

# final report

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## Tagasaste

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## ABSTRACT

Tagasaste [*Chamaecytisus proliferus*] is a leguminous fodder tree that is high in protein all year but cattle only gain weight during the winter and spring. This project investigated the reasons for the poor liveweight performance of cattle over the summer and autumn period.

- Alkane technology quantified tagasaste intake, which showed cattle decreased their intake during the summer and autumn when phenolic concentrations increased.
- The combined effect of low intake and high phenolic during summer and autumn resulted in poor rumen function.
- Urine in cattle is acidic when the phenolic compounds are high as result of fermentation and metabolism of the phenolic to hippuric acid. Moreover, the dietary cation/anion difference [DCAD] of tagasaste during summer and autumn can also contribute to acidification of the urine.
- Barley, barley/urea and lupin supplements restored rumen function and boosted liveweight gain with lupins producing the greatest effect.
- Predictive liveweight response curves were developed for the different supplements to allow calculation of the benefit/cost of feeding the supplements.
- Tagasaste cannot provide the phosphorus requirement of cattle during summer yet extra P either as extra fertiliser for tagasaste or directly as a lick did not produce an economic liveweight response in cattle.
- Producers with tagasaste have quickly adopted lupin supplementation.
- The value of the beef industry in the Dandaragan shire in the West Midland has increased an average of 24% per year over a 14 year period largely due to the introduction of tagasaste.
- A web page has been established at Murdoch University that links with the Department of Agriculture web page and provides access to FarmNotes: [www.vet.murdoch.edu.au/tag/](http://www.vet.murdoch.edu.au/tag/)
- This project has solved the poor growth in summer and provides information to target markets more effectively.

## EXECUTIVE SUMMARY

Tagasaste [aka tree-lucerne], *Chamaecytisus proliferus*, is a high-protein fodder browse tree originally from the Canary Islands, that can produce high liveweight gains in cattle at high stocking rates during the winter and spring. In Australia, and predominantly in Western Australia, the area planted to tagasaste has increased to more than 100,000 ha as farmers are rapidly adopting tagasaste as a useful feed source. Moreover, the total area suitable for planting tagasaste is 1.5 million hectares, if the establishment and productivity is proven to be cost-effective. The planting and establishment of tagasaste has now entered a phase where it is expanding rapidly in areas north of Geraldton. In contrast, the West Midland region that saw rapid expansion of tagasaste in the '80's and 90's, producers are now investigating the benefits of incorporating perennial pasture. During the early stages of development of tagasaste, scientists and producers expected cattle would gain weight over the summer and autumn. However, it is now known cattle will not gain weight over the summer and autumn, but dry cattle will at least maintain weight thus not requiring costly supplements in the same manner as cattle grazing annual pastures. The objectives of this project were two-fold [i] to investigate strategies to increase cattle production in summer and autumn, and [ii] to establish the underlying reasons for the lack of productivity.

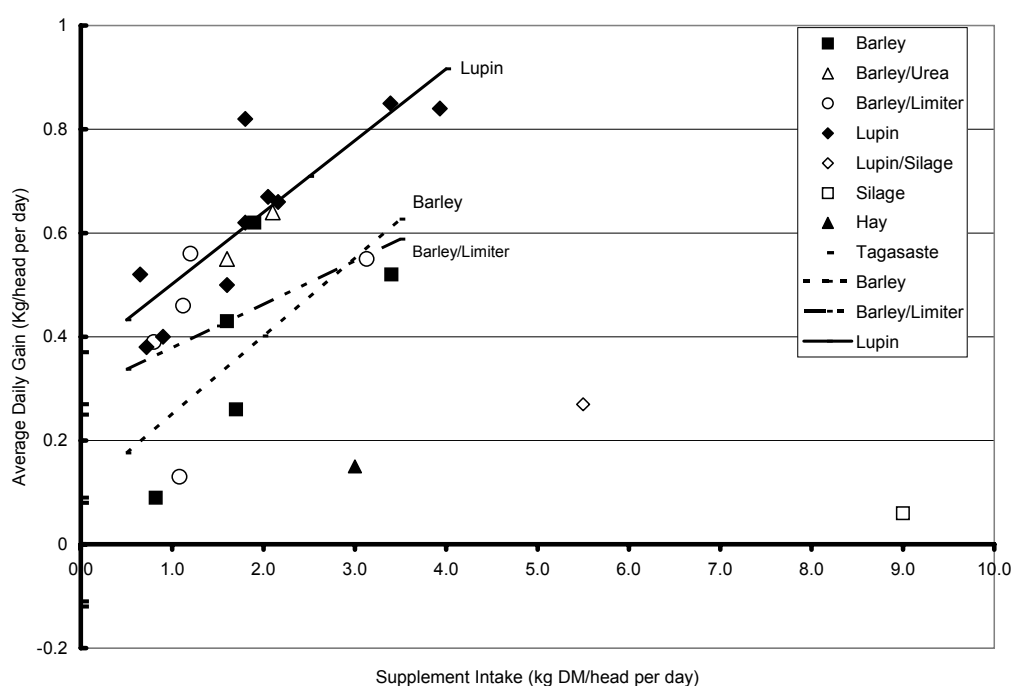
There is anecdotal evidence that weaners will gain weight and cows will suckle calves when browsing tagasaste growing over a water table. In all other instances, however, cattle do not grow when browsing tagasaste during the summer and autumn, irrespective of the conditions, ie. hot and dry, relatively cool and dry, or early break-of-season. As a first step to explain this lack of growth, tagasaste intake by cattle was monitored under practical field conditions by extending alkane capsule technology. The alkane profile established that cattle have high tagasaste intakes during the winter and spring but low intakes of tagasaste during summer/autumn. The reasons for the low intake are not obvious from the chemical analysis of tagasaste that showed the crude protein content did not fall below 14% and *in vitro* calculated metabolisable energy not below 9.7 MJ ME/kg DM from *in vitro* DMD% of 68%. During active plant growth in August and September protein and energy values were as high as 30% and 12.8 MJ ME/kg DM, respectively. These protein and energy values are normally associated with high growth rates in cattle. However, concentration of phenolic compounds peaks during summer and autumn and then decreases markedly during winter and spring. These phenolic peaks were associated with the low intakes and along with the increase in wax content of the leaves and the dietary cation anion differences in the rumen are possibly plant "defence" mechanisms to stop over grazing during the dry periods. From our studies the identification of acid urine in cattle browsing tagasaste in the summer and autumn suggests the fermentation of the phenolic compounds that increase significantly at this time of the year is partly responsible for the poor performance of the cattle.

Supplements of barley and urea that were equivalent in energy and nitrogen to lupins produced lower average daily gain [Figure 1]. Essentially lupins produced much higher [almost twice as much] rates of gain than did barley, with and without urea, for the same rate of supplementation. In contrast, hay and silage [even with some lupins] were not effective supplements for cattle browsing tagasaste during summer as these feeds were eaten in preference to the tagasaste. The relationship of liveweight response to barley, barley/urea, hay, silage and lupin supplement was quantified to allow farmers to choose their level of production according to the market that they intend to supply. Producers can use these response equations as a tool for assessing the economic potential of feeding supplements over the summer and autumn period to meet specific markets. For example, cattle producers

can predict that supplementing one kg of lupins per head per day to cattle browsing tagasaste during summer would produce 0.5 kg of gain per day; a good background rate for cattle entering feedlots.

The phosphorus content of tagasaste decreases during summer to levels below the recommended requirement for cattle. Phosphorus supplementation of both the tagasaste itself through superphosphate and directly to the animal via mineral licks increased liveweight gain in cattle browsing tagasaste fertilised with 300kg superphosphate/ha per year. This increase in liveweight response was measured in the second year after the application of higher rates of superphosphate fertiliser. However, there was no economic benefit in yearling steers and heifers browsing tagasaste fertilised with up to 300 kg super phosphate /ha per annum or supplementing them with a mineral lick with P.

**Figure 1:** The relationship between intake [kg DM/head per day] and average daily gain in cattle browsing tagasaste with a range of energy and N supplements



In the studies investigating the response to phosphorus, feed-on-offer [FOO] was not a useful concept for estimating amount of tagasaste available for stock to eat. While FOO has proved useful for predicting effective stocking rates and productivity on annual pastures, there is a need to develop a new indicator of potential tagasaste utilisation before producers can effectively use tagasaste to its full potential.

Cattle excreted acid urine during the summer and alkaline urine during the spring. The acid urine was not compensating metabolic acidosis as the plasma ketones and free fatty acids in these cattle were normal. We suggest that acid urine is due to excretion of hippuric acid; which is a metabolic product from phenolic degraded by rumen microbes and liver to benzoic acid. Subsequently the liver conjugates benzoic acid with the amino acid, glycine to form hippuric acid that can act as a drain on the amino acid pool that compromises protein metabolism in cattle. Thus, excretion of hippuric acid in large amounts will decrease the

efficiency of protein metabolism in cattle and may be a contributing factor to the poor growth rates during summer and autumn.

Cattle in this project were regularly monitored for rumen function as assessed by ruminal ammonia concentration as an indicator of protein metabolism, volatile fatty acids [VFA] as an indicator of energy metabolism and branch-chain VFA's and urinary purine derivatives as a measure of microbial nitrogen supply from the rumen. Ruminal ammonia was below optimal levels in cattle browsing tagasaste during summer and autumn but reached quite high levels in the spring. Similar seasonal effects were seen in other indicators of rumen function such as branch-chain VFA's that were lower in summer and autumn than in winter and spring. Moreover, cattle browsing tagasaste had low numbers of ruminal bacteria and urinary allantoin that indicated poor rumen microbe synthesis. These seasonal changes in rumen function were indicative of low rates of dietary protein fermentation and microbial protein synthesis due to a combined effect of low feed intake and rumen dysfunction.

The lack of growth in cattle browsing the tagasaste during summer is reflected in the poor rumen function during that time. Lupin supplementation was the most effective way of correcting the problems of rumen function because they were readily fermented in the rumen leading to optimal rates of ammonia and particularly branch-chain VFA's. Lupins were more effective in increasing all of the indicators of rumen function than barley and barley/urea and did not lower rumen pH as far from neutral or as readily as the cereal grain supplements. This pattern of changes in the rumen biochemistry gives a number of reasons for the liveweight responses to the N/E supplements and the poor response to the P supplements in yearling cattle browsing tagasaste.

Tagasaste staggers is a sporadic, sometimes lethal clinical syndrome observed in cattle browsing tagasaste. We suggest that tagasaste staggers is due to tremoragens which are phytotoxins causing significant but transient [ie. 15 – 30 minutes] increases in L-lactate and glucose due to the rapid and localised twitching of muzzle and flank muscles. Tagasaste staggers is not associated with lack of magnesium in the diet or in the plasma as was anecdotally reported.

Producers were clearly pleased to have this simple, practical and now proven way to overcome the lack of weight gain over the late summer and autumn period. Up to 50% [est. 100] of the beef businesses using tagasaste have adopted the technology to the extent that they are now using lupins as supplements when they need to meet particular market weights and times.

Four FarmNote and ProGraze articles and at least 3 refereed publications will be produced to extend and report the findings from this project. A web page has been established at Murdoch University that links with the Department of Agriculture web page and provides access to FarmNotes: [www.vet.murdoch.edu.au/tag/](http://www.vet.murdoch.edu.au/tag/).

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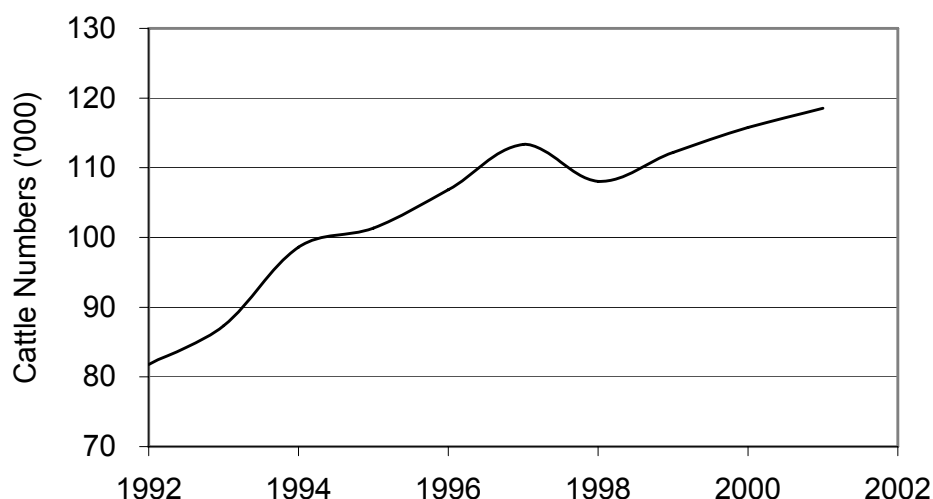
# 1 TAGASASTE (*CHAMAECYTISUS PROLIFERUS*): INTAKE STUDIES AND SUPPLEMENTARY FEEDING IN TAGASASTE BROWSING SYSTEMS

## 1.1 Background to project and the industry context

Tagasaste (*Chamaecytisus proliferus*) is an evergreen perennial shrub, which is native to the Canary Islands was introduced to southern Australia in 1879. Tagasaste has been used as a feed source by local farmers for centuries to maintain their livestock (Snook 1986) but it was not until the early 1980's that potential was recognised in Western Australia by Sir James McCusker. Approximately 850,000 ha of the agriculture region of Western Australia and 435,000 ha in South Australia and Victoria have been classified as suited to growing tagasaste (Lefroy *et al.* 1997). The criteria for suitability included deep sandy soils with annual rainfall > 350 mm.

Cattle numbers in this region increased by 45% between 1992 (81,800 hd) to 2000 (115,800 hd) (Figure 1.1). During this period there has been a reduction in sheep numbers from 2.2 m hd down to 1.7 m hd. A lot of the increase in cattle numbers would be due to the introduction of tagasaste. The value of the beef industry in the Dandaragan shire increased an average of 24% per year over a 14 year period largely due to the introduction of the fodder tree tagasaste.

**Figure 1.1:** Cattle numbers in the West Midland shires of Western Australia



Cattle production systems on tagasaste include breeders and backgrounding weaners or ex-pastoral cattle for the live export trade or for feedlots. In 1994 there was an estimated 40,000 ha planted to tagasaste and in 2001 there is more than 100,000 ha.

The establishment of tagasaste in the West Midlands region of Western Australia has increased the productive potential of the deep infertile sands in this area. Stocking pressure has increased from 1 - 2 DSE/ha of sheep grazing improved pastures to 8 - 10 DSE/ha of cattle browsing tagasaste. Analysis of the edible leaf and stem through the year shows that

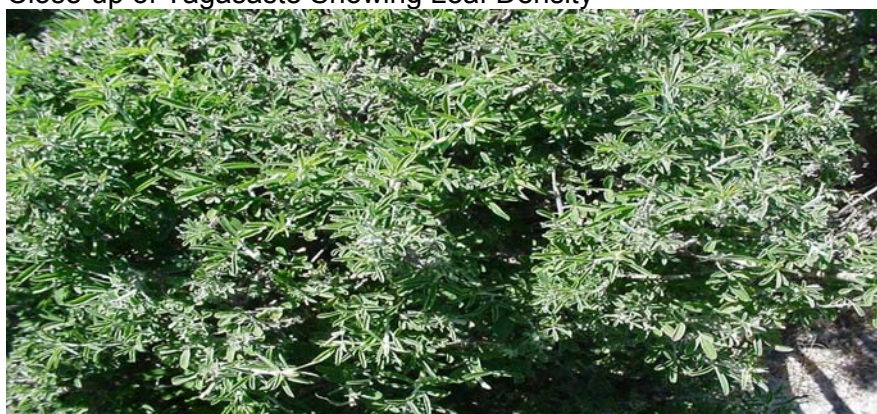
crude protein ranges from 14 - 30% and metabolisable energy from 9 –12.5 MJ ME/kg would suggest live-weight gains in cattle year-round. In reality though, gains are generally only achieved for 6 - 7 months each year with small live-weight losses or maintenance for the balance of the year.

Tagasaste in the West Midlands is ideally situated for flexible cattle production systems including carrying breeders and producing weaners through to finishing on tagasaste or backgrounding steers or heifers for either the feedlot industry or the live export trade. Tagasaste has a significant advantage over a pasture based system in that over late summer into autumn on a pasture based system dry cattle would be fed supplements to stop or reduce liveweight loss whereas on a tagasaste system supplements are generally not required for non-pregnant cattle. The feeding management system is to plant tagasaste in long rows with 10 to 15 m between the rows. Ideally, tagasaste is kept low, in a so-called 'broccoli' form as shown in the figure 1.2. The inter-row can contain a number of volunteer species or it can be planted to serradellas and other species. During winter and spring the inter-row can be a significant source of feed, and when dry during late October through to January, the dry feed can still be significant as seen in figure 1.2. From February onwards there is virtually no feed in the inter-row and cattle are entirely dependent on tagasaste for nutrients. Tagasaste must be trimmed or cut every 2 – 4 years or it will grow to heights that are not effective for feeding cattle [some parts of the tagasaste shown in Figure 1.2 are already at this height]. Beef producers can keep the tagasaste low by regular cutting, thereby allowing cattle to readily feed on the short leafy material and the regrowth (Figure 1.3).

**Figure 1.2:** Tagasaste Managed in Rows of 'Broccoli-form' for Feeding Cattle



**Figure 1.3:** Close-up of Tagasaste Showing Leaf Density



## 2 INTAKE STUDIES AND SUPPLEMENTARY FEEDING IN TAGASASTE (CHAMAECYTISUS PROLIFERUS) GRAZING SYSTEMS

### 2.1 Introduction

One of four key objectives of the project “*Intake studies and supplementary feeding in tagasaste grazing systems*” was to quantify intake of tagasaste throughout the year and under various supplementation and integrated management systems. This was to assist in determining whether the lack of growth of animals grazing tagasaste during the summer and autumn period, as reported from previous studies (Edwards *et al.* 1996; McNeill *et al.* 1996; Edwards *et al.* 1997a, b, c; Edwards *et al.* 2000), was due to an inadequate intake of tagasaste. The aim of this component of the project therefore was to investigate how much tagasaste animals were eating at various times of the year and how this varied from site to site in relation to (for example) season, depth to water table and fertiliser treatment.

Intake was to be quantified in cattle involved with the grazing studies central to the project (ie. supplementary feeding and phosphorus supplementation), as well as in sheep in pen feeding studies involving fistulated sheep. This was partly to validate the alkane-based methods being used in the cattle studies but also to assess the relationship of voluntary feed consumption to animal production and production responses to supplements or alternative feeds.

Initially the intake of tagasaste by cattle was to be determined using the *n*-alkane method in January, March and September for the supplementary feeding experiment at “Dunmar” (Chapter 3) and in an MRC-funded PIRD at ‘Cantabilling Springs’ in March only. Subsequently studies were continued at ‘Dunmar’ in 1999 and 2000 and further cattle studies were undertaken at ‘Tagasaste Farm’ in 1998 and 1999 as part of the mineral supplementation experiment (Chapter 4).

The *n*-alkane technique (Dove and Mayes 1991) is predicated on the fact that alkanes, which are long carbon chain molecules present as a component of plant cuticular wax, are indigestible markers that are relatively easily analysed. Generally plants are dominated by odd-chain alkanes and plant species tend to have their own unique profile of alkanes, allowing for discrimination between plant species. The technique involves inserting a controlled release device (CRD) containing known concentrations and release rates of alkanes of carbon-chain length 32 & 36 ( $C_{32}$  &  $C_{36}$ , respectively) into the rumen of intact sheep or cattle. The animals are then left to graze normally for about 10 days whilst the CRD starts releasing and  $C_{32}$  and  $C_{36}$  reach a steady state concentration in the rumen. In 6 – 8 days faecal samples are then collected from the dosed animals on at least 2 separate days between 10 and 18 days post-insertion and at different times on these days (to account for any diurnal variation in release). These faecal samples are then dried, ground, combined for each animal, chemically extracted and analysed chromatographically for their *n*-alkane profiles. Feed samples (eg. tagasaste, pasture species, weeds etc) are also collected at the time of faecal sampling and submitted to the same analytical procedures to determine their *n*-alkane profiles. Based on the profile of *n*-alkanes in the faeces and the profile in the plant material available for consumption, an estimate can be made of the diet composition for each animal. In addition, using the faecal concentration of  $C_{32}$  or  $C_{36}$ , plus the release rates of

these components from the controlled release device, it is possible to then determine the total dry matter intake by is illustrated in Figure 2.1.

While the technique is relatively widely used in grazing studies its use has not yet been fully validated for shrub species such as tagasaste. Nevertheless, it was not envisaged that the technique would have difficulty providing relevant and useful data on intake in this system. Its major weakness in relation to the current project is that it relies on dietary components to have alkanes as part of their chemical composition.

**Figure 2.1:** The method of calculating daily herbage intake using alkanes

$$\text{Daily herbage intake (kg DM/day)} = \frac{\left( \frac{Faecal_{odd}}{Faecal_{even}} * DailyDose_{even} \right)}{\left( Herbage_{odd} - \frac{Faecal_{odd}}{Faecal_{even}} * Herbage_{even} \right)}$$

Where *Faecal* or *Herbage* refers to the concentration (mg/kg) of the relevant alkane and *odd* and *even* refers to the carbon chain length of that alkane. *Daily Dose* refers to the release rate (mg/day) of C<sub>32</sub> or C<sub>36</sub> from the alkane CRD.

While this is the case for the forage species under study, the supplements being utilised in the 'Dunmar' supplementary feeding experiment would not contain sufficient concentrations of alkanes to be useful. A feed marker, lithium chloride (LiCl) technique was used in conjunction with alkanes to ascertain the intake of both the forage (tagasaste, annual species) and supplement components of the diet (see Chapter 3).

The initial intention for the sheep work was to study intake by transporting a group of rumen fistulated animals from site to site. At each site edible leaf and stem material would be collected by hand and fed to the animals whilst measuring intake, dry matter and organic matter digestibility, nitrogen balance, digesta flow rate and rumen parameters. This protocol was modified in consultation with the TTAG committee linked to the project. Instead of rushing into studies of the voluntary intake of tagasaste and (perhaps) finding no differences due to the selected sites having the same 'quality' tagasaste, a 'survey' of tagasaste quality would initially be carried out to find suitable sites for animal studies in Year 2 of the project. The proposal was to sample tagasaste from different sites, each with potentially different 'quality' tagasaste (ie. palatability and therefore intake) water stress (ie. due to water table or row spacing & inter-row), fertiliser history, grazing management (ie. sheep vs cattle; set stocked vs rotation), time since grazing and latitude/longitude (ie. across the state and W.A. vs S.A./Vic.). The collected material would then be analysed by near infrared spectroscopy (NIR) for crude protein, lignin, ash, NDF, ADF and total phenolic using calibrations set up during the previous MRC-funded project (UWA.007), plus for *in vitro* dry matter digestibility (IVD) by the pepsin-cellulase method. On the basis of differences occurring between any of these 'quality' factors, sites were to be selected for more intensive study in Year 2 (eg. intake via alkanes, pen feeding, etc).

The *n*-alkane based field studies of intake at 'Dunmar', 'Cantabilling Springs' and 'Tagasaste Farm' were therefore intended to feed appropriate information into the rest of the data sets (ie. liveweight gain, supplement intake, rumen parameters, etc) for these experiments to help with interpretation of growth performance of the experimental treatments. On the other hand, the sheep-based pen and field studies were intended to answer some more fundamental

questions about the relationship between voluntary feed intake and digestibility of tagasaste, rumen fermentation, rumen ecology and the response to supplements and the nutritive value of freshly harvested tagasaste. Both sets of data would help clarify reasons for the production seasonality consistently found with tagasaste.

## 2.2 Materials & Methods

### *2.2.1 Standard method for tagasaste sampling*

A standard method for tagasaste sampling was established early in the project to facilitate reproducible collections irrespective of the person sampling. The method was based on the technique used in the Martindale Research Project and UWA.007:

- walk a diagonal transect of the plot or paddock (ie. the unit of interest) and stop 10 times at random.
- at each stop collect about 20 x 10cm growing tips into a paper bag from a shrub/tree on either side of the row (total of 20 shrubs/trees per plot or paddock).
- record the wet weight of the sample.

N.B. This should result in ~100g wet weight of tagasaste; adjust sampling from 'broccoli' tagasaste to result in similar sample size.

### *2.2.2 Standard method for tagasaste processing*

An experiment was run to establish the most appropriate method for handling tagasaste samples between collection from a plot or paddock and sending for analysis (NIR or 'traditional' laboratory analysis). By default the method of choice was that used during the Martindale Research Project and UWA.007, however it was felt that a rigorous procedure should be followed to test a few different methods rather than sticking with a method of convenience which may not be the most appropriate. This experiment compared collection of edible leaf material into liquid nitrogen, dry ice or a car fridge as soon as possible after picking and freeze vs oven drying of samples.

Tagasaste samples were collected from plots 6 to 11 of the mineral supplementation trial at 'Tagasaste Farm' according to the standard protocol above. Approximately 300g of fresh leaf material was collected from each plot, thoroughly mixed and then divided into six equal portions of ~50g. For each plot two of these portions were then placed in plastic bags and immediately snap frozen in liquid nitrogen on site. Another two portions were also placed in plastic bags and placed on dry ice to rapidly freeze. The final two portions were placed into papers bags and kept cool in an 'Engel' car fridge set on the freeze cycle. On return to the laboratory in Bunbury the 36 samples were stored at -20°C until further processing. For each pair of samples (ie. two liquid N frozen samples from plot 6, two dry ice frozen samples from plot 11, etc) one sample was subsequently dried at 55°C in a forced draft oven and the other was freeze-dried. Thus, for each of the 6 plots there were 6 samples as follows:

- Liquid nitrogen frozen; oven dried.
- Liquid nitrogen frozen, freeze-dried.
- Dry ice frozen; oven dried.

- Dry ice frozen; freeze-dried.
- Cooled and frozen at  $-20^{\circ}\text{C}$ ; oven dried.
- Cooled and frozen at  $-20^{\circ}\text{C}$ ; freeze-dried.

The samples were oven dried at  $55^{\circ}\text{C}$ , ground to pass a 1mm sieve, packed into plastic screw-capped vials and sent to Dr Peter Flinn at the Pastoral and Veterinary Institute, Hamilton, Victoria for analysis by Near Infrared Reflectance Spectroscopy (NIR) for crude protein, lignin, ash, NDF, ADF and total phenolic using the calibrations set up during the previous MRC-funded project (UWA.007). Sub-samples were also retained for determination of *in vitro* dry matter digestibility (IVD) in the Department of Agriculture WA nutrition laboratory by the pepsin-cellulase method.

### **2.2.3 Tagasaste ‘survey’**

As indicated in the Introduction, a ‘survey’ of tagasaste quality was planned to find suitable sites for animal studies in Year 2 of the project. The plan was to sample tagasaste from different sites, each with potentially different ‘quality’ tagasaste (ie. palatability and therefore intake) due to water stress (ie. due to water table or row spacing & inter-row), fertiliser history, grazing management (ie. sheep vs cattle; set stocked vs rotation), time since grazing and latitude/longitude (ie. across the state and W.A. vs S.A./Vic.). The following sites were selected:

- ‘Dunmar’ Supplementary Feeding Trial x 4 plots.
- ‘Tagasaste Farm’ Mineral Trial x 5 plots.
- ‘Cantabilling Springs’ x water table effect (ie. up a slope from surface water).
- ‘Joanna Plains’ x 2 paddocks and a site with variation from high to low water table.
- Badgingarra Research Station x 4 plots plus an irrigation site (ie. to simulate no water stress).
- Johns (Coorow, WA) x 2 paddocks (different row spacing of tagasaste).
- Brown (Moora, WA) x 4 plots of differing row spacing which are being grazed by sheep and their water table levels studied.
- Whyne (Binu, WA) x 2 paddocks (Northern extreme of commercial tagasaste).
- Martindale Pty Ltd (New Norcia, WA) x 2 paddocks from long term studies.
- Rogers (Tammin, WA) x 2 paddocks (Wheat Belt tagasaste on an old cropping site).
- Bessell-Brown (Katanning, WA) x 2 paddocks (Sheep grazing).
- Pearce/Adams (Albany, WA) x 2 paddocks (South-Western extreme of commercial production).

- Hockey (Esperance, WA) x 2 paddocks *Eastern extreme*
- Brown (Esperance, WA) x 2 paddocks *of West Australian*
- Kleinig (Esperance, WA) x 2 paddocks *production*
- Brookman (Keith, SA) x 1 paddock (South-East South Australia).
- Bradey (Edenhope, Victoria) x 1 paddock (Western Victoria).

The first sampling was to occur in March/April 1998, however this did not eventuate due to delays establishing the standard sampling and handling protocols (above), followed by substantial opening rains. It was decided at this point that we had missed the window of opportunity for Autumn 1997 (ie. tagasaste at its most water-stressed), so the first sampling was rescheduled for September/October 1998. This sample set, which represented the best (ie. spring) quality tagasaste, was followed in January 1999 with a second sampling as an indicator of declining tagasaste quality (ie. water stress increasing).

The samples were analysed as indicated earlier.

## 2.2.4 Intake Studies

The *n*-alkane technique of Dove and Mayes (1991) was used to quantify the intake of forage (ie. tagasaste and other plant material) by cattle in the supplementary feeding (ie. 'Dunmar') and phosphorus supplementation ('Tagasaste Farm') grazing experiments. Intake by cattle was also determined in an associated supplementary feeding PIRD project at 'Cantabilling Springs'.

Captec alkane controlled release capsules (CRC's; Captec (NZ) Ltd, Auckland, New Zealand) containing *n*-dotriacontane ( $C_{32}$ ) and *n*-hexatriacontane ( $C_{36}$ ) were administered orally to test animals on day 0 of an intake trial. In two of the trials test animals then had faecal samples collected per rectum on day 9 (am) and 12 (pm), followed again on day 19 (am) and 20 (pm). This was an attempt to gain two sets of intake information from a single CRC. However this procedure was abandoned when it was found that many of the CRCs had released all or most of their payload prior to the last samples being collected (day 20), thereby rendering the final samples useless for intake determination. In all other trials faecal samples were only collected on two days between days 12 and 16 post administration. Faecal samples were collected into plastic examination gloves that were subsequently turned inside-out and tied at the wrist. The glove was then labelled with an indelible black marker pen with tag number, date and approximate time and frozen at  $-20^{\circ}\text{C}$ .

Samples were later removed from the gloves, placed in labelled aluminium trays and dried to constant weight at  $55^{\circ}\text{C}$ . Pairs of samples for any one animal for a collection period were then ground to pass a 1mm sieve and bulked. Samples were collected from different times on different days to try to account for potential diurnal variation that may occur in faecal release rate of *n*-alkanes. The final dried, ground and bulked faecal samples were then submitted to CSIRO Animal Production (now CSIRO Livestock Industries) for analysis (see below).

On the same days over which faecal samples were being collected plant materials were also collected to determine their *n*-alkane profiles. These profiles are essential for interpretation

of faecal alkane profiles as they provide the plant basis for which the faecal profiles are ultimately derived. Approximately 100g wet weight of all plant species present in a plot were collected, transported back to Perth in an 'Engel' car fridge set on the freeze cycle and frozen at  $-20^{\circ}\text{C}$ . These samples were subsequently processed ready for alkane determination by the same method as that used for faecal samples.

**Extraction and Purification of Alkane:** Bulkied faecal samples or individual plant samples submitted for analysis were processed as follows to determine their n-alkane profile:

- 1 g of oven dried faeces or 2 g of oven dried plant material was accurately weighed into large culture tubes (Pyrex - Bilby Cat no. 1636/44).
- Internal standard solution was added by pipette into the tube and weighed. Internal standard is 125 mg of  $\text{C}_{34}$  (tetratricontane) dissolved in 50 g of  $\text{C}_{11}$  (undecane). These weights must be recorded to calculate the  $\text{C}_{34}$  factor.
- Approximately 10 ml of ethanolic KOH was then added (slightly in excess to moisten entire sample). [46.29075 g KOH in 550 mls of redistilled ethanol. This solution is made fresh for each batch of samples].
- Tubes are then stoppered using PTFE (to finger tightness) and heated in an oven overnight at  $75^{\circ}\text{C}$  to saponify.
- Samples, heptane and water are then placed into a water bath and maintained at  $50^{\circ}\text{C}$  throughout the duration of the experiment.
- Approximately 8 mls of warm heptane and about 5 mls of warm distilled water are then added, the tubes capped and mixed thoroughly by shaking. Phases are allowed to separate, which can be assisted by centrifuging at low speeds.
- Top phase is transferred with a pasteur pipette into a tablet vial (Crown Corning 8127). Tablet vial is placed in the sample concentrator and the sample dehydrated to dryness.
- This extraction is repeated twice more using two lots of 5 mls of heptane (water is not added again). Heptane extracts are pooled in the same tablet vial and evaporated to dryness.
- A silica gel column is prepared by cutting a Gilson pipette safety plug in half and removing the outer layer of wax paper. The plug is pushed into a 5ml Gilson pipette tip. To this plugged pipette is added sufficient silica gel so as to allow a gap of about 1cm at the top. A new tablet vial is then placed under the column and heptane added until it begins to drip into the tablet vial.
- The dry residue is taken up in the tablet vial in about 2 mls of heptane and transferred to the top of the silica gel column by a pasteur pipette.
- The sample is eluted with 3 X 2ml lots of the heptane, rinsing the original tablet vial with the eluent before adding it to the column. Finally, the column is rinsed through with 3 X 2 mls of heptane.

- The collected column eluent is then evaporated to dryness before being taken up in 0.5 - 0.8 mls of heptane and transferred to a GC auto sampler vial using a pasteur pipette. The samples are then run on a GC under the following conditions:
  - Column HT5. 0.1µm film, 6 m x 0.53 mm id. (SGE part no 051580)
  - Injector temp. 300°C
  - Detector temp. 300°C
  - Column head pressure app 10kP
  - Septum bleed app 0.6mL/min
  - Split vent flow app 6.0mL/min
  - Initial T 150°C
  - Initial time 0
  - Rate 5°C/min
  - Final T 250°C
  - Final time 0
  - Injection volume 1µL

Calculations to convert the GC outputs into mg alkane/kg sample are as follows:

$$\text{mg alkane/kg sample} = \frac{\text{area (Cx)}}{\text{area int. std}} \times 1000 \times \frac{\text{wt int. std (g)}}{1000/\text{wt sample (g)}} \times \text{fraction of C}_{34}$$

These values were then run through a diet selection algorithm using the Diet Selection Calculator, EatWhat (CSIRO, version 1.2, 1996) to provide an estimate of the diet composition of the animal represented by a particular faecal sample. When a satisfactory and realistic diet composition is determined this information is used in the equation (Figure 2.1), along with the faecal and plant alkane profiles, to determine the dry matter intake of the animal in question:

### ***2.2.5 Statistical Analyses***

Statistical analyses were performed using a General Linear Model in SAS System for Windows (SAS Institute Inc., Cary, NC, USA; version 8.00).

## **2.3 Results & Discussion**

### ***2.3.1 Standard method for tagasaste sampling and processing***

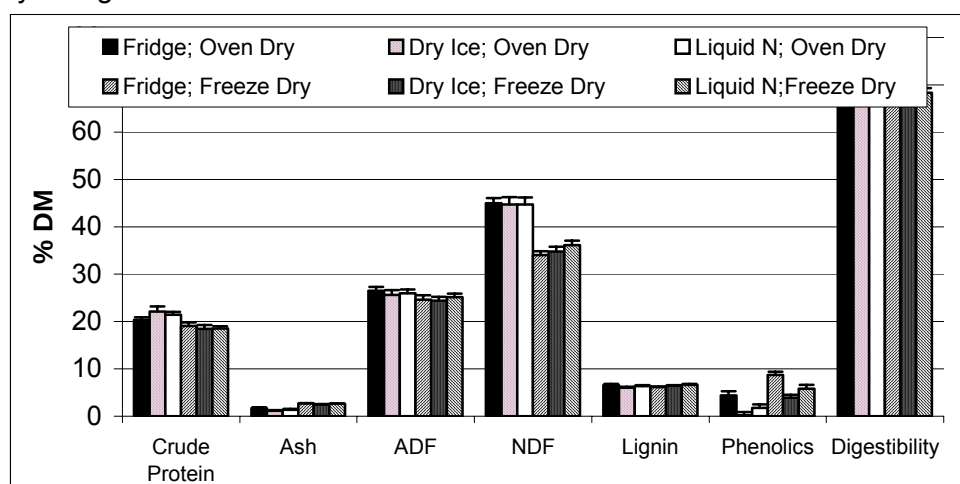
Sample freezing and initial storage method had no effect on crude protein, acid detergent fibre (ADF), neutral detergent fibre (NDF) or lignin levels in tagasaste leaf and edible stem or on the *in vitro* digestibility of tagasaste leaf and edible stem material (Figure 2.2). Ash concentration was significantly lower for dry ice frozen tagasaste than for material that had been kept cool and frozen in a standard freezer on return to Perth, but liquid nitrogen frozen samples were not different from either of the other sample treatments. Similarly, the concentration of total phenolic was affected by freezing method, with concentrations for dry ice frozen samples less than those snap frozen in liquid nitrogen which in turn were less than those kept cool and frozen later. This effect may be important if we were vitally interested in the absolute level of total phenolic in tagasaste samples, however the relative differences between samples of tagasaste were of greatest interest in this project. Thus with this compromise in mind, it was considered that the simplest sample freezing and storage

method was adequate for this project. Any samples collected specifically to quantify the level of total phenolic would, however necessitate use of either dry ice or liquid nitrogen. Reasons for higher phenolic values for tagasaste samples stored using the standard methods are unclear. Some effort was made to investigate this issue by checking the calibration data set used to establish the NIR calibrations, however it was not possible to replicate concentrations measured for this data set and the issue remained unresolved.

Drying method did have a marked effect on some nutritional parameters of tagasaste, especially NDF and phenolic levels and to a lesser extent crude protein and ash levels (Figure 2.2). Oven drying also resulted in higher total phenolic concentrations being measured than if samples were freeze dried, however it is again unclear why this is the case.

Despite these significant effects of sample storage and processing, it was considered that the improved accuracy of the parameters being measured was not worth the extra time and effort required for snap freezing of samples in the field and freeze drying of samples back in the laboratory. Consequently it was decided that all further samples for the project would be collected into paper bags, kept cool in an 'Engel' car fridge set on the freeze cycle until they could be put into a  $-20^{\circ}\text{C}$  freezer. Samples would then be oven dried at  $-55^{\circ}\text{C}$  ready for grinding and further analysis.

**Figure 2.2:** Effect of sample processing (freezing and drying) on chemical composition and digestibility of tagasaste leaf and stem material.



### 2.3.2 Tagasaste 'survey'

Tagasaste leaf and edible stem material was collected in early December 1998, plus samples collected at other times from various plots and paddocks at 'Tagasaste Farm'. These samples were sent to FeedTest, Pastoral and Veterinary Research Institute, Hamilton, Victoria for analysis by NIR. Some differences were noticed between properties, particularly in total phenolic concentrations, but it was also noted that many samples did not fit the existing calibration curve for the NIR. Consequently we were not fully confident in the values being presented and were not prepared to instigate intake studies based on potentially inaccurate information. The inaccuracy in the NIR calibrations is a consequence of the calibrations being set up with a narrow data set, based on samples from a single fertiliser response trial in project UWA.007. Whilst the data were adequate for samples from this trial

site and one other within this project, they were found not to adequately cover the full range of tagasaste across Western Australia and South Australia/Victoria.

It is technically feasible (and potentially relatively easy) to 'fix' or update the calibrations by adding the wet chemistry determined proximate analyses into the NIR dataset along with their NIR spectra. This was not done as part of the current project partly due to inadequate resources. Moreover, any 'fix' would be of academic interest only and not a useful practical outcome of this project. In addition, there has not been a call for tagasaste samples to be analysed for chemical composition on a regular basis through this commercial laboratory. It was considered more appropriate to concentrate our efforts on intake studies in the two major grazing experiments central to the current project, particularly in light of the interesting and confusing production results coming out of the mineral supplementation experiment at 'Tagasaste Farm'.

### ***2.3.3 Intake Studies***

One of the major objectives of this project was to "quantify intake of tagasaste throughout the year and under various supplementation and integrated management systems." This was largely to test the hypothesis that the lack of growth of animals grazing tagasaste during the summer and autumn period is due to an inadequate intake of tagasaste.

Intake studies with fistulated sheep were foreshadowed in the original project proposal as one strategy to address these issues. Whilst these studies were considered important aspects of the project, adequate site selection for such studies was not possible for the reasons explained above. Instead more alkane-based intake studies than originally planned were undertaken at 'Tagasaste Farm' to try to tease out some of the anomalous production results being noticed across the three different paddocks or blocks (see Chapter 4).

Thus, intake of tagasaste and other dietary components were measured at a number of strategic times of the year by cattle in the two core experiments of the project - the nitrogen and energy supplementary feeding studies at 'Dunmar' and the experiment at "Tagasaste Farm" where we were studying the phosphorus and mineral requirements of cattle grazing tagasaste – plus the 'Cantabilling Springs' tagasaste PIRD.

### ***2.3.4 Concentration of Alkanes***

One of the interesting features to emerge from the alkane analyses was the very high concentration of  $C_{31}$  in tagasaste leaf and edible stem material in autumn (Table 2.1). This is consistent across sites, with 3405mg of  $C_{31}$ /kg dry matter in February 1998 and 2878mg in March from 'Dunmar' and 3603 in April from 'Cantabilling Springs'. The concentration of  $C_{31}$  in tagasaste is even more intriguing when other data is considered.  $C_{31}$  concentrations from tagasaste leaf and edible stem collected at Badgingarra Research Station varied dramatically with season - from 393mg/kg DM in September and October '95 to 2000 in April '95 and January '96 and 4826 in April '96. Similarly, material collected from The University of Western Australia's Shenton Park Field Station (UWA.007) had 2000-2500 mg  $C_{31}$ /kg DM in May '96 and ~600mg/kg DM in October of the same year. This magnitude of variation is not seen within a species for annual grasses or clovers, and to our knowledge has not been reported in other perennial species. It is interesting to speculate that this may be another component of tagasaste's summer drought/water stress defence mechanism (ie. increasing the wax layer on its leaves to reduce transpiration and thereby water loss?).

**Table 2.1:** Levels of *n*-alkanes in the cuticular wax of tagasaste and other plant species (mg of alkane/kg of plant)

| Plant species      | C <sub>25</sub> | C <sub>26</sub> | C <sub>27</sub> | C <sub>28</sub> | C <sub>29</sub> | C <sub>30</sub> | C <sub>31</sub> | C <sub>32</sub> | C <sub>33</sub> | C <sub>34</sub> | C <sub>35</sub> | C <sub>36</sub> |
|--------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Tagasaste (Sep'95) | 22              | 6               | 35              | 7               | 172             | 12              | 393             | 5               | 8               | 0.4             | 2.0             | 2.0             |
| Tagasaste (Feb'98) | 10              | 9               | 51              | 19              | 349             | 64              | 3405            | 55              | 120             | 0.0             | 1.7             | 1.7             |
| Capeweed           | 6               | 7               | 28              | 14              | 248             | 37              | 1221            | 22              | 193             | 97              | 49              | 2.0             |
| Ryegrass           | 8               | 5               | 29              | 9               | 219             | 13              | 196             | 5               | 23              | 254             | 1.4             | 2.7             |
| Gum Leaves         | 15              | 12              | 27              | 3               | 6               | 0.7             | 4               | 0.5             | 0.8             | 126             | 0.2             | 1.2             |

### 2.3.5 Calculations

A major hurdle was encountered early in the processing of intake data for this project. Once faecal and plant alkane concentrations have been determined for a particular intake trial (eg. August 1998 at 'Tagasaste Farm') 'all' that is required to convert this raw data into actual dry matter intake for individual animals is that a prediction is made of the proportion of available dietary components that are actually consumed by the animal (eg. 41% tagasaste, 7% capeweed, 32% grasses, 20% clover). Intake is then calculated based on these proportions and the level of C<sub>31</sub> and C<sub>32</sub> (in this instance) in the plant material being consumed and in the faeces. The prediction software used for these intake calculations (EatWhat Diet Selection Calculator) was unable to distinguish adequately between tagasaste and some other dietary components for the animals in these trials. Much time was therefore spent trying to resolve this issue, which incidentally is not unique to tagasaste. A similar problem is being addressed by other workers at CSIRO Livestock Industries in Perth in relation to capeweed's dominance of other species in intake calculations but is not one for which very good or simple solutions are available.

Essentially it was necessary to select a subset of the available alkanes which represent unique alkane profiles for each plant species under consideration for inclusion in a diet. This should then allow EatWhat to distinguish between species and calculate the true diet composition from a faecal alkane profile. Typically an alkane profile includes C<sub>23</sub> through to C<sub>36</sub>, however we were initially only using C<sub>27</sub>, C<sub>28</sub>, C<sub>29</sub>, C<sub>31</sub>, C<sub>33</sub> & C<sub>35</sub>, with the major emphasis on C<sub>31</sub> due to its high concentration in tagasaste. Although it is theoretically possible to select an appropriate subset of alkanes 'by eye', it is far more satisfactory to develop a statistical basis for deciding which subset of alkanes should be used for the analysis. After consultation with Dr Hugh Dove, one of the developers of the alkane technique for grazing ruminants, we tried Cononical Variance statistics to identify a unique set of alkanes for distinguishing tagasaste from other dietary components, based on the studies of Dove et al. (1999). For some of the intake trials extra plant samples then had to be collected and submitted for alkane analysis to provide more data on which to utilise the Canonical Variance statistics. These extra analyses were necessary because initially only one sample of each plant type (eg. tagasaste, capeweed, grass, etc), formed by combining material from each plot being used for grazing, was analysed for its alkane profile for each of these studies. In order to use the statistical approach to resolve the calculations it is necessary to have data from more than one sample of any material analysed. Furthermore, 'raw' alkane data must be transformed by reducing them by the mean value and dividing by the standard deviation of a set of samples, so that loadings can be compared with one another.

We have now (finally) been able to use this rather complicated technique to reanalyse some of the data sets for intake at 'Dunmar' and 'Tagasaste Farm', however there is still a great deal of intuition and experience needed in interpreting the alkane and intake data. This is the reason it has taken so long to process the many samples that were collected for this project and is also why all of the data has not yet been reanalysed.

### **'Tagasaste Farm'**

Intake studies were carried out in both 1998 and 1999. In 1998 studies were carried out in March, to coincide with a period of poor animal growth and plant quality, and again in August to coincide with maximal animal growth and (presumably) tagasaste intake. In 1999 studies were carried out again in March, in August to coincide with a period of higher animal growth and (presumably) tagasaste quality and again in early September to coincide with a period of maximal animal growth and tagasaste quality. The reason for the extra measurements in 1999 was to improve our understanding of the intriguing interactions between treatments and blocks/paddocks occurring at this site (ie. large production differences between "Pea Trial", "Erregulla" & "Pennant" paddocks – see Chapter 4 for more details).

Following manipulation of the raw alkane data to accommodate Canonical Variance statistics EatWhat predicted the animals were eating 100% tagasaste. This was in contrast to predictions of 25-85% tagasaste intake (and therefore 15-75% intake of 'other' species) prior to manipulation of the data. Common sense had told us that the latter predictions were not correct, because although 'other' species (eg. mainly capeweed and grasses) had been collected during the course of the intake study they were only a very minor component of the sward (<1%) at this time of the year when interrow 'pasture' is mainly white sand and a few bits of dry annual plant species. This, in fact, was the first indication that using alkanes to predict intake in animals grazing tagasaste may be difficult and led to the Canonical Variance approach to resolving diet selection. Predictions of species intake in the August 1998 samples prior to Canonical Variance analysis indicated 0-30% tagasaste intake, 0-25% intake of grasses and 63-76% intake of capeweed. Following manipulation of the data EatWhat predicted a more realistic average mix of 30% tagasaste, 16% mixed grasses, 52% capeweed and 2% blue lupins. While the proportion of capeweed in the diet still appears to be high it should be remembered that these measurements were made in August (ie. late winter) when there is typically abundant inter-row pasture available in tagasaste paddocks or plots and capeweed is usually a very high proportion of this 'pasture' mix. One clear limitation to being more confident in these species intake proportions is that we did not make any estimate of the actual proportion of the individual species in the 'pasture' sward. This would effectively have provided a ground truthing of the final EatWhat outputs and provided more confidence in the results.

The actual dry matter intake for 1998 studies at 'Tagasaste Farm' (Table 2.2) indicates realistic levels of intake for the animals at both times. During the March estimates animals on these plots were approximately 250kg and only maintaining their liveweight (dry matter intake at 1.5 - 3% of body weight). In August the same animals were 300kg plus and were gaining weight at over 1kg/head per day (intake at 2 - 3% of bodyweight). This is in contrast to calculations done prior to using Canonical Variance statistics to sort out the species intake issues (above), which are presented for interest on the last row of Table 2.2 and were of the order of 6% of bodyweight! The intake data also indicates that animals subjected to the Extra Superphosphate (300kg/ha/year) plus a mineral lick minus P treatment had significantly higher intake of dry matter (tagasaste only in this case) than animals in the other treatments. These results must be taken with a great deal of caution, however as this

treatment was in the “Pea Trial” block at ‘Tagasaste Farm’, whereas the other treatments were all in the “Erregulla” block. Animals in “Pea Trial” block have consistently outperformed those in the other blocks. No differences were evident between treatments at the August intake measurement.

**Table 2.2:** Tagasaste intake (kg DM/head per day) ( $\pm$  s.e.) by cattle browsing tagasaste with and without phosphorus in their mineral supplements and with differing levels of superphosphate application to the tagasaste at ‘Tagasaste Farm’, March and August 1998 (values are expressed as kg DM/head per day)

| Period | Standard superphosphate (SP) (100kg/ha per an.) | Standard SP (100kg/ha per an) + mineral lick - P | Standard SP (100kg/ha per an) + mineral lick + P | Extra SP (300kg/ha per an) + mineral lick - P* | Extra SP (300kg/ha per an) + mineral lick + P | P     |
|--------|-------------------------------------------------|--------------------------------------------------|--------------------------------------------------|------------------------------------------------|-----------------------------------------------|-------|
| March  | 4.0 $\pm$ 0.40 <sup>a</sup>                     | 4.0 $\pm$ 0.39 <sup>a</sup>                      | 6.5 $\pm$ 0.84 <sup>ab</sup>                     | 7.8 $\pm$ 0.76 <sup>b</sup>                    | 4.4 $\pm$ 0.59 <sup>a</sup>                   | 0.001 |
| August | 9.3 $\pm$ 0.64                                  | 6.7 $\pm$ 0.77                                   | 6.69 $\pm$ 0.56                                  | 8.45 $\pm$ 0.91                                | 9.47 $\pm$ 0.59                               | 0.178 |
| August | 23.6 $\pm$ 1.63                                 | 18.9 $\pm$ 1.89                                  | 17.8 $\pm$ 2.22                                  | 18.8 $\pm$ 1.48                                | 23.4 $\pm$ 2.62                               | **    |

\* N.B. The Extra SP + mineral lick - P treatment had significantly higher intake than the Standard SP, Standard SP + minerals - P & Extra SP + minerals + P, however this treatment was in the “Pea Trial” block, whereas the other treatments were all in the “Erregulla” block. Given that animals in “Pea Trial” block have consistently outperformed those in the other blocks the differences between this treatment and the others should be interpreted with caution.

\*\* These values are from ‘raw’ alkane data and assuming 100% tagasaste intake, not manipulated to accommodate Canonical Variance statistics.

The data in Table 2.3 provide a comparison of the relative intake by animals of one treatment between the three ‘blocks’ (“Pea Trial”, “Erregulla” and “Pennant”) in August 1998. This comparison, which involved extra intake measurements over those originally planned, was undertaken to try to explain the production differences, which were becoming evident between blocks/paddocks at this site. Although the difference in intake of cattle in the “Pennant” block were 25% higher than those in “Pea Trial” Trial block the cattle in “Pea Trial” trial actually gained more weight than those in “Pennant”. The small number of animals in this statistical analysis would be the reason for that this difference in intake was not significant ( $P = 0.179$ ). This difference in performance was not identified. There is, however a trend towards a difference between blocks, but the low number of animals studied per block may not have allowed any differences to reach significance. This issue was studied in greater detail in the 1999 intake trials at this site when 5 animals were studied in each of the 15 plots at March, June and September intake trials. This equates to 30, 20 and 25 animals for each of the three blocks. Canonical variance analysis of these samples has not been completed.

**Table 2.3:** Tagasaste intake (kg DM/head per day) by cattle browsing tagasaste on plots with standard superphosphate application but in three different ‘blocks’ of the experimental site at ‘Tagasaste Farm’, August 1998 (values are expressed as kg DM/head per day)

| Block             | Standard superphosphate (SP) (100kg/ha per an) | N  |
|-------------------|------------------------------------------------|----|
| “Pea Trial” Trial | 8.9 $\pm$ 0.96                                 | 5  |
| “Erregulla”       | 9.3 $\pm$ 0.64                                 | 10 |
| “Pennant”         | 11.25 $\pm$ 0.46                               | 5  |

### ‘Dunmar’

Intake studies were carried out in 1998, 1999 and 2000 at the ‘Dunmar’ supplementary feeding experiment. In 1998 studies were carried out in January/February prior to supplementation beginning so that these data could be used as a covariate in future analyses. Subsequent measurements were carried out in March/April, May (both during supplementation) and September 1998 (after supplementation had ceased) to track intake relative to growth performance and intake of supplements.

Intake estimates for the Jan/Feb, March/April and May trials are presented in Table 2.4. It is important to emphasise that these estimates are made with the assumption that intake is of 100% tagasaste as reanalysis of these data using Canonical Variance analysis has yet to be completed. Whilst this is a fair assumption for the January and March/April trials (ie. end of the dry summer; no inter-row pasture remaining) this will not necessarily be the case for the May estimates. These latter estimates were made after the season break, so it is likely that appreciable inter-row pasture would have been available for consumption. This should, however only affect the magnitude of the intake values at these times, not the relative differences between treatments within a collection time (eg. May). Results for the September trial are not presented as no analyses had been attempted prior to resolving the species selection issues now addressed through Canonical Variance analysis.

The Jan/Feb values in Table 2.4 are not different between the treatment groups ( $P=0.156$ ), which is to be expected since the supplementary feeding regimes had not begun at this time and all animals had access to only tagasaste. For the March measurements, faecal samples were collected 9 and 12 days after insertion of the CRD's and subsequently combined for analysis (collection 1). Further samples were collected on days 19 and 20 and also combined for analysis (collection 2). This procedure was employed with the intention of providing a more representative value for the average dry matter intake of the animals by spreading the measurement over a longer time period (eg. by ultimately combining faeces from days 9, 12, 19 and 20 for analysis). The values presented for Collection 1 in Table 1.4 indicate a significant reduction in tagasaste intake for the high supplement group compared to all others ( $P=0.0001$ ). This is the sort of effect that would be expected as animals substitute grain for tagasaste when consuming a large amount of supplements. It is also interesting to note that the level of tagasaste intake by the Control and Low supplement groups was very similar to those measured in Jan/Feb, whereas both the Medium and High supplement groups had lower intakes in March than Jan/Feb. The results for Collection 2 in March show a similar, although lightly less clear, trend in intake. Again, the high supplement group had a significantly lower intake of tagasaste than the Control, Low and Medium groups however, the Low group was also significantly lower than the Controls. The data for this collection period is less reliable than that for Collection 1 as the CRD's had apparently completed their release of  $C_{32}$  and  $C_{36}$  in some animals by days 19 and 20, despite expectations to the contrary. Although the data from these animals has been removed from the analysis, it is possible that other animals had a declining release of  $C_{32}$  and  $C_{36}$  as the CRD's approached extinction, thereby making some data unreliable. The collection protocol was subsequently modified for future intake trials to allow for three faecal collections between days 12 and 18 after inserting the CRD's to avoid sampling from animals after the CRD's have completed their release.

The samples collected for the May measurement at ‘Dunmar’ were analysed using a compromise protocol, since the samples had been collected before this problem was highlighted. That is, only faecal samples from days 10 and 13 were analysed, since another

set from days 17 and 20 may suffer the same decline in C<sub>32</sub> and C<sub>36</sub> found in the March collection 2 samples. Nevertheless it appears that all levels of supplementation have induced a higher level of forage intake over that by the control (unsupplemented) group. This is reflected in the liveweight gains of these respective groups (see Chapter 3).

Further trials were undertaken in March, June and September 1999 and again with the third batch of animals in March 2000. Unlike in 1998, when the first intake measure was made prior to supplementation beginning so that these data could be used as a covariate in future analyses, this was not possible in 1999 due to tight time restrictions at the start of the trial. Unfortunately some of the faecal samples from the March study were lost in floods which devastated Moora in late March, however the salvaged samples, plus those from the June study have been processed in preparation for data analysis. Supplementation of the animals in this experiment ceased on 15<sup>th</sup> July, so the September intake study was performed to complement those in March and June during supplementation.

**Table 2.4:** Estimates of tagasaste intake ( $\pm$  s.e.) using *n*-alkane methods at 'Dunmar' in 1998 (values are expressed as kg DM/head per day)

| Period                                                     | Control                       | Low                            | Supplement<br>Medium            | High                          | P      |
|------------------------------------------------------------|-------------------------------|--------------------------------|---------------------------------|-------------------------------|--------|
| Jan/Feb '98 <sup>#</sup> (no supplements)                  | 2.36 $\pm$ 0.097              | 2.78 $\pm$ 0.321               | 2.94 $\pm$ 0.146                | 3.05 $\pm$ 0.252              | 0.156  |
| March '98 <sup>#</sup> (during supplementation)            |                               |                                |                                 |                               |        |
| - collection 1                                             | 2.54 <sup>a</sup> $\pm$ 0.165 | 2.81 <sup>a</sup> $\pm$ 0.250  | 2.61 <sup>a</sup> $\pm$ 0.184   | 1.35 <sup>b</sup> $\pm$ 0.170 | 0.0001 |
| - collection 2                                             | 4.04 <sup>a</sup> $\pm$ 0.353 | 2.63 <sup>b</sup> $\pm$ 0.370* | 3.30 <sup>ab</sup> $\pm$ 0.325* | 2.33 <sup>b</sup> $\pm$ 0.232 | 0.003  |
| May '98 <sup>#</sup> (during supplementation) <sup>#</sup> | 3.28 <sup>a</sup> $\pm$ 0.309 | 5.92 <sup>b</sup> $\pm$ 0.589  | 5.97 <sup>b</sup> $\pm$ 0.480   | 5.07 <sup>b</sup> $\pm$ 0.441 | 0.0004 |

Different superscripts within a row denote statistical differences ( $P < 0.05$ ).

\* indicates animals in this group were deleted from data set due to premature CRD release.

# values are based on the assumption that animals ate 100% tagasaste

To accommodate the probable requirement for more plant samples from the experimental sites to enable resolution of the different plant components consumed by the cattle (ie. use of Canonical Variance analyses plus possibility that some species had been missed from the collections). We expended considerable extra effort to take samples of every edible component from every plot in 1999. This resulted in an explosion of samples for analysis, as well as greater complexity in analysing the data.

### 'Cantabilling Springs'

A Produced Initiated Research and Development (PIRD) project, titled "*Growing Cattle Using Tagasaste, Lupins and Silage through Autumn*", was undertaken at the 'Cantabilling Springs' property of Neil Mackintosh near Jurien in April 1998 (Milton *et al.* 2000). As part of this project, which was assessing the effectiveness of three different rations supplying 25% of the animals' nutritional requirements, we undertook to measure the dry matter intakes by the cattle using the alkane technique. The experiment, which was using an alternative supplementary feeding strategy to that being studied in the experiments at 'Dunmar', had cattle grazing tagasaste and being supplemented with either pasture silage, pasture silage plus lupins, or lupins only. The alkane technique should be able to account for intake of silage, however will be unable to detect lupins as they are unlikely to have an alkane profile. As with the 1999 data from 'Tagasaste Farm' and all of the data from 'Dunmar', this data from 'Cantabilling Springs' has yet to be reanalysed using Canonical Variance statistics.

## 2.4 Conclusions

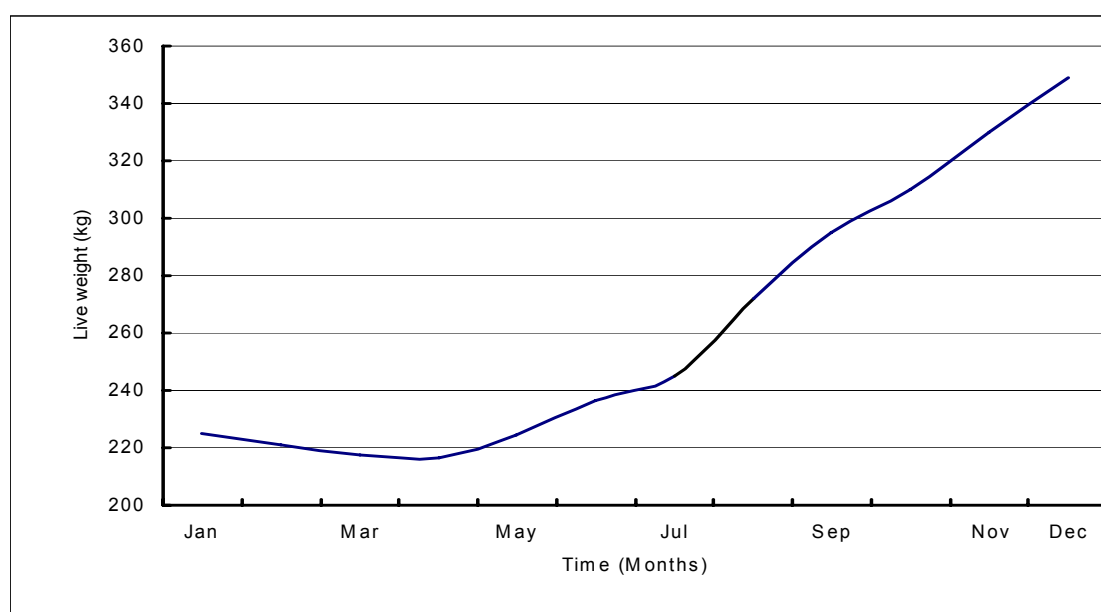
The overall conclusion from these intake studies is that measurement of intake in cattle browsing tagasaste is possible using the alkane technique. Whilst problems were encountered in analysis of the results from these systems these are not unique to a tagasaste system, as similar problems are being encountered on annual pasture environments. The technique is an excellent means of establishing the species composition of intake as well as quantifying that intake in terms of kg dry matter.

## 3 NITROGEN AND ENERGY SUPPLEMENTS TO MAXIMISE THE FLEXIBILITY OF TURN-OFF OF CATTLE BROWSING TAGASASTE OVER THE DRY SUMMER AND AUTUMN PERIOD IN A MEDITERRANEAN ENVIRONMENT

### 3.1 Introduction

The liveweight response of dry weaner cattle browsing tagasaste throughout the year is illustrated in Figure 3.1. The growth of cattle browsing tagasaste is very good over the winter and spring period with daily gains of steers up to 1.5 kg/head per day but over the late summer and autumn weight gains are reduced to maintenance or, perhaps, a small weight loss. The initial production study of cattle browsing tagasaste showed cattle without energy supplements only maintained weight over a 105 day period compared with gains of 0.24 and 0.53 kg/head per day when supplemented with cereal barely grain at 0.75% and 1.5% of body weight [Tudor *et al.* 1997]. In the unsupplemented group, ruminal branch-chain and medium-chain fatty acids, which are the carbon precursors for amino acid synthesis by ruminal bacteria, were lacking. Higher plasma urea-N and creatinine were indicative of endogenous protein mobilisation [Costa *et al.* 1995].

**Figure 3.1:** Liveweight change of cattle browsing tagasaste



Energy supplements readily available in the Western Australia include the cereal grains, oats, barley or wheat and the grain-legume, lupins. In the two regions where tagasaste is most likely to be established, the West Midlands and around Esperance, barley and lupins are likely to be the most cost effective. Energy supplements such as molasses are not economically viable at present because the molasses is imported from Queensland or overseas. With the establishment of a sugar mill or a cotton industry in the Ord it is feasible the price will be lower than current prices because of the closer proximity of the mill; albeit it is still in northern Western Australia. Molasses and cottonseed meal have been very successfully used as supplements with cattle grazing leucaena.

To improve production or increase marketing options of cattle browsing tagasaste through the late summer autumn period a range of supplements have been tested in several trials. This study, which commenced in 1995, includes a series of experiments investigating the liveweight response to protein and energy supplements fed to weaner cattle browsing tagasaste over the summer and autumn period. Those tested were barley [energy supplement], barley plus urea [energy plus a nitrogen source], lupins [energy and protein] and hay [low quality energy and roughage]. Blood and rumen biochemistry were included in the experiments to assist in evaluating the responses to the different supplements. In the generation of the live-weight response curve to supplements data from two other sites have been included. Details from these two sites are detailed at the end of the Material & Methods section.

### ***3.1.1 Hypothesis***

The lack of growth over the summer and autumn period of cattle browsing tagasaste is:

1. Associated with the low concentrations of ruminal volatile acids and nitrogenous constituents, such as ammonia and microbial protein, resulting in poor energy and nitrogen balance and possibly other nutrient deficiencies.
2. Due to inadequate intake of tagasaste

### ***3.1.2 Aims***

In weaned and yearling dry cattle browsing tagasaste:

1. Determine whether supplying appropriate supplements [nitrogen and/or energy] presented as cereal grains or grain legumes will overcome the lack of growth and hence, lead to more acceptable year round live-weight gain in cattle browsing tagasaste.
2. Investigate the live-weight response, ruminal ecology and metabolism and metabolic profile of cattle fed different levels of nitrogen and energy supplements.
3. Investigate the nitrogen and energy requirements of cattle browsing tagasaste over the summer and autumn period.
4. Develop a predictive response curve to N/E supplements which will allow producers to calculate the cost benefit of feeding supplements to meet target

specifications for cattle browsing tagasaste over the dry summer and autumn period.

## 3.2 Material & Methods

### 3.2.1 Experimental Design

Randomised block with four treatments x three replicates with approximately 120 weaner steers or heifers. A series of experiments over a 5-year period investigated the liveweight response in cattle browsing tagasaste to various N and E supplements.

### 3.2.2 Experimental site

#### 'Dunmar'

The nitrogen and energy supplementary feeding experiment was conducted on 55 ha of tagasaste planted at 5-m row spacing in 1988. The property, 'Dunmar' is in the Badgingarra region of Western Australia [Melbourne location 3602, Zone 50, 380139mE, 6638173mN or Lat. 30°24'E, Long. 115°70'S]. 'Dunmar' is approximately 3600 ha with approximately 1600 ha planted to tagasaste with first plantings in 1988. Standard superphosphate was applied annually from 1988 by aerial application at 120 kg/ha per an. The 55 ha of established tagasaste were divided into 12 experimental plots, which varied in area between 3.6 and 5.5 ha. The experimental plots were designed with a holding area at the front of each paddock so cattle could be held after muster and before weighing etc.

#### Cattle

Each year, the experimental cattle were 8-10 month-old steers or heifers weaned in December - January and born on the property to a mixed cross cowherd browsing tagasaste predominantly of Murray Grey or Limousine cows with Murray Grey bulls.

In all the years of experimenting new mobs of weaners were introduced onto the experimental plots in December or early January. Weaners were identified with ear tags and vaccinated with 5 in 1 and drenched with an anthelmintic to control internal parasites. Experimental cattle were weighed and randomly allocated to one of four treatments after stratifying, within sex, on live weight. The mean  $\pm$  s.e. liveweights [kg] for years 1997 – 2000 are shown in Table 3.1.

**Table 3.1:** Initial [ $\pm$  s.e.] liveweights and numbers of steers and heifers in experiments between 1997-'00

|       | Steer |       |            | Heifer |       |            |
|-------|-------|-------|------------|--------|-------|------------|
| Years | n     | kg    | $\pm$ s.e. | n      | kg    | $\pm$ s.e. |
| 1997  | 112   | 247.7 | 2.69       |        |       |            |
| 1998  | 80    | 190.2 | 2.87       | 54     | 176.0 | 2.16       |
| 1999  | 64    | 198.3 | 2.73       | 63     | 199.2 | 2.41       |
| 2000  | 53    | 177.6 | 2.02       | 64     | 176.9 | 2.56       |

Stocking pressure was 2 weaners/ha and was increased with level of grain supplementation to allow for the extra energy from the supplements.

## Treatments

No supplement [Control] or various levels of supplements of either lupin grain, cereal barley, with and without urea, or hay (Table 3.2).

Supplements were fed 3 x week and cattle were weighed every about fortnightly. In the 'Dunmar' study blood and rumen samples from 40 cattle were collected monthly and in the 'Cantabilling Springs' study representative animals were sampled at the end of the experiment.

The 5% urea with 0.5% ammonium sulphate, 1% limestone and 0.5% salt mix [Limiter] has been shown to control intake of cereal grain supplements thus preventing engorgement and ruminal acidosis [May and Barker 1989; Ryan *et al.* 1992] was used to control or regulate the intake of the grain supplement.

**Table 3.2:** Lupin and cereal barley grain supplement intakes [kg DM/head per day] by cattle browsing tagasaste between 1998 – 2000

|      | Control † | Lupin |        |      | Barley   |          |          |
|------|-----------|-------|--------|------|----------|----------|----------|
|      |           | Low   | Medium | High | 0 % Urea | 2 % Urea | Limiter‡ |
| 1997 | 4         | 0.7   | 2.1    | 3.9  |          |          |          |
| 1998 | 4         | 0.9   | 2.6    | 3.5  |          |          |          |
| 1999 | 4         | 1.6   |        |      | 1.6      | 1.6      |          |
| 2000 |           | 1.8   |        |      | 1.9      | 2.1      | 1.2      |

† Cattle browsed tagasaste with no supplements

‡ Limiter: The 5% urea with 0.5% ammonium sulphate, 1% limestone and 0.5% salt mix was used to control or regulate the intake of the grain supplement and control acidosis [May and Barker 1989; Ryan *et al.* 1992].

## Supplements

The cereal barley with virginiamycin [Eskalin, Pfizer]® and  $\pm$  urea supplements and the lupin grain supplements were fed in a loose mix or pelleted form. The cereal barley with Limiter was fed as loose mix. This was done as it is necessary to adapt the cattle to the high urea levels and this was achieved by changing the concentration of urea and ammonium sulphate in the mix every four days [May and Barker 1989]. Lupins and barley were cracked before pelleting or feeding in a loose mix form.

The supplements were fed in self-feeders on Monday, Wednesday and Friday and the cattle were weighed on Tuesdays. At the start of the supplementary feeding all cattle were fed hay to adapt them to the self-feeders and to ensure the animals were not empty and thus over eat when the grain supplements were fed.

The problems of acidosis from rapid or over-consumption [Zorilla-Rios *et al.* 1991] of cereal grains high in starch can be overcome by regulating the rate of consumption of the grain. To control or regulate the intake of cereal grain supplements fed every 2 or 3 days, two different additives were used to the grain supplement prior to feeding.

1. Virginiamycin included at the recommended concentration of 20 ppm in a loose mix or pelleted.

Five % urea, 0.5% ammonium sulphate, 1% limestone and 0.5% salt [Limiter]. This system relies on the high urea and ammonium sulphate making the grain carrier relatively unpalatable so animals only eat small quantities. The cattle were adapted to the high urea by changing the concentration of urea and ammonium sulphate in the mix every four days [May and Barker 1989].

### ***3.2.3 Tagasaste edible feed-on-offer [FOO]***

The quantity and quality of tagasaste and inter-row pasture were assessed monthly. This was done by visually estimating quantity of edible leaf material on the tagasaste and inter-row pasture and weed. To calibrate visual estimates with actual FOO a number of tagasaste bushes were visually estimated then stripped to establish prediction equation between visual estimate and actual DM yield [Chapter 4: FOO]. The pasture predictions were calibrated by cutting a number of quadrates after visual estimates.

### ***3.2.4 Measurements***

Unfasted individual liveweight was recorded about fortnightly and blood and rumen liquor was collected from the same 40 animals [10/treatment] every month. Daily gains were calculated by regressing liveweight against time. Supplements were fed every 2 or 3 days in self-feeders and once per week feeders were cleaned of any residue. Residues were weighed and dried to allow calculations of dry matter fed each week. The blood was taken with lithium-heparinised vacutainer from the jugular vein with a one-inch, 18-gauge needle. The blood was centrifuged at 3000 rpm for 15 minutes with plasma pipetted equally into two x 5-ml tubes using disposable Pasteur pipettes and were frozen prior to analysis. Rumen samples were collected at monthly intervals by inserting a tube of 20 mm ID into the rumen via the oesophagus with the sample drawn by a manually operated pump attached to the tube. The rumen wall was massaged while sampling to ensure mixing of the rumen contents within the rumen cavity.

### ***3.2.5 Statistical Analysis***

The data from this trial have been analysed by fitting a variance component model using residual maximum likelihood [REML]. This technique allows for two variance components to be estimated, in this case variance between animals and variance between plots, with unequal numbers of animals or plots for the treatment effects. In particular, the numbers of animals of each sex were unequal in each paddock. The effects that were examined for significance were effects of level of supplementary feed [Suppl], Sex and interactions between level of supplementary feed and sex.

### ***3.2.6 Data from other studies used in the generation of the response curve.***

Data from two other sites have also been used in the generation of the liveweight response curve to the N/E supplements. These sites were:

- (a) "Tagasaste Farm": Two paddocks with at least 100 weaners per paddock [Commercial feeding]

One hundred and ninety predominantly Brahman cross steers from the northwest of WA with average liveweight [ $\pm$  s.e.] of  $282 \pm 1.5$  kg were backgrounded between November and April to reach target liveweights for the live export trade. The cattle were fed lupin grain supplements at either 1 or 2 kg lupin grain/head per day.

- (b) 'Cantabilling Springs': Four paddocks with four treatments with 80 Brahman cross steers [PIRD].

European infused Brahman cross steers aged about 16 months with average liveweight of  $346 \pm 2.03$  kg browsed tagasaste with supplements of; i] no supplement [Control], ii] 2 kg lupin grain, iii] 1 kg lupin grain + 4.5 kg silage, or iv] 9.0 kg silage. The supplements were calculated to supply 25% of their metabolisable energy requirement to support liveweight gains of 1 kg/head per day [Milton *et al.* 2000].

### 3.3 Results & Discussion

#### 3.3.1 Animal health

1997: Two steers fed lupin supplements died; the 1<sup>st</sup> was fed the High level of supplements and died from acidosis only about 2 weeks after starting the supplements. The 2<sup>nd</sup> animal was fed the Low level of supplements but it was not possible to post mortem the animal as it was decomposed.

1998: There were no deaths or health problems in 1998.

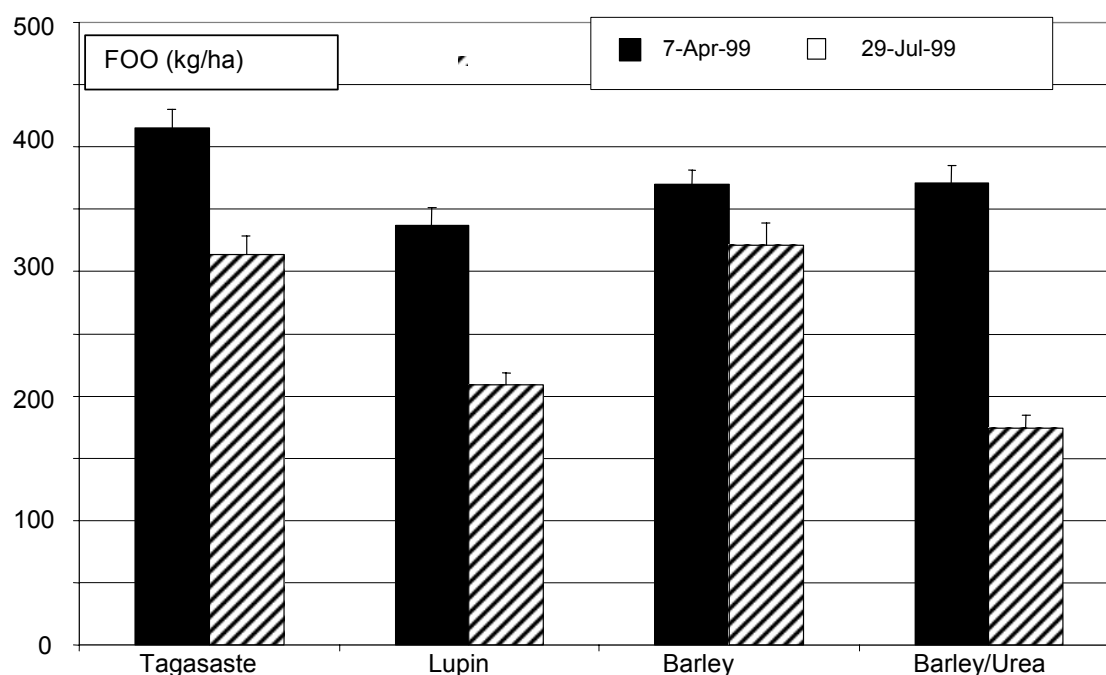
1999: Three animals died in 1999. Two were fed 2 kg/head per day of a cereal barley + 2% urea and died from acidosis even though virginiamycin was included in the mix. One animal from the lupin treatment died, possibly from acidosis.

2000: Three animals died in 2000. Two were fed lupin grain at 2 kg/head per day and died from acidosis [Allen *et al.* 1998] and the third was fed barley with Limiter. It was not possible to post mortem the third animal, as the body was too decomposed when it was found. The four stages used to adapt the animals to the high urea and ammonium sulphate had been completed so death could have resulted from urea poisoning or from acidosis. There was no other health problems during the year.

#### 3.3.2 Feed on Offer and quality of tagasaste biomass

During the late summer and autumn months there was no pasture in the inter-row. Pasture during the winter and spring consisted mainly of weeds such as capeweed and some annual grasses. However, the total FOO at 'Dunmar' never approaches the productive dry matter load in terms of kg per hectare that is observed in annual pasture ie. 2000 kg/ha [Figure 3.2]. Nevertheless, cattle do gain live weight during July when provided solely with tagasaste even though the FOO is estimated at less than 500 kg/ha. In fact the July biomass as indicated by FOO is significantly less than that assessed in April, when cattle are only maintaining live weight on tagasaste. Therefore, it is difficult to support the notion of FOO as a useful index for stocking rate or productivity of tagasaste [or possibly all tree legumes] from this data.

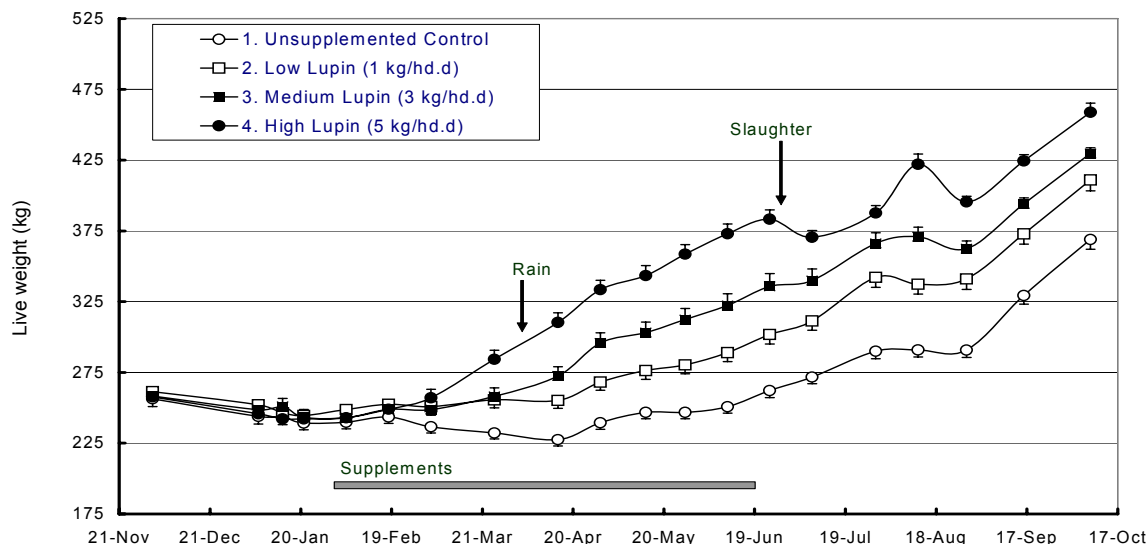
**Figure 3.2:** Food-on-Offer estimates of tagasaste plots at 'Dunmar'



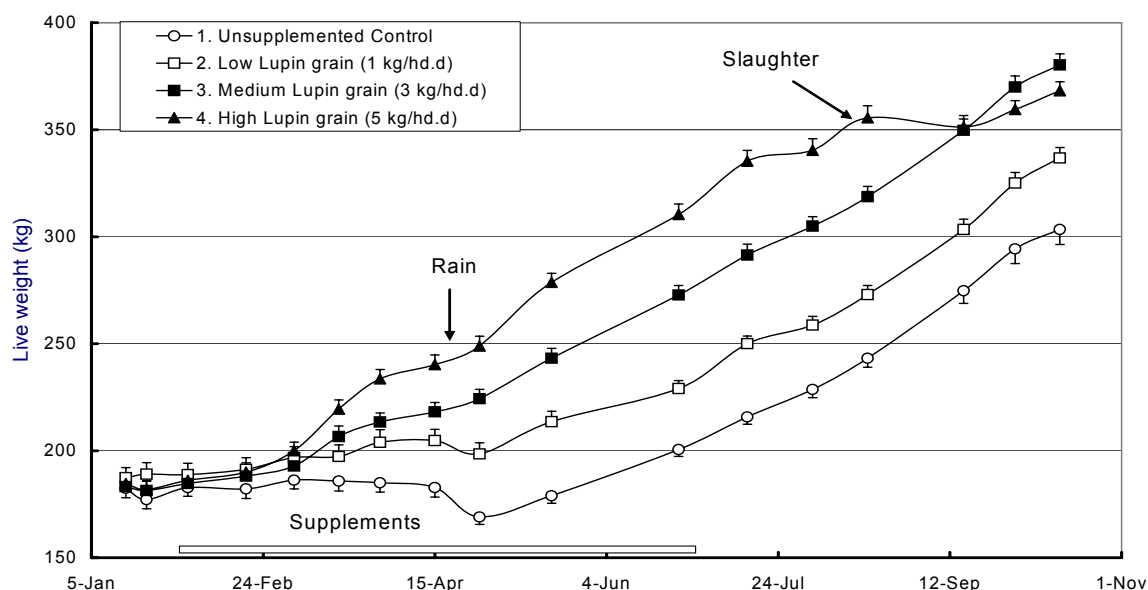
### 3.3.3 Liveweight change

The liveweight response to feeding supplements is illustrated in Figures 3.3, 3.4, 3.5, and 3.6 for trials in 1997, 1998, 1999 and 2000 respectively. In 1997, the supplements consisted of lupin grain fed at three different levels, 1, 3, and 5 kg/head per day and were fed from the end of January to the 19<sup>th</sup> June. Cattle that had reached slaughter weights were removed from the plots after the 19<sup>th</sup> June, leading to a dip in the mean live weights for that treatment group [Figure 3.3]. The response to lupin supplements was repeated in 1998, including removing cattle at slaughter weights [Figure 3.4]. When lupin grain was fed as a supplement there was a linear response in the liveweight gain to level of supplement fed with cattle fed 0, 0.7, 2.2 and 3.4 kg/head per day gaining 0.25, 0.38, 0.66 and 0.85 kg/head per day. The utilisation of the lupin grain supplement was excellent with feed conversions of the supplements at 1.9, 3.3 and 4.0 kg supplement per kg feed. These responses are better than that recorded in feedlots. The higher level of supplementation resulted in 13 out of 26 cattle fed the high levels of supplements reached target slaughter weight for the domestic market by June 25<sup>th</sup> 1997, and 28<sup>th</sup> August 1998.

**Figure 3.3:** The liveweight change of cattle browsing tagasaste with three levels of lupin grain supplements [1997]



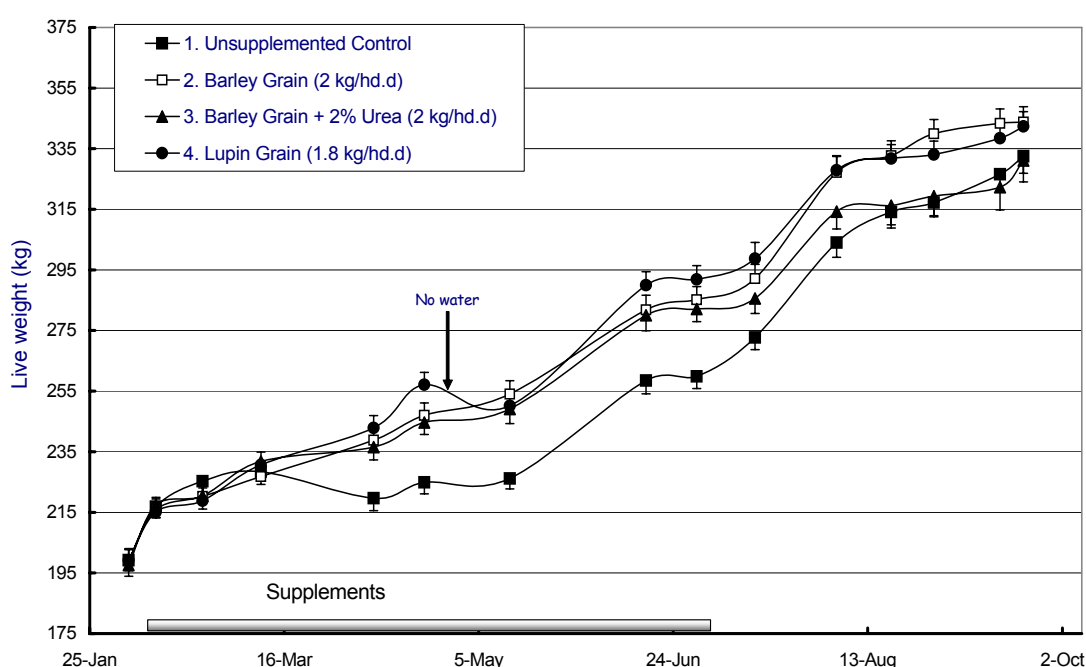
**Figure 3.4:** The liveweight change of cattle browsing tagasaste with three levels of lupin grain supplements [1998]



The rainfall in 1999 was 140% above the long term average with the months of March and May particularly heavy. This rain pattern would have a marked influence on the growth of the tagasaste with new growth starting after the March rain. Cattle like new growth of tagasaste so the response to the supplements may be different to other years. In 1999 barley grain, with either 2% or 5% urea in the Limiter system as extra nitrogen, were compared with lupin and the unsupplemented control treatment. This was to test the hypotheses that, i] the protein in the tagasaste leaf material was sufficient for animals to gain weight and that the animal was only deficient in energy for good liveweight gains or, ii] that protein in the tagasaste leaf was insufficient and extra nitrogen was required. The average daily gain

during the supplementary feeding period for the Control, barley with 2 or 5% urea or lupins were 0.37, 0.50, 0.43 and 0.55 kg/head per day, respectively. The supplement intakes to support these daily gains were all 1.6 kg DM/head per day. The lower than expected response to the lupin supplement is probably due to severe dehydration as a result of lack of water for 2 - 3 days as a result of break in the water line. Fortunately the temperature at the time was only about 27°C so no deaths were recorded but the poorer growth of the lupin supplemented cattle after the incidence suggests they had been severely dehydrated with long term effects on their ability to grow. Prior to water shortage the cattle fed the lupin supplements were starting to kick head of those cattle fed barley with 2 or 5% urea [Figure 3.5].

**Figure 3.5:** The liveweight change of cattle browsing tagasaste with lupin grain, cereal barley grain, with and without, 2% urea or 5% urea, 0.5% ammonium sulphate, 1% limestone and 0.5% salt [1999]



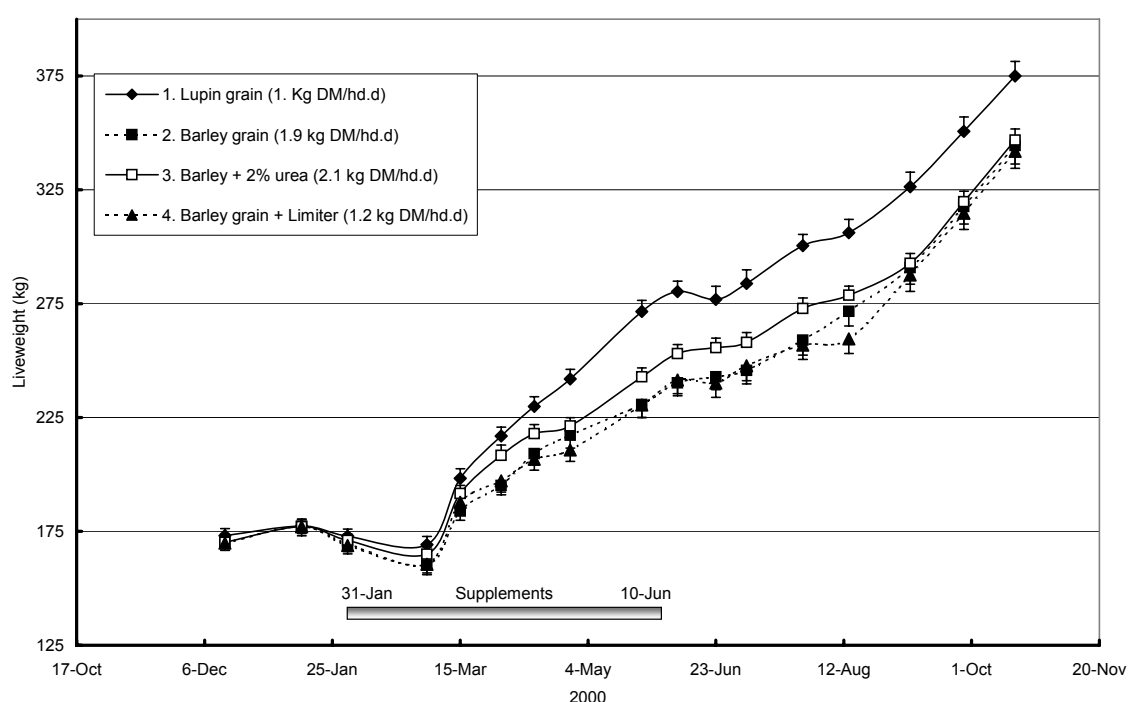
In each of these four years of trials, cattle in the unsupplemented groups feeding on tagasaste alone followed the same pattern of liveweight response, ie. only maintained or lost a small amount of weight depending on the timing of the break of season in each year. It is important to note that unsupplemented cattle did in fact lose weight after rain suggesting that either the cattle chased 'green pick' in the interrow or the tagasaste changed in palatability during this time. Nevertheless, once tagasaste commenced its winter and spring growth, the live weights of the cattle increased [Figures 3.3, 3.4, and 3.5]. Cattle in the unsupplemented Control treatment exhibited compensatory growth, which was sufficient to enable the cattle to catch up with the barley-supplemented group from August onwards [Figure 3.5].

In 2000, the trial did not include a control, unsupplemented tagasaste group as we had confirmed the pattern of liveweight response clearly in the previous trials. Here the response to lupins [1.8 kg/ head per day] was compared to iso-energetic [2kg/head per day barley] and iso-nitrogenous and energetic [barley per head/day + 2% urea] supplements, and iso-energetic and NPN excess 2kg barley/head per day and 5% urea in the Limiter group]. In

this direct comparison, lupins gain performed better than the barley or barley/urea supplements [Figure 3.6].

During 2000, there were several large rainy periods during the summer eg. mid January and March, and the rain contributed to a slow response to supplements. At the end of the trial period and several months after the end of supplementary feeding the lupin supplemented group sustained its weight advantage but there was no difference between the barley supplemented groups with or without NPN [Figure 3.6]

**Figure 3.6:** The liveweight change of cattle browsing tagasaste with lupin grain, cereal barley grain, with and without, 2% urea or 5% urea [2000]



The liveweight gains show the cattle fed 1.8 kg lupin grain DM/head per day gained weight at 0.93 kg/head per day with FCR of 1.8. This compared with cattle fed 1.9 kg DM barley grain [0.66 and 2.7, respectively], 2.1 kg DM barley + 2% urea [0.71 and 2.7, respectively] or 1.2 kg DM barley + 5% urea [Limiter] [0.62 and 1.1 respectively]. These data show the benefit of plant protein [lupin grain] over the NPN in urea and, or urea and ammonium sulphate although the low intake [not expected] of barley and Limiter makes it difficult to compare with the lupin grain or the barley treatments.

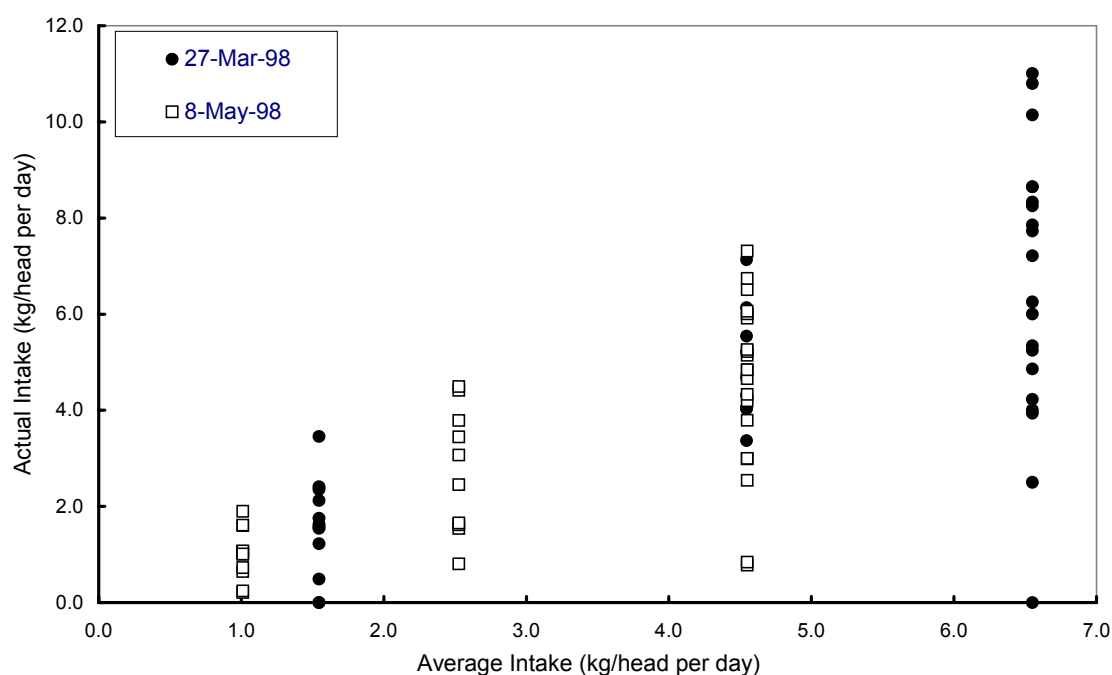
Energy is considered the major driver of growth in cattle, with protein playing a lesser role [AFRC 1993]. There is no indication from the tagasaste analysis or from the lower intakes during summer and autumn [Chapter 2] that protein would be limiting growth in these cattle during any of the trials. Surprisingly the liveweight response has been much greater to protein/energy [lupins] than to energy [barley] in these trials. Moreover, non-protein nitrogen in the form of urea was not as effective as protein-N in lupins. So notwithstanding the protein content of tagasaste, protein is still limiting growth of cattle during summer and autumn. The limitation appears to be a combination of reduced intake, and interestingly a failure of degradation of tagasaste protein that cannot be compensated by nitrogen assimilation post-

ammonia into microbial protein. In fact NPN does not play an effective role as a source of nitrogen over and above that supplied directly as protein-N in barley.

### 3.3.4 Supplement intake

To measure supplement intake by individual animals, lithium chloride [LiCl] marker was mixed with the grain supplements (Kahn 1994) and fed to one replicate of each treatment eight and 15 weeks after starting the supplements. All animals with access to the grain supplement were bled 24 hrs after feeding. Lithium concentration in the blood was determined by atomic absorption spectrophotometry at 670.8 nm using propane/air mixture. The individual intakes measured using LiCl at 'Dunmar' in 1998 [27 March and 8 May] are illustrated in Figure 3.7 and for 1999 [10<sup>th</sup> March and 17<sup>th</sup> June] in Figure 3.8.

**Figure 3.7:** Use of lithium chloride to estimate intake of barley or lupin supplements in cattle browsing tagasaste at 'Dunmar' [1998]

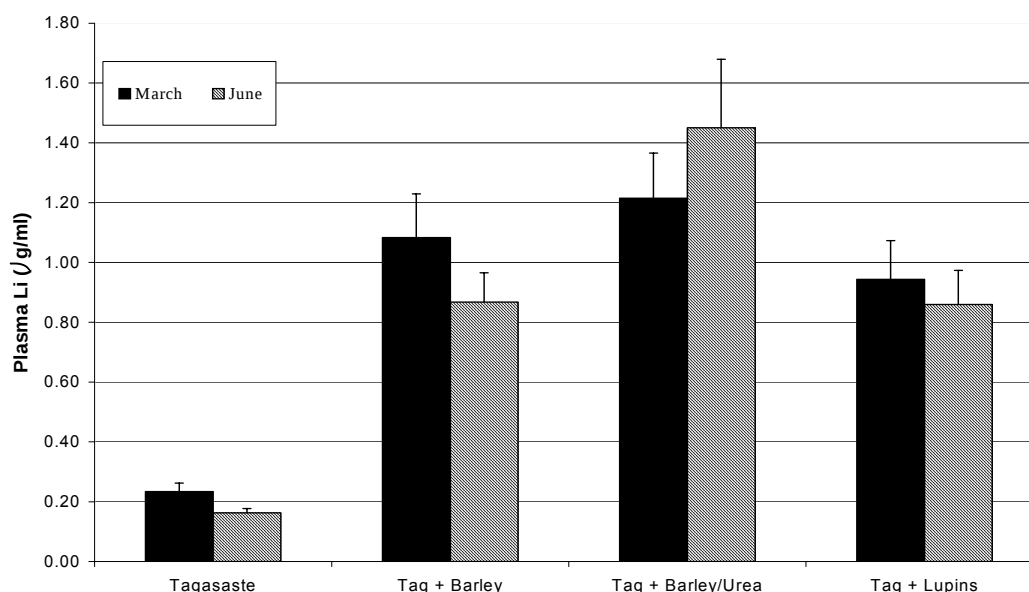


Although the between animal variation was worse early in the supplementary feeding period in 1998 [27 March] compared with later [8 May] the variation was still extreme and questions the relevance or validity of the average intakes calculated by dividing total daily consumption by number of animals. These data confirm views that even though all animals may have access to feed and time to eat and consumption is not necessarily the same for all animals.

Supplement intake by individual animals, measured using LiCl, are presented as mean plasma Li values on the 10<sup>th</sup> March 1999 and 17<sup>th</sup> June 1999 [Figure 3.8].

The low level of lithium recorded in the cattle browsing tagasaste is background noise. The variation in lithium levels in the cattle fed the supplements would be the variation in intake of the various supplements at the two times of sampling.

**Figure 3.8:** Plasma lithium as an indicator of intake of barley or lupin supplements in cattle browsing tagasaste at 'Dunmar' [1999]



### 3.3.5 Supplement response graph

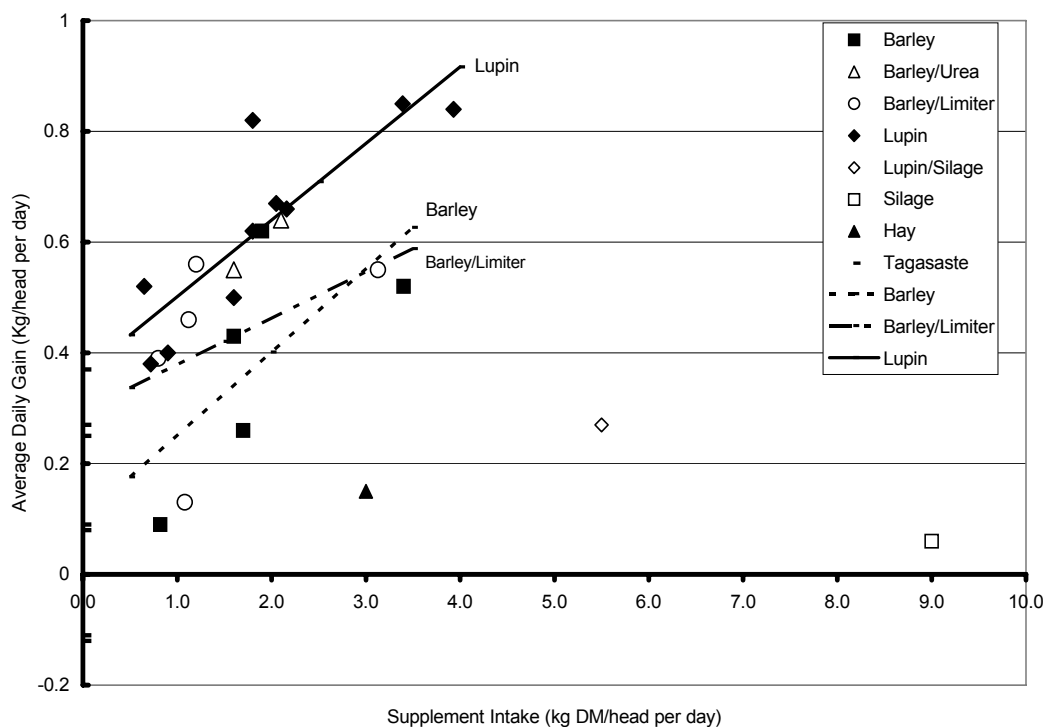
A producer with cattle browsing tagasaste over the summer and autumn period may see the opportunity of targeting a live export ship or feedlot but needs to ensure the weights of the animals will reach the specified market liveweight. This experiment provides the necessary information to allow calculation of how much grain, and therefore the cost of the exercise, to achieve a desired target liveweight.

Supplementary feeding can be defined as providing one or more nutrients to enhance the nutritional adequacy of a daily ration. In this case the rumen microbial population were supplemented with energy and/or protein or NPN to assist in the digestion of the ingested feed.

The data used in the response graph [Figure 3.9] is taken from replicated experiments at 'Dunmar', a non replicated Producer Initiated Research Demonstration [PIRD] at 'Cantabilling Springs' and a Department of Agriculture WA non replicated trial with producers cattle at 'Tagasaste Farm'. The data from these experiments are used in a response chart where average daily gain [ADG] is related to intake of supplements [Figure 3.9] and shows the large variability when feeding the different supplements. The best-fit trendline for Lupins, Barley and Barley with Limiters are illustrated in Figure 3.9 to show the response to lupins and that the response to Barley supplements is dependent on the level of N and energy in the supplement. There were insufficient data points to calculate a best-fit trendline for the Barley with 2% urea but the responses were similar to the response to lupin supplement.

These equations provide beef producers with the tools to calculate a cost benefit of supplementing cattle browsing tagasaste over the dry summer and autumn period to target specific market specifications. The next phase is to write a FarmNote that includes this cost/benefit tool and provides instructions to farmers on how to apply this tool to their particular set of circumstances.

**Figure 3.9:** The relationship between DM intake [kg DM/head per day] and average daily gain [kg/head per day] in cattle browsing tagasaste with a range of N and Energy supplements



The relationship between supplement intake and growth rate were described in the equations:

$$\text{Lupin} - \text{ADG [kg/head per day]} = 0.138 \text{ DMI [kg/head per day]} + 0.363 \quad [R^2 \text{ 0.73}]$$

$$\text{Barley} - \text{ADG [kg/head per day]} = 0.150 \text{ DMI [kg/head per day]} + 0.10 \quad [R^2 \text{ 0.45}]$$

$$\text{Barley/Limiter} - \text{ADG [kg/head per day]} = 0.084 \text{ DMI [kg/head per day]} + 0.30 \quad [R^2 \text{ 0.76}]$$

The slopes of the lupin and barley lines are similar but are very different to the slope of the Barley Limiter line. At lower intakes the response to the Barley Limiter supplements is similar to response to the lupins suggesting the initial supply of N, either protein – N or NPN, is sufficient to provide the necessary requirements of the microbial population. However, as more N is supplied to the microbial population the response shows that the microbial population performs better with protein – N than NPN. The greater liveweight responses to protein rather than energy supplements reported here is similar in type [ie. protein meal greater than grain] and scale [peaking at 1 kg/hd.d] to that predicted by McLennan *et al.* [1995].

The silage, lupin/silage and hay data are not close to any of the grain or lupin points. The silage and lupin/silage data are from the ‘Cantabilling’ Experiment where the liveweight of the Brahman cross steers were at least 100 kg heavier than the steers and heifers used in the other experiments. When supplements such as silage or hay were used the cattle consumed the roughage supplements in preference to the less palatable tagasaste.

The influence supplements have on the performance of the cattle and tagasaste intake can be seen in Table 3.3. In this table, data from the 1998 lupin supplement experiment at 'Dunmar' are used to compare tagasaste intake measured using alkane technology [Chapter 2, Table 2.4] and the predicted intakes using GrazFeed version 4.1.3. The data used in the GrazFeed model is that recorded during the period when supplements were fed.

**Table 3.3:** Tagasaste intake measured with alkanes or predicted using GrazFeed and performance of cattle browsing tagasaste with four levels of lupin supplements

|           |     |                 |                 | Predicted total intake | Tagasaste DM Intake |          |            |
|-----------|-----|-----------------|-----------------|------------------------|---------------------|----------|------------|
| Treat's   | LW  | Growth rate     | Supplement DMI  | GrazFeed               | Alkane              | GrazFeed | Difference |
|           | kg  | kg/head per day | kg/head per day | kg/head per day        | kg/head per day     |          |            |
| Control   | 206 | 0.21            | 0               | 3.4                    | 3.3                 | 3.4      | 0.1        |
| Lupin –L  | 224 | 0.37            | 0.7             | 4.1                    | 2.7                 | 3.4      | + 0.7      |
| Lupin – M | 245 | 0.69            | 2.2             | 3.7                    | 3.0                 | 1.5      | - 1.5      |
| Lupin – H | 263 | 0.92            | 3.4             | 4.7                    | 1.8                 | 1.3      | - 0.5      |

LW is the average liveweight of the cattle during the supplementary feeding period.

Growth rate is the rate of gain calculated by regressing LW against time when the supplements were fed.

Predicted Total Intake from GrazFeed using DM%, ME and CP analysis of the supplements and tagasaste and the average LW and performance of the cattle.

Alkane intake is the average of the two collections measured in March [Table 2.4].

Tagasaste intake calculated from GrazFeed is the total intake minus the supplement intake.

The Difference in Tagasaste intake is the predicted Tagasaste intake minus the calculated tagasaste intake.

When the average liveweight and the growth rate was included in the GrazFeed prediction the intake of tagasaste measured with the alkane technology [Chapter 2, Table 2.4] and the predicted intake using GrazFeed were very similar and only differed by 3%. However, when the lupin supplements were fed the predicted intakes of tagasaste using GrazFeed were significantly different. In the case of the Low level of supplementation GrazFeed predicted the cattle would eat 26% more tagasaste than actually measured using alkanes. If we accept the accuracy of GrazFeed to predict intake [Control treatment] then the good growth of 0.37 kg/head per day with 2.7 kg tagasaste and 0.7 kg Lupin/head per day shows the N and energy have improved the efficiency of fermentation of the rumen microflora enough to stimulate intake of tagasaste. With the two higher levels of lupin supplementation GrazFeed actually predicted lower intakes of tagasaste than actually measured using the alkanes.

The average daily gain, both during the supplementary feeding period and post feeding, along with the slaughter data of 89 of the 112 cattle and the cost benefit [\$ carcass value – cost of supplements] of feeding lupins during the dry summer and autumn is presented in Table 3.4.

The data shows the cattle supplemented with the Medium and High levels of lupin grain had significantly higher returns than those fed a low level of supplement which was higher than the returns for cattle browsing tagasaste with no supplement. The post-feeding supplements period shows the cattle browsing tagasaste with no supplements gained weight at similar rates as cattle that had been fed supplements. The very good gains recorded with animals that had been fed the high levels of supplements was recorded with only 7 seven animals as the other cattle had been slaughtered toward the end of the supplementary feeding period. The slaughter data shows that carcasses of the cattle fed the high level of supplements were higher [\$2.11/kg carcass weight] compared with those fed the Medium level of supplements

[\$1.85], Low [\$1.43] and no supplements [\$1.33]. This variation is mainly due to the time of year when the cattle were slaughtered. In the 90's in WA there was a price premium for any cattle slaughtered between the months of May – July, early August which is simply a supply and demand scenario, ie. there were not a lot of cattle around during those months and this made feedlotting an economic proposition. In the tagasaste experiment the High supplement cattle gained sufficient weight that they satisfied the weight and fat specifications for the domestic trade. The majority of the High treatment cattle were slaughtered in June and early August.

**Table 3.4:** The liveweight, intake and slaughter data of cattle browsing tagasaste and supplemented with four levels of lupin grain [1997]

|                                                        | Control                   |                           | Lupin                     |                          |
|--------------------------------------------------------|---------------------------|---------------------------|---------------------------|--------------------------|
| Supplement Kg DM/head per day                          |                           | Low<br>0.7                | Medium<br>2.1             | High<br>3.9              |
| Initial LW [kg]                                        | 239                       | 245                       | 243                       | 242                      |
| Ave. daily gain when supplements fed [kg/head per day] | 0.27                      | 0.52                      | 0.67                      | 0.84                     |
| LW end of supplements [kg]                             | 290 <sup>a</sup>          | 342 <sup>b</sup>          | 366 <sup>bc</sup>         | 388 <sup>c</sup>         |
| Daily gain post supplements [kg/head per day]          | 1.15 <sup>a</sup><br>[28] | 1.02 <sup>a</sup><br>[24] | 1.20 <sup>a</sup><br>[19] | 1.57 <sup>b</sup><br>[7] |
| Final LW [kg]                                          | 435                       | 444                       | 430                       | 423                      |
| Hot Standard Carcase [kg]                              | 210.6 <sup>a</sup>        | 221.0 <sup>ab</sup>       | 224.1 <sup>b</sup>        | 221.7 <sup>ab</sup>      |
| P8 Backfat [mm]                                        | 6.3                       | 7.0                       | 6.1                       | 7.3                      |
| Dressing [%]                                           | 48.6 <sup>a</sup>         | 49.9 <sup>a</sup>         | 52.2 <sup>b</sup>         | 52.5 <sup>b</sup>        |
| Price [\$/kg HSCW]                                     | 1.33                      | 1.43                      | 1.85                      | 2.11                     |
| Value carcase [\$]                                     | 281 <sup>a</sup>          | 317 <sup>a</sup>          | 413 <sup>b</sup>          | 466 <sup>c</sup>         |
| Supplement intake [kg DM/head per day]                 | 0                         | 0.7                       | 2.1                       | 3.9                      |
| Total supplement [kg AD†/head per day]                 | 0                         | 143                       | 440                       | 697                      |
| Supplement cost‡ [\$/head]                             | 0                         | 23                        | 70                        | 111                      |
| Return [\$/head]                                       | 281 <sup>a</sup>          | 294 <sup>ac</sup>         | 343 <sup>bcd</sup>        | 355 <sup>bd</sup>        |
| Benefit of feeding supplement [\$/head]                |                           | 14                        | 62                        | 75                       |

Means in same row with different superscripts are significantly different at least at  $P < 0.05$ .

Figures in brackets in the daily gain row are number of animals contributing to data.

† Air dry [90% dry matter]

‡ Price of lupins [2001] \$160/t

In the economic benefit calculation the price for lupins was \$160/t which was the cost the year the experiment was conducted. The prices for beef are currently significantly higher than the prices paid in 1997 but there has also been a move away from peak prices between May and early August as there are more cattle available during these periods with the increase in feedlotting in the WA. The price for lupins is currently \$275/t. If the value of the carcase were \$3.50/kg HSCW then the Low, Medium and High lupin supplement treatments would have lost \$3, \$74 and \$153, respectively. If there was a 30<sup>c</sup>/kg carcase benefit to the supplement treatments then the cost benefit to feeding the supplements would be + \$ 60 for the Low level of feeding but the two higher levels would have lost \$11 and \$90 per carcase. Even though when calculating return costs of labour, distributing grain etc were not included it would appear that the benefits of feeding supplements have changed over the years. In the 90's feeding supplements with the aim of slaughtering mid-year would appear to be very beneficial but this need to be reassessed with the current high price of supplements and the more even spread of prices paid for beef across the year.

### 3.3.6 Tagasaste Beef Quality

There is no information on the eating quality of beef from cattle browsing tagasaste. Significantly however, beef was being supplied to the local domestic market from cattle grown on tagasaste with no adverse comments. Not long after the commencement of the project started anecdotal comments were passed “that meat from cattle browsing tagasaste was not fit for the drover’s dog”. This prompted action with the plan to establish a series of public “tasting” sessions at rural shows and exhibitions.

Striploins from experimental cattle in the tagasaste project were purchased when cattle were slaughtered and these meat samples were compared with beef purchased from grain and pasture finished beef. These meat samples were offered for tasting at following venues:

1. Dowerin Agricultural & Machinery Show
2. Jerac, Agriculture Research Expo “Focused on the Science of Farming”, Jerramungup
3. Farming 2000, Dandaragan
4. Moora Show.
5. Mingnew

The striploins were vacuum-packed and aged for 14 days and then cooked on a high-heat BBQ. Participants were asked to rate the samples for tenderness [6 point scale], flavour [6 point scale] and then to indicate the sample they preferred in a single-blinded test. A total of 1,256 samples were tested over the 5 venues.

Before commencing the public meat testing a pre-testing taste trial was conducted at the Departments Head Office in South Perth with aged Striploins from beef finished on tagasaste or Lotfed on high grain diets. The results of 14 panellists that had previously been involved in “taste” tests are presented in Table 3.5.

**Table 3.5:** Tenderness results ( $\pm$  SEM) and pH of beef tested in Perth by a trained panel prior to the Dowerin Field Day.

|                  | Sample A  | Sample B  | Sample C  | Sample D  |
|------------------|-----------|-----------|-----------|-----------|
| Treatment        | Tagasaste | Tagasaste | Grain-fed | Grain-fed |
| Tenderness score | 5.43      | 5.07      | 5.21      | 5.57      |
| SEM.             | 0.137     | 0.305     | 0.214     | 0.172     |
| pH               | 5.59      | 5.58      | 5.63      | 5.59      |

The grand mean results of for the tagasaste and grain-fed beef were 5.25 and 5.39, respectively. These results show the beef from steers finished on tagasaste not distinguishable from grain-fed beef, which is considered to be the most reliable way of producing tender beef.

The results of a “tasting” test of beef from cattle browsing tagasaste or lot-fed on high grain diets at the Dowerin Machinery Show with 406 people are presented in Table 3.6. The “tasters” were asked to score the meat samples on tenderness, flavour and preferred.

**Table 3.6:** Tenderness results ( $\pm$  SEM) and pH of beef tested on the general public at the Dowerin Field Day.

|               | Tenderness |        | Flavour   |        |
|---------------|------------|--------|-----------|--------|
|               | Tagasaste  | Lotfed | Tagasaste | Lotfed |
| Total numbers | 401        | 406    | 402       | 386    |
| Score         | 5.26       | 5.10   | 5.23      | 4.98   |
| SEM           | 0.042      | 0.046  | 0.043     | 0.047  |

In the preferred rating 256, or 61% preferred beef from tagasaste compared with meat from the grain-fed beef, 162. Similar results were recorded at the other venues and show that meat from tagasaste is as good as any beef and the anecdotal evidence that “the meat was not fit for the drover’s dog” was obviously an isolated incidence. A frequent comment at the “tasting” sessions was that meat from tagasaste beef had a stronger flavour than the meat from the grain-fed beef. This could be considered to be an attribute for tagasaste beef in much the same way as “gamey” flavoured meat.

### **3.3.7 Rumen Biochemistry**

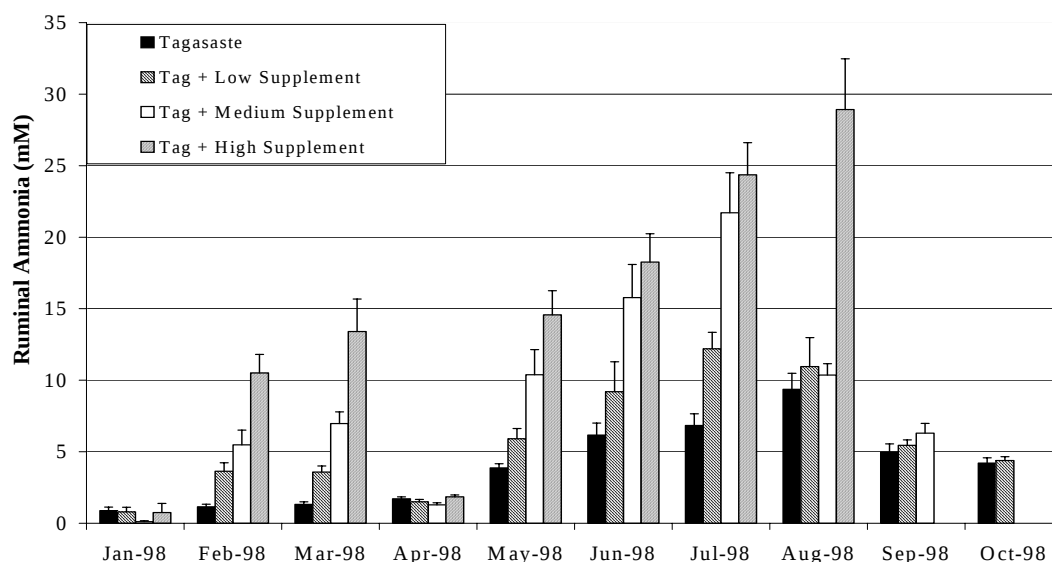
Tagasaste contains relatively high concentrations of protein throughout the year; especially given that 14% protein is considered adequate for growth and 10% adequate for maintenance in cattle. In fact the protein content of tagasaste did not decline below 14% at all of our sites at any month of the year. On this basis, tagasaste should provide sufficient protein for growth and cattle browsing tagasaste should not respond to protein supplements unless there is a failure of protein intake or protein ruminal and systemic metabolism.

Protein in the diet of ruminants is degraded to peptides and amino acids by a limited number of species of ruminal bacteria with protease and peptidase activity. Peptides and amino acids are degraded rapidly by bacterial peptidases and deaminases to ammonia. The level of branch-chain VFA’s in the rumen fluid is an index of the amino acid degradation in the rumen. The concentrations of proteins, peptides and amino acids are very low in the rumen fluid.

Ruminal ammonia concentration is one of the major indicators of protein degradation in the rumen. Ruminal ammonia is a key intermediate in the pathways of protein degradation in the rumen, standing at the cross-over point between degradation of dietary protein and non-protein nitrogen, and its assimilation into microbial amino acids and subsequently protein. Moreover, the ammonia pool in the rumen is relatively small and turns over rapidly. Consequently the amount of ammonia in the pool can vary greatly. This is reflected in a wide range of ruminal concentration from the barely detectable, to what is a consensus of optimum [about 3 – 5 mM] to the overtly toxic [greater than 50 mM].

In cattle browsing tagasaste, ruminal ammonia increased in a dose-dependent manner with increasing level of supplementation of lupins [Figure 3.10].

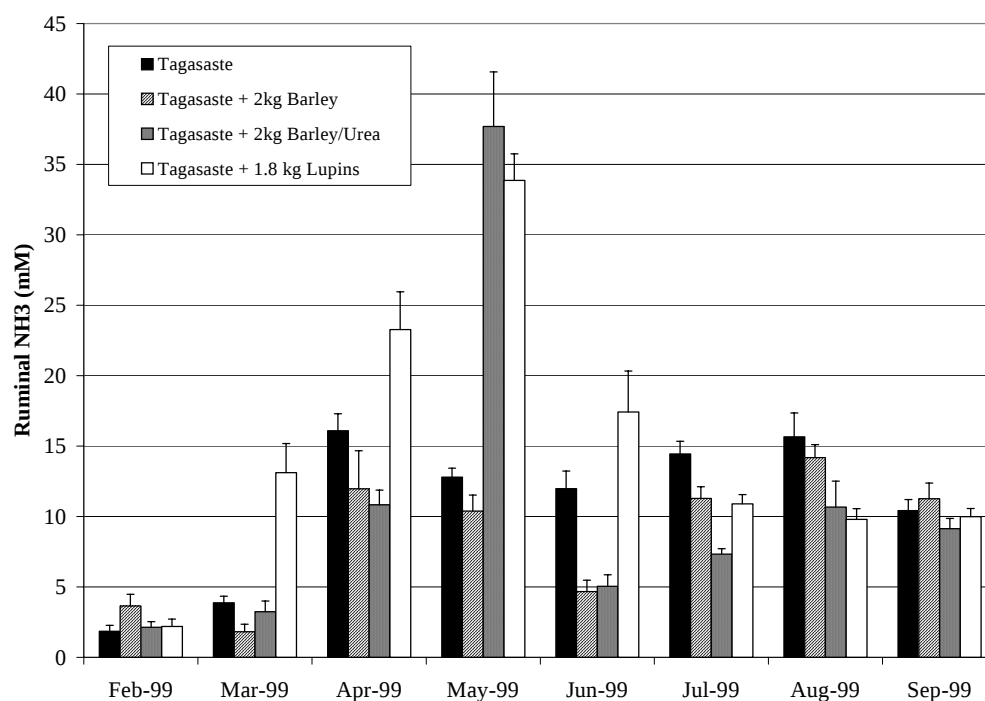
**Figure 3.10:** Ruminal ammonia concentrations in cattle browsing tagasaste and receiving lupin supplements at 'Dunmar' in 1998



This shows that cattle were both eating the lupins and readily degrading the lupin protein to ammonia in the rumen. That is, there was no impediment to appetite or to fermentation by rumen bacteria. The very low rumen ammonia concentration in April is due to not being able to adhere to the normal feeding and weighing schedule. The cattle were fed the supplements two days before the sampling day rather than the usual practice of one day. This resulted in the supplements being completely consumed the day before the sampling, and therefore the ruminal ammonia did not accurately reflect the lupin intake [Figure 3.10].

Ruminal ammonia was below optimum in cattle browsing tagasaste during the summer and autumn; ie. < 3 mM and did not increase to optimal levels until after the break-of-season when this group of cattle began to increase in liveweight. These levels do not support optimum incorporation of N into microbial protein, and would be associated with sub-optimal protein supply during these periods of the year. In contrast those cattle receiving the highest supplementation rate grew at the fastest rate and had the highest ruminal ammonia concentration. In fact there was a strong association between the rate of increase in liveweight in cattle from the lupin-supplemented treatment and the treatment-related ruminal ammonia concentrations. Ruminal ammonia in cattle receiving the highest level of supplements was extremely high [greater than 15 mM; Figure 3.10] in the winter months of June through to August. These cattle may have been consuming large amounts of tagasaste as well as the full supplemental rate of lupins during these periods to reach such high levels of ammonia in the rumen.

**Figure 3.11:** Ruminal ammonia concentrations in cattle browsing tagasaste and receiving either Lupin, Barley, and Barley/Urea supplements at 'Dunmar' in 1999



In the 1999 trial at Dunmar, ruminal ammonia concentrations in cattle receiving lupin supplements was significantly [ $P < 0.001$ ] increased at all samplings compared with cattle in all other treatments groups except for the May sampling of the barley/urea group for the period of supplementation i.e. up to the end of June [Figure 3.11]. This further support for the fact that ruminal ammonia appears to be a good indicator of performance in cattle receiving lupin supplements during the summer/autumn period. Ruminal ammonia concentrations in cattle receiving barley plus urea supplements were not significantly elevated relative to cattle in the tagasaste treatment except for the May sample [Figure 3.11]. This is in contrast to the elevated ruminal ammonia concentration in cattle receiving barley/urea supplements in earlier experiments [1995]. Barley supplementation did not significantly increase ruminal ammonia concentrations relative to the tagasaste treatment group. However, the liveweight did increase significantly in cattle from both of the barley, and the barley/urea treatments compared with cattle from the tagasaste treatment group. The protein-N from the barley fed at 2 kg/head per day did not contribute to a significant rise in ruminal ammonia.

Ruminal ammonia concentrations in cattle browsing tagasaste increased significantly [ $P < 0.001$ ] in the April sampling after the break-of-season and remained higher throughout the period of this study through to September. There was record rainfall in March and May which would stimulate plant growth and influence intake of tagasaste. During the period of fastest growth of cattle in this treatment group, the ruminal ammonia concentrations were always high, and in fact were often higher than in cattle from the other groups after supplementation had ceased. These elevated concentrations indicate that these cattle were readily eating the available tagasaste, and nitrogen supply was not limiting liveweight gain.

Thus ruminal ammonia appears to be a good indicator of cattle performance on the lupin supplementary regime during the summer/autumn period. Moreover, in the winter and spring continuing the supplementation of lupins results in very concentrations of ruminal ammonia. During this period of the year, protein from the growing tagasaste is effectively degraded to ruminal ammonia with concentration being optimal for microbial synthesis.

Nitrogen metabolism was compared with microbial nitrogen supply in a separate study where fistulated cattle were either browsing tagasaste, or consuming a ration of lucerne hay of similar protein and energy content to the tagasaste or pasture hay *ad lib* [Table 3.6].

**Table 3.5:** Composition of pasture hay, lucerne hay and tagasaste dietary treatments used in tagasaste during late summer/autumn [March]

|                                 | Dietary Treatment |             |           |
|---------------------------------|-------------------|-------------|-----------|
|                                 | Lucerne Hay       | Pasture Hay | Tagasaste |
| Digestible Dry Matter [%]       | 77.8              | 69.7        | 80.89     |
| Metabolisable Energy [MJ/kg DM] | 11.2              | 9.9         | 11.62     |
| Crude Protein [%]               | 19.3              | 10.9        | 16.49     |

Lucerne hay and pasture hay samples were analysed by Independent Lab Services and the Department of Agriculture Bunbury laboratory supplied the figures for tagasaste composition from previous analyses performed on tagasaste [Table 3.5]. The samples were collected from 'Tagasaste Farm' at Lancelin, approximately 50km southwest of the Badgingarra Research Station. Fifteen samples collected in March 1997 were averaged to provide the above values. Since the soil and rainfall at Lancelin is similar to Badgingarra, these results were accepted as representative of the tagasaste composition used in this trial.

**Table 3.6:** Daily amount of metabolisable energy and protein intake in the pasture hay, lucerne hay and tagasaste during late summer/autumn [March]

|                     | Dietary Treatment |               |              |
|---------------------|-------------------|---------------|--------------|
|                     | Lucerne Hay*      | Pasture Hay** | Tagasaste*** |
| ME [MJ/d]           | 78.4              | 72.59         | 69.72        |
| Crude Protein [g/d] | 1351              | 1130          | 989.4        |

\* Lucerne hay dry matter intake was assumed to be 7kg/d for these calculations

\*\*Pasture hay intake was calculated to an average of 12.2kg/d that was the value used for these calculations.

\*\*\*Tagasaste intake was assumed to be 6kg/d DM for these calculations.

In any measure of microbial nitrogen supply it is important to establish intakes as a basis for interpretation of subsequent microbial nitrogen comparisons. The calculated average daily intake of the steers fed pasture hay *ad lib* was 12.2 kg/d. At this level of intake, the daily

supply of metabolisable energy and crude protein was similar for the two dietary treatments. Using the unconfirmed estimate of 6 kg/d DM intake [Edwards *et al.* 1997b], tagasaste appeared to provide slightly lower levels of metabolisable energy and crude protein.

**Table 3.7:** Microbial numbers in the rumen and the estimated microbial nitrogen supply in cattle browsing tagasaste or eating lucerne hay or pasture hay during late summer/autumn [March]

|                                             | Lucerne Hay       | Pasture Hay       | Tagasaste         |
|---------------------------------------------|-------------------|-------------------|-------------------|
| Microbial Numbers [/ml]                     | $8.9 \times 10^9$ | $8.6 \times 10^9$ | $4.7 \times 10^9$ |
| Estimated Microbial Nitrogen Supply [g/day] | $124.4 \pm 20.0$  | $134.1 \pm 19.9$  | $56.4 \pm 8.7$    |

The cattle grazing tagasaste were the only animals to lose a significant amount of weight during the trial. This loss of weight is confounding when compared with the nutritional composition of the tagasaste. This tagasaste had quite a favourable composition of 16.49 % crude protein and 11.62 MJ/kg DM of metabolisable energy, which should maintain weight if the cattle are consuming 4.5 kg DM [ARC 1980]. However, there are a number of anti-nutrients whose concentrations are high during summer/autumn, which may be responsible for the loss of live weight. The concentration of phenolic in tagasaste increases during summer/autumn to approximately 8 % dry matter [McNeill *et al.* 1994]. Phenolic have been associated with a decrease in voluntary intake, resulting from the interaction of the phenolic compounds with salivary proteins causing astringency [Cheeke and Shull 1985; Minson 1990]. These high concentrations may make the tagasaste unpalatable, in which case the cattle may not be eating enough to supply sufficient substrate for the microbes in the rumen to replicate. Tagasaste intake is indeed minimal during summer/autumn [Edwards *et al.* 1997a, Varvikko and Khalili 1993]. Edwards *et al.* [1997a] found that dry matter intake reduced from 25 kg/d during winter/spring to 6 kg/d in summer/autumn.

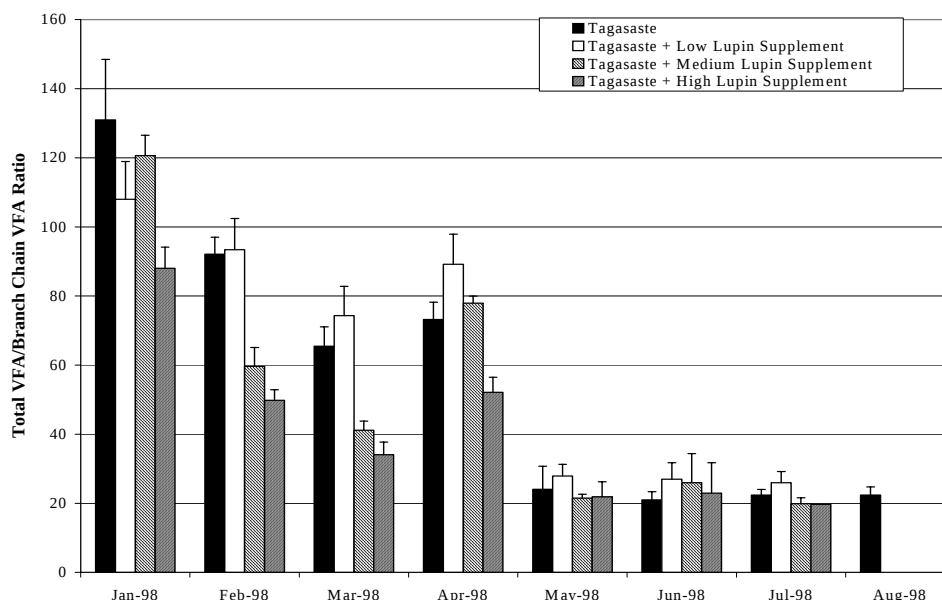
Despite the pasture hay and tagasaste treatments showing similar ruminal ammonia concentrations, the number of bacteria per ml of rumen fluid is much lower for the tagasaste. Using protozoal concentration as an index, Leadbetter [1995] also reported a decrease in ruminal microbial mass during summer/autumn. Apart from ammonia, the other important nutrient required for microbial growth is carbohydrate, which is digested to yield ATP, the primary source of energy for rumen microbes. Carbohydrate fermentation releases volatile fatty acids into the rumen, therefore ruminal VFA concentration can be used to indirectly determine the amount of energy supplied to the microbes. Ruminal VFA concentration in steers grazing tagasaste during March was below the level required to sustain energy requirements. The reduced number of microbes may therefore be a result of insufficient energy supply as well as nitrogen. However, energy supplementation alone does not appear to promote growth. Cattle supplemented with an energy supplement [barley grain] responded by simply substituting the energy supplement for tagasaste. Much more success has been achieved with combined supplements of both nitrogen and energy. Nitrogen supplementation stimulates intake [Preston and Leng 1987]. Nitrogen and energy supplementation stimulates the intake of tagasaste and therefore provides more protein to the animal. The lack of growth may therefore be due to a limiting supply of both protein and energy. The results obtained clearly display a dramatic reduction of both bacterial concentration in the rumen and microbial nitrogen supply to the duodenum in cattle grazing tagasaste. Microbial synthesis was not limited by ruminal ammonia alone, since this was comparable to the pasture hay diet, which produced higher bacterial numbers. Moreover, the ruminal ammonia concentration was above the suggested optimum of 2.8mM. Rather, microbial synthesis appeared to be limited by the supply of ATP, suggesting that the dietary

carbohydrate supply in tagasaste was insufficient. Despite a high crude protein content, the concentration of ruminal ammonia was comparatively low in steers grazing tagasaste.

The calculated supply of microbial nitrogen on the lucerne hay and pasture hay diets in the tagasaste trial was more than double that of the lucerne hay and oaten hay/concentrate diets in the total urine collection trial [Table 3.7].

Supply of microbial protein is a fundamental source of essential and non-essential amino acids in the small intestine of ruminants. While dietary protein that escapes ruminal degradation can supplement protein supply to the small intestine, it is the microbial protein that provides the majority of amino acids and the overall protein quality for metabolism. One of the principal limiting factors to microbial growth is the supply of branch-chain volatile fatty acids, normally derived from the breakdown of the branched-chain amino acids, valine, leucine, and isoleucine. There is a seasonal variation in the ratio of total volatile fatty acids [VFA] to branch-chain VFA in the rumen of cattle browsing tagasaste with the summer/autumn ratio 4-5 fold higher than the winter/spring ratio [Figure 3.12]. Again the April sampling reflected only the tagasaste intake. Hence, branch-chain VFA could be limiting the synthesis of microbial protein during the summer and autumn period in cattle browsing tagasaste. Lupin supplementation supplied a source of branch-chain VFA, thereby decreasing the ratio during summer/autumn [Fig 3.12] and indicating that these supplemented animals may have a greater supply of microbial protein to support liveweight gain. Tagasaste during the winter and spring is very productive both in growth and resulting liveweight gain, and the ratio of total to branch-chain VFA's is consistent with the supply of branch-chain VFA's supporting optimal microbial synthesis [Figures 3.12 & 3.13]. This explanation presumes that lupin protein is readily degraded in the rumen in the presence of tagasaste protein that is not as readily degraded. Hence lupins are a much better source of precursors for microbial growth than tagasaste protein. There are a number of factors that could limit the degradation of tagasaste protein during summer and autumn, factors that change or diminish during winter and spring. For example, the solubility of the protein may decrease in summer as a protection for the plant against water shortage. Decreased solubility of protein decreases the extent of degradation in the rumen [Ørskov 1992] and increases the proportion of the protein bypassing the rumen. However, the decreased rumen degradation is limiting microbial growth as seen from comparisons with lucerne and pasture hay. Thus there is insufficient overall supply of microbial protein in comparison to tagasaste protein that by-passed rumen degradation. This implies a problem of quality of protein as well as quantity for liveweight gain of cattle.

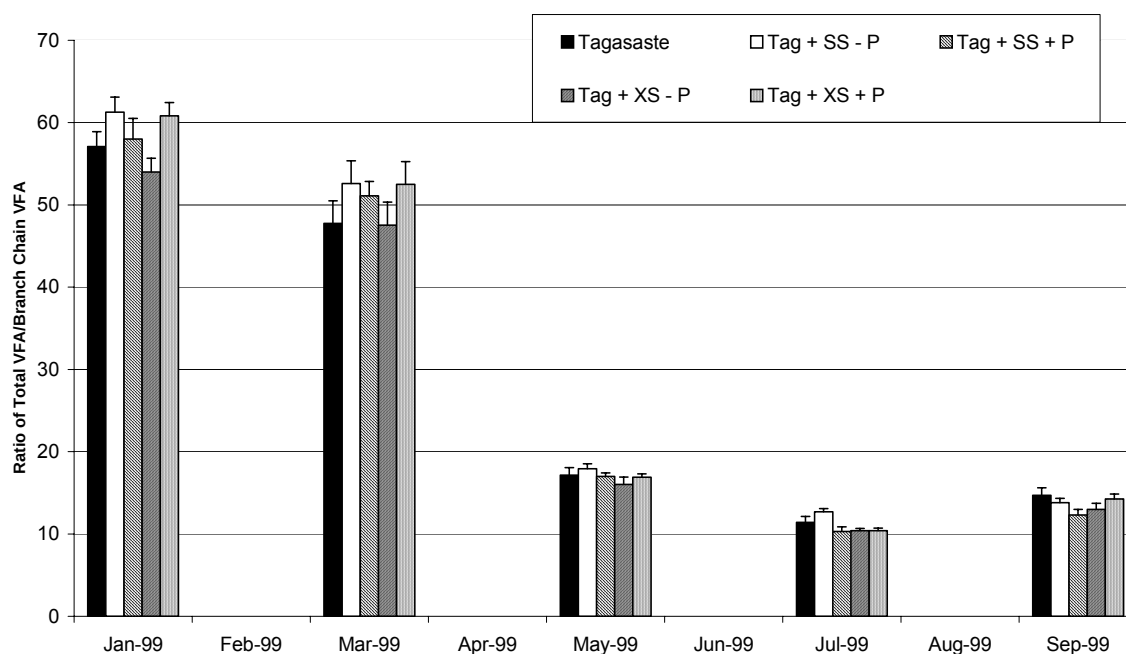
**Figure 3.12:** Ratio of Total Volatile Fatty Acids to Branch-Chain Volatile Fatty Acids in Cattle Browsing Tagasaste and Receiving Lupin Supplements at 'Dunmar'



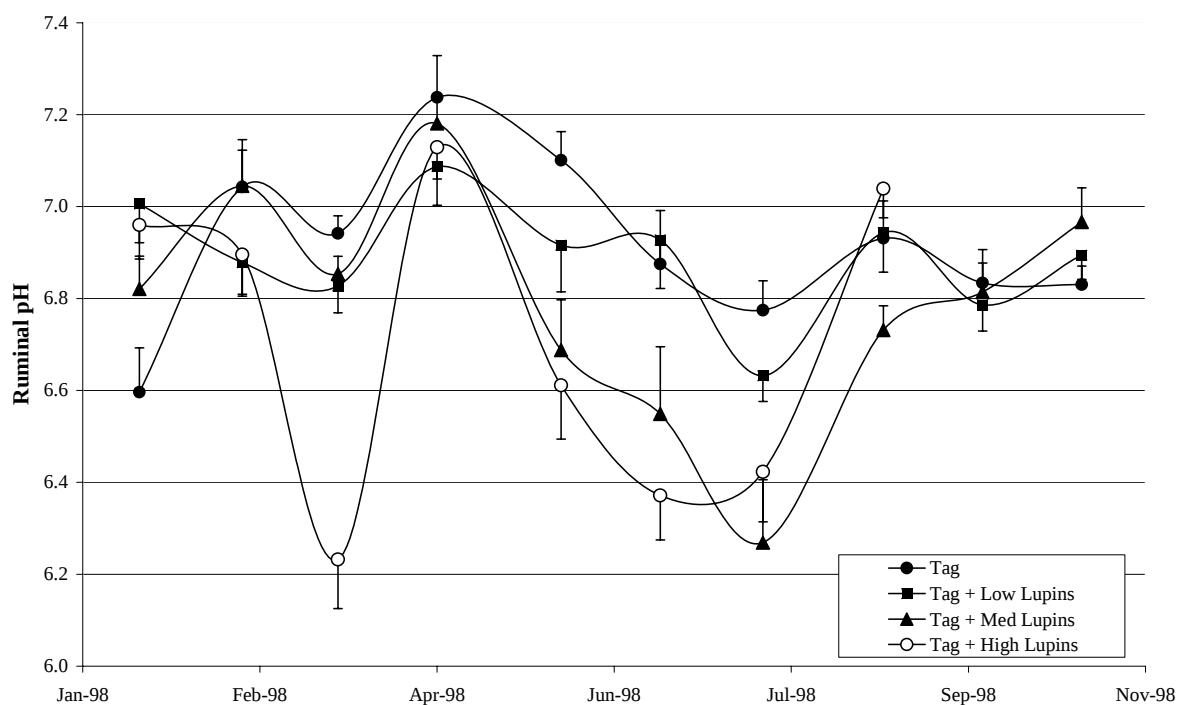
Another interpretation consistent with the data is that cattle eat insufficient tagasaste during the summer and autumn period to supply optimal concentrations of ruminal ammonia and branch-chain VFA's to ensure adequate supply of microbial protein. Thus the problem is essentially one of supply of sufficient precursors of microbial protein synthesis to fuel growth. This interpretation assumes that there are no inherent anti-nutrients in the tagasaste itself during this period. It begs the question as to why cattle do not eat sufficient tagasaste. Factors such as the concentration of phenolic compounds could be limiting intake, thereby limiting ruminal metabolism. Phenolic compounds bind to protein in the rumen, causing a decrease in protein solubility and a decrease in crude protein degradation in the rumen [Cheeke and Shull 1985; Minson 1990]. This in turn results in a reduced concentration of ruminal ammonia, the major nitrogenous substrate for microbial growth. The phenolic may be decreasing crude protein degradation sufficiently so that there is inadequate substrate to support optimal microbial growth, thus causing the decrease in live weight.

Ruminal pH again corresponded to the intake of lupin supplements with pH decreasing with increasing intake [Figure 3.14]. The quantity of lupins supplemented and eaten did not result in overt acidosis, as is reflected with the pH remaining above 6.0. This range of pH would not disadvantage cellulolytic bacteria known for their pH sensitivity. So acidity per se, would not be limiting degradation of cellulose in tagasaste. Thus the overall picture is one of the rumen not operating at an efficiency or capacity to support growth during the summer and autumn period. Tagasaste is very productive during winter and spring, and all of the indicators assessed for ruminal function show that there are no limitations for liveweight gain during this period. However, there is a consistent picture of poor rumen function during the summer and autumn period in cattle browsing tagasaste. This can be overcome by supplementing with lupins or barley, with or without urea. Importantly, lupin supplementation was more effective than barley or barley/urea in improving the indicators of rumen function.

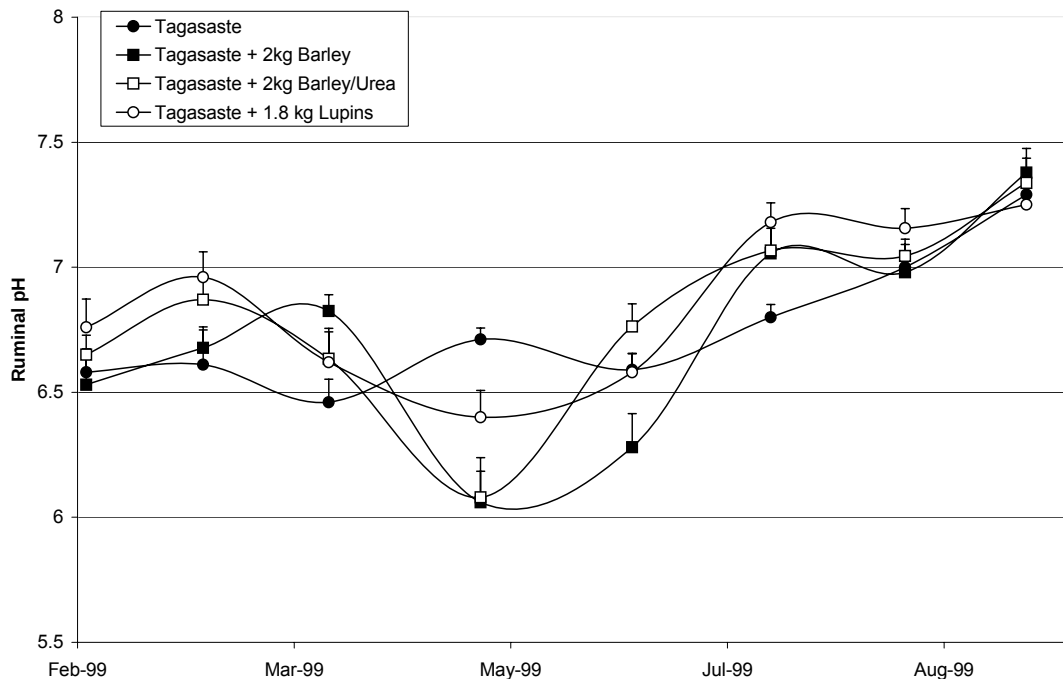
**Figure 3.13:** Ratio of total volatile fatty acids to branch-chain volatile fatty acids in cattle browsing tagasaste and receiving lupin supplements at 'Tagasaste Farm'



**Figure 3.14:** Ruminal pH in cattle browsing tagasaste and receiving lupin supplements at 'Dunmar' [1998]

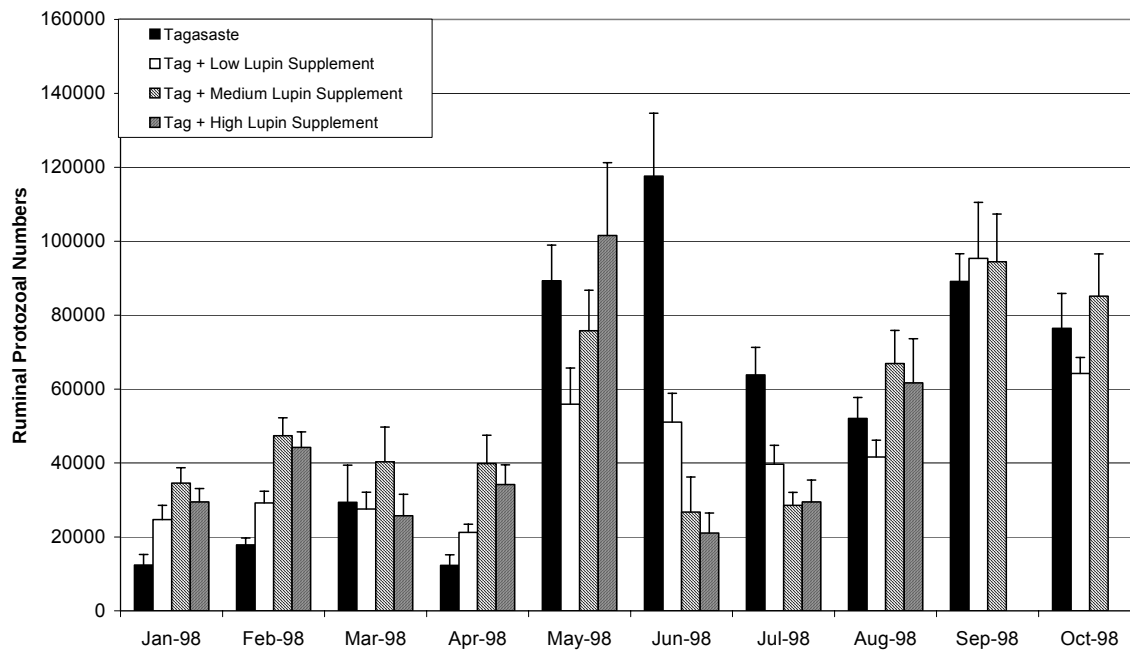


**Figure 3.15:** Ruminal pH in cattle browsing tagasaste and receiving lupin, barley or barley/urea supplements at 'Dunmar' [1999]



The protozoal numbers in the rumen vary according to season more than through treatment effects [Figure 3.16].

**Figure 3.16:** Effect of lupin supplementation and season on ruminal protozoal numbers in cattle browsing tagasaste and receiving lupin supplements at 'Dunmar'



Protozoal numbers are low during the summer and autumn in cattle browsing tagasaste and increase significantly during the winter and spring [Figure 3.16]. The seasonal effect on ruminal protozoal numbers has been observed previously in Western Australia [Purser and Moir 1966], but the reasons have not been fully elucidated.

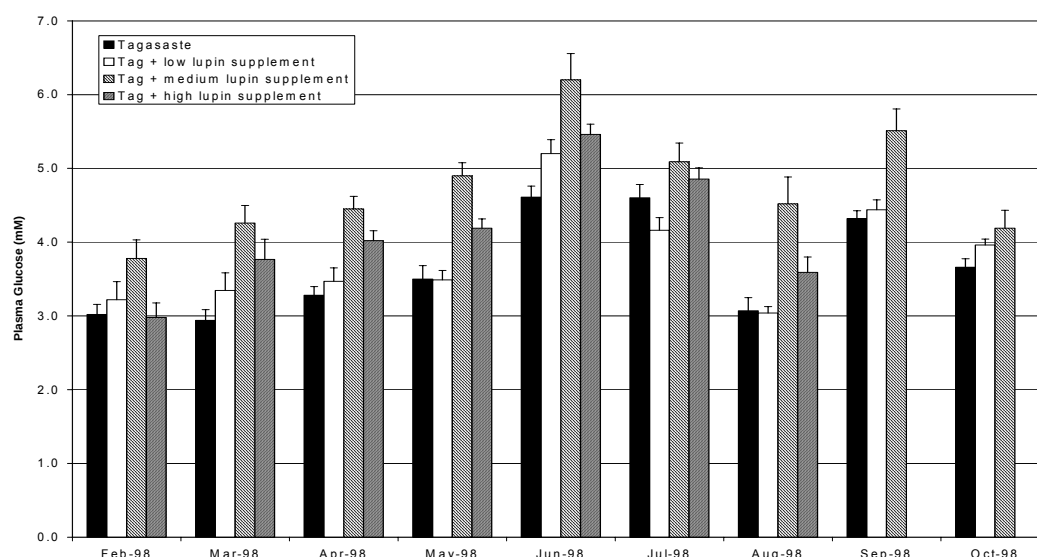
### 3.3.8 Blood Biochemistry

The metabolic profile of plasma metabolites and enzymes were monitored to provide a clear picture of the overall health of the cattle browsing tagasaste, and the metabolic consequences of supplementation.

Several plasma enzymes were monitored to test whether there were any components of tagasaste that were causing tissue damage in cattle at any time of the year. Aspartate amino transferase [AST], an indicator of general liver and muscle damage, was not elevated outside the normal range at any time of the year, in any animal.  $\gamma$ -Glutamyl transferase [GGT], an indicator of biliary and reticuloendothelial cell damage was not elevated either. Finally, creatine kinase [CK], an indicator of muscle damage was only slightly elevated in those cattle that had been bumped or did not settle in the crush at time of sampling. There was no long-term elevation of CK in any animal. This pattern of enzyme activity showed that there is no undescribed component of tagasaste that is likely to cause some toxic effect or metabolic aberration.

Plasma glucose and urea were monitored as indicators of general energy and protein metabolism respectively. Plasma glucose concentrations were within the normal range for cattle and were consistent with adequate glucose homeostasis occurring in cattle from each treatment irrespective of the season [Figure 3.17]. Thus during periods when the microbial protein supply may be limiting in unsupplemented cattle ie. summer and autumn, there is no indication that these animals could maintain energy homeostasis even during the high temperatures. Supplementation with lupins increased the plasma glucose concentrations compared with unsupplemented animals but this was always within the normal range. Nevertheless, this could be interpreted that supplemented cattle more easily maintained their plasma glucose levels.

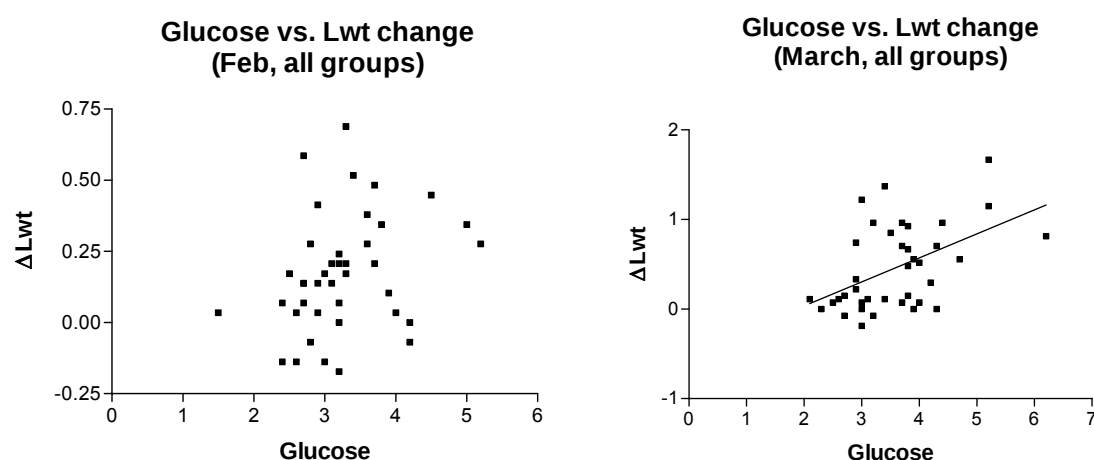
**Figure 3.17:** Plasma glucose concentrations in cattle browsing tagasaste and receiving lupin supplements at 'Dunmar'



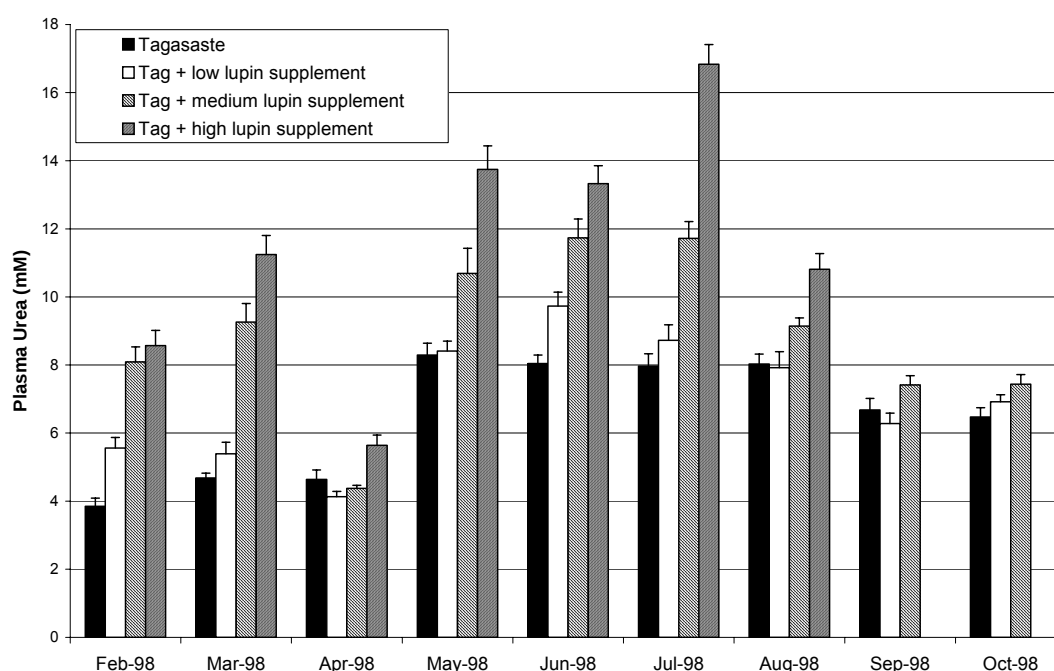
Results shown are the means  $\pm$  SEM of 10 animals in each treatment.

Plasma glucose concentrations were correlated with average daily liveweight gain in cattle during the effective supplementation period of March but not before supplementation in February [Figure 3.18]. This is supportive of the concept that supplemented cattle more easily achieve homeostasis without mobilisation of glucose reserves in the form of glycogen and amino acids.

**Figure 3.18:** Relationship between average daily liveweight gain and plasma glucose in cattle browsing tagasaste at 'Dunmar' during February and March



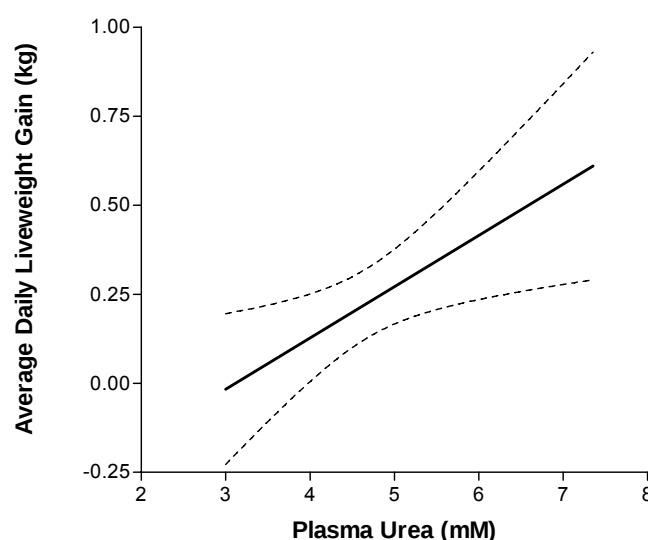
**Figure 3.19:** Plasma urea concentrations in cattle browsing tagasaste and receiving lupin supplements at 'Dunmar'



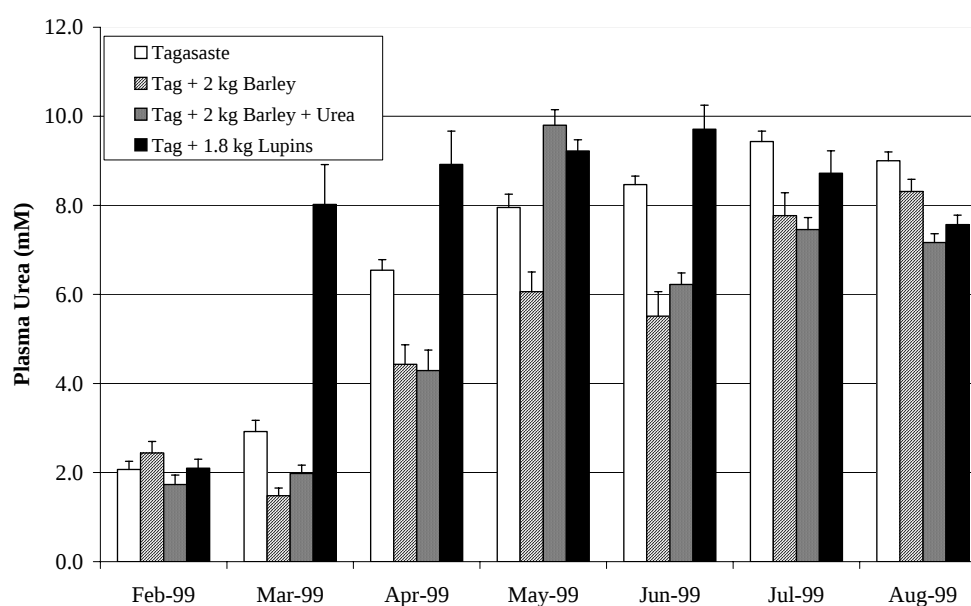
Results shown are the means  $\pm$  SEM of 10 animals in each treatment.

Plasma urea concentrations followed a similar pattern to the ruminal ammonia profiles at 'Dunmar' [Figure 3.19]. This is not surprising since the urea pool in plasma can be sourced from turnover of amino acids from microbial protein and that ruminal ammonia that is directly absorbed across the rumen wall in proportion to ruminal concentrations.

**Figure 3.20:** Relationship between average daily liveweight gain and plasma urea in cattle browsing tagasaste at 'Dunmar' during March. Results shown are for 40 animals



**Figure 3.21:** Plasma urea concentrations in cattle browsing tagasaste and receiving either lupin, barley or barley/urea supplements at 'Dunmar'. Results shown are the means  $\pm$  SEM of 10 animals in each treatment



The pattern of plasma urea does show the effectiveness of lupin supplementation during the summer and autumn period but also the effectiveness of tagasaste protein itself during the winter and spring. Moreover, the plasma urea concentrations are correlated with supplement intake and average daily gain during March, the period of greatest effect of the lupins. [Figure 3.20]. This correlation between ADG and both energy and protein metabolism [Figures 3.18 & 3.20] supports the effectiveness of lupin supplementation for cattle browsing tagasaste, notwithstanding the protein content of tagasaste.

### 3.4 Conclusions

Dry cattle browsing tagasaste will only maintain weight, or perhaps may lose some weight over the dry summer and autumn period even though the composition of the edible leaf and stem suggests animals should gain at a minimum of at least 0.5 kg/head per day. However, even though cattle browsing tagasaste over the summer/autumn period do not gain weight they are at an advantage over cattle grazing annual pastures as the latter animals would have to be supplemented to stop losing weight. Supplementing with lupin grain or barley, with and without urea, will increase liveweight gain over this dry period and is a very effective way of targeting markets during periods when animal's performance is poor. Cattle respond better to lupin grain supplements than barley, with or without urea. Supplementing with low levels of lupin grain (~ 1 kg/head per day) stimulates tagasaste intake whereas high levels of supplementation result in substitution. Rumen ammonia, plasma urea, ruminal pH and VFA's supports the findings of low intake and rumen dysfunction and cattle have low ruminal bacteria that indicates poor rumen microbial synthesis.

## 4 SUPERPHOSPHATE AND MINERAL LICK REQUIREMENTS TO OPTIMISE CATTLE GROWTH WHEN BROWSING TAGASASTE IN A MEDITERRANEAN ENVIRONMENT

### 4.1 Introduction

Early studies at New Norcia (Southern 1988) suggested tagasaste did not require superphosphate. But further studies at 'Dunmar', near Badgingarra, by the Martindale Research Project and MRC project UWA.007, have shown a large response in tagasaste growth and total live-weight gain in cattle to superphosphate on the deep leached sands. The results at 'Dunmar' showed plant production plateau at a lower superphosphate level (~160 kg superphosphate/ha per year) than the live-weight response in cattle which plateau between 200 - 300 kg superphosphate/ha per year. It was proposed the different responses were due to a good fertiliser history at New Norcia compared with "virgin" land at 'Dunmar' but it is unclear if the animal response could be achieved more cost effectively by supplementing with a mineral P lick.

Phosphorus is the second most plentiful mineral in the body and plays an essential role in body functions. It combines with calcium in the formation of bones and teeth and is required in the digestive and metabolic processes in the animal for converting feed into energy and the building and repair of body tissues. The rumen micro-organisms requirement for P must be considered separate to the animals' requirement as low P diets lowers feed intake without

influencing dry matter digestibility. Insufficient dietary P reduces intake and slows down normal skeletal development, growth and reproductive function in cattle. There is considerable conjecture in the scientific literature as to the most appropriate method of establishing the P status of the animal. Little (1972) suggested that as the skeleton stored some 80% of P in the body then rib bone composition (ash, Ca and P) was the most appropriate measure to identify P the status of the animal. Wadsworth *et al.* (1990) suggests that blood inorganic phosphate was highly correlated with liveweight gain during the growing the season but there was no correlation during the dry season. McCosker and Winks (1994) suggest using BiP and faecal N and P can make an effective diagnosis.

The mineral supply for cattle grazing tagasaste is not known. Tagasaste leaf was analysed for Ca, P, K, Mg, Na, Cl, Cu, Zn, Mn, Fe, NO<sub>3</sub>, B, S and Mo. This was done with purpose of establishing the mineral levels in tagasaste relative to cattle requirements for those minerals.

The effect of different sources of phosphorus, ie. fertiliser versus direct P supplementation on live-weight, rib bone and faecal phosphorus, BiP and PUN and rumen metabolic profile will be quantified in grazing cattle. Dicalcium phosphate will be used in the manufacture of the lick as fertiliser grade phosphorus supplements such as MAP and DAP contain significant levels of fluorine and cadmium. Changes in nutrient density of the tagasaste will also be monitored by NIR analysis for protein, energy, and phenolic. Frozen samples will also be collected on a monthly basis to quantify any changes in P content of leaves and solubility of leaf protein with season.

#### **4.1.1 Hypothesis**

1. Superphosphate fertiliser will supply the P requirements of not only tagasaste but also cattle browsing that tagasaste.
2. Supplying additional phosphorus in the form of a mineral lick will satisfy the animal's requirements.
3. Cattle browsing tagasaste will perform better when supplied with a mineral lick compared with cattle with no mineral lick.

## **4.2 Aims/objectives**

1. Investigate the phosphorus supply for cattle browsing tagasaste.
2. Investigate the trace mineral supply for cattle browsing tagasaste.

## **4.3 Material & Methods**

### **4.3.1 Experimental design**

Three experiments were conducted over a three year period with each experiment having five treatments x 3 replicates with approximately 130 steers and/or heifers (30 animals/treatment; 10/replicate).

### **4.3.2 Experimental area**

The fertiliser and mineral phosphorus (P) lick study was conducted on 75 ha of tagasaste planted as double rows at 5-m row spacing in 1990. The property 'Tagasaste Farm' is in the Lancelin region of Western Australia (Lat. 32°40'S and Long. 116°10'E). The 75 ha of tagasaste were divided into 15 experimental plots each 5 ha in area. The 75 ha of tagasaste were three paddocks, which were close, but not adjacent. To reduce the variation within the experiment the three areas are considered to be blocks with each treatment represented in each block.

Standard superphosphate had been applied for the last 5 years at 100 kg/ha per year. In the 1<sup>st</sup> year (1997), 100 kg had been applied as usual in autumn with the extra 200 kg being applied in September. In subsequent years all the fertiliser was applied in autumn.

### **Cattle**

Each year the experimental cattle were predominantly Brahman cross about 15 months of age at the start. In each of the three years (1998 – 2000) new mobs of cattle were introduced into the experimental plots in January. Cattle were identified with ear tags and vaccinated with 5 in 1 antibacterial and drenched with an anthelmintic to control internal parasites. Experimental cattle were weighed and randomly allocated to one of the five treatments after stratifying, within sex, on live weight.

1998: 147 head with mean initial liveweight ( $\pm$  s.e.) of  $213.6 \pm 1.90$  kg

1999: 152 head with mean initial liveweight of  $210.1 \pm 1.83$  kg

2000: 159 head with mean initial liveweight of  $234.2 \pm 2.35$  kg

The skeletal phosphorus status of the animals was determined by rib-bone P analysis of selected treatment animals at the start and end of the experiment (Little 1972). In addition BiP, faecal P and N was analysed to measure dietary P intake.

Animals from each treatment were bled, and rumen samples collected, about every three months to monitor metabolic profile and rumen energy and N status. Note: Because of the problems with blue lupins in some of the paddocks animals from paddocks with blue lupin infestation were bled for GGT analysis as this is a good indicator of liver damage associated with lupinosis.

### **Treatments**

Cattle graze tagasaste fertilised with superphosphate (SP):

1. Standard SP application (100 kg/ha per year)
2. Standard SP application (100 kg/ha per year) + mineral lick - P
3. Standard SP application (100 kg/ha per year) + mineral lick + P
4. Standard SP application (100 kg/ha per year) + extra SP (200 kg/ha per year) + mineral lick - P
5. Standard SP application (100 kg/ha per year) + extra SP + mineral lick + P

Animals were randomly allocated to treatment groups after stratifying on initial live weight.

The stocking pressure was 2 beasts/ha; ie. 10 animals per plot. As extra plant growth was expected (measured as Feed-On-Offer - FOO) on the high (300 kg) superphosphate treatments (Trs 4 & 5), more animals were allocated to these plots so that each paddock was

grazed to a standard FOO. FOO was assessed every three months and extra cattle were allocated to treatments as required but it is anticipated extra animals will only be required in spring. The extra growth of tagasaste was measured as grazing days and liveweight gain/ha.

### Mineral supplements

The composition of the mineral supplement (Table 4.1) was formulated with Dr John Milton after examination of leaf mineral analysis data that had been collected on this site since 1995. The treatments with, and without P ( $\pm$  P) were formulated by adding, or leaving out the P component. The mineral lick was fed *ad lib*.

**Table 4.1:** Composition of mineral lick used at 'Tagasaste Farm'

| <b>Macro Element</b>  | <b>g/kg as fed</b>  |
|-----------------------|---------------------|
| Sodium                | 130                 |
| Sulphur               | 57                  |
| Magnesium             | 33                  |
| Calcium               | 132                 |
| Phosphorus            | 43                  |
| <b>Trace Elements</b> | <b>mg/kg as fed</b> |
| Copper                | 500                 |
| Zinc                  | 1500                |
| Iodine                | 45                  |
| Cobalt                | 20                  |
| Selenium              | 5.5                 |
| Molybdenum            | 30                  |
| Manganese             | 1500                |
| Iron                  | 2000                |

### Measurements and observations

1. Unfasted liveweight change every 2 – 4 weeks
2. Grazing days
3. Liveweight gain
4. Mineral intake weekly
5. Tagasaste intake using alkane capsules in 2<sup>nd</sup> and 3<sup>rd</sup> year
6. Estimate Feed-on-Offer (FOO) every three months
7. Quality of tagasaste on offer: leaf protein, digestibility, lignin, ADF, NDF, phenolic and minerals; Ca, P, K, Mg, Na, Cl, Cu, Zn, Mn, Fe, NO<sub>3</sub>, B, S and Mo. Collect samples monthly and bulk across 3 months (winter, spring, summer and autumn)
8. Bleed representative animals (10/treatment from 1 block) every three months: blood Pi, plasma GGT, plasma urea, plasma glucose, plasma NEFA, blood glutathione peroxidase, plasma Ca, P, Mg, Cu, Zn

9. Rumen fluid every three months for VFA's, pH, ammonia, protozoa and bacterial numbers from the same animals as the bled for metabolic profile
10. Rib-bone ash, Ca and P and faecal N and P
11. Bore holes sunk in the three paddocks to measure water table depth and soil mineral content down to ten metres depth

## Statistics

The data from this trial has been analysed by fitting a variance component model using residual maximum likelihood (REML). This technique allows for two variance components to be estimated, in this case variance between animals and variance between plots, with unequal numbers of animals or plots for the treatment effects. In particular, the numbers of biopsied animals were not equally represented in each treatment or paddock, and all treatments were not equally represented in each paddock. The effects that were examined for significance were *Breed*, *Biopsie*, *Treatment* and all their interactions. The *Treatment* effect was further subdivided into *Control* (no-lick vs lick), *Control.P* (+P vs -P), *Control.Applic* (standard vs extra) and *Control.P.Applic* (interaction between + or -P and stand vs extra).

Plot 6 (Treatment 3) was recorded as part of the "Pea Trial" paddock and Treatment 3 did not occur in the Erragulla paddock. However, all treatment effects were examined after removing (or adjusting) for the effects of *Paddock* and *Biopsie*.

The following measurements of animal performance were analysed.

|          |                                   |                   |
|----------|-----------------------------------|-------------------|
| N5       | Initial LW (5 Nov)                |                   |
| D3       | LW at start of treatments (3 Dec) |                   |
| M4       | LW 4 March                        | }                 |
| M12      | LW 12 May                         | }                 |
| Change1  | LW change 3 Dec to 4 March        | } D3 as covariate |
| Change2  | LW change 3 Dec to 12 May         | }                 |
| Change 3 | LW change 4 March to 12 May       | }                 |

## 4.4 Results & Discussion

### 4.4.1 Animal Health

1998: Seven steers were culled on the 10<sup>th</sup> of June because of weight loss and failure to adapt to browsing tagasaste. No deaths occurred. One steer developed tagasaste staggers but recovered uneventfully.

1999: During this year it was necessary to supplement cattle in plots 13, 14 and 15 in "Pennant" paddock as they lost more than 12% of their liveweight over the summer –autumn period. Crushed lupins were fed at the rate of a kg per head per day, three times a week from the 10<sup>th</sup> of May to the 5<sup>th</sup> of July when they had regained their starting weight. One heifer was not accounted for in July and was presumed to have died. Six heifers were impregnated by a stray bull and were successfully aborted by injecting them with synthetic prostaglandin when they were from 2- 3months pregnant.

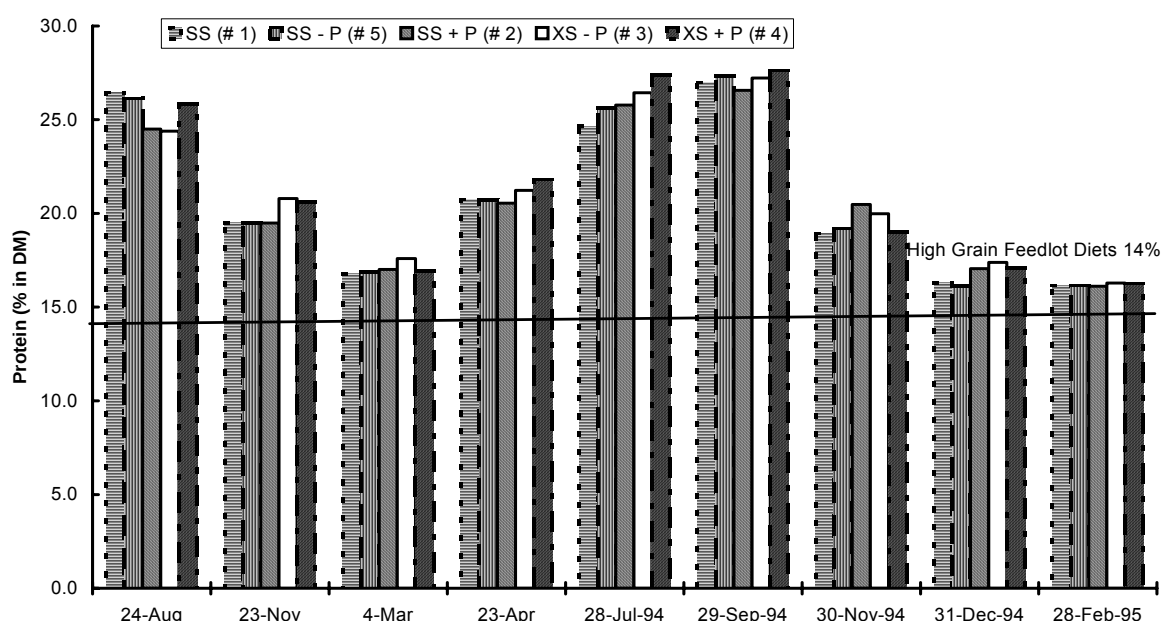
2000: Four animals died during the year, one was destroyed after she broke her leg while being weighed, two died after the speying operation and a fourth death was undiagnosed.

### 4.4.2 Leaf Analysis of Tagasaste

Samples of tagasaste were collected following a randomised pattern over each treatment plot. The analysis of the samples showed seasonal deficiencies in several minerals, when compared with animal requirements for those minerals. In some cases the plant requirement was also limiting and affected the production of tagasaste itself.

Protein content of tagasaste at 'Tagasaste Farm' did not fall below 14% during any part of the year, confirming that protein is always sufficient for growth on analysis (Figure 4.1). Nonetheless, there was a distinct seasonal pattern in the protein content, with peaks of nearly 30% crude protein during the winter and spring and lower levels of 17% crude protein during the summer and autumn (Figure 4.1). The higher proteins in winter and spring were associated with active leaf production by tagasaste.

**Figure 4.1:** Variation in protein content of tagasaste with season and superphosphate fertiliser treatment at 'Tagasaste Farm'. Results are the means of 30 samples per plot



The amino acid profile of the tagasaste during this time is shown in Table 4.2. The total and specific amino acid content increases in winter and spring as compared with summer and autumn. This is the same pattern as the protein profile seen in Figure 4.1. There is a higher concentration of amino acids in the 300 kg superphosphate treatment as compared with the 100 kg which is consistent with the growth profile of cattle on the two treatments. There is no obvious change in the proportion of hydrophobic amino acids that could lead to a decrease in the solubility of the protein in summer. This is one of the explanations we have put forward for the decreased effectiveness of tagasaste protein during that time of the year. Other compounds such as phenolic could still affect tagasaste protein solubility but any effect is not directly due to amino acid composition.

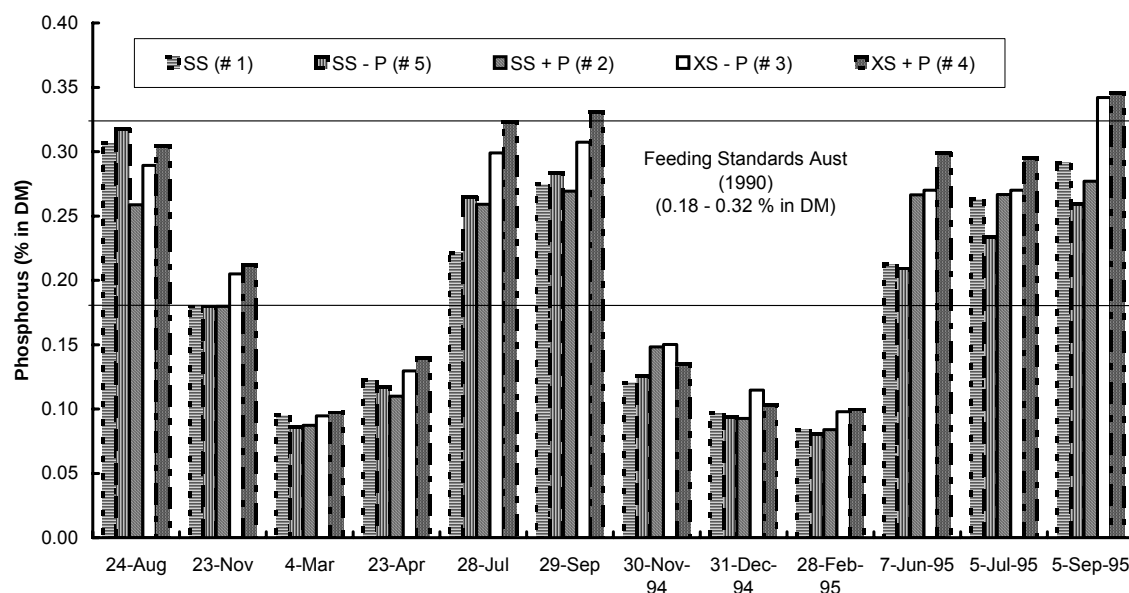
Leaf analysis of phosphorus also showed a distinct seasonal variation. However, in the case of P, the low levels measured during summer and autumn were limiting for animal production according to the Scientific Committee on Agriculture (1990) recommendations for P requirements in cattle (Figure 4.2). Fertiliser application did increase the P content of the leaf especially during the early winter months of June and July (Figure 4.2). Not only did fertiliser increase the P content but also the increase was also in proportion to the rate of application of the fertiliser. However, this proportional effect of fertiliser addition was not apparent during the summer months when the tagasaste was not actively growing (Figure 4.2).

Thus there is a clear rationale for using direct P supplements over and above the supply from tagasaste during the summer when the leaf content of P will not provide sufficient P for cattle requirements. This supplementary effect should not be either required or evident during the winter and spring months.

**Table 4.2:** Amino Acid profile of tagasaste at 'Tagasaste Farm'

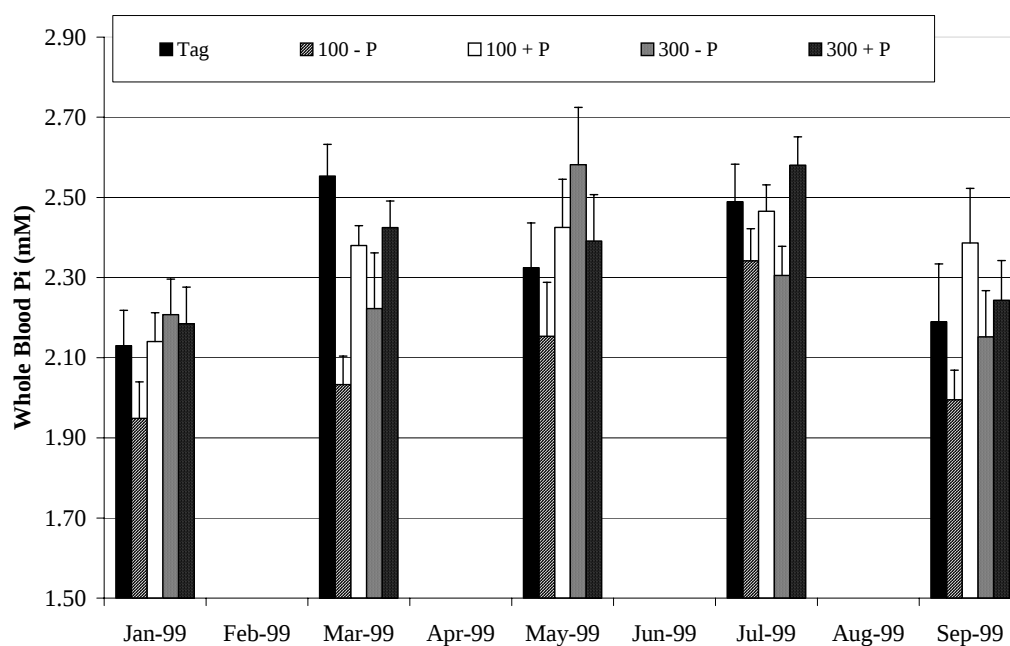
|                | Summer |       | Autumn |       | Winter |       | Spring |       |
|----------------|--------|-------|--------|-------|--------|-------|--------|-------|
| Superphosphate | 300    | 100   | 300    | 100   | 300    | 100   | 300    | 100   |
| kg/ha per year | g/kg   |       | g/kg   |       | g/kg   |       | g/kg   |       |
| Alanine        | 6.88   | 6.65  | 6.12   | 5.84  | 12.17  | 11.54 | 11.81  | 11.05 |
| Arginine       | 4.50   | 4.33  | 4.74   | 4.44  | 9.27   | 8.71  | 10.45  | 9.32  |
| Aspartic acid  | 12.32  | 12.15 | 11.03  | 10.94 | 19.89  | 19.14 | 25.21  | 20.21 |
| Cysteine       | 1.21   | 1.15  | 1.67   | 1.04  | 1.67   | 1.57  | 2.32   | 2.01  |
| Cystine        | 5.20   | 5.66  | 5.13   | 5.42  | 7.82   | 8.30  | 8.06   | 7.93  |
| Glutamic acid  | 10.48  | 10.30 | 9.74   | 9.11  | 19.02  | 18.03 | 19.66  | 17.91 |
| Glycine        | 7.30   | 6.90  | 6.64   | 6.35  | 12.81  | 12.13 | 12.49  | 11.43 |
| Histidine      | 3.15   | 2.84  | 2.81   | 2.61  | 4.22   | 4.12  | 4.74   | 4.36  |
| Isoleucine     | 6.55   | 6.40  | 5.95   | 6.08  | 11.07  | 10.43 | 10.77  | 9.85  |
| Leucine        | 10.99  | 10.58 | 9.81   | 9.60  | 19.05  | 17.90 | 18.46  | 17.14 |
| Lysine         | 10.87  | 10.41 | 10.03  | 9.42  | 16.97  | 15.87 | 17.24  | 15.46 |
| Methionine     | 1.77   | 1.23  | 1.25   | 1.41  | 3.07   | 2.78  | 3.42   | 2.88  |
| Phenylalanine  | 7.28   | 7.02  | 6.71   | 6.84  | 12.55  | 11.93 | 12.13  | 11.27 |
| Proline        | 9.64   | 9.63  | 8.81   | 8.89  | 13.44  | 13.64 | 15.85  | 13.15 |
| Serine         | 6.92   | 6.87  | 6.53   | 6.03  | 10.22  | 9.98  | 10.48  | 10.05 |
| Threonine      | 6.45   | 6.14  | 5.91   | 5.65  | 9.78   | 9.24  | 9.68   | 9.12  |
| Tryptophan     | 0.48   | 0.00  | 0.21   | 0.56  | 0.84   | 1.17  | 1.35   | 1.36  |
| Tyrosine       | 5.97   | 5.52  | 5.81   | 5.34  | 9.59   | 9.03  | 9.26   | 8.57  |
| Valine         | 7.61   | 7.40  | 6.88   | 6.87  | 13.21  | 12.55 | 13.57  | 12.39 |

**Figure 4.2:** Variation in phosphorus content of tagasaste with season and superphosphate fertiliser treatment at 'Tagasaste Farm'. Results are the means of 30 samples per plot



Phosphate concentrations in the cattle did not reflect the mineral intake from the supplements (Figure 4.3). Homeostasis of phosphorus maintains plasma phosphate in the range of 1.5 to 2.6 mM for optimum bone mineralisation and metabolism.

**Figure 4.3:** Plasma phosphate concentrations in cattle with and without mineral supplements browsing tagasaste fertilised at two levels



All of the cattle sampled maintained their phosphate within the homeostatic range indicating that none of these animals was in severe phosphate deficiency. Nevertheless, cattle in the

300kg superphosphate plus phosphate in the mineral supplements had significantly ( $P < 0.001$ ) higher liveweights during summer and autumn than cattle from all of the other treatments.

#### **4.4.3 Bone Phosphorus Levels**

To determine the bone phosphorus status of steers at the start and end of the first trial, rib bone biopsy samples were taken from 4 steers in each of the five treatments on the 6/11/97 and the 23/9/98. Bone phosphorus percentages and compact bone thickness (CBT) were 9.27% and 9.10% and 4.24 mm and 4.6mm respectively. The differences were not significant and none of the steers were phosphorus deficient at either measurement (Little 1972, 1984).

Mean faecal nitrogen and phosphorus levels and blood inorganic phosphorus levels from all treatments for the period January to September 1999 are shown in table 4.2. There were no significant differences in these parameters between treatments, so the data was pooled and compared through the different seasons.

**Table 4.2:** Faecal nitrogen and phosphorus concentrations in cattle browsing tagasaste at 'Tagasaste Farm'

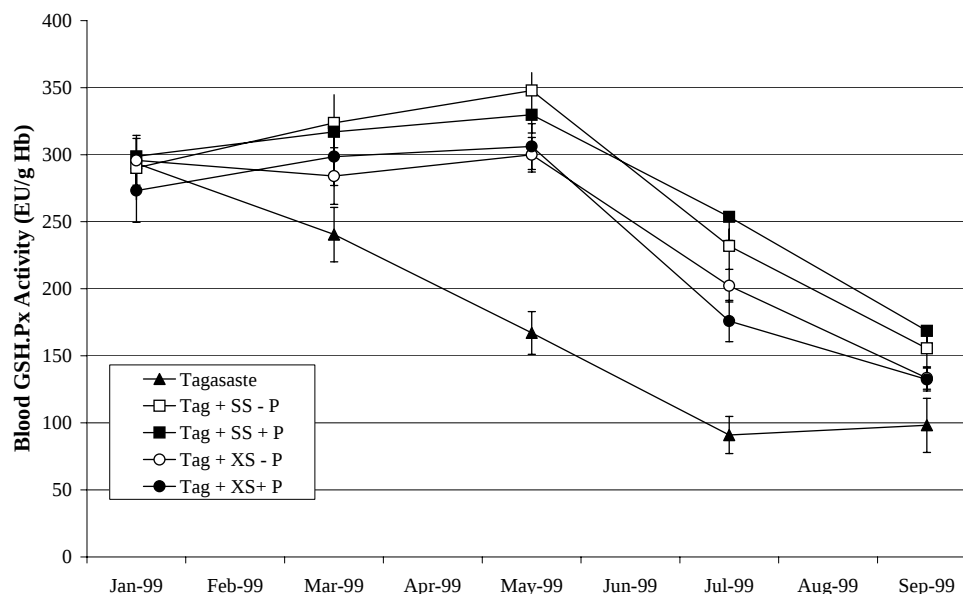
|            | Jan  | March | May  | July | Sept |
|------------|------|-------|------|------|------|
| Faecal N % | 2.08 | 1.48  | 1.32 | 1.84 | 2.23 |
| Faecal P % | 0.25 | 0.22  | 0.25 | 0.46 | 0.76 |

McCosker and Winks (1994) note that under northern Australian conditions the phosphorus status of cattle should be assessed at the end of the pasture growth season. They recommend that three tests in combination namely faecal P and N% and blood Pi should be used to test phosphorus adequacy or deficiency. Based on their guidelines the trial cattle were "adequate" in regard to their phosphorus status during 1999. This confirms the rib biopsy findings taken in 1998.

#### **4.4.4 Selenium Status**

The tagasaste at 'Tagasaste Farm' had been fertilised with superphosphate containing copper, zinc and molybdenum but had not received any selenium fertiliser. The selenium status of these cattle was assessed using whole-blood glutathione peroxidase (GSH.Px) as the indicator (Figure 4.4). GSH.Px activity decreased in cattle not receiving selenium directly through the mineral lick during the period under assay. Cattle receiving selenium in the mineral lick sustained their activity of GSH.Px until the onset of rains that wet the supplement in the troughs resulting in no intake of the supplement from May onwards. At that point, glutathione peroxidase activity declined significantly in all cattle during the early winter period. Decline of activity in cattle not receiving selenium or where the supply from licks had ceased due to rains shows that the soils at 'Tagasaste Farm' were depleted of selenium and the tagasaste itself was not supplying sufficient selenium to sustain the selenium status of the cattle. The decrease in GSH.Px activity in the cattle not receiving any selenium was significant falling from values considered adequate ( $>250$  EU/g Hb) to values such as 50 EU/g Hb where some of the cattle could be responsive to selenium supplementation.

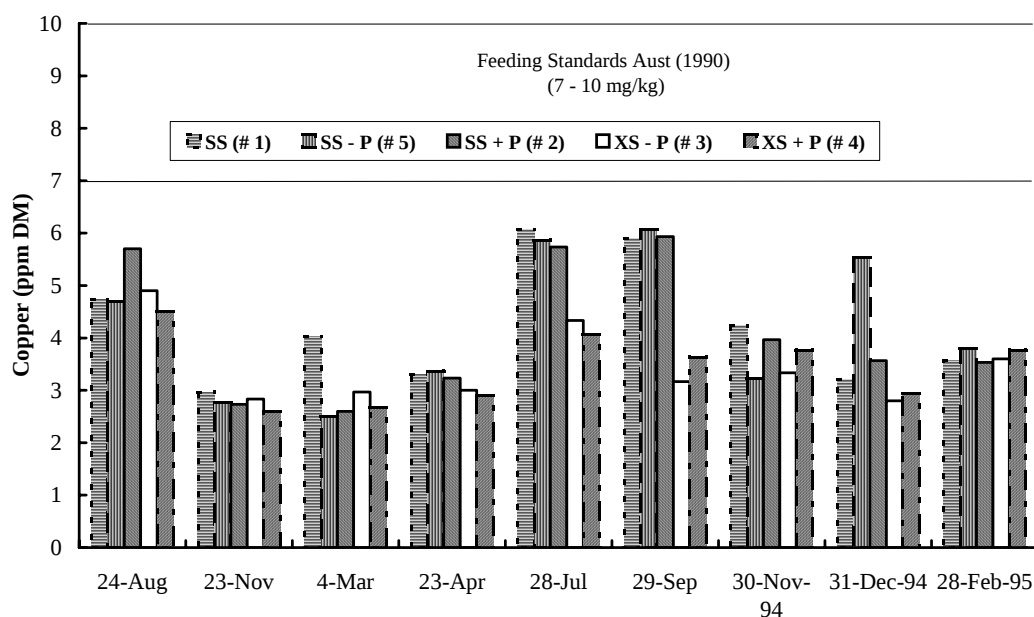
**Figure 4.4:** Glutathione peroxidase activities in blood from cattle with and without mineral supplements browsing tagasaste fertilised at two levels



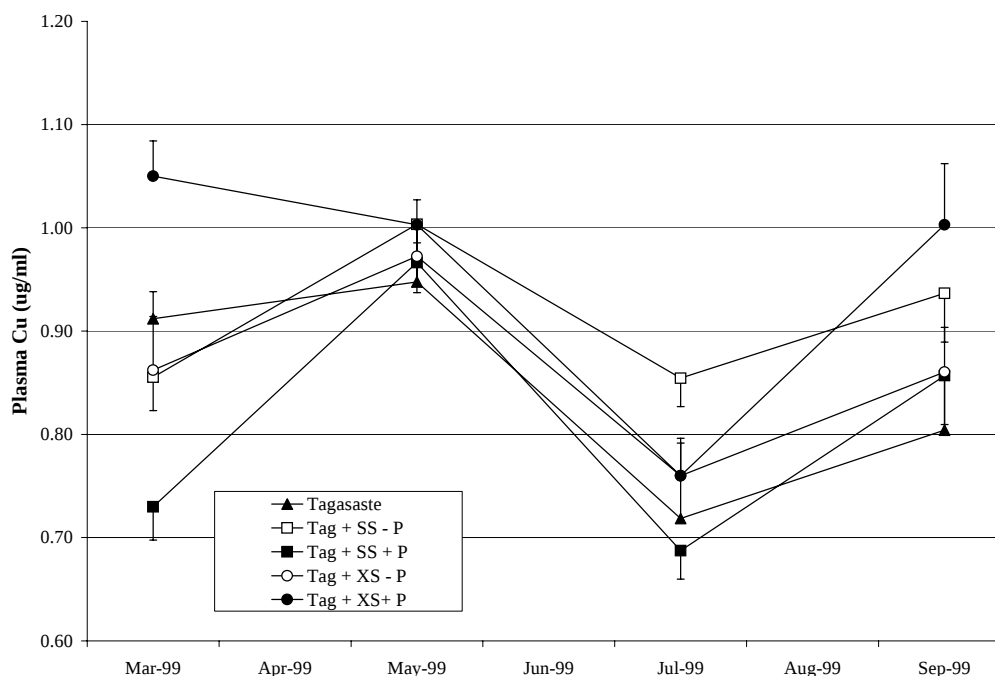
#### 4.4.5 Copper Status

Copper concentrations in edible leaf fraction of tagasaste are shown in Figure 4.5. The fertiliser regime did not include copper or zinc as part of the superphosphate. The concentration of copper in the leaf must exceed 7 ppm DM for adequate supply of copper to cattle. At no stage during our survey has the leaf copper reached this concentration, and this indicates that tagasaste at this site of 'Tagasaste Farm' could be marginal to deficient for copper.

**Figure 4.5:** Copper concentrations in edible leaf from tagasaste at 'Tagasaste Farm'



**Figure 4.6:** Copper concentrations in plasma from cattle browsing tagasaste fertilised at two levels and receiving copper supplements



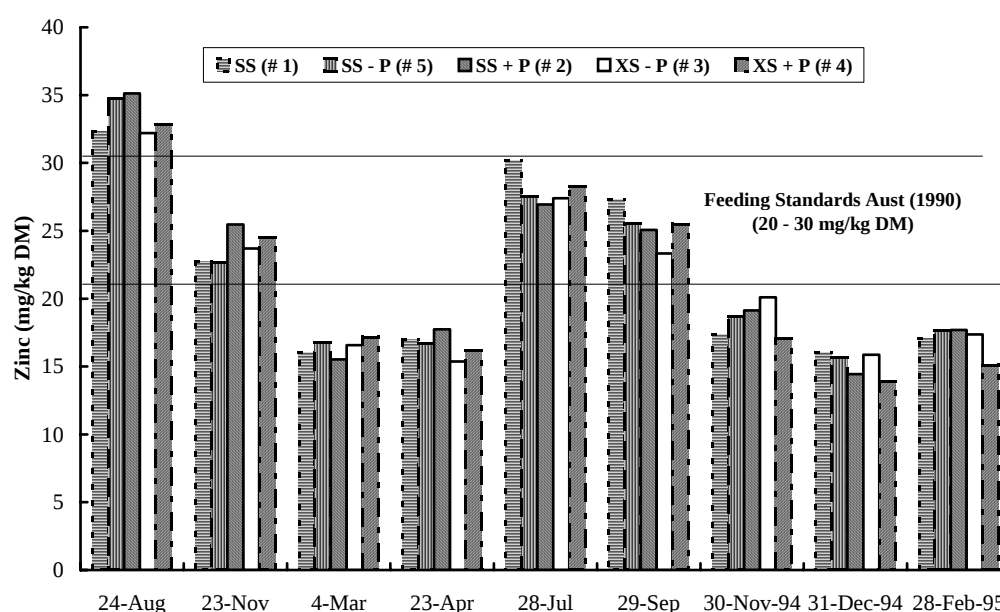
Plasma copper concentrations are shown in Figure 4.6. Plasma copper is not as good an indicator of copper status as liver copper. Nevertheless, the plasma copper concentrations were in fact increasing during period that cattle were taking the mineral lick i.e. February to May and then decreasing in all animals during the period of rapid growth of both cattle and tagasaste in May and June. However, the plasma copper concentrations are not in the deficient range of less than  $0.65 \mu\text{g/ml}$ .

#### 4.4.6 Zinc Status

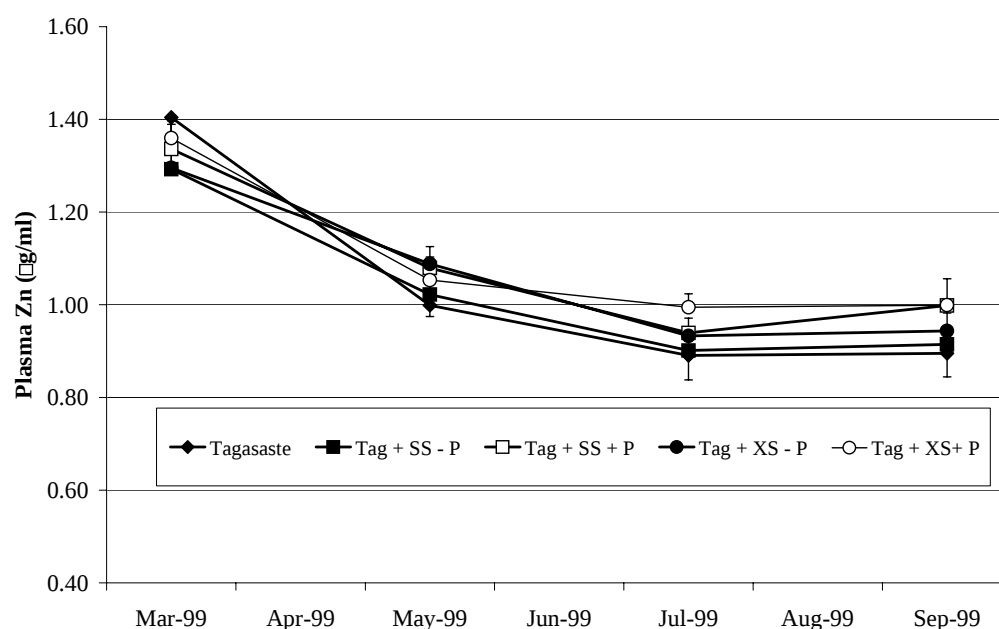
Zinc concentrations in edible leaf fraction of tagasaste are shown in Figure 4.7. The fertiliser regime did not include copper or zinc as part of the superphosphate. The concentration of zinc in the leaf must be at least 20 ppm DM for an adequate nutrient concentration of zinc to cattle. Leaf zinc reached adequate concentration during the winter and spring months of July, August and September but not during the summer and autumn months of January through to April. Thus there is a case for supplementing zinc through a direct mineral lick during the summer and autumn period, based on the leaf tagasaste zinc content.

Plasma zinc concentrations are shown in Figure 4.8. Like plasma copper, plasma zinc is not a good indicator of zinc status. However, plasma zinc concentrations were decreasing in all animals during the period of rapid growth in May and June. This is despite receiving zinc supplementation through the mineral lick. Even allowing for the variation in mineral supplement intake, cattle in this trial should be receiving supplementary zinc over and above the leaf zinc content in tagasaste. As perhaps a reflection of the overall zinc intake, the plasma zinc concentrations were not in the deficient range of less than  $0.65 \mu\text{g/ml}$ .

**Figure 4.7:** Zinc concentrations in edible leaf from tagasaste at 'Tagasaste Farm'



**Figure 4.8:** Zinc concentrations in plasma from cattle browsing tagasaste fertilised at two levels and receiving zinc supplements as part of a mineral lick



#### 4.4.7 Mineral Supplements

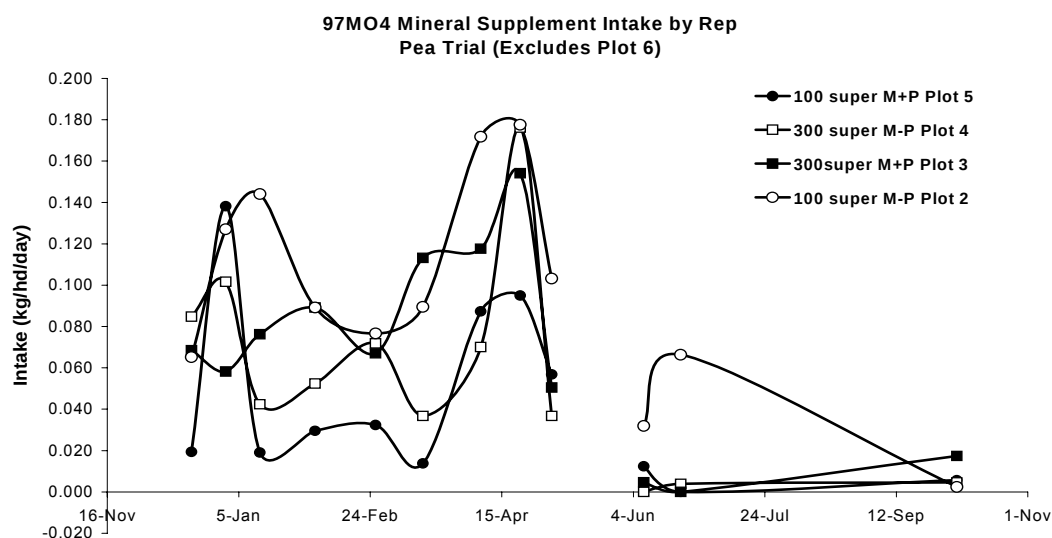
Cattle consumed between 60 – 100 g mineral per day from covered feeders (Figure 4.9) during the dry period but, as soon as the rains started, mineral lick consumption stopped. So during the growing season of the pasture when animals need the extra P supplement animals did not consume any lick. The variation in the intake of the mineral supplements by cattle browsing tagasaste is shown in Figure 4.10.

**Figure 4.9:** Cattle at mineral trough. Note sand with no ground cover [Autumn]

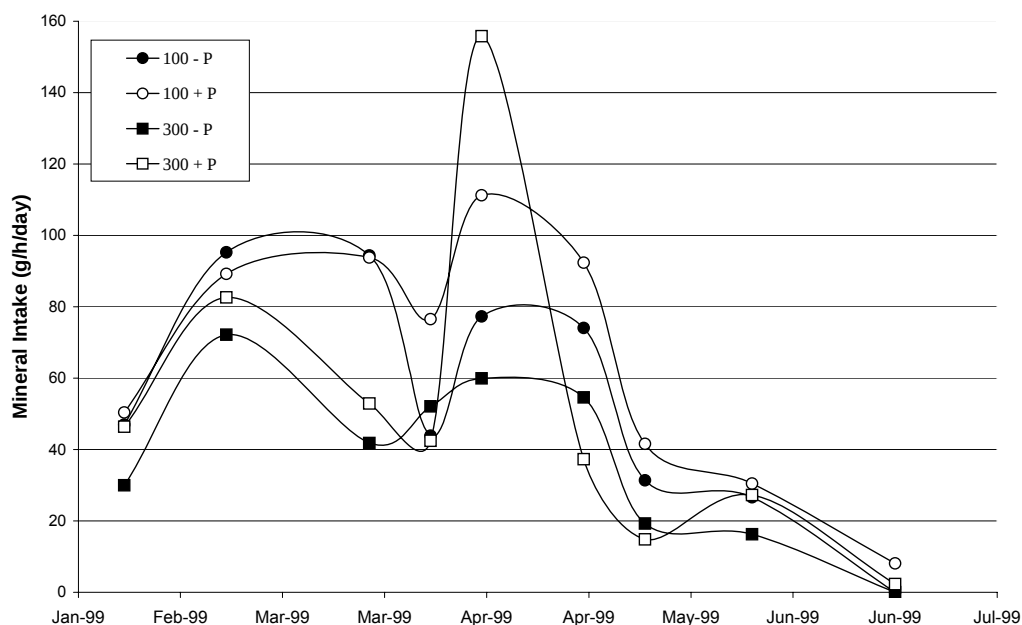


The variation in the intake of the mineral supplements by cattle browsing tagasaste is shown in Figure 4.11. In general the intake of the supplement increased to February and then markedly decreased following unseasonal heavy rain (50 mm) in March. Consumption then peaked in April before fading to zero in June after opening rainfall. The average daily consumption of the mineral supplements in the 100 kg +/- P and 300 kg +/- P treatments to the end of June were 67, 60, 52 and 52 g/hd.d, respectively. The mean intake was 58g/hd/day during the summer and autumn period of 2000 is much lower than the mean of 96g/hd/day in 1999.

**Figure 4.10:** Intake of mineral supplements by cattle browsing tagasaste on "Pea Trial" trial plots at "Tagasaste Farm"



**Figure 4.11:** Intake of Mineral Supplements by Cattle Browsing Tagasaste (Means of 3 replicates)



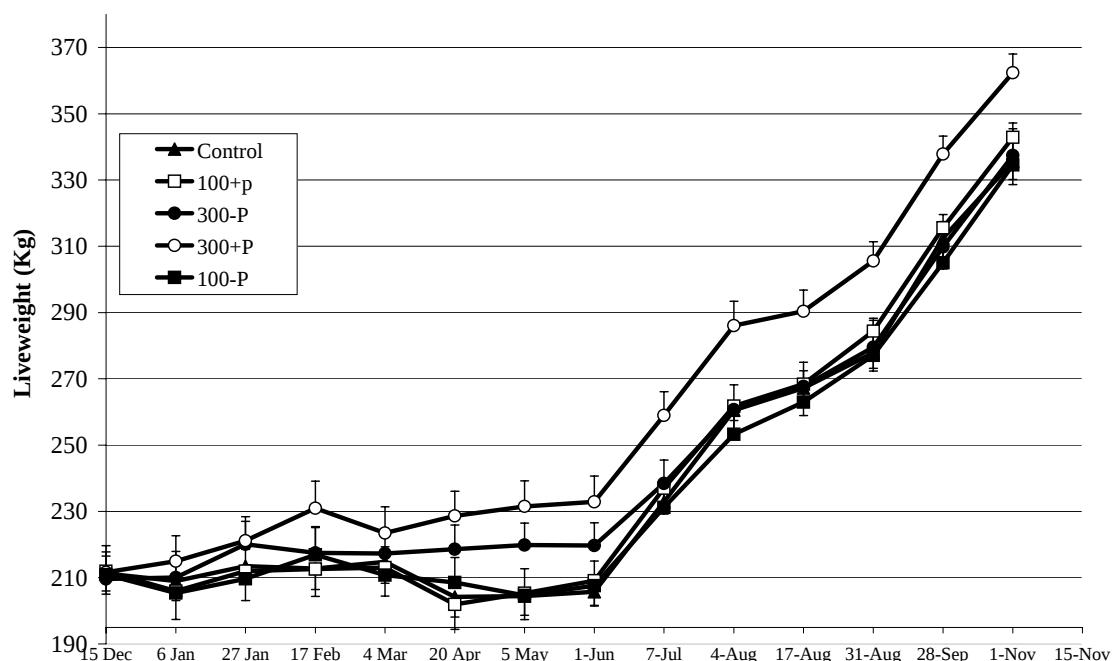
#### 4.4.8 Liveweight change

The liveweight response to extra P, either in extra SS or as a lick, is illustrated in Figure 4.12. The data shows there was response to the mineral P lick over the autumn period in the 300 kg SS treatment and maintained the advantage over the spring period. The cattle browsing tagasaste fertilised at 300kg SS/ha per year and browsing tagasaste with minerals with no P did not lose weight over the autumn period, compared with those animal browsing tagasaste fertilised with 100 kg SS, but did not maintain their LW advantage over spring.

Although the cattle browsing tagasaste fertilised with extra SS (300 kg SS/ha per year) with the mineral P supplement gained more weight than other cattle there was no economic benefits of supplying an extra 200 kg SS. In tagasaste plantations with a reasonable fertiliser history there does not appear to be any benefit in fertilising above 100 kg SS.

Compared with a small weight loss over the autumn period in the cattle browsing tagasaste fertilised with 100 kg superphosphate the cattle browsing tagasaste fertilised at the higher superphosphate (300 kg) level maintained live-weight. All cattle started to gain weight after the break-in the season. The cattle browsing tagasaste fertilised with 300 kg gained about 12 kg live-weight more than those cattle on the same fertiliser level but supplemented with minerals – P between December and 17 February. They maintained this difference over the autumn period. The unexpected negative response to the addition of phosphorus in the mineral lick that was found in 1998 was not repeated in 1999.

**Figure 4.12:** Liveweight gain of cattle browsing tagasaste fertilised at 100 or 300 kg SS/ha per year, with and without, a mineral P lick



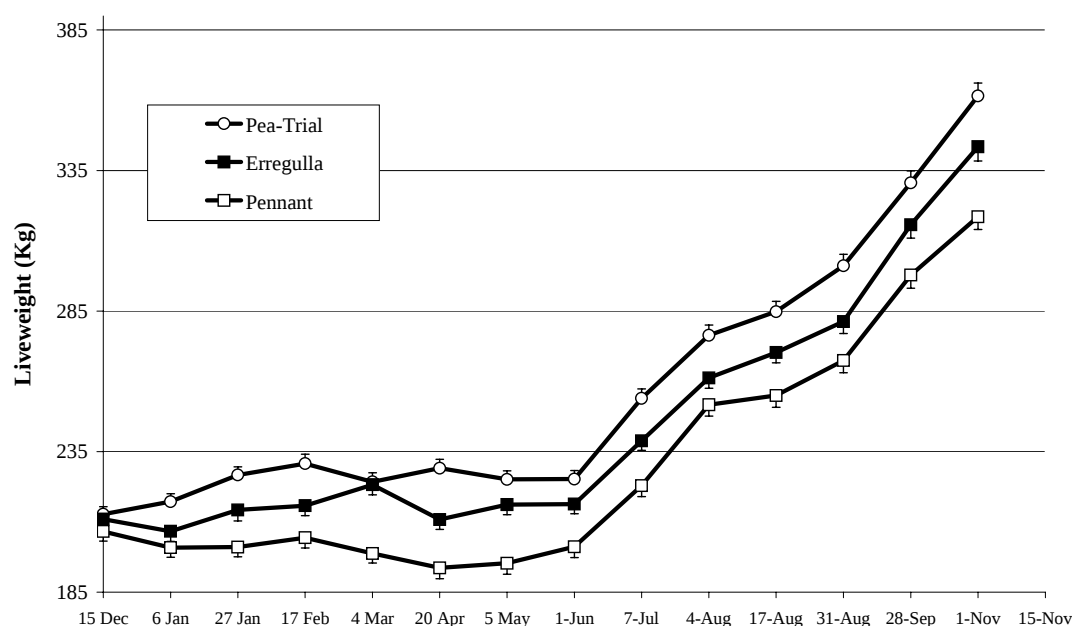
The better performance of cattle browsing tagasaste fertilised with 300 kg superphosphate compared with the 100 kg application was expected and is contrast to the lack of response in the first year (1998). The lack of response to the 300 kg in the first year (1998) was not unexpected as the extra (+ 200 kg) superphosphate was not applied until August 1997. It was anticipated that there would be this improved response to the high superphosphate (300 kg/ha) this year as it was applied in the autumn of 1998, whereas prior to year 1 was not applied until August 1997 (Milestone report 5.6 p14). The difference in live weight of cattle in 300kg superphosphate + additional phosphate was sustained throughout the year. This is in contrast with the cattle from the 300 kg superphosphate but no additional phosphate in the lick. These cattle did sustain live weight better than the lower superphosphate treatments during the summer and autumn (up to June 1). However, their relative advantage disappeared during the period of rapid growth in winter and spring to the point where there was no significant difference between the tagasaste treatment, or other treatments receiving 100kg superphosphate with or without additional phosphate lick.

As occurred in year 1 of the trial, the differences in live-weight gain between replicates was greater than the differences between treatments (Figure 4.12). Cattle in the "Pea Trial"-Trial paddocks again showed the greatest overall liveweight gain. In contrast to 1998, cattle browsing in the "Erregulla" paddocks outperformed those in the "Pennant" paddocks. The problem of large paddock variation in performance of cattle recorded in the 1998 experiment was repeated in this years experiment except that the two paddocks "Erregulla" and "Pennant" have changed position; in 1998 live-weight change in cattle browsing tagasaste was worst in "Erregulla". Figure 4.13 shows the variation in live-weights between each paddock to November 1999 with the best response once again in "Pea Trial".

In an attempt to determine the reason for these differences between paddocks bores will be sunk in the three paddocks to see if there are any obvious variations in the depth of the

underlying water tables. Soil samples will also be collected as the bores are sunk to a depth of 5 - 10 metres. This will allow a check on any measurable differences in soil fertility between the paddocks at tagasaste root zone level.

**Figure 4.13:** Live-weight change of cattle browsing tagasaste in the three replicated plots on 'Tagasaste Farm' in 1999



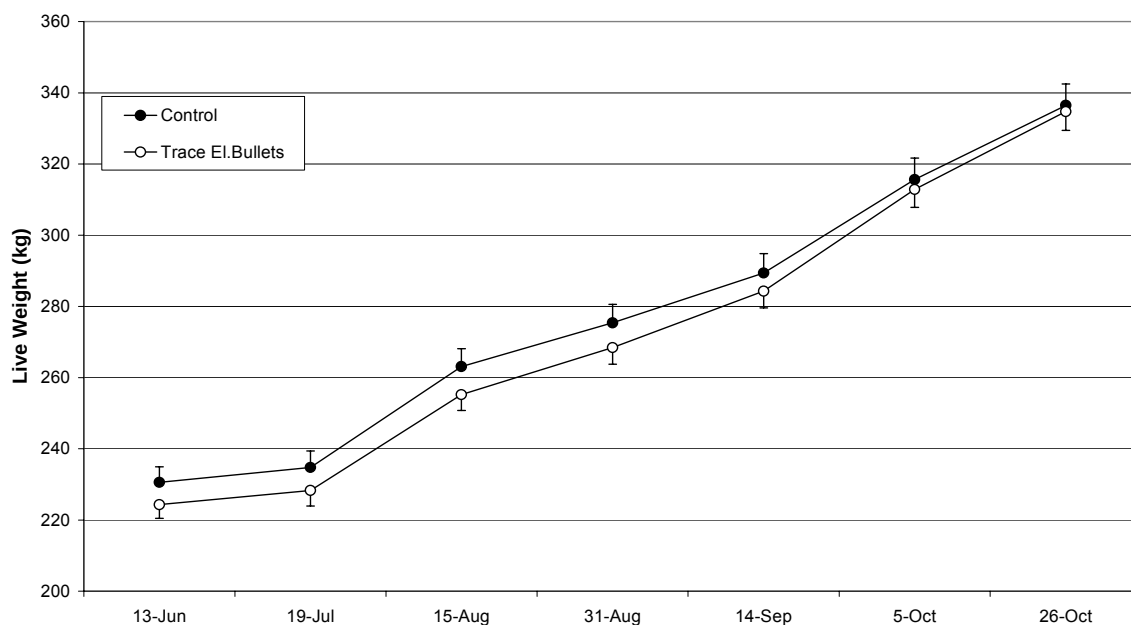
During 1999, it was necessary to supplement the steers in plots 13, 14 and 15 in "Pennant" paddock. The treatments for these 3 plots were Control, 100 kg Superphosphate + P and 100 kg Superphosphate - P respectively. Supplementation was required because on average the cattle on these plots lost 12% of their live-weight since the start of the trial. Lupins were fed at the rate of 1kg /head per day, fed 3 times per week, starting on the 10th of May. Supplementation ceased on the 5<sup>th</sup> of July when they had regained more than their starting weight (mean weight of 215 kg).

#### 4.4.9 Effect of Trace Element Supplementation and Speying on Liveweight Gain

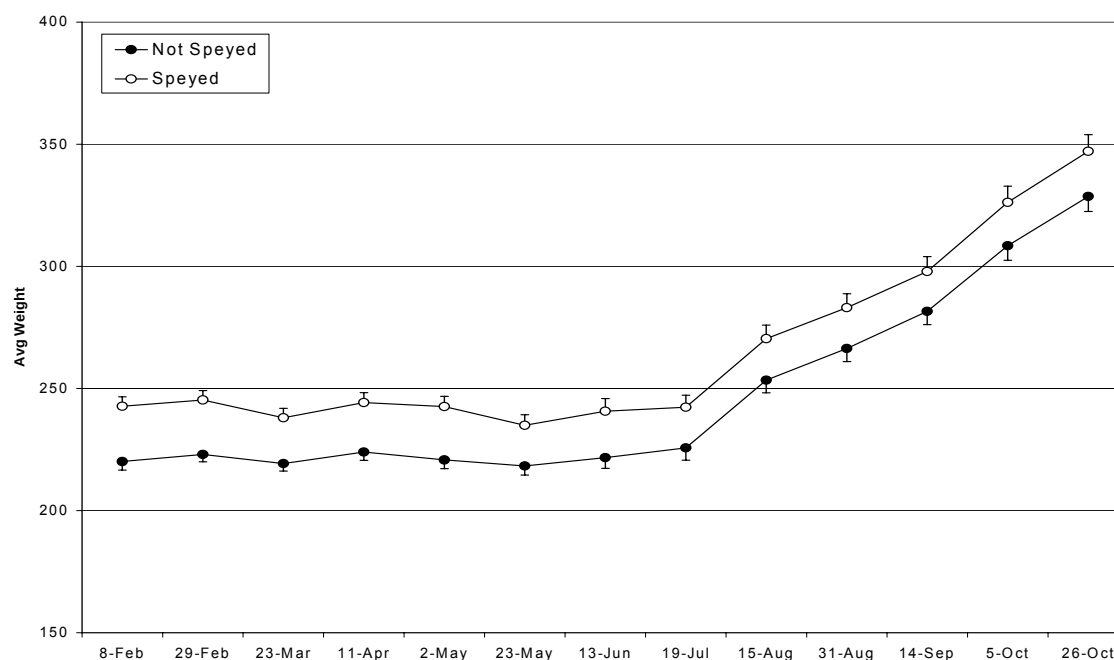
Half the cattle on each plot received copper, selenium and cobalt bullets based on the plant leaf analysis for tagasaste and the plasma values for copper and selenium. The cattle were bulletted on the 13<sup>th</sup> June as the most likely time for a response to trace elements is during the period of rapid plant and animal growth in the winter and spring.

The starting liveweights for the two groups differed by an average of over 6 kg, but at the end of the spring on October 26<sup>th</sup> the weights for the 2 groups had converged. This slightly faster growth in cattle receiving the trace elements was neither significant nor economic (Figure 4.14).

**Figure 4.14:** Effect of trace element supplementation on liveweight change of cattle browsing tagasaste on 'Tagasaste Farm' in 2000



**Figure 4.15:** Effect of speying on liveweight change of cattle browsing tagasaste on 'Tagasaste Farm' in 2000



Heifers that were speyed at the start of the trial were significantly ( $P < 0.001$ ) heavier ( $242.7 \pm 3.8$ ) than those that were not speyed ( $220. \pm 1.3$ ). This weight advantage was sustained throughout the trial by the speyed heifers (Figure 4.15). However, speying did not confer any advantage per se in this trial as speyed heifers did not increase their weight advantage at any stage.

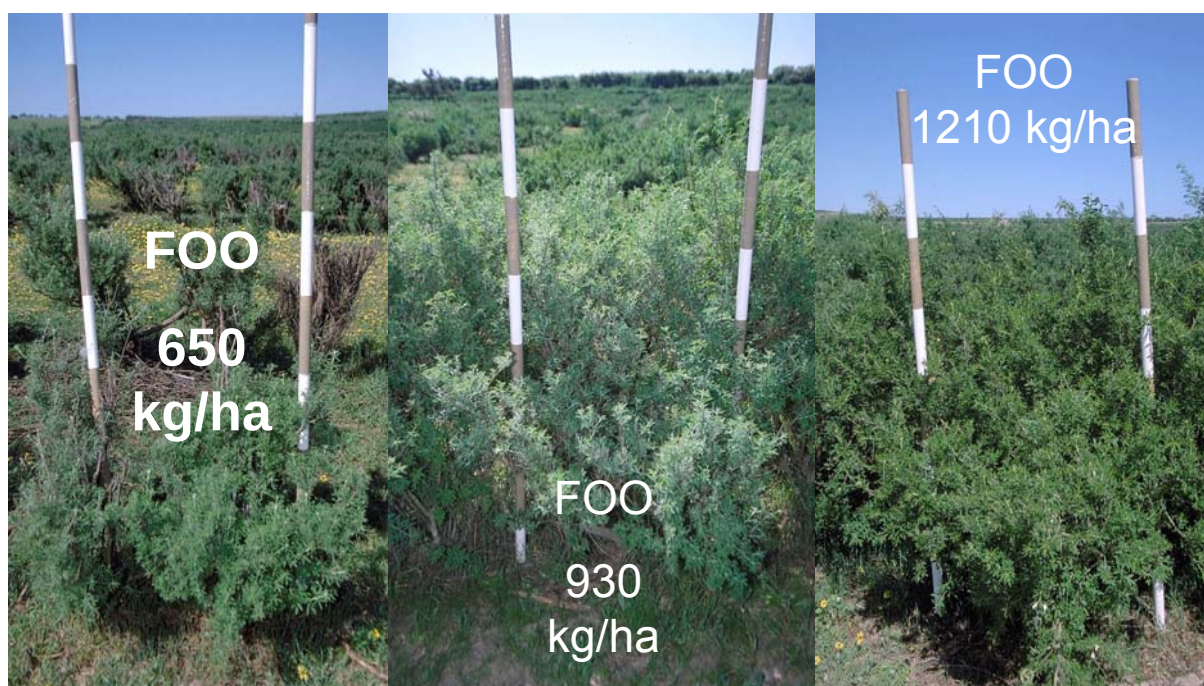
#### 4.4.10 *Food-On-Offer (FOO) and Liveweight Gain*

The relationship between “Food on Offer” (FOO, kg DM/ha) and feed intake of an annual pasture is well understood. During the project, a method was developed for estimating the FOO available to cattle browsing tagasaste during the autumn. FOO was then correlated with the average daily liveweight gain of cattle.

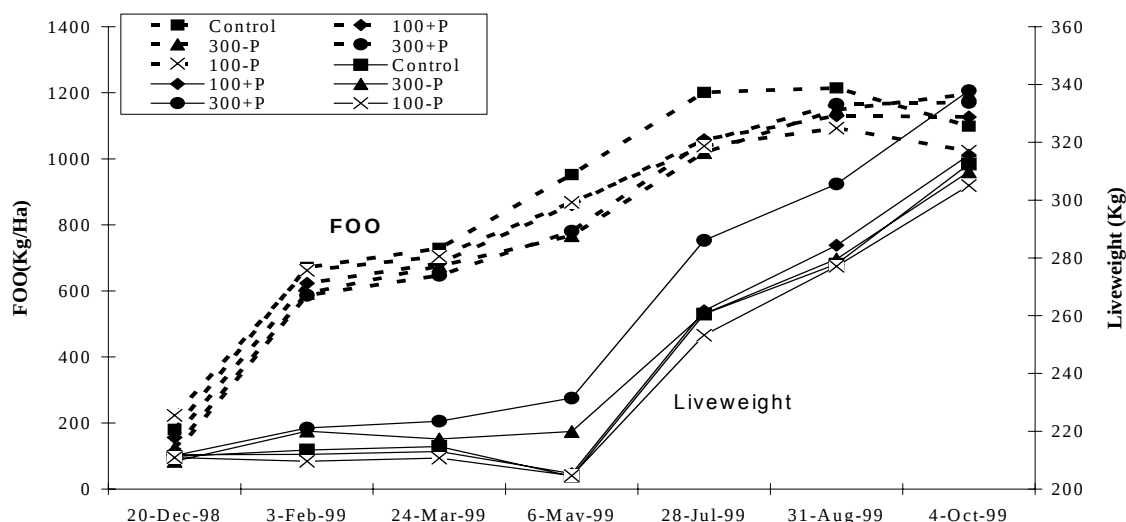
Feed-on-Offer, all leaf material together with about 100mm of stem, was estimated by a visual rating method, based on a one-metre section of row with at least 30 estimates per 5 ha paddock. Calibration of the visual estimates was achieved by measuring the DM yield from a number of one-metre sections of row of known rating. Dry matter content ranged from 24.9% in September to 35.2% in March. FOO was measured every 8 weeks and animals weighed monthly.

FOO in early summer was around 500kg/ha and even though high in CP and of adequate energy, animals grew slowly or maintained weight. As summer progressed, FOO continued to increase, but the animals lost weight until the break of season in May. Following rain, liveweight increased rapidly (0.6 kg/head per day) while the tagasaste grew away slowly (Figure 4.16 and Figure 4.17).

Maximum growth rate of cattle was reached in October, when FOO was at the relatively low level of 1,000kg/ha. This is a little more than half the amount required for maximum intake of annual pastures but similar to other perennial species. As FOO increases the tagasaste shrub simply gets larger, with no associated increase in leaf density. Bite size is not likely to increase greatly as FOO increases.

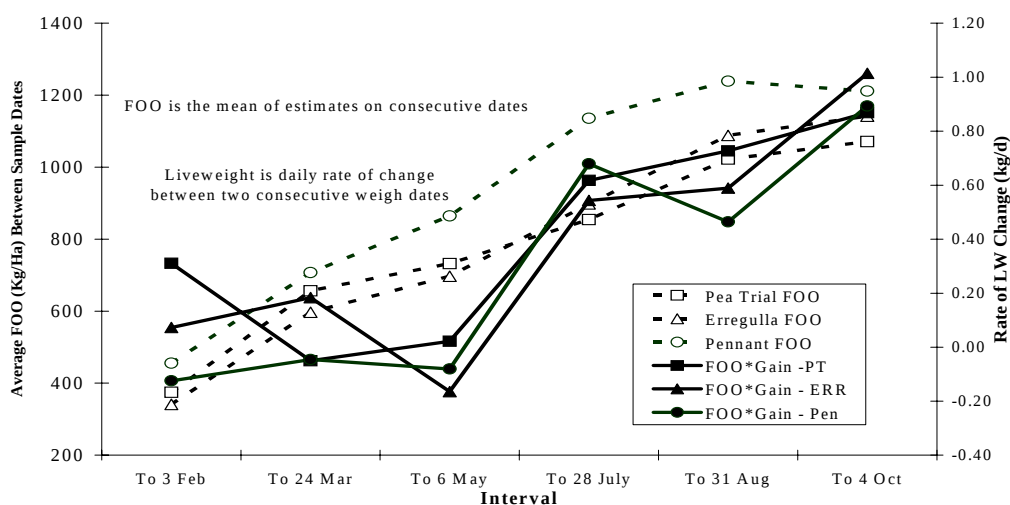


**Figure 4.16:** Relationship between Food-on-Offer (FOO) [y1 axis] and live-weight change [y2 axis] of cattle browsing tagasaste fertilised with superphosphate and supplemented with minerals with or without additional phosphorus



It is not clear from this study whether FOO levels of 1000kg/ha are required in spring to achieve maximum performance. The tagasaste grew away from the animals at all periods of the year, suggesting that possibly the stocking rate chosen was too low. FOO of the fodder shrub tagasaste was not a good indicator of average daily liveweight gain for cattle either during autumn (low growth rates) or during the winter and spring when cattle were growing. In fact animal growth is the ultimate measure of tagasaste availability. Moreover, the palatability and density of small leaf material of tagasaste may be better indicators of true FOO than standard FOO estimates adapted from annual grass/clover pastures.

**Figure 4.17:** Relationship between Food-on-Offer [y1 axis] and live-weight change [y2 axis] of cattle browsing tagasaste in the three replicated plots on 'Tagasaste Farm'



#### 4.4.11 Rumen and Blood Biochemistry

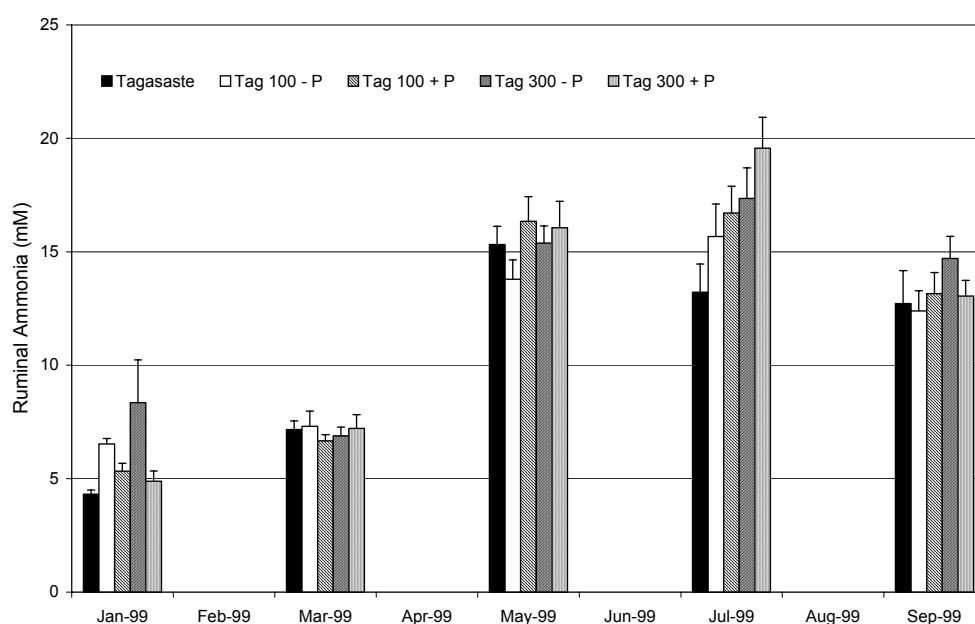
The rumen and blood biochemistry from 'Tagasaste Farm' followed the same seasonal pattern as those from 'Dunmar' but the differences between mineral and fertiliser treatments were not as pronounced as those for the energy and protein supplements. Certainly the differences between the P treatments were not significantly different as they are between the level of lupin supplements.

Nevertheless, during the July sampling when tagasaste is actively growing, the ammonia concentrations do follow the pattern of liveweight response on the plots. Of particular note is the large difference between the summer/autumn concentration of ammonia and that during winter and spring (Figure 4.18). The rumen ammonia concentration in July and September (Figure 4.18) is consistent with the tagasaste intakes (Chapter 2, Table 2.2.).

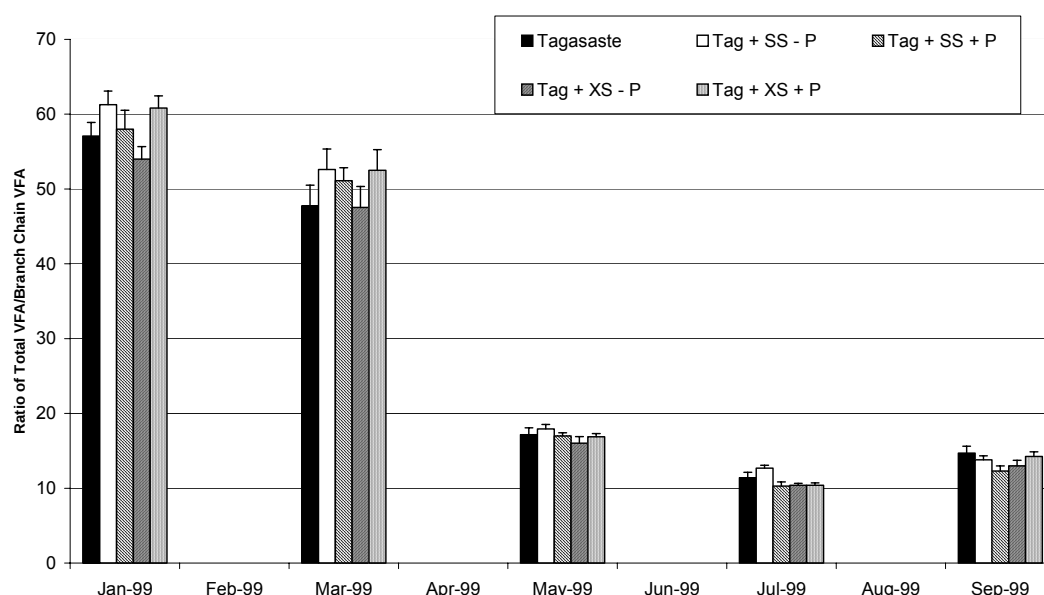
The same pattern of change is evident in ratio of total:branch-chain VFA in cattle receiving the different treatments (Figure 4.19). The logic of argument here is that the precursors of microbial protein supply are limiting during summer and autumn, and thereby limiting the gain. The supply of P is sufficient to enhance the growth weight but the rumen data is not sensitive enough to delineate the order of difference seen in the liveweights.

Both of the ruminal parameters, ammonia and total:branch-chain VFA ratios are consistent with the intake data. In addition, the ratios are consistent with a lack of protein degradation in the rumen to supply branch-chain VFA's. This similar to the pattern seen in the 'Dunmar' trials so the tagasaste effect is not site specific but associated season.

**Figure 4.18:** Ruminal ammonia concentrations in cattle browsing tagasaste and receiving phosphorus supplements at 'Tagasaste Farm'. Results shown are the mean ( $\pm$  SEM) of 15 animals per treatment



**Figure 4.19:** Ratio of total:branch-chain VFA in cattle browsing tagasaste and receiving phosphorus supplements at 'Tagasaste Farm'. Results shown are the mean ( $\pm$  SEM) of 15 animals per treatment



## 4.5 Conclusions

The liveweight response of the cattle browsing tagasaste with two fertiliser levels (100 and 300 kg superphosphate/ha per year) and supplemented with a mineral lick, with and without P, was not as clear and definitive as expected. The lack of response to the extra fertiliser in the 1<sup>st</sup> year [1998] was expected as the extra fertiliser had not been added until August [1997] with the animals starting on the plots in January 1998. In the 2<sup>nd</sup> year [1999] the liveweight advantage with 300kg superphosphate plus P lick was established in the autumn period and sustained throughout the winter and spring. Cattle in the other treatments did not catch-up through compensatory growth during the flush of tagasaste growth. The response to the 300 kg application in 1999 was hypothesised but though the liveweight gain for the cattle with the mineral P lick was above the other treatments the extra liveweight was not significantly different and not economic. What was surprising and unexplainable was the cattle stopped eating the mineral lick when the rains started but the cattle on the 300 kg plus mineral P maintained their weight advantage over the other cattle. On the other hand, the cattle on the 300 kg with minerals but no P actually lost the advantage they gained over the cattle on the 100 kg superphosphate over autumn period. It is as if the extra P gained from the mineral lick over the autumn period was sufficient to maintain the liveweight of the cattle during the rapid growth in spring. The value of FOO as a predictor of carrying capacity and performance of cattle was not a useful index with cattle browsing tagasaste. In summer and autumn when cattle restrict their consumption of tagasaste a value for FOO gives no indication of how the cattle will perform. Part of the reason is that short growing leaves seem to be more palatable and therefore will be eaten compared with older more mature leaves. This is very obvious in spring where, during rapid plant growth and liveweight gain, the young growing leaf in heavily stocked tagasaste paddocks appears to be more attractive to the animal than the older more mature leaves. Ruminal and blood parameters were not sufficiently acute to explain the difference in liveweight observed. These measures did show significant differences between seasons, but not treatment groups. However, analysis of

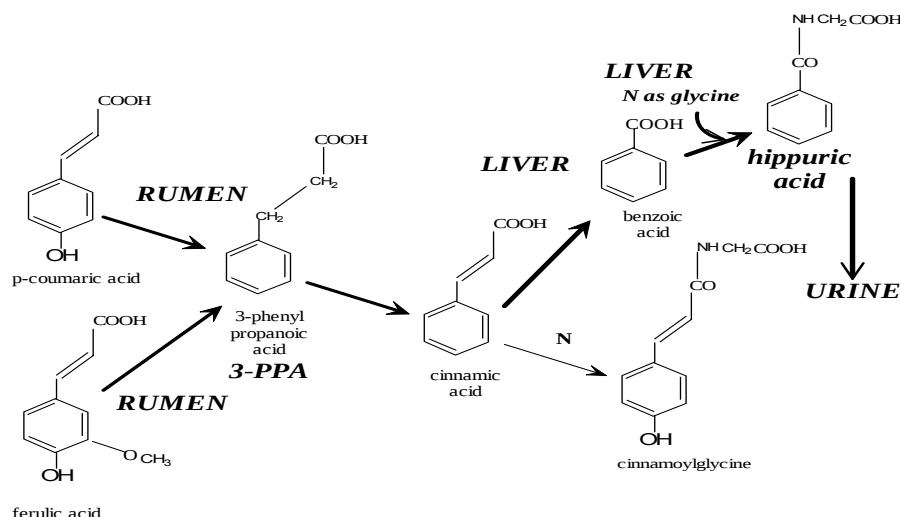
edible leaf from tagasaste provided a broad measure of potential deficiencies of nutrient supplies eg. phosphorus and copper that could lead to liveweight gains through appropriate supplementation.

## 5 IMPLICATIONS FOR ACID URINE IN CATTLE BROWSING TAGASASTE OVER THE SUMMER AND AUTUMN PERIOD IN A MEDITERRANEAN ENVIRONMENT

### 5.1 Introduction

The urinary pH of ruminants is normally above pH 8. Most herbivorous animals have alkaline urine due in large part to high potassium intakes from rapidly growing plants that have high cellular content. A reduction in urinary pH to well below 7 indicates a ruminant's acid-base balance has been sufficiently reduced. In ruminants, acid-base balance can be disturbed during periods of starvation or severely reduced food intake due to fatty acid mobilisation and its associated ketogenesis and possibly ketosis. During these periods the major circulating ketones,  $\beta$ -hydroxybutyrate and acetoacetate are elevated in plasma. Measuring plasma  $\beta$ -hydroxybutyrate can indicate the degree of fat mobilisation if assessed carefully as  $\beta$ -hydroxybutyrate can also result from hydroxylation of butyrate as it crosses the rumen wall. Thus ruminants have a normal circulating  $\beta$ -hydroxybutyrate of around 0.5 – 0.6 mM. Reduction of urine pH is also one means used to protect ruminants against metabolic diseases such as milk fever and urinary calculi. This is normally achieved in the dairy industry by adding anionic salts to the diet, to reduce dietary cation to anion difference (DCAD, 1). In this study we investigated the natural DCAD of tagasaste during the various seasons of the year to see whether the DCAD of tagasaste is sufficiently low to influence the urinary pH. We also tested the hypothesis that the feeding of tagasaste known to rich in absorbable phenolic could achieve a similar result. Absorbed phenolic are converted, by the liver, into organic acids such as hippuric acid that can acidify urine as shown in the Figure 5.1.

**Figure 5.1:** Production of hippuric acid during protein metabolism in cattle browsing tagasaste



### **5.1.1 Hypothesis**

1. Tagasaste forage has a low to negative dietary cation/anion difference (DCAD) during the summer and autumn.
2. Periods where tagasaste has a low to negative DCAD are associated with urine pH below 7.0.
3. Tagasaste has high low-molecular phenolic during summer and autumn that lead to the excretion of hippuric acid that also acidifies urine.

### **5.1.2 Aims/objectives**

1. Investigate the urinary pH in cattle browsing tagasaste.
2. Establish the DCAD values for tagasaste for each season.
3. Identify hippuric acid in the urine from cattle browsing tagasaste.

## **5.2 Material & Methods**

### **5.2.1 Experimental area**

Heifers were browsing 75 ha of tagasaste planted as double rows at 5-m row spacing established in 1990. The property 'Tagasaste Farm' is in the Lancelin region of Western Australia (Lat. 32°40'S and Long. 116°10'E). The 75 ha of tagasaste were divided into 15 experimental plots each 5 ha in area. The 75 ha of tagasaste were three paddocks, which were close, but not adjacent. To reduce the variation within the experiment the three areas are considered to be blocks with each treatment represented in each block.

Standard superphosphate had been applied for the last 5 years at 100 kg/ha per year. In 1997, 100 kg had been applied as usual in autumn with the extra 200 kg being applied in September. In subsequent years all the fertiliser was applied in autumn.

#### **Cattle**

The experimental cattle were predominantly Brahman cross about 15 months of age at the start. In each of the two years (1999 – 2000) new mobs of cattle were introduced into the experimental plots in January. Cattle were identified with ear tags and vaccinated with 5 in 1 antibacterial and drenched with an anthelmintic to control internal parasites. Experimental cattle were weighed and randomly allocated to one of the five treatments after stratifying, within sex, on live weight.

1999: 152 head with mean initial liveweight of  $210.1 \pm 1.83$  kg

2000: 159 head with mean initial liveweight of  $234.2 \pm 2.35$  kg

Urine was collected from heifers from each treatment, about every three months to monitor urinary pH and hippuric acid.

## Treatments

Cattle grazed tagasaste fertilised with superphosphate (SP):

1. Standard SP application (100 kg/ha per year)
2. Standard SP application (100 kg/ha per year) + mineral lick - P
3. Standard SP application (100 kg/ha per year) + mineral lick + P
4. Standard SP application (100 kg/ha per year) + extra SP (200 kg/ha per year) + mineral lick - P
5. Standard SP application (100 kg/ha per year) + extra SP + mineral lick + P

Animals were randomly allocated to treatment groups after stratifying on initial live weight.

The stocking pressure was 2 beasts/ha; ie. 10 animals per plot. As extra plant growth was expected (measured as Feed-On-Offer - FOO) on the high (300 kg) superphosphate treatments (Trs 4 & 5), more animals were allocated to these plots so that each paddock was grazed to a standard FOO. FOO was assessed every three months and extra cattle were allocated to treatments as required but it is anticipated extra animals will only be required in spring. The extra growth of tagasaste was measured as grazing days and liveweight gain/ha.

## Mineral supplements

The composition of the mineral supplement (Table 4.1) was formulated with Dr John Milton after examination of leaf mineral analysis data that had been collected on this site since 1995. The treatments with, and without P ( $\pm$  P) were formulated by adding, or leaving out the P component. The mineral lick was fed *ad lib*.

## Measurements and observations

Urine was measured as soon as possible after collection using a portable pH meter that had had its calibration checked and verified with pH standards just prior to the urine collection itself.

Urine samples were chilled into a portable freezer and kept frozen until assay for hippuric acid. Hippuric acid assay was performed on urine samples by the group at University of Queensland head by Dr David McNeill.

## Statistics

The data from this trial has been analysed for descriptive statistics and for significant differences by using one-way ANOVA on the software package GraphPad.

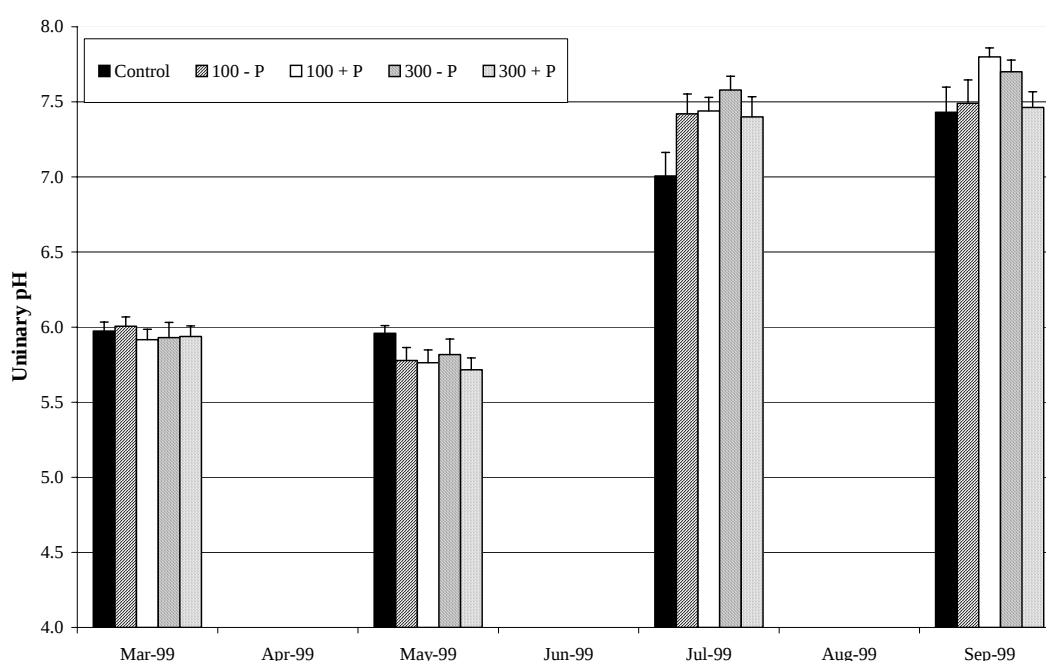
## 5.3 Results & Discussion

Urinary pH values were very low (<6.5) from cattle browsing tagasaste during summer and autumn at 'Tagasaste Farm' (Fig 5.2). These values were not associated with elevated plasma ketones indicative of metabolic acidosis but were associated with elevated urinary hippuric acid concentrations suggesting a direct acidification of the urine. The pH values returned to the more usual alkaline range generally observed in herbivores during the winter and spring (Figure 5.2). Mineral supplementation had no significant effect on urinary pH.

The acid urines were associated with higher concentrations of phenolic in the tagasaste during the summer and autumn. Phenolic compounds can be metabolised to hippuric acid via the rumen and liver, and may explain some of the components of the acid urine.

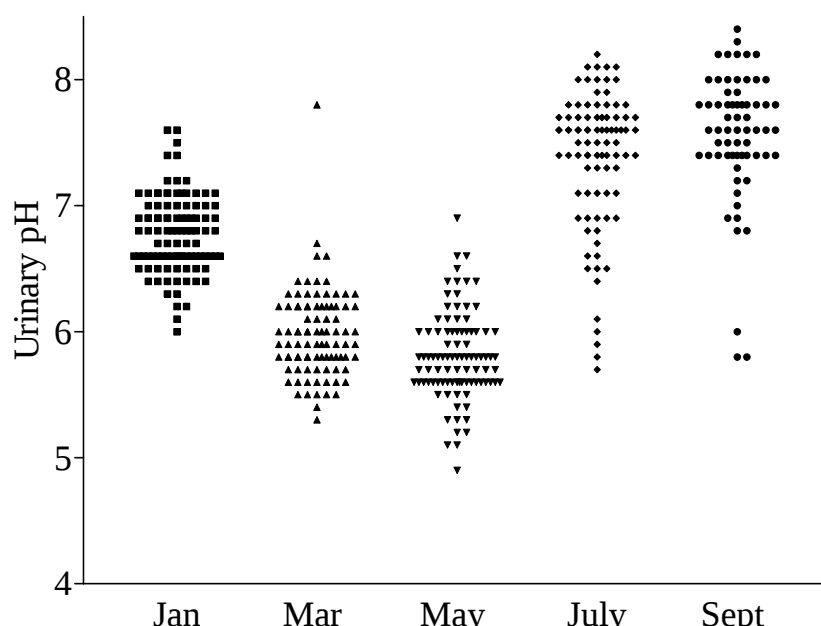
The changes in urinary pH with season are shown in Figure 5.3. The urine pH in cattle browsing tagasaste is around 6 in summer and autumn when animals are not growing (Figure 5.3). Normally, urine pH in cattle grazing pasture is above pH 7. However, there is evidence that shows that in cattle and sheep grazing tropical grass urine pH may fall below 7. The urinary pH is above 7.0 during July and September, the winter and spring months of the year (Figure 5.2).

**Figure 5.2:** Urinary pH in Cattle Browsing Tagasaste and Receiving Mineral Supplements at 'Tagasaste Farm'



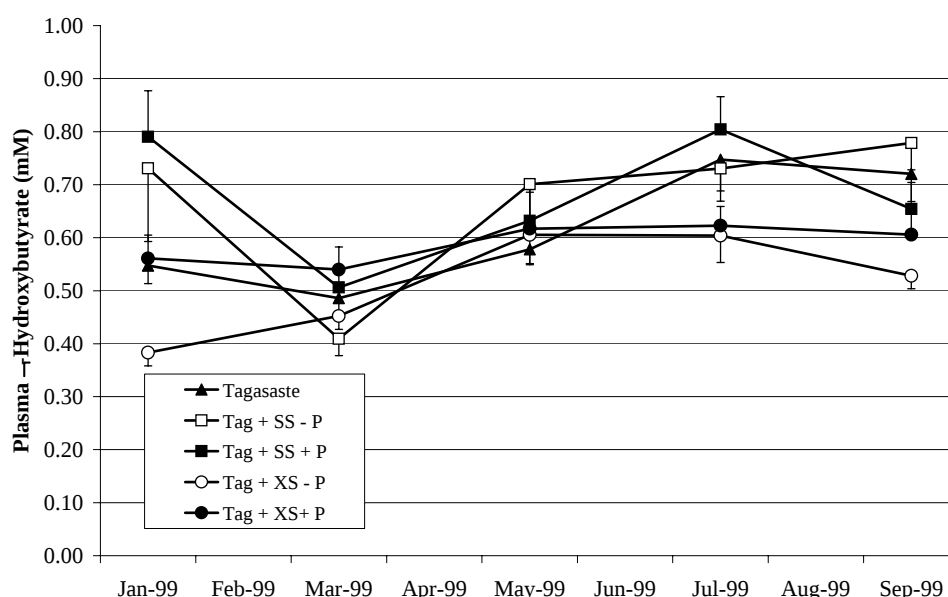
One of the reasons that the urinary pH is lower than 7.0 during the summer and autumn months is that the cattle are eating insufficient tagasaste to maintain their energy requirements and are therefore mobilising their fat reserves. Mobilisation of fatty acids can lead to elevated ketones such as  $\beta$ -hydroxybutyric acid, and an associated mild acidosis.

**Figure 5.3:** Effect of season on urinary pH from cattle at 'Tagasaste Farm'



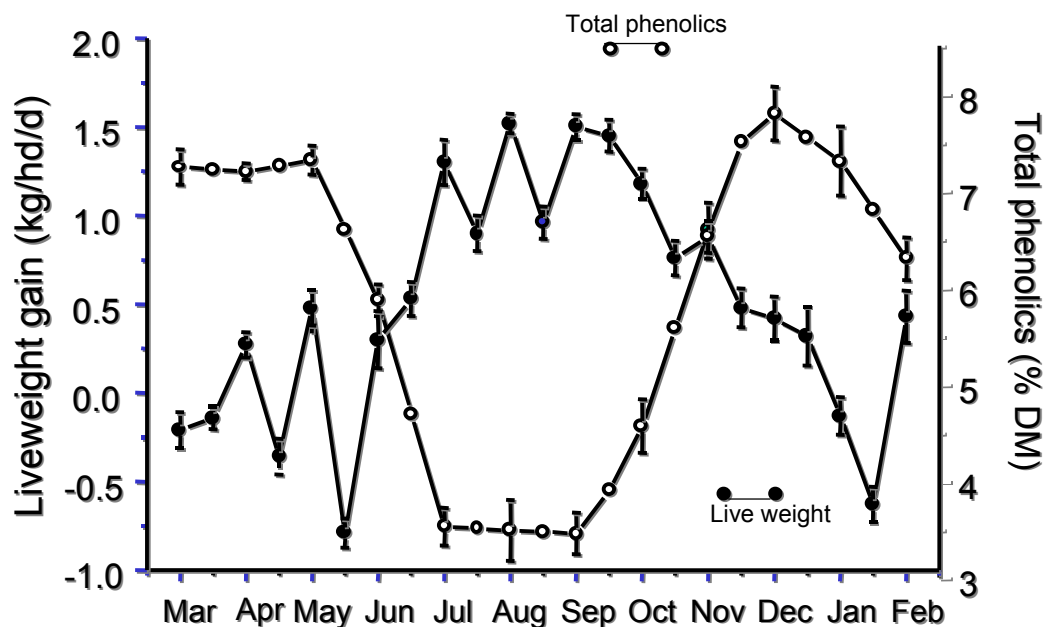
The mild acidosis can be compensated through elevated respirator rate and disposal of acid in the urine resulted in lower pH. However, the concentration of  $\beta$ -hydroxybutyric acid in plasma was not elevated above the normal range (Figure 5.4) during the periods in March and May when the cattle were excreting acid urine. This finding does not support kidney compensating mild acidosis as the reason for lower urinary pH.

**Figure 5.4:** Effect of season on plasma  $\beta$ -hydroxybutyrate from cattle at 'Tagasaste Farm'



Work by Dr Lowry, Tropical Crops and Pasture, CSIRO, Brisbane showed tropical grasses contain phenolic as does tagasaste. The pattern of liveweight gain as compared with total phenolic in tagasaste is presented in Figure 5.5.

**Figure 5.5:** Liveweight gain and total phenolic in cattle browsing tagasaste at 'Tagasaste Farm'



Phenolic compounds were assayed in tagasaste by Borrens and Poppi (1991) who found no trace of the high molecular weight phenolic and polyphenolics in tagasaste. Instead unpublished work by McSweeney et al. has found a high concentration of low molecular weight phenolic glycosides belonging to the flavonoid class of compounds. The two major flavonoids found in tagasaste are apigenin and luteolin both classified as flavones. These are also two most common flavones in plants and they do occur as glycosides in tagasaste. Both of these compounds but particularly luteolin are in the same group as the more studied flavone, quercetin. Phenolic monomers can decrease the solubility of protein by forming a hydrophobic coating around the protein analogous to that seen for tannin-protein complexes. In addition flavones can disrupt energy metabolism through competitive inhibition of the phosphotransferase enzymes and through reduced effectiveness of a series of membrane-associated enzymes and proteins.

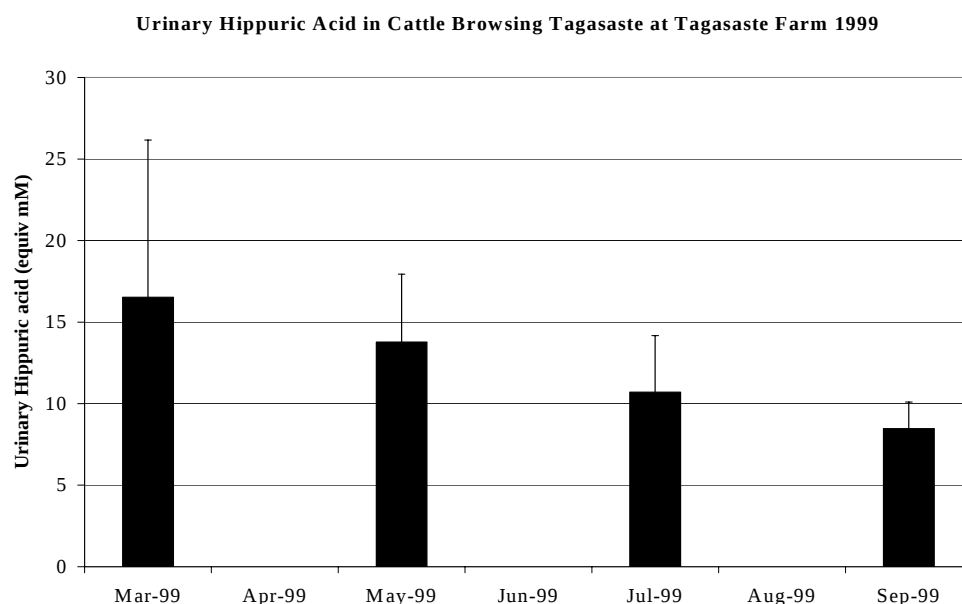
Flavones can also be degraded in the rumen to 3-phenyl propionic acid and cinnamic acid which is absorbed from the rumen, converted in the liver to benzoic acid, conjugated with glycine to form hippuric acid which is excreted in the urine [see diagram below]. This process has two effects. Firstly hippuric acid would directly acidify the urine. Secondly the loss of glycine in the conjugation step would compromise the efficiency of nitrogen metabolism in ruminants.

Thus the failure of growth of cattle browsing tagasaste during the summer and autumn may be due in part to the disruption of protein metabolism (Figure 5.1). This could be associated with the production of hippuric acid and the direct excretion of this acid into the urine, lowering the pH as shown in the diagram below. Thus the acidic urine observed in cattle browsing tagasaste could be associated with the high phenolic concentrations in tagasaste during the summer and autumn.

Urine samples were sent to Dr David McNeill at the University of Queensland to confirm hippuric acid by analysis. The effect of season on the concentration of hippuric acid in the

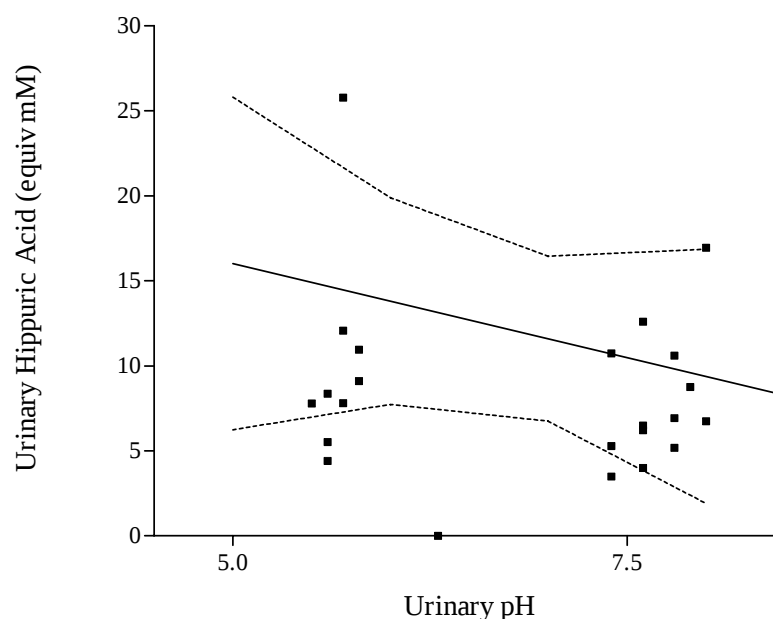
urine is shown in Figure 5.6. Clearly hippuric acid is being excreted in the urine by these cattle at 'Tagasaste Farm'. The concentration of hippuric acid is higher during the periods of high concentrations of phenolic compounds (Figure 5.4). This provides associative evidence that the flavone compounds in tagasaste are being degraded to hippuric acid thereby depleting the glycine pool for protein synthesis.

**Figure 5.6:** Seasonal changes in the concentration of hippuric acid in cattle at 'Tagasaste Farm'



The relationship between the urinary pH and the concentration of hippuric acid in the urine is shown in Figure 5.7. The hippuric acid is sufficiently powerful, or present in high enough concentration to acidify the urine directly. However, the relationship provides only part of the explanation for the low urine.

**Figure 5.7:** Relationship between urinary pH and hippuric acid in cattle at 'Tagasaste Farm'



Another means of lowering urinary pH is through dietary cation anion difference (DCAD). This method is often used in dietary formulation for preparturient dairy cows to assist with the mobilisation of calcium as a preventive measure for milk fever. The leaf composition of various anions and cations is presented in Table 5.1. The mineral composition of tagasaste changes dramatically from January and March through to July and September, particularly in the proportions of  $K^+$  and  $Na^+$  (Table 5.1).

**Table 5.1:** Cation and anion concentrations in tagasaste during different times of the year in 1999

| For the tagasaste diet | Jan.  | Mar.  | May   | July  | Sep.  | SEM   |
|------------------------|-------|-------|-------|-------|-------|-------|
| Urine pH               | 6.77  | 5.95  | 5.81  | 7.37  | 7.58  | 0.146 |
| K                      | 129.0 | 130.7 | 320.3 | 351.3 | 332.5 | 12.42 |
| Na                     | 32.5  | 53.5  | 18.1  | 12.5  | 23.2  | 1.81  |
| Cl                     | 86.1  | 112.6 | 109.2 | 81.5  | 102.4 | 2.67  |
| S                      | 61.3  | 63.2  | 108.3 | 116.3 | 125.3 | 3.38  |
| DCAD                   | 14.1  | 8.4   | 120.9 | 166.1 | 128.1 | 8.11  |

During summer, tagasaste has relatively low  $K^+$  concentrations and this increases more than two-fold during winter. The low  $K^+$  is indicative of low cell turnover and slow growth; possibly a protective measure by the plant against drought and heat during summer. Thus tagasaste is a naturally low DCAD diet during summer and autumn. The reduction in urinary pH could also result from the lower DCAD in the tagasaste during this period. This effect on pH may be additive and not necessarily exclusive of the effect of the hippuric acid on pH. The DCAD is significantly lower during January, March and May than during the winter and spring months of July and September. March is the month with the lowest DCAD and is also the month during which urine pH is acidic. However, it was during May that cattle had the lowest urinary pH when both the DCAD was and hippuric acid were higher than in March. Thus DCAD may have an additive effect on decreasing urinary pH. However, the hippuric acid and DCAD together have a profound effect in lowering urinary pH but they do not provide the total explanation for the urinary pH changes.

## 6 'TAGASASTE STAGGERS' SYNDROME

### 6.1 Introduction

Over the years when mustering cattle browsing tagasaste there have been occasional observations of cattle staggering and collapsing. This symptom has been called "tagasaste staggers". "Tagasaste staggers" have generally been seen in the autumn, early winter months but, recently "staggers" was observed in a mob at Jurien in spring, 1998. "Tagasaste staggers" only ever occurs when animals are under stress while being moved or handled. On all occasions there were no visible signs of anything wrong with the cattle when observed standing or browsing in the paddock. There is no indication the "tagasaste staggers" influences performance of the animals and is considered primarily a "management" problem because cattle exhibiting the symptoms have to be left behind, to be moved at a later date. If effected cattle are left alone they generally recover within 2 - 3 hours, and certainly within 24 hours.

Some observers have suggested a possible involvement of hypomagnesaemia since magnesium has a role in regulation of nerve and muscle activity. Others have suggested that the condition is seasonal occurring mainly after onset of rain particularly in autumn.

What is consistently observed is that affected cattle fall behind when the herd is being moved from one area of tagasaste to the next. If the cattle are pushed, then affected animals become uncoordinated in their movements (ataxic), begin twitching along the flanks and muzzle, and then finally fall straight down on their haunches and hind limbs. When left to recover, generally the cattle will stand up after about 10 –15 minutes and walk along gingerly. Unfortunately cattle collapsing near water holes or dams can get into difficulties, sometimes with fatal results.

This study reports blood values from cattle browsing tagasaste on a property where staggers had been reported.

## 6.2 Material & Methods

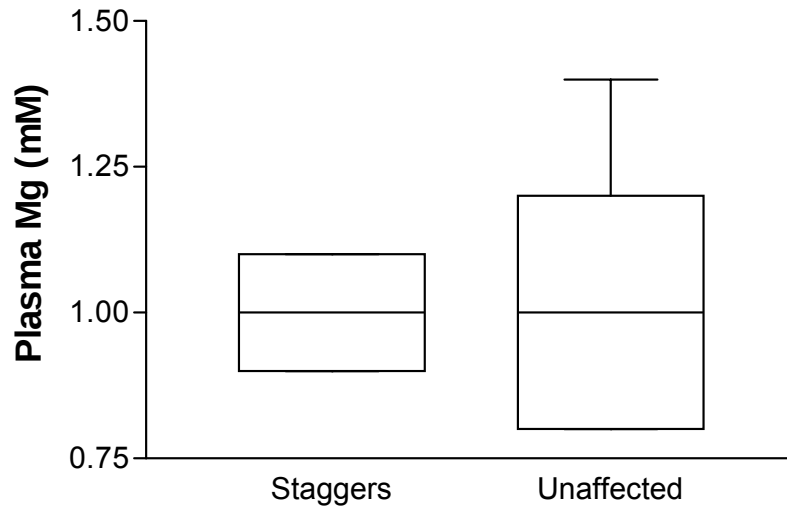
Two hundred and seventy eight Brahman steers with average weight of 330 kg browsed a 60 ha paddock of tagasaste, planted in double rows about 6 m apart, for eight weeks and gained at 0.8 kg/hd.d. Inter-row species included ryegrass, other grasses, radish and capeweed. Water quality was good with 28 grains salt.

Staggers was induced by using men on horseback to drive a herd of cattle known to contain animals that had the condition. Once an animal became ataxic and went down, a team of people took a jugular blood sample and then retreated to allow the animal to recover. Blood was taken from seven such affected animals, then blood was collected from a matching number of unaffected animals in the same herd immediately after the drive. One animal did not recover, a post-mortem was performed by a veterinarian and the central nervous tissue was taken for histopathology that established that there were no lesions where spongiform pathology was present. This study has described some possible causative factors and mode of action.

## 6.3 Results & Discussion

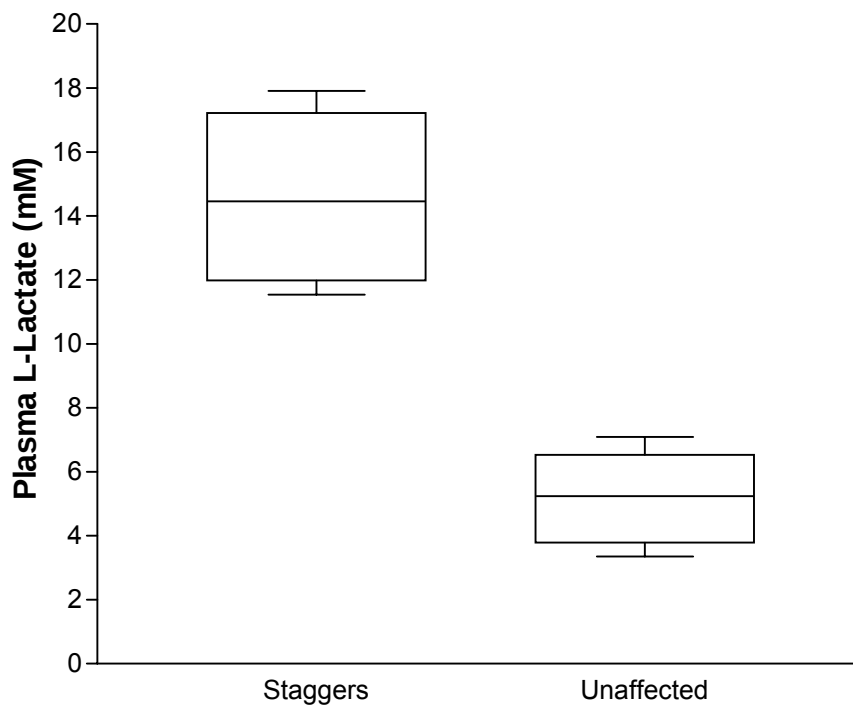
Plasma magnesium concentrations were normal in all the cattle tested (Figure 6.1). The normal range for plasma magnesium 0.8 – 1.2 mM. In fact, unaffected cattle showed a greater range of magnesium in their plasma than affected cattle. This does not support suggestions that tagasaste staggers is due to problems with magnesium supply or function.

**Figure 6.1:** Plasma magnesium in cattle showing a staggering syndrome when browsing tagasaste



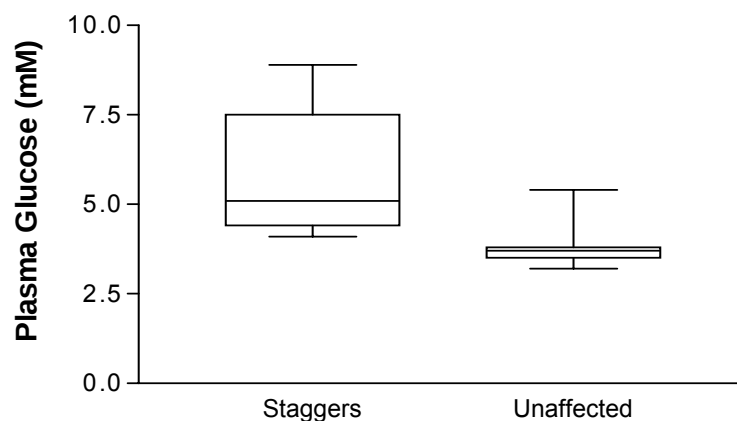
The striking feature of the plasma profile is the significant ( $<0.0001$ ) increase in L-lactate (Figure 6.2). There is no normal range established for L-lactate in cattle but lactate is frequently measured as below 6 mM (Pethick pers. Comm). This increase is consistent with the rapid, trembling muscle contractions high on the flanks and around the muzzle in the affected cattle.

**Figure 6.2:** Plasma L-lactate in cattle showing a staggering syndrome when browsing tagasaste



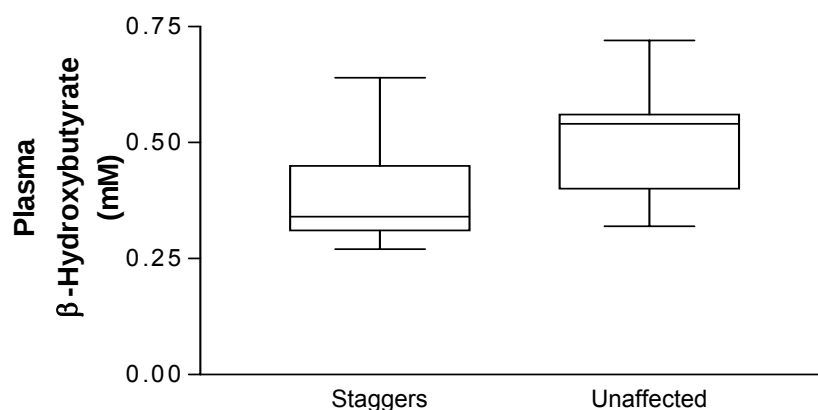
The increase in lactate coincides with significant increases in plasma glucose concentrations in the affected cattle (Figure 6.3). The normal range for plasma glucose in cattle is 2.5 – 4.5 mM. The elevated plasma glucose is consistent with rapid mobilisation of hepatic glucose stores such as glycogen in response to an adrenergic-like stimulus. The duration of the increase was not assessed in this study. The blood samples were taken from the affected cattle while they were roped on the ground, and then the cattle were released. Nevertheless, from the apparently normal recovery, it could be presumed that the glucose and lactate concentrations return to normal relatively rapidly.

**Figure 6.3:** Plasma glucose in cattle showing a staggering syndrome when browsing tagasaste



During the period of tremors, and the in the unaffected cattle that had been moved as part of the mob, and then had blood samples collected, plasma  $\beta$ -hydroxybutyrate concentrations were normal (Figure 6.4). The normal range for  $\beta$ -hydroxybutyrate is less than 600  $\mu$ M. Thus there is no underlying long-term fatty acid mobilisation either due to sustained stress or more specifically due to animals not eating. In fact, if anything, the affected cattle had slightly lower  $\beta$ -hydroxybutyrate in their plasma (figure 6.4) consistent with well-fed cattle.

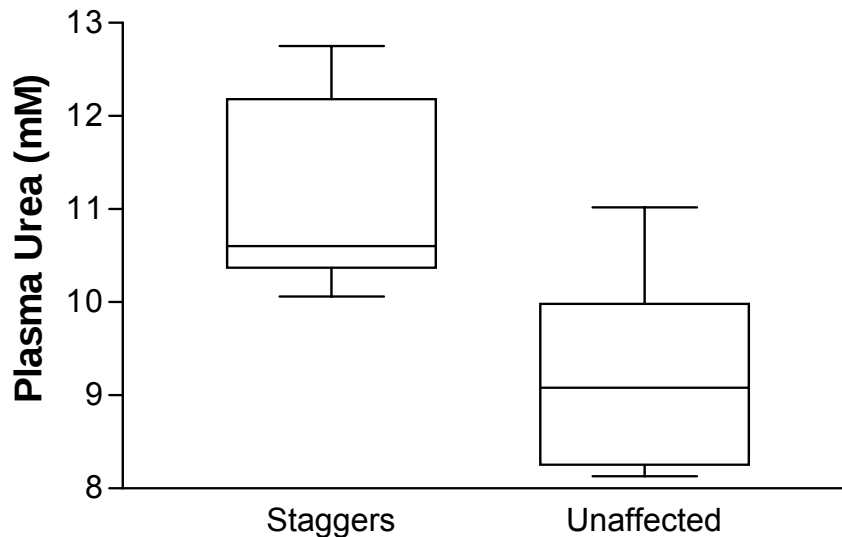
**Figure 6.4:** Plasma  $\beta$ -Hydroxybutyrate in Cattle Showing a Staggering Syndrome when Browsing Tagasaste



The elevated plasma urea concentrations in affected cattle are also consistent with well-fed animals (Figure 6.5). The normal range for plasma urea is 4.1 – 10.5 mM in cattle. In fact both the unaffected and affected cattle exhibit plasma urea concentrations that are normally

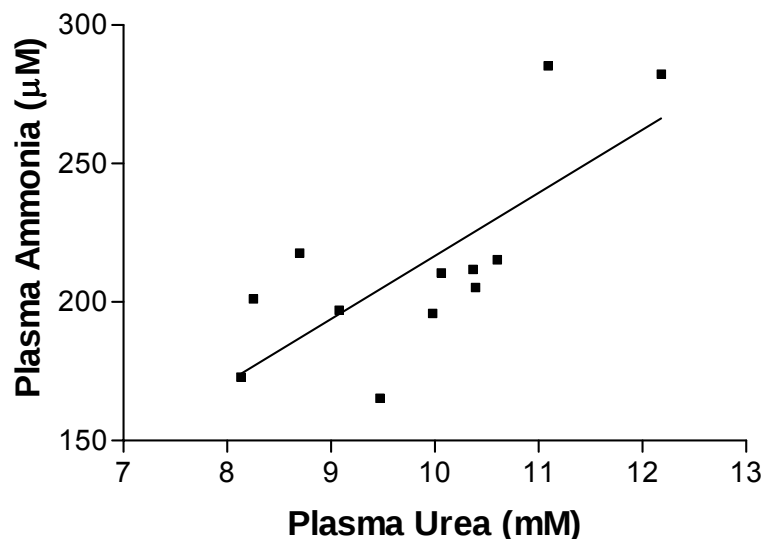
seen in animals on a high protein intake as would be case for cattle browsing tagasaste in October. Moreover, the affected cattle seem to have higher intakes of tagasaste or some other high protein feed from the significantly higher plasma urea concentrations (Figure 6.5).

**Figure 6.5:** Plasma urea in cattle showing a staggering syndrome when browsing tagasaste



The plasma urea concentrations are correlated to plasma ammonia showing that excess nitrogen present as ammonia in the plasma is being cleared as urea by the liver (Figure 6.6). The plasma enzymes  $\gamma$ -glutamyl transferase (GGT) and aspartate transaminase (AST) and glutamate dehydrogenase were all within the normal range for each enzyme ie. 5- 40 U/L for GGT, 60 – 90 U/L for AST and < 25 U/L for GLDH. These activities are indicative of normal liver function so whatever is causing the staggers is not damaging the liver and as a consequence the liver is clearing ammonia nitrogen to urea before it can become neurotoxic.

**Figure 6.6:** Regression relationship between plasma urea and plasma ammonia in cattle showing a staggering syndrome when browsing tagasaste



The uncoordinated movement seen in affected cattle is not due to an underlying myopathy or dystrophy as creatine kinase activities were within the normal range for affected cattle of 50 – 200 U/L with only two outliers in the unaffected group of cattle (Figure 6.7). So the staggering was not caused by muscle damage nor did it cause muscle damage.

**Figure 6.7:** Plasma creatine kinase activity in cattle showing a staggering syndrome when browsing tagasaste



The symptoms seen in the affected cattle during so-called 'tagasaste staggers' are likely to be associated with tremorogens, a group of mycotoxins which are capable of producing tremors and gait abnormalities in animals. Tremorgen literally means, producing tremors. These mycotoxins are highly substituted indole alkaloids and have a common complex ring structure and lysergic acid (its derivative LSD is a well-known hallucinogen) is a member of this chemical group. One example of tremorogens that affect animal production is perennial ryegrass toxicity caused by lolitrem-B. This is a toxicosis that is well known in sheep and cattle in south-eastern Australia and in New Zealand. Another fungus, *Claviceps paspali*, parasitises paspalum grass, *Paspalum dilatatum*, (sometimes known as Dallis grass) and produces the toxins paspalitrem-A and B, paspalanine and lysergic acid. Paspalitremes are usually associated with warm, moist climates where paspalum is most common.

Various derivatives of lysergic acid, including the tremorogenic mycotoxins, are associated with reduced concentrations or antagonism of the inhibitory amino acids gamma-aminobutyric acid (GABA) and glycine. Increased presynaptic neurotransmitter release and prolonged depolarisation may occur and motor end-plate transmission is uncontrolled. This group of toxins also causes vasoconstriction of the cerebral vasculature which may lead to cerebral anoxia. Being indole alkaloids, they will also cause inhibition of monoamine oxidase and thus extend the action of serotonin. Toxicity of these compounds is not well defined but ruminants are most commonly affected by contaminated pasture.

Symptoms seen with tremorogens are usually only seen when animals are excited or undergoing exercise. These may extend from mild tremors and stiffness to seizures. Symptoms usually subside when animals are left undisturbed. This is identical to the symptoms seen in the cattle affected by 'tagasaste staggers'. The metabolic pattern seen in the plasma is consistent with the action of tremorogens. As a consequence we propose that

tagasaste staggers is not due to magnesium deficiency but rather the result of a mycotoxicosis. The sporadic nature of the staggers and lack of direct seasonality of incidence will make it difficult to identify the fungus involved.

## 7 BENEFIT COST ANALYSIS OF USING TAGASASTE IN A CATTLE PRODUCTION SYSTEM

### 7.1 Ex Post Benefit Cost Analysis

BCA Consultant: Jason Kelly, Economist Department of Agriculture WA  
Date: 14 August 2001

### 7.2 Background

The perennial shrub tagasaste has changed the production potential of the deep, infertile sands of the West Midland region from grazing sheep at 2 DSE to grazing cattle at 10 DSE. It has been estimated that 100,000 ha have been planted to tagasaste with considerable adoption occurring in the last few years. Although there has been a rapid acceptance of tagasaste, studies showed that cattle perform for six months of the year but only maintain or lose some condition for the remaining six months.

The Beef Strategy Group identified tagasaste in the West Midland region as one of the most likely means the WA beef industry would be able to expand to the level necessary for its long term viability.

The project was designed to provide information on factors influencing consumption of tagasaste and overcoming the poor growth of cattle over the summer and autumn period, enabling the potential beef production from tagasaste to be realised.

#### 7.2.1 Key Assumptions

While the potentially suitable area is considerable – estimated to be in excess of 1.5 million hectares - it is assumed that only a portion of this area will be planted.

The trading of steers purchased in the pastoral region, grown out on tagasaste, and live exported, is used in this analysis. This appears to be the enterprise best suited to take advantages of the potential from tagasaste. The opportunity cost of production is a self-replacing merino enterprise growing medium type wool.

Tagasaste planting is predicted to continue at current rates of growth, this is partly attributable to the work performed by this project.

|                            |                                                                     |
|----------------------------|---------------------------------------------------------------------|
| Analysis method used       | Investment Analysis incorporating adoption and attribution factors. |
| Derivation of net benefits | Expansion of tagasaste based grazing systems.                       |

|                                |                                                                                   |
|--------------------------------|-----------------------------------------------------------------------------------|
| Scale of Adoption              | Infertile sandy soils, particularly in the West Midlands and South Coast regions. |
| Proportion of benefits project | 60%                                                                               |
| Probability of success         | Good                                                                              |
| Adoption Begins                | 2001                                                                              |
| Peak                           | 80%                                                                               |
| Year                           | 2001                                                                              |
| Ends                           | 2021                                                                              |
| Discount Rate                  | 7 %                                                                               |
| R & D Details                  | CF \$906887<br>Ind \$440587                                                       |

## 7.2.2 Results

The project has a moderate return to investment with a NPV of \$1.6 million, BCR of 2.8 and IRR of 16%. Assumptions are conservative and benefits have been derived based on long-term price projections for beef, wool and sheepmeat. This does not support the ex ante economic investigation which calculated a higher return on investment, principally due to the lower than projected planting's, and inclusion of an opportunity cost.

## 7.2.3 Other considerations

The value of tagasaste planting's in enabling development of a year round production system that supports feedlotting and live export has not been included in this analysis.

At current prices the backgrounding of cattle on tagasaste grazing systems is relatively profitable and should these prices persist in the medium term it is likely adoption could be significantly increased.

Other perennial based grazing systems such as lucerne pose a threat to the further adoption of tagasaste, as they are more readily incorporated into farm rotations and allow for opportunity cropping when seasons and prices are favourable.

Environmental benefits associated with tagasaste such as reducing recharge from the deep sands, stabilisation and erosion control have not been included.

## 7.3 Recommendations

Tagasaste has significantly improved the production potential of the deep infertile sands of the West midlands. This project has provided information that will give producers considering TAG the confidence to make their decision. Producers are now able to predict cattle growth rates on tagasaste with supplementation and compare profitability with alternatives. This is seen as an important step towards increasing adoption and expanding W.A's capability to provide consistent, quality beef through the year.

## 8 GENERAL DISCUSSION

The objectives of this project were two-fold [i] to investigate strategies to increase cattle production in summer and autumn, and [ii] to establish the underlying reasons for the lack of productivity. This project has achieved the first objective by providing a practical and efficient supplementation strategy for increasing beef production on tagasaste as a fodder browse. Lupins proved to be a very effective supplement for producing liveweight response in cattle during summer and autumn. Moreover, there is almost a dose-response effect to lupin supplementation between 1 – 5 kg per head /day. Supplements of barley and urea that were equivalent in energy and nitrogen to lupins produced lower average daily gain [than lupins themselves]. In fact, lupins produced almost twice rate of gain than did barley, with and without urea, for the same rate of supplementation. In contrast, hay and silage [even with some lupins] were not effective supplements for cattle browsing tagasaste during summer as these feeds were eaten in preference to the tagasaste. The relationship of liveweight response to barley, barley/urea, hay, silage and lupin supplement, was quantified to allow farmers to choose their level of production according to the market that they intend to supply. Producers can use these response equations as a tool for assessing the economic potential of feeding supplements over the summer and autumn period to meet specific markets. For example, cattle producers can predict that supplementing one kg of lupins per head per day to cattle browsing tagasaste during summer would produce 0.5 kg of gain per day; a good background rate for cattle entering feedlots.

However, the reasons for the limitation to production were more difficult to elucidate. Importantly, we have established that tagasaste intake is the key limitation to cattle production during summer and autumn. Chemical analysis of tagasaste showed the crude protein content did not fall below 14% and *in vitro* calculated metabolisable energy was above 9.7 MJ ME/kg DM based on an *in vitro* DMD% of 68%. During active plant growth in August and September protein and energy values were as high as 30% and 12.8 MJ ME/kg DM, respectively. These protein and energy values are normally associated with high growth rates in cattle. However, concentration of phenolic compounds peaks during summer and autumn and then decreases markedly during winter and spring. These phenolic peaks were associated with the low intakes and along with the increase in wax content of the leaves and the dietary cation anion differences in the rumen are possibly plant “defence” mechanisms to stop over grazing during the dry periods. From our studies the identification of acid urine in cattle browsing tagasaste in the summer and autumn suggests the fermentation of the phenolic compounds that increase significantly at this time of the year is partly responsible for the poor performance of the cattle. Notwithstanding this explanation, there are still many other possible explanations for the reduction or limitation in intake. The obvious areas to investigate are the concentration of phenolic in the leaf material reducing palatability, the nature of the nitrogen fermentation in the rumen, matching energy supply to protein synthesis during rumen fermentation, and the supply of phosphorus and trace minerals during summer and autumn. Any, or all, or any combination of these factors plus the sheer heat of WA summers could reduce the efficiency of gain in cattle. Moreover, other chemical constituents in tagasaste besides the phenolic compounds assayed in this project also increase during summer, for example, alkanes themselves increase indicating that tagasaste possesses the means to enhance its survival during hot, dry periods.

The phosphorus content of tagasaste decreases during summer to levels below the recommended requirement for cattle. Phosphorus supplementation of both the tagasaste itself through superphosphate and directly to the animal via mineral licks increased liveweight gain in cattle browsing tagasaste fertilised with 300kg superphosphate/ha per year. This

increase in liveweight response was measured in the second year after the application of higher rates of superphosphate fertiliser. However, there was no economic benefit in yearling steers and heifers browsing tagasaste fertilised with up to 300 kg super phosphate /ha per annum or supplementing them with a mineral lick with P. Feed-on-offer [FOO] generally was not a useful concept for estimating amount of tagasaste available for stock to eat. While FOO has proved useful for predicting effective stocking rates and productivity on annual pastures, there is a need to develop a new indicator of potential tagasaste utilisation before producers can effectively use tagasaste to its full potential.

A number of measures were used to assess rumen function in cattle in this project. The measures included ruminal ammonia concentration as an indicator of protein metabolism, volatile fatty acids [VFA] as an indicator of energy metabolism and branch-chain VFA's and urinary purine derivatives as a measure of microbial nitrogen supply from the rumen. Ruminal ammonia was below optimal levels in cattle browsing tagasaste during summer and autumn but reached quite high levels in the spring. Similar seasonal effects were seen in other indicators of rumen function such as branch-chain VFA's that were lower in summer and autumn than in winter and spring. Moreover, cattle browsing tagasaste had low numbers of ruminal bacteria and urinary allantoin excretion both indicative of poor rumen microbial protein synthesis. Thus a combined effect of low feed intake and rumen dysfunction could be the two major determinants of poor production.

Lupin supplementation was the most effective way of correcting the problems of rumen function because cattle readily fermented lupins in the rumen leading to optimal rates of ammonia and particularly branch-chain VFA's. Lupins were more effective than barley and barley/urea in increasing all of the indicators of rumen function and did not lower rumen pH as far or as rapidly as the cereal grain supplements. This pattern of changes in the rumen biochemistry gives a number of reasons for the liveweight responses to the N/E supplements and the poor response to the P supplements in yearling cattle browsing tagasaste.

During this project we had an opportunity to study the problem of tagasaste staggers which is a sporadic, sometimes lethal clinical syndrome in cattle browsing tagasaste. We suggest that tagasaste staggers is due to tremoragens which are phytotoxins causing significant but transient [ie. 15 – 30 minutes] increases in L-lactate [and glucose] leading to muscle cramps preceded by rapid and localised twitching of muzzle and flank muscles. Tagasaste staggers is not associated with lack of magnesium in the diet or in the plasma as was anecdotally reported.

Producers were clearly pleased to have this simple, practical and now proven way to overcome the lack of weight gain over the late summer and autumn period. Up to 50% [est. 100] of the beef businesses using tagasaste have adopted the technology to the extent that they are now using lupins as supplements when they need to meet particular market weights and times.

## 9 COMMUNICATIONS AND PUBLICATIONS

### 9.1 Field days & other meetings

#### 9.1.1 Field Days

1. 14 August 1997: Bruce and Dayle Wynne 'Kirra Plains', Northhampton.  
Proposed tagasaste studies and N & E supplementary feeding studies from 1995 – 1997.
2. 10 October 1997: Danny and Lyn Johns, Coorow  
Proposed study into nitrogen and energy supplementary feeding at 'Dunmar' presented at West Midland Fodder Systems Development Group Field Day.
3. 22 October 1997: JERAC, Jerramungup  
Proposed Tagasaste research and N & E supplementary feeding studies from 1995 – 1997.
4. 5 February 1998: 'Cantabilling Springs', Badgingarra  
PIRD supplementary feeding studies. John Milton.
5. 8 April 1998: Farming 2000, Dandaragan  
Progress in N & E supplementary feeding studies
6. 30 April 1998: 'Cantabilling Springs', Badgingarra  
Preliminary results of superphosphate, mineral lick study at 'Tagasaste Farm'  
7 October 1998: Craig and Donelle Forsyth property in the Irwin shire. the West Midland Fodder Systems Development Group Spring Field Day  
Results from the superphosphate, mineral lick study at 'Tagasaste Farm'..
7. 16 October 1998: Neearra Cattle Field Day, Three springs.  
Tagasaste fertiliser and management.
8. 21 October 1998: JERAC, Jerramungup  
N & E and fertiliser, mineral P supplementary studies
9. 22 October 1998: David and Hayley Cox's 'Shiloh', Esperance.
  - Field Day Nick Costa, Warren Standing and Geoff Tudor presented results on the N/E supplementary feeding experiment, rumen and microbial biochemistry and the value of Feed On Offer assessments in tagasaste browsing systems.
  - Beef Improvement Association in Esperance.
10. 31 March 1999: Bob and Anne Wilson, 'Tagasaste Farm', Lancelin.  
Research update including intake, minerals, Feed On Offer, Wingless grasshoppers and export markets
11. November 1999: Department of Agriculture University of Queensland  
Seminar by Geoff Tudor on Tagasaste project
12. 9 April 2000: Farming 2000, Dandaragan  
N & E and fertiliser, mineral P supplementary feeding studies. Meat tasting.

13. 1 July 2000: *Field Day at Naracoorte, South Australia*  
N & E, fertiliser, mineral P and FOO.

### **9.1.2 Other Meetings**

#### **TTAG**

The first meeting with the members of the TTAG was held in August 1997. At this meeting the research team detailed what they wanted to study. After lengthy discussion and acceptable compromise was agreed upon

The research team met at least twice a year with the TTAG members to review progresses of the projects and discuss concerns and possible variations. These meetings were always very well attended with good open and frank discussion on progress of project.

#### **'Tagasaste Farm'**

March 1999: Met with soil and plant scientists from the University of Western Australia, CSIRO and Agriculture Western Australia were invited to visit the site and to discuss possible reasons for the large variation between paddocks at 'Tagasaste Farm'. After a lengthy discussion, no specific ideas were given to explain the results but it was suggested that holes be dug to measure and monitor water use. It is planned to drill 11 holes across the 3 paddocks to a depth of 15 m, which is below measured root depths of 10 m. Soil samples will also be collected for analysis for pH, P, Ca and Na.

#### **Invited scientists**

Dr Jim Barrow, retired CSIRO: Soil Chemist and Plant Nutritionist

Dr Mike Bolland, AGWEST: Plant Nutritionist, Specialist in plant phosphorus nutrition.

Mr Don Nicholas, AGWEST: Plant Nutritionist,

Dr Zed Rengel, University of Western Australia: Plant Nutritionist

Dongara, WA.

Major Tagasaste meeting with the West Midland Fodder Systems group was held on the 8<sup>th</sup> July 2000. Details of all the experimental work was presented.

Cottesloe Perth.

Overview of whole project to TTAG members and Wayne Pluske, CSBP on 18 November 2000.

#### **Seminar Series**

A series of seminars with peer group scientists with the animal production group at CSIRO were held in 1997, 2000 and 2001.

### **9.1.3 Publications**

#### **Web Page**

A web page with all the information and data from this project has been established at Murdoch University: [www.vet.murdoch.edu.au/tag/](http://www.vet.murdoch.edu.au/tag/). This web page links with the Department of Agriculture FarmNote web page and provides access to anybody searching for tagasaste to either site.

## Prograzier and Farmnotes

- Nitrogen and energy supplements to target markets.
- Superphosphate and mineral licks in tagasaste production systems.
- Variations in tagasaste intake with season of year.
- Tagasaste staggers not a magnesium problem.

## Scientific

### **Australian Society of Animal Production**

Standing, WR, McMullen, GR, Costa, ND, Edwards, NJ, Taylor, EG & Tudor, GD [2000]. Food-on-Offer in tagasaste; what does it mean? *Asian-Aus. J. Anim. Sci.* 13 Supplement July 2000 B: 224.

Taylor, EG, Costa, ND, Edwards, NJ, Standing, WR & Tudor, GD [2000]. The phosphorus and mineral requirements of cattle browsing tagasaste. *Asian-Aus. J. Anim. Sci.* 13 Supplement July 2000 B: 184.

Tudor, GD, Costa, ND, Edwards, NJ, Standing, WR & Taylor, EG [2000]. Improving the performance of cattle browsing tagasaste over the summer and autumn. *Asian-Aus. J. Anim. Sci.* 13 Supplement July 2000 B:114.

### **Nutrition Society**

McNeill, D.M., Tudor, G.D., Komolong, M., Taylor, E.G., Standing, W.R., Costa, N.D. and Pluske, W.m. (2002). Diets based on forage-tree legumes can reduce urine pH in sheep and cattle. *Proc. Nutr. Soc. Aust.* 24: 76.

There was also very good discussions with Professor's Leng, Nolan [UNE] and Ørskov [McCauley Institute] and Dr Geoff Judson [SARDI] on the experimental data from the tagasaste project particularly the superphosphate and mineral lick results from 'Tagasaste Farm'.

## **9.1.4 Bibliography**

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## **10 RESEARCH TEAM**

### **10.1 Scientists**

Dr Geoff Tudor, Project Leader and Principal Investigator – N/E Supplementary Feeding:

Senior Beef Research Officer, Rangelands and Intensive Animal Industries, Department of Agriculture WA. Adjunct A/Professor, Division of Veterinary and Biomedical Sciences, Murdoch University.

Associate Professor Nick Costa, Principal Investigator – Biochemistry & Nutrition: Division of Veterinary and Biomedical Sciences, Murdoch University.

Dr Eric Taylor, Principal Investigator – Fertiliser and mineral lick: Senior Lecturer, Division of Veterinary and Biomedical Sciences, Murdoch University.

Dr Nick Edwards, Principal Investigator – Tagasaste intake: Division of Animal Production, CSIRO.

### **10.2 Graduate Research Assistants**

Ms Ruth Morgan, BSc December 1997 – January 1999.

Ms Kelly Becher, BEnvSc January 1999 – March 2001.

### **10.3 Technical Assistants**

Mr. Warren Standing, Senior Technician, Department of Agriculture WA.

Mr. Geoff McMullen, Senior Technician, Department of Agriculture WA.

Ms. Emma Mitchell, Beef Development Officer, Department of Agriculture WA.

Ms. Jill Lehman, Technician, Department of Agriculture WA.

Ms. Leonarda Paskudzka-Baizer, Technician, Department of Agriculture WA.

### **10.4 Consultants**

Dr John Milton, Senior Research Fellow, Faculty of Agriculture, University of WA.

Mr. Tim Wiley, Agronomist, Department of Agriculture WA.

Mrs Jane Speijers, Senior Biometrician, Department of Agriculture WA.

Mr. Wayne Pluske, Agronomist, Futurefarm, CSBP.

### **10.5 Honour Students**

Ms Elizabeth Leadbetter, Honours in School of Veterinary Science.

“Carbon and Nitrogen Metabolism in Cattle Grazing Tagasaste”.

Ms Linda Kennedy, Honours in Division of Veterinary & Biomedical Sciences.

“Use of Urinary Allantoin as an Indicator of Microbial Protein Supply in Cattle Browsing Tagasaste.”

## 10.6 Technical Transfer Advisory Group (TTAG)

- Kim Glasfurd, Walebing (Chair). Kim runs a sheep, cattle and cropping alley farming and plantation property at Walebing. The cattle enterprise is directed at live export trade plus Kim has a Droughtmaster stud. Phone: (08) 9651 1733
- Danny Johns, Coorow. Danny manages cattle and prime lamb property with tagasaste and other perennial pastures. The cattle are marketed either on the live export market or slaughtered for the local trade.. Phone (08) 9952 5010
- Bob Leeson, Cattaby. Bob manages a cattle property with annual and perennial pastures plus plantation tagasaste. The cattle are generally marketed on the live export trade to Indonesia and Malaysia. Phone (015 474 203)
- Bob Wilson, 'Tagasaste Farm', Lancelin. Bob leases a tagasaste and annual pasture property and specialises on backgrounding cattle for the live export trade to Indonesia and Malaysia. Phone (08) 9655 1055
- Paul Michael, Bolgart. Paul combines cattle and sheep production along with cropping. He has both plantation and alley farming tagasaste and is interested in mixed perennial including salt bush. Phone (08) 9574 2741

Mr Bob Wilson replaced Mr Kim Glasfurd as chairman of the TTAG in early 1999 when Kim's business commitments prevented him from fulfilling his role in the TTAG.

## 10.7 Acknowledgments

We would like to thank CSBP FutureFarm for their kind donation of extra superphosphate for the fertiliser and mineral lick study (Chapter 4) and for the all the mineral analysis of the edible leaf and stem.

We would also like to thank Gallagher Australia for the Solar Powered Energiser unit for the electric fencing on 'Tagasaste Farm' where the fertiliser and mineral lick study was conducted.

## 11 ADDENDUM

### Review of Outcomes from the Tagasaste Project SBEF.015

#### MLA Project COMP.041

### Phase 2

McCausland Associates - 6 November 01

#### 11.1 Introduction

- The purpose of this study is to review the economic, environmental and social impacts of MLA project SBEF.015 entitled 'Intake Studies and Supplementary Feeding in Tagasaste Browsing Systems'.
- The project was carried out between 1997 and 2000 by Agriculture WA with cooperation from scientists at Murdoch University and CSIRO. It originated because cattle feeding on tagasaste, a perennial shrub which had been planted on poor sandy country in the West Midland and Esperance areas of Western Australia, were failing to gain weight during late summer and autumn, even though the foliage was still green and leafy.
- Planting of tagasaste had started in the mid 1980's and had been responsible a three to five fold increase in carrying capacity in the areas where it was planted, but producers were unable to finish cattle when livestock exporters required them in late summer and autumn.

#### 11.2 Project Objectives

- The overall objective of project SBEF.015 was as follows:

By 31 December 2000, to develop grazing and supplementary feeding strategies which overcome the reduction in growth rate that occurs in cattle when grazing tagasaste in late summer and autumn.

#### 11.3 Review Approach

- The approach taken has been to:
  - Read relevant literature on the project, including the project proposal; various milestone reports; an 'Ex Post Benefit Cost Analysis' by Jason Kelly and Geoff Tudor; and a paper being prepared for publication 'Improving the Flexibility of Producing Beef in a Tagasaste Production System'.
  - Discuss the project results by phone with the project leader, producers and others as required. Those interviewed [by phone] are shown in Attachment 1.

- Determine, with the project leader, input data for MLA's on farm project assessment tool. MLA requested that the project results be analysed by Rendell McGuckian Agricultural Consultants using this tool. The input data was discussed with Rob Rendell who, where necessary, added to or adjusted the data to fully reflect farm costs and practices. A summary of the results of his analysis is appended.

## 11.4 Results of the Review

### 11.4.1 *Technical Success*

- The project has been successful and fulfilled its overall objectives. In particular:
  - The project has shown that cattle on tagasaste gain weight at over 1kg/head/day when supplemented with lupin grain during late summer and autumn.
  - The researchers found good evidence that branch chain volatile fatty acids [VFA's] may be a limiting factor for microbial protein supply in cattle browsing tagasaste in late summer and autumn. Branch chain fatty acids arise from protein fermentation and lupins contain the correct amino acid profile such that supplementation with them overcomes this limiting factor.
  - The results have been quickly adopted by industry because:
    - o the results are easy to adopt and lupins are plentiful in WA;
    - o the trials were done on commercial tagasaste properties, with strong producer involvement.
    - o communication of the results has been excellent.

### 11.4.2 *Level of Uptake of the Technology*

#### Current

- It is estimated by the project leader that up to 50% [est. 100] of the beef businesses using tagasaste have adopted the technology to the extent that they are now using lupins as supplements when they need to meet particular market weights and times.
- Lupin supplementation is seen by producers as a proven tool to overcome the lack of weight gain in late summer and autumn. Those interviewed who have not used it indicated that they would adopt the technology when the need arose.

#### Future

- Adoption by up to half the beef producers with tagasaste so soon after the project results were available indicates that the project provided a simple and

economically viable solution to a real need. Hence it is likely that usage will increase to an even higher percentage.

- In addition, the project may encourage more planting of tagasaste because it has successfully provided the solution to one of the main problems associated with the shrub's use as a cattle feed. At present only 100,000 hectares have been planted with tagasaste in WA, whereas an estimated 1.5 million hectares are considered suitable for the shrub.

### **11.4.3      *Financial Benefits***

#### **Analysis by the Researchers**

- The researchers have done a benefit cost analysis on the project, assuming the opportunity cost of running an enterprise where beef were fed on tagasaste and supplemented with lupins was to be unable to run a sheep enterprise on the same land without tagasaste. The analysis included the total cost of the project at \$1.35 million and found a moderate return to investment with a NPV of \$1.6 million, a BCR of 2.8 and an IRR of 16%. The discount rate used was 7%.

#### **On Farm Assessment Tool**

- Rendell McGuckian has run the MLA on farm assessment tool to analyse economic benefit to producers using lupins to supplement cattle browsing tagasaste during late summer and autumn.
- Using the on farm assessment tool, two similar tagasaste properties were compared. Lupin supplementation was used on one of the properties to enable cattle to be sold in a premium livestock export market at 17 months. On the other property there was no supplementation and cattle were sold into a normal [no premium] market at 22 months.
- Based on this analysis, the value to the business which supplemented with lupins was \$7,000. A summary of the results of the analysis is appended.

### **11.4.4      *Environmental Benefits***

- Tagasaste is considered to be beneficial environmentally in that it lowers the water table and provides erosion control. This project is likely to stimulate more tagasaste planting, but any environmental benefit would need to take into account the considerable increase in numbers of beef cattle associated with it.

#### **Social Benefits**

- No social benefits are apparent.

### 11.4.5 *Industry Comment*

#### Benefits

- Producers were clearly pleased to have this simple, practical and now proven way to overcome the lack of weight gain over the late summer and autumn period. Some gave clear examples of the financial gain they had already experienced, while others indicated they would be using lupin supplementation soon.

- Comments included:

*If I hadn't had the supplementary feeding I wouldn't have got any on the boat.*

*When we found out we could overcome the problem by using lupins it was dramatic really. Such a simple solution – immediate, no questions.*

*A boat was coming in February and some lighter steers weren't going to get there. I got them to 360 kgs by February and so got them off 9 months earlier.*

*The project has given confidence to use lupins. Instead of having to theorise about it we can go straight in and use it.*

#### Producer Participation

- Producers appreciated that the trials were done on commercial properties and that they were involved in the research, some as part of a MLA TTAG group overseeing the project with the researchers.

- Comments included:

*Researchers have been strong in getting producers to participate and the project was the one everyone wanted to see done.*

*Being associated with the TTAG was very rewarding and there was good interaction with the researchers.....I'm a great fan of TTAGs for all research – it has to happen – it's a vital principle.*

### 11.4.6 *Communication*

- Those interviewed spoke highly of the communication about this project to producers with tagasaste, through the Departmental publication Tag Talk and other means.

- Comments included:

*A lot of messages only get to the top 5% of operators. In this case it got to 75 to 80%.*

*There's no arms length stuff and they did a pretty good job on this one. They had a lupin forum 12 – 24 months ago and I was impressed with the whole partnership thing.*

#### **11.4.7 More to Learn about Tagasaste**

- Most made unsolicited comments about the continuing need to learn more about how to use tagasaste to maximum advantage.

- Comments included:

*Tagasaste is still developing in ways to use it.*

*There is still a lot to learn about tagasaste.*

*There is still a lot more R&D to be done on tagasaste.*

### **11.5 Conclusions**

- Through this study it is concluded that:
  1. The project has fully met its overall objectives.
  2. Producers with tagasaste consider the results to be of direct relevance to their operation and have quickly adopted lupin supplementation.
  3. Lupin supplementation, fed at levels determined in the project, has resulted in producers meeting premium livestock export markets and turning cattle off 6-9 months earlier.
  4. Researchers on the project were very well integrated with the producers who grow tagasaste, and communication of the results has been excellent.
- From the perspective of a triple bottom line assessment, it is concluded that the project has had:
  - A considerable economic benefit in that it has provided a highly cost effective and simple way to overcome the lack of growth in cattle browsing tagasaste in late summer and autumn.
  - A minimal environmental effect date. In as much as it stimulates more tagasaste plantings in the future, it may have a beneficial effect by lowering the water table and reducing erosion. However the effect of greater cattle numbers would also need to be considered.
  - No apparent social benefit, apart from strengthening the local economy.

## Attachment 1

### People Interviewed

|               |                                                  |
|---------------|--------------------------------------------------|
| Dave Brindal  | Beef Producer, Mingenew, WA                      |
| Nick Costa    | Associate Professor, Murdoch University, WA      |
| Emma Davey    | Beef Development Officer, Gingin, Agriculture WA |
| Craig Forsyth | Beef Producer, Mingenew, WA                      |
| Jason Kelly   | Agricultural Economist, Agriculture WA           |
| Greg Kleinig  | Beef Producer, Esperance, WA                     |
| Ivan Rogers   | Beef Producer, Tammin, WA                        |
| Geoff Tudor   | Project Leader, Agriculture WA                   |
| Bob Wilson    | Beef Producer, Lancelin, WA                      |