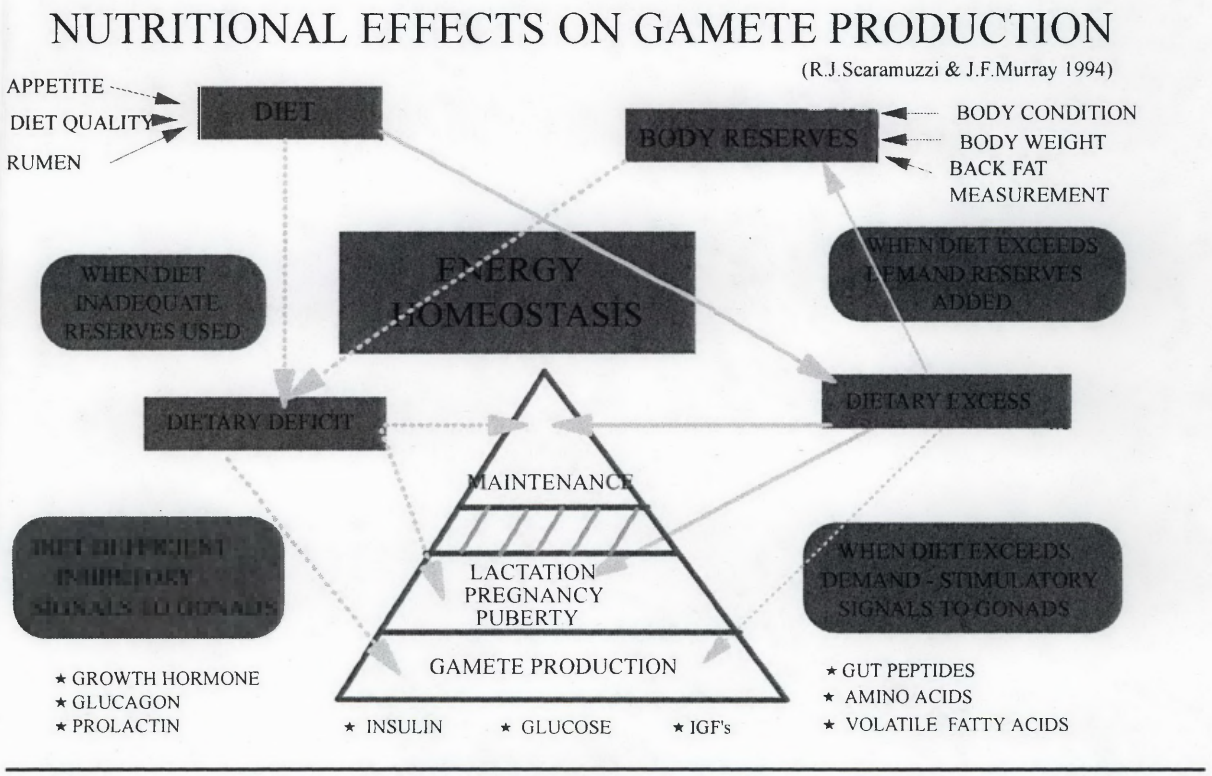
10 BODY CONDITION, REPRODUCTION and DIET INTERACTION

These effects of dietary inadequacies, condition and hormone interactions have been summarised by Scaramuzzi and Murray (1994). They have related the availability of excess nutrients to gamete production only after the other homeostatic mechanisms have been satisfied (see Fig. 23).

Figure 22 Nutrition and fertility



The interactions between body state and reproduction and nutrition have been explained in a number of ways. Frisch and Revelle (1970, 1971) showed a correlation between body weight and body composition with reproductive functioning. Bronson (1985) demonstrated that during adult-hood, low nutrition may impair reproductive success. Schreihofer et al. (1993) proposed that gastro -intestinal peptides, released by specific constituents of food, or their metabolites, may act within the central nervous system to influence the GnRH pulse generator directly.

The initiation of puberty has been associated with the attainment of a critical body weight in a range of diverse animals, human females (Frisch and Revelle (1970), rats (Kennedy and Mitra, 1963), pigs (Hughes, 1982) and sheep (Foster et al. 1985). These observations are consistent with the hypothesis that the onset of puberty depends on the attainment of a critical live weight and composition (particularly related to body fat). Further refinement of this hypothesis led to the proposition of the attainment of a minimum lean muscle to fat ratio (Frisch et al. 1973) and finally to the achievement of a minimum percentage of body fat (Frisch and Mcarthur,1974; Kirkwood and Aherne, 1985).

10.1 Fat deposition

Relative fatness may influence reproduction through steroid metabolism. Fat contains a significant pool of progesterone (Hillbrand and Elsaerser, 1983) and is capable of aromatizing to androgens and oestrogens (Nimrod and Ryan, 1975). Changes in live weight and fatness are associated with a change in oestrogen metabolism; decreasing live weight being correlated with increased oestradiol conversion to catechol oestrogens rather than oestriol (Fishman et al. 1975). The effect of condition score or fatness in a range of animals, on reproductive rates has been shown by numerous experiments. A decrease in sex hormone-binding globulin and a consequent increase in the percentage of biologically active free oestradiol is associated with obesity in women (Nisker et al. (1980) and thus no suppression of oestrus in these subjects, while obese men exhibit enhanced conversion of androgens to oestrogens.

10.2 Fat depletion

Intensively trained female athletes have a high incidence of amenorrhoea, but establish normal menstrual cycles within several months of ceasing training and with out showing a measurable change in live weight or percentage of body fat (Warren, 1980). Sows that are young and light at puberty can exhibit an increase in ovulation rate during their first few oestrus cycles when they are increasing weight and fatness (Kirkwood and Aherne,1985). However, the consistent effect of live weight change and reproductive function has not been clearly established (Warren, 1980; Russell et al. 1984).

10.3 Glucose and amino acid effects

Kennedy and Mira (1963) postulated that food intake and its associated affects on metabolic rate might be the triggering mechanism for changes in reproductive function. Glucose and amino acid administration elicited significant increases in lutenising hormone (LH) and follicle stimulating hormone (FSH) secretion in castrated juvenile monkeys at a steady body weight state: Steiner et al. (1983) and Cameron et al. (1985) support the contention of a energy/protein interaction with reproductive function.

When examining the mechanisms by which growth and nutrition affect gonadotrophin secretion, Foster and Olster (1985) used nutritionally restricted prepubertal lambs. The lambs were fed *ad libitum* to 20 kg live weight and, while maintained at that weight, did not attain puberty. Identically treated overectomised lambs exhibited very low LH secretion. Subsequent *feeding ad libitum* at 28 weeks induced rapid growth and attainment of puberty in several weeks, also LH pulse frequency increased dramatically within 48 h of reinstatement of the *ad libitum* diet as did plasma FSH. However, no change had occurred in live weight. When the lambs were returned to the restricted diet, LH pulse frequency could still be maintained by a constant parentral infusion containing glucose and or amino acids. Metabolic correlates of energy balance, live weight, adiposity or protein reserves may each provide short or long term signals that influence reproductive function and theses signals may differ.

Bucholtz et al. (1996) in testing the hypothesis that mechanisms controlling the secretion of LH are modulated by glucose availability concluded that glucose availability affects LH secretion by acting within the central nervous system at a detection sites(s) peripheral to the GnRH neuron.

10.4 Level of nutrition

The effects of live-weight change in late pregnancy and the condition score (score range 1-5) at calving also affect the time at which *post partum* oestrus occurs. Cows in a condition score equal to, or greater than 2.5, return to oestrus with in 90 d. Cows with condition scores of 2 or

below take longer (Whitman,1977; Wright et al. 1992). The loss of weight after parturition appears to delay the recurrence of oestrus, but not as much as a low condition score at calving. Weight loss before calving or low condition score at calving also delay the return to oestrus (McClure, 1994). If nutrition is not adequate for lactation demands, then ovulation may not occur and even under conditions where ovulation does occur an improvement in the nutritional plane can further increase the number of ovulations. The practice of flushing is well known in domestic animals, especially sheep (Smith,1985) and pigs (Aherne and Kirkwood, 1985).

O'Rourke et al. (1995b) in a study on lifetime productivity from cows in the tropics found that the live weight of 2-year old heifers was lowest when lactating (nutrient drain). Their live weight increased with advancing pregnancy. Failure to lactate was related to nutritional supply from the pasture and failure was highest in the late dry season and early wet season and lowest in the late wet season.

10.5 Ovarian function post-partum

The establishment of the ovarian cycle post–partum involves the re-establishment of the episodic release of LH. The release of LH can be delayed by a negative energy balance and the onset of lactation and suckling (Butler and Smith, 1989). A short luteal phase is common after the first post partum oestrus (Perry et al. 1991).

The inhibition of the pulseatile release of LH caused by an acute energy deficiency has been reported (McClure and Saunders, 1985; Randel, 1990). The intensity and duration of oestrus is critical, especially where artificial insemination (A.I) programs are implemented, but also in the extensive conditions where there can be great distances between cows and bulls. The effect of nutrition on ovarian function is such that, in beef cattle, nutritional restriction can cause follicular development to be suspended at the medium sized stage (Rutter and Manns, 1991, Suzuki et al. 1982). Nutrition also affects fertilisation as up to 40 % of fertilised ova can die between fertilization and Day 16 post-fertilisation, which is before implantation of the developing conceptus takes place (Diskin and Sreenan, 1980; Humblot et al. 1988). It has been suggested that the cause is a lack of glucose (hypoglycemia) in the maternal tissues (Weigelt et al. 1988). If the embryo dies between Day 24 and Day 50 post-fertilisation, the

luteal phase is prolonged and therefore return to oestrus is delayed by 14 days (Ryan et al. 1992). The magnitude of the nutritional effect on pregnancy and fertility gives measurable hormonal responses and pregnancy analysis involving the percentage of cows pregnant while lactating reflects adequate nutrient levels for successful re-establishment of breeding activity. The ratio of these cows compared to those non-lactating and pregnant could be used as critical measurements reflecting the effect of nutritional levels in the herd. Further refinement can be added by measuring the age of the foetus in the lactating animals as an index of time to return to pregnancy. These measurements are cheap to perform, immediate and can also be used to measure the effect of supplementation on a population of cows and to see if improvement to fertility can be affected through changes in nutritional management.

11 REVIEW OF SUPPLEMENTATION IN NORTHERN AUSTRALIA

Supplementation is the provision of small amounts of nutrients to augment the deficiencies occurring in the pasture relative to the animals physiological state so that the animal is more productive when grazing those pastures.

Although there are different opinions as to the effectiveness of the forms of delivering supplementation, *e.g.* water medication, loose mix and block form, these differences in opinion are usually based on the apparent differences in intake of the supplement. Each physical formulation of the supplements has its own advantages and disadvantages. However, the scientific basis for providing supplements is the same regardless of the physical form of the supplement. Dixon et al.(1998b) showed that both urea-based dry lick and M8U(molasses with 8% urea) supplements can lead to large increases in protein supply to the small intestine in heifers fed hay of a quality similar to senesced native pasture.

The manager of 'Anthony Lagoon' chose to use molasses-based multinutrient block supplementation because of the convenience. It was the purpose of this study to try to ascertain if the supplementary regime he chose to use was effective and if its effectiveness was reflected in the pregnancy and lactation status relative to the grass cycle in the dry period. A secondary objective was to examine if there was an intake/physiological status relationship.

There have been many excellent reviews concerning nutrition and reproduction interactions in Northern breeder herds (Holroyd, 1987; Holroyd et al. 1990*a*, 1990*b*, 1988; Anderson, 1990 anderson et al.1988; O'Rourke et al.1992, 1995*a,b,c*; Fordyce et al. 1988,1992,1993,1997; Dixon, 1998; McSweeney et al.1993).

The positive effects of supplements – primarily those providing nitrogen, phosphorus and sulphur, but also the micro-minerals copper, cobalt and selenium – made available to breeding cattle grazing dry pastures of Northern Australia can be summarised under the following headings.

11.1 Mortality of females

Dixon (1998) cites female mortality (see also Section 7.4) rates of 3–7%, whilst O'Rourke et al (1995*a*) reviewed of results from 'Swan's Lagoon' during the period from 1972 to 1992. They found an average mortality rate for 1973–87 cohorts of 1.2% (range 0.5–2.6%) with no pattern for age. For the 1970–72 cohorts, the average mortality was 1.7% (range 0–4.2%), with the overall rate tending to increase with age.

For 1970-72 cohorts, the average wastage rate was 8.9% (2.1-8.3%) with no clear age pattern. This was not the case with the 1973–87 cohorts where an average wastage rate of 9.2% had a clear age pattern. The 2-year-olds had a high rate of 27.5% but 3–7 year-olds had a rate of 2.8–8.9%. The rate increased to 11.9% for 8 year olds and 14.2% for 9 year olds. This wastage and mortality required heifer replacements at the rate of 17.5% and 19.2% in the two herds studied. The life-time production of calves was low, 3.3 calves being reared from 1970-72 cohorts in 4.7 years with an average yearly calving rate of 57.5%. The 1973-87 cohorts were rearing 3.1 calves over 4.9years. However, there was marked variability between individual cows. The cattle were grazing spear grass pastures. 'Kidmans Springs', a harsher environment, had an average breeder mortality rate of 11.5% with a range between years of 5.7–24.8% (Dixon 1998).

Female mortality is often hard to define in the extensive grazing areas owing to an absence of accurate numbers of breeders, incomplete musters, episodic events (drought, botulism, BTEC programs) and unstable breeding herd numbers (Dixon, 1998). Wicksteed (1992) is cited in

Dixon (1998) as concluding that the number of females offspring available for sale, after herd replacement requirements were fulfilled, was the most accurate way to estimate the female mortality. Despite certain inherent difficulties with conditions that can affect this assumption, it is one of the best tools for the commercial property situation. Female sales ratios for north Queensland have been estimated at 30-35%, suggestive of an average female mortality in the beef industry of 10-12%.

11.2 Nutrition and fertility interactions

Numerous studies have examined the effects of liveweight and fertility of breeders, e.g. Doogan et al. 1991; McSweeney et al.1993 and Holroyd et al. (1988). The relationship between live weight and fertility has been found to be curvi-linear in some instances and linear in others (Goddard et al. 1980; Anderson 1990; Meaker 1975 and Buck et al. 1976).

Dixon (1998) interpreted the differences in these relationships as follows:

(a) For first calf and mature age cows with a curvilinear response, the inflection point occurred at approximately 330-350 kg live weights.

(b) Low weight cows had an increase of pregnancy response of between 4–9% units per 10 kg additional increase in body weight in the case of a curvilinear response. If cattle were in the upper range of the low weight category, the pregnancy increase was in the range 0-3% units increase in pregnancy per 10 kg additional live weight gain.

(c) With a linear response over the range of the liveweight in the study, 3-8% increase in pregnancy occurred per each 10 kg of additional live weight gained post calving.

(d) In the harsher environmental areas of 'Mount Bundy' and 'Kidman Springs', the response was 3-4% increase in pregnancy rates for each additional 10 kg live-weight gain post calving for low live-weight cows but less than 1% for high live-weight cows. A pregnancy rate of only 65% was attained even though their live weight was over 400 kg. However, live weight was measured in the mid dry season rather than at mating.

Dixon (1998) concluded that, in cows of less than 340 kg live weight, a 5% improvement in pregnancy rates would be likely to occur for each 10 kg increase in body weight at mating. For cows over 340 kg live weight at mating, the response is more likely to be a 3% increase in

pregnancy rates. Mature cows and first calf cows appeared to respond similarly to increases in live weight where it was possible to compare them over similar live-weight ranges.

(e) Fertility responses are obtained from breeders supplemented through the dry season. Dixon(1998) cited experiments using dry lick containing low level urea- based supplements (10% urea) during the dry season and concluded there was a significant correlation between the reduction in live-weight loss due to feeding supplements during the dry season and increased pregnancy rates the following year.

Recording body weights accompanied by condition scoring would be a consistent method of assessing the true metabolic state of cattle.

(f) Weaning. Early weaning and its effects on the subsequent weight loss of the lactating cow have been discussed by many authors (McSweeney et al. 1993, Schlink 1987, Schlink et al 1988, Smith and Vincent 1972). The benefits of weaning to subsequent conception are thought to act through the removal of the effect of suckling and to reduce the live-weight loss in the cows due to catabolism of protein and fat to maintain milk secretion.

(g) Management practices. Management practices of a 5/8 Brahman herd at 'Swan's Lagoon' from 1986 to 1994 involved:

(i) A three-month controlled mating period starting mid- January

(ii) Weaning 6-8 weeks after the end of mating (calves 5-7 mo.)

(iii) Moderate stocking rate on native pastures, no supplements except to prevent excessive moralities when there was a delayed break to the wet season.

(iv) Pregnancy diagnosis about 7 weeks after the end of mating.

Overall pregnancy rate for the herd was approximately 81 % and ranged from 69 % in 1991 to 94 % in 1990 ('Swan's Lagoon' Annual Reports).

In a second herd of 5/8 Brahman, 3/8 shorthorn cross herd, the management practices included:

(i) Moderate stocking rate (average 1 adult equivalent (AE) per 6 ha)

(ii) Weaning musters twice yearly in April/May and August/September at which time calves over 100 kg live weight were weaned. In dryer years calves were on some occasions be weaned at lower live weight.

(iii) Continuous mating with 4 bulls/100 cows.

(iv) Replacement heifers were selected for post-weaning growth rate and mated as 2 year olds.

(v) Vaccination programs against *Clostridium botulinum* in all cattle, leptospirosis in females, 5 in 1 tick fever in calves and vibrio and BEF in the bulls were carried out.

(vi) Supplementary feeding with M8U only as required to avoid excessive mortalities.

(vii) Cows usually culled at 8 years of age.

On average in the period 1986-94, it was found:

a.	The proportion of cows lactating was:	April	80%
		July/ August	36%
b.	The proportion of first calf cows lactating was:	April	76%
		July/ August	42%
c.	The cows conceiving while lactating was, up to	April	60%
		July/ August	47%
d.	The proportion of first calf cows conceiving while lactating was: up to	April	14%
		July/ August	29%

Source: 'Swans Lagoon' Annual Report (1995)

In cows supplemented to overcome phosphorus deficiency in a different area of North Queensland ('Dagworth', Queenstown), urea supplement was also given in the dry season when necessary and a phosphorus dry lick (Kynophos) was provided in the wet season. Management included vaccination, early weaning and culling. The branding percentages achieved over a 4-year period are given in Table 10.

Table 10 Branding percentages at 'Dagworth' station (1990-94)

Year	1990	1992	1993	1994
P supplement	81%	73%	76%	65%
Control	65%	59%	53%	–

('Dagworth', Georgetown, 1994: Producer Demonstration Site Reports to the MRC)

11.2.1 Reproductive wastage

Embryonic mortality has been recognised as normally being about 25-30% (Holroyd et al.1993), probably as non-viable embryos. Foetal losses may be difficult to reduce below 2%, particularly in high ambient temperatures, especially in poorly adapted cattle and these abortions can occur probably at any stage of pregnancy (Holroyd, 1987).The effect of diet has also been recognised (Thatcher et al. 1995). Therefore the average pregnancy rate following one cycle of natural mating is 75% maximum and to maintain pregnancy to 45 days, cows must cycle a minimum of three times (Fordyce, 1995).When pregnancy has been established for 6 weeks, a mortality to weaning of 12% is reported as normal (Holroyd, 1987), although in better areas, losses as low as 4% have been achieved (O'Rourke et al. 1992).

Diseases, notably leptospirosis, brucellosis, campylobacteriosis and trichomonas are well known infectious causes of abortion. Arboviruses can also affect pregnancies and cause abortion. Some cause abortion due to the induced hyperthermia in the animal. Other causative viruses include IBR and pestivirus (McGowan and Kirkland, 1993).

A significant number of losses occur within one week of birth with no apparent cause, but genetic factors in cow such as poor mothering ability, bottle teats and congenital deformities in the calves may be significant in some breeds and lines of cattle (Fordyce, 1995).

11.3 Other supplements

A major benefit in the pregnancy rates of cattle grazing pastures grown on red earth and basalt soils was found to occur when the elements sodium and sulphur were supplemented. The effect occurred due to the greater live-weights gains by the supplemented animals in the wet season (Producer Demonstration Site at Hughenden; report to MRC, 1992.). Trial results for diverse locations also show the effect of supplements on production, e.g.'Producers adapting to market needs'. The NAPCO story, Beef 94 seminar; Landsdown Townsville (1988); Kidman Springs NT; Mount Sanford. VRD, NT; Central MT Wedge, Central Australia.

11.4 Supplement strategies

Dixon (1998) reported various supplement strategies have evolved and their effects have been reported (e.g. Lindsay, 1983, Lindsay and Loxton,1981; McLennan, 1983; Nicol et al. 1984). These included:

(i) Long-term, low level supplements fed through the dry season

(ii) Crisis supplements fed late in the dry season

(iii) Long-term, low-level supplementation through the growing season

(iv) Spike feeding of breeders in late pregnancy and late in the dry season with M8U or cotton seed meal (CSM) to increase reproductive rate (Fordyce et al.1992; Lindsay et al. 1984)

(v) Production feeding with high levels of supplements of molasses/urea/CSM to finish of animals for sale to defined markets. (Lindsay and Laing, 1994).

11.5 Control of Supplement intake

Numerous strategies have been tried for restricting supplement intakes (Dixon,1998). Supplement intake restriction has been instigated in the belief that an 'economic' level can be achieved and still affect productivity. The attainment of this 'economic' intake has been achieved by the consideration/modification of the following factors.

11.5.1 Palatability

(i) Increased levels of common salt to limit intake.

(ii) Higher levels of urea and ammonium sulphate to limit intake (Hough et al. 1995)

(iii) Attractants such as molasses and palabind that are sweet.

(iv) Improvement in texture, cottonseed meal reduces 'sweating' in mixtures and maintains a suitable texture (Dixon,1998a).

Pasture condition (protein availability), texture and the physiological status of the animal may have more influence on the amount and type of supplement intake than palatability (Leng, pers. com., 1998); Backhouse and Leng, unpublished data, 1998). Dixon et al. (1998a) found that palatability of a dry lick rather than previous experience of cattle affected intake with a low-palatablity, salt-based lick (compared with a more palatable cottonseed-containing lick. The supplements were fed to growing *Bos indicus* x Shorthorn heifers. However, if the protein requirements for heifers at this age for ruminal digestible protein and indigestible (by-pass) protein are considered, it could be just as easily be concluded that the heifers were attempting to satisfy their higher protein needs. Higher protein of better quality (by-pass) may have been the basis of increased CSM intakes. Is this a possible example of intake dictated by animal requirements?

Petherick et al.(1998) examined the intake of lick block (Olsson's 'Dry Season 20% urea'), relative to the siting of these supplements to water. They found that in a small paddock (1800 m x 200 m) the siting of the lick had little effect on the way cattle grazed the paddocks although intakes of supplements were reduced by 29% when the supplements were sited away from water. The duration of each experiment, however, was only 90 days and the significance of this experiment when applied to a large paddock situation is unknown.

11.5.2 Urea nitrogen and phosphorus requirements

The Queensland Department of Primary Industries generally recommends 30 g urea/day in the dry season for weaner and yearling cattle and 45 to 60 g urea/day for breeders. However, the response of growing cattle to molasses and urea in roller drums was found to be variable with

30 g of urea daily providing 0-350 gm /day growth depending on the seasonal conditions (Winks et al.1972; O'Rourke et al. 1992). Fordyce (1995) found that low-level, wet season supplementation of lactating cows with both N and S (as little as 5 g urea/day) may significantly improve conception rates, provided the supplement has been available over the full season and not just in the latter stages of the wet season.

McCosker and Winks (1994) have recommended daily requirements for phosphorus of 5-10 g/d but the requirements for phosphorus will be affected by the level of deficiency, age of the animal and reproductive status.

11.5.3 Distribution of supplements to the herd

Dixon (1998) states 'that economic cost associated with poor intake by some animals in the mob is difficult to assess'. He believes the important factors are:

1. The shape of the animal response curve to increased intakes in the herd
2. The type of supplement, *i.e.* ruminal degradable protein *vs* undegraded protein, type of phosphorus and molasses based.
3. The ability to feed supplements to various herds on an 'as needs' basis, *i.e.* non-lactating, pregnant, lactating and weaners.
4. The type of response required from the different groups, *i.e.* growth compared with pregnancy.

11.6 Dry lick supplementation

Dixon (1998) costed the dry lick system of supplementation at approximately $1.50/month per head. The advantages of dry licks are:

1. Lower cost of ingredients
2. Well-established technology
3. Flexibility, allowing seasonal changes in the mixes

The disadvantages are:

1. Difficult to achieve desirable levels of intake
2. A large proportion of the herd may be non-eaters

3. Sweating of the mix due to the hygroscopic nature of some of the ingredients, especially urea.

4 Potential for urea toxicity especially in the wet.

5. Rain spoilage.

11.7 Supplementation in the form of blocks

Advantages

1. Well-established technology
2. Variety of blocks available
3. Convenience
4. Low labour costs in feeding out
5. Wet season feeding, little rain spoilage and do not need sheds

Disadvantages

1. Cost per unit of nutrient
2. Little opportunity to change mix to control intake
3. Limited opportunity to purchase blocks optimal for specific areas and situations
4. High variability of intake in the mob and, at times, a high proportion of non-eaters.
5. Some situations occur where cattle will not eat the blocks.

Points 2 and 3 above have been addressed by using combinations of formulations within a block, e.g. a primarily urea block plus a mineral block plus a block containing high levels of sulphur. Weaner cattle and cattle in the last last trimester of pregnancy can also be given block supplements containing high concentrations of by-pass protein. By using various combinations of formulations at the same time ample opportunity to change the mix and to optimise the combinations for specific areas is afforded.

12 GENERAL MATERIALS AND METHODS - INTRODUCTION

Nutritional experiments involving the extensive cattle rearing properties in the northern parts of Australia are difficult and costly to undertake. Controlled experiments to examine the effects of single or multiple nutrients of the diet are difficult to design and carry out ***in situ*** in extensive live stock production areas. This is because of the varieties of microclimates experienced in open range conditions. Lack of enclosures and large herds of unmarked cattle together with lack of individual data on factors such as calving dates, time to return to oestrus after parturition, first service conception rates, pregnancy and calving rates have hampered the carrying out of meaningful nutritional trials in these areas.

The north Australian Beef producers have a large beef producing industry concentrated on grass feeding with access to the markets of Asia. The collection of meaningful data that would lead to improved reproductive and survivability rates of cows and calves would have a large and significant effect on the economic viability of these properties. It is critical therefore to develop a better understanding of the effect of nutrition on fertility and to develop ways to improve nutrition. This improvement will have to be able to be measured early enough in the breeding cycle to have an effect on the ensuing years output of calves.

Branding rates are traditionally the way any nutritional intervention is measured. However, this measure does not occur until 15 to 18 months after the establishment of pregnancy. By this time, however, it is to late for nutritional interventions to affect the ensuing pregnancy outcome. The scientific basis for providing supplements in the form of molasses urea multinutrient blocks to overcome deficiencies occurring in the pasture and to increase the intake and digestibility of native dry pastures has been reviewed in Sections 4.2, 5.1.6 and 6.1. Multi-nutritional supplements in a block form provide a very convenient for overcoming pasture deficiencies. Major minerals such as phosphorus and micro-minerals such as cobalt and selenium in deficient areas, as well as urea and by-pass protein, may constitute an appropriate supplement. This method of supplementation has been previously accepted with some skepticism by research workers and extension officers in the field because of the lack of information on individual intake and the factors affecting intake.

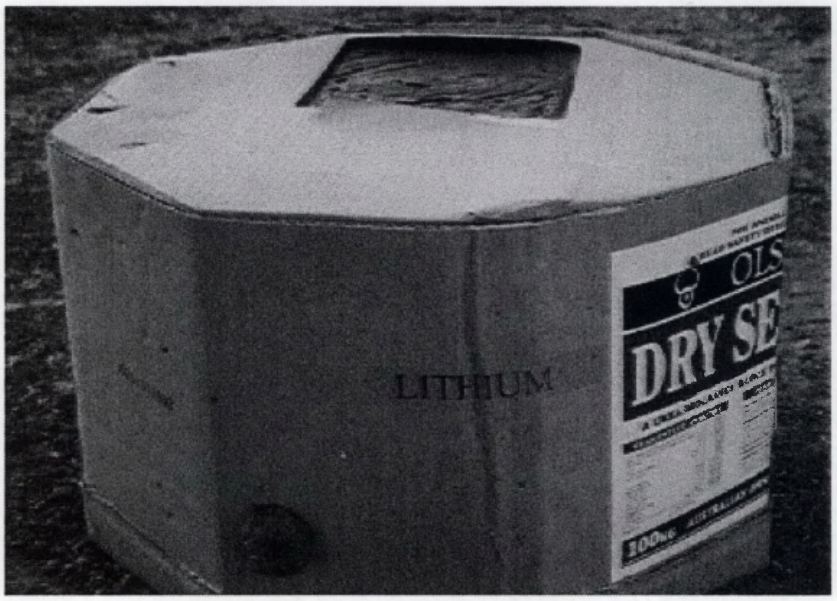
The aim of the undertaken for this degree was to determine a pattern of intake in cows, of molasses-urea multinutrient supplement blocks, by twice providing labeled multinutrient blocks to cattle, at an interval of 10 days. The blocks contained lithium chloride (1% w/w) as a marker which could be detected in the blood of the cattle if they ingested the block. Blood samples were taken randomly from approximately 10% of the cows present on the first bleed and 5 % on subsequent bleeds. The blood was initially taken twice; 10 days apart, on the fourth day after the marker blocks were supplied the cattle. The uneaten labelled block residues were removed on Day 3. The blood urea nitrogen concentrations in sera were also determined in all cattle sampled. On the occasion of the second sampling during Experiment 1, some cows from the first sampling were re-sampled. The protocol was used in the same group of cows in Experiment 2 which was carried out 12 months later when a third sampling from 100 cows was taken. The intake of supplement was again calculated to ascertain if any change in intake pattern had occurred. The intakes of individual animals could be calculated to gain a greater insight into supplementary feed strategy acceptance and to find out if enough of the supplement would be eaten by enough animals in the herd to have a beneficial effect on herd health and ultimately fertility. The second part of Experiment 2 involved examining the blood hormone concentrations of progesterone and 1-7β-oestradiol.

The concentrations of these hormones change during the reproductive cycle. They were selected to see if they could be used to evaluate the effectiveness of supplementation and to determine whether they would correlate with increased ovarian activity caused by supplementation. The hormone levels may also reflect reproductive activity or lack of it. Pregnancy testing was also carried out in Experiment 2, using the 100 blood samples collected. This was done to see if an index of pregnancy could be found that would be useful in determining the time to return to pregnancy, reflecting the effect of supplementation strategies. The reproductive and lactation status of the cattle sampled were then correlated with intake to see if there was any relationship with physiological state and supplement intake. The cattle were weighed at this time and their weights recorded along with those of 25 randomly selected calves. Both experiments were carried out in October and November each year, corresponding with the dry period when green growth was absent and pasture availability was minimal. At this time the cattle, of mixed age, were in 'forward store'

condition, *i.e.* about condition score 3. Early weaning was not practiced on the co-operator's property and bulls were in the herd throughout the year.

Experimental and analytical procedures used for the estimation of lithium in the sera and procedures associated obtaining the results for this thesis are listed later in this chapter. The hormone assay procedures are described in the following chapter.

12.1 Supplement manufacture and application



A commercial process at Olsson Industries produced the molasses-urea multi-nutritional block delivery system. The mixture was poured into cartons and left to set under temperature controlled conditions. The blocks used in Experiments 1 and 2 were formulated using the commercial formulation used to produce 'Dry Season 10%' blocks. The molasses content was decreased by 15 kg and LiCl was added at the rate of 10 kg/tonne of mix. Five L of fenbendazole (10% w/v) in water was also added, becauseLi has a peak concentration in sheep at 12 to 24 h after ingestion of lithium-labelled supplement (Suharyono et al. 1991) and thus might not provide an accurate estimate of supplement intake over a period of several

days. The fenbendazole has been considered to provide an estimate of supplement intake during the previous 48 h (Knox et al. 1992). The aim of including both markers was to obtain an estimate of the supplement intake profile for the 1-3 days before the blood sample was collected.

Unfortunately the analysis method for fenbendazole was not available from the CSIRO in time for this study. Supplementary blocks from the batch made for Experiment1 were used in Experiment 2. The unlabelled 'Dry Season' blocks that were placed around the bore No. 19 on 'Anthony Lagoon' were removed and replaced by the marker blocks. These were left for 3 days and on Day 4 the animals were mustered and bled. This protocol was followed for all samplings.

12.2 Experimental site and animals

The area involved was Bore 19 and the surrounding paddock at 'Anthony Lagoon' in the Northern Territory (see Section 2.2). The experimental animals were in a group of about 2000 mixed ages *Bos indicus* cows. The cattle had had access to commercially available molasses urea blocks (Olsson's Dry Season), for the previous two years and so had previous experience of exposure to the blocks.

The blocks used were commercially formulated, molasses blocks (Olsson's Dry Season) containing 10% urea. A formulation containing 10% urea was used because this is the formulation that had been fed previously. The formulation as stated on the blocks is given in Table 10.

Table 11 **Published formulation of dry season blocks**

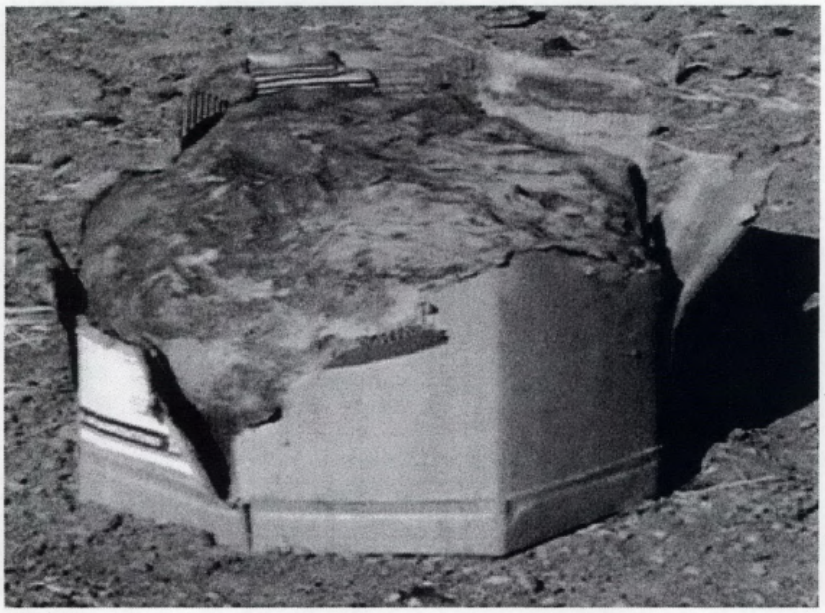
Min crude protein	30.1%
Molasses	46.7%
Urea	10.0%
Cottonseed meal	3.8%
Salt (NaCl)	Min 9.0%
Calcium (Ca)	Min 3.5%
Phosphorus (P)	Min 1.8%
Sulphur (S)	0.1%
Fluorine (f)	Max 0.1%
Iodine (I)	Min 0.038%
Copper (Cu)	Min 0.025%
Cobalt (Co)	Min 0.021%
Ferrous iron (Fe^{3+++})	Min 0.08%
Magnesium (mg)	3.1%

Owing to the extensive conditions of the paddocks at 'Anthony Lagoon', the watering place, 'Bore 19', was selected for the placement of blocks. Bore 19 was used because the cattle at the bore could be mustered into nearby yards with little stress, when it came time to take blood samples and for pregnancy testing. Mustering the cows was easily performed because the yards had watering troughs in them and when outside water was shut off, a high proportion of the cattle were camped in the yards and cattle were mustered in from a radius of 5 km from Bore 19. It was relatively simple to move those outside the yards to join those already inside.

The nearest bore to Bore 19 was 10 km away and the population of cattle using Bore 19 was relatively stable and did not move between bores. The cattle came to the water early in the morning and stayed around the bore for most of the day, moving out at sunset to feed during the night, returning again around daylight. However, about 20% of the cattle moved out during the daylight to feed.

One potential indicator of improved feed utilisation may be a decrease in the number of cattle camped at the bore during the day leading to a decrease in the time they spend camped around the water.

All labeled blocks were placed adjacent to the cattle camp areas within 0.5 km of the bore and they were available exclusively as the supplement for the three days prior to sampling. Ten blocks of 100 kg weight were placed at different sites and were continuously available throughout the 3 days. On the fourth day, the animals were mustered and animals were randomly selected for bleeding. Two hundred and eighty-nine cows were randomly selected for bleeding out of a total population of approximately 2,000 at the first sampling. After the animals were bled they were marked on the back with yellow marking paint. The labeled blocks were replaced with unlabelled blocks for 10 days and then labeled blocks were again put out for 3 days. Some of the cows marked with yellow paint were then again bled to obtain samples for lithium analysis.



12.3 Bleeding technique

The cattle were restrained in a cattle crush by a head bail and venous blood was obtained from the left side jugular vein using a 20 gauge 4cm double-ended needle and collected into a plain vacutainer (vacuum) tube. The blood was placed into an insulated cooler box at 15^0C at the crush side for the duration of the collecting. It was then placed in a cool room at 10^o C for 12 h. The next morning (12–14 h post bleed), tubes were centrifuged and the sera were decanted into plastic vials for freezing until lithium estimations were carried out. Serum urea nitrogen analyses were also carried out on the collected blood. The blood collection in the first experiment took place in September, nearing the end of the dry season. The second bleeding was carried in August of the following year (1996)

13 ANALYTICAL METHODS

13.1 Serum lithium – analysis by atomic absorption spectrophotometry

1. Analysis of lithium concentration in the blood.

2. Preparation of sample:

The sample was removed for the freezer and allowed to come to room temperature, it was then centrifuged at 3,000 ***g*** for 2 min and the serum removed from the sample and placed in a cuvette for analysis by Atomic Absorption spectrophotometry (Perkin-Elmer model 360). The method used was from 'Analytical Methods for Atomic Absorption Spectrophotometry ' published by Perkin- Elmer.

Since the cattle were not weighed, it was estimated that they were 350–400 kg and the intake was computed for both values.

13.2 Calculation of amount of block eaten

If a cow weighed 360 kg and had a conditions score between 2 and 4, it was assumed that the total body water content would be 67% of live weight or 234.2 kg of water. It was also assumed that lithium was distributed evenly between intra- and extra-cellular water.

If the concentration on analysis of lithium is 1.55 ug/ml of serum (1.551 mg / L):
Then in a 360 kg beast, there is 1.551 x 234.2 mg of Li

= 363.2 mg of Li

Now in LiCl, Li = 16.37% of the weight of LiCl;

Thus 363.2 mg of Li represents 2219 mg LiCl and as LiCl is 1% concentration in the block.
This then this represents (2218.7 X 100/1000) g of block eaten,
Thus amount ingested = 222 g of block

13.3 Serum urea nitrogen analysis

Serum urea nitrogen was determined using an Auto-Analyzer with a slight modification of the procedure of Marsh et al. (1965). The procedure is a modification of the carbamido-diacetyl reaction as applied to the determination of urea nitrogen. It is based on the direct reaction of urea and diacetyl monoxime (2,3,-Butanedione-2-oxime) in the presence of thiosemicarbazide under acid conditions. The presence of thiosemicarbazide intensifies the color of the reaction product and enables the determination to be run without the need of concentrated acid reagents. The colored product of the reaction is measured at 520mu in a flow cuvette with a 15 mm. light path.

13.4 PROGESTERONE ASSAY METHOD

The assay was carried out using the radio-immune Assay technique for milk and plasma or sera as described below.

13.4.1 Reagents

Phosphate buffer di-sodium hydrogen orthophosphate (MW 142)	4.33 g
Sodium dihydrogen orthophosphate (MW 156)	3.04 g
Sodium chloride	9.0g
Sodium azide	1.0 g
*Gelatin (add just before using buffer)	1 g/L

Add 900 ml 'nanpoure' water. Adjust pH to 7.0 with 5M NaOH
Make up to 1 L, store at 4°C.
Gelatin was weighed for the volume of buffer being used. It was heated to dissolve it and cooled to room temp before use. Left-over buffer was stored in a refrigerator at 4°C and discarded if not used again within two days.

13.4.2 Progesterone Standards (Sigma)

10 ug /ml in Ethanol. Weigh out 250ug of progesterone. Make to 25 ml in volumetric flask with ethanol (absolute)
1 ug.ml in Ethanol. Take 1ml of above standard and make to 10 ml with ethanol in volumetric flask.

On the day before making up standard curve take 150 µl of this standard into a 1.5 ml Eppendorf pipette.

Dry off ethanol under nitrogen gas

Add 150 µl of above buffer and vortex. Cap and leave ON at 4°C.

Vortex next day before use.

Working standard 25.6 ng/ml. Take 102.4 µl of 1µg/ml stock in buffer + 3,898 µl of buffer.Vortex.

Serially dilute in 2 ml of buffer to give 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2 and 0.1 ng/ml (0 ng/ml buffer only)

13.4.3 Progesterone Antiserum.

1. Antiserum. The progesterone antiserum was produced from sheep by the immunisation of the sheep against 4-Pregen-11Alpha-ol-3,20 - dione-hemisuccinyl-HAS. (C.S.I.R.O. Prospect, NSW.). The anti-serum was tested against other relevant steroids and found to have the following cross reactivities:

11 B-hydroxy progesterone	17.5%
deoxycorticosterone	9.5%
5 B -pregene-3,20-dione	2.1%
3 B -pregene-3,20-dione	2.5%
17a-hydroxy-progesterone	<0.1%

Cholesterol	<0.1%
Cortisol	<0.1%
Pregnenolone	<0.1%
Testosterone	<0.1%
Oestradiol-17 B	<0.1%

The antiserum was added to the assays at an initial dilution of 1: 6000 and was stored in undiluted aliquots in -20°C freezer.

One aliquot was diluted to 1/1000 by adding the required volume of buffer respectively, mixing well (e.g. 5 µl add 5 ml).

A volume of 1/1000 solution was taken and diluted to a working dilution (1/6000) by calculating

1000/6000 x (vol of a/s req) = (vol of 1/1000 to dilute to vol of a/serum required), allowing 5 ml more than required. Stir on stirrer in cold room while adding to tubes.

13.4.4 I-125 Tracer (Amersham IM 140)

5µl aliquot of tracer was taken in a fume hood and dred off MeOH under nitrogen gas. The cpm was checked and total volume of tracer required to give approx. 10,000 cpm per 100 µl was calculated. Take volume of tracer was dried off as above and diluted with phosphate buffer and stirred in a cold room. The final cpm was checked before adding the solution to all tubes.

13.4.5 Dextran coated charcoal

Prepare volume required by calculating:
(no. of tubes) x (250 µl) + 25 ml extra = vol required

Dextran T 70	100mg
Activated Charcoal	1g (Heat ON in oven)*
Phosphate buffer	100 ml

Dissolve Dextran in buffer. Add charcoal. Stir at least 1 hour or ON at 4°C before use.

*Activated carbon, Norit A<100mesh. American Norit Company Co,Inc.

13.4.6 Assay Setup Procedure

Add the following volumes to the appropriate glass tubes. (size 12 x 75 mm)

BLANK TUBES ... 100 µl OVK milk (overectomised cows milk)

300 µl buffer (in triplicate)
STANDARD TUBES... 100 µl of appropriate std.
200 µl buffer
100 µl OVX (in triplicate)
SAMPLE TUBES... 100 µl of sample
300 µl of buffer (in duplicate)
QC TUBES.... 100 µl of QC 4,5, or 6 x 300 µl buffer tubes can be covered and placed at -20°C until extraction if necessary.

13.4.7 Extraction Procedure

1. Add 2 ml of hexane to each of the above tubes
2. Cap and shake tubes for 10 min at -20°C
3. Remove caps and freeze aqueous phase by standing tubes in a mixture of dry ice and ethanol for 1-2 min or liquid nitrogen for several seconds. In a fume hood. Rotate in groups of four tubes, *i.e.* one group being poured off while 2nd group freezing.
4. Pour off hexane into correspondingly labeled glass tubes and dry off in fume hood for at least 2 days to evaporate hexane. Tubes can be covered and stored at -20°C at this stage prior to being assayed as below.

13.4.8 RIA Procedure

DAY 1

1. Add 100 µl of buffer to each dried off tubes. Vortex, cover and leave ON at 4°C.

DAY 2

2. Prepare a 1/6000 a/s dilution and keep on ice.
3. Prepare a tracer dilution to about 10,000 cpm per 100 µl and keep on dry ice. Then with tubes left in the cold room:
4. Add 100µl of buffer to blank tubes only.
5. Add 100 µl of a/s dilution to standard, Sample and QC tubes. Stand for 15 min including time for addition of a/s.
6. Add 100 µl of tracer dilution to **all** of the above tubes plus 3 tubes containing Tracer only.
7. Vortex and leave ON at 4°C for 2 days (approx. 44-46 h)

DAY 3

7. Prepare required volume of charcoal / dextran mixture, cover and stir ON at 4°C.

DAY 4

9. Add 250 ul of charcoal soln. to all tubes except Tracer tubes within 2 min. (The maximum number of tubes per spin is 160)
10. Vortex and incubate tubes for the remainder of 20 min at 4°C
11. Spin at 500 ***g*** for 15 min at 4°c
12. Aspirate and count using protocol 85 or 88 on gamma counter. Keep tubes on ice after spinning until they are aspirated so pellet stays firm.

Sensitivity 0.7ng/ml

Between assay CV	1.2 ng/ml	CV% 18.5
2.6 ng/ml		10.30
5.4 ng/ml		16.90
Intra assay CV	0.5 ng/ml	14.74
	1.9 ng/ml	15.22
	5.1 ng/ml	11.60

Specificity Cross reactivity with other steroids:

11-beta hydroxy-progesterone	17.5%
deoxycorticosterone	9.5%
5-beta-pregnene-3,20-dione	2.1%
3-beta-pregnene-3, 20-dione	2.5%
17-alpha-hydroxy-progesterone	<0.1%
cholesterol	<0.1%
pregnenolone	<0.1%
testosterone	<0.1%
oestradiol-17beta	<0.1%

Reference: Martin et al.(1983)

13.5 OESTRADIOL ASSAY METHOD

Immulite oestradiol is a solid-phase, chemiluminescent immunoassay. The solid phase, a polystyrene bead enclosed within a DPC Immulite Test Unit is coated with a polyclonal rabbit antibody specific for 17B -Oestradiol. The test sample and the oestradiol reagent (alkaline phosphatase-conjugated oestradiol) are simultaneously introduced into the Test Unit and incubated for approximately 60 min at 37^0C with intermittent agitation. Unbound enzyme conjugate is removed by centrifugal wash. Chemi-luminescent substrate is added to the test unit and incubated for a further 10 min.

The chemi-luminescent substrate undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of this intermediate results in the sustained emission of light that provides a window for multiple readings. The

bound complex photon output as measured by luminometer is inversely proportional to the concentration of 17B-Oestradiol. Oestradiol is reported in pmol/L (Mitchell, pers.com).

14 EXPERIMENTAL METHODS

14.1 Experiment 1 (part 1)

In Experiment 1 (part 1), 289 *Bos Indicus* cows were sampled as per Section 10.3 and marked with marking paint on the back. No cows were weighed but they were estimated by the manager to weigh between 350 kg and 400 kg.

14.2 Experiment 1 (part 2)

Cattle were re-sampled as per part 1, 10 days later. One hundred and fifty-five cows randomly selected, of which 81 cows had been previously sampled and 77 cows not previously sampled were bled.

14.3 Experiment 2 (part 1)

Blood sampling took place the following year, as with Experiment 1, approximately 11 months later, at the same bore and using the same population of cattle as the previous year and samples were taken randomly from 100 cows. Marker blocks were again put out for three days and then the cattle were bled on the fourth day.

Blood was taken and handled the same way as in Experiment 1 and lithium concentration was again estimated as for Experiment 1. However individual cattle weights were recorded and used in the calculations of block intake.

14.4 Experiment 2 (part 2)

The blood was also analysed for serum progesterone levels and animals with low progesterone concentrations (< 1 ng) were also examined for 17-Oestradiol concentration.

14.5 Experiment 2 (part 3)

The cattle were weighed and manually pregnancy tested and the results recorded as either early pregnant (minimum 16 weeks), middle pregnant (4-7 months) and late pregnant (more than 7months) and the mammary glands examined for the presence or absence of lactation. Twenty-five calves were also randomly selected and weighed.

Unfortunately the cows and calves were separated in the drafting process, so it was impossible to determine the cow/calf pairs. It was not possible; therefore to use calf size as a measure of time since calving in the cows when they were pregnancy tested. If this could have been done, it would have been useful when analysing the pregnancy test results.

The blood was collected from the left jugular vein as described in Section 10.3.

14.5.1 Statistical proceedures

14.5.2 Intake and physiological status

The intake and physiological status were compared using General Linear Model (GLM) analysis. Analysis of variance, type iii sums of squares, residual analysis, multiple comparisons, residual analysis and tables of least squares means were used.

14.5.3 Intake compared at three stages of pregnancy

A one-way analysis of variance, table of means, multiple range test and variance check were used to determine the significance of stage of pregnancy to block intake.

14.5.4 Serum urea nitrogen

The estimations involving serum urea N concentrations between samplings were analysed using the 'two sample t-test ', assuming unequal variances.

15 RESULTS

15.1 Experiment 1 (parts 1 & 2) – estimation of block intake

From the results above, the intake of block by individual cattle was calculated. Detectable concentrations of lithium were found in serum of 93 % of the herd on 19/9/95 and in 95 % on 3/11/95 indicating that at least small amounts of block had been ingested by the majority of animals in the herd. The mean intake of supplement estimated during part 1 and part 2 of Experiment 1 was 147 (SE 6.7) g/animal per d. The range and patterns of intake of cattle from Experiment 1 are given in Fig. 23.

Figure 23 Distribution of intake among individual cattle in the herd at both bleedings. Animals with similar intakes are grouped into categories and numbers in categories are expressed as a percentage of the cattle sampled. (Note that x-axis is non-linear).

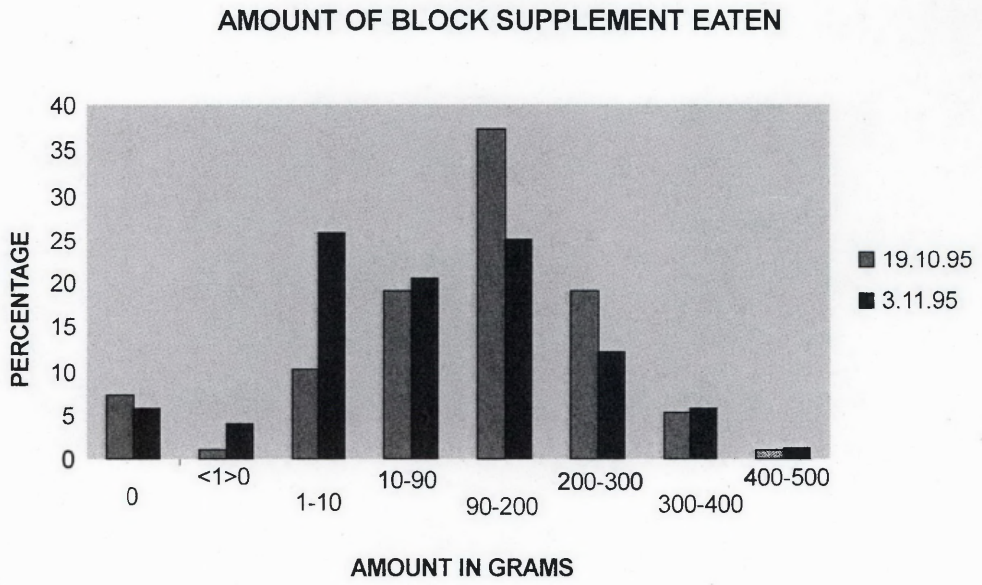
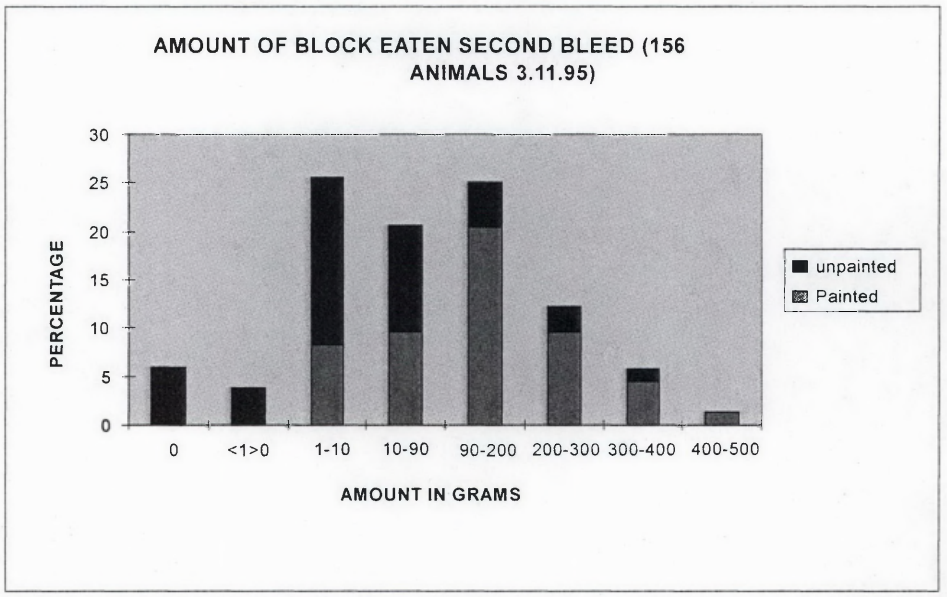


Figure 24 Distribution of block intake among individual cattle in the herd in November 1995. Animals with similar intakes are grouped into categories and numbers in categories are expressed as a percentage of the cattle sampled. (Note that x-axis is non-linear). A group of 289 cattle were sampled at random on 19 October, 1995 and marked ('first sample'). On 3 November 1995, 158 cattle were sampled at random from the herd. Some of these cattle were marked and were therefore designated 're-sampled'.



The percentage found to be eating detectable levels of supplement in part 1 was 93% and in part 2 was 95%.

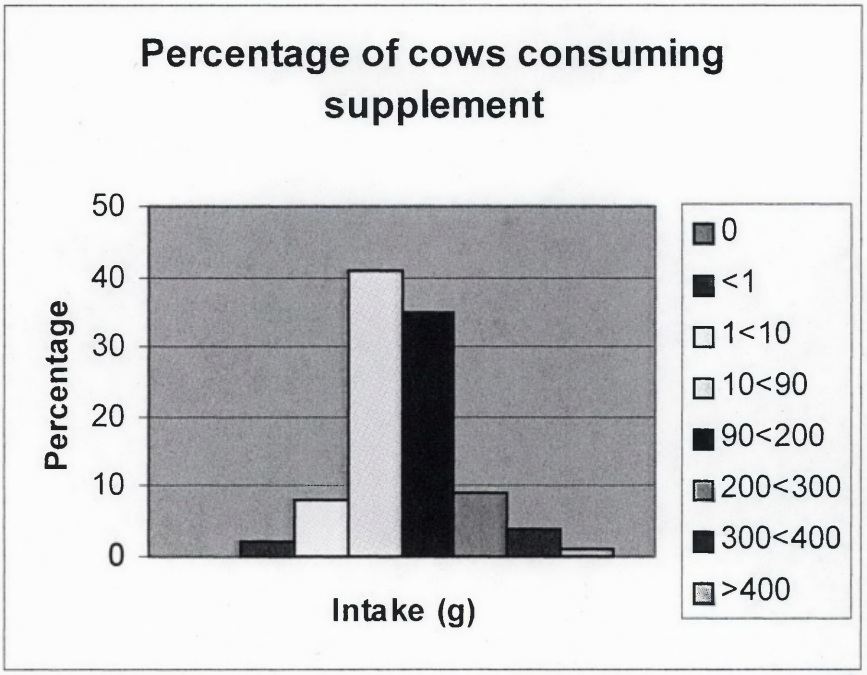
Part 2. Cattle were randomly bred as in Part1 fourteen days later. Cattle that had been bled previously were identifiable and were not purposely selected but if they came into the crush they could be noted. In this sampling 158 animals were bled with 81 being re-bleeds and 77 being previously unsampled. In figure 25 the total distribution is graphed but further broken down to the percentage of cows either re-bled or not bled in part1

15.2 Experiment 2: distribution of cattle eating block

Analysis of blood serum taken from cattle in the herd on Bore 19 'Anthony Lagoon' during November, 1996 after exposure to Li-labeled blocks in November, 1996 indicated that 90 %

of animals were ingesting detectable amounts of the block. Mean intake was 109g/head/d, SE 10 g/head/d. The distribution of block intakes of is given in Fig. 29.

Figure 25 Experiment 2. Distribution of intake (g) among individual cattle in the herd in November, 1996



16 INTAKE ACCORDING TO PHYSIOLOGICAL STATE

Mean block intakes did not differ (P>0.05) between pregnant or non-pregnant groups, or between lactating and non-lactating cattle. These intake estimates are given in Table 12.

Table 12 **Experiment 2. Mean block intake of cattle according to physiological breeding status, i.e. pregnant or non-pregnant and either lactating (wet) or non-lactating (dry)**

	Pregnant		Non-Pregnant	
	Wet	Dry	Wet	Dry
Mean block intake (g)	98	102	133	98
Overall mean (g)	100		116	

16.1 Experiment 2 Intake (g) according to stage of pregnancy

Cows returning a positive pregnancy test in November, 1996 were classified as being in 'early-pregnancy' (<16 weeks), 'mid-pregnancy' (>4<16 mo) or 'late-pregnancy' (>7 mo). The estimated mean intakes of block by cows in these groups did not differ significantly ($p>0.05$) and these are given in Table 13.

Table 13 **Mean block intake of cows in November, 1996 classified as being in 'early-pregnancy', 'mid-pregnancy' or 'late-pregnancy'**

	Early	Mid	Late
Mean intake (g)	76	115	138

16.2 Serum urea nitrogen

Urea-N concentrations in serum samples from Experiment 1 taken 10 days apart (in part 1 and part 2) did not differ significantly ($P>0.05$) and the overall mean was 92.5 mgN/L.

16.3 Pregnancy status

In November 1996, 54 of the 92 female animals' pregnancy tested were pregnant (59 % of those tested) and of those pregnant, 16 % were still lactating. Of the 38 females that were found to be non-pregnant, 39 % were lactating and 61 % were dry.

As indicated above, the 54 cows returning a positive pregnancy test were classified as being in 'early-pregnancy' (<16wks), 'mid-pregnancy'(>16 wks<7 mths) or 'late-pregnancy'(>7mths). In the early pregnant animals, 7 (13 % of the pregnant group) were lactating while 9 (17 %) were not lactating. Of the cows in mid pregnancy, 1 (1.5 %) were still lactating whilst 16 (30%) were non-lactating. In cows in late pregnancy, 1 (1.5 %) was lactating and 20 (37 %) were dry.

16.4 Progesterone level: pregnancy and oestrus

The 92 animals tested were divided into two sub-groups. In the sub-group that had progesterone concentrations greater than 31.8 nmol/L, 40 % were found to be pregnant and 23 % were non-pregnant. In the sub-group with progesterone concentrations below 31.8 nmol/L, 19 % of the 92 animals were pregnant and 19 % were non-pregnant (Table 14). The percentages of cows within these sub-groups that were lactating or dry are also given in Table 14.

Table 14 **Experiment 1: Distribution of pregnancy and lactation with reference to progesterone levels above and below 31.8 nmol/L**

Progesterone (nmol/L)	**Pregnant**		**Non-Pregnant**	
	Wet	Dry	Wet	Dry
>31.8	2	38	10	13
<31.8	8	11	8	11

16.5 17β-Oesradiol assay (Expt 2)

Oestradiol estimations were only carried out on samples from 31 cows that had progesterone concentrations <12.1 nmol/L. The distribution of results is given in Table 15.

Table 15 Oestradiol estimations were only carried out on samples with progesterone <12.1 nmol/L

Oestradiol conc (pmol/L)	0<13	13-17	>17
No. of cows	0	11	21

16.6 Cow weights

In Experiment 2, the average cow weight was 413 kg ± 60 kg.

16.7 Calf weights

In November 1996, 25 calves were selected at random and weighed to give an indication of age spread of calves and the results are given in Table 16.

Table 16 Live weights of 25 randomly sampled calves in November 1996

	75-100 kg	101-200 kg	201-300 kg
Number	4/25	17/25	4/25
%	16	68	16

Formula (Anon) for estimating calf age from liveweight

Calf weight = 25 kg (birth weight) + 25 kg /month of age.

Therefore a 100 kg calf is about 3 months of age. Similarly, a 200 kg calf is about 7 mo of age and a 300 kg calf is about 11 mo of age. Thus, the range of ages of calves weighed was 2–8 mo approx.

17 GENERAL DISCUSSION

The aim of the research work was to establish the intake distribution of dry feed block supplement by cattle in the Northern Territory. The use of LiCl as the labeling agent contained in the supplement, with detection of lithium in the cattle sera, proved an acceptable method of achieving supplement intake profiles. The first two intake experiments, backed up by the results of the third intake experiment gave a useful estimate of the distribution of intakes for the dry season supplement on this property.

The question of whether supplementation is an economical alternative has not been addressed, nor was it proposed that this research would answer that question. The aim of the research was to examine tools that can now be applied to critically answer the question of economics. The hormone profiles of progesterone and oestradiol may be useful in measuring the immediate response to supplementation, even though progesterone is subject to pulsatile fluctuations of about ± 9.5 nmol/ml. (Procknor et al. 1986). The results of these profiles are independent of the fertility of the bulls in the herd, unlike pregnancy rates or branding rates. It was initially envisaged that a single blood test, using both these hormones could identify those animals in anoestrus and oestrus. However, it would take two samplings 10 days apart, *viz.* the first one to screen for low progesterone, low oestrogen animals, then a second sample 10 days later to see if the hormone levels had changed. The problem with this approach is that instead of a single sample, two samplings would be needed. Moreover, the test is still a laboratory test, but the development of a rapid crush side agglutination test would make it cheaper and easier to do. One further problem with this approach is that it has been implied that the animals not breeding in the northern beef herds are in anoestrus due to the ovarian inactivity. The results of the present study did not indicate this to be the case, as low progesterone and low oestradiol serum concentrations did not occur simultaneously, indicating that the lactational anoestrus was caused by another mechanism. Ovarian cysts, high oestradiol

levels/low progesterone levels, while not specifically looked for in this study, did not appear evident when the cattle with low progesterone concentrations were examined for oestradiol levels. The finding of high progesterone levels in cattle that were non-pregnant was interesting and it is worth repeating the experiment to confirm this finding. If the finding is repeatable, then further investigation will be needed to find the mechanism of a possibly high progesterone-induced anoestrus.

The use of pregnancy testing and defining the ratios of ages of conceptus with respect to pregnancy and lactation status is an easy, economical method of judging the immediate effects of supplements on reproductive activity, assuming the cattle are mated with fertile bulls. However, if it was practical, the hormone testing technique would be useful in checking for fertility in bulls in a herd in extensive situations if it is not possible to check the semen quality of the bulls. If a large number of cows are cycling normally, but the pregnancy rate is low, then bull fertility is suspect.

The effect of decreasing the inter-calving interval and moving to seasonal mating has been discussed above and it relationship to the effect of maximising microbial growth in the rumen by providing supplements containing the elements lacking in the pasture has been explored earlier in this thesis.

17.1 Intake estimations

The results obtained from both Experiments 1 (part I) and (part II) and Experiment 2, show that the LiCl protocol is a valid way of finding out intakes of molasses-based supplements in extensive areas. When this study was initially set up, two markers were included, LiCl and Fenbendazole. The LiCl will detect intake within the last 12 – 24 h, whereas the fenbendazole would probably give a reasonable estimate of intake during the previous 48 h (M. Knox, pers. com). Unfortunately, because the fenbendazole assay became unavailable in CSIRO, the assay was not performed. However, the aim of the project was largely attained using the LiCl only: an intake pattern in the herd was determined from the first two blood collections of Experiment 1 and the knowledge thus attained was verified by the second experiment, where the animals true weights were also known. Although tritiated water has been used to assess

intake of nutrients (Nolan et al. 1975) and has the advantage of a longer half-life, the use of radioactivity was considered unacceptable for this study (JV Nolan, pers com.).

Knox et al. (1992) commented on the problem of low and high intakes of Li in the sheep eating a similar molasses formulation of block without urea. He found that with the continued high ingestion rate of blocks, a higher ratio of mean block consumption to plasma Li level occurred than when consumption was low. That is, the plasma Li levels were relatively higher in animals consuming lower amounts of the block than animals consuming higher levels of the block. He attributed this to increased excretion/metabolism of Liwhen high levels of other salts (Na, K) were present from the blocks in higher intakes. However, as the bore water at 'Anthony lagoon' contained high magnesium concentrations, any effect of the magnesium would probably have occurred over all intakes and would not have affected the relative rates. The research, however, has demonstrated the following pattern of consumption:

1. The percentage of animals not eating the block in a 24–72 hour period varied from 5.8 % in September to 3% in November.

2. A large percentage (92 %) of animals ingested between 1g and 500 g of the block in September.

3. In November, 2-3 % of animals ate none of the block.

4. In November, 96-97% were eating 1–500 g of block.

The slight shift in the determined intake pattern from 1995 to 1996 may not be significant and may have been a response to the effect of a further reduction in the nutritional value pasture, or to a difference in the weather pattern in 1996. As sampling took place a month later in 1996 than in 1995, some of the pregnant cattle would have been entering their third trimester and would have been closer to calving.

In the second part of Experiment 1, all the animals that were re-sampled on a random basis were found to be eating the block. The intakes varied, *i.e.* from 1-10 g (15.5% of those sampled), 10–90 g (17.9 %) and greater than 90 g (66.6 %). There was a possibility of carry-over of Li from Experiment 1 (part 1). However, if the half-life of Li in the cattle was from

12-24 h (S. McLennan, pers. com.), the animals would have had to ingest about 2 kg of block in part 1 to have a 'carry over' effect amounting to 2 g in the estimate of intake in part 2. The distribution of intake in Experiment 1 (part 2) was not the same as that found in Experiment 1 (part 1). This may indicate, as Knox et al. (1992) found, that eating patterns differed between days. Even though their experiment was with penned sheep, the animals showed a similar intake pattern to that found with the cattle on 'Anthony Lagoon'. Furthermore, over time these workers found that, although 90 % of the flock were eating appreciable amounts of block each day, on successive days, the 90 % comprised different animals. It would be reasonable to conclude that a similar situation occurred with the cattle. Li was not detectable in analyses of the bore water on 'Anthony Lagoon' and the results in cattle in Experiment 1 that showed that 5–8% of animals had no lithium in their blood confirmed that there was no natural background source of Li in cattle in this region.

The use of 100 µl of sera for the analysis of Li concentration was found to be only just sufficient when Li was included in the supplement at 1 %. In future experiments, LiCl added at 2 % to the supplement would overcome this problem. At these concentrations, palatability should not be affected.

The absolute amounts of nitrogen, sulphur and phosphorus that are 'theoretically' needed have not been commented upon. This is because the amounts needed in this situation by **each** individual animal will be affected critically by the quality of the pasture, the age of the animal, the animal's previous breeding history and itsbreeding status. Because there are so many variables, until the attainment of continuous high levels of fertility with a short time to return to pregnancy, regardless of the climatic conditions, such figures are not meaningful for this area. In this study it was found that 82% of animals in the first sampling had an intake of urea between 1 and 40 g. Twelve months later 84% of animals sampled had an intake of between 1 and 40 g of urea. There is also sulphur and minerals above those in the analysis of the block contained in the molasses in the block.

The method of calculation of intake of the block differed from that used by Suharyono et al. (1991) because, in his experiment, he fed a known number of animals a known amount of feed (pellets) and calculated the intake from the amount of Li in the left-over feed. In the experiment at 'Anthony Lagoon', intake was not known, the absolute number of animals was

not known and the amount of leftover block was not weighed. The physiological basis of calculation of Li intake from the sera of those animals bled was the only method available.

The number of non-eaters of blocks at 'Anthony Lagoon' was lower than values published by some other authors (e.g. Nolan et al.1995). This may be due to the animals being more familiar with the blocks as they had had exposure to them continuously for the last two years. Nolan and Hinch (1997) reviewed aspects of animal behavior and intake and showed that reasons leading to acceptance or avoidance of supplements are complex but include factors such as previous exposure and the positive or negative metabolic effects of the supplement.

17.2 Serum urea nitrogen

Serum urea nitrogen (SUN) reflects the rate of synthesis of urea from ammonia or amino groups from amino acid catabolism or ammonia from intestinal microflora. Urea is excreted by the kidneys and, in healthy ruminants, has been used as a reflection of the amount of ruminally degraded proteins in the diet.

One suggestion is that high levels of urea nitrogen in healthy ruminants can contribute to infertility. Fergusson et al. (1986) suggested that a serum urea nitrogen concentration of greater than 200 mg/L was suspect in healthy cattle on pasture. It is suggested that dairy cattle grazing lush pastures are affected by high blood ammonia levels arising from rapid ruminal degradation of plant protein causing an adverse effect on reproduction. Clinically, dehydration and renal failure cause a rise in SUN, whilst liver failure or diets critically low in protein cause a decrease in SUN. The SUN levels shown in these two experiments, (9.25 ± 2.75), were within the normal range and there was no significant difference between experiments. There was no evidence of dehydration caused by holding the animals in the yards, which was to be expected, as ample water was available.

17.3 Supplement intake in pregnant compared with non pregnant intake

The analysis of intake in pregnant *v.* non-pregnant animals gave no significant difference (100 *v.* 116 g), which was unexpected. The reason for this lack of significance could be due to the

cattle in the herd being in various stages of pregnancy, lactation and non-pregnancy and lactation. Cows in the last trimester need better nutrition than either non-pregnant animals or cows in the first or second trimester of pregnancy and cattle in early lactation need more nutrients than those non-pregnant and non-lactating. The trend of increasing nutritional needs was suggested by the increasing block intake through pregnancy although the increase was not statistically significant. The reason why intake was not found to differ significantly between groups is intriguing and demonstrates why producers have had confusion with the acceptance and application of supplements extensively in dry areas. Perhaps a longer continual period of assessment is needed to clarify this finding. When assessing the benefit of supplementation using the accepted methods of weight gain and branding percentage, these parameters do not reflect the total long-term benefits derived from the use of supplements. Further, the amount of supplementation that should be provided has never been examined to determine definitively the correct level to be used. Depending on the climatic conditions in most years all of the animals suffer starvation. Whether it is economical to provide supplementation and feed sufficient to alleviate this annual deficiency is the real question that has still to be answered.

Experiments during which live weight gain (or even decreased weight loss) is determined over short periods are unlikely to demonstrate the compounding benefits of live weight on reproduction and lactation in females. In the breeding, lactating female, small changes in weight can have significant compounding effects on fertility and therefore, the economics of the enterprise over a long period.

Retrospective parameters of response, such as branding rates are of little or no use when the approach to providing supplement has to be prospective to affect reproduction. Therefore, to determine definitively the amount of supplement to be used, differential pregnancy data should be applied when using various supplements supplying urea, by-pass protein and minerals.

Initially, an experiment should be carried out that would involve providing unlimited amounts of supplements to small groups of cattle on adequate dry matter, (the cost of the supplements would be unimportant at this stage of the investigation). This experiment would indicate the level of supplement needed to make a significant difference to fertility. Then, whether or not

the supplementation is economic can be assessed. The experiment should be a long-term one lasting for at least five years so that the compounding effects of supplementation in the females can become evident. Intake of individuals should be estimated repeatedly during the study to ultimately resolve the confusing effect of large differences in apparent daily variation in intake. Wide differences in intake may not be detrimental at all and daily intakes may not be consistent in individuals, but at this time the causes and extent of individual variation is uncertain. In the extensive areas in Northern Australia, because individual records are non existent, it is difficult to know how long it has been since a particular cow has weaned her calf. However, this is important information as, immediately after weaning has taken place, a cow that has been lactating for 6-8 months will be more nutritionally compromised than a cow that is not lactating and in the first trimester of pregnancy. In fact the cow has become pregnant because she is not nutritionally compromised.

The intake of supplement by animals is more complicated than the supposition that those that eat the most, need the most. A continuous experiment to map individual intakes over an extended period is needed to try to find out which animals need supplement and establish the relationship between need and intake.

The spread of pregnancies also shows that those cows up to three months (approx.) pregnant made up 17% of the herd. These cows would have become pregnant in about late July or early August. Nineteen percent of the herd were in mid term and so became pregnant in about May-June, whilst 2% were mid to late pregnant (April conception) and late pregnant animals were 21% of the group studied. These animals became pregnant in late February and March at the beneficial part of the grass cycle. These results can give an indication as to which animals will be calving at the right time of the year to be in tune with the increase in pasture (see pasture cycle for Anthony Lagoon). The cattle in late pregnancy and 12 weeks post calving, will benefit from supplements that supply not only urea and phosphorus but also by-pass protein. In contrast, those cows in early pregnancy will only need the urea supplements and phosphorus and feeding these cattle extra by-pass protein will be economically wasteful.

The finding that the average intake increased as pregnancy progressed was expected. Within the groups, however, great variation in intake on an individual basis occurred and again no significant difference in intake was found between stages of gestation. The cattle that are

lactating will also benefit from more by-pass protein. It can be seen that if possible, a shift to seasonal calving would be advantageous, not only for reproductive performance and calving synchronisation, but it would also reduce supplement costs by supplying supplements attuned to pasture cycle and the cow's physiological needs.

17.4 Pregnancy and progesterone

The hormone progesterone was originally assayed to identify cows with low progesterone levels. The oestradiol levels in these cows could then be determined to find those with inactive ovaries and so identify those not cycling (low progesterone, low oestradiol). The inference has been that the cows shut down ovarian cyclic activity when nutrition is not adequate. In the present experiment, however, a different scenario was evident. From Table 14 when the results were tabulated according to progesterone profile levels and pregnancy status, the most important result is in the non-pregnant cattle. Here 10% of the lactating non-pregnant cattle and 13% of, non-lactating, non-pregnant cattle have high range progesterone levels, (*i.e.* > 31.8 nmol/L). If a lower range of progesterone concentrations of 12.7-31.8 nmol/L is included, then 27% of these cattle had progesterone levels greater than 13 nmol/L. This represented 66% of the non-pregnant cattle.

These results have to be interpreted carefully due to the pulsatile nature of progesterone secretion (Procknor et al. 1986). Fitzpatrick (1999, pers com) also found similar.high blood progesterone concentrations in non-pregnant, Brahman cattle. He repeated the experiment using ovarectomised cattle and found a similar pattern of progesterone concentrations and concluded that the progesterone was increased due to adrenal stimulation.

However, an alternative explanation of these findings is that it is a survival mechanism in *Bos indicus* cattle that operates when nutrient levels are not sufficient for the establishment of pregnancy. Possibly, after a silent heat, post-partum cows of this breed may continue to secrete progesterone, perhaps by retaining the corpus luteum and delaying the return to oestrus until the nutritional status of the cow improves.When nutritional conditions improve, the corpus luteum may regress and ovarian cyclicity may then be re-established so that the animal is set up to breed again. This scenario would be worth examining further, to ascertain

whether retention of a corpus luteum following pregnancy is a major reason for a prolonged return to service after calving.

Jordon and Swanson (1979) reported that dairy cows on lower protein diets (12% CP) had lower basal serum LH levels and higher progesterone levels. These cows were less responsive to 100 μg of GnRH (gonadotrophic hormone) to bring them into season. The action of oxytocin on the uterus is inhibited by progesterone and stimulated by oestrogen. Thus high levels of progesterone will inhibit the excretion of prostaglandin from the uterus stopping the involution of the corpus luteum. Prolactin, the hormone responsible for milk secretion, was found in rodents to maintain the corpus luteum and in humans, hypoglycemia and strenuous exercise increase both prolactin and growth hormone. Ganong (1983) reports that 15%-20% of women with secondary amenorrhoea have elevated prolactin levels and, when prolactin secretion is reduced, normal menstrual cycles and fertility returns.

Whether the same scenario occurs in cattle is unknown but is worth investigating. It would appear that once cattle are pregnant and calve, lactation would take precedence over conception (as such a mechanism would ensure a better survival of the species). Therefore, by ensuring that most calves born will be nourished to almost breeding age in the herd, the breeding nucleus will be retained. The cost to the herd is loss of yearly breeding. Thus raising a viable calf every 2-3 years would be a better ecological option than breeding every year and loosing 3 out of 4 calves at a young age. This may be a manifestation of a reproductive adaptation of this breed to low protein feed supply.

Hormone assays, although desirable, are almost impossible to carry out on farm at this time. However, the results of pregnancy testing, to ascertain the total pregnancy percentage in a herd, along with the ratio of wet pregnant cattle to dry pregnant cattle, can be used as a very good indicator of the inter-calving interval. The higher the ratio, the sooner the cows are returning to pregnancy. This ratio would then provide another effective measure of the nutrient / ovarian interaction. A low ratio means that nutrition (including that from a supplement) has been of marginal use where a higher or better succeeding ratio indicates that the nutrition /supplement are more effective.

17.5 Pregnancy and oestradiol

Only two cows had progesterone levels less than 3 nmol/ml coupled with low oestradiol levels (<3.5 pg/ml) but their pregnancy status was unknown. Of the cattle of known status, inactive ovaries did not appear to occur. It would seem that the condition of anoestrus caused by non-functioning ovaries did not occur in the animals tested in this herd, perhaps because of the effect of the supplementation, or perhaps because it does not occur in *Bos indicus* cattle to the extent it occurs with dairy breeds.

17.6 Calf live weights

Unfortunately, during the drafting of cows into the crush, the cows / calves combinations became separated and it became impossible to ascertain which calf belonged to which cow. This was unfortunate because, if the relationship between the calves and cows bled had been preserved, the calves would have provided a living record of time from last calving. If the calf is weighed and the cow pregnancy tested the intercalving interval can be ascertained reasonably accurately. For example, from Table 15##, it can be seen that, of the 25 calves weighed, 16 % were between 1 and 3 months, 68 % were between 3 and 5 months and 16 % were between 5 and 7 months old. If the calves could have been traced to their mothers and the cows pregnancy tested, a more accurate assessment of how long the cows took to return to pregnancy could have been made.

With this method, it would be possible to create an approximate 'return to pregnancy' interval for the cow. This would be based on the of recording calf weight, with reference to her gestation length. For example, the number of cows 16 weeks pregnant/ 200 kg, or less, calf weight. This would mean that the cows would have become pregnant about 90 days after the calves were born. For example, if the cow was pregnancy tested and found to be approximately 16 weeks in calf and her calf weighed 200 kg, (approximate calf's age of 7 months), then her inter-calving interval would be approximately 3 months. This scale could then be used to compare with other cattle in the herd. Another practically appropriate time to pregnancy test the cow is at weaning and if the cow and calf unit cannot be identified the lactation /pregnancy status of the cows can be tabulated.

18 CONCLUSIONS

Aspects of the reactions in the rumen, involving bacteria, protozoa and fungi have been considered. This consideration was in relation to cattle grazing dry pastures containing 5-6 MJ/kg DM of metabolisable energy and crude protein values below 60 g/ kg DM. Mechanisms for improving digestibility by maximising microbial protein growth and their relevance to the formulations of supplements were discussed. The effect of increased nutrition in cattle eating dry pastures for the majority of their breeding cycle and possible ways of improving fertility using supplements were also discussed but the mechanisms by which responses have usually been measured were questioned.

The effect of improved conception rates and by implication calving rates, improved weaner survival and the effect on growth rates from improved lactation were also examined. The effect of increased mortality in females is often an unrecognised source of economic loss. The resultant complications of the pregnant state in cattle with low body weights have also been discussed. Supplementation of protein and minerals at strategic times would improve not only productivity, but also the economical use of supplementary formulations aligned to the animals physiological state would be more efficient and decrease the total cost of supplementation. At the present time, however, because of the lack of critical immediate appraisal mechanisms relating to the effect of supplementation, the desired intake and the combinations of constituents that make up the supplements have not been established in the field. Therefore the true economic benefits of the supplements have still not been definitively defined. The long-term effects on reproduction and the multiplying effect of these benefits, have also been discussed in Section 6.1.1.2.1.

The technique of seasonal breeding and the feeding of supplements better attuned to the seasonal deficiencies of the pasture and the animals physiological state could be practiced. This would lead to decreased wastage of supplement. With the identification of a marker for supplement intake and blood derived oestrus cycle hormone levels, a means of assessing the effectiveness of supplementation at the ovarian level has been suggested. If the hormone assay method could be refined to a crush side agglutination test, it could then give the producer the freedom to change to a better formulation at a time that could improve the

occurrence of oestrus and ovulation and the outcome of fertilization and pregnancy maintenance.

However due to the limitations with on site hormone estimations, the application of pregnancy testing, with the differential parameters described before, will provide more information about the breeding state and nutritional status of the herd. If these indexes are combined with a seasonal breeding program then more accurate information is available to the producer about the effectiveness of the supplementation.

The producer could then economically decide if the cost of the expected pregnancies is economically rational. Thus supplements could be changed prospectively depending on the climatic and pasture conditions rather than retrospectively at present. The following years production could therefore be influenced by intervention, at a time that will make a difference to the reproductive rates in females. A strategy for improving female survivability based on seasonal breeding and culling to try to restrict the intercalving interval to 12 months has also been explained.

The results of the investigations involving the hormones progesterone and oestradiol were interesting, but subject to limitations. The delay in returning to oestrus in a number of cattle may be due to the retention of corpora lutea and not to multiple follicles, (cysts), or ovarian inactivity as has been suggested for dairy cattle.The effect of the adrenal gland contributing to increased progesterone levels has to be examined before definitive conclusions can be reached The establishment of patterns of individual intake when correlated to ovarian activity levels has the potential to provide an insight to what is happening in the herd. In extensive areas this tool when used with sampling techniques that have been applied in human epidemiological studies, to use part of the herd, could give insights into the trends in the whole herd. This would provide a novel real way of evaluating nutrition and breeding activity in the population and alleviate the tyranny of trying to assess the whole herd.

Subsequent research should now be carried out on this property involving two parallel herds (say of 100 cows). One herd should be supplemented in a systematic way to provide the main groups of nutrients, *i.e.* protein, both by-pass and urea, macro minerals phosphorus and micro minerals including sulphur, whilst the other should not be supplemented. The measurement of

ovarian activity in conjunction with pregnancy testing in both herds over a number of years would then definitively establish the effects of supplementation and enable evaluation of the economics of supplementation.

The aim of fertility assessment in the female is to show that adverse grazing conditions can be modified to improve the production of quality ova enabling fertilisation and the maintenance of a uterine environment that supports the production of a viable calf. The calf should then get sufficient nutrients (milk) from the cow to thrive, while enabling the cow to return to oestrus in the quickest possible time. Conversely, examining the numbers of ovulations and pregnancies could give an index of the adequacy of nutrition as early as 17 days after impregnation with viable semen.

The usefulness of the measurement of the production of a viable follicle, using hormones progesterone and oestradiol, has been examined and as yet in this context it is impractical.

Pregnancy testing,is economical, easy and gives immediate results. The number of cows non-pregnant and lactating compared to those pregnant and lactating cows is an index of nutritional sufficiency in the cow. If adequate nutrition is occurring more cows will be pregnant and lactating that non-pregnant and lactating.

By applying sampling techniques used in health surveillance programs, the problems of distance and size of populations in the northern herds to a certain degree can be overcome to allow for the collection of worth while experimental data. This could be the tool to facilitate the eventual moving to seasonal calving in theses herds and could bring large economic gains for producers. The establishment of seasonal breeding on a property should take place in one herd at a time (say bore 19 herd in the above case) and then more intensive monitoring could take place to identify the less productive females.

Thankfully, we still have those less productive regions. Some humans have dreams though, to make the deserts bloom and to make the depths of the sea and even Antarctica yield their bounty. As each year goes by, we come closer to developing the technologies that will allow us to realise these dreams. Each year we also feel an increasing need to utilise marginal lands in order to feed our growing populations. With our dreams fulfilled we will, I fear, see a wave of extinction's so vast as to dwarf anything that has gone before. For we will have become the exterminator species that broke all the rules. The one that could take not only all the resources of rich lands, but of poor ones as well.

Tim Flannery - *The Future Eaters*