score measurements during T2.

HEREFORDS

| Animal No. | H69 | H18 | H62 | HNM | H12 | H24 | H9 | H63 |
|---|---|---|---|---|---|---|---|---|
| Date | | | | | | | | |
| 21/2 | 3.5 | 3.25 | 3.25 | 3.5 | 3.25 | 3.5 | 3.25 | 3.25 |
| 28/2 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| 6/3 | 3.75 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| 14/3 | 3.75 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| 27/3 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.75 | 3.5 | 3.5 |
| 3/4 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |

HOLSTEINS

| | F58 | F33 | F34 | F54 | F61 | F53 | F31 | F32 |
|---|---|---|---|---|---|---|---|---|
| 21/2 | 2 | 2.25 | 2.25 | 2.25 | 2.5 | 2.5 | 2.5 | 2.5 |
| 28/2 | 2.25 | 2.25 | 2.5 | 2.5 | 2.5 | 2.75 | 2.5 | 2.5 |
| 6/3 | 2.25 | 2.25 | 2.5 | 2.25 | 2.5 | 2.5 | 2.5 | 2.5 |
| 14/3 | 2 | 2 | 2.25 | 2.25 | 2.5 | 2.5 | 2.25 | 2.5 |
| 27/3 | 2.25 | 2.25 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| 3/4 | 2 | 2 | 2.5 | 2.25 | 2.25 | 2.5 | 2.25 | 2.5 |

**Appendix 9a. Regression analysis for change in condition score in T1.**

***** Regression Analysis for Jerseys *****

Response variate: Condition score
Fitted terms : Constant + week + Animal + week.Animal

*** Summary of analysis ***

| | d.f. | s.s. | m.s. | v.r. |
|---|---|---|---|---|
| Regression | 11 | 2.7507 | 0.250068 | 29.23 |
| Residual | 30 | 0.2567 | 0.008557 | |
| Total | 41 | 3.0074 | 0.073352 | |
| Change | -5 | -0.1804 | 0.036086 | 4.22 |

Percentage variance accounted for 88.3
Standard error of observations is estimated to be 0.0925

*** Estimates of regression coefficients ***

| | estimate | s.e. | t (30) | t pr. |
|---|---|---|---|---|
| Constant | 2.6786 | 0.0630 | 42.50 | <.001 |
| week | 0.0000 | 0.0175 | 0.00 | 1.000 |
| Animal 2 | -0.7679 | 0.0891 | -8.61 | <.001 |
| Animal 3 | -0.6696 | 0.0891 | -7.51 | <.001 |
| Animal 4 | -0.6786 | 0.0891 | -7.61 | <.001 |
| Animal 5 | -0.6786 | 0.0891 | -7.61 | <.001 |
| Animal 6 | -0.6786 | 0.0891 | -7.61 | <.001 |
| week.Animal 2 | 0.0893 | 0.0247 | 3.61 | 0.001 |
| week.Animal 3 | 0.0089 | 0.0247 | 0.36 | 0.721 |
| week.Animal 4 | 0.0000 | 0.0247 | 0.00 | 1.000 |
| week.Animal 5 | 0.0000 | 0.0247 | 0.00 | 1.000 |
| week.Animal 6 | 0.0000 | 0.0247 | 0.00 | 1.000 |

**Appendix 9a. (Cont). Regression analysis for change in condition score in T1.**

***** Regression Analysis Herefords *****

Response variate: Condition score
Fitted terms : Constant + week + Animal + week.Animal

*** Summary of analysis ***

| | d.f. | s.s. | m.s. | v.r. |
|---|---|---|---|---|
| Regression | 15 | 2.7868 | 0.18579 | 7.80 |
| Residual | 40 | 0.9531 | 0.02383 | |
| Total | 55 | 3.7400 | 0.06800 | |
| Change | -7 | -1.0957 | 0.15653 | 6.57 |

Percentage variance accounted for 65.0
Standard error of observations is estimated to be 0.154

*** Estimates of regression coefficients ***

| | estimate | s.e. | t (40) | t pr. |
|---|---|---|---|---|
| Constant | 2.482 | 0.105 | 23.60 | <.001 |
| week | 0.0893 | 0.0292 | 3.06 | 0.004 |
| Animal 7 | 0.170 | 0.149 | 1.14 | 0.261 |
| Animal 8 | 0.384 | 0.149 | 2.58 | 0.014 |
| Animal 9 | 0.330 | 0.149 | 2.22 | 0.032 |
| Animal 10 | 0.045 | 0.149 | 0.30 | 0.766 |
| Animal 11 | -0.571 | 0.149 | -3.84 | <.001 |
| Animal 12 | -0.241 | 0.149 | -1.62 | 0.113 |
| Animal 13 | -0.054 | 0.149 | -0.36 | 0.721 |
| week.Animal 7 | -0.0804 | 0.0413 | -1.95 | 0.058 |
| week.Animal 8 | -0.1161 | 0.0413 | -2.81 | 0.008 |
| week.Animal 9 | -0.1339 | 0.0413 | -3.25 | 0.002 |
| week.Animal 10 | 0.0089 | 0.0413 | 0.22 | 0.830 |
| week.Animal 11 | 0.0714 | 0.0413 | 1.73 | 0.091 |
| week.Animal 12 | 0.0446 | 0.0413 | 1.08 | 0.286 |
| week.Animal 13 | -0.0179 | 0.0413 | -0.43 | 0.667 |

**Appendix 9a. (Cont). Regression analysis for change in condition score in T1.**

***** Regression Analysis for Holsteins *****

Response variate: Condition score
Fitted terms : Constant + week + Animal + week.Animal

*** Summary of analysis ***

| | d.f. | s.s. | m.s. | v.r. |
|---|---|---|---|---|
| Regression | 15 | 3.5513 | 0.23676 | 10.80 |
| Residual | 40 | 0.8772 | 0.02193 | |
| Total | 55 | 4.4286 | 0.08052 | |
| Change | -7 | -0.3457 | 0.04939 | 2.25 |

Percentage variance accounted for 72.8
Standard error of observations is estimated to be 0.148

*** Estimates of regression coefficients ***

| | estimate | s.e. | t (40) | t pr. |
|---|---|---|---|---|
| Constant | 2.196 | 0.101 | 21.77 | <.001 |
| week | 0.0536 | 0.0280 | 1.91 | 0.063 |
| Animal 15 | 0.045 | 0.143 | 0.31 | 0.756 |
| Animal 16 | -0.357 | 0.143 | -2.50 | 0.017 |
| Animal 17 | 0.339 | 0.143 | 2.38 | 0.022 |
| Animal 18 | -0.152 | 0.143 | -1.06 | 0.294 |
| Animal 19 | 0.339 | 0.143 | 2.38 | 0.022 |
| Animal 20 | 0.000 | 0.143 | 0.00 | 1.000 |
| Animal 21 | 0.188 | 0.143 | 1.31 | 0.196 |
| week.Animal 15 | 0.0446 | 0.0396 | 1.13 | 0.266 |
| week.Animal 16 | -0.0357 | 0.0396 | -0.90 | 0.372 |
| week.Animal 17 | -0.0536 | 0.0396 | -1.35 | 0.183 |
| week.Animal 18 | 0.0625 | 0.0396 | 1.58 | 0.122 |
| week.Animal 19 | -0.0179 | 0.0396 | -0.45 | 0.654 |
| week.Animal 20 | 0.0357 | 0.0396 | 0.90 | 0.372 |
| week.Animal 21 | -0.0268 | 0.0396 | -0.68 | 0.502 |

**Appendix 9a. (Cont). Regression analysis for change in condition score in T1.**

Conclusion:

The critical value for $t_{(40)}$ is 2.704. The significance of the term 'week' for the Herefords means that they had a change in condition score greater than zero. Jerseys and Holsteins did not change significantly in condition score over the trial period.

**Appendix 9b. Test of model suitability for condition score in T2 using Genstat.**

***** Analysis of variance for condition scores *****

Variate: score

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Animals.Breed.Ration stratum | | | | | |
| Breed | 1 | 30.094401 | 30.094401 | 741.58 | <.001 |
| Ration | 1 | 0.235026 | 0.235026 | 5.79 | 0.033 |
| Breed.Ration | 1 | 0.344401 | 0.344401 | 8.49 | 0.013 |
| Residual | 12 | 0.486979 | 0.040582 | 6.54 | |
| Animals.Breed.Ration.Weeks stratum | | | | | |
| Weeks | 5 | 0.271484 | 0.054297 | 8.75 | <.001 |
| Lin | 1 | 0.006151 | 0.006151 | 0.99 | 0.324 |
| Quad | 1 | 0.095905 | 0.095905 | 15.45 | <.001 |
| Cub | 1 | 0.008161 | 0.008161 | 1.31 | 0.256 |
| Deviations | 2 | 0.161268 | 0.080634 | 12.99 | <.001 |
| Breed.Weeks | 5 | 0.198568 | 0.039714 | 6.40 | <.001 |
| Breed.Lin | 1 | 0.089775 | 0.089775 | 14.46 | <.001 |
| Breed.Quad | 1 | 0.030138 | 0.030138 | 4.86 | 0.031 |
| Breed.Cub | 1 | 0.007142 | 0.007142 | 1.15 | 0.288 |
| Deviations | 2 | 0.071513 | 0.035757 | 5.76 | 0.005 |
| Ration.Weeks | 5 | 0.011068 | 0.002214 | 0.36 | 0.876 |
| Ration.Lin | 1 | 0.000489 | 0.000489 | 0.08 | 0.780 |
| Ration.Quad | 1 | 0.000748 | 0.000748 | 0.12 | 0.730 |
| Ration.Cub | 1 | 0.003066 | 0.003066 | 0.49 | 0.485 |
| Deviations | 2 | 0.006765 | 0.003383 | 0.54 | 0.583 |
| Breed.Ration.Weeks | 5 | 0.073568 | 0.014714 | 2.37 | 0.050 |
| Breed.Ration.Lin | 1 | 0.025237 | 0.025237 | 4.07 | 0.048 |
| Breed.Ration.Quad | 1 | 0.000033 | 0.000033 | 0.01 | 0.942 |
| Deviations | 3 | 0.048298 | 0.016099 | 2.59 | 0.061 |
| Residual | 60 | 0.372396 | 0.006207 | | |
| Total | 95 | 32.087891 | | | |

*

## Appendix 9b(Cont). Test of model suitability for condition score in T2 using Genstat.

*****Table of means*****

| Breed | Hereford | Friesland |
|---|---|---|
| | 3.4896 | 2.3698 |

| Ration | Nil | supplement |
|---|---|---|
| | 2.9792 | 2.8802 |

| Weeks | 0.00 | 1.00 | 2.00 | 3.00 | 5.00 | 6.00 |
|---|---|---|---|---|---|---|
| | 2.8437 | 2.9844 | 2.9687 | 2.9062 | 2.9844 | 2.8906 |

| Breed | Ration | Nil | supplement |
|---|---|---|---|
| Hereford | | 3.4792 | 3.5000 |
| Friesland | | 2.4792 | 2.2604 |

| Breed | Weeks | 0.00 | 1.00 | 2.00 | 3.00 | 5.00 | 6.00 |
|---|---|---|---|---|---|---|---|
| Hereford | | 3.3437 | 3.5000 | 3.5312 | 3.5312 | 3.5312 | 3.5000 |
| Friesland | | 2.3438 | 2.4688 | 2.4063 | 2.2813 | 2.4375 | 2.2813 |

| Ration | Weeks | 0.00 | 1.00 | 2.00 | 3.00 | 5.00 | 6.00 |
|---|---|---|---|---|---|---|---|
| Nil | | 2.9063 | 3.0313 | 3.0000 | 2.9688 | 3.0313 | 2.9375 |
| supplement | | 2.7812 | 2.9375 | 2.9375 | 2.8437 | 2.9375 | 2.8437 |

| Breed | Ration | Weeks | 0.00 | 1.00 | 2.00 | 3.00 | 5.00 |
|---|---|---|---|---|---|---|---|
| Hereford | Nil | | 3.3125 | 3.5000 | 3.5000 | 3.5000 | 3.5625 |
| | supplement | | 3.3750 | 3.5000 | 3.5625 | 3.5625 | 3.5000 |
| Friesland | Nil | | 2.5000 | 2.5625 | 2.5000 | 2.4375 | 2.5000 |
| | supplement | | 2.1875 | 2.3750 | 2.3125 | 2.1250 | 2.3750 |

| Breed | Ration | Weeks | 6.00 |
|---|---|---|---|
| Hereford | Nil | | 3.5000 |
| | supplement | | 3.5000 |
| Friesland | Nil | | 2.3750 |
| | supplement | | 2.1875 |

*** Standard errors of differences of means ***

| Table | Breed | Ration | Weeks | Breed Ration |
|---|---|---|---|---|
| rep. | 48 | 48 | 16 | 24 |
| d.f. | 12 | 12 | 60 | 12 |
| s.e.d. | 0.04112 | 0.04112 | 0.02785 | 0.05815 |

| Table | Breed Weeks | Ration Weeks | Breed Ration Weeks |
|---|---|---|---|
| rep. | 8 | 8 | 4 |
| s.e.d. | 0.05463 | 0.05463 | 0.07725 |
| d.f. | 33.46 | 33.46 | 33.46 |

Except when comparing means with the same level(s) of

| | | | |
|---|---|---|---|
| Breed | 0.03939 | | |
| d.f. | 60 | | |
| Ration | | 0.03939 | |
| d.f. | | 60 | |
| Breed.Ration | | | 0.05571 |
| d.f. | | | 60 |

Conclusion:

The non significance of the term 'linear' means the linear model is not suitable for comparative assessment of change in height in this data.

T- Tests for differences between means(within breed comparison) using the formula:

$$t = \frac{d}{SE(difference)}$$

showed no significant differences between means(P>0.05),i.e., there was no significant change in condition in either breed.

**Appendix 10a. Dosing gun output in T1.**

| | Sample | | | |
|---|---|---|---|---|
| | 101.6 | 101.2 | 102.3 | 102.5 |
| | 102.0 | 102.1 | 102.1 | 102.3 |
| | 101.9 | 101.2 | 101.8 | 102.1 |
| | 101.5 | 101.1 | 102.0 | 102.0 |
| | 101.9 | 101.4 | 101.8 | 101.0 |
| | 102.4 | 101.8 | 101.3 | 101.7 |
| | 102.1 | 102.5 | 101.0 | 101.7 |
| | 101.3 | 102.3 | 100.8 | 102.7 |
| | 102.6 | 102.8 | 101.7 | 101.2 |
| | 102.2 | 101.7 | 101.9 | 101.9 |
| | 99.2 | 101.1 | 101.6 | 101.6 |
| | 100.8 | 100.7 | 102.2 | 102.0 |
| | 100.9 | 102.3 | 101.0 | 101.9 |
| | 100.7 | 103.2 | 101.7 | 101.6 |
| | 102.0 | 102.2 | 102.0 | 102.0 |
| | 102.5 | 100.7 | 102.5 | 102.4 |
| | 101.5 | 101.7 | 103.3 | 101.9 |
| | 101.5 | 102.7 | 102.6 | 100.4 |
| | 102.3 | 100.4 | 102.0 | 102.2 |
| | 101.2 | 102.6 | 103.2 | 101.9 |
| | 102.1 | 101.7 | 102.7 | 100.9 |
| | 102.0 | 103.0 | 102.9 | |
| | 101.0 | 101.7 | 102.7 | |
| Mean | 101.83 | | | |
| CV (%) | 0.07 | | | |
| N | 90 | | | |

## Appendix 10b. Dosing gun output in T2.

| | Sample | | | |
|---|---|---|---|---|
| | 102.1 | 100.1 | 101.6 | 101.4 |
| | 102.2 | 100.3 | 100.7 | 102.2 |
| | 102.8 | 100.7 | 102.5 | 101.5 |
| | 102.1 | 101.1 | 101.9 | 102.7 |
| | 102.7 | 100.9 | 101.2 | 102.8 |
| | 101.5 | 102.0 | 102.1 | 101.8 |
| | 100.4 | 100.5 | 101.4 | 100.3 |
| | 101.5 | 100.9 | 101.8 | 101.1 |
| | 100.8 | 101.9 | 102.9 | 101.0 |
| | 101.0 | 100.5 | 102.7 | 101.3 |
| | 100.1 | 102.7 | 101.5 | 101.5 |
| | 101.0 | 102.6 | 102.7 | 101.9 |
| | 100.2 | 100.6 | 101.1 | 102.5 |
| | 101.9 | 101.7 | 102.1 | 102.2 |
| | 101.2 | 101.5 | 101.7 | 101.7 |
| | 101.0 | 102.2 | 101.0 | 100.6 |
| | 100.7 | 102.3 | 102.1 | 100.3 |
| | 101.4 | 102.0 | 102.7 | 100.5 |
| | 102.6 | 102.5 | 100.3 | |
| X | | 101.5 | | |
| CV% | | 0.1 | | |
| N | | 75.0 | | |

**Appendix 11. Estimated quantities and variation in alkane(mg/kgDM) administered to each animal.**

| | C32( g/kg) | T1 Dose(mg/animal) | T2 Dose(mg/animal) |
|---|---|---|---|
| SAMPLE | | | |
| 1 | 90 | 488 | 487 |
| 2 | 89 | 483 | 481 |
| 3 | 98 | 531 | 530 |
| 4 | 93 | 503 | 502 |
| 5 | 84 | 453 | 452 |
| 6 | 84 | 455 | 453 |
| 7 | 99 | 540 | 538 |
| 8 | 90 | 488 | 486 |
| 9 | 93 | 508 | 506 |
| 10 | 89 | 483 | 481 |
| MEAN | 91 | 493 | 492 |
| SE | 1.5 | 9 | 8 |
| CV(%) | 1.7 | 1.72 | 1.72 |

**Appendix 12. Herbage alkane concentrations in T1 and T2.**

| T1. | | | | | | | MEAN | SE |
|---|---|---|---|---|---|---|---|---|
| Day | 1 | 2 | 3 | 4 | 5 | 6 | | |
| c31 | 0.106 | 0.110 | 0.089 | 0.116 | 0.086 | 0.109 | 0.103 | 0.004 |
| c32 | 0.010 | 0.008 | 0.007 | 0.008 | 0.008 | 0.009 | 0.008 | 0.000 |
| c33 | 0.200 | 0.227 | 0.204 | 0.234 | 0.198 | 0.224 | 0.215 | 0.005 |
| c35 | 0.172 | 0.225 | 0.210 | 0.226 | 0.208 | 0.224 | 0.211 | 0.007 |
| T2 | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | | |
| c31 | 0.106 | 0.105 | 0.100 | 0.072 | 0.107 | * | 0.096 | 0.006 |
| c32 | 0.021 | 0.009 | 0.014 | 0.009 | 0.025 | * | 0.013 | 0.002 |
| c33 | 0.211 | 0.232 | 0.212 | 0.144 | 0.216 | * | 0.200 | 0.014 |
| c35 | 0.206 | 0.250 | 0.220 | 0.142 | 0.216 | * | 0.205 | 0.016 |

**Appendix 13a. Faecal alkane concentrations(mg/kgDM) in T1.**

| | | | Day | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| JERSEYS | Animal No. | Alkane | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | INTRO | C31 | 231 | 299 | 111 | 341 | 206 | 254 | 360 |
| | | C32 | 204 | 320 | 146 | 397 | 283 | 291 | 321 |
| | | C33 | 440 | 633 | 303 | 759 | 516 | 575 | 696 |
| | | C35 | 404 | 659 | 422 | 813 | 549 | 627 | 684 |
| | ISMAY | C31 | 345 | * | * | 318 | 307 | 296 | 260 |
| | | C32 | 355 | * | * | 279 | 285 | 234 | 265 |
| | | C33 | 723 | * | * | 604 | 587 | 532 | 533 |
| | | C35 | 638 | * | * | 564 | 578 | 501 | 507 |
| | INTEREST | C31 | 217 | 251 | 146 | 188 | 184 | 333 | 168 |
| | | C32 | 216 | 179 | 131 | 232 | 218 | 284 | 221 |
| | | C33 | 461 | 220 | 285 | 478 | 457 | 660 | 454 |
| | | C35 | 463 | 251 | 270 | 504 | 423 | 655 | 524 |
| | ILVA | C31 | 412 | * | 202 | 246 | * | 287 | 96 |
| | | C32 | 316 | * | 167 | 248 | * | 245 | 252 |
| | | C33 | 725 | * | 367 | 458 | * | 513 | 469 |
| | | C35 | 616 | * | 338 | 424 | * | 427 | 376 |
| | DONGA | C31 | 237 | 213 | 137 | 228 | 287 | 280 | 73 |
| | | C32 | 239 | 177 | 227 | 229 | 246 | 223 | 105 |
| | | C33 | 460 | 386 | 410 | 438 | 537 | 304 | 219 |
| | | C35 | 424 | 354 | 475 | 435 | 494 | 475 | 288 |
| | DOLPHIN | C31 | 223 | * | 210 | 363 | 132 | 25 | * |
| | | C32 | 263 | * | 220 | 248 | 313 | 86 | * |
| | | C33 | 474 | * | 400 | 723 | 384 | 171 | * |
| | | C35 | 486 | * | 429 | 696 | 383 | 297 | * |

## Appendix 13a(Cont). Faecal alkane concentrations(mg/kgDM) in T1.

| | | | Day | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| HOLSTEINS | Animal No. | Alkane | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | F29 | C31 | 194 | 325 | 57 | 143 | 333 | * | * |
| | | C32 | 220 | 230 | 105 | 262 | 266 | * | * |
| | | C33 | 514 | 682 | 231 | 503 | 646 | * | * |
| | | C35 | 547 | 675 | 241 | 536 | 641 | * | * |
| | F30 | C31 | 253 | 309 | * | 312 | 302 | * | * |
| | | C32 | 273 | 258 | * | 293 | 289 | * | 237 |
| | | C33 | 517 | 608 | * | 630 | 684 | * | 241 |
| | | C35 | 477 | 609 | * | 587 | 637 | * | 407 |
| | F28 | C31 | * | 338 | 199 | 174 | 221 | 143 | 110 |
| | | C32 | * | 222 | 223 | 182 | 162 | 253 | 124 |
| | | C33 | * | 677 | 498 | 521 | 525 | 471 | 339 |
| | | C35 | * | 668 | 557 | 600 | 589 | 472 | 384 |
| | F26 | C31 | 475 | 232 | 236 | 238 | 275 | 285 | 284 |
| | | C32 | 263 | 212 | 167 | 182 | 235 | 211 | 198 |
| | | C33 | 797 | 551 | 550 | 442 | 565 | 591 | 598 |
| | | C35 | 720 | 605 | 604 | 434 | 554 | 590 | 617 |
| | F33 | C31 | 171 | 184 | 155 | 228 | 371 | 332 | 99 |
| | | C32 | 220 | 230 | 153 | 320 | 343 | 284 | 193 |
| | | C33 | 447 | 410 | 311 | 599 | 742 | 581 | 285 |
| | | C35 | 509 | 449 | 326 | 665 | 748 | 582 | 368 |
| | F32 | C31 | 135 | 122 | * | 250 | 199 | 384 | 144 |
| | | C32 | 126 | 129 | * | 206 | 165 | 323 | 241 |
| | | C33 | 300 | 311 | * | 491 | 365 | 740 | 473 |
| | | C35 | 323 | 374 | * | 461 | 383 | 701 | 527 |
| | F34 | C31 | * | 273 | 245 | 307 | 225 | 247 | 209 |
| | | C32 | * | 247 | 321 | 277 | 244 | 249 | 332 |
| | | C33 | * | 596 | 588 | 654 | 509 | 562 | 518 |
| | | C35 | * | 640 | 641 | 681 | 540 | 597 | 563 |
| | F31 | C31 | 217 | 249 | 346 | 208 | 287 | 255 | 204 |
| | | C32 | 178 | 214 | 290 | 177 | 225 | 210 | 276 |
| | | C33 | 491 | 519 | 674 | 479 | 576 | 480 | 446 |
| | | C35 | 500 | 542 | 689 | 504 | 586 | 420 | 458 |

**Appendix 13a(Cont). Faecal alkane concentrations(mg/kgDM) in T1.**

| | | | Day | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| HEREFORDS | Animal No. | Alkane | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | HNM | C31 | 382 | 241 | 407 | 325 | 257 | 347 | 280 |
| | | C32 | 255 | 257 | 267 | 209 | 167 | 281 | 346 |
| | | C33 | 752 | 509 | 741 | 603 | 475 | 763 | 830 |
| | | C35 | 745 | 746 | 674 | 532 | 467 | 801 | 813 |
| | H62 | C31 | 224 | 361 | * | * | 340 | 318 | 397 |
| | | C32 | 191 | 256 | * | * | 252 | 218 | 276 |
| | | C33 | 471 | 681 | * | * | 652 | 616 | 721 |
| | | C35 | 443 | 633 | * | * | 607 | 564 | 646 |
| | H69 | C31 | 326 | 421 | 274 | 318 | 408 | 345 | 236 |
| | | C32 | 229 | 249 | 188 | 223 | 274 | 264 | 204 |
| | | C33 | 660 | 807 | 545 | 652 | 752 | 663 | 564 |
| | | C35 | 658 | 780 | 580 | 642 | 700 | 608 | 530 |
| | H12 | C31 | 233 | 383 | 251 | 268 | 325 | * | 458 |
| | | C32 | 221 | 288 | 205 | 202 | 278 | * | 298 |
| | | C33 | 506 | 668 | 580 | 513 | 622 | * | 747 |
| | | C35 | 511 | 603 | 524 | 477 | 607 | * | 630 |
| | H24 | C31 | * | 410 | 426 | 396 | 288 | 286 | 279 |
| | | C32 | * | 234 | 261 | 259 | 233 | 219 | 159 |
| | | C33 | * | 907 | 846 | 767 | 667 | 687 | 563 |
| | | C35 | * | 785 | 845 | 780 | 744 | 694 | 558 |
| | H18 | C31 | 82 | 368 | 105 | 338 | 273 | 365 | 289 |
| | | C32 | 89 | 248 | 81 | 234 | 189 | 260 | 241 |
| | | C33 | 284 | 704 | 268 | 703 | 555 | 683 | 673 |
| | | C35 | 378 | 701 | 297 | 720 | 570 | 660 | 674 |
| | H9 | C31 | 137 | 264 | 25 | 385 | 336 | * | * |
| | | C32 | 108 | 201 | 164 | 269 | 207 | * | * |
| | | C33 | 280 | 577 | 409 | 730 | 654 | * | * |
| | | C35 | 304 | 570 | 368 | 711 | 632 | * | * |
| | H63 | C31 | 285 | 385 | 242 | 425 | 96 | 415 | 393 |
| | | C32 | 192 | 208 | 138 | 215 | 160 | 209 | 193 |
| | | C33 | 598 | 759 | 416 | 787 | 454 | 753 | 715 |
| | | C35 | 564 | 700 | 475 | 706 | 497 | 648 | 633 |

## Appendix 13b. Faecal alkane concentrations(mg/kgDM) in T2.

| | | | Day | | | | | |
|---|---|---|---|---|---|---|---|---|
| HOLSTEINS | Animal No. | Alkane | 1 | 2 | 3 | 4 | 5 | 6 |
| | F32 | C31 | 189 | 282 | 244 | 192 | 301 | 280 |
| | | C32 | 166 | 196 | 161 | 154 | 216 | 199 |
| | | C33 | 510 | 576 | 507 | 416 | 608 | 552 |
| | | C35 | 539 | 591 | 532 | 456 | 626 | 566 |
| | F61 | C31 | 288 | 249 | 329 | 345 | 251 | 205 |
| | | C32 | 208 | 159 | 179 | 212 | 294 | 200 |
| | | C33 | 605 | 493 | 631 | 660 | 848 | 490 |
| | | C35 | 643 | 655 | 640 | 666 | 919 | 533 |
| | F33 | C31 | 228 | * | * | 262 | 82 | 227 |
| | | C32 | 202 | * | * | 217 | 173 | 194 |
| | | C33 | 532 | * | * | 620 | 241 | 488 |
| | | C35 | 580 | * | * | 640 | 437 | 515 |
| | F34 | C31 | 204 | 215 | 303 | 281 | 254 | 218 |
| | | C32 | 150 | 159 | 229 | 234 | 225 | 223 |
| | | C33 | 451 | 454 | 645 | 624 | 604 | 607 |
| | | C35 | 474 | 475 | 679 | 696 | 685 | 686 |
| | F58 | C31 | 469 | 247 | 362 | 325 | 156 | 317 |
| | | C32 | 293 | 185 | 265 | 259 | 104 | 250 |
| | | C33 | 857 | 534 | 771 | 612 | 403 | 622 |
| | | C35 | 807 | 566 | 755 | 637 | 472 | 633 |
| | F31 | C31 | 188 | 204 | 141 | 346 | 287 | 239 |
| | | C32 | 146 | 148 | 222 | 226 | 178 | 230 |
| | | C33 | 410 | 433 | 472 | 686 | 602 | 605 |
| | | C35 | 421 | 444 | 564 | 667 | 625 | 686 |
| | F53 | C31 | 415 | 204 | 145 | 308 | 313 | 374 |
| | | C32 | 201 | 130 | 87 | 194 | 193 | 209 |
| | | C33 | 880 | 482 | 309 | 658 | 663 | 736 |
| | | C35 | 913 | 544 | 341 | 705 | 724 | 747 |
| | F54 | C31 | 248 | 268 | 271 | 257 | 191 | 304 |
| | | C32 | 125 | 166 | 162 | 160 | 176 | 167 |
| | | C33 | 480 | 536 | 574 | 527 | 472 | 621 |
| | | C35 | 468 | 556 | 599 | 565 | 534 | 653 |

## Appendix 13b(Cont). Faecal alkane concentrations(mg/kgDM) in T2.

| | | | Day | | | | | |
|---|---|---|---|---|---|---|---|---|
| HEREFORDS | Animal No. | Alkane | 1 | 2 | 3 | 4 | 5 | 6 |
| | H63 | C31 | 223 | | 364 | 396 | 572 | 358 |
| | | C32 | 160 | 114 | 228 | 242 | 250 | 219 |
| | | C33 | 532 | 444 | 795 | 827 | 792 | 740 |
| | | C35 | 615 | 511 | 849 | 859 | 749 | 774 |
| | H69 | C31 | 288 | 310 | * | 283 | 381 | 129 |
| | | C32 | 181 | 201 | * | 204 | 224 | 107 |
| | | C33 | 603 | 612 | * | 641 | 807 | 343 |
| | | C35 | 628 | 620 | * | 704 | 872 | |
| | H62 | C31 | * | * | 232 | 366 | 359 | 292 |
| | | C32 | * | * | 144 | 224 | 199 | 166 |
| | | C33 | * | * | 475 | 693 | 770 | 653 |
| | | C35 | * | * | 498 | 664 | 817 | 691 |
| | H24 | C31 | 184 | 149 | 84 | 470 | 238 | 351 |
| | | C32 | 90 | 125 | 78 | 280 | 120 | 194 |
| | | C33 | 359 | 430 | 250 | 633 | 468 | 749 |
| | | C35 | 378 | 533 | 305 | 571 | 468 | 782 |
| | HNM | C31 | 254 | 360 | * | 198 | 214 | 371 |
| | | C32 | 198 | 204 | * | 114 | 125 | 227 |
| | | C33 | 561 | 714 | * | 405 | 429 | 771 |
| | | C35 | 620 | 728 | * | 438 | 467 | 832 |
| | H9 | C31 | 345 | 280 | 262 | 319 | * | * |
| | | C32 | 215 | 160 | 169 | 261 | * | * |
| | | C33 | 702 | 582 | 583 | 672 | * | * |
| | | C35 | 717 | 609 | 629 | 697 | * | * |
| | H12 | C31 | 299 | 283 | 305 | 349 | 298 | 336 |
| | | C32 | 213 | 183 | 206 | 221 | 175 | 235 |
| | | C33 | 633 | 605 | 597 | 726 | 671 | 688 |
| | | C35 | 670 | 641 | 615 | 759 | 718 | 701 |

## Appendix 14a. Estimated intake(g/kgLW$^{0.75}$) using C31, C33 and C35 in T1.

| JERSEYS | Day | | | | | | |
|---|---|---|---|---|---|---|---|
| **C31** | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Animal No. | | | | | | | |
| INTRO | 113.0 | 89.9 | 70.6 | 81.4 | 67.7 | 83.1 | 111.8 |
| ISMAY | 102.2 | * | * | 124.4 | 115.6 | 140.8 | 103.4 |
| INTEREST | 89.7 | 136.1 | 101.3 | 69.8 | 73.4 | 108.2 | 65.1 |
| ILVA | 139.1 | * | 26.9 | 99.4 | * | 122.1 | 34.4 |
| DONGA | 105.1 | 132.9 | 59.5 | 105.3 | 127.4 | 139.6 | 69.6 |
| DOLPHIN | 86.0 | * | 98.4 | 167.2 | 39.6 | 27.3 | * |
| **C33** | | | | | | | |
| INTRO | 102.3 | 92.4 | 97.9 | 88.6 | 83.9 | 92.1 | 103.0 |
| ISMAY | 103.7 | * | * | 111.7 | 105.1 | 118.2 | 102.3 |
| INTEREST | 91.0 | 55.7 | 86.6 | 92.2 | 80.8 | 99.2 | 102.7 |
| ILVA | 113.9 | * | 108.4 | 87.8 | * | 102.1 | 88.5 |
| DONGA | 97.2 | 112.6 | 90.0 | 96.4 | 112.8 | 65.2 | 106.6 |
| DOLPHIN | 88.7 | * | 89.4 | 159.1 | 57.5 | 100.1 | * |
| **C35** | | | | | | | |
| INTRO | 90.2 | 94.6 | 143.3 | 93.9 | 88.3 | 99.7 | 98.6 |
| ISMAY | 87.5 | * | * | 100.6 | 100.7 | 107.4 | 94.4 |
| INTEREST | 92.8 | 49.4 | 94.7 | 88.7 | 90.5 | 102.6 | 88.5 |
| ILVA | 91.6 | * | 95.9 | 78.4 | * | 80.4 | 67.2 |
| DONGA | 86.4 | 99.3 | 104.5 | 93.5 | 99.7 | 106.7 | 145.5 |
| DOLPHIN | 89.3 | * | 94.8 | 147.8 | 56.1 | 194.5 | * |

## Appendix 14a(Cont). Estimated intake(g/kgLW$^{0.75}$) using C31, C33 and C35 in T1.

| | Day | | | | | | |
|---|---|---|---|---|---|---|---|
| **C31** | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Animal No. | | | | | | | |
| HNM | 128.6 | 72.0 | 131.4 | 135.0 | 133.0 | 100.3 | 60.7 |
| H62 | 99.7 | 126.0 | * | * | 119.0 | 132.0 | 129.4 |
| H69 | 120.5 | 151.4 | 124.1 | 120.2 | 127.3 | 108.0 | 92.9 |
| H12 | 89.0 | 118.7 | 107.0 | 118.0 | 100.9 | * | 143.0 |
| H24 | * | 168.7 | 152.7 | 140.1 | 106.7 | 114.5 | 169.4 |
| H18 | 71.0 | 128.3 | 108.4 | 123.8 | 124.4 | 119.2 | 97.7 |
| H9 | 104.6 | 109.7 | 10.3 | 122.2 | 145.0 | * | * |
| H63 | 116.5 | 156.7 | 146.0 | 172.8 | 39.7 | 173.9 | 180.9 |
| **C33** | | | | | | | |
| HNM | 120.1 | 73.7 | 111.0 | 116.8 | 114.8 | 108.0 | 92.7 |
| H62 | 101.5 | 112.0 | * | * | 108.3 | 121.2 | 109.4 |
| H69 | 113.5 | 126.8 | 124.5 | 113.7 | 97.7 | 86.2 | 99.9 |
| H12 | 94.2 | 95.8 | 122.7 | 106.9 | 91.8 | * | 105.3 |
| H24 | * | 184.9 | 144.3 | 128.3 | 123.1 | 138.6 | 163.6 |
| H18 | 135.4 | 115.9 | 141.4 | 124.7 | 121.2 | 104.9 | 113.4 |
| H9 | 103.1 | 117.5 | 98.4 | 109.3 | 133.3 | * | * |
| H63 | 118.6 | 146.1 | 113.6 | 147.4 | 104.4 | 144.2 | 150.0 |
| **C35** | | | | | | | |
| HNM | 115.8 | 115.0 | 96.2 | 97.4 | 109.4 | 112.0 | 88.3 |
| H62 | 91.8 | 99.8 | * | * | 96.6 | 105.6 | 93.1 |
| H69 | 116.9 | 136.4 | 117.8 | 118.9 | 109.5 | 98.3 | 110.9 |
| H12 | 93.2 | 82.7 | 105.4 | 95.5 | 87.1 | * | 83.5 |
| H24 | * | 147.4 | 140.3 | 127.7 | 138.2 | 136.9 | 157.3 |
| H18 | 196.1 | 112.5 | 158.7 | 125.4 | 122.4 | 98.0 | 110.9 |
| H9 | 111.7 | 112.9 | 84.4 | 103.2 | 124.1 | * | * |
| H63 | 107.2 | 127.5 | 132.0 | 123.6 | 114.6 | 114.9 | 123.6 |

## Appendix 14a(Cont). Estimated intake(g/kgLW$^{0.75}$) using C31, C33 and C35 in T1.

| | Day | | | | | | |
|---|---|---|---|---|---|---|---|
| **C31** | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Animal No. | | | | | | | |
| F29 | 83.2 | 111.9 | 77.4 | 65.8 | 87.3 | * | * |
| F30 | 72.3 | 94.2 | * | 84.0 | 94.4 | * | 36.0 |
| F28 | * | 104.8 | 83.1 | 117.9 | 134.6 | 58.6 | 108.4 |
| F26 | 114 | 93.8 | 127.2 | 86.4 | 85.3 | 103.3 | 113.5 |
| F33 | 91.1 | 78.2 | 91.1 | 82.5 | 98.2 | 92.0 | 62.8 |
| F32 | 98.1 | 99.2 | * | 97.7 | 89.0 | 93.2 | 77.5 |
| F34 | * | 115.8 | 83.0 | 112.2 | 96.7 | 106.3 | 69.1 |
| F31 | 119.5 | 102.5 | 96.8 | 116.7 | 109.6 | 95.3 | 64.0 |
| **C33** | | | | | | | |
| F29 | 83.2 | 111.9 | 77.4 | 65.8 | 87.3 | * | * |
| F30 | 72.3 | 94.2 | * | 84.0 | 94.4 | * | 36.0 |
| F28 | * | 104.8 | 83.1 | 117.9 | 134.6 | 58.6 | 108.4 |
| F26 | 114.0 | 93.8 | 127.2 | 86.4 | 85.3 | 103.3 | 113.5 |
| F33 | 91.1 | 78.2 | 91.1 | 82.5 | 98.2 | 92.0 | 62.8 |
| F32 | 98.1 | 99.2 | * | 97.7 | 89.0 | 93.2 | 77.5 |
| F34 | * | 115.8 | 83.0 | 112.2 | 96.7 | 106.3 | 69.1 |
| F31 | 119.5 | 102.5 | 96.8 | 116.7 | 109.6 | 95.3 | 64.0 |
| **C35** | | | | | | | |
| F29 | 87.7 | 107.7 | 79.4 | 69.3 | 84.4 | * | * |
| F30 | 64.4 | 92.1 | * | 75.4 | 84.6 | * | 63.2 |
| F28 | * | 109.4 | 74.3 | 100.7 | 118.3 | 59.8 | 94.8 |
| F26 | 97.6 | 103.0 | 140.3 | 82.6 | 81.3 | 100.7 | 115.2 |
| F33 | 104.2 | 85.0 | 93.9 | 91.2 | 96.8 | 90.1 | 82.4 |
| F32 | 104.7 | 122.1 | * | 88.2 | 92.1 | 85.2 | 86.1 |
| F34 | * | 123.3 | 89.8 | 115.1 | 101.2 | 111.8 | 74.2 |
| F31 | 119.5 | 105.3 | 98.2 | 122.4 | 109.6 | 79.7 | 64.0 |

## Appendix 14b. Estimated intake(g/kgLW$^{0.75}$) using C31, C33 and C35 in T2.

| | Day | | | | | |
|---|---|---|---|---|---|---|
| **C31** | 1 | 2 | 3 | 4 | 5 | 6 |
| Animal No. | | | | | | |
| F32 | 78 | 101 | 108 | 86 | 98 | 99 |
| F61 | 94 | 108 | 130 | 113 | 55 | 67 |
| F33 | 87 | * | * | 94 | 35 | 91 |
| F34 | 101 | 101 | 98 | 88 | 83 | 71 |
| F58 | 115 | 94 | 96 | 88 | 107 | 89 |
| F31 | 90 | 97 | 42 | 110 | 117 | 71 |
| F53 | 130 | 94 | 100 | 95 | 98 | 109 |
| F54 | 128 | 100 | 104 | 100 | 64 | 114 |
| **C33** | | | | | | |
| F32 | 104 | 99 | 107 | 90 | 94 | 93 |
| F61 | 95 | 101 | 118 | 102 | 94 | 78 |
| F33 | 98 | * | * | 107 | 49 | 93 |
| F34 | 108 | 102 | 100 | 95 | 95 | 97 |
| F58 | 99 | 98 | 99 | 78 | 137 | 82 |
| F31 | 94 | 99 | 69 | 103 | 117 | 88 |
| F53 | 132 | 108 | 102 | 98 | 99 | 102 |
| F54 | 117 | 95 | 106 | 98 | 77 | 112 |
| **C35** | | | | | | |
| F32 | 113 | 104 | 116 | 102 | 99 | 97 |
| F61 | 103 | 144 | 122 | 105 | 105 | 87 |
| F33 | 110 | * | * | 113 | 95 | 101 |
| F34 | 117 | 110 | 108 | 109 | 112 | 113 |
| F58 | 94 | 106 | 98 | 83 | 169 | 86 |
| F31 | 99 | 103 | 86 | 102 | 125 | 103 |
| F53 | 141 | 127 | 117 | 108 | 112 | 106 |
| F54 | 115 | 101 | 114 | 108 | 91 | 121 |

**Appendix 14b(Cont). Estimated intake(g/kgLW$^{0.75}$) using C31, C33 and C35 in T2.**

| HEREFORDS | | | | Day | | |
|---|---|---|---|---|---|---|
| **C31** | 1 | 2 | 3 | 4 | 5 | 6 |
| H63 | 81 | * | 94 | 97 | 145 | 97 |
| H69 | 109 | 105 | * | 93 | 118 | 79 |
| H62 | * | * | 111 | 113 | 126 | 123 |
| H24 | 126 | 68 | 61 | 100 | 122 | 109 |
| HNM | 81 | 116 | * | 114 | 112 | 106 |
| H9 | 106 | 117 | 101 | 77 | * | * |
| H12 | 95 | 106 | 101 | 108 | 118 | 97 |
| **C33** | | | | | | |
| H63 | 94 | 112 | 99 | 97 | 89 | 96 |
| H69 | 109 | 99 | * | 102 | 119 | 104 |
| H62 | * | * | 109 | 101 | 130 | 133 |
| H24 | 116 | 98 | 91 | 61 | 114 | 112 |
| HNM | 86 | 109 | * | 111 | 106 | 105 |
| H9 | 102 | 116 | 108 | 78 | * | * |
| H12 | 96 | 108 | 93 | 108 | 129 | 95 |
| **C35** | | | | | | |
| H63 | 114 | 136 | 109 | 103 | 85 | 103 |
| H69 | 117 | 102 | * | 116 | 133 | 92 |
| H62 | * | * | 117 | 98 | 142 | 145 |
| H24 | 126 | 129 | 116 | 56 | 116 | 120 |
| HNM | 98 | 114 | * | 124 | 120 | 117 |
| H9 | 107 | 125 | 121 | 83 | * | * |
| H12 | 105 | 118 | 98 | 116 | 142 | 98 |

**Appendix 15a. Analysis of variance for alkane estimated intake in T1 using Genstat (1995).**

```
***** Analysis of variance *****

Variate: Average intake across days

Source of variation      d.f.       s.s.       m.s.     v.r.  F pr.
Method                      2       34.3       17.1     0.11  0.898
Breed                       2     7503.1     3751.5    23.63  <.001
Method.Breed                4      105.5       26.4     0.17  0.955
Residual                   57     9048.4      158.7
Total                      65    16691.2

***** Tables of means *****

Variate: Average intake.

   Method        1        2        3
            104.0    103.1    102.2

    Breed        1        2        3
             98.2     92.9    116.9
     rep.       18       24       24

   Method    Breed        1        2        3
        1              98.5     92.7    119.4
             rep.         6        8        8
        2              97.6     92.7    117.7
             rep.         6        8        8
        3              98.4     93.5    113.8
             rep.         6        8        8

*** Standard errors of differences of means ***

Table                Method       Breed      Method
                                                Breed
rep.                     22     unequal     unequal
d.f.                     57          57          57
s.e.d.                             4.20X        7.27  min.rep
                       3.80        3.93        6.80  max-min
                                   3.64        6.30  max.rep

 (No comparisons in categories where s.e.d. marked with an X)

***** Stratum standard errors and coefficients of variation *****

Variate: average

   d.f.          s.e.         cv%
     57         12.60        12.2
```

**Appendix 15a(Cont). T - Tests for differences in intake between breeds in T1.**

The significants of the F value for breed ($p<0.01$) means there were breed differences in intake.

Herefords vs Holsteins.

$$t_{(57)}=\frac{116.9-92.9}{3.64}$$

Conclusion:

The calculated t value of 6.59 is significant ($P<0.01$), ie, the Hereford had intake significantly higher than the Jerseys.

Jerseys vs Holsteins.

$$t_{57}=\frac{98.2-92.9}{3.93}$$

The calculated t value of 1.38 is not significant ($p>0.05$), ie, the Jerseys and the Herefords had the same ADG.

Herefords vs Jerseys.

$$t_{57}=\frac{116.9-98.2}{3.93}$$

Conclusion:

The calculated t value for 4.75 is significant ($p<0.01$), ie the Herefords had higher ADG.

## Appendix 15b. Analysis of variance for intake in T2 (Genstat 1995).

***** Analysis of variance *****

Variate: Average intake across days.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Method | 2 | 1254.75 | 627.38 | 13.48 | <.001 |
| Breed | 1 | 591.36 | 591.36 | 12.71 | 0.001 |
| Additive | 1 | 13.72 | 13.72 | 0.29 | 0.591 |
| Method.Breed | 2 | 71.74 | 35.87 | 0.77 | 0.471 |
| Method.Supp | 2 | 8.68 | 4.34 | 0.09 | 0.911 |
| Breed.Supp | 1 | 139.41 | 139.41 | 3.00 | 0.093 |
| Method.Breed.Supp | 2 | 2.02 | 1.01 | 0.02 | 0.979 |
| Residual | 33 | 1535.54 | 46.53 | | |
| Total | 44 | 3617.23 | | | |

***** Tables of means *****

Variate: average intake.

| Method | 1 | 2 | 3 |
|---|---|---|---|
| | 98.5 | 101.0 | 110.8 |
| rep. | 24 | 21 | |

| Breed | 1 | 2 |
|---|---|---|
| | 100.1 | 107.3 |
| rep. | 24 | 21 |

| Supp. | 1 | 2 |
|---|---|---|
| | 102.9 | 104.0 |
| rep. | 24 | 21 |

| Method | Breed | 1 | 2 | 3 |
|---|---|---|---|---|
| 1 | | 93.6 | 104.3 | |
| | rep. | 8 | 7 | |
| 2 | | 98.0 | 104.4 | |
| | rep. | 8 | 7 | |
| 3 | | 108.6 | 113.3 | |
| | rep. | 8 | 7 | |

| Method | Supp | 1 | 2 |
|---|---|---|---|
| 1 | | 98.5 | 98.5 |
| | rep. | 8 | 7 |
| 2 | | 100.5 | 101.6 |
| | rep. | 8 | 7 |
| 3 | | 109.8 | 111.9 |
| | rep. | 8 | 7 |

**Appendix 15b (Cont). Analysis of variance for intake in T2 (Genstat 1995).**

```
*** Standard errors of differences of means ***

Table                Method       Breed    Additive      Method
                                                            Breed
rep.                     15     unequal     unequal     unequal
d.f.                     33          33          33          33
s.e.d.                                                     3.65  min.rep
                       2.49        2.04        2.04        3.53  max-min
                                                           3.41  max.rep

Table              Method
                  Additive
rep.               unequal
d.f.                    33
s.e.d.                3.65  min.rep
                      3.53  max-min
                      3.41  max.rep
Except when comparing means with the same level (s) of
 Method               3.66  min.rep
                      3.54  max-min
                      3.42  max.rep

***** Stratum standard errors and coefficients of variation *****

Variate: average
    d.f.          s.e.          cv%
      33          6.82          6.6
```

**Appendix 15b (Cont). T-tests for differences in intake in T2.**

The significants of the F values ($p<0.05$) for breed and method means that there were differences in estimated intake in both breed and method of estimation.

Comparison of Methods:

**C31 vs C33.**

$$t_{(33)} = \frac{101 - 98.5}{2.49}$$

Conlusion:

The calculated t value of 1.004 is not significant ($p<0.05$), ie, there was no difference between C31 and C33 estimated intake.

**C31 vs c35.**

$$t_{(33)} = \frac{110.8 - 98.5}{2.49}$$

The calculated t value of 4.94 is significant ($p<0.01$), ie, C35 had high estimates of intake compared to C31.

**C33 vs C35.**

$$t_{(33)} = \frac{110.8 - 101.0}{2.49}$$

The calculated t value of 3.94 is significant, ie, C35 had higher estimated intake compared to C33.

**Appendix 15b (Cont). T-tests for differences in intake in T2.**

The significance of the F ratio for 'breed' means there were breed differences in intake.

**Hereford vs Holstein**

$$t_{(33)} = \frac{107.3 - 100.1}{2.04}$$

The calculated t value of 3.53 is significant ($p<0.01$), ie, the Herefords had higher intake than the Holsteins.

## Appendix 16. T test for differences in intake between T1 and T2 using C33 data

Herefords.

$$t_{(84)} = \frac{117.7 - 104.4}{\sqrt{8.41 + 5.29}}$$

The calculated t value of 3.58 is significant ($p<0.05$), ie, there was a drop in intake in Herefords in T1 compared to T2.

Holsteins.

$$t_{(93)} = \frac{92.7 - 98.0}{\sqrt{7.29 + 4.84}}$$

The calculated t value of 1.52 is not significant (p.0.05), ie, there was no change in intake in Holsteins in T1 compared to T2.

**Appendix 17a. Estimated digestibility(%) using C35 in T1.**

| | Day | | | | | | |
|---|---|---|---|---|---|---|---|
| Animal No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| JERSEYS | | | | | | | |
| INTRO | 46.3 | 65.2 | 48.4 | 70.8 | 59.2 | 63.6 | 66.3 |
| ISMAY | 64.2 | * | * | 60.1 | 61.0 | 55.8 | 56.3 |
| INTEREST | 52.5 | 16.5 | 22.1 | 56.0 | 48.5 | 65.0 | 57.5 |
| ILVA | 63.1 | * | 36.8 | 48.6 | * | 48.9 | 42.7 |
| DONGA | 48.6 | 39.4 | 53.6 | 49.8 | 55.2 | 53.6 | 26.7 |
| DOLPHIN | 54.6 | * | 49.2 | 66.7 | 43.7 | 28.7 | * |
| HOLSTEINS | | | | | | | |
| F29 | 59.0 | 65.9 | 13.3 | 58.3 | 64.3 | * | * |
| F30 | 53.8 | 62.7 | * | 61.5 | 64.1 | * | 46.7 |
| F28 | * | 65.6 | 59.7 | 62.2 | 61.6 | 53.3 | 0.4 |
| F26 | 67.7 | 62.5 | 62.4 | 49.7 | 59.5 | 61.7 | 63.1 |
| F33 | 56.4 | 51.3 | 34.7 | 65.4 | 68.7 | 61.2 | 41.5 |
| F32 | 34.1 | 42.5 | * | 52.3 | 43.7 | 66.9 | 57.7 |
| F34 | * | 64.3 | 64.3 | 66.1 | 58.6 | 62.1 | 60.1 |
| F31 | 55.7 | 58.7 | 66.5 | 56.0 | 61.5 | 48.2 | 52.1 |
| HEREFORDS | | | | | | | |
| HNM | 68.6 | 68.6 | 65.8 | 58.1 | 52.9 | 70.5 | 70.8 |
| H62 | 50.6 | 63.9 | * | * | 62.6 | 60.1 | 64.5 |
| H69 | 65.1 | 69.8 | 61.1 | 0.6 | 66.9 | 62.7 | 57.9 |
| H12 | 56.5 | 62.4 | 57.5 | 53.8 | 62.6 | * | 63.8 |
| H24 | * | 69.9 | 71.7 | 69.8 | 68.6 | 66.7 | 59.8 |
| H18 | 43.0 | 67.0 | 28.9 | 67.7 | 60.5 | 65.2 | 65.8 |
| H9 | 30.4 | 60.5 | 41.5 | 67.4 | 63.9 | * | * |
| H63 | 60.1 | 66.9 | 53.6 | 67.1 | 55.5 | 64.7 | 63.9 |

**Appendix 17b. Estimated digestibility(%) using C35 in T2**

| | Day | | | | | |
|---|---|---|---|---|---|---|
| Animal No. | 1 | 2 | 3 | 4 | 5 | 6 |
| HOLSTEINS | | | | | | |
| F32 | 58 | 61 | 57 | 51 | 63 | 60 |
| F61 | 64 | 64 | 64 | 65 | 73 | 57 |
| F33 | 60 | * | * | 64 | 49 | 56 |
| F34 | 53 | 53 | 66 | 66 | 66 | 66 |
| F58 | 70 | 60 | 68 | 64 | 53 | 63 |
| F31 | 47 | 50 | 59 | 65 | 63 | 66 |
| F53 | 73 | 58 | 36 | 67 | 67 | 68 |
| F54 | 52 | 59 | 62 | 60 | 58 | 64 |
| HEREFORDS | | | | | | |
| H63 | 62 | 56 | 71 | 72 | 68 | 69 |
| H69 | 63 | 63 | * | 67 | 72 | 28 |
| H62 | * | * | 55 | 65 | 70 | 66 |
| H24 | 42 | 57 | 29 | 60 | 52 | 69 |
| HNM | 63 | 67 | * | 49 | 52 | 71 |
| H9 | 67 | 62 | 63 | 66 | * | * |
| H12 | 65 | 64 | 62 | 69 | 67 | 66 |

**Appendix 18a. Analysis of Variance for digestibility in T1 (Genstat 1995).**

```
***** Analysis of variance *****

Variate: Digestibility.

Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Breed                      2     248.25     124.12    4.37  0.027
Residual                  19     539.41      28.39
Total                     21     787.66

***** Tables of means *****

Variate: digest

Grand mean  56.4

    Breed         1         2         3
              51.4      56.8      59.8
     rep.         6         8         8

*** Standard errors of differences of means ***

Table                 Breed
rep.                unequal
d.f.                     19
s.e.d.                3.08X min.rep
                      2.88  max-min
                      2.66  max.rep

 (No comparisons in categories where s.e.d. marked with an X)

***** Stratum standard errors and coefficients of variation *****

Variate: digest

   d.f.          s.e.          cv%
     19          5.33          9.4
```

The significance of the F-value ($p<0.05$) means there were breed differences in digestibility.

**Appendix 18a (Cont). Test for breed differences in digestibility in T1**

Herefords vs Jerseys

$$t_{(19)} = \frac{59.8 - 51.4}{2.88}$$

Conclusion.

The calculated t value of 3.15 is significant ($p<0.01$), ie, the Herefords had higher digestibility compared to Jerseys.

Holsteins vs Jerseys.

$$t_{(19)} = \frac{56.8 - 51.4}{2.88}$$

The calculated t value of 1.875 is not significant ($p<0.05$), ie, there was no difference in digestibility between the Holsteins and the Jerseys.

Herefords vs Holsteins.

$$t_{(19)} = \frac{59.8 - 56.8}{2.66}$$

Conclusion:

The calculated value of 1.13 is not significant ($p<0.05$), ie, there were no differences in digestibility between the Herefords and the Holsteins.

**Appendix 18b. Analysis of variance for digestibility in T2.**

***** Analysis of variance *****

Variate: Digestibility.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Breed | 1 | 5.11 | 5.11 | 0.28 | 0.607 |
| Supplement | 1 | 7.20 | 7.20 | 0.40 | 0.542 |
| Breed.Supplement | 1 | 1.76 | 1.76 | 0.10 | 0.761 |
| Residual | 11 | 200.17 | 18.20 | | |
| Total | 14 | 214.25 | | | |

***** Tables of means *****

Variate: Digestibility.

Grand mean 61.0

| Breed | 1 | 2 |
|---|---|---|
| | 60.5 | 61.6 |
| rep. | 8 | 7 |

| Supplement | 1 | 2 |
|---|---|---|
| | 61.7 | 60.3 |
| rep. | 8 | 7 |

*** Table of means for Breed. Supplement cannot be calculated
(contains mutually non-orthogonal components).

*** Standard errors of differences of means ***

| Table | Breed | Supplement |
|---|---|---|
| rep. | unequal | unequal |
| d.f. | 11 | 11 |
| s.e.d. | 2.21 | 2.21 |

***** Stratum standard errors and coefficients of variation *****

Variate: Digestibility.

| d.f. | s.e. | cv% |
|---|---|---|
| 11 | 4.27 | 7.0 |

The non-significants of the F-ratios means the re were no significant treatment effects on digestibility.

## Appendix 19. Sampling time and Concentration of $Cr_2O_3$(ppm) in faeces.

| | Supp | | | Nonsupp | | |
|---|---|---|---|---|---|---|
| TIME(hrs) | | | | | | |
| HEREFORDS | H62 | H18 | H24 | H69 | H63 | HNM |
| 8 | 798.8 | 334.7 | 566.7 | 536.7 | 403.6 | 391.0 |
| 12 | 1200.4 | 799.0 | 1926.5 | 2082.0 | 1019.8 | 1766.2 |
| 16 | 2428.2 | 2353.9 | 2411.8 | 3137.1 | 2273.2 | 3073.8 |
| 20 | 3569.5 | 2431.1 | 2250.6 | 3339.1 | 2845.9 | 3171.1 |
| 24 | 2999.8 | 2352.7 | 1869.3 | 2112.7 | 2489.3 | 3181.4 |
| 28 | 2406.1 | 1954.8 | 1503.5 | 1817.3 | 2184.1 | 1970.5 |
| 32 | 1974.3 | 2213.3 | 1288.2 | 1637.7 | 1714.1 | 1844.9 |
| 36 | 1503.1 | 1800.6 | 1060.5 | 1460.4 | 1395.4 | 1415.7 |
| 40 | 1269.7 | 1626.5 | 911.7 | 1142.4 | 1081.4 | 1157.1 |
| 44 | 971.8 | 1187.7 | 756.0 | 1070.6 | 1009.2 | 1069.1 |
| 48 | 728.4 | 843.1 | 581.7 | 716.5 | 650.5 | 809.4 |
| 54 | 539.4 | 695.1 | 407.9 | 531.0 | 507.8 | 503.0 |
| 60 | 417.3 | 558.7 | 341.6 | 469.9 | 354.9 | 428.5 |
| 66 | 298.5 | 348.6 | 283.9 | 322.6 | 281.6 | 360.3 |
| 72 | 148.4 | 247.6 | 164.6 | 279.6 | 154.6 | 309.1 |
| 80 | 94.0 | 112.5 | 84.6 | 79.7 | 81.9 | 102.7 |
| 96 | 34.0 | 64.1 | 40.6 | 82.7 | 50.2 | 51.4 |
| HOLSTEINS | | | | | | |
| | H58 | F53 | F31 | F34 | F54 | F61 |
| 8 | 295.1 | 215.5 | 255.3 | 869.2 | 194.2 | 531.7 |
| 12 | 2900.0 | 1129.9 | 1168.4 | 4987.0 | 845.4 | 1002.2 |
| 16 | 2707.8 | 1817.5 | 3230.2 | 7862.7 | 1943.5 | 2280.1 |
| 20 | 3639.3 | 2343.1 | 3377.8 | 5615.3 | 3349.3 | 2541.9 |
| 24 | 3514.5 | 2856.5 | 2707.3 | 2739.3 | 2099.0 | 2653.1 |
| 28 | 2414.8 | 2414.8 | 2414.8 | 1788.4 | 2127.6 | 2401.9 |
| 32 | 1706.7 | 1765.2 | 1647.1 | 1530.6 | 1356.9 | 1595.2 |
| 36 | 2328.4 | 1509.0 | 1621.7 | 1420.1 | 1184.5 | 1639.4 |
| 40 | 1145.0 | 1043.5 | 1246.5 | 680.5 | 1048.4 | 1392.3 |
| 44 | 1077.5 | 844.5 | 1162.2 | 452.4 | 1058.0 | 1168.4 |
| 48 | 1058.0 | 373.1 | 733.9 | 380.4 | 766.9 | 1153.5 |
| 54 | 878.2 | 527.9 | 675.9 | 233.3 | 409.3 | 661.5 |
| 60 | 758.6 | 372.0 | 473.7 | 233.4 | 500.8 | 561.7 |
| 66 | 591.4 | 139.4 | 384.8 | 139.6 | 294.0 | 426.4 |
| 72 | 367.4 | 262.3 | 308.9 | 122.9 | 189.8 | 583.1 |
| 80 | 244.1 | 105.5 | 136.1 | 98.2 | 90.0 | 155.3 |
| 96 | 72.8 | 35.2 | 30.3 | 26.2 | 49.1 | 66.6 |

## Appendix 20. Summary of Model parameters from non-linear iterative procedures of Blaxter *et al.* (1956) (G) and Dhanoa *et al.* (1985) (D)

| BREED | SUPP | A_D | K1_D | K2_D | N_D | A_1 | K1_G | K2_G | T_T |
|---|---|---|---|---|---|---|---|---|---|
| H | 1 | 24471.48 | 0.07 | 0.19 | 9.40 | 424514.84 | 0.08 | 0.08 | 7.3 |
| H | 1 | 17245.47 | 0.06 | 0.19 | 11.69 | 20542.99 | 0.06 | 0.08 | 7.4 |
| H | 1 | 28546.85 | 0.06 | 0.18 | 5.53 | 3898.70 | 0.05 | 0.34 | 9.2 |
| H | 0 | 7492.990 | 0.05 | 0.36 | 29.76 | 28537.25 | 0.08 | 0.11 | 7.3 |
| H | 0 | 17241.74 | 0.07 | 0.19 | 10.82 | 38765.09 | 0.08 | 0.09 | 8.1 |
| H | 0 | 10984.30 | 0.06 | 0.30 | 21.67 | 6343.47 | 0.05 | 0.17 | 6.1 |
| HF | 1 | 11920.27 | 0.05 | 0.33 | 32.38 | 12541.69 | 0.06 | 0.12 | 7.5 |
| HF | 1 | 12072.01 | 0.06 | 0.25 | 17.71 | 115935.12 | 0.08 | 0.08 | 8.3 |
| HF | 1 | 13624.53 | 0.06 | 0.33 | 44.78 | 5301.32 | 0.05 | 0.37 | 11.2 |
| HF | 0 | 18465.65 | 0.08 | 0.45 | 50.05 | 384322.75 | 0.14 | 0.15 | 7.6 |
| HF | 0 | 10877.08 | 0.06 | 0.24 | 17.81 | 345376.99 | 0.08 | 0.08 | 8.2 |
| HF | 0 | 10969.65 | 0.05 | 0.22 | 15.78 | 5106.16 | 0.04 | 0.20 | 10.5 |

N.B. Supp = 1; No Supp = 0. H = Hereford. HF = Holstein.

## Appendix 21. Test for relationship between k1D and k1G for $Cr_20_3$ data.

Dependent Variable: K1G

Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Value | Prob>F |
|---|---|---|---|---|---|
| Model | 1 | 0.00377 | 0.00377 | 8.268 | 0.0165 |
| Error | 10 | 0.00456 | 0.00046 | | |
| C Total | 11 | 0.00833 | | | |

| | | | |
|---|---|---|---|
| Root MSE | 0.02136 | R-square | 0.4526 |
| Dep Mean | 0.07084 | Adj R-sq | 0.3979 |
| C.V. | 30.15080 | | |

Parameter Estimates

| Variable | DF | Parameter Estimate | Standard Error | T for H0: Parameter=0 | Prob > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -0.053161 | 0.04356374 | -1.220 | 0.2503 |
| K1D | 1 | 2.112142 | 0.73455493 | 2.875 | 0.0165 |

## Appendix 21 (Cont). The relationship between K1G and K1D on $Cr_2O_3$ data.

The significants of the F-ratio means that there was a significant linear relationship between the two constants. The regression accounted for 45.26% of the variation.

The non significants of the term 'intercept' means the intercept is not significantly different from zero.

**T-test for regression coefficient.**

$$t_{(10)} = \frac{2.112142 - 1}{0.73455493}$$

Conclusion:

The calculated t rario of 1.514 is not significant ($p<0.01$), ie, the slope is not different from 1.0.

## Appendix 21 (Cont). Analysis of Variance for K1D and K1G on Cr2O3.

General Linear Models Procedure

Dependent Variable: K1G

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > Model |
|---|---|---|---|---|---|
| | 3 | 0.00150994 | 0.00050331 | | 0.59 |
| 0.6386 | | | | | |
| Error | 8 | 0.00682421 | 0.00085303 | | |
| Corrected Total | 11 | 0.00833414 | | | |

| R-Square | C.V. | Root MSE | K1G Mean |
|---|---|---|---|
| 0.181175 | 41.22820 | 0.02920661 | 0.07084134 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| BREED | 1 | 0.00028420 | 0.00028420 | 0.33 | 0.5797 |
| SUPP | 1 | 0.00086490 | 0.00086490 | 1.01 | 0.3434 |
| BREED*SUPP | 1 | 0.00036084 | 0.00036084 | 0.42 | 0.5337 |

Dependent Variable: K1D

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > Model |
|---|---|---|---|---|---|
| | 3 | 0.00010278 | 0.00003426 | | 0.37 |
| 0.7776 | | | | | |
| Error | 8 | 0.00074274 | 0.00009284 | | |
| Corrected Total | 11 | 0.00084552 | | | |

| R-Square | C.V. | Root MSE | K1D Mean |
|---|---|---|---|
| 0.121559 | 16.41217 | 0.00963546 | 0.05870927 |

**Appendix 21 (Cont). Analysis of variance for k1D and k2G.**

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| BREED | 1 | 0.00000172 | 0.00000172 | 0.02 | 0.8951 |
| SUPP | 1 | 0.00000268 | 0.00000268 | 0.03 | 0.8694 |
| BREED*SUPP | 1 | 0.00009838 | 0.00009838 | 1.060.3334 | |

Summary Table of Means.

| Level of BREED | Level of SUPP | N | K1G Mean | K1G SD |
|---|---|---|---|---|
| H | 0 | 3 | 0.06898091 | 0.01964520 |
| H | 1 | 3 | 0.06296873 | 0.01667288 |
| HF | 0 | 3 | 0.08968118 | 0.05020042 |
| HF | 1 | 3 | 0.06173454 | 0.01510307 |

| Level of BREED | Level of SUPP | N | K1D Mean | K1D SD |
|---|---|---|---|---|
| H | 0 | 3 | 0.05575235 | 0.00904832 |
| H | 1 | 3 | 0.06242339 | 0.00886813 |
| HF | 0 | 3 | 0.06072184 | 0.01335784 |
| HF | 1 | 3 | 0.05593951 | 0.00569393 |

The non significance of the F-ratios means there were no breed, diet or breed*diet effects on k1.

## Appendix 22. Sampling times and faecal alkane concentrations (mg/kg)

| Herefords | | | | | | |
|---|---|---|---|---|---|---|
| | Supp | | | Non-Supp | | |
| Time(Hrs) | H62 | H24 | H18 | H69 | HNM | H63 |
| 8 | 65 | * | 42 | 84 | 61 | 53 |
| 12 | 25 | 13 | 81 | 127 | 132 | 40 |
| 16 | 159 | 175 | * | * | 173 | 136 |
| 20 | 101 | 335 | 151 | 392 | 236 | 196 |
| 24 | 81 | * | * | 121 | 200 | 150 |
| 28 | * | * | * | * | * | * |
| 32 | * | 154 | 209 | * | * | * |
| 36 | * | 50 | * | 83 | * | * |
| 40 | 8 | 100 | 168 | 70 | * | * |
| 44 | * | * | * | * | * | * |
| 48 | * | * | * | * | * | * |
| 54 | 56 | 51 | * | * | 53 | 36 |
| 60 | * | * | * | * | * | * |
| 66 | * | * | 125 | 26 | 58 | 85 |
| 72 | 82 | 35 | 52 | * | * | * |
| 80 | * | * | * | * | 54 | * |
| Holsteins. | | | | | | |
| | F61 | F34 | F54 | F31 | F58 | F53 |
| 8 | * | 74 | 34 | * | 28 | 118 |
| 12 | * | 189 | 110 | * | 202 | 136 |
| 16 | * | 171 | * | * | * | 130 |
| 20 | * | * | 153 | 200 | 153 | * |
| 24 | * | * | * | 159 | * | * |
| 28 | 175 | 196 | * | * | * | * |
| 32 | 127 | 161 | * | 196 | 135 | 188 |
| 36 | 128 | * | * | 173 | * | * |
| 40 | * | 133 | 141 | 188 | * | * |
| 44 | * | * | * | * | * | * |
| 48 | * | * | * | * | * | * |
| 54 | 41 | 127 | * | * | 158 | 126 |
| 60 | * | 124 | 125 | 41 | * | * |
| 66 | * | * | 52 | * | 78 | 42 |
| 72 | * | * | * | * | * | * |
| 80 | * | * | * | * | 41 | * |

## Appendix 23. Summary of parameters using nonlinear iterative procedures on C32 data.

| OBS | BREED | SUP | AD | K1D | K2D | ND | A1 | K1G | K2G | TT |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | HEREFORD | NS | 355.04 | 0.027136 | 0.26775 | 13.9305 | 326.77 | 0.027341 | 0.14425 | 6.30268 |
| 2 | HEREFORD | S | 3112.58 | 0.054389 | 0.09228 | 5.7787 | 2866.34 | 0.031655 | 0.03722 | 2.83824 |
| 3 | HOLSTEIN | NS | 524.49 | 0.047046 | 0.24135 | 10.2011 | 759.52 | 0.059729 | 0.11312 | 6.13222 |
| 4 | HOLSTEIN | S | 409.66 | 0.029726 | 0.19064 | 8.7639 | 849.38 | 0.044178 | 0.08147 | 6.49284 |

NS = NO SUPPLEMENT. S = SUPPLEMENT

## Appendix 24. Test of relationship between K1D and K1G using C32 data.

Dependent Variable: K1G

Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Value | Prob>F |
|---|---|---|---|---|---|
| Model | 1 | 0.00004 | 0.00004 | 0.125 | 0.7577 |
| Error | 2 | 0.00060 | 0.00030 | | |
| C Total | 3 | 0.00063 | | | |

| | | | |
|---|---|---|---|
| Root MSE | 0.01728 | R-square | 0.0587 |
| Dep Mean | 0.04073 | Adj R-sq | -0.4120 |
| C.V. | 42.43074 | | |

Parameter Estimates

| Variable | DF | Parameter Estimate | Standard Error | T for H0: Parameter=0 | Prob > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 0.030206 | 0.03101736 | 0.974 | 0.4328 |
| K1D | 1 | 0.265818 | 0.75275370 | 0.353 | 0.7577 |

The non signifcance of the F ratio means there was no significant relationship between the two constants.

**Appendix 25a. k1 values using the graphical procedure on C32 data.**

| OBS | BRED | SUP | ANIM | MODEL | TYPE | DEPVAR | RMSE | A1 | K1 | LN |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | HF | 0 | 34 | | | LN | 0.05345 | 6.17143 | -.0321 | -1.0 |
| 2 | HF | 0 | 54 | | | LN | 0.46182 | 6.06942 | -.0272 | -1.0 |
| 3 | HF | 0 | 61 | | | LN | 0.16358 | 6.74810 | -.0559 | -1.0 |
| 4 | HF | 1 | 31 | | | LN | 0.45931 | 6.63991 | -.0409 | -1.0 |
| 5 | HF | 1 | 33 | | | LN | 0.20043 | 7.38448 | -.0603 | -1.0 |
| 6 | HF | 1 | 58 | | | LN | 0.43730 | 5.98435 | -.0252 | -1.0 |
| 7 | H | 0 | HNM | | | LN | 0.34832 | 5.68427 | -.0238 | -1.0 |
| 8 | H | 0 | 63 | | | LN | . | 6.12000 | -.0467 | -1.0 |
| 9 | H | 0 | 69 | | | LN | 0.02845 | 5.66219 | -.0358 | -1.0 |
| 10 | H | 1 | 18 | | | LN | 0.32463 | 6.16435 | -.0260 | -1.0 |
| 11 | H | 1 | 24 | | | LN | 0.19045 | 6.02600 | -.0354 | -1.0 |
| 12 | H | 1 | 62 | | | LN | . | 4.72000 | -.0133 | -1.0 |

NB. Supp = 1 ; Non-supp = 0., H = Hereford ; HF = Holstein.

**Appendix 25a(Cont). Analysis of variance on K1 (Graphical procedure) using C32 data.**

General Linear Model Procedure for K1

Class Level Information

| Class | Levels | Values |
|---|---|---|
| BREED | 2 | F H |
| SUP | 2 | 0 1 |

Number of observations in data set = 12

Dependent Variable: K1

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 0.00049416 | 0.00016472 | 0.82 | 0.5168 |
| Error | 8 | 0.00160043 | 0.00020005 | | |
| Corrected Total | 11 | 0.00209459 | | | |

| R-Square | C.V. | Root MSE | K1 Mean |
|---|---|---|---|
| 0.235920 | -40.15349 | 0.0141441 | -0.03522497 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| BREED | 1 | 0.00030656 | 0.00030656 | 1.53 | 0.2509 |
| SUPP | 1 | 0.00003488 | 0.00003488 | 0.17 | 0.6872 |
| BREED*SUPP | 1 | 0.00015272 | 0.00015272 | 0.76 | 0.4077 |

General Linear Models Procedure

Least Squares Means

| BREED | K1 LSMEAN | Std Err LSMEAN | Pr > \|T\| H0:LSMEAN=0 |
|---|---|---|---|
| HF | -0.04027932 | 0.00577429 | 0.0001 |
| H | -0.03017063 | 0.00577429 | 0.0008 |

| SUP | K1 LSMEAN | Std Err LSMEAN | Pr > \|T\| H0:LSMEAN=0 |
|---|---|---|---|
| 0 | -0.03692991 | 0.00577429 | 0.0002 |
| 1 | -0.03352004 | 0.00577429 | 0.0004 |

| BREED | SUP | K1 LSMEAN | Std Err LSMEAN | Pr > \|T\| H0:LSMEAN=0 |
|---|---|---|---|---|
| HF | 0 | -0.03841683 | 0.00816607 | 0.0015 |
| HF | 1 | -0.04214180 | 0.00816607 | 0.0009 |
| H | 0 | -0.03544298 | 0.00816607 | 0.0025 |
| H | 1 | -0.02489827 | 0.00816607 | 0.0158 |

Lack of significance of the ratio means there was no significant treatment effects on K1.

**Appendix 25 (Cont). Analyses within breed on ln[C32] using the graphical procedure.**

---------- BREED=Holstein ----------

Model: MODEL1
Dependent Variable: LN

Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Value | Prob>F |
|---|---|---|---|---|---|
| Model | 1 | 4.49296 | 4.49296 | 36.850 | 0.0001 |
| Error | 19 | 2.31656 | 0.12192 | | |
| C Total | 20 | 6.80952 | | | |

| | | | |
|---|---|---|---|
| Root MSE | 0.34918 | R-square | 0.6598 |
| Dep Mean | 4.70476 | Adj R-sq | 0.6419 |
| C.V. | 7.42177 | | |

Parameter Estimates

| Variable | DF | Parameter Estimate | Standard Error | T for H0: Parameter=0 | Prob > \|T\| |
|---|---|---|---|---|---|
| INTERCEPT | 1 | 6.104024 | 0.24277129 | 25.143 | 0.0001 |
| TIME | 1 | -0.030356 | 0.00500060 | -6.070 | 0.0001 |

---------- BREED=Hereford ----------

Dependent Variable: LN

Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Value | Prob>F |
|---|---|---|---|---|---|
| Model | 1 | 7.99133 | 7.99133 | 46.879 | 0.0001 |
| Error | 39 | 6.64818 | 0.17047 | | |
| C Total | 40 | 14.63951 | | | |

| | | | |
|---|---|---|---|
| Root MSE | 0.41288 | R-square | 0.5459 |
| Dep Mean | 4.54146 | Adj R-sq | 0.5342 |
| C.V. | 9.09124 | | |

Parameter Estimates

| Variable | DF | Parameter Estimate | Standard Error | T for H0: Parameter=0 | Prob > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 5.785659 | 0.19281895 | 30.006 | 0.0001 |
| TIME | 1 | -0.026541 | 0.00387640 | -6.847 | 0.0001 |

$$t_{(58)} = \frac{0.030356 - 0.026541}{\sqrt{0.0038764^2 + 0.005^2}}$$

The calculated t value of 0.60 is not significant (p>0.05), ie, there was no difference between breeds in K1.