THE ONSET OF PUBERTY AND HERBAGE INTAKE IN DIFFERENT SELECTION LINES OF ANGUS CATTLE

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This thesis is dedicated to my parents

José Ramón Martínez and

Elisa Mercedes Marecos de Martínez

ABSTRACT

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Puberty onset and herbage dry matter intake was measured in four lines of Angus cattle selected for High and Low EBV-600 day weight (HG and LG respectively) and High and Low EBV-milk (HM and LM respectively). The heifers were generated on four industry farms in the North Island of New Zealand.

Heifers from the HG line were younger at puberty (438.6 ± 9.3 days $P < 0.01$) than heifers from the HM (459.4 ± 7.1 days) and LM (476.1 ± 7.8 days) lines. No difference in age at puberty between HG and LG (455.1 ± 11.8 days) lines were found. Average weight at puberty across all genetic lines was (349.6 ± 9.9 kg). There was no difference among the four genotypes for this trait.

Ninety-five percent of the animals reached puberty by the end of the trial and there was no difference in the percentage of animals reaching puberty by genetic line. Animals coming from Farms 1 and 3 tended to reach puberty earlier than animals coming from Farms 2 and 4. There was no differences in pregnancy rate among the genetic lines and the overall pregnancy rate was 90 %.

Intake was measured on two occasions using n-alkanes (M1 and M2 respectively) and the pre- and post-grazing technique (M3 and M4 respectively). Average liveweight (LW) and estimated herbage intake at M1 was 240.2 ± 0.4 kg and 3.47 ± 0.1 kg DM respectively. The values at M2 were 287.2 ± 1.9 kg LW and 6.50 ± 0.36 kg DM. No differences in estimated herbage intake among the genetic lines were detected in M1 or

M2. In M3 heifers had an average LW of 247.1 ± 0.7 kg and mean estimated intakes of 4.86 ± 0.26; 4.17 ± 0.26; 4.37 ± 0.26 and 3.00 ± 0.26 kg DM for the HG, LG, HM and LM lines. The LM line having a significantly ($P < 0.05$) lower estimated intakes than the other lines. Average LW at M4 was 272.5 ± 0.6 kg. Animals from the LM (7.28 ± 0.19 kg DM) line had significantly ($P < 0.05$) higher intakes than animals from the LG (6.52 kg ± 0.18 DM) and HM (6.71 ± 0.18 kg DM) lines. Intakes from the HG (6.99 ± 0.18 kg DM) animals was not significantly different from intakes of the other genetic lines.

In general the HG heifers outperformed the heifers from the other lines in puberty onset and feed conversion efficiency. However, in a self-replacing beef cowherd, the higher maintenance cost and lower milk production of dams from the HG line should be considered. Combining the growth characteristics of the HG lines with an appropriate level of milk production into a selection index would produce animals with the adequate combination of genes for a self-replacing beef cowherd.

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CHAPTER 1

INTRODUCTION

In beef production enterprises there are two major areas which influence the efficiency of the system: reproductive performance and feed intake for growth and maintenance.

Reproductive performance has been identified as the most important economic trait in the beef cattle industry (Baker *et al.*, 1987) and some reports estimate that it can be four times economically more important than growth or end product traits (Evans *et al.*, 1999). Unfortunately, despite the great economic value of the herd's reproductive performance, it is of low heritability (< 10 %) and there is little opportunity to improve it by direct selection (Baker *et al.*, 1987). The low heritability and the high age at which records are collected are responsible for the low genetic progress in reproductive efficiency. However, age at puberty can be used as an indirect method to improve reproductive performance since this trait possesses a heritability in the range of 0.32 to 0.49, is collected at an earlier age and presents a moderate negative genetic correlation with pregnancy rate (Morris & Wilson, 1997).

Age at puberty is particularly important on beef production systems that use restricted breeding seasons and in which pastures are the main source of nutrients (Martin *et al.*, 1992). Similarly, it directly affects the over all efficiency of the productive system by determining the beginning of the mating season, calf performance and lifelong productivity of the cows.

One factor strongly influencing puberty onset is the genetic make up of the animal and both between and within breed differences for age and weight at puberty have been reported (Martin *et al.*, 1992; Hall *et al.*, 1995).

The commercial breeder usually faces the situation in which he/she has to choose between maternal lines (seeking the long term effects of improving maternal traits) or paternal lines (seeking the short term benefits from sale of carcass) (Nicoll, 1999). Thus, it would be important to determine the efficiency (in terms of DM intake to first oestrous) with which lines selected for growth or maternal ability reach puberty as well as the age at which they attain sexual maturity.

In order to do this, Estimated Breeding Value for milk (EBV-milk) and Estimated Breeding Value for growth (EBV-600 day weight) can be used. The EBV's refer to the predicted breeding worth of an animal based on performance records of the animal itself, its ancestors and relatives (i.e., progeny, sibs, etc). (Simm, 2000).

The EBV-600 day weight is at present the best indicator of the likely maintenance cost of the mature animal, while EBV-milk reflects the fact that milk yield is associated with higher metabolic requirements throughout the year (not just lactation). Both EBVs' are ultimately related to the animals' maintenance requirements, which is an economically important trait (Ridler, 1999) considering the fact that 65 to 75 % of total feed consumption is required by the breeding herd, and that 50 % of this intake is required just for maintenance (Montaño-Bermudez *et al.*, 1990).

Therefore, the aim of this study was to determine the effect of the genetic make-up of beef heifers on two economically important traits, age at puberty and feed intake from weaning to puberty on pastoral systems.

CHAPTER 2

LITERATURE REVIEW

The economic and biological role of early puberty in seasonal production systems is reviewed. Different puberty definitions were found in the literature, however, most of them were not appropriate for the purpose of this study. A definition of puberty is presented and the reasons behind the concept are shown.

The physiological changes leading to puberty and the influence of the genetic make up of the animal on puberty onset and reproductive ability are also presented. The last section reviews the literature on the use of n-alkanes as an indirect measure of herbage intake in grazing animals.

2.1. THE IMPORTANCE OF AN EARLY PUBERTY

The onset of puberty is one of the most important factors affecting the economic performance of the cow-calf system, especially in systems with seasonal breeding. Early pubertal animals will be bred early and therefore wean a heavier calf than late pubertal heifers (Izard & Vandenbergh, 1982). This and other advantages of an early puberty are discussed in this section.

2.1.1. Raising animals to calve at two years of age

Age at puberty is important when heifers are raised to calve at 2 years of age, especially when a restricted breeding season is used (Ferrel, 1982).

Animal production in grazing systems is largely determined by the seasonal supply of nutrients from pastures (quantity and quality), and animal growth often follows the same curvilinear pattern of pasture growth (Lowe, 1970). Therefore heifers that don't reach puberty in a given mating period must be kept unproductive until the next mating period (usually 12 months) in order to breed.

Results from a trial carried out to measure the difference in performance between heifers which calved at two years of age (2Y) vs heifers which calved at three years of age (3Y) are presented in Table 2-1. The data presented is the cumulative performance of the cows after 12 years, under the culling criteria of eliminating cows failing to conceive in two successive years.

Table 2-1 Cumulative performance of cows managed to calf first at two or at three years of age

Age of cows (year)	Age at first calving (years)	Breeding seasons	Pregnancies	Calves born	Calves alive at weaning	Weight of weaned calf (kg)
5	2	3.37	2.94	2.84	2.55	498.4
	3	2.55	2.19	2.15	1.99	398.5
8	2	5.59	4.93	4.75	4.41	878.5
	3	4.64	4.07	3.97	3.75	779.5
12	2	8.09	7.12	6.80	6.30	1240.9
	3	6.95	5.91	5.71	5.36	1102.7

Source: Núñez-Dominguez *et al.*, 1991.

It can be seen in the table above that there is a consistent advantage for the 2Y group over the 3Y group at all ages and in all aspects analysed. After 12 years, the cumulative production of both groups showed that the 2Y cows presented 1.1 more breeding

seasons, 1.2 more pregnancies, 1.1 more calves, weaned 0.9 more calves and produced 138.2 kg extra calves than the 3Y cows (Núñez-Dominguez *et al.*, 1991).

It should be noted that difficulties at calving are higher for cows calving at two years of age than for cows calving at three years of age (Núñez-Dominguez *et al.*, 1991). In order to avoid potential problems, bulls from small sized breeds (i.e., Jersey) and/or bulls with high Calving Ease (DIR) EBV should be used in the first mating (Baker & Carter, 1982; Baker *et al.*, 1990).

Successful biological efficiency expressed as total kg of calf weaning weight per unit of winter feed required, is highly dependent on the pregnancy rate of the yearling heifers (McMillan & McCall, 1991). To reach the target mating liveweight at 14-16 months, yearling mated heifers should receive a higher pasture allowance than heifers first mated at two years of age. To accommodate the higher intakes of the heifers calving at two years of age, the herd size (mixed age cows and replacements) should be reduced by 3 % throughout the winter period according to McMillan & McCall (1991). The resulting lower number of productive cows plus the lower liveweight of calves born out of two-year-old-calving animals requires pregnancy rates of around 80 % in yearling heifers to be as biologically efficient as the cows first calving at three years of age (McMillan *et al.*, 1992).

2.1.2. The relationship between puberty and pregnancy rate

Age at puberty is negatively correlated (desirable direction) with pregnancy rate (number of pregnancies divided by number of mating years) (Morris & Cullen, 1994). A trial with two beef cattle herds presenting a 12 % difference in calving rate showed that heifers from the herd selected for high calving rates had an earlier sexual maturity than heifers originating from the herd selected for low calving rates (Mackinnon *et al.*, 1990).

Further evidence to support the relationship between early onset of puberty and improved pregnancy rate can be seen in the data of Morris & Cullen (1994), in which there is a negative relationship (i.e., desirable) between the standardised age at first oestrus (SFO) and pregnancy rate (PR), as shown in Figure 2-1.

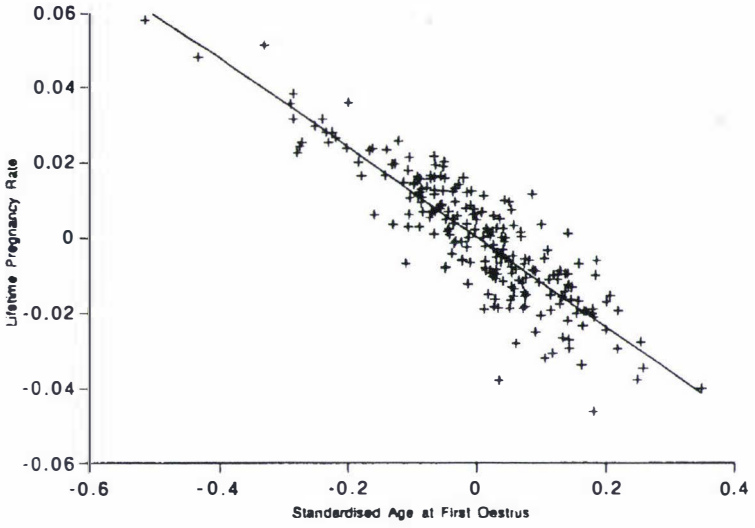

Figure 2-1 Sire expected progeny difference (EPD) for pregnancy rate (up to five mating years) related to standardised age ate first oestrus in daughters.

Source: Morris & Cullen, 1994.

It can be seen in Figure 2-1 that as age at first oestrus decreases, pregnancy rate increases. The authors concluded that 0.1 genetic standard deviation (3.0 days) reduction in SFO would increase pregnancy rate in 0.0108 %.

In a cow-calf system, the number of dams to produce a given number of calves per year will vary according to the reproduction efficiency of the herd. Fewer dams of high fertility can produce the same number of calves than a larger number of dams of low fertility (Baker *et al.*, 1987). This presents a significant impact on the efficiency of the system since empty or pregnant cows have similar maintenance requirements regardless their physiological state.

Higher reproductive rate means that more calves will be available for the market and the system becomes more efficient as the cow feed maintenance costs are distributed among a higher number of offspring.

2.1.3. Calving date and lifetime production

First calving date in beef heifers has a positive relationship with subsequent calving dates. In other words, heifers that calve early in the calving season tend to maintain this characteristic throughout their productive life (Lesmeister *et al.*, 1973).

Table 2-2 shows the results of an experiment in which a herd of heifers were classified into six groups according to their calving dates (Lesmeister *et al.*, 1973).

In order to have an accurate classification, the expected calving season was divided into 21 days increment, beginning 283 days after the first day of the mating season.

Table 2-2 Initial and subsequent calving group of cows, weaning weights, weaning age and average daily gain of their calves (19 years average data).

Initial calving group (21 day periods)	Subsequent calving group. Average of the cow's productive life	Weaning weight (kg)	Weaning age (days)	Average daily gain (kg)
Early	1.4	222	241	0.80
1	1.5	210	227	0.80
2	1.5	196	210	0.79
3	1.8	172	190	0.76
4	1.7	160	176	0.75
Late	1.7	168	153	0.80

Source: Adapted from Lesmeister *et al.*, 1973.

Thus, four groups of 21 days were formed, plus two extra groups at the tails, designated as "Early" and "Later" groups, in which heifers which calved earlier (i.e., < 283 days) or later (i.e., > 367 days) than expected were included (Lesmeister *et al.*, 1973).

Therefore, a cow had an initial calving group all of her life based on the birth date of her first calf and subsequent calving groups according to the calving dates in later calving seasons (Lesmeister *et al.*, 1973).

Table 2-2 shows that a cow with an initial calving group of 1 (i.e. gave birth between 283 and 304 days from the beginning of the mating season), presented an average calving group of 1.5 (i.e., gave birth at an average of 314.5 days) at the end of her productive life (Lesmeister *et al.*, 1973).

Similarly, a cow originally classified in the "Late" group (i.e., gave birth > 367 days from the beginning of the mating season) presented an average calving group of 1.7 at the end of her productive life (Lesmeister *et al.*, 1973).

It would be tempting to think that the cows that calve late in the season will also wean their calves later, thus having a shorter period to recover and cycle again. That is not the case however, because the trial required all the calves to be weaned at the same time, regardless their age, thus allowing all the cows to start the next mating season under the same conditions (Lesmeister *et al.*, 1973).

Similar results were found under New Zealand conditions. Early calving cows which in the first season presented an advantage of 19 days over their later calving herdmates, calved again 2 days earlier in the following season (Morris, 1998). Late calving cows presented an over-all reduction of 2-3 % in pregnancy rate mainly because they cycled after the breeding season has ended. However, it is not advisable to use calving date within a herd as a selection criteria to improve reproductive performance since the response is likely to be slow (Morris, 1998).

Under a system where calves are weaned at a particular time rather than at a particular weight or age, cows that calve early can wean a heavier and more mature calf than cows that calve late in the season as seen in the data above (Lesmeister *et al.*, 1973).

It was also demonstrated (data not shown) that heifers calving as two-year-olds calve significantly ($P<0.05$) earlier throughout their subsequent productive life than heifers first calving as three-year-olds (Lesmeister *et al.*, 1973).

2.1.4. Improved pregnancy rates as heifers

Successful reproductive performance is attained when heifers experience two or three oestrous cycles before the breeding season as shown by the data presented in Table 2-3. Heifers mated at their pubertal oestrus (O1 group) presented lower pregnancy rates when compared with heifers mated at their third oestrus (O3 group). Based on these findings it is important that heifers attain puberty at 12 months of age if they are to conceive at two years of age (Patterson *et al.*, 1992; Schillo *et al.*, 1992).

Table 2-3 Pregnancy rate, age and weight at puberty and at breeding for heifers bred at their first or third oestrus.

Treatment	Age at puberty (days)	Weight at puberty (kg)	Age at breeding (days)	Weight at breeding (kg)	Pregnancy rate (%)	Heifers repeating oestrus (%)
O1‡	322[a]	295[a]	322[a]	295[a]	57[a]	43[a]
O3‡‡	339[a]	306[a]	375[b]	326[b]	78[b]	22[b]

Source: adapted from Byerley *et al.*, 1987.

‡O1: heifers bred at their pubertal oestrus.

‡‡O3: heifers bred at their third oestrus.

[a,b] values with different letters in the same column are statistically different ($P<0.05$).

Table 2-3 shows that heifers in the O3 group presented higher pregnancy rates than heifers of the O1 group (Byerley *et al.*, 1987). Also, the proportion of heifers returning

to oestrus after being bred to a fertile bull was higher in O1 than in O3 (Byerley *et al.*, 1987).

There was no statistical difference between both groups in age or weight at puberty, but heifers in the O3 group were older and heavier at breeding than heifers in the O1 group. Weight at breeding however did not influence rate of pregnancy as revealed by a within-treatment covariate analysis (Byerley *et al.*, 1987).

Conversely, age at breeding affected pregnancy rate for heifers on the O1 treatment but not for heifers on the O3 treatment. In other words pregnancy rate is expected to improve with age in the O1 group but not in the O3 group.

The physiological reasons for sub-fertility on heifers mated at their first oestrus are not clear. Heifers in the O1 group presented a normal cycling activity since they were showing behavioural oestrus, presented a palpable *corpus luteum* and had an increased concentration of blood serum progesterone (Byerley *et al.*, 1987).

2.2. PUBERTY DEFINITIONS

There are many definitions of puberty in the literature, some of them are:

- Puberty can be defined as the process of attaining sexual maturity and the ability to produce an offspring. In female farm animals, it is generally considered that they have reached puberty by the time they had their first oestrus (McLeod & Phillips, 1998).

- Puberty can be defined as the age of the first expressed oestrus with ovulation (Bearden & Fuquay, 2000).

- Puberty can be defined as the time of first standing oestrus and ovulation accompanied by a functional *corpus luteum* development and a rise in blood progesterone (Bellows & Short, 1994).

None of these definitions are completely satisfactory and throughout this thesis the definition of puberty will be taken as the time of the first ovulation accompanied by the development of a *corpus luteum* and a rise in blood progesterone level. The reasons for the inadequacy of the previous definitions are presented in sections 2.5.1 and 2.5.2.

2.3. MECHANISMS REGULATING THE ONSET OF PUBERTY

The events leading to sexual maturity can be broadly classified into those involving physiological aspects of the hyphotalamic-pituitary-gonadal axis and those related to the metabolic status of the animal. They are discussed in the following sections.

2.3.1. Physiological mechanisms leading to puberty

The onset of puberty is the result of a series of complex events that take place within the reproductive endocrine axis (Schillo *et al.*, 1992).

Figure 2-2 is a schematic representation of the hypothalamic-pituitary-ovarian axis and the interaction of the various endocrine and neuronal mechanisms.

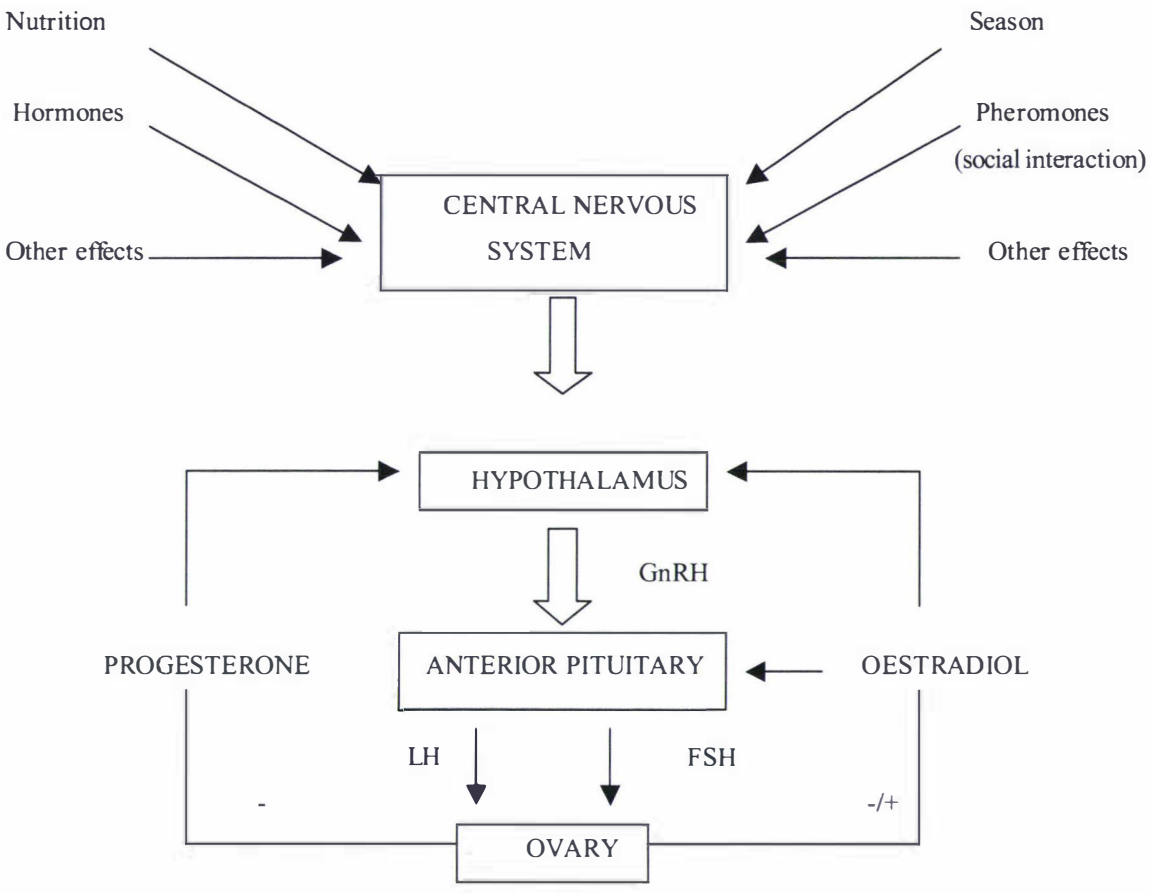

Figure 2-2 Schematic representation of the reproductive axis showing neuronal and endocrine interactions.

(+) positive feedback from oestradiol on the hypothalamus and anterior pituitary.

(-) negative feedback from ovarian steroids (progesterone and oestradiol) on the hypothalamus and anterior pituitary.

Source: Schillo *et al.*, 1992.

Environmental (nutrition, social interaction, season, etc) and internal factors (hormones, enzymes, nutrients) affecting puberty onset convey into the central nervous system (Bucholtz *et al.*, 1996; Cunningham *et al.*, 1999). This information is integrated in the hypothalamus, which is in charge of the production of gonadotropin-releasing hormone (GnRH) (Schillo *et al.*, 1992; Darwash *et al.*, 1999). To produce GnRH, the hypothalamus seems to be stimulated by aspartate/glutamate neurotransmitters (I'Anson *et al.*, 1993).

Pulsatile releases of GnRH react with the pituitary gland, which in turn originates pulsatile releases of LH and follicle-stimulating hormone (FSH). These gonadotropins control folliculogenesis and other ovarian functions (Gonzalez-Padilla *et al.*, 1975; Armstrong *et al.*, 1981; Schillo *et al.*, 1992; I' Anson *et al.*, 1993). Follicular development is characterised by two or three waves of follicular growth in the majority of bovine cycles. A wave of follicular growth in turn is characterised by the simultaneous growth of a group of follicles out of which one will become dominant and the others will regress (Darwash *et al.*, 1999).

The mechanisms that regulate the reproductive physiology of heifers, function long before first oestrus, as indicated by the fact that luteinizing hormone (LH) pulses are detectable in the peripheral circulation of heifers as early as one month of age (Schillo *et al.*, 1992). Moreover, calves 30 days old respond to super-ovulation treatment and can release cleaved ova (Seidel Jr. *et al.*, 1971). The presence of LH in a young animals' blood however, is not enough for the transition from infantile to pre-pubertal status. Two other events seems necessary for this to happen:

1. The number of GnRH receptors in the pituitary gland must increase in order to respond to GnRH pulses coming from the hypothalamus. The increase in GnRH receptors is age related and is a necessary step for the production of higher amounts of LH (Schillo *et al.*, 1992).

2. The gonadotropins (FSH and LH) need to be produced at high enough concentrations and at a high enough frequency to initiate follicle growth, oocyte maturation and ovulation (Schillo *et al.*, 1992; McLeod & Phillips, 1998).

Fulfilment of these pre-requisites (maturation theory) does not mean immediate puberty as there are other factors affecting puberty onset and these will be discussed in Section 2.4.2.

Ovarian steroids (progesterone and oestradiols) also regulate gonadotropin release through feedback mechanisms. Low concentrations of oestrogen exerts a negative feedback on LH release. This effect is probably exerted at the level of the central nervous system (hypothalamus) and the pituitary gland as seen in Figure 2-2 (gonadostat theory) (Day *et al.*, 1984; Schillo *et al.*, 1992; Darwash *et al.*, 1999).

Conversely, high concentrations of oestrogen (> 1 ng/ml), a characteristic of the follicular phase of the oestrous cycle stimulates the production of LH, which in turn induces ovulation (Day *et al.*, 1984; Schillo *et al.*, 1992).

The main function of the *corpus luteum* is to secrete progesterone which is crucial to prepare the uterus to sustain the early embryo. In a 21 day oestrous cycle, the average duration of the luteal phase is 14 days (Darwash *et al.*, 1999). Progesterone, also seems to act at the hypothalamus level by negatively affecting GnRH production in a way that a reduction in the pulse frequency emission of LH is observed. This phenomenon was observed in sheep, however, in cattle it has not been proven yet (Figure 2-2) (Schillo *et al.*, 1992).

In cattle, the feedback mechanism exerted by the ovarian steroids on the pituitary gland and the hypothalamus seems to be completely developed by between three and five months. Likewise, LH can be found in the peripheral blood of the heifers at a very young age (i.e. 1 month old) (Schillo *et al.*, 1992). Young animals however, seem to be very sensitive to the negative feedback effect of oestradiol on LH secretion. This is confirmed by an increase in LH concentration in calves that were castrated and by the suppression of LH release when the same ovariectomized cows were injected with oestradiol (Day *et al.*, 1984; Schillo *et al.*, 1992).

The responsiveness to negative oestradiol feedback decreases with age (Day *et al.*, 1984) and is associated with a reduction in the number of unoccupied oestradiol receptors in both the hypothalamus and the anterior pituitary (Schillo *et al.*, 1992).

Therefore it was hypothesised that this response reduction to oestradiol negative feedback, permitted the emission of LH to such levels that it would stimulate the development of ovarian follicles to the pre-ovulatory stage (Day *et al.*, 1984; Schillo *et al.*, 1992).

Once this pre-ovulatory stage is over, the heifer enters for the first time into the luteal phase, which is characterised by high plasma progesterone concentration. This increase in plasma progesterone plus the presence of a palpable *corpus luteum* are sure signals that the heifer has reached puberty (Schillo *et al.*, 1992).

It must be remembered however, that even though the hypothalamic-pituitary axis is functional (as are the feedback mechanisms and receptors), follicle growth does not proceed to the pre-ovulatory and ovulatory phase because of the low frequency pulses in LH release. This is caused by the high sensitivity of the pituitary-hypothalamic axis to the negative oestrogen feedback mechanism characteristic of very young animals (Schillo *et al.*, 1992).

2.3.2. Mechanisms related to the metabolic status of the animals

Several metabolic signals have been identified as links between nutritional status and reproductive function. Some of the metabolites necessary to trigger the events leading to the first ovulation are described below.

For puberty to occur, metabolic fuels such as glucose circulating in the blood stream must be adequate. Low levels of glucose are detected by receptors situated at the brain stem level inhibiting the production of LH through the inhibition of GnRH secretion (Hall *et al.*, 1992; Bucholtz *et al.*, 1996; Cunningham *et al.*, 1999).

Likewise it has been proposed that a "critical body fat level" is necessary to attain puberty. The fat level is directly associated with the production of the amino-acid leptin

which is thought to be one of the metabolic substances detected by the central nervous system to assess the body's metabolic status. Even though the presence of leptin seems necessary for the physiological processes leading to puberty to initiate, it has not been proved conclusively that leptin alone can trigger puberty (Cunningham *et al.*, 1999).

The amino-acid tyrosine is also responsive to the nutritional status of the animals and as feed level increases tyrosine levels also increase both in the peripheral circulation and at brain level. Thus it was also suggested that tyrosine acted as a nutritional signal on the central nervous system, however, trials with sheep failed to prove that tyrosine acted as the sole signal metabolite triggering puberty (Hall *et al.*, 1992).

It is conclusive that under-nutrition retards puberty onset, however, based on the evidence it seems that there are a number of metabolites interacting with the central nervous system. It is unlikely that a single metabolite would be responsible for triggering the mechanisms leading to puberty.

2.3.3. Ovarian development

In heifers, the ovaries grow rapidly from birth until four months of age reaching a plateau between five and eight months. Following this period, the size of the ovaries increase again until onset of puberty. Folliculogenesis follows the same pattern. The most distinctive feature in the ovaries of pre-pubertal heifers is the enlargement of the follicles (Schillo *et al.*, 1992).

The main source of oestradiol in pre-pubertal animals is the dominant follicle, and the steroid concentration is in the range found in adult animals during the oestrous cycle (Darwash *et al.*, 1999).

2.4. HORMONAL MECHANISMS OF THE OESTROUS CYCLE

The hormonal patterns in the ovulation cycle are presented in Figure 2-3.

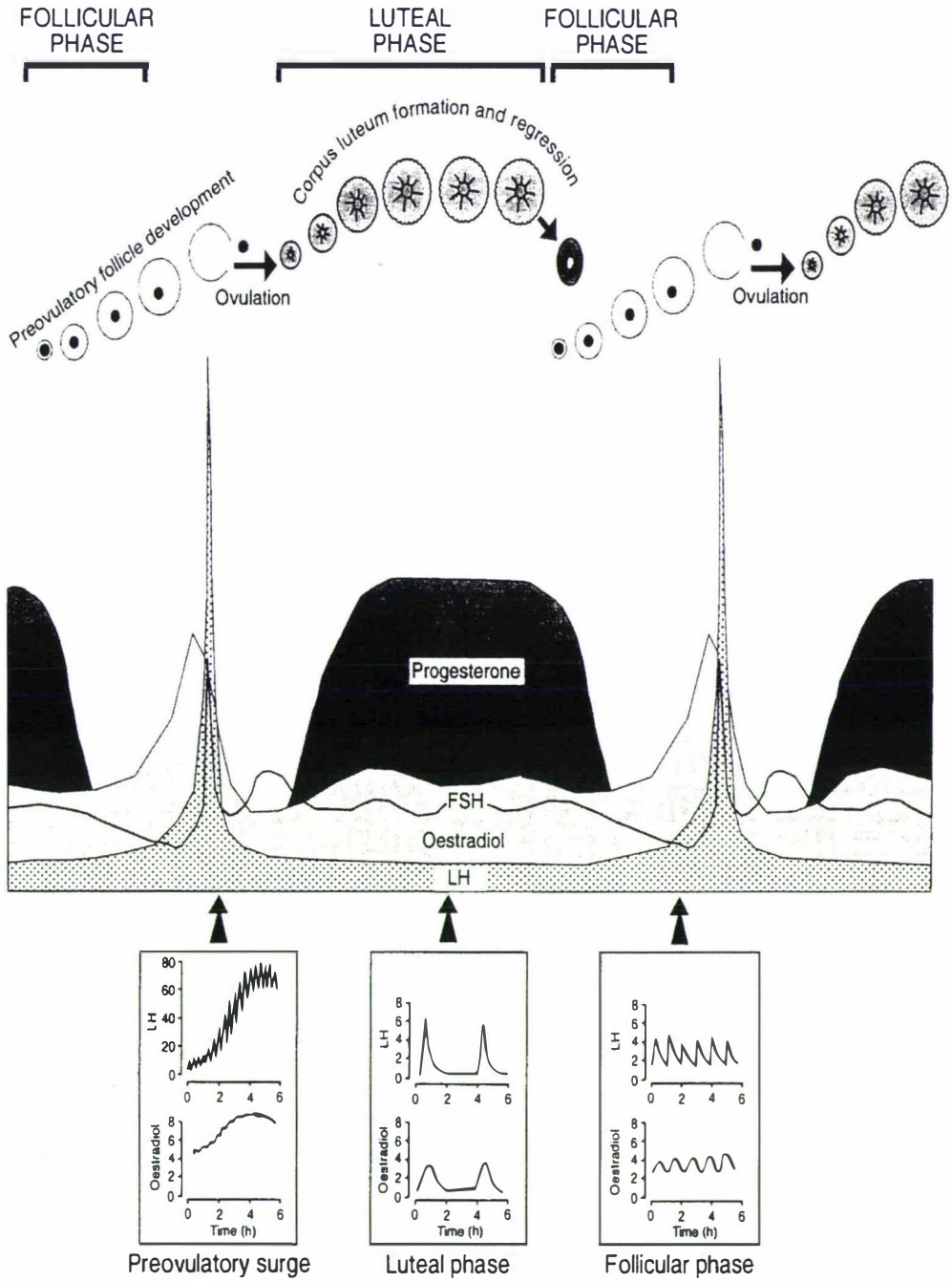

Figure 2-3 Hormonal mechanisms of the oestrous cycle.

Source: McLeod & Phillips, 1998.

Progesterone levels are low at the time of oestrus but rapidly increase three to five days after ovulation. Two to three days later, progesterone concentration reaches a plateau and remains elevated until the regression of the *corpus luteum* begins. At this stage, progesterone concentration returns to basal levels (McLeod & Phillips, 1998) (Figure 2-3).

These changes in progesterone concentration influence the secretion of GnRH hormones from the pituitary gland due to a negative feedback mechanism, as was seen in the previous section (Day *et al.*, 1984; Darwash *et al.*, 1999). This feedback mechanism begins in the ovaries and is initiated by a decline in progesterone concentration levels due to the regression of the *corpus luteum.* As progesterone concentration declines, the negative feedback effect on the hypothalamus decreases; oestrogen begins to increase and as a consequence GnRH is released which in turn stimulates the production of LH (Figure 2-2) (McLeod & Phillips, 1988; Schillo *et al.*, 1992; Darwash *et al.*, 1999). There is still controversy over whether peak oestrogen concentration occurs prior to oestrus, after oestrus onset or coincides with it (Darwash *et al.*, 1999).

It can be seen in Figure 2-3 that the concentration of LH during the luteal phase is very low and progressively increases, reaching its peak at ovulation. Luteinizing hormone pulses occur at a frequency of only one every 8-12 hours during the luteal phase while progesterone concentration is high; however, as progesterone level declines following luteal regression, the frequency and amplitude of LH secretion goes up. The increase in LH pulse frequency continues during the follicular phase of oestrus until they are occurring at 40-60 minutes intervals immediately before the onset of the pre-ovulatory LH surge (McLeod & Phillips, 1988).

Plasma FSH remains constant during the luteal phase of the oestrous cycle. In the follicular phase however, there is a decline on plasma FSH that begins at the time of the *corpus luteum* regression and continues until the time of the pre-ovulatory surge. There

is a simultaneous surge of FSH and LH at ovulation as seen in Figure 2-3 (McLeod & Phillips, 1988).

In sheep and cattle there is a second hormonal surge 18-24 hours after the pre-ovulatory surge; this second surge is probably a consequence of the abrupt decline on the negative feedback effect of oestradiol on the hypothalamus and anterior pituitary (Figure 2-2) (McLeod & Phillips, 1988; Schillo *et al.*, 1992).

2.5. PUBERTY AND FIRST BEHAVIOURAL OESTRUS

Time of first ovulation and time of first observed behavioural oestrus are two separate events indicating the transition from infantile to pubertal status and the receptivity to the male respectively (Bearden & Fuquay, 2000).

They are largely unrelated events and strictly speaking puberty is the time of the first ovulation accompanied by a rise in blood progesterone (McLeod & Phillips, 1998) and behavioural oestrus can be manifested at either side of the ovulation episode. There are typically three possible scenarios related to the ovulation time. Heifers ovulate and show overt oestrus at the same time; heifers show overt oestrus but don't ovulate and heifers that ovulate but don't present overt oestrus. The last two situations will be discussed in sections 2.5.1 and 2.5.2 respectively.

2.5.1. Non-pubertal oestrus

There are situations in which pre-puberal heifers show behavioural oestrus not followed by ovulation and formation of a *corpus luteum* (Nelsen *et al.*, 1985). This phenomenon is called non-puberal oestrus (NPE).

An experiment designed to determine age at puberty included the utilisation of sterile marker bulls to identify heifers showing behavioural oestrus. Animals presenting a mark were analysed for blood plasma progesterone and rectally palpated to detect the formation of a *corpus luteum*. In the first year of the experiment 13 % of the animals showing overt signs of oestrus were not ovulating; while in the second year 22 % of the animals showed the same phenomena. Figure 2-4 shows the distribution of animals showing overt oestrus accompanied by physiological signs of sexual maturity and animals presenting NPE (Nelsen *et al.*, 1985).

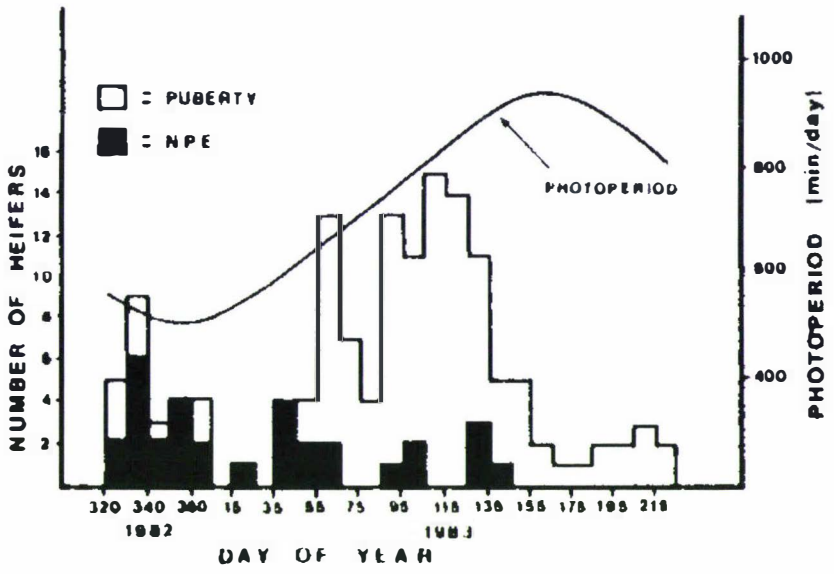

Figure 2-4 Distribution of pubertal and non-pubertal oestrus.

Source: Nelsen *et al.*, 1985.

This experiment was carried out in the Northern Hemisphere, and it can be seen in Figure 2-4 that most of the NPE episodes occurred during the winter months (i.e., December-February). Since NPE occurs mainly at the beginning of the reproductive season, it can be hypothesised that younger animals are more prone to show this behaviour than older animals or that there is an influence of photoperiod on NPE manifestation (Nelsen *et al.*, 1985; Bellows & Short, 1994).

In some heifers certain physiological changes involved in the sexual maturation process, provokes external signs of oestrus before all the changes leading to puberty are completed. Even though NPE is not associated with any abnormalities or dysfunctions and is probably caused by waves of follicle growth in pre-pubertal animals, it is important to consider its occurrence in herds under experimental conditions (Nelsen *et al.*, 1985; Schillo *et al.*, 1992). The occurrence of NPE can bias the results of an experiment where behavioural oestrus is the only criteria for age at puberty determination, therefore, other measurements (i.e., serum progesterone) must be carried out to increase accuracy of puberty estimation (Nelsen *et al.*, 1985).

2.5.2. Silent oestrus

Ovulation not preceded by external signs of heat is called silent oestrus (Darwash *et al.*, 2001). Silent oestrus has been studied more in dairy breeds than in beef breeds and in adult animals than in peri-pubertal animals. Gonzalez-Padilla *et al.* (1975) reported that 50 % of Angus heifers (n= 6) did not present overt oestrus despite the presence of a *corpus luteum*. Silent oestrus in some animals seems to be related to differences in the sensitivity of the hypothalamus to oestradiol challenges (Darwash *et al.*, 2001). Thus, some animals require higher amounts of oestrogen to express overt oestrus than others.

2.6. INFLUENCE OF THE DAMS' GENETIC MAKE-UP ON THE SYSTEM

New Zealand beef production system is characterised by high replacements rates of approximately 20 % (Charteris *et al.*, 1998). In a national breeding herd of approximately 1.525.000 animals (Beef New Zealand, 2002) it represents 305.000 heifers coming into the production system annually. Considering the high number of

replacement animals it is important to evaluate the genetic make-up of heifers entering the breeding herds.

In a self-replacement breeding herd and without the control of sex of the offspring, choice of breed and sires within breed must consider maternal performance of female offspring as well as growth performance of male offspring and surplus females (Baker & Carter, 1982).

Genetic x environment interactions are distinctly evident in reproductive and maternal performance (Baker & Carter, 1982; Montaño-Bermudez & Nielsen, 1990) thus, cow weight (mature size) and level of milk production should be optimised rather than maximised and this optimum will depend on the environment, management system, the cows' re-breeding performance and breeding objectives (Baker & Morris, 1984; Irgang *et al.*, 1985).

Favourable outcomes on the mating of yearling heifers depend on the animals reaching puberty at around 12 months of age (Patterson *et al.*, 1992) which is influenced by environment and nutrition (Baker & Carter, 1982). Large, late maturing breeds or lines will perform well in adequate environments where nutrition is not limiting at any time. As nutrition becomes limiting or environment becomes more nutritionally challenging, earlier maturing animals will be more appropriate.

2.6.1. Mature size and reproduction

Animals selected for higher growth rates have larger mature size than animals not selected for this trait, consequently, fast growing females kept as replacements will require more energy for maintenance than females from slower growth rate lines (Koch *et al.*, 1982; MacNeil *et al.*, 1984). Maintenance comprises around 60 -75 % of the total

energy requirements of the cow, her calf and replacement (Baker *et al.*, 1987; Montaño-Bermudez *et al.*, 1990; Morris *et al.*, 1991).

Heifers selected for fast growth rates present significantly higher dystocia and calf mortality rates than heifers not selected for this trait (Koch *et al.*, 1982). These undesirable correlated responses are associated with longer gestation periods and higher calf birth weights (Koch *et al.*, 1982; Irgang *et al.*, 1985). Nelson & Beavers (1982) reported an increase of 2.5 % in assisted calving per kg increase in birth weight. The negative effects of high growth rate are present mainly in heifers calving at two years of age and are negligible in mature cows (Nelson & Beavers, 1982; Baker & Morris, 1984). On the other hand, under feedlot conditions it has been shown that animals from high growth rate lines were significantly more efficient (53.8 vs 51.1 Mcal ME/kg gain) at converting feed into meat than animals not selected for this trait. Likewise, the selected animals produced more lean and less fat on carcasses of equal weight than the non-selected animals (Koch *et al.*, 1982).

Farmers are demanding increasingly bigger animals because they attain bigger carcass weights and are more efficient in converting grass to meat than smaller animals (Vargas *et al.*, 1999). However, mature size as measured by frame size (FS) presents a negative relationship with female fertility traits such as age at puberty and re-breeding efficiency as shown by a trial carried out with Brahman cattle of three different FS (Vargas *et al.*, 1999).

The three groups of animals were managed as a single herd except at the time of mating when the dams were separated and mated to sires of similar FS. During the experimental period they were offered *ad libitum* quantities of good quality forages and supplements (Vargas *et al.*, 1999). Table 2-4 summarises the results of the trial.

Table 2-4 Least squares means for different reproductive and productive traits of Brahman heifers and cows of different mature size.

Trait	Frame size	Parity		
		First	Second	Third or greater
Calving rate (%)	Small	93.5 [a]	65.8 [a]	93.5 [a]
	Medium	88.5 [a]	69.0 [a]	78.5 [b]
	Large	97.3 [a]	41.0 [b]	79.8 [b]
Production per cow (kg)	Small	143.3 [a]	121.8 [a]	140.6 [a]
	Medium	161.9 [a]	115.4 [a]	150.3 [a]
	Large	102.9 [b]	80.5 [b]	176.8 [a]

Source: Vargas *et al.*, 1999.

[a]Values with different letters within a column and within a trait are statistically different (P>0.05).

It can be seen that there is no statistical difference in calving rate (the percentage of cows exposed during the breeding season that subsequently calved) between first parity heifers of different mature size. The lack of difference is due to the fact that heifers were joined at two years of age when the majority of animals were already cycling. On the second parity group however, there was a significant difference between animals of different frame size (FS). Calving rate of large FS animals was 25 % lower than that from medium and low FS. Adult cows (third or greater parity) of small mature size also performed better than their contemporaries of medium and large mature size (Vargas *et al.*, 1999).

Production per cow defined as weight of calf weaned per cow exposed is a function of calving rate, calf survival rate and calf weaning weight. There was an influence of FS on production per cow on first and second parity cows. Large FS heifers and cows presented a lower performance than medium and small FS heifers and cows. This difference disappeared in the third or greater parity dams and no statistical difference was found among groups (Vargas *et al.*, 1999).

Puberty was attained at 633, 626 and 672 days for small, medium and large frame animals respectively. There was no statistical difference in age at puberty between the small and medium FS dams, large FS animals however, attained puberty at a significantly older age.

Large frame size animals presented a significantly poorer performance in most of the aspects considered in the trial during the first and second year of reproduction. As the large Brahman cows matured, they seem to overcome the negative effects imposed by their large FS. Thus after they stopped growing, the nutritional regimen provided in the experiment seemed to more closely match their demands for maintenance and lactation (Vargas *et al.*, 1999).

Consequently, it can be concluded that the poor reproductive performance of the High FS animals was not exclusively a consequence of fertility problems as shown by the high pregnancy rates of first parity heifers in all groups.

Re-breeding performance was more a function of nutritional management rather than low fertility *per se*. Therefore it may be possible to overcome the disadvantages of the large FS cows through increased nutrition, but the cost associated with this added nutrition may be too large (Vargas *et al.*, 1999). It must also be considered the higher fixed maintenance cost of large animals.

The small FS animals are easier to manage in this sense; however, their reduced growth potential and consequently light carcass weights makes them unattractive for the meat industry (Vargas *et al.*, 1999).

The recommended cow size is probably in between these extreme points. A medium size cow will be relatively easy to feed and maintain in adequate body condition in order to obtain good reproductive performance under commercial conditions and will also produce steer progeny with acceptable carcass weight (Vargas *et al.*, 1999).

2.6.2. Milk yield and reproduction

Milk production can be altered via selection and crossbreeding and between 60 to 66 % of the weaning weight variation is due to milk yield (Willham, 1972; Robison *et al.*, 1978). The phenotypic correlations between these two traits is between 0.5 and 0.8 (Irgang *et al.*, 1985).

An increase of 1 kg day^{-1} of milk is associated with an increase of 0.28 kg DM day^{-1} in feed consumption during lactation. The increment in DM consumption is not only to support the higher milk yield but to cover higher maintenance requirements per $kg^{0.75}$ of the higher yielding cows (Montaño-Bermudez & Nielsen, 1990).

The most important aspects to asses when deciding the desired level of milk production in a cow-calf operation are the environment and the production system. Adequate environmental conditions will allow for higher animal performance and vice versa.

When level of nutrition is inadequate, the dam attempts to maintain milk production levels according to her genetic potential at the expense of body reserves. Consequently, reproductive performance may be adversely affected by high milk yields in environments that cannot supply adequate nutrition (Mackinnon *et al.*, 1990; Martin *et al.*, 1992).

Selection for milk production presents a positive relationship with puberty. It was shown that breeds that were selected for milk production attained puberty at younger ages and lighter weights than breeds of the same mature size that were selected solely for meat production (Laster *et al.*, 1979). Similar results were obtained by Morris *et al.* (1992) in a New Zealand study. In an earlier experiment in New Zealand, Pleasants *et al.* (1975) also found that dairy and dairy x beef crosses reached puberty at earlier ages and heavier weights than pure Angus heifers.

A study carried out to determine the biological efficiency of cows with different potential for milk production (estimated 205 day milk production of 1718, 1532 and 1157 kg for the High, Medium and Low milk yielding cows respectively) but similar mature size and growth rate (Clutter & Nielsen, 1987) revealed that there were no statistical differences between reproductive parameters such as pregnancy rate, calving interval and calf-crop percentage among the three lines (Montaño-Bermudez & Nielsen, 1990). As opposed to this study, Hetzel *et al.* (1989) and Mackinnon *et al.* (1990) found

a negative relationship between reproductive performance and milk production. However, the trial reported by these authors was carried out in a tropical environment characterised by nutritional restrictions during the autumn-winter months as opposed to the trial by Montaño-Bermudez & Nielsen (1990) were cows received a high quality diet throughout the year. It is probable that the antagonism between milk production and reproductive performance is expressed more frequently under low nutritive conditions. Environmental effects on puberty and reproductive performance have been reported by several authors (Pleasants *et al.*, 1975; Gregory *et al.*, 1979; Morris *et al.*, 1993b; Morris *et al.*, 1993c).

2.7. MANAGING THE HEIFERS TO CALVE AT TWO YEARS OLD

The growth and adequate development of replacement heifers from birth to puberty is a fundamental step in the over-all efficiency of a closed cow-calf production system. To maximise returns in cow-calf production systems, developing heifers should be managed to calve at 2 years of age (Bagley, 1993). This requires an adequate management of the forage-animal system to ensure that the heifers cycle for the first time at least 42 days before the beginning of the mating season. Nutritional management during the early developmental stage of growing heifers will influence their lifetime productivity (Ferrel, 1982).

2.7.1. Growth before weaning

It was demonstrated that calves that suffered energy restrictions before weaning (< 7 months of age) required 14 to 18 additional months to compensate to 70 to 80 % of the controls. Restrictions before weaning are very difficult to overcome, this situation is aggravated if the deficient elements are proteins (Bagley, 1993). In calves suckling

cows however, this situation is not likely to occur unless serious illness prevents the dams from nurturing her offspring.

2.7.2. Post-weaning growth

Increasing daily liveweight gain of post-weaning heifers from 0.42 kg day^{-1} to 0.61 kg day^{-1} hastened puberty onset. Nearly 10 % more heifers in the higher liveweight gain treatment reached puberty before the start of the breeding season (70.9 vs 61.3 %) (Buskirk *et al.*, 1995).

Ferrel (1982) in a study comprising six different breeds showed that very high (0.8 kg day^{-1}) or low (0.4 kg day^{-1}) post-weaning daily gains delayed puberty onset in heifers by 7 and 22 days respectively when compared to a group of heifers growing at 0.6 kg day^{-1}. The average age at puberty for the moderate growth group was 365 days. Similar results were obtained in a previous study by Short & Bellows (1971) who found that heifers growing to gain 0.68 kg day^{-1} reached puberty at younger and heavier weights than heifers growing at 0.23 or 0.45 kg day^{-1}.

The above studies indicate that in order to avoid the negative effects of under or overfeeding, the ideal post-weaning weight gain of heifers should be around 0.6-0.7 kg day^{-1}. It is important to take into account that the results reported in this section are from trials that used a constant liveweight gain during all the study period. Therefore the recommended weight gains are valid if they are to be applied during all the development stage of calves.

This point is emphasised because time of gain and rate of gain at different times seems to influence puberty onset. These aspects are discussed in the next section.

2.7.3. Time and rate of gain

Some studies suggest that puberty onset is determined by the total amount of growth achieved during the post-weaning period rather than by specific rates of gain or period (time) of growth (Clanton *et al.*, 1983).

Table 2-5 summarises the results of a trial in which three different feeding strategies were applied. Each treatment was divided into two separate feeding periods called 1st and 2nd half. In treatment 1, heifers were fed to gain 0.91 kg day^{-1} in the 2nd half of the feeding trial but 0 kg day^{-1} in the 1st half.

In treatment 2 heifers were fed to gain 0.45 kg day^{-1} in both periods and treatment 3 required that heifers gain 0.91 kg day^{-1} in the 1st half but 0 kg day^{-1} in the 2nd half.

Table 2-5 Puberty onset as influenced by different rates of gain and different periods of growth.

	Treatment 1		Treatment 2		Treatment 3	
Period	1st half	2nd half	1st half	2nd half	1st half	2nd half
Daily gain (kg)	0	0.91	0.45	0.45	0.91	0
Initial weight (kg)	185		184		185	
Final weight (kg)	281[a]		278[a]		285[a]	
Age at first oestrous (d)	403[a]		394[a]		392[a]	

Source: Clanton *et al.*, 1983.

[a]Values with the same letter within a row are statistically no significant (P>0.05).

It can be seen that heifers from the three treatments attained puberty at the same time even though they were gaining weight at different times and at different rates during the post-weaning period (Clanton *et al.*, 1983).

Similar results were obtained in a trial where two groups of calves were fed to gain 0.45 and 0.91 kg day^{-1} respectively during the first part of their developing stage (from 143 days of age to 243 days of age). At the end of the first stage, both groups were merged

and fed a common diet formulated to produce daily weight gains of 0.91 kg. There was no statistical difference on age at puberty between both groups at the end of the trial. However, heifers that gained 0.91 kg day^{-1} during period 1 and 2 were significantly heavier at puberty than heifers that gained 0.45 kg day^{-1} during Period 1 and 0.91 kg day^{-1} during period 2 (Dufour, 1975).

These results suggest that there is much flexibility in the feeding policy of growing heifers, as long as they are fed to attain a target weight at the beginning of the mating season (Clanton *et al.*, 1983; Schillo *et al.*, 1992).

There are different nutritional management options to rear replacements heifers and it is clear that providing certain constrains are met, there is enough flexibility in the rearing of calves. However, it would be advisable to rely on a system were an adequate and *constant* liveweight gain is obtained during the development stage in order to avoid unnecessary stress to the growing animals or unexpected situations (like feed shortages, dry spells, diseases, etc) that could compromise the development of the animals.

To calculate the necessary daily liveweight gains for growing calves, the following equation can be used.

$$\text{Daily gain required} = \frac{\text{Target weight - Weaning weight}}{\text{Days to breeding}}$$

It is important to remember that heifers should cycle ideally 2 or 3 times before mating in order to ensure high pregnancy rates. Therefore it will be necessary to subtract between 42 to 63 days from the "days to breeding" value in order to ensure that the target weight is reached prior to mating.

Nicoll (1987) suggested that growing heifers of Angus breed should reach 250 kg at the beginning of the mating season. Attaining this objective can lead to a pregnancy rate of around 85 % in a 42-day mating period.

2.8. MEASURING HERBAGE INTAKE

In grazing systems it is important to determine the amount of feed ingested by the animals. Feed intake measurements are important since they can be use to predict animal performance (Dove & Mayes, 1991) and they represent a major input cost to the production system (Archer *et al.*, 1999). This measurement is relatively easy in indoor feeding studies where feed given is weighed and intake is calculated by the difference between feed offered and feed rejected. However, intake measurements in free-ranging animals is difficult and several approaches have been developed (Reeves *et al.*, 1996).

Two different techniques will be reviewed in this section, the direct pre- and post grazing methodology and the indirect n-alkane methodology.

2.8.1. Intake measurement using the n-alkane technique

Intake of herbage by animals at pasture can be calculated by the ratio between faecal excretion and forage indigestibility (Malossini *et al.*, 1996).

The equation on which this method is based is:

$$\text{Intake} = \frac{\text{Faecal output}}{\text{1- digestibility}} \qquad \text{Eq: 1}$$

It can be seen in equation 1 that total faecal collection is necessary to calculate intake. However, total faecal collection from free-ranging animals is a laborious method and has the potential to disturb the animals' normal behaviour and thus affect herbage intake (Dove & Mayes, 1996). It was latter discovered that by the simultaneous use of two markers (one internal and one external) this problem could be overcome (Malossini *et al.*, 1996).

Faecal output can be estimated from the dilution in faeces of an orally administered external marker which must be totally indigestible and absent from the diet (Dove & Mayes, 1996), while digestibility can be estimated from the concentration of an internal marker naturally present in the diet (Malossini *et al.*, 1996). Thus the combination of an internal and external marker provide the information required in Equation 1.

Internal markers are natural compounds found within plant material, meaning that they are not administered to the animals. The use of indigestible plant components as markers for digestibility determinations in grazing ruminants allows for the measurement of individual *in vivo* digestibility values. This presents a considerable advantage over methods relying on *in vitro* digestibility values to calculate intakes, such those based on the use of chromium oxide.

External markers on the other hand should be absent from the diet and are dosed to the animals. Some internal markers however, are present in such small amounts that they can be dosed to the animals and used as external markers (i.e. even-chained n-alkanes) (Beek *et al.*, 1997).

The ideal marker (internal and external) should feature the following characteristics:

- Should be easy to identify and analyse (Dove & Mayes, 1991).
- Should be indigestible (inert) in the digestive tract (Dove & Mayes, 1991).
- Should not interfere with the digestive process (Beek *et al.*, 1997).
- Should mix homogeneously with the diet to be marked and travel along with it through the digestive tract (Beek *et al.*, 1997).

Nutritionists have long sought for an internal marker having the above characteristics. Several naturally present markers such as chromogen, lignin, acid-insoluble ash and indigestible acid detergent fibre have been suggested and evaluated (Dove & Mayes,

1996). None of these markers however, gained acceptance as some are degraded or modified within the digestive tract (lignin, indigestible fibre), while others are in very low concentrations in feeds or are difficult to analyse (acid-insoluble ash) (Beek *et al.*, 1997). Probably the best internal marker to date are the alkanes present in plants cuticular wax (Mayes *et al.*, 1986).

2.8.1.1. Using n-alkanes as markers.

The alkanes are single bonded, straight chained, saturated aliphatic hydrocarbons, usually present in the cuticular film of wax that coats the surface of plant leaves (Dove & Mayes, 1991; Beek *et al.*, 1997).

Plants contain mixtures of n-alkanes having chain lengths ranging from 21 to 35 carbons, those most abundant being C_{29}, C_{31} and C_{33} (Figure 2-5). Even-chain alkanes are present in very small concentrations in plants (Beek *et al.*, 1997). There are marked plant species differences in the concentration of n-alkanes which permits differentiation of one plant from another (Dove & Mayes, 1996).

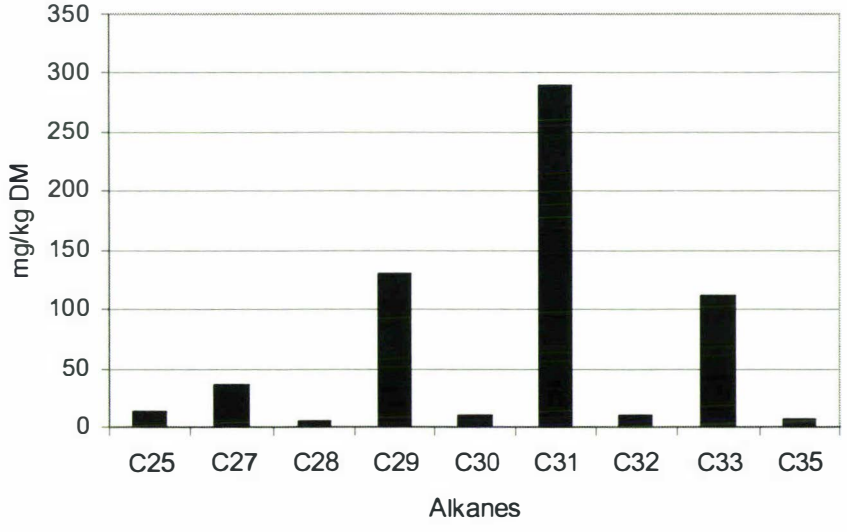

Figure 2-5 Average concentration of alkanes in tropical and temperate grasses. (Data from Dove & Mayes, 1991).

Over 30 years ago, similarities were found between the pattern of alkanes extracted from cattle faeces and the pattern of alkanes found in the forage they had consumed. It was established that n-alkanes were almost indigestible and that indigestibility increased with chain length. This property suggested the possibility of using cuticular alkanes as digestibility markers (Beek *et al.*, 1997).

The n-alkane technique is a special application of the double marker method and is based on the combined use of and odd-chain n-alkane naturally present in the cuticular waxes of plants, and a commercially available even-chain n-alkane as the external marker (Malossini *et al.*, 1996).

Grazing animals consume the naturally occurring odd-chain alkanes, which can be used as internal markers to determine forage digestibility. The external markers are represented by the adjacent synthetic even-chain alkanes, which are dosed to determine faecal output (Dove & Mayes, 1991). As a result intake can be estimated by using both the internal and external markers, providing that their recoveries in the faeces are similar (Dove & Mayes, 1991).

2.8.1.2. Advantages of n-alkanes over other markers

The alkane methodology can overcome some of the deficiencies of previous methods to measure intake and has proved to be very accurate under a range of different conditions, as seen in Table 2-6 (Dove & Mayes, 1996). It is important to notice that more variability has been reported in cattle than in sheep due to a lower recovery and more erratic behaviour of alkanes in cattle (Dove & Mayes, 1996).

Table 2-6 Comparison of known herbage intake of sheep and cattle with estimated intakes using dosed C_{32} alkane and natural C_{33} alkane as markers.

Source	Animals and conditions	Known intake (g day^{-1} DM)	Discrepancy (g day^{-1} DM)
Mayes *et al.*, 1986.	30 kg lambs, fresh herbage	579	Nil
Mayes *et al.*, 1986.	10 week old lambs, milk plus herbage	112-273	0.4
Mayes *et al.*, 1986.	Mature beef cows, fresh herbage	4000	70.0
Dillon & Stakelum, 1989.	Dairy cows, late lactation, fresh herbage	1418	90.0
Dove *et al.*, 1991.	Mature sheep, perennial grass	913	0.2
Vulich *et al.*, 1991.	34 kg lambs, fresh herbage	778	20.0

Source: Dove & Mayes, 1991; Dove & Mayes, 1996.

The high accuracy in the estimation of intake using alkanes is based on the following characteristics:

- Alkanes are largely indigestible.
- Relatively easy to analyse.
- High repeatability.
- Higher accuracy.
- Lower dosages per day (Malossini *et al.*, 1996; Beek *et al.*, 1997).

2.8.1.3. Choosing an n-alkane

There are between 8 to 15 n-alkanes that can be used as markers. Some important aspects to consider when choosing n-alkane pairs to measure intake are:

1. As chain length increases, the percentage of the alkane recovered in the faeces increases and the difference in the recovery of adjacent alkanes decreases (Reeves *et al.*, 1996; Beek *et al.*, 1997) (Figure 2-6).

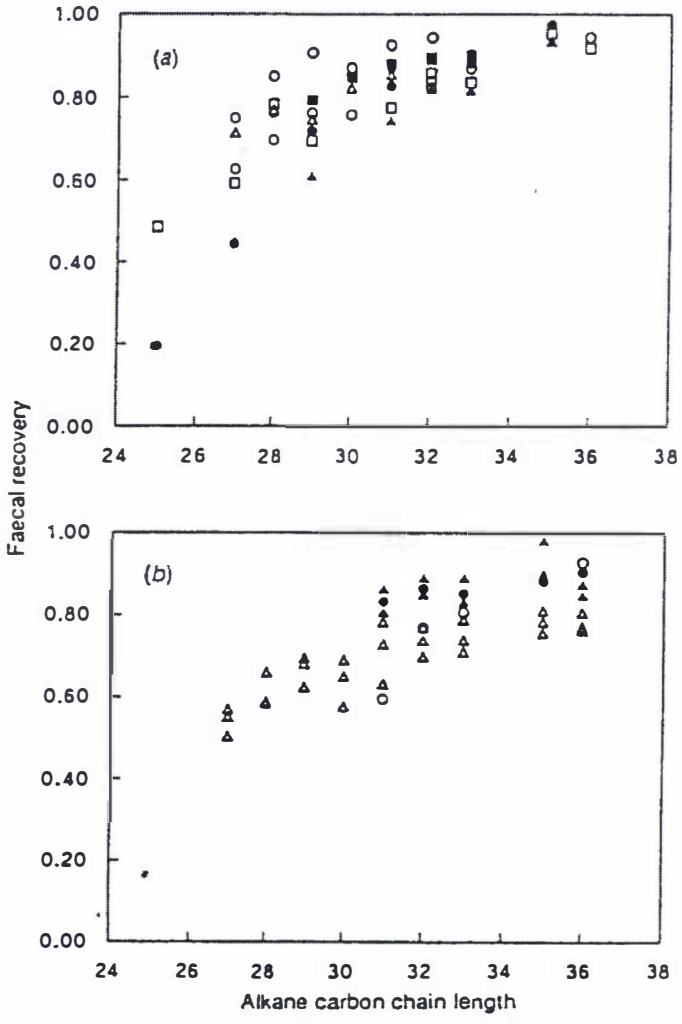

Figure 2-6 Relationships between the estimated faecal recoveries of n-alkanes and their carbon chain lengths in sheep (a) and cows (b).

Source: Dove & Mayes, 1991.

2. Considering the relationship between chain length and recovery level, it would appear that the best alkane is C_{35}. Its concentration in forages however, is too low (Figure 2-5) for accurate measurements (Beek *et al.*, 1997).

3. When choosing an internal-external pair, the faecal recoveries should be similar. The larger the percentage difference in recovery, the higher the error in estimating intake and for every unit of discrepancy between the pairs in faecal recovery, there

is 1.25 % error in estimated intake. Taking these factors in consideration, the more suitable alkanes seem to be C_{31} and C_{33} as internal markers and C_{32} as external marker (Reeves *at al.*, 1996; Beek *et al.*, 1997; Hameleers & Mayes, 1998).

2.8.1.4. Recovery of alkanes in the faeces

Alkanes are not completely recovered in faeces, and as the alkane chain length increases, so does the recovery in faeces. However, even for C_{35} and C_{36} alkanes the recovery is not complete (Dove & Mayes, 1991; Duncan *et al.*, 1999).

This has led an important consideration when using the n-alkane technique. If both, the odd and even-chain alkanes have similar recovery rates, the subsequent errors cancel out (Reeves *et al.*, 1996). Thus Equation 2 below is derived from Equation 1.

$$\text{DM intake} = \frac{(F_i / F_j) \times D_j}{H_i - (F_i / F_j) \times H_j} \qquad \text{Eq: 2}$$

H_i : herbage concentration of the internal n-alkane.

F_i : faecal concentration of the internal n-alkane.

H_j : herbage concentration of the dosed n-alkane.

F_j : faecal concentration of the dosed n-alkane.

D_j : daily dose amount of the external n-alkane.

The reason for this is that for any given value of H_i, H_j and D_j, Intake is a function of F_i / F_j (i.e., the ratio between the faecal recoveries of the natural and dosed alkanes). If the recoveries of the two n-alkanes are R_i and R_j respectively, the corrected faecal concentrations will be (F_i/R_i) / (F_j/R_j). However, if R_i is similar to R_j, the ratio (F_i/R_i) / (F_j/R_j) becomes F_i / F_j (Malossini *et al.*, 1996).

It is obvious from Table 2-7 that the assumption of a single recovery value for different alkanes will lead to errors, therefore, recovery values corresponding to the particular alkane being used should be considered in the calculations (Dove & Mayes, 1991).

Table 2-7 Alkane recovery values from different animal species and different diets.

Source	Obs.	C_{27}	C_{29}	C_{31}	C_{32}	C_{33}	C_{35}	C_{36}
Hameleers & Mayes, 1998.	Dairy cows RG[1] & WC[2].	0.75	0.76	0.82	0.86	0.83	0.88	nd[3]
Dove & Mayes, 1991.	Sheep. RG[1]. n=8	0.59	0.69	0.77	0.85	0.83	0.95	0.92
Mayes *et al.*, 1986.	Sheep. RG[1] & barley	0.71	0.74	0.85	0.88	0.89	0.93	nd[3]
Berry *et al.*, 2000	Dairy cattle. Forage mix .	nd[3]	nd[3]	0.76	0.87	0.85	nd[3]	0.86

[1]RG: rye grass, [2] WC: white clover, [3]nd: no data.

No information on puberty onset and herbage dry matter intake for animals selected for High and Low EBV-600 day and High and Low EBV-milk under pastoral conditions were found in the literature. Thus, the literature review was based on general principles governing puberty onset, the effect of the genetic make-up of animals on reproduction traits and the role of the environment in regulating the expression of the genes. The use of an increasingly accepted methodology to measure herbage intake in free-ranging animals was also reviewed.

CHAPTER 3

MATERIALS AND METHODS

3.1. EXPERIMENTAL LAYOUT

The present experiment aimed to determine age and weight at puberty of four genetic lines of Angus heifers. Intakes were also estimated on four occasions during the trial, twice using the n-alkane technique and twice using the pre- and post-grazing technique. The experimental layout is presented in the chart below.

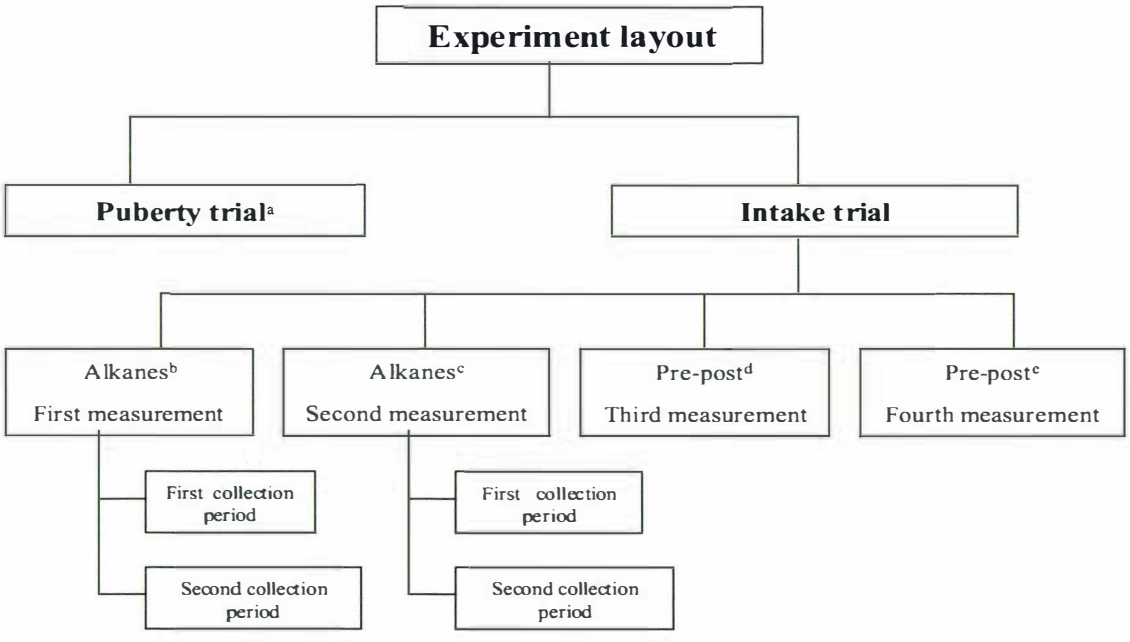

Figure 3-1 Experimental layout.

[a] Section 3.4, [bc] Section 3.5.1, [de] Section 3.5.2.

3.2. BACKGROUND TO ANIMALS

Four experimental groups were formed with progeny derived from industry bred Angus cows that were mated with bulls selected for either High or Low EBV-200 day milk (HM and LM respectively) and High or Low EBV-600 day weight (HG and LG respectively) (Table 3-1).

Table 3-1 Number of animals by genetic line and farm of origin.

	Genetic lines			
Farm	HG[1]	LG[2]	HM[3]	LM[4]
1	3	12	9	9
2	10	2	7	0
3	11	6	5	0
4	0	0	10	11

[1]HG: High EBV-600 day weight , [2]LG: Low EBV-600 day weight , [3]HM: High EBV-200 day milk , [4]LM: Low EBV-200 day milk.

The dams from which the calves were bred were evenly distributed between four farms on the East Coast of the North Island (New Zealand). Dams were assumed to be of similar breeding value so as to reduce the direct genetic effects and ensure that the differences in performance between the animals of the different lines were primarily determined by the genetic merit of the sire.

Between three and five bulls were used within each genetic line. They were mated to at least 30, oestrus synchronised cows, by artificial insemination. Calves originating from these matings were born between June and October 2001. The animals were weighed at birth and identified according to the sire line (Morris *et al.*, 2000).

The heifers were weaned and weighed in March - April 2002, at an average age of 6.5 months and then transferred to Massey University's Keebles farm. The trial started on 1

May with the arrival of 74 heifers and on May 15 sixteen heifers arrived. The last group of five heifers were added to the experiment on 16 July.

3.3. ANIMAL MANAGEMENT

The four genetic lines were run as a single herd from the time they arrived at Keebles until the mating period. An exemption to this management was during the herbage intake measurement with both the n-alkane and the pre- and post-grazing technique. From 1 May 2002 until 25 December 2002, the heifers were rotationally grazed on 21.67 ha of flat country predominantly composed of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture divided in 7 paddocks of different size. At the beginning of mating, the heifers were moved to a different area of the farm in which they remained until the end of the trial.

Mating of the heifers started on the 25 November, when the heifers were at an average age of 15.5 months (± 25.2 days) and 352.62 ± 0.98 kg. Mating continued for three cycles. The heifers were weighed at the beginning of mating, at the end of the first mating cycle and at the end of mating.

During mating, heifers were divided into two groups by randomly allocating equal number of individuals from each line into each group. Two yearling bulls were joined with each group of heifers. The four bulls were of equal genetic merit and, at the end of the first three weeks of mating, the bulls were switched between groups.

Animals were treated with Eprinex (Eprinomectin 5mg/mL. Merial-Holland) at the recommended dose of 1ml per 10 kg LW every 6 weeks for the control of nematodes and external parasites.

On 9 December, 5 ml of Selovin-5 (Sodium selenate 5mg/mL. Bomac Laboratories Ltd. Manukau City, New Zealand) was administered sub-cutaneously to all the heifers, since a representative blood sample analysis revealed a deficiency of this element.

The heifers were weighed on arrival from the different farms and then every 28 days. The animals were weighed within two hours of being removed from the paddocks.

3.4. PUBERTY DETERMINATION

Puberty onset was studied in the 95 animals that participated in the trial. One animal died of unknown causes before reaching puberty, another animal was a freemartin and three other heifers presented ovary hypoplasia. Data from these animals were excluded from the statistical analysis.

3.4.1. Tail painting and rectal palpation

To determine first overt oestrus, the heifers were painted with a strip of commercial tail paint (Fil Tail Mark. Mount Maunganui, New Zealand) approximately 20 cm long and 7 cm wide. The paint strip started at around the first coccygeal vertebra and extended cranially to cover the area usually exposed to rubbing by mounting heifers (Macmillan & Curnow, 1977). The tail painting began on 1 May and, initially, the paint was applied generously following the direction of hair growth. However, since the heifers had a dense hair coat due to the winter season it was difficult to detect the paint being rubbed off. Therefore, on the 25 June, the paint was removed and the animals were re-painted with the enamel applied sparingly and against the direction of hair growth. This procedure facilitated the paint being disturbed by oestrus behaviour. All the heifers were

tail-painted with blue enamel. After the first heat they were tail-painted with red and for their next heat the colour was changed to yellow.

No male animals were used to detect cycling heifers, since the presence of entire or castrated males hastens the onset of puberty in heifers (Izard & Vandenbergh, 1982; Small *et al.*, 2000). Thus, if male animals had been used for oestrus detection, this trial would not replicate the situation on commercial farms, in which heifers are raised separated from the males until the beginning of the mating season.

The heifers' tail paint was checked weekly and those animals with broken, removed or rubbed tail paint (Macmillan & Curnow, 1977) were palpated *per rectum* to determine ovarian activity. Heifers with large ovaries containing either *corpora lutea* or large follicles with the uterine horns having a good tone were identified and classified as having active ovaries (AO). By comparison, heifers with small, smooth ovaries with no follicular development and small toneless uterus were also identified and classified as having inactive ovaries (IO). However, since it takes several days for a *corpus luteum* to form, manual palpation was used as a tool to classify heifers into AO or IO rather than accurately determining oestrus activity. Oestrus activity was determined by means of blood plasma progesterone analysis as described in the following section.

Heifers were also tail painted at the beginning of mating to determine mounting activity. Tail paint disturbance was recorded weekly.

3.4.2. Blood plasma progesterone

To determine the first ovulation, blood samples were taken every 7 days for plasma progesterone analysis (Day *et al.*, 1984). Blood sampling began on 1 May, at an average age of 8 months and finished when an animal presented high progesterone (i.e. > 1 ng ml^{-1}) concentration on two consecutive cycles (Day *et al.*, 1984).

Blood samples (6-7 ml) were collected by venipuncture of the coccygeal vein using heparinised Vacutainer tubes and immediately stored in ice (Buskirk *et al.*, 1995; Small *et al.*, 2000). Plasma was harvested from the samples within 6 hours of collection (Buskirk *et al.*, 1995) after centrifugation at 4 °C and 3050 rpm for 25 min. Two separate 1.5 ml aliquots of plasma were stored at – 20 °C in plastic microtubes for enzyme-linked immunosorbent assay (ELISA) (Morris & Cullen, 1994).

The onset of the first behavioural oestrus was the reference point to determine which samples to analyse. Once behavioural oestrus was established by the tail paint being rubbed off, four plasma samples preceding this event and three samples following this event were thawed and analysed.

Considering that progesterone concentration follows a curvilinear pattern during the oestrous cycle and that blood samplings were carried out at fixed calendar intervals, it was necessary to analyse 7 samples (4 pre- and 3 post-overt oestrus) to ensure the detection of a cycle (Figure 3-2).

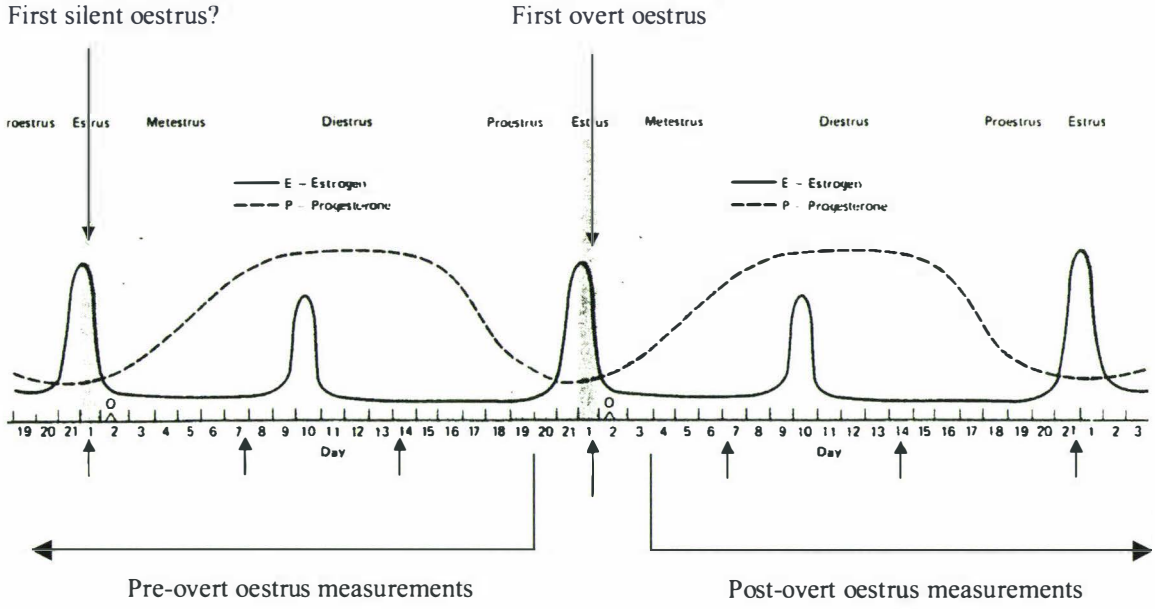

Figure 3-2 Blood sampling procedure to determine time of first ovulation.

Source: adapted from Sorensen Jr., 1979.

Note that the samplings were performed every 7 days and that one sampling preceding the overt oestrus is not shown in the graphic due to space limitations.

Whenever a sampling coincided with an overt oestrus; that sample was discarded since it would not show any increase in progesterone (Figure 3-2).

Figure 3-2 shows that the pre-oestrus measurements were designed to detect animals that had already cycled 21 days prior to the overt oestrus but have not shown any external signs (silent oestrus), while the post-oestrus measurements were to identify either "normal" animals in which ovulation and behavioural oestrus occurred at the same time, or animals which presented an overt oestrus but did not present a palpable *corpus luteum* accompanied by a rise in plasma progesterone (non-pubertal oestrus).

An animal that had at least one blood sample containing an elevated (i.e., > 1ng/ml) progesterone concentration at each side of the overt oestrus episode was considered to be puberal providing that a high progesterone concentration was detected at the next cycle. Animals that presented a high progesterone concentration for a given oestrous cycle but failed to repeat it in the next cycle underwent further analysis until two consecutive cycles gave high progesterone concentration (Day *et al.*, 1984). Age at puberty was considered to be the date at which the first sample showing a high progesterone concentration was taken (Day *et al.*, 1984). Weight at puberty was taken as the averaged value of the two weights at either side of the oestrus episode (Hall *et al.*, 1995; Garcia-Muñiz *et al.*, 1997).

3.4.2.1. Elisa test

The enzyme-linked immunosorbent assay (ELISA) methodology is based on the principle that antibodies have specific affinity for the antigens that provoked their synthesis. Antibodies that recognise a particular molecule are coated into a polymer support (e.g. micro-wells) into which the sample is added. If the molecule of interest is

present, it will bind to the coated antibody. After washing excess antigen and other proteins a labelled enzyme is used to mark the antigen-antibody conjugate. The amount of labelled enzyme that attaches to the antigen is proportional to the concentration of antigen in the sample. Finally, a colourless substrate is added that reacts with the labelled enzyme to produce a colour reaction. The concentration of the molecule of interest can then be read by using a colorimeter (Stryer, 1995).

Blood plasma progesterone concentration was measured using ELISA test-kits (Ridgeway Science Ltd., Cirencester, UK) as described by Sauer *et al.* (1986) and Groves *et al.* (1990). The assay kit was brought to room temperature and the aluminium foil was stripped-off from the wells to be used. Ten microlitres (μl) of standards prepared with charcoal-stripped anoestrus cow plasma containing 0.5; 1.0; 2.0; 5.0; 10.0; and 20.0 ng of progesterone /litre were used to create a standard curve. Standards were assayed in duplicate in adjacent columns. Quality control were done by using four other wells in duplicate to measure standards containing high and low progesterone concentrations as well as conjugate alone without substrate (Ausubel *et al.*, 1991). The coefficient of variation within and between the ELISA assays were 8.6 and 10.5 % respectively.

Duplicate of samples of plasma (10 μl) were added into the remaining wells. Two hundred microlitres of enzyme-linked label (progesterone-11α-glucoronide-alkaline phophatase) was then added and the plates were incubated at room temperature for 2.5 hours. The wells were rinsed three times with substrate buffer and tap dried on paper each time. Alkaline phosphatase substrate (p-nitrophenol phosphate liquid substrate system; Ridgeway Science Ltd., Cirencester, UK; 200 μl) was added and the plate was left for a second incubation period of approximately 1 hour until colour development (Ausubel *et al.*, 1991).

Colour intensity was read using a SLT 340ATC (Austria) colorimeter set at a wavelength of 550 nm. The results were then analysed using the PRISM computer program (GraphPad Software Inc., San Diego, USA), which creates a non-linear regression (standard curve) equation using the optical density values of the progesterone standards. Progesterone concentration in the samples was determined by fitting their optical values in the regression equation. The limit of sensitivity defined as twice the standard deviation of the optical densities of wells containing progesterone-free anoestrus cow plasma was 0.1 ng/ml.

3.5. HERBAGE INTAKE MEASUREMENTS

Herbage dry matter intake was measured on four occasions, twice using the n-alkane technique and twice using the pre- and post-grazing method.

3.5.1. Intake measurements using the n-alkane technique

In June 2002 (First Intake Measurement) 90 animals participated in the trial and they were given capsules containing synthetic n-alkanes to measure herbage intake (Dove & Mayes, 1991; Dove & Mayes, 1996). In September 2002 (Second Intake Measurement) there were 95 animals, however the heifers were heavier and had higher theoretical intakes. Therefore, in order to use the same paddocks as in the First Intake Measurement (M1), only 80 animals randomly selected within line were used in the Second Intake Measurement (M2).

The literature was reviewed to find a typical intake variance (σ^2) value in experiments using n-alkanes. This value was used along with the least squared differences (LSD) in estimated intake to obtain the number of necessary samples. This preliminary statistical

analysis indicated that at least 17 animals per line were needed to detect a 10 % difference in intake (Trout & Marini, 1984), which was the criteria used in both M1 and M2. Figure 3-3 is a schematic representation of the procedures.

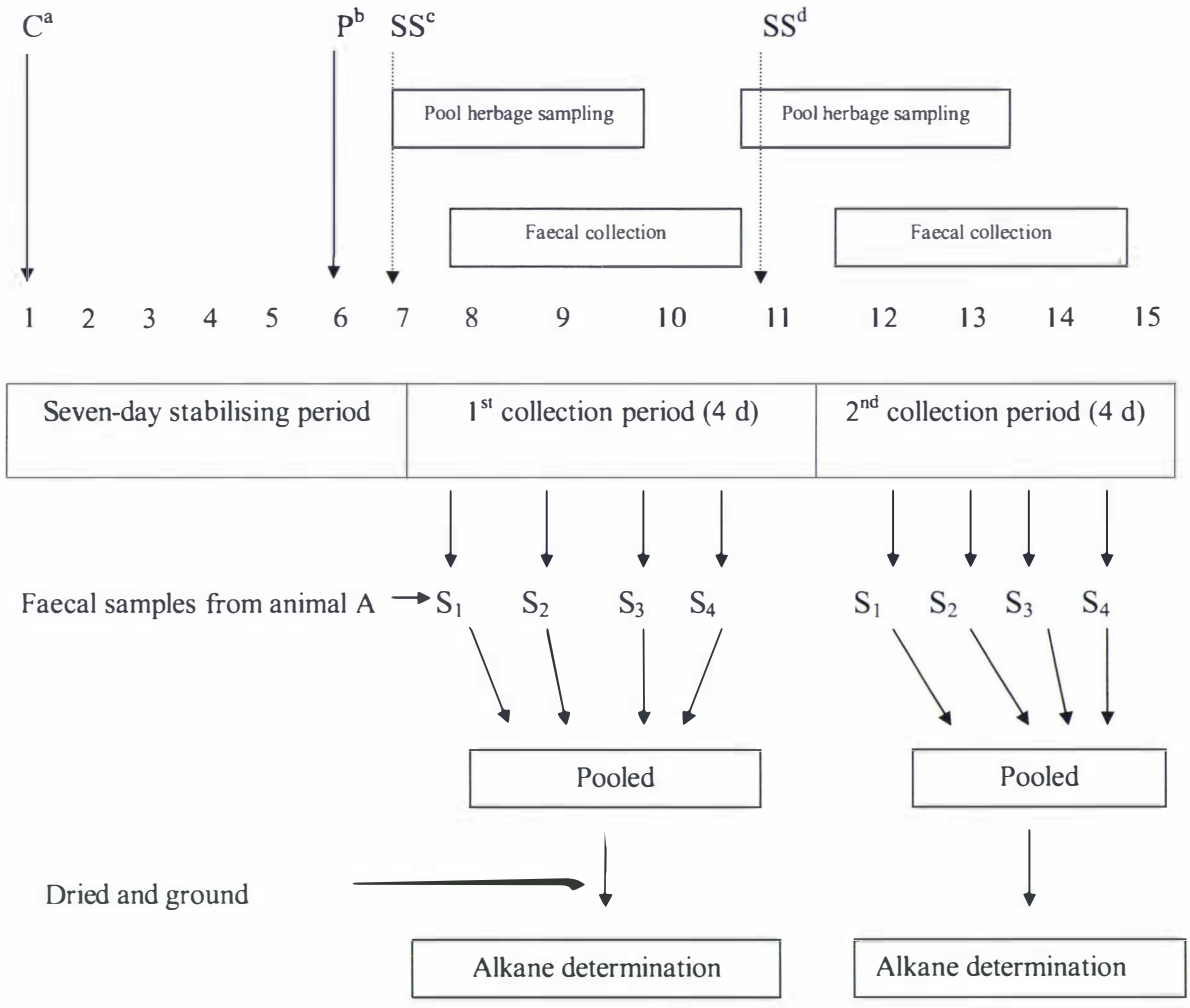

Figure 3-3 Schematic representation of the procedures used in the first and second herbage intake measurements using n-alkanes.

C^{a}: n-alkane capsule insertion., P^{b}: heifers moved to paddocks prepared for intake measurement, SS^{cd}: single herbage sample taken on days 7 and 11, S_{1-4}: faecal sample from day 1 to 4.

Herbage sampling and drying methodology to determine n-alkanes profiles differ in the literature and there is not a definitive protocol. Thus, two different herbage sampling techniques were performed in both M1 and M2 (Section 3.5.1.4) to investigate the

influence of the sampling technique on the herbage intake estimates. Also in M2, two different herbage drying techniques were tested (Section 3.5.1.4).

The procedures that follow are common to M1 and M2 and will be described only once unless they differ in some aspects.

3.5.1.1. Alkane capsules insertion

Eight days prior to the beginning of the faecal collection period, controlled release alkane capsules (CRC, Captec Ltd., Auckland, New Zealand) containing synthetic C_{32} and C_{36} alkanes were administered into the rumen of the experimental animals using a specific capsule applicator.

Two different size capsules were used, one for 100-300 kg cattle and the other for 300-650 kg cattle. The expected C_{32} and C_{36} mean release rate for the small capsules was 200 and 211.5 mg day^{-1} respectively. The value for the bigger capsules was 350.5 mg day^{-1} for both alkanes. The capsules released at the specified rate for a period of 20 ± 3 days.

In order to track regurgitated capsules back to their correspondent animals, numbers from 1 to 90 (M1) were indented in the plastic capsules. At the time of insertion, each numbered capsule was related to the animals' tag number (Caldas, 2000). In M2 the capsules were numbered from 100 to 180 to distinguish between capsules from M1 and M2 in the event of a regurgitated capsule being found.

After capsule insertion the animals were kept together for half an hour on an adjacent yard to detect capsule regurgitation (Caldas, 2000) and to monitor them for any signs of discomfort. One capsule was damaged by a heifers' teeth during the insertion procedure in M1 and it was replaced.

During the following 5 days the heifers were checked in the morning and in the afternoons for excess salivation, throat inflammation or other signs of distress. Nothing irregular was observed during this period and no regurgitated capsules were found in the paddock.

3.5.1.2. Faeces collection

After capsule insertion (Day 1), seven days were allowed for stabilisation of n-alkanes in the faeces (Vulich *et al.*, 1991) (Figure 3-3). On Day 6 the heifers were moved to the paddocks prepared for intake measurement. Faecal collection started on Day 8 and comprised two consecutive 4-day periods (days 8 to 11 and 12 to 15) named First and Second Collection Period respectively (Caldas, 2000). At least three faecal samples per animal were collected in each Collection Period.

Starting at 7:00 am, the heifers were observed daily for 4-h and approximately 40 grams of faeces were collected from each individual immediately after defecation. Thus, at the end of the first and second collection periods, there were approximately 720 faecal samples (90 animals x 8 days) in M1 and 640 (80 animals x 8 days) samples in M2.

Faecal samples from individual animals were pooled within each collection period since Vulich and Hanrahan (1995) reported no differences in herbage intake estimation when n-alkane profiles resulting from the analysis of daily samples or a pool of samples were used in the calculations.

The fresh faecal samples were kept in plastic bottles at 2-4 °C until the end of each collection period. At this point the faeces in each bottle were thoroughly mixed and equal amounts (volume based) (Vulich and Hanrahan, 1995) of faeces were extracted to obtain a single representative sample for the period. Thus, the four faecal samples from a given animal in the first collection period were reduced to a single faecal sample by

the pooling procedure. The same was done with the faecal samples of the second collection period.

Pooled faecal samples from the First and Second Collection Period were analysed for n-alkanes concentration using gas chromatography (Herd *et al.*, 1996) and the results were used to calculate individual herbage intake.

3.5.1.3. Intake paddocks

Two adjacent paddocks were used for intake measurements using the n-alkane technique. Herbage mass targets for the beginning of the intake experiments were set taking in consideration the heifers liveweight (LW) and liveweight gain (LWG) during the previous month. These data were used in energy requirement equations (See Section 3.5.2.2) to calculate the amount of megajoules of metabolisable energy (MJME) necessary to maintain the previous month performance. The MJME values were then transformed to DM intake values.

In M1 and M2 the heifers entered the first of the two paddocks two days prior to the beginning of the collection period and remained there until the desired post-grazing residual was achieved (First Collection Period). The animals were subsequently moved to the next paddock and remained there until the completion of the trial (Second Collection Period). Each paddock was split in two, using portable electric tapes, and half of the heifers were randomly allocated to each side. This was done in order to facilitate visual observation and faeces collection. Four people were necessary to collect the faecal samples from the experimental animals.

3.5.1.4. Herbage sampling for alkane profiles

One of the pre-requisites of the n-alkane technique is the obtaining of representative samples of the diet ingested by the experimental animals (Mayes *et al.*, 1986; Vulich *et al.*, 1993; Malossini *et al.*, 1996). Herbage sampling began one day earlier than the faecal sampling and consequently finished one day earlier (Figure 3-3) in each Collection Period. This was done considering that it takes approximately 24 h for herbage to pass trough the digestive tract of ruminant animals (Van Soest, 1994) and to ensure that faecal samples reflected the diet being ingested by the animals (Vulich & Hanrahan, 1995; Berry *et al.*, 2000).

Two different herbage collection procedures were used in M1 and M2 to determine if the n-alkane profiles were affected by the collection method. In the first procedure, a single herbage (S) sample was obtained on Days 7 and 11 for Collection Period 1 and 2 respectively (Figure 3-3). This procedure assumed that the alkane profile resulting from the analysis of a herbage sample taken on a given day was representative of the animals' diet throughout the Collection Period. The sample was obtained by hand-plucking at least 60 herbage sub-samples at the same height that the animals were observed to be eating (Morris *et al.*, 1993a). The sub-samples were thoroughly mixed and a single sample (400 grams of fresh material) from each Collection Period was obtained and preserved at 2 °C for later analysis.

In the second procedure a pooled herbage (P) sample was obtained by hand-plucking at least 60 herbage sub-samples on a daily basis throughout Collection Periods 1 and 2. As in the previous procedure the samples were taken at the same height that the animals were observed to be grazing which resulted in samples being taken at increasingly lower levels on the vertical axis as the grazing days increased. The daily collected samples were kept refrigerated at 2 °C and at the end of each collection period the four herbage

samples were thoroughly mixed and a single sample (400 grams of fresh material) representing four grazing days was obtained (Vulich *et al.*, 1993; Cosgrove *et al.*, 1998).

In M1, the S and P herbage samples were oven-dried at 70 °C (Herd *et al.*, 1996) and then ground to 0.1 mm particles for analysis of n-alkane concentration (Mayes *et al.*, 1986). In M2, two different drying techniques were used, thus, the final herbage sample was divided in two, half was oven-dried and the remaining half was freeze-dried (Vulich *et al.*, 1993).

The herbage and faecal n-alkane concentrations were used to determine herbage intake using the equation (Dove & Mayes, 1991):

Daily herbage intake (Kg DM /day cow) $$I = \frac{(F_i/F_j)*D_j}{H_i - (F_i/F_j)*H_j}$$

Where

Dj: is the daily dose, or average release rate (mg/day) from the synthetic alkane.

Hj and Fj: herbal and faecal concentrations (mg/kg DM) of the natural even chain alkanes.

Hi and Fi: herbal and faecal concentrations (mg/kg DM) of the natural odd chain alkanes.

3.5.2. Intake measurement using the pre-post grazing technique

Herbage intake was measured in August (Third Intake Measurement) and October (Fourth Intake Measurement) using the "Difference Method" (Frame, 1993) in a 3.3 ha paddock divided into four lanes of approximately the same area (0.834 ha).

Twenty animals per line were randomly selected and allocated at random to each of the lanes. Average LW per line was available to calculate theoretical intakes.

Intake was measured during four and six consecutive 24 hour periods in the Third Intake Measurement (M3) and in the Fourth Intake Measurement (M4) respectively. Prior to the intake measurement, the heifers were allowed to graze for 24 hours in the first strip of grass to ensure that they would not go under the portable electric fences.

A fresh area of herbage was offered to the animals each day and a back fence was used to avoid re-grazing. The pre-grazing herbage mass was measured using a rising plate meter (Jenquip. Fielding, New Zealand) immediately before the animals entered a new strip of grass and the post-grazing herbage mass was measured immediately after the animals were moved to the next strip. The rising plate meter (RPM) readings were used in regression equations to predict herbage mass. Intake was then obtained as the difference between the pre- and post-grazing values (Reeves *et al.*, 1996).

3.5.2.1. Regression equations

Herbage mass was calculated using the double-sampling technique, which involves the sampling of a given population by two different methods. The first method provides accurate information (direct measurement) but it is labour intensive, while the second method (indirect measurement) provides less precise data but is fast, less labour and time demanding and requires less resources. The regression between the paired readings is used to predict direct values from a large number of indirect measurements (Frame, 1993).

Herbage within a 0.09 m^2 metal quadrat was cut at ground level with a battery-operated shearing handpiece. The samples were then washed and oven-dried at 80 °C until constant weight (Khadem *et al.*, 1993; Realini *et al.*, 1999). Prior to cutting, a reading was performed using the RPM. Forty-eight, randomly selected sites, representing the

whole range of herbage heights were measured (Frame, 1993; Murphy *et al.*, 1995; Realini *et al.*, 1999). The paired readings were used to develop a simple linear regression relating RPM readings with herbage mass (kg DM ha^{-1}).

Pre-grazing herbage mass was determined by performing 100 readings with the RPM before the animals entered the allocated area and using the average reading value on the regression equation. Similarly, one hundred RPM readings were performed immediately after the animals were moved to the next grazing strip to determine the post-grazing herbage mass. The same regression equation was used to determine both the pre- and post-grazing values in M3.

In M4, separate regression equations for the pre- and post grazing events were obtained. The post-grazing regression equation in M4 was calculated by cutting 24 quadrats on the second and on the fourth day of the experiment, immediately after the animals were moved to a new grazing strip. The 48 paired readings were used to obtain the regression equation.

3.5.2.2. Pasture area allocation

The daily area of grazing strip offered to each genetic line was a function of (a) the energy requirements (MJME day^{-1}) expressed as kg DM day^{-1} necessary to obtain 1 kg live weight gain and (b) pre and post-grazing herbage mass.

A pre-grazing pasture cover of at least 2500 kg DM ha^{-1} and a residual cover of 1800 kg DM ha^{-1} were constraints in the procedure, to ensure that intake was not limited by pasture mass availability (Reardon, 1977; Marsh, 1979; Reid, 1986).

Metabolisable energy requirement was calculated using the equations by the AFRC (1993).

$$Mmp\ (MJ\ d^{-1}) = ((F + A) / k) \times \ln (B / (B-R-1)) \qquad \text{Eq: 1}$$

Where

- Mmp (MJ d^{-1}) : megajoules of metabolisable energy requirements for maintenance and production per day.
- F: fasting metabolism.
- A: activity allowance.
- k and B: derived parameters to predict energy retention.
- R: energy retention (MJ d^{-1}), scaled by fasting metabolism.

Fasting metabolism (F) and activity allowance (A) are calculated with Equations 2 and 3 as follows:

$$F\ (MJ\ d^{-1}) = C1\ (0.53\ (W / 1.08)^{0.67}) \qquad \text{Eq: 2}$$

$$A\ (MJ\ d^{-1}) = 0.0071 \times W \qquad \text{Eq: 3}$$

Where

- C1 : correction factor (C1 equals 1.15 for bulls and 1.0 for other cattle).
- W : liveweight.
- The factor 1.08 converts liveweight to fasted liveweight.
- The value of 0.071 assumes an horizontal movement of 200 metres, 12 hours standing and 6 position changes.

The values for B, k and R in Equation 1 are given in Equations 4, 5 and 6 respectively.

$$B = k_m / (k_m - k_f) \qquad \text{Eq: 4}$$

$$k = k_m \times \ln (k_m / k_f) \qquad \text{Eq: 5}$$

$$R = E_f / E_m \qquad \text{Eq: 6}$$

Where

k_m : efficiency of utilisation of metabolisable energy (ME) for maintenance. The value k_m is given by the expression.

$$k_m = (0.35 \times 18.8 \text{ MJ kgDM}^{-1}) + 0.503 \qquad \text{Eq: 7}$$

k_f : efficiency of utilisation of ME for weight gain. The value k_f is given by the equation

$$k_f = (0.78 \times (ME / GE)) + 0.006 \qquad \text{Eq: 8}$$

Where

- ME : metabolisable energy of the diet (MJME / kg DM).
- GE : gross energy value of the diet (MJ / kgDM).

The term E_f found in Equation 6 is the net energy retained in the growing animal. It is determined using the following formula.

$$E_f = EVg \times \nabla W \qquad \text{Eq: 9}$$

Where

EVg : is the energy value of tissue lost or gain (MJ kg^{-1}); which in turn is given by the expression:

$$EVg = C2\ (4.1 + 0.0332W - 0.000009W2) / ((1-C3) \times 0.1475\ \nabla W)) \qquad \text{Eq: 10}$$

Where

- C2 corrects for mature body size and sex of the animal.
- C3 corrects for plane of nutrition.
- ∇W: live weight gain or loss ($\pm$ kg day^{-1}).

The average LW of each genetic line was used in the above formulas to calculate the MJME day^{-1} requirements. The daily energy requirement was then multiplied by 20 to

obtain the energy requirement of the whole group and, finally, this value was transformed to kg DM day^{-1}.

3.6. SWARD MEASUREMENTS

3.6.1. Botanical composition

Botanical composition was determined in the four intake measurements by cutting the herbage at ground level using a battery-operated shearing handpiece (Hernandez Garay, 1995). At least 12 sites were sampled following an imaginary line crossing from one corner of the paddock to the other.

The samples were mixed and a sub-sample of approximately 80 gr fresh material was manually dissected into grass leaf, clover, weeds and dead material. The separate components were oven-dried (80 °C) until constant weight and expressed as a percentage of the total dry matter (Salles, 2002). Leaf material with > 50 % dead tissue was classified as dead material, otherwise it was classified as leaf material (Salles, 2002).

3.6.2. Herbage nutritive value

Herbage samples for Near Infrared Reflectance Spectroscopy (NIRS) were taken in the four intake measurements.

Herbage samples for analysis were collected following an imaginary diagonal line crossing from one corner of the paddock to the other. At least 60 hand plucked samples from 12 different sites across the paddock were taken at the same height as that observed being grazed by the animals (Morris *et al.*, 1993a; Cosgrove *et al.*, 1998;

Salles, 2002) on a daily basis during the experimental period. The samples were kept at 3 °C and at the end of the trial they were thoroughly mixed and approximately 300 gr fresh herbage was oven-dried at 70 °C until constant weight. The dried sample was ground and analysed using NIRS with calibrations based on wet chemistry methods (Corson *et al.*, 1999).

3.7. STATISTICAL ANALYSIS

All data were analysed using the computer package SAS version 8.2. (SAS Inst. Inc., Cary, NC. USA). Herbage intake data determined with the n-alkane technique was analysed using the general linear models procedure (GLM) with genetic line and farm of origin as the main affects to explain the variation in intake. Herbage intake using the pre- and post-grazing technique was analysed using a repeated measures analysis in the GLM procedure. Estimated herbage intake was analysed using liveweight (LW) as a covariate considering the differences in LW between the animals from the different lines. Fixed effects were genetic line and farm of origin.

Live weight, and live weight change were analysed using the GLM procedure in a model set for repeated measures analysis (Gill & Hafs, 1971; Small *et al.*, 2000). The model used and the repeated measures analysis prevented the use of birth date as a covariate for sequential LW measurements

Age and weight at puberty was analysed using the MIXED procedure. The model included line and farm of origin as fixed effects. Birth date and LW were used as covariates for the analysis of weight and age at puberty respectively (Laster *et al.*, 1979; Morris *et al.*, 1993a).

Fisher's exact test was used to analyse percentage of animals reaching puberty by line and pregnancy rates (Izard & Vandenbergh, 1982). Number of pregnancies in each

mating cycle was analysed using planned contrast in the GENMOD procedure (SAS, 1990).

CHAPTER 4

RESULTS

This chapter presents the results obtained in the trial designed to determine age and weight at puberty of four lines of Angus heifers that were selected for high and low EBV-600 day (HG and LG respectively) and high and low EBV-milk (HM and LM respectively). Sections 4.4 and 4.5 show the results from two intake measurements using n-alkanes (Measurement 1 and 2). Finally Sections 4.6 and 4.7 present the results of the two intake measurement using the pre- and post-grazing technique (Measurements 3 and 4). The main objective of the last four intake measurements was to detect differences in herbage dry matter intake among the selection lines.

4.1. LIVEWEIGHT

Heifers were weighed monthly (within 2 hours of being removed from pasture) between 1 May 2002 and 28 January 2003. Data from 19 heifers were not available on 1 May, therefore, liveweight (LW) and liveweight gain (LWG) repeated measures analysis began with data collected from the 30 May onwards (Figure 4-2). Weight at puberty was analysed using data collected from 1 May onwards to include animals that had cycled.

The average age of heifers from the various farms at the beginning of the trial was significantly ($P < 0.001$) different (282.1 ± 2.1; 291.0 ± 2.9; 319.5 ± 2.8 and 249.2 ± 2.6

days for Farms 1, 2, 3 and 4 respectively). Repeated measures analysis procedure made it unfeasible to use birth date as a covariate for sequential LW measurements. Liveweights shown in Figure 4-1 are not adjusted for birth date. The omission of birth date in the repeated measures model does not affect the analysis of liveweight gain (LWG).

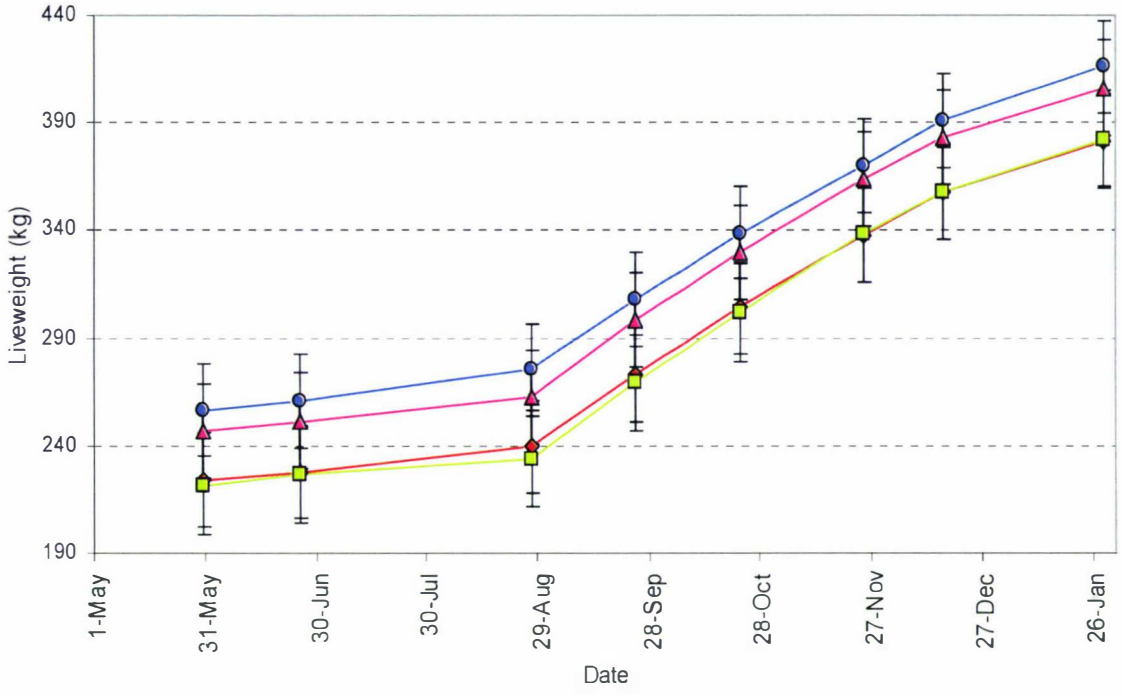

Figure 4-1 Monthly liveweight (kg) of the High EBV-600 day (--●--) Low EBV-600 day (--▲--), High EBV-milk (--◆--) and Low EBV-milk (--■--) heifer lines. Vertical bars represents standard errors of the means.

Differences in LW between the genetic lines at the beginning (30 May) and at the end (28 January) of the trial were analysed using birth date as a covariate. The analysis of variance showed that birth date significantly ($P < 0.001$) affected LW at the beginning the trial. The LW ranking of the animals from the different genetic lines as a consequence of the difference in birth date was maintained throughout the trial since LWG was similar across genetic lines (Table 4-1). However, there was a significant

($P < 0.001$) interaction in LWG between genetic line and season (month) of year as shown in Figure 4-2.

Table 4-1 Liveweight increase (kg) and liveweight gain (kg day^{-1}) during the experimental period (243 days) (means ± SEM).

	Liveweight increase	Average liveweight gain
High EBV-600 day	160.2[a]	0.66[a]
Low EBV-600 day	158.7[a]	0.65[a]
High EBV-milk	157.7[a]	0.65[a]
Low EBV-milk	161.2[a]	0.66[a]

[a]Means with different superscript are statistically different ($P < 0.001$).

Figure 4-2 presents the LWG of the four genetic lines and the Time*Line interaction during the trial.

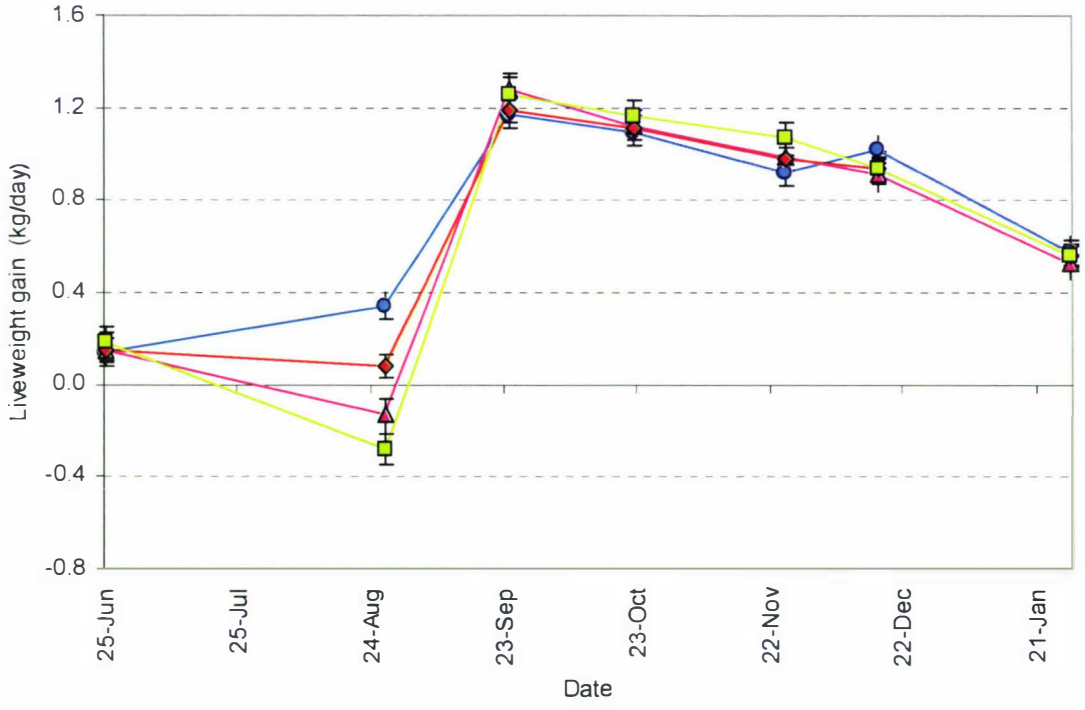

Figure 4-2 Liveweight gain (kg/day) of the High EBV-600 day (--●--), Low EBV-600 day (--▲--), High EBV-milk (--◆--) and Low EBV-milk (—■--) genetic lines. Vertical bars are the standard errors of the means.

On the 24 August, heifers from the HG line had significantly ($P < 0.05$) higher growth rates (0.34 ± 0.06 kg/day) than heifers from the LG (-0.13 ± 0.07 kg/day), HM (0.08 ± 0.05 kg/day) or LM (-0.28 ± 0.07 kg/day) lines. Heifers from the HM line had a significantly ($P < 0.05$) higher growth rate than LG and LM lines. There was no statistical difference in growth rate between LG and LM lines during this time of year.

Heifers from the HG line tended to grow faster than all the other lines during September, December and January; however, during the months of October and November, the HG line was slightly outperformed by LG, HM and LM. Only on the 24 August did the difference in LWG among the different lines reached statistical significance. The differences in growth rate among lines did not reach statistical significance at any of the other dates.

4.2. AGE AND WEIGHT AT PUBERTY

From the 95 animals that started the experiment; one animal died prior to puberty onset, another was a freemartin and three heifers presented ovary hypoplasia. These animals were removed from the puberty trial.

Four animals did not reach puberty by the end of the trial and they were not included in the statistical analysis to determine age and weight at puberty, thus, age and weight at puberty onset was analysed as a truncated trait with data from 86 animals. The percentage of heifers that reached puberty by genetic lines was analysed with a Fishers' exact test using data from the 90 heifers in the trial (Table 4-4).

Table 4-2 shows age and weight at puberty of the different genetic lines.

Table 4-2 Age (days) and weight (kg) at puberty by genetic line (means ± SEM).

Line	Age at puberty	Weight at puberty
High EBV-600 day	438.6 ± 9.3[a]	340.7 ± 9.5[a]
Low EBV-600 day	455.1 ± 11.8[ab]	344.1 ± 11.1[a]
High EBV-milk	459.4 ± 7.1[b]	361.4 ± 8.2[a]
Low EBV-milk	476.1 ± 7.8[b]	352.5 ± 10.8[a]

[ab]Means with different superscripts within a column are statistically different ($P < 0.05$).

Heifers from the HG line were significantly ($P < 0.01$) younger at puberty than heifers from the HM and LM lines. There was no statistical difference in age at puberty between the HG and LG lines. Heifers from the LG line were younger at puberty than heifers from the HM and LM lines, however, the differences between the three lines were not statistically significant. No statistical differences in weight at puberty were detected among the genetic lines (Table 4-2).

Age and weight at puberty of animals coming from the different farms were not statistically different from each other (Table 4-3).

Table 4-3 Age (days) and weight (kg) at puberty by farm of origin (means ± SEM).

Farm of origin	Age at puberty	Weight at puberty
Farm 1	447.9 ± 10.5[a]	357.9 ± 9.2[a]
Farm 2	482.7 ± 14.9[a]	363.4 ± 8.2[a]
Farm 3	457.4 ± 16.5[a]	321.9 ± 26.4[a]
Farm 4	427.5 ± 24.5[a]	357.2 ± 28.1[a]

Means with different superscripts within a column are statistically different ($P < 0.05$).

Age at puberty between Farms 1 and 2 and between Farms 2 and 4 tended to be different ($P < 0.07$). Likewise, weight at puberty between Farms 2 and 3 tended to be different ($P < 0.08$).

The interaction of all levels of Line by all levels of Farm could not be tested due to the nature of the experimental design (Table 3-1). Therefore, the Line*Farm interaction was performed without Farm 4 and the LM line. The analysis of variance showed no interaction between farm of origin and genetic line on age at puberty. However, a strong interaction was observed with weight at puberty (Figure 4-3).

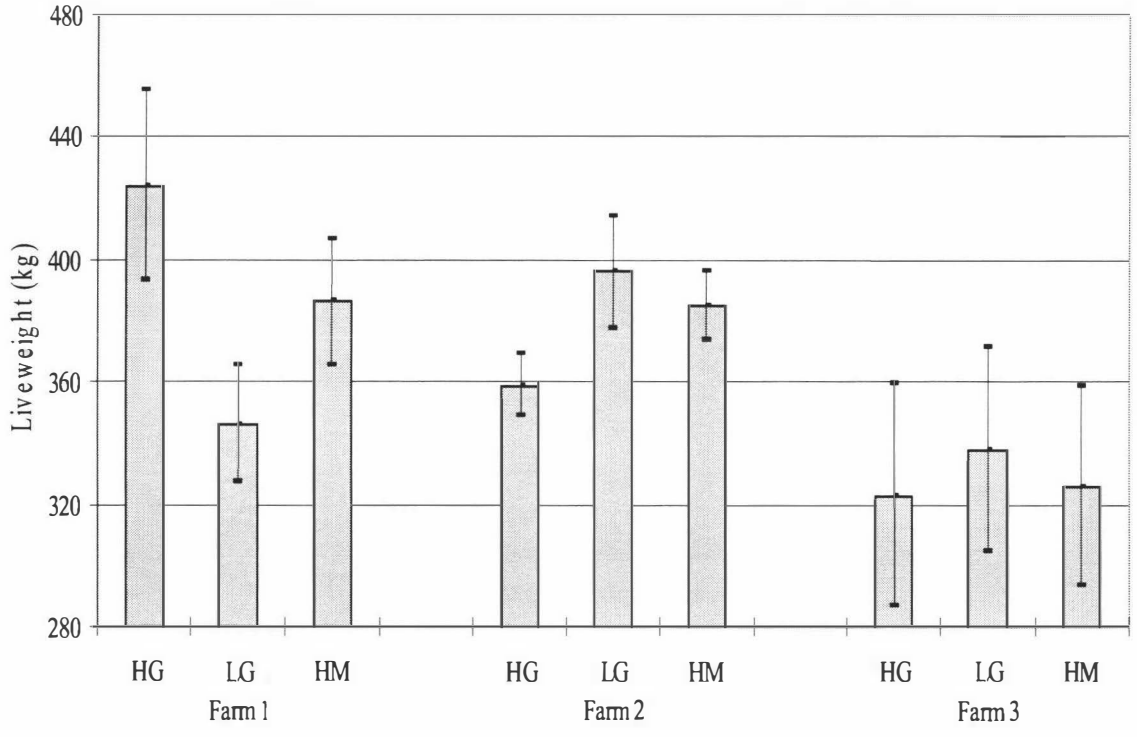

Figure 4-3 Weight at puberty (kg) of the High EBV-600 day (HG), Low EBV-600 day (LG) and High EBV-milk (HM) by farm of origin. Vertical solid lines are the standard errors of the means.

Within Farm 1, HG (424.2 ± 31.2 kg) and HM animals (385.8 ± 20.9 kg) were significantly ($P < 0.05$) heavier at puberty than LG animals (345.9 ± 19.1 kg). In Farm 2, heifers from the LG (395.7 ± 18.3 kg) and HM (384.8 ± 11.0 kg) line were significantly ($P < 0.05$) heavier at puberty than heifers from the HG (359.4 ± 10.1 kg)

line. There was no statistical difference in weight at puberty among animals coming from Farm 3.

Animals from the HG line coming from Farm 1 were significantly ($P < 0.01$) heavier at puberty than animals from the same line coming from Farm 2 and Farm 3 (323.3 ± 36.2kg). Likewise, LG animals coming from Farm 2 were significantly ($P < 0.01$) heavier at puberty than LG animals coming from Farm 1 and Farm 3 (337.6 ± 33.6 kg). Finally, animals from the HM line coming from Farm 3 (326.1 ± 32.5 kg) were significantly ($P < 0.01$) lighter at puberty than animals from the same genetic line coming from Farms 1 and 2.

Averaged across all genetic lines, 86 (95.5 %) out of 90 heifers reached puberty. Table 4-4 shows the number of animals reaching puberty by genetic line.

Table 4-4 Number of heifers per line and number of heifers reaching puberty.

Line	HG[1]	LG[2]	HM[3]	LM[4]
N	22	19	30	19
Pubertal	22	19	28	17
% Pubertal	100[a]	100.0[a]	93.3[a]	89.5[a]

Values with different superscripts within a row are statistically different ($P < 0.05$).
[1]HG: High EBV-600 day , [2]LG: Low EBV-600 day , [3]HM: High EBV-milk , [4]LM: Low EBV-milk.

All heifers from the HG and LG line reached puberty. Two heifers from each of the HM and LM lines failed to cycle by the end of the trial. There was no statistical difference among lines in the proportion of animals attaining puberty.

There was a noticeable difference in the percentage of animals from the different farms attaining puberty within each month of the year (Figure 4-4). Cumulative percentage of animals that reached puberty by November 2002 for Farms 1, 2 and 3 were 35.3; 15.8 and 45.0. No animals from Farm 4 were pubertal by this date. In the month of December, 58.8; 63.2; 50.0 and 92.3 % of heifers from Farms 1, 2, 3 and 4 cycled,

giving a cumulative percentage of 94.1; 79.0; 95.0 and 92.3 % respectively. The percentage of animals reaching puberty in January 2003 was 5.9; 21.1; 5.0 and 7.7 % for Farms 1, 2, 3 and 4 respectively. The differences in cumulative percentage of animals reaching puberty by genetic line, showed no statistical significance. The high increase in the percentage of animals reaching puberty across all lines in the month of December, followed a selenium injection on the 9 December.

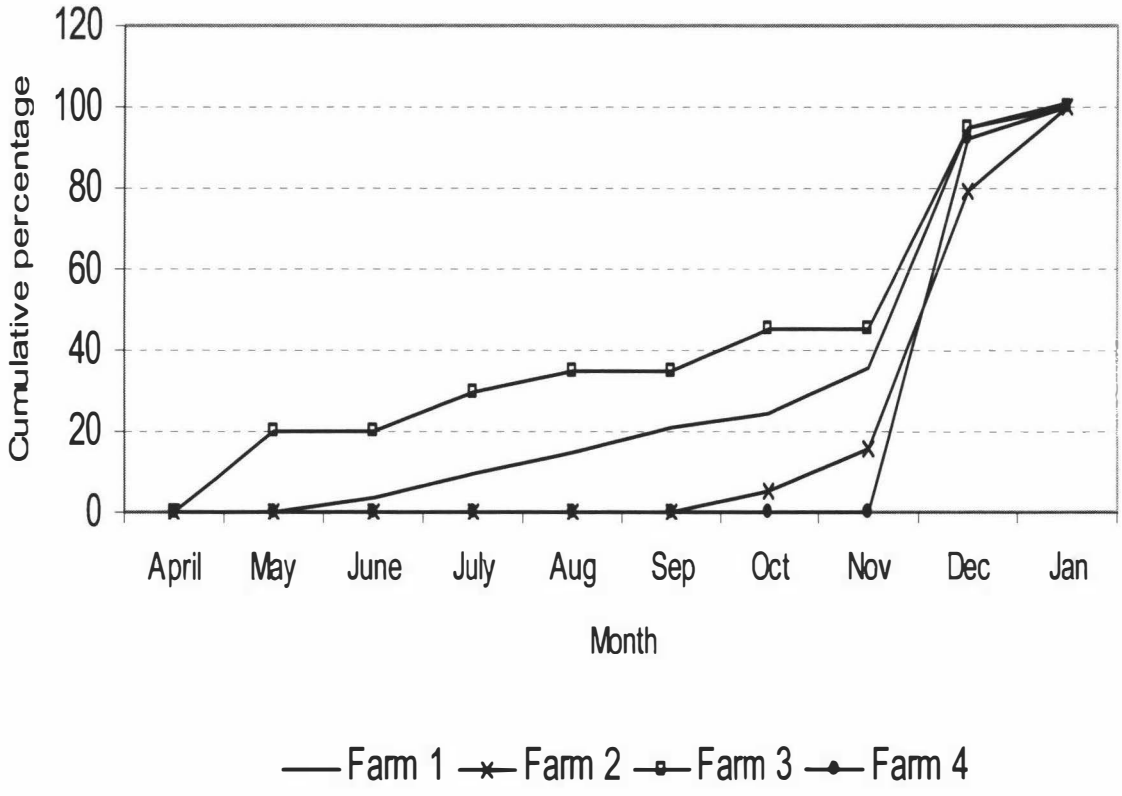

Figure 4-4 Cumulative percentage of animals attaining puberty by farm of origin.

The relationship between age and weight at puberty was explored by fitting a simple linear regression to the data. The adjusted R-square values for the HG, LG, HM and LM lines were 0.54; 0.76; 0.55 and 0.24 respectively. Adding farm of origin and sire to the model did not improve the accuracy of the regression.

The scatter plot and the studentized residuals suggested that a non-linear function would describe the data more accurately (Fernandez, 1992). The quadratic regressions between age and weight at puberty for the different genetic lines are presented in Figure 4-5.

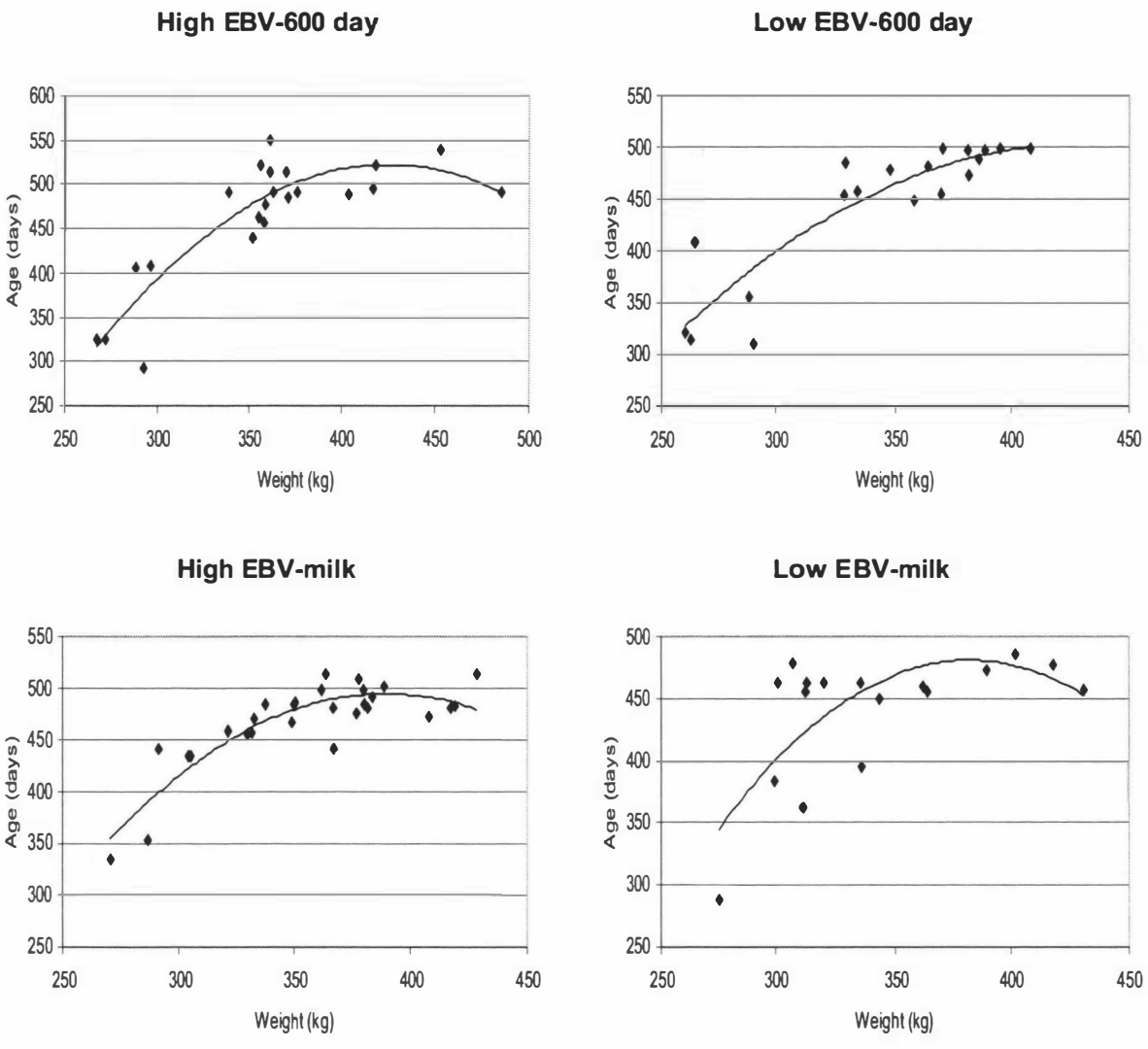

Figure 4-5 Quadratic regression between liveweight (kg) and age (days) at puberty for the High EBV-600 day, Low EBV-600 day, High EBV-milk and Low EBV-milk lines.

The quadratic function in the regressions indicates that weight and age at puberty increases over time until they reach a plateau after which an inverse relationship between age and weight at puberty occurs. In other words, once the plateau is reached age at puberty is reduced as weight increase and similarly weight at puberty is reduced

as age increase (Figure 4-5). However, this is hard to explain from a biological point of view, thus, a different equation with a square root term was fitted to the data. This equation will originate a curve in which the response variable (weight) increases with the independent variable (age), but the rate of increase becomes smaller and smaller until the response variable approaches a constant value called the asymptote. The quadratic regressions shown in Figure 4-5 presented R-square values of 0.77, 0.78, 0.76 and 0.39 for the HG, LG, HM and LM lines respectively. The corresponding values using the alternative equations are shown in Table 4-5. Both equations present similar R-square values, but the equation with the square root term is more adequate from a biological point of view, since the curve never goes downwards (Graybill & Iyer, 1994).

Table 4-5 presents relevant statistics for the regression equations using the square root term, separated by genetic line.

Table 4-5 Equations and statistics from the regressions relating age at puberty (age) to weight at puberty (lw) by genetic line.

Lines	Equation[2]	Adj R-sq	n	Signif.[1]
High EBV-600 day	age = -11.3 lw + 465.9 $lw^{0.5}$ – 4284.3	0.77	22	s
Low EBV-600 day	age = -6.8 lw + 293.0 $lw^{0.5}$ – 2612.9	0.78	19	s
High EBV-milk	age = -13.2 lw + 521.6 $lw^{0.5}$ – 4652.1	0.78	28	s
Low EBV-milk	age = -16.4 lw + 642.9 $lw^{0.5}$ –5789.1	0.41	17	s

[1]significant at $P < 0.05$

[2]It was used the expression $lw^{0.5}$ instead of $\sqrt{lw}$ in the equations.

The non-linear function improved significantly the accuracy of the regression equations as seen in the adjusted R-square values. All the models were statistically significant.

4.3. PREGNANCY RATE

Eighty-one animals out of 90 were pregnant at the end of the mating period giving a pregnancy rate of 90 % across all genetic lines. There were no differences among lines in pregnancy rate.

Table 4-6 Percentage of pregnant heifers by mating cycle.

Line	HG[1]	LG[2]	HM[3]	LM[4]
% Pregnant 1 Cycle	28.6[a]	33.3[a]	32.0[a]	23.5[a]
% Pregnant 2 Cycle	52.4[a]	55.6[a]	36.0[a]	52.9[a]
% Pregnant 3 Cycle	19.0[a]	11.1[a]	32.0[a]	23.5[a]

Values with different superscripts within a row are statistically different ($P < 0.05$).
[1]HG: High EBV-600 day , [2]LG: Low EBV-600 day , [3]HM: High EBV-milk , [4]LM: Low EBV-milk.

The percentage of pregnant heifers by mating cycle was analysed using planned contrast in the GENMOD procedure. No differences between lines were detected in the analysis.

4.4. INTAKE ESTIMATION USING N-ALKANES, FIRST MEASUREMENT

The first experiment with alkanes started on the 18 June 2002 with the four lines selected divergently for high (n=23) and low (n=20) EBV-600 day (HG and LG respectively) and high (n=30) and low (n=17) EBV-milk production (HM and LM respectively).

4.4.1. Herbage measurements

Intake was measured during two consecutive 4-day periods named First Collection Period (18-21 June) and Second Collection Period (22-25 June) performed in two adjacent paddocks named C1 and C2 respectively.

The pre-grazing herbage mass in C1 and C2 at the beginning of the trial was 3412 and 2554 kg DM ha^{-1}, respectively. The corresponding post-grazing values at the end of each collection period was 1618 and 1423 kg DM ha^{-1} for C1 and C2 respectively. The pre- and post-grazing values were chosen with the objective of maintaining the previous month LWG (over-all mean 63.1 ± 4.5 g /day).

The botanical composition of herbage on offer and the nutritive value of herbage from samples taken during the First and Second Collection Periods are presented in Table 4-7 and Table 4-8 respectively.

Table 4-7 Herbage botanical composition of the paddocks used in the First (18-21 June) and Second (22 –25 June) Collection Periods.

Collection period	Botanical composition (%)			
	Grass	Clover	Weeds	Dead matter
1	58.35	16.14	3.04	22.46
2	56.89	27.06	2.99	13.05

Table 4-8 Herbage nutritive value from the First (18-21 June) and Second (22-25 June) Collection Periods.

Collection period	Dry matter (%)	Crude protein (%)	OMD (%)	ME (MJ/kg DM)
1	94.6	28.6	82.4	11.3
2	94.5	29.9	84.1	11.6

OMD : organic matter digestibility.
ME: metabolisable energy.

The variation in botanical composition (Table 4-7) between paddocks C1 and C2 was not reflected in herbage nutritive value (Table 4-8).

4.4.1.1. N-alkane profiles and sampling procedure

Alkane profiles from single (S) and pooled (P) herbage samples taken during C1 and C2 are presented in Table 4-9.

Table 4-9 Alkane concentration (g/kg DM) of herbage samples collected by taking a single or pooled herbage sample. Average of First (18-21 June) and Second (22-25 June) Collection Periods.

Alkanes	Single sample	Pooled sample
C_{31} (g /kg DM)	231.64	256.94
C_{32} (g /kg DM)	9.99	11.40
C_{33} (g /kg DM)	76.93	84.39

In general, the n-alkane profiles obtained from a S herbage sample presented lower concentration of alkanes than that obtained with the P herbage sample.

4.4.2. Intakes

Herbage intake was estimated with the C_{31}:C_{32} and the C_{32}:C_{33} pairs of alkanes using the equation of Dove and Mayes (1991). Preliminary results showed that herbage intake estimation using the C_{32}:C_{33} n-alkanes were closer to the theoretical intakes. Thus only results using the C_{32}:C_{33} pairs were reported here.

Two different herbage sampling procedures were used in the trial resulting in two different alkane profiles as seen in Table 4-9, therefore, two intake results were obtained.

Theoretical intakes based on LW at the beginning of the trial and LWG during the trial (average across lines 0.160 kg day^{-1}) were calculated using formulae by the AFRC (1993) (Section 3.5.2.2). Average metabolisable energy values (MJME/ kg DM) used in the calculations were obtained from data shown in Table 4-8.

Data from three animals were deleted from the analysis because they were outside $\pm$ 2 standard deviations from the mean. Herbage intake was analysed with and without the animals that failed to produce at least three faecal samples (n= 5). Deleting the five

animals from the analysis did not influence the outcome, therefore, the results reported are from the analysis of 87 animals (Table 4-10).

Table 4-10 Liveweight (kg), herbage intake (kg DM) estimates using a single (S) and pooled (P) grass sample and the C_{32}:C_{33} alkane pairs plus theoretical (T) intake (kg DM) based on published formulae for the different selection lines of heifers (means ± SEM).

Lines	LW[1]	Estimated intake		Theoretical	Difference	
		S	P	Intake[2]	S – T	P - T
HG[3]	257.0 ± 0.4 [a]	3.84 ± 0.10[a]	3.56 ± 0.10[a]	3.01 ± 0.13[a]	0.83[§]	0.55[§]
LG[4]	249.0 ± 0.5 [b]	3.53 ± 0.13[a]	3.27 ± 0.13[a]	2.94 ± 0.16[a]	0.59[§]	0.33
HM[5]	226.8 ± 0.4 [c]	3.79 ± 0.08[a]	3.51 ± 0.07[a]	2.98 ± 0.10[a]	0.81[§]	0.53[§]
LM[6]	228.0 ± 0.5 [d]	3.83 ± 0.10[a]	3.55 ± 0.10[a]	3.26 ± 0.13[a]	0.57[§]	0.29

[a,b,c] Means with different letter within a column are statistically different (P < 0.05).

[§] Indicates statistical difference (P < 0.05) between estimated and theoretical intakes.

[1]LW: liveweight , [2]Theoretical intakes calculated using LW at the beginning of the trial and LWG during the trial in the AFRC (1993) equations.

[3] HG: High EBV-600 day , [4] LG: Low EBV-600 day , [5] HM: High EBV-milk , [6] LM: Low EBV-milk.

Table 4-10 presents mean estimated intake by line using the C_{32}:C_{33} n-alkanes, separated according to grass sampling procedure. Theoretical intakes and the difference between the two estimations are also presented.

Heifers from the HG line were significantly heavier ($P < 0.001$) than heifers from all the other lines. Heifers from the LG line were significantly heavier than HM and LM heifers ($P < 0.001$) and heifers from the HM lines were heavier than heifers from the LM line ($P < 0.05$). There were no statistical difference in LWG among heifers from the different lines during the intake measurement.

There were no statistical differences in estimated intake between lines using alkane profiles from the S or P sampling procedure. However, estimated intake using the alkane profile from the P grass sampling procedure showed less deviation from the theoretical intakes than results obtained using the S sampling procedure. The difference between theoretical and estimated intakes using the n-alkanes from the S sampling procedure were statistically different ($P < 0.05$) for all lines. There was no statistical

difference between theoretical and estimated intakes derived from the P sampling procedure in the LG and LM lines; the differences were statistically significant ($P < 0.05$) for the other two lines (Table 4-10).

4.5. INTAKE ESTIMATION USING N-ALKANES, SECOND MEASUREMENT

The second experiment with n-alkanes started with the capsules insertion on the 17 September. Faecal samples started on the 24 September and ended on the 1 October. Twenty one animals from the HG line, 19 from the LG line, 23 from the HM line and 17 from the LM line were used in the trial.

4.5.1. Herbage measurements

Herbage intake measurement comprised two consecutive 4-day collection periods (First and Second Collection Periods respectively) performed in two adjacent paddocks named C1 and C2 respectively.

The pre-grazing herbage mass in C1 and C2 was 3495 and 3601 kg DM ha^{-1} and the corresponding post-grazing herbage mass was 2227 and 2216 kg DM ha^{-1}. The pre- and post-grazing values were chosen to maintain the previous months LWG (over-all lines mean 1.2 ± 0.06 kg /day).

The botanical composition of herbage on offer and the nutritive value of herbage samples taken during the trial are presented in Table 4-11 and Table 4-12 respectively.

Table 4-11 Herbage botanical composition of the paddocks used in the First (24-27 September) and Second (28 September –1 October) Collection Periods.

Collection period	Botanical composition (%)			
	Grass	Clover	Weeds	Dead matter
1	67.10	19.76	11.90	1.24
2	50.94	44.09	1.12	3.75

Table 4-12 Herbage nutritive value from the First (24-27 September) and Second (28 September –1 October) Collection Periods.

Collection period	Dry matter (%)	Crude protein (%)	OMD (%)	ME (MJ/kg DM)
1	94.1	25.9	> 85.0	12.6
2	94.5	26.3	> 85.0	12.1

OMD : organic matter digestibility.
ME: metabolisable energy.

Paddock C2 (Second Collection Period) had more clover (+24.33 %) and less weeds (-10.78 %) than paddock C1 (First Collection Period). However, the differences in botanical composition between Paddocks C1 and C2 were not reflected in the herbage nutritive value. Both paddocks had high quality herbage samples.

4.5.1.1. N-alkane profiles and sampling procedure

The average (First and Second Collection Periods) n-alkanes concentration of the herbage samples used in the calculations, separated by sampling procedure and drying method is shown in Table 4-13.

The freeze-dried (F) and oven-dried (O) procedures resulted in similar n-alkanes concentration when a single (S) forage sample was analysed. The F samples from pooled sample (P) procedures however, presented a higher concentration of n-alkanes than the P-O samples.

Table 4-13 N-alkanes concentration in herbage obtained by a single or pooled sampling procedure and freeze- or oven-dried.

	Oven-dried		Freeze-dried	
Alkanes	Single	Pooled	Single	Pooled
C_{31} (g /kg DM)	192.4	183.3	191.5	198.8
C_{32} (g /kg DM)	9.7	7.7	10.3	13.3
C_{33} (g /kg DM)	66.5	62.5	65.8	66.2

The O-S sample presented higher n-alkanes concentration than the O-P sample. The opposite happened in the freeze-drying procedure; the S-F sample presented lower n-alkanes concentration than the P-F sample.

4.5.2. Intakes

Preliminary results showed that intake estimation using the C_{32}:C_{33} alkane pairs were closer to the theoretical intakes than intakes estimated using the C_{31}:C_{32} alkane pairs. Therefore, only results using the C_{32}:C_{33} n-alkanes will be presented.

Two different sampling procedures and two different herbage drying methods were used to determine the n-alkane profile of the animals' diet, resulting in four different estimations of intake.

The estimated intakes were compared against theoretical intakes calculated using published formula (AFRC, 1993). Animal LW at the beginning of the trial and their LWG during it (1.2 kg day^{-1}) were included in the theoretical calculations plus the average metabolisable energy values of the herbage samples (Table 4-12).

Estimated intakes calculated with two different herbage collection procedures and two herbage drying techniques plus theoretical intakes are presented in Table 4-14.

Table 4-14 Liveweight (kg) and herbage intake (kg DM) estimates using the C_{32}:C_{33} alkane pairs and single (S) or pooled (P) grass sample oven-dried (O) or freeze-dried (F). Theoretical intake (kg DM) based on published formulae for the different selection lines of heifers (means ± SEM).

Lines	LW[1]	Estimated intake				Theoretical Intake[2]
		Oven-dried		Freeze-dried		
		S	P	S	P	
HG[3]	308.1 ± 1.9[a]	5.48 ± 0.29[a§]	5.47.± 0.26[a§]	5.70 ± 0.29[a§]	6.12 ± 0.35[a§]	7.21 ± 0.28[a]
LG[4]	298.5 ± 2.1[b]	6.23 ± 0.31[a]	6.14 ± 0.28[a]	6.37 ± 0.30[a]	6.98 ± 0.37[a]	6.17 ± 0.26[b]
HM[5]	272.9 ± 1.6[c]	5.69 ± 0.26[a§]	5.67 ± 0.24[a§]	5.86 ± 0.26[a§]	6.37 ± 0.32[a]	7.28 ± 0.22[a]
LM[6]	269.4 ± 2.0[c]	5.84 ± 0.33[a§]	5.86 ± 0.30[a§]	6.05 ± 0.32[a]	6.55 ± 0.40[a]	7.11 ± 0.31[a]

[a.b.c] Means with different letter within a column are statistically different ($P < 0.05$).

[§] Indicates statistical difference ($P < 0.05$) between estimated and theoretical intakes.

[1] LW: liveweight, [2] Theoretical intakes calculated using LW at the beginning of the trial and LWG during the trial in the AFRC (1993) equations.

[3]HG: High EBV-600 day , [4] LG: Low EBV-600 day , [5] HM: High EBV-milk , [6] LM: Low EBV-milk.

Data from 8 animals were deleted from the analysis because they were outside ± 2 standard deviations from the mean. All the animals produced at least 3 faecal samples during each of the collection periods.

Heifers from the HG line were significantly ($P < 0.05$) heavier than heifers from all the other lines at the beginning of the trial. Heifers from the LG line were heavier ($P < 0.05$) than the HM and LM heifers and there were no statistical difference in LW between the last two lines. There were no statistical difference in LWG among heifers from the different lines during the intake measurement.

There were no statistical differences in intake between the genetic lines regardless herbage sampling procedure or herbage drying method. However, estimated intake between the HG and LG lines tended to be different when the n-alkane profiles obtained from the S-O ($P < 0.06$) samples (single sample that was oven-dried) and the P-F ($P < 0.07$) samples (pooled samples that were freeze-dried) were used in the calculations.

4.6. INTAKE ESTIMATION USING PRE- AND POST-GRAZING, THIRD MEASUREMENT

The first experiment to determine herbage intake using the pre- and post grazing technique started on the 15 August and finished on the 18 August. Twenty animals from each genetic line were randomly selected for the trial.

4.6.1. Herbage measurements

The paddock used for the pre- and post-grazing trial was divided into four lanes of equal area named 18-A, 18-B, 18-C and 18-D respectively. The herbage botanical composition and nutritive value of the different lanes are shown in Table 4-15 and Table 4-16 respectively.

Table 4-15 Herbage botanical composition of the lanes used in the pre- and post grazing trial.

	Botanical composition (%)			
	Grass	Clover	Weeds	Dead matter
Lane 18-A	80.14	6.38	0.00	13.48
Lane 18-B	82.23	2.97	0.00	14.80
Lane 18-C	74.38	8.68	0.31	16.63
Lane 18-D	81.56	2.00	0.00	16.43

Table 4-16 Herbage nutritive value from lanes 18-A, 18-B, 18-C and 18-D.

	Lanes			
	18-A	18-B	18-C	18-D
Dry matter (%)	92.2	93.4	93.0	92.5
Crude protein (%)	26.7	26.1	25.5	27.2
OMD[1] (%)	82.8	81.0	79.9	83.4
ME[2] (MJ/kg DM)	11.6	11.4	11.2	11.7

[1] OMD : organic matter digestibility.

[2] ME: metabolisable energy.

Average herbage mass in lanes 18-A, 18-B, 18-C and 18-D at the beginning of the trial was 2818, 2447, 2714 and 2636 kg DM ha^{-1}, respectively. The post-grazing values at the end of the trial were 1920, 1870, 1937 and 2014 kg DM ha^{-1} for the same lanes. Target pre-and post-grazing herbage mass (2500 and 1800 kg DM ha^{-1} respectively) chosen to achieve 1 kg of LWG during the trial were met.

4.6.2. Regression equation and intakes

The regression line relating the rising plate meter (RPM) reading and herbage mass is shown in Figure 4-6.

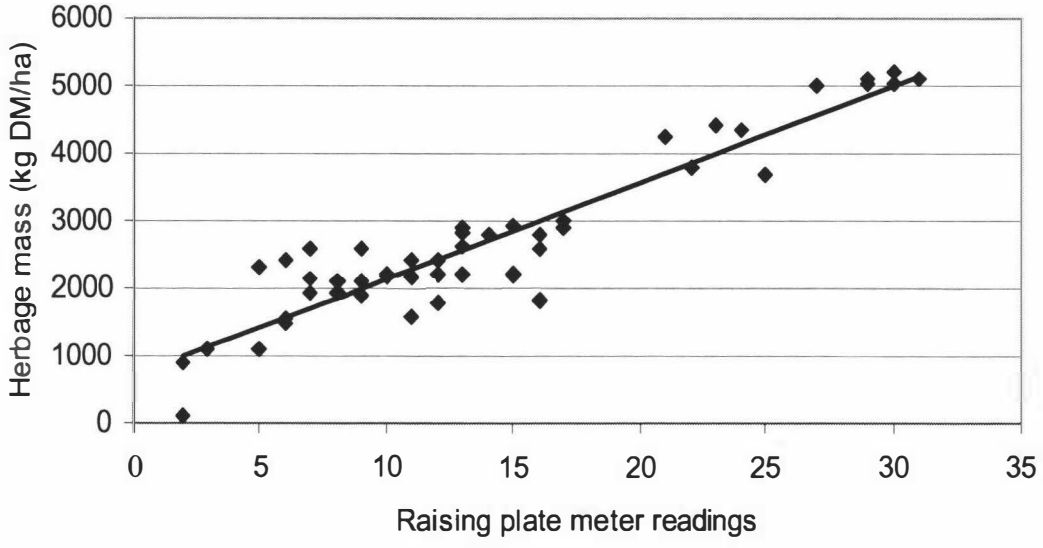

Figure 4-6 Simple linear regression between the rising plate meter readings and herbage mass (kg DM/ha).

The simple linear regression equation (± SE of the slope and intercept) relating RPM to herbage mass (kg DM ha^{-1}) is:

Herbage mass = 142.9 (± 7.7) x RPM + 721.9 (± 123.9)

The R-square for the equation was 0.88 and the coefficient of variation was 15.71 %.

Results presented in Table 4-17 are the mean LW at the beginning of the trial, LWG during the trial, estimated herbage intake and theoretical herbage intake. The difference between the estimated and theoretical values are also presented.

Table 4-17 Liveweight, liveweight gain, estimated herbage intake and theoretical herbage intake plus difference between estimated and theoretical values for the selection lines of heifers (means ± SEM).

Lines	LW[1] (kg)	LWG[2] (kg day^{-1})	Estimated intake (kg DM)	Theoretical intake[3] (kg DM)	Difference	
					(kg DM)	%
HG[4]	264.5 ± 0.7^{a}	0.39 ± 0.13^{a}	4.86 ± 0.26^{a}	3.61 ± 0.35^{a}	1.25§	34.62
LG[5]	257.8 ± 0.7^{b}	-0.05± 0.14^{b}	4.17 ± 0.26^{a}	3.13 ± 0.40^{a}	1.04§	33.22
HM[6]	237.6 ± 0.7^{c}	0.41 ± 0.13^{a}	4.37 ± 0.26^{a}	3.20 ± 0.33^{a}	1.17§	36.56
LM[7]	228.5 ± 0.7^{d}	-0.14 ± 0.15^{b}	3.00 ± 0.26^{b}	2.11 ± 0.30^{b}	0.89§	42.18

^{abcd}Means with different superscripts within a column are statistically different ($p < 0.05$).

§ Indicates statistical difference ($P < 0.05$) between estimated and theoretical intakes.

[1]LW: liveweight , [2]LWG: liveweight gain , [3] Theoretical intakes calculated using LW at the beginning of the trial and LWG during the trial in the AFRC (1993) equations.

[4]HG: High EBV-600 day , [5]LG: Low EBV-600 day , [6]HM: High EBV-milk , [7]LM: Low EBV-milk.

Genetic line ($P < 0.001$) significantly affected LW of the animals at the beginning of the trial. Difference in LW were statistically significant across lines.

Liveweight gain during the experiment was significantly affected by genetic line ($P < 0.01$). Heifers from the HG and HM line gain weight while heifers from LG and LM line lost weight ($P < 0.05$). There was no statistical difference in performance between the HG and HM lines and between the LG and LM lines.

Heifers from the HG, LG and HM lines had significantly higher estimated herbage intake than animals from the LM line ($P < 0.001$). There was no statistical difference in estimated herbage intake between the HG, LG and HM lines (Table 4-17).

Theoretical intakes were calculated using LW at the beginning of the trial and LWG during the trial (Table 4-17) in the AFRC (1993) equations (Section 3.5.2.2). Average MJME content of the forage used in the calculations were taken from data in Table 4-16.

Heifers from the HG, LG and HM line presented significantly higher theoretical intakes than heifers from the LG line ($P < 0.05$). There was no statistical difference in theoretical intake between HG, LG and HM lines (Table 4-17). Estimated and theoretical intakes were below expected intakes considering the pre- and post grazing values during the trial.

Herbage intake estimated using the pre- and post-grazing technique overestimated intake by 36.6 % over all lines when compared against theoretical intakes. A t-test showed that the difference between estimated and theoretical intakes were statistically different ($P < 0.05$) across lines (Table 4-17).

4.7. INTAKE ESTIMATION USING PRE- AND POST-GRAZING, FOURTH MEASUREMENT

The second experiment to determine herbage intake using the pre- and post-grazing technique started on 9 October and continued until 14 October. Twenty animals per line were randomly selected for this intake measurement.

4.7.1. Herbage measurements

The same lanes (i.e., 18-A, 18-B, 18-C and 18-D) used in the first pre- and post-grazing measurement were used in the second pre- and post-grazing measurement. The herbage

botanical composition and nutritive value of the different lanes is shown in Table 4-18 and Table 4-19 respectively.

Table 4-18 Botanical composition of the lanes used in the pre and post- grazing trial.

	Botanical composition (%)			
	Grass	Clover	Weeds	Dead matter
Lane 18-A	83.46	9.08	0.0	7.45
Lane 18-B	78.35	8.06	2.20	11.38
Lane 18-C	82.74	1.87	2.23	13.16
Lane 18-D	81.84	5.85	1.11	11.19

Table 4-19 Herbage nutritive value from lanes 18-A, 18-B, 18-C and 18-D.

	Lanes			
	18-A	18-B	18-C	18-D
Dry matter (%)	93.0	93.7	93.0	94.1
Crude protein (%)	25.7	25.7	25.3	26.1
OMD[1] (%)	83.4	84.2	85.0	84.5
ME[2] (MJ/kg DM)	11.7	11.8	11.9	11.8

[1]OMD : organic matter digestibility.
[2]ME: metabolisable energy.

Average herbage mass in lanes 18-A, 18-B, 18-C and 18-D at the beginning of the trial was 2705, 2492, 2439 and 2709 kg DM ha^{-1} respectively. The post-grazing values at the end of the trial were 1898, 1932, 1827 and 2045 kg DM ha^{-1} for the same lanes. The target pre- and post-grazing herbage mass of 2500 kg DM ha^{-1} and 1800 kg DM ha^{-1} was met. The pre- and post-grazing parameters were selected to ensure a LWG of at least 1 kg day^{-1} during the trial.

4.7.2. Regression equations and intakes

The regression equation and relevant statistics plus the regression line relating the RPM reading to herbage mass for the pre- and post grazing events are presented in Table 4-20 and Figure 4-7.

Table 4-20 Regression equations, r-square and coefficient of variation (CV) for the pre- and post-grazing models.

	Regressions	R-square	CV (%)
Pre-grazing	HM = 148.5 ± 9.2 x RPM + 507.8 ± 191.4	0.84	11.16
Post-grazing	HM = 172.5 ± 17.3 x RPM + 488.8 ± 255.0	0.68	24.93

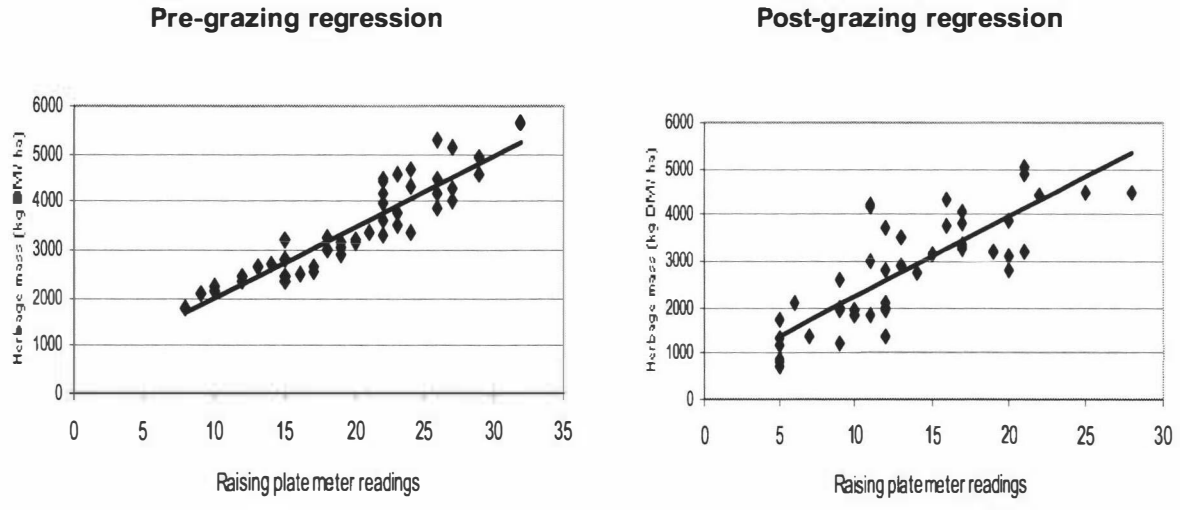

Figure 4-7 Simple linear regression between the rising plate meter readings and herbage mass (kg DM/ha) for the pre- and post-grazing events.

The slopes and intercepts from the pre- and post-grazing equations were statistically different from each other ($p < 0.001$).

Results presented in Table 4-21 are mean LW at the beginning of the trial, LWG during the trial, estimated herbage intake and theoretical herbage intakes plus the difference between the estimated and theoretical values for the different selection lines of heifers.

Genetic line significantly affected LW at the beginning of the trial ($P < 0.01$). Heifers from the HG line were significantly heavier than heifers from all the other lines. There was no statistical difference in LW between LG and HM heifers and both lines were significantly heavier than heifers from LM line.

Table 4-21 Liveweight, liveweight gain, estimated herbage intake and theoretical herbage intake plus difference between estimated and theoretical values for the different genetic lines of heifers (means ± SEM).

Lines	LW[1] (kg)	LWG[2] (kg day^{-1})	Estimated intake (kg DM)	Theoretical intake[3] (kg DM)	Difference (kg DM)	Difference %
HG[4]	293.0 ± 0.7^{a}	1.20 ± 0.05^{a}	6.99 ± 0.18^{ab}	8.12 ± 0.40^{a}	-1.13§	-13.91
LG[5]	275.1 ± 0.7^{b}	1.04 ± 0.07^{b}	6.52 ± 0.18^{b}	8.41 ± 0.39^{ab}	-1.89§	-22.47
HM[6]	274.2 ± 0.6^{b}	1.17 ± 0.05^{ab}	6.71 ± 0.18^{b}	8.47 ± 0.37^{ab}	-1.76§	-20.77
LM[7]	247.5 ± 0.7^{c}	1.16 ± 0.07^{ab}	7.28 ± 0.19^{a}	9.52 ± 0.42^{b}	-2.24§	-23.52

^{abc}Means with different superscripts within a column are statistically different ($P < 0.05$).

§ Indicates statistical difference ($P < 0.05$) between estimated and theoretical intakes.

[1]LW: liveweight , [2]LWG: liveweight gain , [3] Theoretical intakes calculated using LW at the beginning of the trial and LWG during the trial in the AFRC (1993) equations.

[4]HG: High EBV-600 day , [5]LG: Low EBV-600 day , [6]HM: High EBV-milk , [7]LM: Low EBV-milk.

Heifers from the HG line gained weight at a significantly higher rate than heifers from the LG line ($P < 0.05$). The difference in growth rate between the HG, HM and LM animals were not statistically significant. There was no statistical difference in growth rate between LG, HM and LM animals.

Animals from the LM line had significantly higher estimated herbage intake than animals from the LG and HM lines ($P < 0.05$). There were no significant differences in estimated herbage intake between LM and HG lines. High EBV-600 day heifers presented higher intake than LG heifers followed by HM heifers, however, the differences were not significant.

Theoretical herbage intake was calculated with the AFRC (1993) equations (Section 3.5.2.2) using LW at the beginning of the trial and LWG during the trial (Table 4-21). Average MJME content of the forage used in the calculations were from data in Table 4-19. Animals from the LM lines presented the highest theoretical intake, followed by

animals from the HM and LG line, however, the differences in intake among them was not significant. Statistically significant differences between LM and HG lines were detected ($P < 0.01$). There were no statistical differences in theoretical herbage intake among HG, LG and HM.

The pre- and post-grazing technique underestimated herbage intake by 20.16 % over all lines when compared against theoretical intakes. A t-test showed that the difference between estimated and theoretical herbage intake was statistically different ($P < 0.05$) across farms (Table 4-21).

CHAPTER 5

DISCUSSION

This study aimed to measure differences in age and weight at puberty of four lines of Angus cattle divergently selected for High and Low EBV-600 day weight and High and Low EBV-milk. Differences in intake among the different lines were also quantified. Both traits are of economic importance to farmers as they influence reproductive efficiency and reflects DM intake for maintenance and production.

Puberty traits and intake measurements results will be discussed separately in this chapter. No data were found in the literature about genetic lines within a breed that were divergently selected for the traits studied here and their correlation with puberty or dry matter intake. Therefore, comparisons with other studies reported in this section will be from trials were the Angus breed was used alone or in crosses with other breeds unless otherwise stated.

The accuracy of herbage mass intake prediction using the n-alkanes and the pre- and post- grazing technique will be compared along with the accuracy and appropriateness of the different methodologies used in the n-alkane procedures. Finally, the differences in herbage mass intake among the different genetic lines will be discussed.

5.1. PUBERTY

5.1.1. Age and weight at puberty

In the present study, the average age (457.3 ± 83.4 days) and weight (349.6 ± 91.8 kg) at puberty across all genetic lines were higher than other New Zealand and overseas findings. There are several reasons that may have contributed to the difference between the current study and other reports in the literature.

Pleasants *et al.* (1975) reported mean age and weight at puberty in Angus cattle of 374 ± 83 days and 201.3 ± 38.7 kg during a three year trial. Morris *et al.* (1992), in a breeds comparison trial involving ten breeds and crosses reported age and weight at puberty of 411 ± 52 days and 245 ± 33 kg respectively for Angus cattle. Age and weight at puberty of 339 ± 21 days and 307 ± 15 kg were found in Angus cattle by Gregory *et al.* (1979) in a germ plasm evaluation trial in the USA. Puberty in these studies was defined as the first behavioural oestrus determined by the paint mark of vasectomized bulls equipped with a chin ball mating harness. In the current trial puberty was determined by the concentration of progesterone in blood samples. Nelsen *et al.* (1985) found that between 13-22 % of heifers showing behavioural oestrus were not ovulating as determined by basal levels of plasma progesterone. It can be concluded that using behavioural oestrus as the criteria to define puberty can be misleading in the sense that animals classified as pubertal are not yet ovulating.

Likewise in the studies presented above, male animals were used to detect puberty. Zalesky *et al.* (1984) found that the presence of bulls hastened the resumption of oestrous cycles in adult cows after parturition, while Izard & Vandenbergh (1982) and Small *et al.* (2000) found that bull exposure increased the percentage of animals reaching puberty at a given age by 32.1 and 35 % respectively. In the current study no

male animals were used to detect behavioural oestrus for the reasons expressed in Section 3.4.1.

In conclusion, the large differences in age and weight at puberty between the present trial and the others reported in this section may be partially explained by the differences in experimental design and in puberty definition. It should be noted also that the Angus cattle used in the current trial are different from the Angus cattle of 30 years ago. The modern Angus breed is a heavier and later maturing animal, thus any comparisons should also take into account this characteristic. Other factors that may have delayed puberty onset in the current experiment are presented in Section 5.1.3.

5.1.2. Genetic make-up and its effect on age and weight at puberty

It was postulated that in the current trial the HM and LM lines would reach puberty at an earlier age and lighter weight than the HG and LG lines, however, the opposite happened in this study. The lines selected for mature size (i.e. HG and LG) reached puberty at a younger age and lighter weight than the lines selected for milk ability (i.e. HM and LM).

This is not in agreement with a crossbreeding experiment reported by Morris *et al.* (1992) in which crosses containing dairy breeds (Jersey x Angus and Friesian x Angus) were younger (338 and 362 days respectively) at puberty than Hereford x Angus (396 days) crosses. Similar results were reported by Laster *et al.* (1979) in a crossbreeding trial in the USA, in which sire breeds mated to Angus and Hereford dams ranked in age at puberty from youngest to oldest as follows: Jersey, Gelbvieh, Brown Swiss, Red Poll, South Devon, Hereford-Angus, Maine Anjou, Simmental, Limousin, Charolais and Chianina. Martin *et al.* (1992) concluded that breeds selected for milk production reached puberty at younger ages than breeds of the same mature size that were selected

solely for meat production and that genetic associations with milk production can offset the relationship between mature size and puberty.

Weight at puberty ranking in the current experiment is also in disagreement with the results presented by Martin *et al.* (1992) who found that fast growing breeds of large mature size tended to reach puberty at heavier weights than slower gaining breeds of smaller mature size. It should be emphasised that the studies reported here are between-breeds comparisons and that no within-breeds data for the traits analysed in the current study were found in the literature.

One factor that may explain the results in the current trial is that heifers were obtained by artificial insemination of commercial dams of similar genetic merit. The use of commercial dams to originate the HG, LG, HM and LM lines equalised the maternal effects across all genetic lines. Herd (1990) reported that the phenotypic response to selection has a direct component (genetic) and a maternal component (i.e. mothering ability, milk production). Moreover, Laster *et al.* (1979) observed a correlation of – 0.88 between milk yield and puberty and Gregory *et al.* (1979) reported a significant maternal effect on age and weight at puberty. Ferrel (1982) suggested that maternal effects determined some of the differences observed in age and weight at puberty among different genotypes. Thus it can be hypothesised that the use of males and females of the same genetic characteristics (i.e. HM x HM) would have produced results similar as those that were postulated in the current study. This idea is reinforced by the fact that additive genetic effects where reduced to half in the present study. Herd (1990) found that direct genetic effects were responsible for 0.85 of the variation in liveweight at yearling age and that maternal effects accounted for 0.11 of the variation.

In conclusion, the use of HM males on HM female animals would probably originate offspring that reach puberty at a younger age and lighter weight than all the other lines providing each of the HG, LG and LM animals is a homozygous individual. The HM

line would be followed by the LM line, LG line and the latest maturing breed would be expected to be the HG line as was hypothesised.

5.1.3. Environmental effects

Animals coming from Farms 1 and 3 tended to reach puberty earlier in the season than animals from Farms 2 and 4 (Figure 4-4). The results in the present trial suggests an important effect of farm of origin on age and weight at puberty that were not related to either age or weight differences between animals coming from the different farms at the beginning of the trial as determined by covariance analysis (Table 4-3). Thus, the exact nature of the environmental effects influencing on the animals coming from the different farms was not clear since assessing environmental effects was not an objective of the trial.

However, a mineral deficiency was suspected and a representative number of blood samples (3 samples per farm) were analysed on the 15 October. All the samples had low (< 77 nmol/litre) concentration of selenium. Blood samples taken at the beginning of the trial (i.e. 30 May) were also analysed to determine if the animals developed the deficiency at Keebles or if they were already deficient on arrival. Selenium content in blood samples from Farms 1 (n=3) and 3 (n=3) were adequate (i.e. > 85 nmol/litre). Two samples from Farm 2 had adequate levels of Se and one sample showed low levels of the element. All samples from Farm 4 (n=3) showed selenium deficiency. The number of samples and the sample type (i.e. serum) were in accordance with standard recommendations of assessing selenium deficiency (Clark & Ellison, 1993).

Selenium deficiency has been associated with several reproductive disorders and conception rates improved when dairy cattle with selenium deficiency were supplemented with this element (Grace, 1994). Selenium metabolism and functions are complex, due to its association with Vitamin E and other trace minerals (Laflamme &

Hidiroglou, 1991; Grace, 1994). More than ten protein compounds containing selenium in several tissues including the ovaries have been isolated, but so far the function of only a few has been established (Basini & Tamanini, 2000). The mechanisms by which selenium affects reproductive performance has not been established (Wichtel *et al.*, 1996; Basini & Tamanini, 2000) and the complex interactions involved in the selenium metabolism is reflected by the different minimum blood concentration recommended by New Zealand and overseas research (Wichtel *et al.*, 1994).

Laflamme & Hidiroglou (1991) studied the effect of selenium supplementation on replacement heifers and found no difference in age at puberty between supplemented and un-supplemented heifers; but significantly higher pregnancy rates occurred in animals that received selenium (58.3 %) than in animals that did not receive the mineral (33 %). However, the trial started at weaning (8 months), so selenium levels were normal prior to the trial commencement as opposed to the current trial in which the heifers were already deficient at or near weaning (Farm 4). No other trials were found in the literature in which selenium was investigated as a factor affecting puberty.

However, laboratory trials using granulosa cells strongly suggests that selenium is a vital element that triggers the mechanisms leading to puberty. Granulosa cells are specialised structures in the ovarian follicles that are responsible for the production of oestrogen which is a key hormone responsible for the initiation of puberty (Sorensen Jr., 1979; Day *et al.*, 1984; Schillo *et al.*, 1992; Darwash *et al.*, 1999). The follicle-stimulant hormone (FSH) is the most important regulator of granulosa cell growth (Basini & Tamanini, 2000).

Basini & Tamanini (2000) investigating the role of selenium in the follicles' granulosa cells found that the presence of selenium significantly improved the growth and viability of granulosa cells from small follicles when compared with control cells that were incubated in the absence of selenium. Moreover, selenium significantly augmented

the effects of follicle-stimulating hormone (FSH), as measured by the biological activity of the granulosa cells from small follicles (selenium plus FSH increased biological activity of granulosa cells by 200 % above the controls). Granulosa cells from small follicles produced significantly higher amounts of oestrogen in the presence (520 pg/ml) of selenium than in the absence (80 pg/ml) of it. Likewise, oestrogen metabolism was significantly higher in granulosa cells coming from large follicles treated (1050 pg/ml) and untreated (700 pg/ml) with selenium after 144 hours of incubation (Basini & Tamanini, 2000). Interestingly, FSH induced the production of oestrogen by the granulosa cells coming from small follicles only in the presence of selenium. No interaction between selenium and FSH in the production of oestrogen was detected in granulosa cells coming from large follicles (Basini & Tamanini, 2000).

The findings in the work by Basini & Tamanini (2000) indicates that selenium plays a fundamental role in the growth and viability of granulosa cells as well as in the synthesis of oestrogen. It also suggests that selenium is more critical in the biochemistry of small follicles than in large follicles. Thus, it can be suggested that selenium deficiency can delay puberty onset in developing heifers especially if the deficiency was established early in their life. For details about the mechanisms by which selenium enhances oestrogen production and stimulates granulosa cells multiplication and viability the reader is referred to Basini & Tamanini (2000), and to Kamada & Ikumo (1997) for the role of selenium on the synthesis of progesterone by luteal cells.

It is impossible from the current study to conclude that selenium deficiency affected puberty since all the animals were injected with selenium once the deficiency was detected (hence there was not a control herd). However, the Farm*Line interaction on weight at puberty (Figure 4-3), the distribution of animals attaining puberty by farm of origin (Figure 4-4), the selenium concentration in blood plasma from the 30 May and the evidence presented by Basini & Tamanini (2000) suggest that selenium did affect puberty; especially in animals coming from Farms 2 and 4.

A hypothesis to explain the trend in pubertal status shown in Figure 4-4 is that animals coming from Farms 1 and 3 started cycling early in the season while selenium body reserves were adequate. The animals that cycled were probably the oldest, heaviest and/or more precocious in the herd. As the season advanced, more animals from these farms depleted their selenium reserves and, even though they may have reached threshold ages or weights to become pubertal, they were held back due to low selenium status. No animals from Farm 4 cycled until they were injected with selenium, which may be a reflection that they were already deficient at the commencement of the trial. The fact that a small proportion of animals from Farm 2 cycled before the selenium injection also reflects that not all heifers coming from that farm presented low levels of the mineral. It is also necessary to consider the "bull effect" on the heifers (mating began on the 25 November) and the possible interaction of Selenium*Bulls on puberty. Small *et al.* (2000) concluded that the presence of the bull hastened puberty onset in heifers that were already close to puberty (pubescent).

It was concluded that environmental effects affected age and weight at puberty in the current trial, but the exact nature of it was not identified. Environmental effects are profusely documented in the literature. Morris *et al.* (1993b) found marked differences in age and weight at puberty between animals of similar genetic merit coming from different locations. Animals coming from Goudies were 412 days old and weighed 276 kg at puberty. The numbers at Templeton were 390 days and 269 kg and at Tokanui 377 days and 279 kg. Arije & Wiltbank (1974) reported year of birth effects on age and weight at puberty, Pleasants *et al.* (1975) and Gregory *et al.* (1979) reported maternal effects and Morris *et al.* (1993b) reported the effects of age of dam on age and weight at puberty.

Berge (1991) concluded that pre-weaning (< 7 months age) nutrient shortages were difficult to overcome and that a very reduced compensatory growth could be expected regardless of the severity of food restriction. Likewise, it was concluded that pre-

weaning energy restrictions required between 14 to 18 additional months for the restricted calves to compensate to 70-80 % of the controls (Bagley, 1993).

5.1.4. Environmental x genetic interactions

It was not possible to fully test interactions using animals from the LM line because they were not present in all farms. Likewise, Farm 4 was not tested for interactions because it had empty cells in the genetic lines column (Table 3-1). Birth date was used as a covariate in the analysis to remove the significant difference in age between animals from the different farms. Analysis of the data without Farm 4 and LM line showed a significant genotype x environmental interaction on weight at puberty. Over all farms, animals coming from Farm 3 tended to be lighter at puberty than animals from Farms 1 and 2. Genetic lines ranked differently in age at puberty depending on farm of origin (Figure 4-3).

Morris *et al.* (1993b) in their trial to determine environmental interactions with reproductive traits found that in a good environment (i.e. Tokanui), the difference among several genotypes in important reproductive traits were reduced in comparison to the differences observed among the same genotypes in more challenging environments (i.e. Goudies). The authors concluded that this was one of the factors responsible for the interactions observed. Data presented in Figure 4-3 shows that the mean weight at puberty of animals coming from Farm 3 presented less variation than the mean weight at puberty of animals coming from Farms 1 and 2. Extrapolating the conclusions of Morris *et al.* (1993b) to the current study it can be hypothesised that nutritional conditions were best at Farm 3 than at Farms 1 and 2. Again it is not possible be conclusive due to lack of information on the pre-weaning management and nutritional conditions on the farms of origin.

5.1.5. Regression curves

The correlation between age and weight at puberty for the present study ranged from a low of 0.39 for the LM line to a high of 0.78 for the LG line. Laster *et al.* (1979) and Morris *et al.* (1992) reported correlations of 0.90 and 0.74 respectively between age and weight at puberty.

The low correlation between age and weight at puberty in the LM line may reflect the fact that animals from the LM line were only raised on Farms 1 (n=9) and 4 (n=8). Nutritional conditions in Farm 4 apparently affected the performance of the heifers as was discussed in Section 5.1.3.

5.2. INTAKE ESTIMATIONS

Intake was measured on four occasions in the present trial, twice using the n-alkane technique and twice using the pre- and post-grazing technique. The First and Second measurement using n-alkanes will be referred to as M1 and M2 respectively. In M1 two herbage sampling procedures were used, a single sample (S) and a pooled sample (P). In M2 a single (S) and a pooled (P) herbage sampling procedure were combined with two herbage drying techniques; oven-drying (O) and freeze-drying (F) (Section 3.5.1.4). The Third and Fourth measurements using the pre- and post-grazing technique will be referred to as M3 and M4 respectively.

The four intake measurements were analysed using LW as a covariate to account for the differences in LW among heifers from the different lines.

The four estimations of intake were compared against theoretical intakes as described in Section 3.5.2.2. It should be stressed that one limitation of this approach (theoretical) is that the same mathematical model used to predict intake was applied to all of the

heifers, therefore differences in feed conversion efficiency, grazing ability, social interaction, appetite and other factors affecting feed intake were not taken into account.

5.2.1. Intakes using the n-alkane technique

In M1 estimated herbage intake calculations using the P samples showed no differences between the estimated and theoretical intakes for the LG and LM lines (Table 4-10). In M2 estimated herbage intake calculation using the P-F samples showed no statistical differences between the estimated and theoretical intakes for the LG, HM and LM lines (Table 4-14). In general the n-alkanes were precise intake estimators when compared against theoretical intakes.

There were no differences in intake among the four genetic lines in either M1 or M2 regardless of herbage sampling procedure or herbage drying technique. However in M2, heifers from the HG line tended ($P < 0.06$) to present lower estimated intakes than LG lines. Heifers from the HG line presented intakes of 1.98 kg 100 kg LW^{-1} in contrast to 2.33; 2.33 and 2.43 kg 100 kg LW^{-1} of the LG; HM and LM lines. In M1 the estimated intakes of the heifers from the HG, LG, HM and LM lines were 1.17; 1.18; 1.31 and 1.42 kg 100 kg LW^{-1}. Considering that there were no statistical differences in LWG during both M1 and M2 measurements, these values suggests that the HG lines are the most efficient in converting grass into tissue and that LM line has the lowest efficiency.

In a previous study at Massey University, Khadem *et al.* (1993) using intraruminal chromic oxide controlled release capsules in Hereford x Friesian heifers weighing 365 kg and growth rate of 0.04 kg day^{-1} reported estimated dry matter intakes of 1.56 kg 100 kg LW^{-1}. This is similar to the average across line estimated intake of 1.55 kg 100 kg LW^{-1} found in M1 in the current study with 240 kg LW heifers gaining 0.16 kg day^{-1}. In a second intake measurement Khadem *et al.* (1993) reported that the same heifers (412 kg LW) gaining 1.70 kg day^{-1} had an estimated herbage intake of

2.42 kg 100 kg LW^{-1}. This is also similar to the results in M2 in the current trial in which heifers (average 287.2 kg) growing at 1.2 kg day^{-1} had an average across lines estimated herbage intake of 2.26 kg 100 kg LW^{-1}. Morris *et al.* (1993a) using intraruminal chromic oxide controlled release capsules in 223 kg Charolais x Angus steers with a LWG of –0.42 kg day^{-1} reported estimated dry matter intakes of 1.3 kg 100 kg LW^{-1}. In a subsequent measurement the authors reported that the same steers (334 kg LW) gaining 1.47 kg day^{-1} had an estimated herbage intake of 2.6 kg 100 kg LW^{-1}. The estimated herbage intakes reported in the present study are in accordance to previous trials performed under New Zealand pastoral conditions. No intake estimations for the genetic lines used in the present trial were found in the literature.

5.2.1.1. Herbage sampling procedure

In M1, estimated herbage intake using the P herbage sample was closer to the theoretical intake than estimated intakes using the S herbage sample (Table 4-10). In M2, the estimated intake using the P-F herbage sample was closer to the theoretical intake than S-O; P-O and S-F herbage samples (Table 4-14). Vulich *et al.* (1993) found that n-alkane concentration in predominantly rye grass swards varied on a daily basis and that this variation was related to the part of the plant being sampled and to the phenological state of the sward. Thus, from a biological point of view, a better herbage intake estimation can be obtained using a pool of daily samples rather than a single herbage sample. It can be concluded that the P herbage samples in this trial reflected more accurately the n-alkane concentration of the heifers diet than the S herbage samples in both (M1 and M2) herbage intake measurements.

5.2.1.2. Herbage drying procedure

Herbage samples processing would be greatly simplified if the samples could be oven-dried instead of freeze-dried prior to grinding (Dove & Mayes, 1991). Oven-drying the rush *Juncus effusus* at 100 °C reduced its alkane concentration by 50 %; however, this temperature did not affect the alkane analysis of perennial ryegrass (Dove & Mayes, 1991). Oven-drying lucerne samples at 100 °C also markedly reduced the concentration of n-alkanes in the sample, but there was only a slight reduction when the samples were dried at 70 °C (Dove & Mayes, 1991). Thus, drying temperature greatly affects the concentration of alkanes in herbage samples and there seems to be a temperature*species interaction.

In M2, herbage samples from predominantly perennial ryegrass pastures (59 %) were freeze-dried and oven-dried at 70 °C. Comparison of the drying techniques in the S samples showed no statistical differences in n-alkane concentration between the two methods regardless of the n-alkane chain length (data not shown). The difference in n-alkanes concentration between the O and F samples was 0.9 and 0.7 g/ kg DM for the C_{31} and C_{33} n-alkanes respectively; while the concentration of the C_{32} n-alkane was 0.6 g/ kg DM lower in the O sample than in the F sample (Table 4-13).

The differences in n-alkanes concentration between O and F samples that were obtained by the analysis of the P samples were much higher. However, these differences were probably caused by errors in the pooling procedure rather than by the drying method. The S herbage samples were obtained by thoroughly mixing a herbage sample obtained in a given day and dividing it in two portions; one was oven-dried and the other was freeze-dried. The P herbage samples on the other hand involved the daily collection of herbage samples during the Collection Period (i.e. 4 days). The four samples were thoroughly mixed and divided in two portions; one was oven-dried and the other freeze-dried (Section 3.5.1.4). The preparation of the P samples involved more steps and

working with a larger herbage sample (4 samples x 400 g each) than the preparation of the S herbage sample (1 x 400 g). This was probably responsible for the differences in n-alkane composition between the two final herbage portions (i.e. . the one that was oven-dried and the one that was freeze-dried).

It was concluded in the present trial that oven-drying herbage samples of predominantly ryegrass swards at 70 °C did not affect the n-alkane analysis. It was also concluded that the collection of daily samples better reflects the diet of the animals; however, the pooling procedure in this trial proved unsatisfactory. Another way of ensuring a homogeneous sample (especially when working with large quantities of herbage) is by cutting the herbage samples in 5 cm long pieces prior to pooling.

5.2.2. Intakes using the pre- and post grazing technique

In M3 and M4 estimated intakes using the pre- and post-grazing technique were averaged-across-all-lines 36.6 % higher and 20.2 % lower than the theoretical intakes. The differences between estimated and theoretical intakes were statistically significant in both M3 and M4 measurements (Table 4-17 and Table 4-21). Taking the theoretical intakes as a parameter, herbage intake estimation using the pre- and post-grazing technique were not accurate intake estimators in the current trial. Nevertheless, the ranking of genetic lines in dry matter intake estimation are expected to be precise.

There are several potential sources of error in the estimation of herbage mass using the rising plate meter (RPM). Operator variability, paddock contour, percentage of dead material and treading damage are important factors affecting the accuracy of the estimations (Lile *et al.*, 2001). In the current trial the same person operated the RPM, the paddock was reasonably flat and the amount of dead material was on average 15.3 and 10.8 % in M3 and M4 respectively. One of the factors contributing to the inaccuracy of the measurements was related to treading damage, which was exacerbated

by rain and by the small grazing area allocated to the heifers every 24 hours because of the trial requirements (i.e. strip grazing). In M3 it rained during the 4 days (15-18 August) of the experiment and in M4 it rained during three days out of the six days of the experiment (9-14 October). The difference between the pre- and post-grazing herbage mass determined by the RPM increases as grass in the post-grazed paddocks were covered in mud and abnormally compressed due to trampling, especially in M3.

Thomson *et al.* (2001) concluded that the RPM was operated differently when taking farm measurements and plot measurements. They suggested that to calibrate the RPM (i.e. plot measurement to produce the regression equation), it is placed carefully on pasture so that the only force acting on the sward canopy is the weight of the disc on a vertical axis. However, when paddocks were assessed, the RPM was usually pressed with a greater force over the grass and at an angle, compressing the pasture to a greater extent causing an underestimation of herbage mass. The "trampling effect" was not so severe in M4, thus, it is possible that the concepts presented by Thomson *et al.* (2001) were responsible for the low intakes found in M4.

The high herbage intake overestimation in M3 was not only the result of trampling; the use of a single regression equation for the pre- post grazing readings probably also contributed. In M4 where two separate equations were used for the pre- and post-grazing events, herbage intake was underestimated. However, the deviation from the theoretical intakes were lower using separate regression equations than using a single regression equation relating RPM readings with herbage mass.

Herbage intake measurements in M1 and M2 showed no statistical difference in intake among heifers from the different lines. However, in M3 heifers from the LM line showed significantly lower intakes than heifers from the other genetic lines; while in M4 heifers from the LM line presented statistically higher intakes than heifers from the HG line. The lack of consistency between the results of the four intake measurements

was probably caused by differences in LWG among the genetic lines during the measurements. In M1 and M2 there was no statistical differences in LWG among animals from the different lines during the measurement period as opposed to significant LWG differences among the genetic lines in M3 and M4 (Table 4-17 and Table 4-21).

5.2.3. Intake measurements implications

The analysis of M1 and M2 showed no differences in intake among the four genetic lines under similar LWG. However, animals from the HG line had lower dry matter intakes per 100 kg LW^{-1} than all the other lines in both M1 and M2. Heifers from the LG, HM and LM ranked from lowest to highest dry matter intake per 100 kg LW^{-1} in that order. These results suggests that HG animals were the most efficient in the trial and that LM animals presented the lowest efficiency with the LG and HM lines in between these extremes.

The analysis of M3 and M4 should be interpreted with more caution since it is a less reliable method to estimate intakes. The results shows significant differences in estimated intakes among the genetic lines, but the differences were related to LWG.

CHAPTER 6

CONCLUSIONS

The original hypothesis proposed at the beginning of this trial was that genotypes with milk production genes (i.e. HM and LM) would reach puberty at younger ages and lighter weights than animals selected solely for growth (i.e. HG and LG). Results from this study however, showed that heifers from the HG line were 37.5, 20.8 and 16.5 days younger at puberty than heifers from the LM, HM and LG line. Only the LM and HM were statistically different from HG in age at puberty. There was no differences in weight at puberty among the different lines.

It should be noted that the HG, LG, HM and LM progeny were generated from commercial dams. Several authors have reported the effects of direct and maternal effects on animal performance. If sires and dams of the same selection lines (i.e. HM x HM) were used, then the original hypothesis may have been accepted. Another limitation of the experimental design was the use of animals raised in four different environments which could have significantly influenced pubertal traits.

There was no genetic line differences in the number of animals reaching puberty, in the number of animals pregnant, nor in the percentage of animals pregnant at each oestrus cycle. It was concluded that no differences in reproductive performance existed among the genetic lines up to the time of pregnancy diagnosis.

Animals in the current study were older and heavier at puberty than other values reported in the literature. Differences in experimental design and puberty definition

among this and other trials were probably responsible for part of these differences. The highest accuracy in puberty onset determination can be obtained by the analysis of blood plasma progesterone. Most of the puberty data reported in the literature relied on the observation of tail-paint marks or visual observation of oestrus manifestations; those methods are not accurate and tend to reduce the age at which puberty occurs. Environmental effects have also been detected in the current trial and they may have also contributed to the differences. It must be stressed however, that the Angus cattle used in the current study are different from the Angus strains reported in earlier experiments. The modern Angus is a heavier, later maturing animal than the Angus of 30 years ago. Any comparisons should take into account this characteristic.

There was a possibility in the current trial that selenium deficiency in animals originating from Farms 2 and 4 had a role in delaying puberty onset. It was not possible to be conclusive on the matter since the experiment was not designed with the purpose of assessing the effects of selenium on age at puberty. No other trial was found in the literature describing the effects of selenium deficiency at or around the time of weaning on puberty. The results in this trial suggest the presence of such an effect, which makes it an interesting topic for future research.

No differences in herbage intake among animals from the different selection lines were observed when using n-alkanes as an indirect estimator of intake. Statistical differences in estimated intake among lines were detected using the pre- and post-grazing technique. The results in the n-alkanes measurements and the pre- and post-grazing measurements are in line with the LWG patterns during the intake estimations. In the n-alkane measurements there were no statistical differences in LWG among the genetic lines as opposed to the significant differences in LWG during the pre- and post-grazing measurements. Even though there were no statistical differences in intake in M1 and M2; animals from the HG line showed the lowest intake in both trials which suggests that this line has a marginally higher feed conversion efficiency than the other lines. It

was interesting to observe that estimated intakes were similar across lines in both n-alkanes measurements and that intakes were different across lines in both pre- and post-grazing measurements. Grazing management was different in the n-alkane and in the pre- and post-grazing trial. It would be interesting to determine the performance of these genetic lines under different sward surface heights (i.e. 5 vs 10 cm) and grazing management conditions and to include grazing behaviour studies in future trials.

Results in the current trial indicate that daily herbage samples pooled at the end of the measurement were more accurate to estimate herbage intake than single herbage samples taken at the beginning of the trial. No difference in estimated herbage intake using freeze-dried or oven-dried samples were detected. Thus, when technical and cost constraints are considered as a limitation, the use of pooled herbage samples that are oven-dried can produce accurate herbage intake estimations.

Overall, under the management conditions imposed on the heifers in the current study, the HG performed better both in terms of age at puberty and in feed conversion efficiency. However, in a system where female animals are kept as replacements and male animals are sold at weaning, it would be important to consider the higher maintenance requirements of the HG dams and possible lower milk production resulting in a lower weaning weight of their offspring. Considering the advantage of the HG line in the traits investigated in this study and the disadvantages related to their larger mature size and lower mothering ability an index combining growth rate and an adequate level of milk yield would produce animals that will better suit a self replacing beef cowherd. Alternatively the use of HG sires on HM dams would produce suitable animals to be kept as replacements, these HG x HM cows could be crossed again with HG sires and all the progeny can be sold.

Whatever selection criteria is chosen the farmer needs to carefully match that criteria with the intended destiny or eventual use of the progeny from the herd matings. Results

in the current study showed that there is sufficient genetic variability among the selection lines, which offer the farmers flexibility when choosing from the diverse genetic lines and to exploit complimentarity.

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