# Efficiency of microbial protein production in cattle grazing tropical pastures

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# Declaration of Originality

*I certify that the substance of this thesis has not already been submitted, either in whole or in part, for a degree at this or any other university.*

*I certify that any help received in preparing this thesis and all sources used, have been acknowledged in this thesis.*

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February 2003

# Abstract

Microbial protein (MCP) provides a significant proportion of the total protein supply to the ruminant. Some reports indicate low efficiencies of microbial protein production (eMCP) in ruminants consuming tropical forages. Thus this research determined eMCP in cattle grazing a range of tropical pasture types over varying seasonal conditions and examined strategies to increase eMCP under these conditions.

A method was developed to estimate MCP production in grazing ruminants by using purine derivative (PD) concentration in urine and intravenously administered markers to estimate total urine output. In Experiment 4.1, five Friesian (*Bos taurus*) and five high-grade Brahman (> 75 % *B. indicus*) steers were fasted for 7 d in metabolism crates to determine the endogenous excretion of PD in the urine. The excretion of endogenous PD in urine of high-content *B. indicus* steers (190 µmol/kg $W^{0.75}$) was *ca.* half that for *B. taurus* steers (414 µmol/kg $W^{0.75}$) indicating the need to adjust the equation proposed by Chen & Gomes (1995) to estimate MCP production, when *B. indicus* cattle are used. In Experiments 5.1 and 5.2, when CrEDTA was continually infused intravenously, Cr recovery in urine of steers was relatively constant over time and with varying diet quality (*ca.* 90 %) indicating its suitability as a urine output marker. In contrast, intravenously infused lithium sulphate was unsuitable, as urinary recovery of Li was variable and was influenced by feed quality and duration of infusion.

In Experiments 6.1 - 6.7, eMCP was determined in association with a range of pasture and animal parameters in six to eight rumen fistulated, high-grade Brahman steers grazing seven different pasture types, including: tropical native grass ($C_4$) pasture (major species *Heteropogon contortus* and *Bothriochloa bladhii*) in the early wet, the wet/dry transition and the dry season; introduced tropical grass ($C_4$), creeping bluegrass (*Bothriochloa insculpta* cv. Bisset); introduced tropical legumes ($C_3$), Dolichos lablab (*Lablab purpureus* cv. Highworth) and butterfly pea (*Clitoria ternatea* cv. Milgarra); and the temperate grass ($C_3$) pasture, ryegrass (*Lolium multiflorum* cv. Tetila). A large range in values for eMCP was recorded, i.e. 26 - 209 g MCP/kg digestible organic matter intake (DOMI). Even the highest quality

tropical grasses were associated with eMCP values well below the range of 130 - 170 g MCP/kg DOMI given in the feeding standards (e.g. SCA (1990), AFRC (1993), and NRC (1996b)). Tropical legumes had associated eMCP values that were within the extant feeding standards' range while ryegrass values were well above this range. Estimated rumen degradable protein (RDP) supply (42 - 525 g/kg DOMI) appeared to be the major factor affecting eMCP across the range of pasture types studied, with RDP supply for tropical grass pastures inadequate (i.e. < 130 g/kg DOMI) to meet requirements for eMCP in the extant feeding standards' range. Total non-structural carbohydrate concentration in plucked pasture leaf (42 - 157 g/kg DM), rumen fluid and particle dilution rate (4.5 - 11.7 %/h and 2.0 - 4.4 %/h, respectively), protozoal concentration in rumen fluid (0.21 - 3.5 x $10^5$/ml) and rumen fluid pH (6.1 - 6.8) were poorly correlated with eMCP.

In Experiments 7.1 - 7.3, four treatment diets were fed in a latin square design to eight high-grade Brahman steers in metabolism crates and four rumen fistulated, high-grade Brahman steers in pens to determine the effect of quantity and source of RDP on eMCP. The treatments included a control diet of pangola grass (*Digitaria eriantha*) hay, and hay with supplements estimated to provide 150 g (urea or casein) or 300 g RDP/kg DOMI (casein). Efficiency of microbial protein production was increased from 123 g MCP/kg DOMI on the control diet to 167 g MCP/kg DOMI when RDP was provided at the highest rate, which was also associated with a four-fold increase in the concentration of branched-chain volatile fatty acids in the rumen fluid. However, source of RDP (urea or casein) had no effect on eMCP (109 - 115 g MCP/kg DOMI). Mean daily rumen ammonia-nitrogen concentration ranged from 73 mg/l in steers consuming the control diet to 277 mg/l for the highest level of RDP supplementation. Treatment did not affect *in vivo* NDF digestibility (599 g/kg DM) or the rate (0.037 /h) or extent (potential degradable fraction: 636 g/kg) of *in situ* disappearance of pangola grass hay OM. In addition, rumen particle dilution rate was unaffected by treatment (2.2 %/h) and rumen fluid dilution rate, although showing some treatment differences (4.8 - 6.2 %/h), was poorly correlated with eMCP.

It was concluded that eMCP was well below the extant feeding standards' range when cattle consumed tropical grass pastures and that RDP was the primary limiting nutrient. Furthermore, even when minimum RDP requirements were met, there was still a large variation in eMCP indicating that other factors were then limiting.

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I dedicate this work to my parents, Bob and Heather, who have always been there to provide love and support.

# Publications

Bowen MK, McCosker, K, McLennan, SR, & Poppi DP (2003) The development of a novel method to estimate microbial protein production in grazing Brahman crossbred cattle. *Tropical and Subtropical Agroecosystems* **3**, 365-367.

Bowen MK, McLennan SR, & Poppi DP (2001) Rumen fluid dilution rates in cattle grazing tropical pastures. *Journal of Animal Science* **78 (Suppl 1)**, 423.

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# List of Abbreviations

| | |
|---|---|
| ADF | Acid detergent fibre |
| ADIN | Acid detergent insoluble nitrogen |
| BB | Creeping bluegrass pasture in the mid-wet season (studied in Experiment 6.4) |
| BCFA | Branched-chain volatile fatty acids |
| BP | Butterfly pea pasture (studied in Experiment 6.6) |
| CNCPS | Cornell Net Carbohydrate and Protein System |
| CP | Crude protein |
| Cr | Chromium |
| CRD | Controlled release device |
| CrEDTA | Chromium complexed with ethylenediamine tetraacetic acid |
| DAPA | Diaminopimelic acid |
| dg | Degradability of protein in the rumen, expressed as a proportion of the total crude protein supplied in the diet |
| DM | Dry matter |
| DMD | Dry matter digestibility |
| DMI | Dry matter intake |
| DOMI | Digestible organic matter intake |
| eMCP | Efficiency of microbial crude protein production |
| FDR | Fractional disappearance rate |
| FME | Fermented metabolisable energy |
| FO | Faecal output |
| FOM | Organic matter actually fermented in the rumen |
| FOR | Fractional outflow rate |
| GFR | Glomerular filtration rate |
| HPLC | High performance liquid chromatograph |
| IADF | Indigestible acid detergent fibre |
| ICP-AES | Inductively coupled plasma atomic emission spectrometer |
| IVDMD | *In vitro* dry matter digestibility |
| IVOMD | *In vitro* organic matter digestibility |

| | |
|---|---|
| $K_s$ | Ammonia saturation constant |
| Li | Lithium |
| LiCl | Lithium chloride |
| LL | Dolichos lablab pasture (studied in Experiment 6.5) |
| LW | Liveweight |
| LWG | Indicates non-fistulated steers used to monitor liveweight gain/loss on grazed pastures in Experiments 6.1 - 6.7 |
| MCP | Microbial crude protein |
| ME | Metabolisable energy |
| MN | Microbial nitrogen |
| MP | Metabolisable protein |
| N | Nitrogen |
| NDF | Neutral detergent fibre |
| NDIN | Neutral detergent insoluble nitrogen |
| $NH_3$ | Ammonia |
| NPD | Native $C_4$ grass pasture in the dry season (studied in Experiment 6.3) |
| NPEW | Native $C_4$ grass pasture in the early wet season (studied in Experiment 6.1) |
| NPN | Non-protein nitrogen |
| NPT | Native $C_4$ grass pasture in the wet/dry transitional period (studied in Experiment 6.2) |
| NSC | Non-structural carbohydrate |
| OF | Oesophageal fistulated |
| OM | Organic matter |
| OMADR | Organic matter apparently digested in the rumen |
| OMD | Organic matter digestibility |
| OMI | Organic matter intake |
| OMTDR | Organic matter truly digested in the rumen |
| P | Phosphorus |
| P = / < / > | Probability of significant difference between treatments. All values are given as P = x, unless P < 0.001. The other exception is when protected lsd tests have been used, in which case probability is stated as P < or > 0.05. |

| | |
|---|---|
| PB | Purine bases |
| PD | Purine derivatives |
| RDP | Rumen degradable protein |
| RF | Rumen fistulated |
| RG | Annual ryegrass pasture (studied in Experiment 6.7) |
| rpm | Revolutions per minute |
| S | Sulphur |
| SC | Structural carbohydrate |
| SD | Standard deviation |
| SE | Standard error |
| TDN | Total digestible nutrients |
| TNSC | Total non-structural carbohydrates |
| UDP | Undegraded dietary protein |
| VFA | Volatile fatty acids |
| WSC | Water soluble carbohydrate |
| Yb | Ytterbium |

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# Chapter 1

# General introduction

## 1.1 BEEF CATTLE PRODUCTION FROM TROPICAL PASTURES IN NORTHERN AUSTRALIA

The gross value of beef production in Australia is approximately $4.8 billion (ABARE, 2000), and as such, makes an important contribution to Australia's economy. Northern Australia contributes almost half of this amount and supports around 10 million beef cattle representing 54 % of the national herd (Gramshaw & Lloyd, 1993).

Beef cattle production in northern Australia is largely based on extensive grazing of tropical pasture with native pastures being the predominant forage type. The major native pasture communities of economic importance are black speargrass, brigalow, *Aristida/Bothriochloa* and Queensland bluegrass in the higher rainfall zones, and Mitchell grass, mulga, gidgee, channel and spinifex in the more arid, inland areas (Weston & Harbison, 1985; Gramshaw & Lloyd, 1993). Areas of sown, exotic grasses and legumes also exist in northern Australia although these areas are relatively small compared to those of native pasture. About 5 million ha of northern Australia has been sown with exotic (hereafter introduced) grasses and legumes, with spreading and naturalisation of some of these species on a further 5 million ha (Gramshaw & Lloyd, 1993).

In northern Australia, seasonal rainfall distribution results in rapid growth of pasture during the spring/summer period, which then declines in quality as the season advances and the plants mature and senesce. At the onset of the dry period, the pastures are largely mature and dry and consequently have very low digestibilities and levels of nitrogen (N), phosphorus (P) and sulphur (S) well below those recommended for cattle (Winks, 1984; Dixon & Doyle, 1996). The pattern of cattle growth from these pasture systems closely follows the seasonal pattern of pasture growth with weight loss commonly occurring during the dry season followed by wet season weight

gain (Winks, 1984). Thus annual cattle liveweight gains on pasture-based systems in northern Australia are low compared to those on temperate pastures, and vary from 70 - 160 kg/year on native pastures up to *ca.* 200 kg/year on introduced pastures (Gramshaw & Lloyd, 1993; Bortolussi *et al.* 1999; Tyler & Shaw, 1999).

Furthermore, wet season liveweight gains are commonly much lower than those equivalent to the genetic potential of the cattle, which are *ca.* 1.5 kg/d when measured over sustained periods (t'Mannetjie, 1982). A review of growth experiments in northern Australia by Winter *et al.* (1991) showed the average wet season liveweight gain to be around 0.7 kg/d with growth rates rarely exceeding 1 kg/d on most pastures. This growth rate is also well below that possible on temperate pastures during the growing season (Boom & Sheath, 1998, 1999; Kitessa & Nicol, 2001).

The lower nutritive value of tropical grasses, as compared to temperate species, is attributable to greater lignification, higher cell wall levels and lower levels of soluble carbohydrates, N, P and S in the tropical species (Minson & McLeod, 1970; Minson, 1971; Norton, 1982). On average, tropical grasses are 13 units of digestibility lower than temperate grasses (Minson & McLeod, 1970; Minson, 1971). Tropical legumes are of higher quality than tropical grasses by virtue of their high protein and usually lower cell wall content (Van Soest, 1994). Nevertheless, they are also generally of lower quality than their temperate counterparts due to their higher lignin content (Van Soest, 1994).

The low annual weight gains, being a product of low wet season gains and often high dry season losses, frequently result in northern cattle reaching market weight at ages greater than 3.5 years (Poppi & McLennan, 1995). Strong markets, such as the U.S. manufacturing beef market, currently exist for these older cattle. However, higher priced markets for beef cattle, including the domestic, European, Korean and Japanese markets demand younger, heavier carcasses (Cheffins, 1996; Loxton, 1997, R. Cheffins, personal communication 2002). New markets are also emerging, particularly in Asia, which demand a high quality product meeting stringent specifications for age, weight and fat distribution. In response to this increased demand for high quality beef, Meat Standards Australia (MSA) has recently been developed to specify stringent carcass requirements and growth rate targets for the

domestic market. Loxton (1997) states that the demand for younger carcases is likely to continue to increase for Australia's major domestic and export beef markets, resulting in a maximum cattle age at turnoff to market of 2 years.

It is apparent that, in northern Australia, the cattle growth rates required to meet the high quality markets are often difficult to achieve from the native or introduced tropical pastures without nutritional intervention (Tyler & Shaw, 1999). Surveys have shown that while some cattle producers are meeting the high quality market specifications with pasture production systems, the necessary growth rates are not widely or regularly achieved (Bortolussi *et al.* 1999; Tyler & Shaw, 1999).

Grain finishing of cattle in feedlots is currently a major means of achieving the target weight and age specifications for the high quality beef markets. However, there is potential for these high growth rates to be achieved through manipulating nutrient supply to the animal from existing pasture systems. Considerable research effort focussing on the prevention of dry season weight loss has resulted in supplementation strategies which allow liveweight to be maintained, or only slightly reduced, during the dry season (Poppi & McLennan, 1995). However, strategies to increase liveweight gain of cattle during the wet season are less common. Nutritional strategies that have been used to increase liveweight gain during the wet season include the use of introduced grasses and legumes, the planting of forage crops or speciality pastures, and supplementation of the animal.

## 1.2 THE POTENTIAL TO INCREASE GROWTH RATES THROUGH ADDITIONAL PROTEIN SUPPLY

### 1.2.1 Nutritional requirements of the ruminant and growth response to energy and protein supply

Growth rates of cattle on pasture can be increased most efficiently by identifying the primary limiting nutrient to growth and correcting this deficiency. When identifying the first limiting nutrient for the ruminant animal, the requirements of both the animal tissues and the rumen microbes need to be considered. For maintenance and growth, the animal tissues require a source of amino acids for protein production, and a source of energy, which can be in the form of volatile fatty acids (VFA), glucose, lipids or

amino acids. A range of minerals is also required by the animal tissues. The animal's requirement for protein and energy can be quantified as metabolisable protein (MP) and metabolisable energy (ME). The reticulo-rumen (hereafter rumen) is an important source of both energy and protein for the host animal tissues. The energy from the rumen is largely supplied in the form of volatile fatty acids, which are the products of microbial fermentation, while protein is supplied in the form of microbial crude protein (MCP), which represents the protein of the microbes themselves. The growth of rumen microbes requires a source of energy (e.g. structural carbohydrate (SC) and non-structural carbohydrate (NSC)), N (or rumen degradable protein (RDP)), and specific minerals including S and P.

The feeding standards (eg. AFRC (1993)) usually consider energy to be the first limiting nutrient for growth of the ruminant animal. This supposition has arisen partially from the fact that an increase in ME intake by the animal will also result in an increase in MP supply, as a consequence of the stimulation of MCP production in the rumen. Equations in the feeding standards allow the adequacy of the MP supply to be verified for the level of growth or production expected for the given energy supply. According to predictions of AFRC (1993), in most situations, all the MP requirements of the animal can be met by MCP. The exceptions are young calves less than *ca.* 200 kg liveweight (LW), bulls with high rates of gain, and lactating cows producing more than about 7 or 8 kg of milk per day. These classes of animal require feed protein that has escaped rumen degradation (hereafter undegraded dietary protein (UDP)), in addition to MCP, to meet their MP needs.

Black & Griffiths (1975) clarified the relationship between energy and protein supply to the tissues, and liveweight gain of the animal, by stating that, for a given energy supply, N retention by the animal increases linearly in response to increasing protein supply before plateauing when the energy supply becomes limiting. This implies that large increases in N retention, or growth rate, can be obtained by increasing energy supply, while increasing protein supply will result in relatively smaller increases in growth, the rate of which will be influenced by the biological value of the protein. However, there are difficulties in determining the tissue response to nutrients supplied in the diet, due to the impact of the rumen on supply of substrate to the intestines,

preventing prediction of the exact proportions and amounts of nutrients absorbed from a particular diet (Poppi, 1990).

According to the above arguments, increasing the ME intake of cattle should have the greatest effect on their growth rate. As discussed by McLennan *et al.* (1999), the most effective way to increase ME intake in a grazing situation is to increase the total dry matter intake (DMI) of the animals. Some strategies to increase the DMI of pasture include:

1) removing any specific nutrient deficiencies such as N or mineral deficiencies that occur at the rumen or animal tissue level;

2) increasing the allocation, or supply, of pasture through management procedures or through planting improved pasture species which have a longer growing season or greater dry matter (DM) production (e.g. buffel (*Cenchrus ciliaris*) in south-east and central Queensland); and

3) replacing part or all of the existing pasture base with one that has an inherently higher intake (e.g. tropical legumes; speciality pastures such as winter and spring temperate cereals (e.g. oats and barley), or temperate pasture grasses (e.g. ryegrass), in the subtropics; or the summer and autumn grazing of tropical forage crops (e.g. sorghum) in all regions).

Providing a supplement high in ME content is another strategy that could be employed to increase the ME intake. However, from a series of experiments with cattle grazing tropical pastures, Bolam (1998) concluded that improving cattle growth rates through providing high-ME supplements was unfeasible due to substitution of the basal forage and the large supplement intake required.

When strategies for increasing energy intake have been exploited to their potential for a particular region, the question remains as to whether the animal will respond to additional protein supply to the tissues. Although previous discussion indicates that protein should not be the primary limiting nutrient for growth on fresh forage such as wet season tropical grass pasture, various researchers have observed responses to

proteins supplied postruminally to ruminants consuming fresh forages (Black *et al.* 1979; Barry, 1980, 1981; Fraser *et al.* 1991). In addition, supplements consisting of rumen-escape proteins have improved the growth rate of sheep (Poppi *et al.* 1988) and cattle (Anderson *et al.* 1988; Goedecken *et al.* 1988; Klopfenstein, 1996) when consuming temperate pasture diets.

While fewer experiments have been conducted with ruminants consuming wet season tropical pasture, there is evidence that a growth response to increased supply of intestinal protein would also be expected under these conditions. Such evidence comes from experiments by Mbongo *et al.* (1994) who reported an increase in liveweight gain of 240 g/d (from the baseline of 780 g/d) when Belmont Red steers (272 kg LW) grazing setaria (*Setaria sphacelata*) pasture (120 g crude protein (CP)/kg DM in extrusa) in summer/autumn were supplemented with 150 g/d of formaldehyde-treated casein. In addition, Bolam (1998) increased liveweight gain of Brahman crossbred steers (213 kg LW) grazing pangola grass (*Digitaria eriantha*) pasture (120 g CP/kg DM in extrusa) by 210 g/d (from the baseline of 830 g/d) by supplementing with 250 g/d fishmeal, which is a source of rumen escape protein. Furthermore, collation of a number experiments by McLennan *et al.* (1995) revealed that liveweight gain responses were obtained by supplementation of cattle consuming relatively high quality tropical forages with protein meals. However, it was emphasised by the authors that protein meals, such as cottonseed meal, provide RDP and energy, in addition to UDP.

It is possible that the observed responses to additional intestinal protein supply are due to the provision of one, or several, limiting amino acids to the tissues. Alternatively, the amino acids may be acting as gluconeogenic precursors and, as such, may cause a response by increasing the supply of glucose, and thus energy, to the tissues.

### 1.2.2 Strategies to increase the supply of protein to the animal tissues

If cattle growth rates on wet season tropical pasture can be increased with additional intestinal protein supply, the challenge is to find the most efficient, practical and economical way of supplying it. Alternatives include the addition of a legume to the pasture, provision of a UDP supplement, provision of an energy supplement to

increase MCP synthesis or strategies to increase the efficiency of microbial protein production (eMCP) in the rumen.

A review by Poppi & McLennan (1995) suggested that, contrary to popular belief, incorporation of legumes of high CP content in the diet of grazing animals had only a marginal effect on intestinal protein supply per unit of DMI due to extensive protein degradation in the rumen and the lack of available energy for the microbes to capture the ammonia ($NH_3$) released. However, the legumes increased total protein intake through stimulating a higher total DMI.

Providing a protein supplement, which is partially undegradable in the rumen, is another strategy used to increase protein supply to the tissues. However, a limitation to the use of protein supplements for grazing cattle in Northern Australia is their high cost, with the benefit/cost ratio influenced by fluctuations in the cost of the supplements in relation to the market price for beef cattle. Furthermore, protein supplements such as cottonseed meal contain a significant amount of non-protein available energy, which can lead to an energy substitution effect when the quality of the basal diet is high (Poppi & McLennan, 1995).

Provision of energy supplements may also indirectly increase the supply of protein to the animal by increasing $NH_3$ uptake by the rumen microbes and thus increasing MCP production. This would be a relevant strategy when ruminants are consuming temperate pastures and tropical legumes where CP content is greater than 210 g/kg digestible organic matter intake (DOMI), (Poppi & McLennan, 1995).

In addition to the strategies outlined above, increasing eMCP in the rumen would be another approach to increase MP supply to the animal tissues. Efficiency of MCP production is an expression of the quantity of MCP produced per some measure of the energy supply to the rumen microbes (see later, section 2.2). Microbial protein represents a significant portion of the ruminant's total protein supply, with microbial nitrogen (MN) comprising *ca.* 40 % of the non-ammonia-N entering the small intestine with high protein diets and 60 % or greater when the animal is consuming low protein diets (Owens & Zinn, 1988). Additionally, rumen microbes are capable of synthesising all of the essential as well as the non-essential amino acids, are

considered to have a reasonably constant amino acid composition, and to have a high biological value of *ca.* 0.8 (McDonald *et al.* 1988). The dependency of the ruminant on MCP as a protein source means that the eMCP can have a significant effect on protein supply to the animal tissues and thus on animal production. Furthermore, as MCP is effectively the least expensive source of protein for the animal, maximising eMCP should be a key strategy when attempting to increase intestinal protein supply.

# Chapter 2

# Efficiency of microbial protein production in cattle grazing tropical pastures: a review

## 2.1 INTRODUCTION

This review examines the potential to increase protein supply to cattle on tropical pasture-based systems through increasing eMCP in the rumen. The factors believed to influence eMCP are reviewed and measured values for eMCP in cattle grazing tropical forages are documented and evaluated. Finally, techniques available to estimate eMCP in cattle under grazing conditions are considered.

## 2.2 EFFICIENCY OF MICROBIAL PROTEIN PRODUCTION

Protein is synthesised by rumen microbes from the amino acids, small peptides and $NH_3$ released into the rumen by degradation of protein in the feed or from endogenous sources. The quantity of MCP produced in the rumen varies with the quantity of available N and energy (SCA, 1990; AFRC, 1993; NRC, 1996b).

Due to the dependence of MCP synthesis on the supply of fermentable energy to the rumen, the eMCP is generally expressed in relation to some measure of energy supply. A number of units are used to quantify eMCP in the literature, including:

- g MCP/MJ fermentable metabolisable energy (FME) intake;
- g MCP/kg feed organic matter apparently or truly digested in the rumen (OMADR and OMTDR, respectively);
- g MCP/kg total digestible nutrients (TDN); and
- g MCP/kg DOMI.

The use of TDN and DOMI in the expression of eMCP does not take into account the inability of most rumen bacteria to utilise protein, fat or lipid as an energy source, and their requirement for carbohydrate as the primary energy source for growth (Russell *et*

*al.* 1992). However, TDN and DOMI are commonly used due to ease of measurement.

Feeding systems give optimum values for eMCP which are based on summarised literature values. When the various feeding systems are compared on a common basis (g MCP/kg DOMI) as in AFRC (1992), it can be seen that there is a range in the values adopted: 130 in the U.K. system (ARC, 1984), 165 in the Nordic system (Madsen, 1985), 161 in the West German system (Ausschuss fur Bedarfsnormen, 1986), 126 in the French system (INRA, 1988), 95 - 170 in the Australian system (SCA, 1990), 130 in the U.S. system (NRC, 1996b), and *ca.* 130 - 160 g MCP/kg DOMI in the most recent AFRC manual (AFRC, 1993). Excluding values for silage diets, all these eMCP values generally fall within the range of 130 - 170 g MCP/kg DOMI which will hereafter be referred to as the extant feeding standards' range.

Many of the more recent versions of the feeding systems recognise that eMCP is not a constant and may vary according to the diet type. The Australian feeding standards (SCA, 1990) give efficiency values (g MCP/kg DOMI) for three broad types of diets: 170 for first growths of temperate legumes, grasses and green forages, fresh or dried; 130 for all other fresh and dried forages, and mixed diets; and 95 for silages. The AFRC (1993) recommends the use of an adjustment factor for level of feeding, or rumen outflow rate, while Level 1 of the NRC (1996b) model considers pH effects by using an adjustment factor for diets containing less than 40 % forage. Level 2 of the NRC (1996b) model includes aspects of the Cornell Net Carbohydrate and Protein System (CNCPS) described by Russell *et al.* (1992), Sniffen *et al.* (1992) and Fox *et al.* (1992), which predicts microbial growth from feed carbohydrate and protein fractions as well as their digestion and passage rates.

Reported values for eMCP range from 50 to 370 g MCP/kg DOMI (Corbett, 1987; SCA, 1990; Purser & Hogan, 1992) and appear to vary with forage type, diet quality and season. The lowest efficiency values have been found with silage diets. SCA (1990) states that a possible contributing factor to the low eMCP on silage diets, is that the calculations have overestimated the energy that has been available to, and used by, the microbes due to inclusion of VFA and lactic acid content in the organic

matter (OM) component. The greatest eMCP values have been found for first growths of temperate grasses or legumes (SCA, 1990).

The stoichiometry of ruminal fermentation indicates that the theoretical maximum yield of microbial OM is *ca.* 360 g/kg OM actually fermented in the rumen (FOM), which is approximately equivalent to 225 g MCP/kg FOM (Corbett, 1987; SCA, 1990). Corbett (1987) calculated that MCP yields in practice are unlikely to exceed 180 g MCP/kg FOM, or 312 g/kg OMADR, with higher values indicating an improbably high efficiency of use of energy and nutrients from fermentation for microbial growth. He further suggested that values greater than the above can be attributed to errors in estimating either digesta flow rates or the microbial biomass.

The average value of OMADR as a fraction of DOMI in animals given a wide variety of diets is 0.65 (ARC, 1980). Using this conversion factor, the maximum MCP yield in the rumen would equate to *ca.* 200 g MCP/kg DOMI. However, Corbett (1982) found the value for OMADR as a fraction of DOMI to vary with organic matter digestibility (OMD) such that the OMADR/DOMI ratio could be predicted from the calculation: 0.90 (± 0.02) *x* OMD. Thus, the maximum possible MCP synthesis, expressed per kg DOMI, would be greater on pastures of high digestibility than for those of lower quality.

The term "microbial protein", when referring to measures of eMCP, can be misleading. This is because, traditionally, estimates of MCP flow have been made using markers solely for rumen bacteria. In addition to bacteria, protozoa and anaerobic fungi contribute to the microbial fraction leaving the rumen. However, there is evidence to suggest that protozoa make only a very minor contribution to the microbial protein flow to the small intestine, particularly with high fibre diets, due to their long generation times and their ability to attach to the rumen wall and feed particles (Weller & Pilgrim, 1974) and to migrate from the reticulum to the rumen (Murphy *et al.* 1985). The contribution of anaerobic fungi to the microbial fraction leaving the rumen would be expected to vary according to their biomass on a particular diet, but this has not been estimated (Dewhurst *et al.* 2000). Thus, consideration of the factors which influence eMCP can be largely restricted to those which impact upon the bacterial population of the rumen.

## 2.3 FACTORS INFLUENCING THE EFFICIENCY OF MICROBIAL PROTEIN PRODUCTION

At the rumen level, a number of factors may influence the eMCP. These factors can be grouped under four major areas of: substrate availability, rumen dilution rate, intraruminal recycling of protein and pH of rumen contents. These factors generally influence eMCP by affecting the growth rates, and thus maintenance energy requirements, of bacteria (Dewhurst *et al.* 2000). Maintenance energy is the energy required to maintain cells in a live state, with important maintenance costs to bacteria including: motility, cellular turnover, production of extracellular molecules, active transport of solutes, inefficient phosphorylation, energetic uncoupling and lysis of cells (Harmeyer, 1986). As demonstrated by Stouthamer & Bettenhaussen (1973), the maintenance energy requirement is dependent on the growth rate of bacteria with slower growth rates resulting in proportionally greater maintenance energy requirement than faster growth rates. Greater maintenance requirements decrease the nutrients available for microbial growth and thus reduce cell yields (Stouthamer & Bettenhaussen, 1973).

### 2.3.1 Substrate availability

As for all cells, the most important substrates required by the rumen microbes for growth are energy and N. Microbial growth and fermentation processes in the rumen also require an adequate supply of minerals. However, on most diets, minerals will generally be supplied in sufficient quantities for the rumen microbes, or will be recycled from the body's endogenous supply (Durand & Kawashima, 1980). The important exception is S which may become limiting on low quality diets, such as dry season tropical pastures, which are also limiting in N. Sulphur is particularly important for the activity of cellulolytic bacteria, ciliate protozoa and anaerobic rumen fungi (Komisarczuk-Bony & Durand, 1991) and it is usual to ensure that the N/S supply in feed supplements meets microbial requirements (SCA, 1990).

#### *2.3.1.1 Type of energy substrate: structural vs. non-structural carbohydrate*

Energy sources for the rumen microbes can be broadly divided into two classes of carbohydrate, being the slowly degraded SC and the more readily fermentable NSC. The SC are derived from plant cell walls and include hemicelluloses and cellulose. The polymer, lignin, is closely associated with SC in the cell wall and may thus

reduce digestibility of SC components (Jung, 1985, 1988; Jung & Vogel, 1986). The NSC components include organic acids (carbohydrate precursors), mono- and oligo-saccharides ("sugars"), starches and fructans derived from the cell contents and pectic substances, galactans and β-glucans derived from the cell wall (Hall, 2000). The amounts of SC and NSC in the feed source may affect microbial maintenance requirements because of differences in rates of fermentation and rates of passage, and due to effects on rumen pH. These aspects will be discussed in later sections. In addition, individual bacterial species differ in their inherent maintenance requirements and the balance of species, and thus total population maintenance requirement, can be altered by the form of the energy substrate (Van Soest, 1994).

The SC and NSC carbohydrate proportions in ruminant diets can be influenced by feed type (forage vs. concentrates) as well as by forage type including species, seasonal effects and other environmental factors such as temperature, shading, defoliation and sward density (Smith, 1973).

Tropical grasses contain greater amounts of SC than temperate grasses (Norton, 1982). The increased concentration of SC in tropical grasses is partly associated with their specialised anatomy required as part of the $C_4$ photosynthetic pathway. However, the concentration of SC also appears to be temperature related, with temperate grasses ($C_3$ photosynthetic pathway) also showing increased cell wall content when grown under higher temperature conditions (Norton, 1982). Little is known about the SC content in the cell walls of different species of tropical legumes. However, it is known that tropical legumes, like tropical grasses, tend to have higher lignin content than temperate species. Lignin content in tropical grasses and legumes is higher in the stems than the leaves, this difference being especially pronounced for legumes (Norton, 1982).

Tropical grasses contain less NSC, on average, than temperate species (Smith, 1973). There are also differences in the type of NSC accumulated. Tropical grasses, and all legumes, characteristically accumulate sucrose and starch, in contrast to temperate grasses which accumulate sucrose and fructosans (Norton, 1982; Hall, 2000). In addition, legumes contain greater amounts of pectic substances than grasses. While

sucrose and fructosans are readily soluble in water, starches can have a variable solubility depending on their amylopectin content (Norton, 1982). However, all of these NSC components would be expected to be rapidly and extensively degraded by rumen microbes particularly as, unlike the SC, they appear unaffected by lignification (Hall, 2000).

Fulkerson & Donaghy (2001) have reviewed the factors affecting NSC levels in ryegrass (*Lolium* spp.) pasture (a temperate $C_3$ species). The authors demonstrated that changes in the period and/or intensity of solar radiation, through changes in day length, cloud cover, shading or sward density, influence photosynthesis and NSC content in the plant. NSC is utilised by the plant in the respiration process and as respiration exhibits an exponential increase with increasing temperature, the NSC reserves in plants are reduced under conditions of high ambient temperatures (Fulkerson & Donaghy, 2001). Factors that reduce growth, such as mild moisture stress, N deficiency or low temperatures, allow NSC to accumulate, while defoliation causes initial NSC depletion followed by restoration during regrowth (Smith, 1973; Fulkerson & Donaghy, 2001). As a result of these effects, spring plant growth of temperate species commonly contains greater quantities of NSC than autumn growth (SCA, 1990; Dove & Milne, 1994). However, in contrast to the above findings for temperate grasses, tropical grasses have been found to show little variation in NSC content with changing environmental conditions (Smith, 1973; Wilson & t'Mannetjie, 1978).

In the Australian feeding standards (SCA, 1990), the SCA reviewed the available literature and concluded that changes in plant water-soluble carbohydrate (WSC) content would be a major factor causing observed seasonal changes in eMCP. The SCA (1990) postulated that a reduced plant WSC content would result in a decrease in the readily available supply of energy for the rumen microbes which, as a result, could be less efficient in capturing N. Dove & Milne (1994) presented evidence to support this case in an experiment with ewes grazing perennial ryegrass (*Lolium perenne*) pasture in spring/summer and in autumn. The eMCP in the ewes grazing the spring/summer pasture was almost twice that estimated for the ewes grazing the autumn pasture and it was argued that the differences were due to the WSC content of the consumed herbage. However, the effect of season was confounded in this

experiment with lactation and rumen fluid dilution rate, making it difficult to clearly discern the controlling influence on eMCP.

Bolam *et al.* (1998) investigated the effect of supplemental NSC on eMCP in cattle consuming tropical forage (rhodes grass (*Chloris gayana*) hay; CP: 69 g/kg DM, OMD: 560 g/kg). The eMCP on basal diet was low (62 - 92 g MCP/kg DOMI) and was increased up to within the extant feeding standards' range (130 - 170 g MCP/kg DOMI) with grain or molasses supplements fed at levels of at least 1.5 % LW. However, the increase in eMCP tended to be linear and did not show a rapid increase when lower amounts of fermentable carbohydrate were supplied (e.g. 0.5 % LW).

#### *2.3.1.2 Quantity of rumen-available nitrogen supplied*

The quantity of N available in the rumen has a major effect on both total MCP yield and eMCP. If N is limiting, the total amount of carbohydrate digested in the rumen is depressed and thus the total microbial yield (g MCP/d) is reduced (Hoover & Stokes, 1991). However, a low supply of N in the rumen would also be expected to have a negative effect on eMCP. According to Hespell (1979), extensive fermentation of carbohydrates can occur in cultures with inadequate N, but microbial growth will be limited due to energetic uncoupling, which results in low MCP production per unit of carbohydrate digested.

The rumen microbes may utilise $NH_3$, branched chain volatile fatty acids (BCFA), amino acids and peptides in cellular protein synthesis, which is discussed further in section 2.3.1.3. As reviewed by Stern (1986), the proportion of microbial N derived from ruminal $NH_3$-N has been reported to range from 40 - 100 %, varying with diet and rumen conditions. Thus it is evident that $NH_3$ in the rumen liquor is a key intermediary in microbial degradation and synthesis of protein.

*In vitro* studies showed maximum microbial growth to occur when the $NH_3$-N concentration was 50 - 80 mg/l (Allison, 1970; Satter & Slyter, 1974). By comparison, *in vivo* studies showed maximum microbial growth to be attained at concentrations of rumen $NH_3$-N ranging from 20 - 240 mg/l (Hume *et al.* 1970; Miller, 1973; Slyter *et al.* 1979; Okorie, 1981). Such optimum $NH_3$-N concentrations

are considerably higher than those predicted from $NH_3$ saturation constant ($K_s$) measurements for isolated rumen species. Schaefer *et al.* (1980) reported that for most species studied, the $K_s$ was such that a growth rate of 95 % of maximum would be achieved at 14 mg $NH_3$-N/l. Furthermore, as discussed by Hoover (1986), the optimal $NH_3$-N concentration for maximising the rate of fermentation can differ from that required for maximal MCP synthesis.

As reviewed by Durand (1989), such wide variations in optimal $NH_3$-N concentrations can be explained by different microbial requirements for growth and fermentative activities and also by the different pathways of $NH_3$ incorporation, which are in turn influenced by N source and $NH_3$ concentrations. In addition, the type of energy substrate influences the microbial requirement for $NH_3$, with the optimal $NH_3$ concentration varying with the amount of readily fermentable carbohydrate available (Hoover, 1986). The bacteria's capacity for protein synthesis and $NH_3$ uptake increases with increasing rates of carbohydrate fermentation due to more energy and carbon skeletons being available for amino acid synthesis. Furthermore, as discussed by Hoover (1986), the $NH_3$ requirements of microbes that are adherent or intimately associated with feed particles may be greater than those of the free-floating organisms in the rumen fluid.

According to the various feeding standards, which suggest that the amount of MCP synthesised generally falls within the range of 130 - 170 g/kg DOMI, then it can be inferred that the RDP requirements of the microbes should also be within the range of 130 - 170 g RDP/kg DOMI. McMeniman & Armstrong (1977) suggested that to optimise the eMCP the diet should contain at least 170 g RDP/kg of rumen degradable OM. However, the efficiency with which the microbes can capture the available RDP will no doubt affect the optimal proportion of RDP to DOMI. Although the efficiency of capture of rumen-available N is not known precisely, SCA (1990) suggested it to be around 0.8 for non-protein nitrogen (NPN). However, N recycled to the rumen via saliva or across the rumen wall can compensate for the inefficient capture of NPN to some degree. In particular, on low protein diets, recycled N may contribute significantly to the total N used by the microbes, as the rate of passage of urea through the rumen wall and thus into the rumen is inversely related to rumen $NH_3$

concentrations and positively influenced by supply of readily fermented carbohydrates (Durand, 1989).

The CP content of a plant can be used as a rough indicator of the supply of RDP to the animal. A review of published values by Minson (1990) found the mean CP content of tropical grasses to be lower than that for temperate grasses (100 vs. 129 g CP/kg DM). Furthermore, 21 % of the tropical grass samples studied contained less than 60 g CP/kg DM, below which microbial growth has traditionally been expected to decline (Langlands, 1987). However, tropical legumes were found to have a high CP content similar to their temperate counterparts, at 166 and 175 g CP/kg DM, respectively.

In addition, the SCA (1990) indicates that the degradability of protein (dg) may be lower in tropical compared with temperate grasses (0.65 vs. 0.78) and legumes (0.70 vs. 0.81). However, data collected by McLennan *et al.* (1997) for a tropical pasture (predominantly buffel grass) in northern Australia indicated that RDP represented more than 75 % of the CP in the diet of oesophageal-fistulated (OF) steers throughout the *ca.* 2-year sampling period.

McLennan *et al.* (1997), in their study on buffel grass pasture, found the highest protein degradability (0.96) in the early wet season when pasture was green and lush. Total CP content of the diet selected by OF steers remained above 60 g/kg DM for most of year, dropping below only during the dry winter/spring period. The authors calculated that the diet contained at least 130 g RDP/kg DOMI for most of the wet season but declined below this level during the drier months. However, some caution should be exercised in interpreting these values as the calculation of protein degradability involved an assumed constant value for rumen particulate matter passage rate (0.02/h). As passage rates would be expected to vary considerably with the quality of the pasture, this assumption may have introduced some error to the estimates of RDP supply/DOMI in the diet. This applies particularly to those samples of higher quality which would be expected to have passage rates > 0.02/h (SCA, 1990) and thus lower protein degradability and RDP supply than that calculated. If this were the case, it would imply that the tropical pasture diet consumed by the cattle

in the above experiment may have been even more marginal in RDP supply (< 130 g/kg DOMI) throughout the year than that estimated by the authors.

The buffel grass pasture studied by McLennan *et al.* (1997) is an important pasture type in the grazing lands of northern Australia, and characteristically grows on some of the more fertile soils found in areas classified as brigalow pasture communities. Thus, these findings have important implications for eMCP in cattle grazing tropical pastures throughout the northern grazing industry, particularly those pasture communities found in areas of lower soil fertility which would be expected to have even lower CP content and thus RDP supply.

Due to the difficulties in accurately determining the degradability of protein in the rumen, e.g. the requirement for rumen fistulated animals for determination of *in sacco* protein degradability and rumen dilution rates, there appears to be a dearth of experiments measuring protein degradability in conjunction with associated measurement of eMCP. This is particularly the case for tropical pastures for which there are far fewer experiments measuring both parameters.

It can be inferred that if the quantity of RDP supply is limiting on $C_4$ grass pastures then a response in eMCP should occur to RDP supplementation. Bolam *et al.* (1998) supplemented cattle consuming a mid-quality, tropical forage (rhodes grass hay; CP: 69 g/kg DM, OMD: 560 g/kg) with cottonseed meal as a source of additional RDP. The eMCP on the basal diet was low (62 g MCP/kg DOMI) and was increased up to within the extant feeding standards' range (130 - 170 MCP/kg DOMI) with RDP supplementation, but only when the supplement intake was at a very high intake of 2 % LW.

#### *2.3.1.3 Source of rumen-available nitrogen*

There has been considerable discussion in the literature regarding the effect on eMCP of providing a true protein source of RDP rather than an NPN source. While it is generally accepted that the growth of bacteria fermenting NSC is stimulated by peptides and amino acids, fibre-digesting bacteria are believed to primarily use $NH_3$ for MCP synthesis (NRC, 1996b). The CNCPS assumes that bacteria fermenting SC do not utilise peptides or amino acids and that all of their N must come from $NH_3$

(Russell *et al.* 1992). However, at the time, Russel *et al.* (1992) suggested that modifications to the CNCPS rumen submodel were underway to accommodate the recognised requirement of cellulolytic bacteria for BCFA. A review by Dijkstra *et al.* (1998) indicated that the quantitative relationship between microbial growth and peptides, amino acids and BCFA concentrations, has not been examined in detail. In particular, the response by rumen microbes to these substrates *in vivo*, and their interaction with energy substrate type and supply, is not clearly understood.

It could be inferred that when forage diets are fed, and in particular, tropical forages which are expected to be low in NSC and high in SC content, the SC-fermenting bacteria will play an important role in determining the rate of substrate breakdown and overall eMCP. Thus, the requirement of SC-fermenting bacteria for non-$NH_3$-N substrates requires more detailed examination as does the response in eMCP to availability of peptides, amino acids and BCFA.

***Amino acids, peptides and true protein***

Van Soest (1994) stated that growth of the SC-fermenting bacteria, *Ruminococcus flavafaciens, Fibrobacter succinogenes* and *Butyrivibrio fibrisolvens*, is stimulated by provision of amino acids, as is that of the pectinolytic species *Succinovibrio dextrinosolvens* and *Lachnosira multiparus*. In addition, *R. albus* and the three SC-fermenting bacteria mentioned above are believed to have a requirement for BCFA (Van Soest, 1994), which can be derived from the breakdown of amino acids in the rumen.

Hungate (1966) summarised the results of available experiments and concluded that a number of SC-fermenting bacteria are capable of assimilating at least some amino acids. Since that publication, pure culture studies have shown that SC-fermenting bacteria can use peptides and amino acids (Ling & Armstead, 1995; Atasoglu, 1996; Wallace *et al.* 1999) and that the growth of SC-fermenting bacteria is stimulated by these N sources (Cotta & Russell, 1982; Cruz Soto *et al.* 1994). Wallace *et al.* (1999) found that while $NH_3$ is essential for the growth of celluloytic bacteria, these bacteria still incorporate about half of their cell N from pre-formed amino acids in rich medium. Notably, Cruz Soto *et al.* (1994) found the growth rate of cellulolytic

bacteria to be stimulated by provision of peptides and amino acids when cultures were grown on cellobiose but not cellulose, thus indicating that the type of substrate may influence the response to true protein sources. The authors concluded that on cellulose substrate, the growth rate of cellulolytic bacteria was limited by the degradation rate of the energy source rather than the availability of peptides and amino acids when $NH_3$ was available.

While it has been shown that rumen microbes as a population have no absolute requirement for amino acids, peptides or proteins (Virtanen, 1966; Salter *et al.* 1979) numerous *in vitro* and *in vivo* studies have demonstrated protein, peptides and amino acids to stimulate microbial growth and fermentation.

Maeng *et al.* (1976) and Maeng & Baldwin (1976b) found small amounts of amino acids markedly stimulated microbial growth when mixed rumen bacteria were incubated *in vitro* with glucose, starch, and cellobiose as energy sources. Furthermore, when small amounts of amino acids were added to rumen contents from a cow fed a purified diet with urea as the sole N source, rumen microbial yields per kg carbohydrate digested were increased considerably (Maeng & Baldwin, 1976a). Atasoglu *et al.* (1999) found peptides and amino acids to support increased microbial growth and fermentation rates when included in an *in vitro* incubation of rumen fluid taken from four sheep receiving a mixed diet. Similar results were found by Fujimaki *et al.* (1992) who obtained a greater microbial yield in an *in vitro* study, using rumen fluid taken from a goat consuming a mixed diet, when an amino acid mixture was substituted for 25 % of the urea-N.

Merry *et al.* (1990) used a continuous culture system to compare supplementation of a basal diet of barley and straw (50:50, w/w) with either fish meal or urea. While the extent of fibre digestion was significantly increased with true protein supplementation, eMCP was unaffected. Extent of fibre digestion was also increased when non-urea-N sources, rather than urea-N, were supplied in continuous culture systems with a basal diet of mixed grass hay and grains (Griswold *et al.* 1996). Broudiscou *et al.* (1999) used a continuous culture system to compare the supplementation of $NH_3$-treated wheat straw with either urea and ammonium sulphate, or casein. Fibre degradability was not affected by treatment while MN

flows and eMCP were only slightly increased by supplementation levels up to 50 g CP/kg DM, with no treatment difference. It was concluded that microbial growth was not limited on this feed source by N supply, whether in the form of inorganic, amino acid or peptide N, nor by a deficiency in BCFA.

In contrast to the results of Merry *et al.* (1990) and Broudiscou *et al.* (1999), both urea and a true protein source (sunflower meal) were found to increase MCP synthesis and eMCP in a continuous culture system fermenting the semi-arid shrub *Ulex parviflorus* (CP: 90 g/kg DM; *in vitro* OMD (IVOMD): 440 g/kg) with the largest effect coming from the true protein source at equivalent N intakes (Molina Alcaide *et al.* 1996).

Similar to the *in vitro* experiments, *in vivo* trials have shown conflicting results when amino acids, peptides, and protein have been compared to $NH_3$-N as a substrate for rumen microbes on both 100 % forage and mixed diets. Hume *et al.* (1970) supplemented sheep consuming a virtually protein-free diet (containing cellulose, starch and sucrose) and with casein infused abomasally, with four levels of urea. The eMCP was increased from 9.1 g MCP/100 g OMTDR when N was most limiting to 13.3 g MCP/100 g OMTDR when N was in excess of requirements (recalculated, using data provided in the paper, as 81 g MCP/kg DOMI and 116 g MCP/kg DOMI, respectively). However, eMCP was below the extant feedings standards' range at all levels of supplementation and the authors concluded eMCP may have been limited by a deficiency of S or protein pre-cursors. In a second experiment, Hume (1970a) increased eMCP in sheep by *ca.* 36 % (17.1 to 23.3 g MCP/100 g OMTDR; recalculated as 145 and 165 g MCP/kg DOMI, respectively), by substituting casein for BCFA and 50 % of urea-N on a protein-free diet. Interestingly, when gelatin was substituted for BCFA and 50 % of urea-N on the same diet, the increase in eMCP was smaller, *ca.* 16 %. The authors concluded that the gelatin diet may not have supplied the ideal mix of amino acids for the microbes, due to being limiting in several amino acids, including methionine.

Ben-Ghedalia *et al.* (1978) reported findings in agreement to Hume (1970a) when replacing 10 % of the urea-N in a purified diet (containing cellulose, starch and glucose) with maize gluten as a true protein source. The flow of microbial OM to the duodenum in sheep was significantly greater in sheep on the maize-gluten compared

with the urea alone treatment (147 vs. 122 g OM/d). The authors suggested that this increase in MCP flow was caused by an increased eMCP although eMCP did not differ significantly between treatments (31.3 vs. 26.5 g MN/kg OMTDR). However, in contrast to the results of Hume (1970a), Ben-Ghedalia *et al.* (1978) found no response in microbial flow or eMCP when casein was added to the purified diet. The authors postulated that as casein is very soluble in the rumen, the preformed peptides or amino acids necessary for stimulation of microbial growth may not have been present for a long enough period or in sufficient concentrations to be effective.

Redman *et al.* (1980) studied the effect of true protein supply to steers consuming a more natural, low quality roughage (3 kg oaten chaff; 48 g CP/kg DM) fed at 3-hourly intervals. Urea or casein was fed as a pelleted supplement, isonitrogenously. The eMCP were similar for the control, urea and casein diets (*ca.* 21 g MN/kg OM apparently digested in the stomach; recalculated as *ca.* 103 g MCP/kg DOMI) indicating that the eMCP was not limited by the supply of $NH_3$-N or by peptides and amino acids in the rumen.

There are several possible explanations for the variable responses to true protein supplements in the experiments of Hume (1970a), Ben-Ghedalia *et al.* (1978) and Redman *et al.* (1980), which may have affected the results obtained. Firstly, although values were not supplied in each of the reports, estimates indicated that RDP supply/DOMI varied between the experiments. Secondly, the proportion of RDP from NPN, that was replaced as true protein, varied amongst the experiments. It is possible that the total quantity of true protein provided may not have been sufficient in some cases to produce a response. Thirdly, intake was kept constant in each of the three experiments. If intake had been allowed to increase as a possible effect of true protein supplementation under *ad libitum* feeding conditions, an increase in fractional outflow rate (FOR), (Estell & Galyean, 1985) may have resulted in an additional increase in eMCP. Finally, all of the diets contained some starch and sugar components and the amount and type of NSC substrate, as well as the interaction with the type of SC substrate supplied in each case, may have influenced the response in eMCP to true protein supply.

Additional experiments have been conducted with more practical feed substrates. The effect of ruminally infused solutions of peptides, amino acids or $NH_3$, on microbial activity in the rumen of sheep consuming grass hay (125 g CP/kg DM), was studied by Cruz Soto *et al.* (1994). There was no effect of treatment on rate or extent of OM digestion or on MN flow (g/d). However, the total CP supply was already high and it is possible that sufficient protein precursors were already present for maximal MCP synthesis. In support of these findings, and with lower quality roughage (*ca.* 30 and 60 g CP/kg DM), Kropp *et al.* (1977a,b) found no response in eMCP when they substituted soybean meal for urea as a supplement to cattle. However, in contrast to these experiments, Amos & Evans (1976) increased MCP synthesis in wethers consuming low quality bermuda grass (*Cynodon* spp.) hay (86 g CP/kg DM) when sunflower meal supplement, but not an isonitrogenous quantity of urea, was provided.

In addition to studies on purified diets and forage diets, a number of experiments have been conducted on mixed forage/concentrate diets and on silage. When sheep fed mixed diets were supplemented with an amino acid mixture substituting for 25 % of urea (Fujimaki *et al.* 1989), culturable counts of cellulolytic and amylolytic bacteria in rumen fluid tended to increase, although not significantly. Acid detergent fibre (ADF) digestion rate was not affected by amino acid supplements but amino acid supplemented diets were associated with a two to five fold increase in N incorporation, *in vitro*, into washed cell suspensions of mixed rumen bacteria. McAllan (1991) found that supplementation of steers consuming a mixed diet with urea plus fish meal significantly enhanced the digestibility of structural sugars over supplementation with urea alone. Increases in MCP flow were found in cattle consuming silage diets in response to provision of true protein (Rooke & Armstrong, 1989). Newbold *et al.* (1991) also reported increases in MCP flow, in response to dietary protein, in sheep consuming silage diets.

As noted by Cruz Soto *et al.* (1994) the published *in vitro* and *in vivo* experiments designed to study the effects of true protein supplementation on MCP production have often utilised mixed diets or diets containing some level of starch or soluble sugars. This has precluded clear extrapolation of the results to 100 % forage diets. For example, although Redman *et al.* (1980) set up an experiment to study the effect of supplementing cattle with a true protein source of RDP on low quality roughage, the

experimental diet contained a significant quantity of starch present in the pellet and possibly as oat grain in the oaten chaff. Cruz Soto *et al.* (1994) noted that, as for their own experiment (see earlier) the diets or substrates associated with a response to rumen degradable, true protein often had a substantial component of rapidly degraded carbohydrate. Thus accordingly, the authors suggested that stimulation of microbial growth by peptides and amino acids would be unlikely to occur on slowly fermented carbohydrate diets where bacterial growth rate would be limited by the energy supply rather than the rate of synthesis of amino acids.

This hypothesis was supported by the experiment of Chikunya *et al.* (1996) where sheep receiving diets of grass hay or molassed sugar beet pulp were supplemented with 9.3 g N/kg DM added as either urea or casein. A response to the true protein supply in microbial numbers and yield occurred on the rapidly degraded sugar beet pulp diet, but not on the more slowly degraded grass hay diet.

However, the hypothesis of Cruz Soto *et al.* (1994) was also tested by Wang *et al.* (1997) and Carro & Miller (1999), who recorded results inconsistent with the above authors. Wang *et al.* (1997) found peptides to increase microbial growth in an *in vitro* study in which rumen fluid, taken from a sheep consuming a mixed diet, was incubated with different carbohydrate sources including straw, starch or glucose. Carro & Miller (1999) used an *in vitro* semi-continuous culture system (RUSITEC) to study the effect of supplementing a fibre basal diet with different nitrogen forms. They found that where neutral detergent fibre (NDF) provided the only carbohydrate source, the replacement of $NH_3$ by various non-$NH_3$ sources of N resulted in an increased fibre digestibility and VFA production, as well as an increased flow of MN and eMCP. The proportion of MN derived from $NH_3$ decreased successively in the order: $NH_3$ > amino acids > peptides > protein treatments, indicating preferential uptake of peptides without passage through the $NH_3$ pool. The eMCP (g MN/kg organic matter apparent disappearance) was greater for the non-$NH_3$-N forms than for the $NH_3$ treatment, with peptides and protein treatments supporting higher efficiencies than the amino acid treatment. The authors concluded that N forms other than $NH_3$ are required for optimal growth of fibre-digesting bacteria and for maximum fibre digestion.

However, Newbold (1999) suggested that the division of rumen bacteria into those that ferment SC or alternatively NSC may be overly simplistic. The author refers to the work of Carro & Miller (1999) where distinct differences in the responses of solid-attached and liquid-associated bacteria were found. The solid-attached bacteria failed to show any consistent response to added amino acids, peptides or protein, while the yield of liquid-associated bacteria increased and the proportion of their cellular N derived from $NH_3$ decreased when pre-formed amino acids were added. Newbold (1999) hypothesised that these results reflect differences in the type of bacteria present in the two pools or, alternatively, differences in the fermentation by similar bacteria on the different energy sources present in the two pools. He further suggested that the stimulation in fibre breakdown when pre-formed amino acids are fed may result from a stimulation of the non-cellulolytic partners of the microbial consortia associated with the fibre rather than a direct effect on the cellulolytic organisms *per se*.

In addition to the general studies investigating the effect of mixed amino acids on microbial growth, several studies have investigated the effect of methionine alone. The hemicellulolytic species *Bacteriodes ruminicola* has been found to have a nutritional requirement for methionine when grown with $NH_3$ as a major N source (Pittman & Bryant, 1964). Furthermore, Patton *et al.* (1968) reported that methionine had a marked stimulating effect on microbial lipid synthesis.

Loosli & Harris (1945) and McLaren *et al.* (1965) found that substitution of methionine for isonitrogenous amounts of urea-N increased the retention of absorbed N in lambs fed mixed basal rations. Loosli & Harris (1945) measured a corresponding increased rate of gain in lambs given the methionine treatment. However, such responses could also be due to post-ruminal effects if a proportion of the methionine was undegraded in the rumen. More recently, White *et al.* (2000) estimated that 1 g of potentially rumen-degradable methionine increased intestinal MN flow by 18 % in sheep consuming mixed diets. Although, the authors questioned whether this result was reproducible as they had not observed this effect in subsequent experiments and stated that no other microbial responses to methionine had been reported with the type of mixed diets used in the experiment.

Allison (1969) stated that at least part of dietary methionine and cysteine are catabolised and S from these and other organic sources passes through a free $H_2S$ (hydrogen sulphide) pool which is utilised for amino acid biosynthesis. It could be inferred that diets S-deficient for the rumen microbes may show a response in MCP synthesis due to the supply of S-amino acids. However, authors of the above reports stated that it was unlikely their respective experimental diets were deficient in sulphur for the rumen microbes.

***Higher volatile fatty acids***

BCFA are isoacids including isobutyrate, isovalerate, and 2-methylbutyrate and are formed from the oxidative deamination of amino acids:

$$\text{valine} + 2H_2O \longrightarrow \text{isobutyrate} + NH_3 + CO_2$$
$$\text{leucine} + 2H_2O \longrightarrow \text{isovalerate} + NH_3 + CO_2$$
$$\text{isoleucine} + 2H_20 \longrightarrow \text{2-methylbutyrate} + NH_3 + CO_2$$

(Van Soest, 1994).

It has been shown that BCFA are essential factors for the growth of many SC-fermenting rumen bacteria (Bryant, 1973). Van Soest (1994) lists the following SC-fermenting bacteria as requiring a source of BCFA: *R. albus, R. flavafaciens, F. succinogenes* and *Butyrivibrio fibrisolvens*. More specifically, Robinson & Allison (1969) found *Bacteroides ruminicola* (a hemicellulolytic species) to synthesise isoleucine from 2-methylbutyrate.

SC-fermenting bacteria utilise the BCFA by essentially the reverse of the reactions described above, that is, by reductive carboxylation and then amination to form the branched chain amino acids (valine, luecine, and isoleucine). Isoacids may also be used by NSC fermenting species for long-chain fatty acid synthesis and, sometimes, for amino acid synthesis (Van Soest, 1994).

The oxidative deamination of amino acids, required to form BCFA, requires the transfer of hydrogen via NADH (reduced form of nicotinamide adenine dinucleotide). As the rumen is an anaerobic environment, this oxidative process is not favoured.

Furthermore, because of their saturated side chains, the isoacids and their associated amino acids are among the more hydrophobic substances within the VFA and amino acid groups, respectively (Chen *et al.* 1987), causing peptides containing such amino acids to be metabolised more slowly. It has been suggested that this combination of factors may lead to isoacid production, from dietary and intraruminally recycled amino acids, becoming limiting for cellulolytic fermentation. (Van Soest, 1994).

Furthermore, Bryant *et al.* (1975) concluded that the major fibrolytic bacterial species cannot ferment amino acids to provide the BCFA they require and have to rely upon their supply in the feed or from the fermentative action of other microorganisms. When carbohydrate availability is low some bacterial species tend to ferment amino acids and excrete BCFA rather than incorporate these amino acids into proteins (Wallace & Cotta, 1988). Thus, for diets that are high in fibre with slow rates of fermentation, an additional supply of protein may stimulate fibrolytic bacterial growth through the fermentation of amino acids by non-fibrolytic bacteria and through the subsequent increased supply of BCFA (Dijkstra *et al.* 1998). In support, Russell *et al.* (1992) suggested that a BCFA deficiency could be encountered if high-forage diets are low in true protein and NPN is used as a N supplement. In deference to their belief that BCFA are required by the SC-fermenting rumen bacteria, Russell *et al.* (1992) saw the need to include a modification to the current rumen sub-model for the CNCPS to accommodate the requirement of cellulolytic bacteria for BCFA for growth.

However, in contradiction to the arguments suggesting that a response to BCFA will occur on high fibre diets, Van Soest (1994) suggested that a response to isoacid supplementation would require a rate of carbohydrate fermentation sufficient to create a microbial need for the cofactors. He concluded that providing BCFA would be ineffective on low-quality diets, even when protein supply is marginal, because recycling of the respective carbon skeletons would be sufficient due to the relatively slow rate of fermentation.

Although most interest and experiments have concentrated on the effects of the BCFA isobutyrate, isovalerate and 2-methylbutyrate, it is possible that phenylsubstituted fatty acids, as well as n-valerate, acetate and other amino acid carbon skeletons may

also have a stimulating effect on SC-fermenting bacteria. In fact, W.C. Ellis (personal communication 2001) suggested that the failure of some experiments to show a response to BCFA mixes may have been due to the exclusion of other higher VFA from the mix, which may also have been limiting for the SC-fermenting bacteria.

It has been shown that the phenylsubstituted fatty acids, phenylacetate, hydroxyphenylacetate, and indoleacetate, are suitable substrates for reductive carboxylations and can be utilised for the biosynthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan, respectively (Allison, 1965, 1969; Allison & Robinson, 1967; Kristensen, 1974). Such reductive carboxylation pathways, which are present in some rumen bacteria, appear to occur in addition to the biosynthesis of these aromatic amino acids from a common precursor, chorismate (Morrison & Mackie, 1997). Sauer *et al.* (1975) provided evidence to support the presence of both routes of aromatic amino acid biosyntheses with mixed rumen contents, but the quantitative importance of the two pathways remains unclear (Morrison & Mackie, 1997).

The availability of branched-chain and phenylsubstituted fatty acids for rumen bacteria modulates the flux of glucose carbon into amino acids. Allison *et al.* (1984) found that supplementation of growth medium with isovaleric acid effectively blocked glucose carbon flow into leucine. Similarly, the provision of phenylacetate and 2-methylbutyrate reduced the utilization of glucose carbon for phenylaline and isoleucine biosynthesis, respectively. These findings provide further evidence of the ability of microbes to use BCFA and phenylsubstituted fatty acids as precursors for amino acid synthesis.

Van Soest (1994) suggested that n-valerate is also required by some bacteria for growth, and specifically identifies *F. succinogenes*, a SC-fermenter. Valerate can be formed by the reduction of proline to delta-aminovaleric acid, which is then converted to valeric acid (Hungate, 1966). Alternatively, valerate can be formed from propionyl CoA and acetyl CoA. In addition, Hungate *et al.* (1964) concluded that acetate stimulated the growth of several groups of ruminal bacteria, especially when $NH_3$ was the main source of N. Similar to the process of utilisation of BCFA and

phenylsubstituted fatty acids, acetate is reductively carboxylated and then aminated to form alanine.

Allison (1969) proposed that carbon skeletons of amino acids other than those discussed above may be synthesised by reductive carboxylation reactions. Preliminary studies by Robinson & Allison (1969) indicated that *Bacteriodes ruminicola* (hemicellulolytic species) synthesises the glutamate carbon skeleton by reductive carboxylation of succinate. Allison (1969) also postulated that histidine may be synthesised from imidazole acetate acid. However, the results of Sauer *et al.* (1975) support the contention that histidine biosynthesis proceeds via a different pathway.

It is possible that the previously mentioned responses of SC-fermenting bacteria to the supply of proteins, peptides and amino acids may be in part due to the supply of BCFA and other higher VFA, as their breakdown products, rather than the direct assimilation of these substrates. A number of experiments have studied the effects of direct additions of higher VFA to attempt to isolate the effect of these factors alone.

Russell & Sniffen (1984) found that when inocula were obtained from a cow fed timothy hay, incubations containing isovalerate and 2-methylbutyrate supported more efficient conversions of carbohydrate to bacterial protein than those not receiving the added acids. However, additions of valerate and isobutyrate had no effect. In contrast to the results for timothy hay, inoculum comprising 60 % concentrate did not show significant response to addition of the isoacids.

Addition of various mixtures of higher VFA to rations for ruminants has in some cases had a positive effect on diet digestibility, nitrogen retention, appetite or animal performance (Hungate & Dyer, 1956; Lassiter *et al.* 1958a,b; Hemsley & Moir, 1963; Cline *et al.* 1966; Felix *et al.* 1980a,b; Papas *et al.* 1984). This effect has often been attributed to enhancement of microbial growth or cellulose digestion.

Hume (1970b) provided some insight into the effect of BCFA on eMCP in an experiment with sheep given a protein-free diet and casein infused abomasally. Treatments were either urea as an NPN source or urea plus a BCFA mixture. The

BCFA mix (1.23 % of diet DM) included: 27.0 % isobutyric, 26.3 % 2-methylbutyric, 29.9 % isovaleric, and 16.8 % n-valeric acids with proportions designed to correspond to those of their amino acid analogues found in mixed rumen MCP (Purser & Buechler, 1966). The addition of the mixture of BCFA increased daily MCP production by *ca.* 10 g, but not eMCP (13.0 g MCP/100 g OMTDR; recalculated as 115 g MCP/kg DOMI).

However, contrasting results were obtained by Gunter *et al.* (1990) who supplemented beef heifers consuming grass hay with 4 and 5-carbon BCFA. The BCFA was provided as a commercial product, IsoPlus, containing 82 % calcium salts of isobutyrate, mixed 5-carbon VFA and stearate, and were encased in gelatin capsules and dosed through the rumen cannulae once daily. The BCFA supplement had no effect on MN flows, or eMCP. It was postulated that more frequent dosing was required to maintain higher concentrations of BCFA throughout the day.

***Conclusions***

Although conflicting results have been reported, there is evidence to indicate that true protein supplements may produce increases in eMCP on both forage and mixed diets. However, the exact nature of the requirements of specific microbe species, and the interaction with feed substrate and supply, has not been clearly elucidated. In addition, the relative importance of peptides, amino acids, BCFA and other higher VFA as precursors for individual microbe species and groups of species is also not clearly understood. It appears that further work is required to more clearly define these relationships. This would allow more confident recommendations for inclusion of true protein, or precursors such as BCFA, on various diet types.

#### *2.3.1.4 Rate and synchrony of substrate supply*

On many forage diets the rate of availability of RDP in the rumen exceeds that of energy (Beever, 1993a). In addition to decreasing the efficiency of microbial capture of ruminally degraded N, this lack of synchrony in substrate supply could theoretically decrease the rate and extent of microbial fermentation and growth and thus decrease eMCP (Beever, 1993b).

The lack of synchrony of RDP and carbohydrate supply is potentially high on silage-based diets where the ensiling process causes a reduction in the readily fermentable OM available for the rumen microbes, but an increase in readily available N due to extensive protein degradation. This has been suggested to be the cause of the low eMCP measured for many silage diets (Beever, 1993a). Rooke *et al.* (1987) provided evidence for the negative effect of energy and protein imbalance in the rumen on MCP synthesis in an experiment where nutrients were infused into the rumens of cows consuming grass silage diet. While isonitrogenous infusions of urea or casein produced no significant increases in MCP synthesis or eMCP, intraruminal infusion of glucose syrup with or without casein stimulated a significant increase in the extent of MCP synthesis. However, eMCP was only increased when glucose syrup and casein were co-infused and, in a subsequent, experiment Rooke & Armstrong (1989) demonstrated that the supply of true protein was the cause of the increases in eMCP, once the energy/protein imbalance was corrected with supply of readily fermentable energy.

Similar results to those described above were obtained by Chamberlain *et al.* (1993), and Kim *et al.* (1999a,b) who measured increases in MCP synthesis when readily fermentable energy sources were intraruminally infused in sheep or cattle consuming silage-based diets. Furthermore, the type of NSC used as an energy supplement appears to be important, as demonstrated by Chamberlain *et al.* (1993), who found that sugars, particularly sucrose, were superior to starch for the microbial fixation of N in the rumen when sheep were consuming silage diets. Unfortunately eMCP was not measured in these experiments.

Lack of synchronisation of N and energy supply may also occur when ruminants graze lush, green pasture in which N is characteristically readily available but the SC more slowly degraded (Beever & Siddons, 1986). Similar to the situation on silage diets, an increased supply of readily available carbohydrate on lush pasture diets could theoretically increase the degree of N/energy synchrony and thus lead to an increase in eMCP. As previously discussed (section 2.3.1.1) Dove & Milne (1994) found eMCP in sheep grazing perennial ryegrass pasture during spring/summer was more than double that during autumn (255 vs. 117 g MCP/kg OMADR). They argued that the differences in efficiency were due to a greater WSC content in the consumed

herbage during the spring/summer period, although season was confounded with physiological state of the animals and with rumen dilution rates. In addition, Trevaskis *et al.* (2001) found that providing sheep consuming annual ryegrass (*Lolium multiflorum*) or kikuyu grass (*Pennisetum clandestinum*) with a readily available source of carbohydrate timed to coincide with peak rumen $NH_3$ concentration, increased MCP flow from the rumen, although eMCP synthesis was not determined. However, in contrast to these results, Lee *et al.* (2002) found no increase in eMCP (average 106 g MCP/kg OMADR) in steers consuming perennial ryegrass containing elevated concentrations of WSC (243 g/kg DM) compared to one with intermediate WSC concentrations (161 g/kg DM).

Poppi & McLennan (1995) have discussed the potential of supplying energy supplements to ruminants grazing pasture in order to increase eMCP. They concluded that energy supplements would be most effective when there is rapid $NH_3$ production and thus loss of N from the rumen. It was determined that this was likely to occur when CP content of the pasture exceeded 210 g CP/kg DOM, and thus involve temperate pastures, especially in spring, some of the tropical legumes and perhaps tropical grasses in the early growth stage.

Decreasing the rate of forage protein breakdown in the rumen is another strategy to increase the synchrony of energy and N supply to the microbes. Beever *et al.* (1987) established that pre-treatment of white clover pasture with formaldehyde increased eMCP (418 vs. 274 g MCP/kg OMADR) and the overall capture of RDP in the rumen of cattle. However, this procedure had little practical application. Studies of tannin-containing legumes have demonstrated similar effects in increasing absorption of amino acids in the small intestine through slowing the release of N (see review Beever, 1993b). However, the potential to exploit this process may be limited by the possible adverse effects that increasing tannin content in the diet may have on other digestive processes.

Asynchrony of N and energy supply is also likely to be pronounced when rapidly available RDP supplements, such as urea, are provided in conjunction with low quality fibrous feeds which have slowly released fermentable energy. Although there is a dearth of information regarding measured values for eMCP under such

conditions, a range of digestive and animal performance parameters have been monitored. Tudor & Morris (1971) found that more frequent ingestion of urea increased the efficiency of use of urea as a N supplement for sheep fed low quality roughages. Similarly, Romero (1976) found that the efficiency of use of urea and the DOMI of cattle consuming low quality roughage increased in association with increasing frequency of urea ingestion. However, Henning *et al.* (1993) suggested that the ability of ruminants to recycle N back to the rumen, as well as the ability of bacteria to store excess energy as polysaccharides for later use, may reduce the importance of synchrony of energy and N supply in the rumen on low quality diets. This may help to explain the findings of Rush & Totusek (1975) who found that the frequency of ingestion (three times per day vs. three times per week) of urea-containing supplements by range cattle had little influence on apparent urea utilisation, as indicated by weight changes.

A number of methods have been devised to minimise the assumed negative effects of rapidly released N in supplements for low quality pasture. Biuret, formed by heating urea, is more slowly degraded in the rumen than urea. However, due to its unpalatability, the period of adaptation required, its lack of solubility and its expense, it has not been used in the Australian beef industry. A recent trial by Loest *et al.* (2001) found that replacing half the N from urea with biuret in cooked molasses blocks for cattle consuming low-quality prairie hay, had no advantage over urea alone in increasing intake and digestibility of the diet. Furthermore, rumen $NH_3$ concentrations did not support the hypothesis of a sustained release of N from biuret. The authors suggested that the biuret may pass through the gastrointestinal tract unhydrolysed and/or that a period of greater than 3 weeks may be required for ruminal microbes to acquire the metabolic capacity to hydrolyse biuret.

As an alternative strategy, bentonite has been included in supplements containing a rapidly degraded N source, such as urea to slow the rate of release of N in the rumen. Bartos *et al.* (1982) found that bentonite could absorb $NH_3$ from rumen fluid and release it later. The binding capacity of bentonite was 6 - 12 mg $NH_3$-N/g bentonite, and was assumed to be related to ion exchange. Britton *et al.* (1978) found that complexing protein with sodium bentonite had a positive effect on N utilisation and liveweight gain in ruminants in a number of experiments with both sheep and cattle.

However, these results were not supported by those of Eady *et al.* (1990) who found that the production of sheep consuming either mulga (*Acacia aneura*) or lucerne (*Medicago sativa*) pellets with addition of a N and mineral mix, was not increased by the addition of sodium bentonite or zeolite.

A number of experiments have been conducted in an attempt to verify the importance of synchrony of energy and N supply in determining eMCP. Herrera-Saldana *et al.* (1990) and Sinclair *et al.* (1993, 1995) used combinations of energy and protein supplements of varying degradability to formulate diets either synchronous or asynchronous in the supply of energy and N to the rumen. All experiments found eMCP to be increased in animals fed the synchronous diets. Conflicting evidence was provided by Salter *et al.* (1983) and Casper *et al.* (1990) who supplemented either straw or lucerne hay diets, respectively, with protein and energy sources of varying degradability. They found that attempting to synchronise energy and protein supply had no effect on the efficiency of capture of N from the degradable protein but, unfortunately, did not measure eMCP.

However, the various experiments described above have used different feed ingredients to formulate diets either synchronous or asynchronous in energy and N supply, with a possible confounding effect of feed type. Other groups have attempted to overcome this problem by supplying the same dietary ingredients but altering the feeding pattern or pattern of infusion into the rumen. Henning *et al.* (1993) and Kim *et al.* (1999b) infused readily available N and/or energy sources intraruminally to create different patterns of supply on straw and silage based diets, respectively. These groups found no effect on eMCP or MCP flow, respectively. However, Kim *et al.* (1999a) found a marked increase in MCP flow in cows when malto-dextrin was infused synchronously with feeding of the basal diet of grass silage and concentrates, rather than asynchronously.

As already discussed, urea recycling to the rumen may be a possible explanation for the lack of effect of energy and N synchronisation observed on low quality diets. In addition, negative effects of supplements containing readily available carbohydrate on rumen pH (discussed in section 2.3.4) may explain the lack of effect of synchronising diet energy and N supply in some cases. Rooke & Armstrong (1989) suggested that

synchronisation becomes more important at higher rumen pH (e.g. 6.7 - 6.8) due to a more rapid absorption of $NH_3$ into the blood, which could lessen the time available for bacteria to utilise this source of N.

It is evident that the importance of synchrony of substrate supply in determining eMCP in the rumen is still not clearly understood. The experimental approach used has confounded interpretation in some cases. Where dietary ingredients have been altered, it is not possible to identify whether effects on eMCP have been due to an effect of N/energy synchrony or to effects of level and type of ingredients *per se*. Furthermore, it is possible that the type of feed base, e.g. silage, fresh forage with high RDP content, or low quality roughage supplemented with RDP, as well as type of supplement, influences the extent to which synchronisation of substrates is important.

### 2.3.2 Dilution rate in the rumen

Dilution rate is the rate of passage, or FOR, of fluid or particles from the rumen. Rumen dilution rate effects eMCP by moderating microbial growth (Isaacson *et al.* 1975). Theoretically, there are three possible reasons for an increase in eMCP due to dilution rate. Firstly, a higher dilution rate may select for a proportional increase in microbial species with a higher growth rate and thus lower maintenance energy requirement (Baker & Dijkstra, 1999). Secondly, a reduction in the mean age of the bacterial population due to increased dilution rate is expected to result in lower rates of autolysis and predation (Van Soest, 1994). Thirdly, increasing dilution rate can result in depression of protozoal numbers and thus reduce predation of bacteria by protozoa (Meng *et al.* 1999).

*In vitro* studies using continuous fermentation of mixed bacterial cultures have generally found Michaelis-Menton type saturation curves to provide a good fit for the relationship between microbial cell yield efficiency and fluid dilution rate (Stouthamer & Bettenhaussen, 1973; Isaacson *et al.* 1975; Russell & Cook, 1995). More recently, Meng *et al.* (1999) found a quadratic model to be the most appropriate for describing the relationship between fluid dilution rate and eMCP for diets of fibrous carbohydrate, non-fibrous carbohydrate, and protein in a single-effluent continuous culture system.

While traditional laboratory chemostats apply only a liquid dilution rate, due to the use of soluble feed components, the rumen involves two major dilution rates applying to fluid and solid pools. The passage rate of liquid from the rumen is controlled by dietary, osmotic, neural and hormonal factors (Owens & Goetsch, 1986). Infusion of solutes into the rumen (Harrison *et al.* 1975) or their inclusion in the diet (Potter *et al.* 1972; Hemsley, 1975; Wiedmeier *et al.* 1987) increases the fluid FOR in proportion to the solute load. In contrast, the FOR of rumen fluid is not affected when water alone is infused into the rumen (Harrison *et al.* 1975). Thus, it appears that the FOR of fluid from the rumen is a function of those dietary factors associated with increased rumen osmolality and/or increased flow of saliva into the rumen.

The rate of particle outflow from the rumen has been found to be a function of particle size, with experiments with sheep and cattle indicating that a reduction in particle size to less than 1.2 mm by mastication, rumination and digestion generally precedes exit from the rumen (Poppi *et al.* 1980, 1981). However, the mean particle length required for ruminal exit can be modified by the level of feed intake, frequency of feeding and diet composition (Owens & Goetsch, 1986). The density of particles has also been found to be an important factor influencing rate of escape of from the rumen (DesBordes & Welch, 1984). More recent experiments by de Vega & Poppi (1997) showed that neither extent of digestion nor origin of particles (grass or legume) had an influence on the fractional outflow rate of these same particles from the rumen of sheep. Instead, the results indicated that the type of diet being consumed (grass or legume) had greatest influence on rates of passage of both particulate and liquid phases.

An increase in animal intake has been shown to be an important factor in increasing both liquid and solid pool dilution rates (Estell & Galyean, 1985) and has been associated with increased eMCP synthesis in a number of experiments (Chen *et al.* 1992a; Djouvinov & Todorov, 1994). In addition, environmental temperatures can have an effect, with prolonged exposure of ruminant animals to cold increasing both fluid and particle FOR and also eMCP (Kennedy *et al.* 1976; Kennedy & Milligan, 1978a).

Fluid FOR measured in cattle grazing temperate pastures have been as high as 25 %/h in the early growing season (McCollum & Galyean, 1985). However, the available literature for cattle fed on tropical pasture diets give a range of values for fluid FOR which are much lower at *ca.* 4 - 9 %/h (Poppi *et al.* 1981; Kennedy, 1982; Hunter & Siebert, 1985b; Cafe & Poppi, 1994; Mullik, 1999). The lowest values were found for cattle consuming stem fractions of pangola grass and Rhodes grass forage (Poppi *et al.* 1981). If such low dilution rates are characteristic of tropical pastures in general, this would imply that eMCP would also be low on tropical compared to temperate pasture diets. However these values for tropical pastures were recorded with animals consuming hay diets, predominantly pangola grass, and may not be representative of a range of other tropical pasture species or of fresh forage consumed under grazing conditions.

There is some debate about the relative importance of fluid and particle FOR with regard to their effect on eMCP. Owens & Goetsch (1986) state that because rumen microbes can be associated with ruminal solids and the ruminal wall, as well as the liquid/small particle pool, eMCP may not always increase with increasing fluid FOR. Furthermore, the authors suggested that eMCP may be more responsive to changes in the passage rate of solid material as the passage rate for the liquid fraction is generally already high. This theory, that particle FOR is of greater importance in determining eMCP than fluid FOR, is supported by a series of experiments using continuous culture fermenters with high concentrate diets. These experiments found that increases in liquid dilution rate resulted in only modest, pH-dependent increases in eMCP, while increases in solids passage rate increased eMCP more consistently (Crawford *et al.* 1980; Hoover *et al.* 1982, 1984; Shriver *et al.* 1986). Similarly, in an experiment with lactating dairy cows consuming mixed forage/concentrate diets, Rode & Satter (1988) showed eMCP to be positively related to solids turnover rate in the rumen, regardless of ruminal liquid dilution rate. However, when there was no change in solids turnover rate, eMCP was stimulated by an increase in liquid turnover rate. In contrast to these results, relationships of both fluid and particle FOR with eMCP have been observed in other *in vivo* experiments (Kennedy *et al.* 1976; Kennedy & Milligan, 1978a; Djouvinov & Todorov, 1994).

As water is the vehicle for transport of digesta out of the rumen, it could be expected that the FOR of fluid must be the major determinant of the FOR of the particulate matter in the rumen. This hypothesis is supported by the experiments of de Vega & Poppi (1997) who fed sheep varying diets including low and high quality hays, pelleted lucerne, and concentrate, and found strong a relationship between FOR of CrEDTA (chromium complexed with ethylenediamine tetraacetic acid; fluid marker) and FOR of Yb (ytterbium; particle marker), indicating that particle turnover rate is influenced by the water turnover rate. Thus it could be inferred that any changes in eMCP would be related to the FOR of both the rumen fluid and particles, with the relative importance of fluid and particle FOR being related to the degree of association of microbes with the two main rumen pools as well as the relative size of the pools.

A review of the literature indicates that measurement of both fluid and particle FOR parameters, in conjunction with determination of eMCP, has been rare. The dilemma of the relative importance of fluid and particle FOR was avoided in experiments conducted by Kennedy (1982) where eMCP was expressed relative to the retention time of the microbes themselves. In experiments with cattle consuming alfalfa and pasture hays, the author found eMCP to have a relationship of exponential decline with increasing microbial retention time in the rumen.

In contrast to those experiments described above, some experiments have found no effect of dilution rate on eMCP. Mathers & Miller (1981) found no effect of rumen fluid dilution rate on eMCP for sheep given a range of diets varying from 100 % hay to 100 % concentrate. Furthermore, in twenty-three experiments carried out with grazing sheep Corbett *et al.* (1982) found the mean fluid FOR of 22 %/h (SD ± 6 %/h) was not significantly related to eMCP. However, this mean represents a very high value for fluid FOR and would likely be close to the plateau of any response curve effect of fluid FOR on eMCP.

Rumen dilution rates have been artificially increased in a number of *in vivo* experiments with corresponding increases in eMCP. Harrison *et al.* (1975, 1976) showed that intraruminal infusion of solutes increased dilution rate, with an associated

increase in eMCP. This strategy was tested on a more practical level by Mullik (1999) who increased eMCP, in steers given pangola hay (85 g CP/kg DM), from 76.5 to 118.7 g MCP/kg DOMI when urea was added (19 g/kg hay DM), and to 140.5 g MCP/kg DOMI with urea and salt (0.15 % LW). Correspondingly, fluid dilution rates were 6.27, 6.61 and 7.91 %/h, respectively, showing a relationship trend between eMCP and dilution rate. However, these levels of salt are extremely high and not commercially practical for increasing eMCP. In addition, Hvelplund (1991) suggested the need for caution when adding extra minerals to the rumen, as feeding large amounts of mineral salts can cause hypertonic rumen fluid which may inhibit fermentation by creating excess osmotic pressure. Evidence of such a negative effect was found by Dewhurst & Webster (1992), in experiments with sheep, where high dietary sodium concentrations were associated with reduced MCP flow.

Ohajuruka *et al.* (1991) showed the addition of animal or vegetable fat, up to 5 % of dietary DM, increased liquid dilution rate linearly from 11.1 to 12.1 %/h but neither the source nor amount of fat influenced eMCP. Furthermore, dietary inclusions of natural mineral buffers such as bentonite, zeolite and vermiculite have been suggested to increase the dilution rate of rumen fluid (Bartos *et al.* 1982). However, there appears to have been few trials to examine the effect of these substances on dilution rate and eMCP in practical feeding situations. Erasmus & Prinsloo (1989) supplemented dairy cattle consuming a hay and concentrate diet with 3.6 % vermiculite and found no effect of vermiculite on dilution rate.

The body of evidence indicates that rumen fluid and particle dilution rates can affect eMCP in ruminants. Low values for rumen fluid FOR have been observed in ruminants consuming tropical forages fed as chaffed hays, but there is a dearth of information concerning expected fluid and particle FOR, and their relation to eMCP, for ruminants grazing a range of tropical forage species and over varying seasonal conditions. Various strategies such as addition of mineral salts or fats to the diet have been proposed as methods to increase dilution rate and thus eMCP. However, currently, there are no practical strategies for increasing eMCP through dilution rate manipulations.

### 2.3.3 Intraruminal recycling

Studies of intraruminal recycling of N indicate that up to half of the MCP present in the rumen may be degraded *in situ* resulting in a decreased eMCP (Leng & Nolan, 1984; Hvelplund, 1991; Nolan, 1993). As discussed by Baker & Dijkstra (1999), the recycling of MCP in the rumen can involve bacteriophage-mediated lysis of microbes, starvation of microbes, lysis of fungal thalli after zoospore release, and uptake of bacteria by protozoa.

Baker & Dijkstra (1999) suggest that the major contribution to microbial turnover in the rumen is likely to be related to protozoal activities, as the breakdown of bacterial protein *in vitro* was reduced by approximately 90 % upon the removal of protozoa in a number of studies. Apart from their effect on N recycling, protozoa could theoretically decrease eMCP through competition with bacteria for substrates (Baker & Dijkstra, 1999). The negative effect of substrate competition would be due to the lower efficiency of ATP utilisation by protozoa compared to bacteria (Nuzback *et al.* 1983). In addition, the contribution of protozoa to MCP flowing from the rumen is much less than that of bacteria due to their mechanisms for retention in the rumen, as already discussed (section 2.2).

Defaunation of the rumen has been shown to increase intestinal protein supply and MCP flow in a number of studies (Ushida *et al.* 1984, 1986; Kayouli *et al.* 1986; Meyer *et al.* 1986). A review by Jouany (1996) indicated that MCP flow to the duodenum can be almost doubled in some cases by defauantion of ruminant animals. Jouany *et al.* (1988) summarised values from the literature and found the increase in eMCP, due to defaunation of sheep, to range from 16 to 125 % when eMCP was expressed as g MN incorporated/kg OM fermented in the rumen. The greatest increases in eMCP were associated with diets more favourable to protozoal growth.

In a more recent study, eMCP was increased from 20.0 to 37.8 g MN/kg OMTDR by defaunating sheep consuming lucerne haylage and barley concentrate (Koenig *et al.* 2000). However, the authors stated that the difference may have been over-estimated due to the change with defaunation in the purine:N ratio in the total microbial population. Further recent evidence comes from an experiment with Holstein heifers, where feeding fats in conjunction with a corn silage and concentrate diet decreased

total protozoal counts and tended to increase eMCP (19.6 vs. 16.3 g MN/kg carbohydrate truly digested (Oldick & Firkins, 2000).

A review by Jouany *et al.* (1988) indicated that defaunation usually increases eMCP more than MCP flow. The authors suggested that a possible explanation for this is the reduced degradation of cell walls and thus reduced organic matter fermented in the rumen as a consequence of removal of protozoa from the ecosystem. In fact, on low quality diets high in plant cell wall content, the increase in eMCP brought about by defaunation has been found in some cases to be offset by the concomitant impairment of fibre degradation such that overall MCP flow (g MCP/d) is not increased (Jouany *et al.* 1988).

A number of methods have been used to achieve defaunation under research settings, including the use of chemicals or oils that are toxic to protozoa. In a practical setting, protozoal numbers in sheep have been decreased due to supplementation with saponin-rich plants (Navas-Camacho *et al.* 1993). Feeding moderate levels of unsaturated fat has also reduced protozoal numbers, especially on low forage diets (Firkins, 1996; Jouany, 1996; Dewhurst *et al.* 2000). In addition, high concentrate diets with large amounts of starch may decrease protozoal numbers due to their sensitivity to low pH (Mendoza *et al.* 1993; Franzolin & Dehority, 1996). However, refaunation occurs with ease once defaunated animals are exposed to contact with other ruminants. Thus, none of these methods have been found successful in maintaining defaunation, in a practical setting, for extended periods of time (Jouany, 1996).

In addition to the use of antiprotozoal agents, increasing the dilution rate of the rumen, as previously discussed, has been suggested to reduce intraruminal recycling due to a younger bacterial population and thus increased resilience to predation by protozoa (Van Soest, 1994). Furthermore, increased dilution rates may lead to increased washout of protozoa from the rumen (Meng *et al.* 1999). It is likely that the nature of the diet, and thus the ability of the protozoa to prevent their washout from the rumen, would contribute to the magnitude of the effect of dilution rate on protozoal numbers and intraruminal recycling in the rumen.

In conclusion, there is substantial evidence to suggest that defaunation increases eMCP through reducing intraruminal N recycling. However, practical means of achieving defaunation of ruminants for extended periods of time under grazing conditions have not been developed.

### 2.3.4 Rumen pH

Rumen pH can decrease under conditions of high concentrate feeding and when ruminants consume lush temperate pastures with high levels of readily available NSC. In such conditions, the rate of carbohydrate fermentation is rapid and acid production is faster than its absorption from the rumen. In addition, silage consumption can also decrease rumen pH due to high levels of lactic acid and VFA already present in the feed. Salivary secretions and dietary additives buffer pH change in the rumen but in some cases may not be sufficient to prevent rumen pH decline. The amounts and type of saliva secreted can be influenced by diet type, with high concentrate diets having lower levels of mastication, and thus salivation, than high roughage diets. Beever (1993a) commented on the variation amongst forages with regard to buffering capacity, suggesting that legumes have a greater buffering capacity than grasses, owing to their inherently high content of protein and soluble ash.

It is well established that low rumen pH is associated with reduced digestion of proteins, cellulose, hemicellulose, and pectins, but with less effect on starch (Hoover *et al.* 1984; Shriver *et al.* 1986; Strobel & Russell, 1986; Calsamiglia *et al.* 2002). It has been shown both *in vitro* (Terry *et al.* 1969; Stewart, 1977) and *in vivo* (Mould & Orskov, 1983/84) that the optimal pH for microbial digestion of fibre is in the range of 6.6 - 7.0, with digestion severely reduced at pH < 6.2 and negligible at pH < 6.0.

In addition, *in vitro* studies have indicated that eMCP can decrease significantly when pH falls below 6.5 (Russell & Drombrowski, 1980; Strobel & Russell, 1986; de Veth & Kolver, 2001). Furthermore, de Veth & Kolver (2001) found a negative linear relationship between eMCP and the time spent at suboptimal pH. The decline in eMCP at low pH can be partially explained by a reduction in carbohydrate utilisation (Strobel & Russell, 1986). In addition, low pH is associated with increased production of lactic acid (Russell & Drombrowski, 1980; Strobel & Russell, 1986). While lactic acid itself is not detrimental to microbial growth, this would imply a

lower energy yield per unit of carbohydrate fermented due to lactate pathways yielding less ATP than those leading to acetate or propionate as end-products (Strobel & Russell, 1986). However these experiments have often found protein synthesis to be reduced to a greater extent than that attributable to ATP production, indicating that ATP was being used for non-growth, or energy spilling reactions, particularly at $pH \leq 6.0$. Strobel & Russel (1986) stated that such energy spilling reactions would occur at low pH due to the additional energy required to expel protons and thus maintain the membrane potential of microbes.

However, other *in vitro* studies have shown that eMCP was either not affected, or increased, with decreasing pH (Hoover *et al.* 1984; Shriver *et al.* 1986; Calsamiglia *et al.* 2002). In particular, Shriver *et al.* (1986) and Hoover *et al.* (1984) found an interaction between pH and solid and liquid dilution rates, with eMCP declining with increasing pH but increasing with increased FOR of digesta from the rumen.

There is a dearth of information documenting eMCP changes with pH in *in vivo* experiments. Recently, Kolver & de Veth (2002) reviewed data from twenty-three separate studies involving lactating dairy cows consuming diets based on fresh pasture. Mean daily ruminal pH across studies ranged from 5.6 - 6.7. Within studies, a high MN flow from the rumen was associated with low ruminal pH. The authors concluded that in cows fed high quality pasture MCP flow was not affected when mean ruminal pH decreased to 5.8. However, eMCP was not determined and may have shown a different relationship with pH than total MCP flow from the rumen. It can be postulated that if eMCP had been decreased at low pH, as expected from *in vitro* studies, this could be offset by an increased total fermentable energy supply in the form of ruminally degradable carbohydrate, consistent with conditions that cause the lower pH values. An increased supply of fermentable energy for the microbes could explain an increased total MCP production.

In conclusion, it appears that the relationship of pH with eMCP *in vivo* may be complicated by interactions with substrate type, and possibly rumen dilution rates. Changes in rumen pH over a range of tropical grass and legume species, and with season, have not been well documented. However, low rumen pH would not be expected on tropical grass diets, with their lower NSC levels compared to their

temperate counterparts (see section 2.3.1.1), and thus would not be expected to affect digestion parameters or eMCP.

## 2.4 THE EFFICIENCY OF MICROBIAL PROTEIN PRODUCTION IN CATTLE GRAZING TROPICAL PASTURES

From the previous discussion it appears that tropical grasses possess many of the attributes likely to be associated with low eMCP, including low levels of RDP and NSC, and low dilution rates. Such attributes are associated with the lower nutritive value of tropical grasses compared to temperate species, and reflect the fundamental anatomical differences, of both leaf and stem, between $C_3$ and $C_4$ species (Norton, 1982). As described by Kennedy (1995), when compared to temperate species, tropical grass leaf has more vascular bundles per unit of cross-sectional area, a more closely-packed cell structure, and suberised, thick-walled bundle sheath cells. The stems of tropical grasses also contain more vascular bundles than temperate grasses. The specialised anatomical structure found in $C_4$ species, in particular the increased vascularity, is associated with a high resistance to both mechanical and microbial degradation. This, in turn, leads to longer retention times in the rumen and reduced voluntary intake and digestibility (Norton, 1982).

The very rapid maturation of tropical grasses after the onset of the growing season compounds the effects of already low digestibility, RDP and NSC levels, and the low rumen dilution rates. The effects of ageing on the content and nature of the NSC, SC, protein and minerals in the tropical plant have been reviewed by Norton (1982) and McLennan *et al.* (1988). These can be summarised as:

1) a marked increase in the cell wall constituents in the leaf and stem at the expense of the more digestible cell contents;

2) increased lignification and a greater degree of crystallinity and polymerisation of the structural carbohydrates;

3) a decline in the plant leaf : stem ratio thus increasing the SC content of the plant as a whole;

4) a decline in whole plant protein content after flowering;

5) a probable increase in the association of plant protein with fibre as the plant matures, thus decreasing N availability for the rumen microbes; and

6) a decrease in mineral content in plant tissue.

An examination of the literature reveals that there has been limited attempt to determine eMCP in tropical grasses over a range of maturity phases and, in particular, few studies have examined ruminants grazing fresh pasture. In addition, there are no reliable values for pure stands of tropical legume.

Early work conducted by Kennedy (1982) found eMCP in Brahman crossbred and Hereford steers consuming tropical pasture hay (a mixture of predominantly siratro (*Macroptilium purpureum*) and green panic (*Panicum maximum*); 70 g CP/kg DM) to be low at 83 g MCP/kg OM apparently digested in the stomach (recalculated as 72 g MCP/kg DOMI). This value was lower than that recorded with the same steers consuming lucerne hay (160 g MCP/kg OM apparently digested in the stomach; recalculated as 125 g MCP/kg DOMI). However, data re-calculated by SCA (1990) from experiments of Hart & Leibholz (1983) and unpublished studies, indicated eMCP to be just within the extant feeding standards' range for cattle consuming cut and dried paspalum grass (*Paspalum dilatatum*; early, mid and late; average 105 g CP/kg DM) at 131 g MCP/kg DOMI, but lower for kikuyu grass (early and mid; average 154 g CP/kg DM) at 100 g MCP/kg DOMI.

McMeniman *et al.* (1986b) and unpublished studies (cited in SCA (1990)) provided estimates of eMCP in sheep grazing Mitchell grass (*Astrebla* spp.) and mulga/grassland association pastures in south-west Queensland over a 4-year period. The eMCP on Mitchell grass and mulga/grassland pastures after winter rain fell within or above the extant feeding standards' range at 173 and 184 g MCP/kg DOMI, respectively. During the dry season, the eMCP was significantly depressed on the Mitchell grass pasture (87 g MCP/kg DOMI) but within the extant feeding standards' range on the mulga/grassland pasture (133 g MCP/kg DOMI). However, while it could be assumed that the pastures in this sub-tropical, semi-arid region would consist

largely of $C_4$ species, McMeniman *et al.* (1986a) showed that up to 550 g/kg of the diet DM on the Mitchell grass pastures after winter rain, and 880 g/kg of the diet DM on mulga/grassland association pastures after winter rain, consisted of $C_3$ pasture species. The $C_3$ pasture species included forbs, and in the mulga/grassland pasture, up to 430 g/kg diet DM of mulga oats (*Monochather paradoxus*) and mulga Mitchell (*Thyridolepis mitchelliana*) grasses. In addition, up to 60 g/kg of the diet DM consisted of mulga on the mulga/grassland pasture after winter rain. The diet on dry season mulga/grassland pasture also consisted of largely $C_3$ pasture species and browse (240 g/kg mulga oats and mulga Mitchell grasses, 70 g/kg forbs, 350 g/kg mulga and 150 g/kg DM other browse species). The only diet consisting of a majority of $C_4$ species was that consumed by sheep on dry season Mitchell grass pasture (*ca.* 1000 g $C_4$ grasses/kg DM), which did indeed fall below the extant feeding standards' range for eMCP.

More recently, Prior *et al.* (1998) compared eMCP in Brahman crossbred steers for a number of tropical grass hays, with annual ryegrass hay. While eMCP with ryegrass was 185 g MCP/kg DOMI, the values were much lower for the tropical species, viz. 117, 85 and 95 g MCP/kg DOMI, for young buffel grass, mature buffel grass, and black speargrass (*Heteropogon contortus*), respectively. It is of note that the CP concentrations in the tropical grasses (64, 28 and 53 g CP/kg DM, respectively) were much lower than in the ryegrass hay (127 g CP/kg DM). King *et al.* (1999) also compared eMCP in Brahman crossbred cattle when consuming either annual ryegrass or pangola hay. Similarly, they found eMCP from pangola grass hay (50 g CP/kg DM) was much lower than that from ryegrass hay (194 g/kg DM) viz. (87 vs. 191 g MCP/kg DOMI).

Low eMCP values in the same order as those measured by Prior *et al.* (1998) and King *et al.* (1999) have been reported from the same laboratory when *B. indicus* crossbred steers consumed rhodes grass hay (62 g MCP/kg DOMI; Bolam (1998) and 58 g MCP/kg DOMI; Marsetyo *et al.* (2002)) or pangola grass freshly harvested (72 g MCP/kg DOMI; Mullik (1999)) or as hay (44 and 77 g MCP/kg DOMI; Mullik (1999)).

Other groups have also measured low eMCP in *B. indicus* and *B. indicus* crossbred cattle when consuming tropical grass forages. Shem *et al.* (1999) measured eMCP in cattle given a range of tropical grasses grown in Tanzania including freshly harvested Guatemala grass (*Tripsacum fasciculum*), setaria grass, napier grass (*Pennisetum purpureum*) and Rhodes grass, the latter both freshly harvested and as hay. After recalculation, the eMCP values were found to be well below the extant feeding standards' range at 72, 31, 53, 75, and 50 g MCP/kg DOMI, respectively. Nsahlai *et al.* (2000) also found low eMCP on freshly harvested napier grass (recalculated as 90 g MCP/kg DOMI) and dried napier grass (recalculated as 114 g MCP/kg DOMI).

When comparing results across the various experiments, an important consideration is the different methodologies used to measure MCP flow. While the experiments of Kennedy (1982), Hart & Leibholz (1983) and McMeniman *et al.* (1986b) estimated MCP flows using traditional methods of marker flow measurements taken at the abomasum, all of the more recent experiments cited above estimated MCP flows using the method based on excretion of purine derivatives (PD) in the urine (see later, section 2.5.1). There is also variation in the equation used to estimate MCP flow from urinary PD excretion. In particular, all experiments other than that of Nsahlai *et al.* (2000) utilised the equation of Chen & Gomes (1995) to calculate MCP flow. This equation takes into account an endogenous value for PD excretion, as determined for *B. taurus* cattle. However, Nsahlai *et al.* (2000) used a modification of the equation of Chen & Gomes (1995), incorporating measured values for endogenous PD excretion for Zebu and Zebu x Friesian cattle (Osuji *et al.* 1996). The smaller endogenous PD excretions measured by Osuji *et al.* (1996) for *B. indicus* and *B. indicus* crossbred cattle would result in larger values for estimated MCP flow when compared to the equation of Chen & Gomes (1995).

Nevertheless, despite the different methodologies used to estimate MCP flow in ruminants consuming tropical forages, with one exception, all values for eMCP have fallen below the extant feeding standards' range of 130 - 170 g MCP/kg DOMI. Thus it appears that there is potential to increase the supply of total protein to ruminants grazing tropical pastures through increasing eMCP up to the extant feeding standards' range. However, there is a dearth of knowledge concerning the variation in eMCP across tropical grass and legume species and how these vary with seasonal conditions.

In addition, there is a major deficiency in information relating to estimation of eMCP for ruminants grazing pasture rather than consuming hays or cut forages, and this warrants further investigation. If the low values reported above for eMCP from tropical forages can be rigorously confirmed under grazing conditions, then it would be important to investigate further the potential to increase eMCP through supplementation or management strategies.

## 2.5 TECHNIQUES TO ESTIMATE MICROBIAL PROTEIN PRODUCTION IN CATTLE UNDER GRAZING CONDITIONS

The cattle industry of northern Australia is based on rangeland and grassland production systems, from the breeding phase through to the feed-on, and to some extent, the finishing phase. These tropical pasture systems are often very heterogenous and consist of a number of plant species, which vary with land system and soil type. The quality and quantity of the available pasture varies with season and rainfall. Under field conditions, ruminants selectively graze species and plant parts and thus may select a diet considerably higher in quality than that on offer (Stobbs, 1975; Corbett, 1976).

The nutritive value of feedstuffs consumed by ruminant animals is commonly determined in metabolism studies, generally involving the feeding of chaffed hays and the measurement of total feed intake, as well as faecal and urinary output. However, the determination of digestibility and MCP synthesis under such conditions will not give an accurate estimation of the quality of the diet selected by cattle when grazing topical forages. Hays cut from mature tropical pastures will be low in N and digestibility and thus lower in quality than that available earlier in the wet season, when pasture yields are low. Furthermore, the feeding of chaffed hays, often at 90 % of *ad libitum* intake, minimises potential for selection of plant species and parts.

Techniques exist which allow estimation of faecal output, intake, digestibility and diet composition under field conditions. These involve the use of faecal markers such as Yb, chromic oxide and n-alkanes; OF animals to obtain representative samples of the diet selected; and new techniques still under development, including use of n-alkane profiles and near-infrared spectroscopy (NIRS) to predict aspects of diet quality. However, no satisfactory methods have been developed which allow the routine

estimation of MCP synthesis under grazing conditions. The various options available to allow estimation of MCP synthesis in grazing cattle are reviewed below.

### 2.5.1 Measurement of urinary purine derivatives

Microbial protein flow to the small intestines has traditionally been determined with the use of microbial markers, such as nucleic acids, diaminopimelic acid (DAPA), aminoethylphosphoric acid, $^{32}P$, $^{35}S$ and $^{15}N$. These methods require the use of post-ruminally cannulated animals, methods of achieving prolonged, steady intraruminal infusion of isotopes, and procedures for measuring digesta flow and for separation of rumen microbes. In addition to the tedious and complicated nature of these methods, the surgical invasion involved can cause some changes in the physiology of digestion, can reduce feed intake, and limits the number of experimental animals that can be used (MacRae & Wilson, 1977; Wenham, 1979).

The measurement of urinary excretion of PD is an alternative, non-invasive, technique for estimating MCP flow to the small intestine in ruminants. This method is based on the principle that the nucleic acids leaving the rumen are essentially of microbial origin (Smith & McAllan, 1970; McAllan & Smith, 1973a,b). These microbial nucleic acids undergo extensive degradation in the small intestine and are hydrolysed into purine nucleosides and free bases, which are absorbed from the intestinal lumen, metabolised and excreted in the urine as PD (Chen & Gomes, 1995).

In sheep and goats, a small proportion of the absorbed purines can be converted into tissue nucleic acid via a salvage pathway (Smith *et al.* 1974; Razzaque *et al.* 1981; Chen *et al.* 1990b; Belenguer *et al.* 2002). The remaining purines are metabolised to their metabolic end-products, hypoxanthine, xanthine, uric acid and allantoin, which are excreted in the urine. In cattle, there is negligible salvage of purines due to very high activities of xanthine oxidase in most tissues including the blood (Chen *et al.* 1990c). Xanthine oxidase diverts practically all of the absorbed purines away from the salvage pathway by degrading hypoxanthine to form xanthine and then uric acid, which can subsequently be oxidised in the presence of uricase to form allantoin. Both uric acid and allantoin cannot be incorporated into tissue nucleic acids. Thus in cattle the PD measured in the urine are allantoin and uric acid, with smaller to negligible amounts of xanthine and hypoxanthine.

In addition to purines originating from microbial flow from the rumen, there is an endogenous fraction in the urine coming from tissue nucleic acid turnover, which must be accounted for. The higher concentrations of endogenous PD excretion in cattle compared to sheep and goats (Chen *et al.* 1990c; Belenguer *et al.* 2002) have been explained by the higher xanthine oxidase concentrations in cattle tissue, which would prevent salvage of tissue nucleic acids to any major extent (Chen *et al.* 1990c).

A large number of experiments comparing the urinary PD excretion method with measurements of microbial marker flow in post-ruminally cannulated animals have confirmed that the daily urinary excretion of total PD is related to the duodenal flow of purine bases (PB) estimated using flow markers (Djouvinov & Todorov, 1994; Perez *et al.* 1996, 1997; Martin-Orue *et al.* 2000). However, the dual phase marker system, used as the reference method in the measurement of duodenal flows of PB, is itself not free of error (Faichney, 1975). The assumption that the flow of markers and microbes exist in two simple phases can lead to error (France & Siddons, 1986) and the location of intestinal cannulas may influence the accuracy of nutrient flow estimates (Palmquist *et al.* 1993).

Response models between duodenal flow of PB and urinary PD excretion have been developed for sheep (Chen *et al.* 1990b; Balcells *et al.* 1991), cattle (Verbic *et al.* 1990) and goats (Belenguer *et al.* 2002). Chen & Gomes (1995) detail the widely used equation, developed by Verbic *et al.* (1990) for calculating MN supply from urinary PD excretion in cattle. Absorption of microbial purines (X; mmol/d) is first calculated using measured values for excretion of PD in the urine (Y; mmol/d) and the metabolic body weight (kg) of the animal:

$$X = (Y - 0.385\ W^{0.75}) / 0.85.$$

The value of 0.85 represents the recovery of absorbed purines as PD in the urine. The component, $0.385\ W^{0.75}$, represents the net endogenous contribution of PD to total excretion. MN flow can then be determined according to the equation:

$$MN\ (g\ N/d) = (70\ X) / (0.116 \times 0.83 \times 1000)$$
$$= 0.727\ X.$$

The following assumptions are made:

1) the digestibility of microbial purines is 0.83,
2) the N content of purines is 70 mg N/mmol, and
3) the ratio of purine N : total N in mixed rumen microbes is taken as 11.6 : 100.

However, a number of experiments have found some variation in the parameters assumed as constants in the equation of Chen & Gomes (1995).

***Recovery of absorbed purine derivatives in the urine***

Although urinary excretion is the primary route for disposal of PD from the plasma, it has been suggested that a portion of the PD may be disposed of by non-renal routes such as the irreversible loss of PD via salivary flow into the rumen or as a direct transfer across the gut wall (Chen *et al.* 1990a; Prasitkusol *et al.* 2002). However, Kahn & Nolan (2000) and Kahn *et al.* (2001) demonstrated that in sheep the loss of allantoin via these mechanisms was negligible. In lactating animals, a proportion of the total PD has been shown to be secreted with the milk (Kirchgessner & Windisch, 1989).

In the equation used to calculate PD absorption (Chen & Gomes, 1995), a constant value for recovery of absorbed PD in the urine of cattle is assumed (0.85, as determined by Verbic *et al.* 1990). However, Verbic *et al.* (1990) cautioned that the recovery rate obtained using intragastrically nourished animals may not be widely applicable to animals receiving normal diets, where the loss of PD into the digestive tract may be greater.

However, later trials confirmed that recovery rates of PD in the urine for cattle fed normally are similar to those found by Verbic *et al.* (1990), i.e. 0.86 (Vagnoni *et al.* 1997) and 0.84 (Orellana Boero *et al.* 2001). However, Orellana Boero *et al.* (2001) found that urinary PD recovery increased with level of infusion level of yeast PB,

from 0.67 on a basal diet to 0.82 at the highest level of PB infusion. They suggested that the differences in digestibility between yeast-RNA and duodenal PB might explain differences in recovery estimations. Thus Orellana Boero *et al.* (2001) concluded that this aspect deserved further attention as all published models are based on the response to infusion of microbial extracts (*Torula* yeast or *Pruteen*), with the assumption that duodenal PB and yeast-RNA have the same digestibility.

In addition, there has been some discussion in the literature as to whether the recovery of absorbed PD in the urine differs for different species of cattle. However, recent research has found that the recovery of supplied exogenous PB in the urine was 0.83 for 5/8 *B. indicus* content cattle (Ojeda & Parra, 1999) and 0.85 for Kedah-Kelantan (*B. indicus*) cattle (Pimpa *et al.* 2001) and thus similar to values reported above for *B. taurus* cattle.

***Ratio of purine-nitrogen : total nitrogen in mixed rumen microbes***

Experiments with both sheep (Perez *et al.* 1997, 1998) and cattle (Martin-Orue *et al.* 2000) and also from semi-continuous fermenters (Carro & Miller, 2002) have shown liquid-associated bacteria to have a higher PB : N ratio than solid-attached bacteria. Thus the estimation of MCP production may be biased if a single microbial fraction is used as a reference. However, the contribution of liquid-associated and solid-attached bacteria to duodenal flow is not well documented and is influenced by diet and rumen environment (Perez *et al.* 1998). The difficulty in obtaining a microbial sample representative of the total biomass flowing to the intestines and in obtaining a consistent and reliable sample of particle-bound bacteria are major obstacles to more clearly defining the of ratio purine-N : total N in the mixed rumen microbes.

***Endogenous purine derivative excretion in the urine***

As stated earlier, a fraction of the urinary PD originates from the endogenous nucleic acid turnover in the animal tissues. The equation of Chen & Gomes (1995) uses a constant value of 385 μmol/kg $W^{0.75}$ for endogenous excretion of PD in cattle. This value was based on the mean of all measurements made in the laboratory of Verbic *et al.* (1990; 493 μmol/kg $W^{0.75}$) with adjustment downwards of 0.22 for the proportion of salvage of exogenous purines. This latter value was also estimated by Verbic *et al.* (1990) from the abomasal infusion of microbial extract (*Pruteen*), and interpreted as

utilisation of exogenous purines in the intestinal mucosa. The value for endogenous PD excretion was determined in experiments using *B. taurus* cattle (Verbic *et al.* 1990). While other authors using *B. taurus* cattle have generally measured values for endogenous PD excretion in the same order as that found by Verbic *et al.* (1990), those using pure-bred *B. indicus* and *B. indicus* crossbred cattle have recorded much lower values (see Table 2.1). The literature documented in Table 2.1 indicates that while age, sex or physiological state (preruminant calves, steers and lactating cows) appear to have little effect on endogenous PD excretion in cattle (Chen *et al.* 1990c), the cattle species appears to be an important variable with recent research showing that, in general, *B. indicus* content cattle have values for endogenous PD excretion that are around half those reported for *B. taurus* cattle.

However, the method used to estimate endogenous PD excretion may also influence the values obtained. A number of experiments measuring endogenous PD excretion in *B. taurus* cattle have used the technique of total nourishment of animals by intragastric infusion, where microbial fermentation in the rumen is eliminated while still maintaining a normal nutritional status (Fujihara *et al.* 1987; Chen *et al.* 1990c; Verbic *et al.* 1990). In this technique the animals are totally nourished by volatile fatty acids and casein infused into the rumen and abomasum, respectively. Other researchers have used techniques of rumen emptying (Giesecke *et al.* 1993) or extrapolation of purine infusion/excretion regression relationships to zero purine input (Beckers & Thewis, 1994). While values obtained by each of the above methods are within the same general order for *B. taurus* cattle (see Table 2.1), Orellana Boero *et al.* (2001) used the technique of isotopic labelling of exogenous purines and found the endogenous PD excretion in Friesian cows to be around half that found by others. These workers suggested that their method may be more representative of the situation occurring in ruminants fed normal diets as there is no experimental evidence to suggest that endogenous losses would be equivalent to the basal excretion rate when there is no exogenous input of purine bases.

**Table 2.1. Comparison of published estimates of endogenous purine derivative (PD) excretion in the urine of cattle**

| Species, breed and class of cattle | *n* | Endogenous excretion of total PD (μmol/kg $W^{0.75}$) | Details of experiment | Reference |
|---|---|---|---|---|
| ***B. taurus* cattle** | | | | |
| Friesian steers | 2 | 456 | Totally nourished by intragastric infusion | Fujihara *et al.* (1987) |
| Friesian x Hereford and Friesian steers (6), calves (3) and cow | 10 | 514 | Totally nourished by intragastric infusion | Chen *et al.* (1990c) |
| Friesian steers | 2 | 428 | Totally nourished by intragastric infusion | Verbic *et al.* (1990) |
| | | 493 | Mean of all values found in laboratory for cattle | Verbic *et al.* (1990) |
| | | 385 | Mean value with adjustment for 0.22 salvage proportion at normal rates of feeding | Verbic *et al.* (1990) |
| Angeln steers | 5 | 560 | Rumen emptying | Giesecke *et al.* (1993) |
| Belgian Blue bulls | 2 | 531 | *Torula* yeast infused and linear regression extrapolated to zero purine input | Beckers & Thewis (1994) |
| Friesian cows | 2 | 236 | Isotopic labelling of exogenous purine bases | Orellana Boero *et al.* (2001) |
| ***B. indicus* cattle** | | | | |
| Boran bulls | 3 | 172 | Fasting (20 d) | Osuji *et al.* (1996) |
| Kedah-Kelantan males | 6 | 275 | Fasting (6 d) | Liang *et al.* (1999) |
| Kedah-Kelantan males | 3 | 147 | Purine bases infused and linear regression extrapolated to zero purine input | Pimpa *et al.* (2001) |
| ***B. indicus* x *B. taurus* cattle** | | | | |
| Boran x *B. taurus* steers | 3 | 108 | Fasting (20 d) | Osuji *et al.* (1996) |
| 3/8 *B. indicus* males | 6 | 268 | Fasting (7 d) | Ojeda & Parra (1999) |
| 1/2 *B. indicus* males | 6 | 294 | Fasting (7 d) | Ojeda & Parra (1999) |
| 5/8 *B. indicus* males | 6 | 269 | Fasting (7 d) | Ojeda & Parra (1999) |

The majority of studies involving *B. indicus* content cattle have been based on the fasting technique. This method involves the prolonged starvation of animals and the associated measurement of urinary PD excretion during the fasting period. However, it is possible that the metabolic activities of the animals, or the rate of degradation of tissue nucleic acids, and thus the excretion of PD in the urine, could be altered by nutritional restriction. Furthermore, there is no evidence that duodenal flow of PB is discontinued during fasting. Nevertheless, results obtained by Pimpa *et al.* (2001), by regressing the linear relationship of purine infusion/excretion to zero purine input, have confirmed the low values found for *B. indicus* content cattle using the fasting method.

As no experiments have compared *B. indicus* and *B. taurus* species using the same methodology, it cannot be determined as to whether the differences in the endogenous PD excretion are due to species of cattle, or to the methodology used.

### 2.5.2 Estimation of urine output in grazing animals

The use of the urinary PD excretion technique to estimate MCP production in ruminant animals is believed to have advantages over the digesta flow methods due to its simplicity and utilisation of non-invasive sampling techniques. However, while easily implemented in experiments using animals housed in metabolism crates, it is much more difficult to use the urinary PD method under field conditions. This is because of the requirement for complete collection of urine over a period of greater than 5 d to reduce error associated with variations in urine volume (Chen & Gomes, 1995). Non-surgical devices for collection of urine from free-grazing male (Betteridge & Andrewes, 1986) and female (Stillwell *et al.* 1983) cattle have been developed. However, these are cumbersome to use and may cause disturbance to the normal behaviour of the animals. Thus there is requirement for an alternative method of either estimating total urine output, or negating the need for it, in order to apply the PD excretion method in the field.

If a technique using either spot (single sample in time) urine or blood samples could be developed, the PD method could be extended to provide a more practical index of MCP supply under field conditions. The use of the PD concentration in a single spot plasma sample, as an index of MN supply in sheep and cattle, was investigated by a

number of groups including Chen *et al.* (1991, 1992b, 1995a) and Dapoza *et al.* (1999). While little diurnal variation in plasma PD concentration has been observed, the variability in glomerular filtration rate (GFR) with diet type and between animals resulted in poor correlation of plasma PD concentration with daily output of PD in the urine.

For a method based on spot urine samples to be possible, the concentration of PD in the spot sample needs to be measured in conjunction with an internal or external marker so that the effects of urine volume can be accounted for. The use of creatinine as an internal marker has been investigated. Creatinine is a natural metabolic waste-product formed from the break-down of creatine phosphate in the muscles. This method is based on the assumption that creatinine is excreted in the urine at a constant proportion of metabolic liveweight (Chen *et al.* 1995a,b; Faichney *et al.* 1995). Thus, a daily excretion of creatinine per kg $W^{0.75}$ is assumed and is multiplied by the individual animal $W^{0.75}$ and the ratio of PD:creatinine in spot urine samples, to give an estimate of daily PD excretion (Chen *et al.* 1995b).

Chen *et al.* (1992b) and Chen *et al.* (1995a), in studies with cattle and sheep, respectively, found the ratio of PD : creatinine in urine spot samples to show little diurnal variation and to be highly correlated with daily output of urinary PD. However, Puchala & Kulasek (1992), Faichney *et al.* (1995), Shingfield & Offer (1998) and King *et al.* (1999) found that the variation in creatinine excretion was too great for sufficient accuracy in prediction of daily PD excretion. Faichney *et al.* (1995) found creatinine excretion to vary considerably between individual animals and with diet type, and Manuel *et al.* (1996) and Bolam (1998) showed creatinine excretion to be linearly related to DOMI, N balance and growth rate of animals. Furthermore, Dapoza *et al.* (1999) found creatinine excretion in urine and milk to be affected by physiological state, being higher in pregnant than in lactating ewes.

The use of external markers to estimate urine output has the potential to give more accurate results than the use of creatinine, but has largely been untested in ruminant animals. In order to be successful as a urinary output marker, a substance must be non-toxic, not cause any physiological affects, not be metabolised by the body, be small enough to be freely filtered through the glomerular membrane, not be bound by

plasma protein, not be reabsorbed, secreted, synthesised or metabolised by the kidney, be quantitatively excreted in the urine and be easily analysed in the plasma and urine (Hladky & Rink, 1986; Lote, 1994).

Mayes *et al.* (1995) suggested that orally-administered markers may have potential as urinary output markers if they are completely absorbed, quantitatively excreted in the urine, and are absent from the diet. Substances which may be suitable are the phenolic compounds, orcinol or salicylic acid, which are found in particular plant types (Mayes *et al.* 1995). However, to the author's knowledge, there have been no investigations into the suitability of such substances as urine output markers.

Intravenously administered markers have been more commonly used in human and animal studies. The basic procedure is to infuse the marker intravenously, at a steady rate, until the plasma concentration reaches equilibrium at which point urinary excretion rate matches the infusion rate. The excretion rate of the marker can then be assumed as equal to the known infusion rate and PD excretion can be determined by multiplying the daily marker infusion rate by the PD : marker ratio in the urine. Procedures to remove the necessity for continuous infusion involve injecting a single dose of the marker and measuring its disappearance rate from the plasma, or from the plasma and urine. However, this method relies on the assumption that the fractional disappearance rate (FDR) of the marker is related to the clearance rate of the PD from the plasma.

Inulin, a polyfructose, is an external marker which has been used in human medicine to measure GFR and kidney function, as it meets the basic requirements for an external marker (Gaspari *et al.* 1997). Although used extensively in humans, limited information is available on the use of this marker in ruminant animals. Furthermore, inulin is expensive and the analytical procedure to determine its concentration is laborious (Gaspari *et al.* 1997; Palnes Hansen *et al.* 1997).

***The potential of intravenously administered CrEDTA as a urine output marker***

Chromium complexed with ethylenediamine tetraacetic acid (CrEDTA) has also been used to investigate kidney function in human medicine and in monogastric research (Palnes Hansen *et al.* 1997). In addition, a number of researchers have investigated

various aspects of CrEDTA use in ruminant animals. In particular, Downes and McDonald (1964) injected a single dose of $^{51}$CrEDTA into the jugular vein of sheep and detected no $^{51}$Chromium (Cr) in the rumen liquor or faeces. Over 90 % of the $^{51}$Cr was recovered in the urine, with 86.5 % of the dose appearing in the urine within 24 h after injection.

Stacy & Thorburn (1966) compared renal clearances in sheep of inulin and $^{51}$CrEDTA administered by intravenous infusion and found the mean of the clearance ratio of $^{51}$Cr to inulin to be 0.95 ± (SD) 0.03. It was suggested that part of the difference between the two markers in recovery might have been due to binding of the metal chelate by plasma proteins, as 1.5 - 2 % of the $^{51}$CrEDTA in plasma could not be removed by dialysis. However, the authors stated that unlike most EDTA chelates used in biological work, the Cr(III) complex of EDTA is stable and inert, and thus unlikely to be involved in exchange reactions with other metal ions *in vivo*. An example of such an exchange reaction is the formation of CaEDTA, which has a deleterious effect on the renal proximal tubules (Stacy & Thorburn, 1966). The authors provided evidence confirming the inertness of CrEDTA, referring to prior experiments conducted with rats in their laboratory, where prolonged infusion with CrEDTA failed to affect kidney function or to cause any histological abnormalities. Further experiments with sheep (Stacy & Thorburn, 1966) demonstrated that the renal clearance of CrEDTA was independent of its concentration in plasma, as clearance was not affected when the amount of CrEDTA was markedly increased from 0.004 to 1 mg Cr/100 ml plasma.

Following the evidence provided by these initial experiments, $^{51}$CrEDTA has been used to estimate GFR in both sheep (Kennedy & Milligan, 1978b) and cattle (Norton *et al.* 1979). More recently, King *et al.* (1999) investigated the suitability of using the FDR of CrEDTA from plasma to reflect differences in the clearance rate of allantoin and thus allow estimation of MCP production in cattle. The ratio of estimated allantoin clearance to actual measured clearance was not constant, suggesting that the FDR of CrEDTA from plasma would not offer a simple field based method for estimating urinary allantoin excretion.

***The potential of intravenously administered lithium sulphate as a urine output marker***

Lithium (Li) has been used in human medicine to treat manic depression, as a compliance indicator in nutritional research and to determine proximal and distal tubular reabsorption of sodium and water. In humans, *ca.* 90 - 97 % of ingested Li is recovered in the urine (Trautner *et al.* 1955; Heijnen *et al.* 1996) with 30 - 60 % of the Li excreted within 6 - 8 h after ingestion of a single dose (Trautner *et al.* 1955). A review by Suharyono (1992) has indicated that in monogastric animals ingested Li is rapidly absorbed from the gut and excreted mainly in the urine, with the remainder either excreted in faeces or detected in the sweat and saliva.

Various groups have investigated the suitability of Li as a marker for estimating rumen fluid volume in ruminants. In studies with sheep, Harrison *et al.* (1963), Ulyatt (1964) and Walker & Hawley (1965) found that Li administered as lithium sulphate was unsuitable as a rumen fluid marker due to its rapid absorption from the rumen and subsequent recycling back to the rumen in saliva. The urine was a major route of excretion. After ingestion, Li has been detected in milk (Suharyono, 1992), bone (Birch & Hullin, 1972), various body organs (kidney, liver and cerebrum), wool and hair (Mocsenyi *et al.* 1987). However, despite these apparent limitations in the use of Li as a rumen fluid marker in ruminants, Cole & Hutcheson (1985) and Cole (1991) used lithium sulphate to estimate rumen fluid volume in beef steers and lambs, respectively.

Suharyono (1992) investigated the use of lithium chloride (LiCl) as a marker for estimating dietary supplement intake in sheep. The recovery of Li from the body 72 h after a single intraruminal or intravenous dose was 70 %, with more Li being excreted via the urine than in faeces (54 and 15 %, respectively, for intraruminal injection; and 52 and 17 %, respectively, for intravenous injection). After both intraruminal and intravenous administration, Li was detectable in the blood plasma, rumen fluid, urine and faeces of sheep. Suharyono (1992) concluded that intraruminally administered Li is absorbed into the blood stream by direct passage across the rumen wall and the lower gut. Li present in the plasma can be re-circulated to the rumen in saliva, returned to the gut post-ruminally, deposited elsewhere in the body or excreted in the urine and faeces.

However, contrary to the results cited above, recent research by Schonewille & Beynen (1999) found the average recovery of Li in the urine of goats over 9 d, while feeding LiCl in a pelleted concentrate, was 99 %. They thus concluded that LiCl had potential as a urine output marker in goats.

After review of the available literature, it is evident that no clear conclusion can be drawn as to the suitability of Li as a urine output marker in ruminant animals. In addition to contradictory experimental results, the issue is further complicated by evidence that the nature of the Li salt could affect Li kinetics in the animal, with Morrison *et al.* (1971) finding LiCl and lithium carbonate to differ in absorption characteristics when administered to rats.

### 2.5.3 Conclusions

The use of the urinary PD excretion technique in conjunction with an external marker, such as Cr or Li, appears to have potential for estimating urine output under field conditions. However, further validation of the equations used to estimate MCP synthesis is required for *B. indicus* cattle, in particular, determination of the most appropriate endogenous PD excretion component for this species. CrEDTA and lithium sulphate, administered intravenously, appear promising as urine output markers but have not been previously used for this purpose.

## 2.6 CONCLUSIONS

As reviewed in Chapter 1, the higher priced markets for Australian beef cattle are increasingly demanding younger, heavier carcasses. However, the necessary growth rates required to meet these market specifications are not widely or regularly achieved off pasture-based systems in the tropics. Responses to increased tissue protein supply have been achieved with beef cattle grazing wet season pastures in northern Australia. Low eMCP has been recorded on tropical forages, indicating an opportunity to increase the protein supply to animal tissues through supplementation strategies or other nutritional intervention aimed at increasing this avenue of protein supply. A number of factors have been found to influence eMCP including substrate supply, rumen dilution rate, intraruminal recycling and rumen pH, and these factors could be targeted as part of a strategy to increase eMCP.

However, there is a lack of scientific information documenting the variation in eMCP across a wide range of tropical grass and legume species, and over a range of seasonal conditions, in particular, for grazing animals. Thus further investigation is required in order to confirm the low eMCP values that have been measured in ruminants consuming chaffed tropical pasture hays, for grazing animals. Investigation of associated pasture parameters, and animal digestion and metabolic factors, should enhance the understanding of the variables influencing eMCP on such forages and should reveal opportunities for its future manipulation or for more strategic intervention with supplements. The measurement of urinary PD in spot samples, in conjunction with intravenously infused Cr or Li as a urine output marker, has potential as a technique to allow estimation of MCP synthesis under grazing conditions and thus provide more accurate values for eMCP for beef production systems relevant to northern Australia.

# Chapter 3

# General materials and methods

## 3.1 INTRODUCTION

Experimental and analytical procedures that have been used in a number of experiments are described in this chapter. Those procedures pertaining to a single experiment only are described in the relevant chapter as are individual variations on this general methodology.

## 3.2 ETHICAL CLEARANCE

Animal ethics clearance was obtained before conducting all experiments. Applications were submitted to the University of Queensland Animal Ethics Committee for all experiments conducted at the University of Queensland's Mt Cotton Research Farm. Clearance was obtained from the Department of Primary Industries Animal Ethics Committee (Bribie LAEC) for all experiments conducted at Brian Pastures Research Station, Gayndah.

## 3.3 EXPERIMENTAL SITES AND ANIMALS

Experiments 4.1, 5.1 and 7.1 - 7.3 were conducted in the animal house facilities at the University of Queensland's Mt Cotton Research Farm in south-east Queensland (latitude $27^0$31'S, longitude $153^0$14'E, elevation 24 - 53 m). Experiments 5.2 - 5.4 and 6.1 - 6.9 were conducted at Brian Pastures Research Station, 18 km ESE of Gayndah in south-east Queensland (latitude $25^0$39'S, longitude $151^0$44'E, elevation 131 m).

Eight rumen fistulated (RF) steers were used in a number of the experiments described in Chapters 5, 6 and 7. These steers were high-grade Brahman (> 75 % *B. indicus* content). The steers underwent surgery to insert permanent rumen cannula (Bar Diamond, 10 cm i.d.) at *ca.* 12 months of age and 280 kg. Further details of animal particulars and of other experimental animals used in the various experiments are described in the relevant chapters.

## 3.4 EXPERIMENTAL PROCEDURES

### 3.4.1 Jugular infusion of CrEDTA and lithium sulphate

***Preparation of CrEDTA solution***

CrEDTA solution was made by dissolving AnalaR chromium trichloride hexahydrate ($CrCl_3.6H_20$) and ethylenediamine tetraacetic acid (EDTA) in distilled water and mixing together. The solution was boiled gently for *ca.* 1 h and then the excess EDTA neutralised with 1M calcium chloride and the pH brought to 7.4. The solution was then made to the correct volume and left to stand overnight prior to filtering through No. 4 Whatman filter paper (particle retention 20 μm). This concentrated CrEDTA solution (*ca.* 12 mg Cr/ml, 10 ml) was injected through single use Millex syringe filters (0.22 μm) into 500 ml bags containing isotonic saline solution (0.9 % sodium chloride).

***Preparation of lithium sulphate solution***

Lithium sulphate solution was made by dissolving $LiSO_4.H_20$ in the required volume of water to achieve *ca.* 14 mg Li/ml. Ten ml of this concentrated solution was injected through single use Millex syringe filters (0.22 μm) into 500 ml saline bags already containing 10 ml concentrated CrEDTA solution.

***Catheterisation***

Steers were prepared for catheterisation by shaving the entire neck area, on both sides, with a 1 mm blade. The catheterisation procedure was conducted while the animals were secured in a veterinary crush. Five minutes (min) prior to catheterisation, the steers were injected with ilium xylazil-20 (Rompun) (e.g. 0.6 ml for 450 kg steers). Nose pliers were then used to pull the neck around tightly and a 12 gauge, 2-inch hypodermic needle inserted into the jugular vein.

Single lumen polyethylene tubing (i.d. 0.80 mm, o.d. 1.20 mm) had been pre-cut to the desired length (*ca.* 2.5 m) with the jugular-entry end cut on a $45^0$ angle. This tubing was inserted through the hypodermic needle, 30 cm into the jugular vein. The hypodermic needle was then removed and a 21 gauge, "blunted" needle was pushed on to the end of the tubing. A 10 ml syringe was used to infuse *ca.* 5 ml heparin-

saline solution (200 IU/ml) into the jugular vein via the catheter tubing and then to draw blood back through the tubing to check for correct catheter function. The catheter tubing was refilled with heparin-saline solution and a sterile injection port inserted as a temporary stopper. The injection port was bound in place with Leukoplast tape.

A small square of Leukoplast tape was folded around the catheter tubing at the entry site to the jugular vein and fixed with Superglue to the neck of the animal. The entire neck area was then sprayed liberally with adhesive (Aussie Grip Spray Adhesive). Once the adhesive was tacky, Elastoplast tape was used to bandage the catheter tubing securely to the neck of the animal. The extra tubing (that which protruded from the bandage and including the stoppered end) was enclosed in a protective patch and also stuck securely to the neck of the animal with Elastoplast tape and adhesive spray. The steers were then returned to their paddock or pen (depending on the experiment) and allowed to recover from the effects of the Rompun for the remainder of the day.

***Connection of catheter tubing to the infusion pumps***

On the morning following catheterisation, each steer's catheter tubing was attached to a small battery-operated, peristaltic infusion pump (Plate 1). The pump was either held in frames above metabolism crates (Experiment 5.1 and 5.2; Plate 2) or in packs worn by the steers (Experiments 5.3 and 6.1 - 6.7; Plate 3). A 500 ml bag containing isotonic saline solution (0.9 % sodium chloride) was placed in the pump and connected to small silastic laboratory tubing (i.d. 2.64 mm, o.d. 4.88 mm) that was used as the pump tubing. The pump tubing was connected via a joiner to a three-way tap, which was also connected to the catheter tubing. Saline solution was infused into the jugular vein for the remainder of the day on which the catheter tubing was connected to the pumps.

***Infusion of CrEDTA and lithium sulphate***

On the morning following pump connection (second day of infusion), the saline bags were exchanged for 500 ml saline bags containing CrEDTA, and in some cases lithium sulphate (the concentrations are provided in the relevant chapters), at a recorded time. The marker solution was then infused continuously over 6 - 7 d

(depending on the experiment) at a rate of 16.5 – 20.5 ml/h. Infusion bags were replaced each morning during the infusion period at a recorded time.

### 3.4.2 Rumen dosing of Yb marker

***Manufacture of Yb Marker***

The concentration of Yb in the marker doses was varied for each experiment and is described in the relevant chapter. Yb marker was made by soaking 3 mm ground hay (Rhodes grass (*Chloris gayana*) for Experiments 6.1 - 6.7 and pangola grass (*Digitaria eriantha*) for Experiment 7.2) in $YbCl_3.6H_20$ solution for *ca.* 24 h in large trays lined with black plastic. Sufficient water was added to the hay to make a slurry which was mixed thoroughly a number of times during the soaking period. The hay was then squeezed out by hand and placed into individual aluminium containers. All liquid remaining in the large trays was passed through a 125 μm sieve so that particles greater than 125 μm could be removed and added to the retained hay. The soaked hay was then oven dried at 60 - $70^0$C for 48 h after which the hay was bulked and remixed. The marker doses were made by wrapping 10 g (Experiment 7.2) or 20 g (Experiments 6.1 - 6.7) of oven-dried, Yb-marked hay in paper towel and securing with tape.

***Yb marker dosing procedure***

The Yb-marked hay was dosed into the rumen of RF steers. The dosing procedure involved first cutting the tape and then pushing the packet into the posterior ventral sac of the rumen where the packet was broken apart with the fingers. When three packets were inserted per dosing time (Experiment 7.2), each packet was inserted into a different site in the rumen, one being the posterior ventral sac.

### 3.4.3 Rumen dosing with controlled release devices

Controlled release devices (CRD), including Alkane CRC (Captec, New Zealand) and a chromic oxide ($Cr_2O_3$) CRD (Captec, New Zealand) were placed in the rumen of the RF cattle in a number of experiments (alkane CRD in Experiments 6.1 - 6.7 and $Cr_2O_3$ CRD in Experiment 5.4). The concentrations of the marker components in the capsules are provided in the relevant chapters. One CRD per steer was inserted by pushing the capsule, wings first, into the posterior ventral sac of the rumen. A nylon

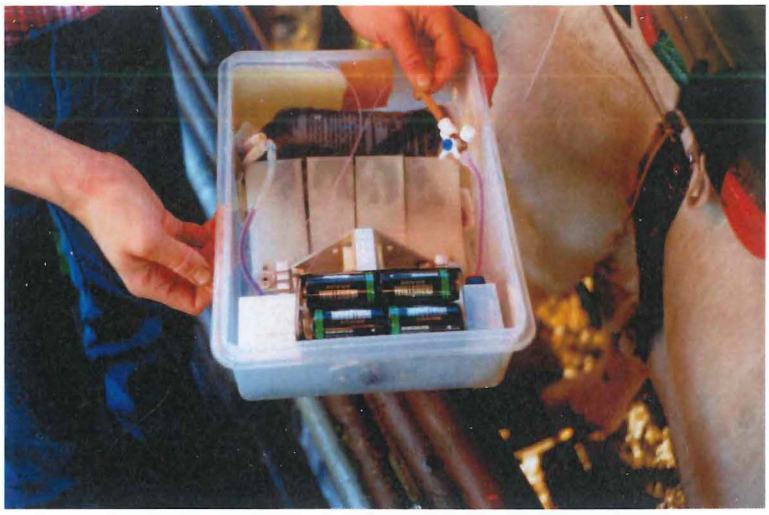

**Plate 1.** Battery-operated, peristaltic pump used to continuously infuse CrEDTA and lithium sulphate solutions intravenously.

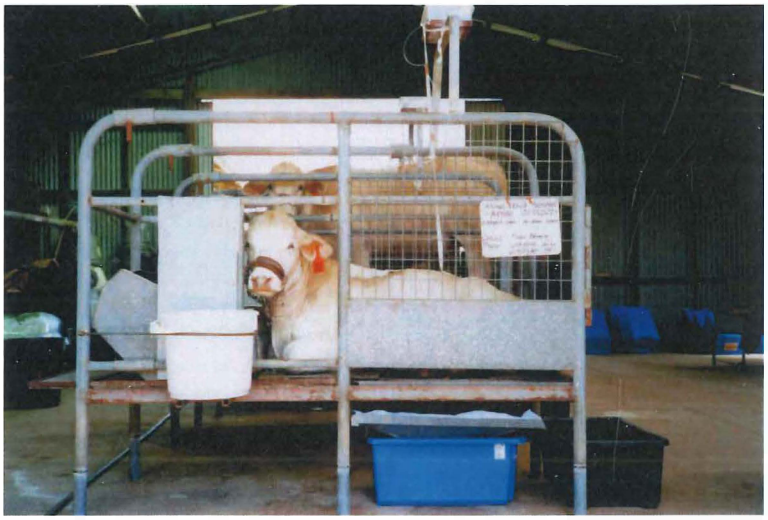

**Plate 2.** Continuous intravenous infusion of CrEDTA and lithium sulphate in metabolism crates.

**Plate 3.** Continuous intravenous infusion of CrEDTA and lithium sulphate under grazing conditions.

cord was tied at one end to the CRD and at the other end to a hook in the cannula bung to facilitate ease of CRD withdrawal. The linear delivery rate of each CRD was measured daily during the sampling period by withdrawing the CRD through the rumen cannula and measuring CRD core displacement with vernier callipers. On each occasion, core displacement was measured at four points of the circumference ($0^0$, $90^0$, $180^0$ and $270^0$) to find an average displacement value. Reinsertion involved pushing the CRD, wings first, back into the posterior ventral sac of the rumen. This procedure was conducted at the same time each day and was carried out as quickly as possible to minimise the time the CRD was out of the rumen. During withdrawal and reinsertion of the CRD, care was taken to minimise disturbance to the releasing end.

At completion of the sampling period, the CRD was removed from each steer and destructively subsampled so that one clean, undamaged segment of core was obtained. The linear height and weight of each core segment was found and the segments were then stored in the freezer for later analysis.

### 3.4.4 Rumen dosing of CrEDTA

A single dose of concentrated CrEDTA solution (*ca.* 12 mg Cr/ml) was injected through the rumen cannula into at least four different sites in the rumen of RF steers and washed in with *ca.* 200 ml of water. The volume of CrEDTA solution injected was varied for each experiment (Experiments 6.1 - 6.7 and 7.2), so that the dose rate would approximate 0.67 g Cr/100 kg average LW of the RF steers. Apparatus identical to that used for rumen fluid sampling (section 3.4.9) was used to inject the solution. The preparation of CrEDTA solution was as described for the jugular infusion procedure (section 3.4.1).

### 3.4.5 Metabolism crate collections of urine and faeces

***Urine Collection***

Total urine output from steers was collected in bins placed under metabolism crates and containing either no acid (Experiment 5.2) or *ca.* 150 - 300 ml of 20 % sulphuric acid (Experiments 4.1, 5.1, 7.1) so that pH was kept below 3 over each 24 h period of collection. The urine bins were replaced with clean bins each day at *ca.* 08.00 h and the daily urines were weighed and subsampled.

Prior to commencement of jugular infusions (Experiments 5.1 and 5.2) a background spot urine sample (*ca.* 250 ml) was collected from each steer. This sample was frozen for later use in standard curve preparation for Cr and Li analyses. In some experiments (Experiment 4.1, 5.1 and 5.2) additional urine subsamples were taken to monitor specific gravity of the urine.

***Faecal collection***

Total faecal output from steers was collected in bins positioned behind the metabolism crates (Experiments 4.1, 5.1, 5.2 and 7.1). When collections were made for feed digestibility calculations, a daily subsample (5 or 10 % of total daily wet faeces weight) was bulked into a large plastic bag for each individual steer over the duration of the collection period. The ends of the plastic bags were tied and the bulk samples were stored in a freezer during the collection period. At the end of the collection period each bulk sample was defrosted and mixed thoroughly before taking duplicate subsamples for DM determination ($60^0$C until samples reached constant weight) and a further subsample for freeze-drying and later chemical analyses. Daily subsamples were also taken for DM determination ($60^0$C until samples reached constant weight) during some of the experiments (Experiment 4.1, 5.1 and 5.2).

Prior to commencement of jugular infusions (Experiments 5.1 and 5.2), a background spot faecal sample (one large handful) was collected for each steer. This sample was frozen for later use in standard curve preparation for Cr and Li analyses.

### 3.4.6 Field collection of urine and faecal spot samples

***Urine collection***

Spot urine samples were collected from the RF steers during grazing trials (Experiments 5.4 and 6.1 - 6.7) at times designated by the sampling schedule (detailed in the relevant chapters). A team of four collectors approached the six to eight steers used in the various experiments while they were grazing or resting in the paddock. Any urination events were collected at this time by holding a plastic 250 ml container, held in a steel receptacle with attached handle, beneath the steers. The steers were then walked to portable panel yards situated in the test paddock. Here, urine sampling continued from those steers that had not yet given a sample, while other sampling and

dosing procedures were conducted, as detailed for the relevant experiments. The time of each individual collection was recorded and the spot urine samples were placed on ice until further processing could occur.

Prior to commencement of jugular infusions (Experiments 6.1 - 6.7) or $Cr_2O_3$ capsule insertion into the rumen (Experiment 5.4), a background spot urine sample (*ca.* 250 ml) was collected from each steer. This sample was frozen for later use in standard curve preparation for Cr and Li analyses.

***Faecal collection***

Spot faecal samples were collected from RF steers (Experiments 6.1 - 6.7) at times designated by the sampling schedule (detailed in Chapter 6). Spot faecal samples were also collected from OF steers (Experiments 6.1 - 6.7) on the days of extrusa sampling (see Chapter 6) and from non-fistulated steers in Experiment 6.7. Spot samples were collected at the time of defecation by holding an aluminium tray beneath the tail, or by collecting samples fresh from the ground or per rectum. Each faecal sample was placed on ice and then frozen (-20$^0$C). The RF-steer faecal samples were freeze-dried prior to Yb, OM, N, ADF and alkane analysis and nylon bag incubation. The OF-steer faecal samples were oven dried prior to n-alkane analysis. The faeces from non-fistulated steers were chilled prior to conducting worm egg counts.

Prior to commencement of marker dosing for the RF steers (Experiments 6.1 - 6.7), a background spot faecal sample (one large handful) was collected from each steer. This sample was frozen and then freeze-dried for later use in standard curve preparation for Yb analyses.

### 3.4.7 Processing urine samples for analysis

Subsamples were collected for PD analysis by pipetting 5 ml of urine, either acidified (Experiments 4.1, 5.1, 6.2 and 7.1) or non-acidified (Experiments 6.1 and 6.3 - 6.7), into a 50 ml graduated tube containing 45 ml of 0.1 M ammonium phosphate ($NH_4H_2PO_4$) buffer. The sample was mixed and then frozen for later PD analysis.

Urine subsamples taken for Cr and Li analysis were either acidified (Experiments 5.1 and 6.2) or non-acidified (Experiments 5.2, 5.4, 6.1 and 6.3 - 6.7) and were simply frozen until analysis could occur.

### 3.4.8 Serum sampling

Whole blood samples, *ca.* 10 ml, were taken via either a jugular catheter (Experiments 5.2 and 6.1 - 6.7) or from the tail vein of experimental steers (Experiment 5.3). The samples were placed in iced water until centrifugation at 2500 - 2800 rpm for 10 min. The serum was removed from centrifuged samples and frozen until analyses could occur.

### 3.4.9 Rumen fluid sampling from RF steers

***Rumen fluid sampling procedure***

Rumen fluid sampling (Experiments 5.3, 6.1 - 6.7 and 7.2) involved removing the cannula bung and inserting a sampling probe into the rumen. The sampling probe consisted of a length of PVC pipe (1.5 cm i.d.), the end of which was perforated with holes and covered by nylon stocking (usually the "reinforced toe" section). A piece of plastic tubing was inserted into the PVC pipe with the outer end attached to a 60 ml syringe. Rumen fluid was drawn, via suction, out of at least three different sites in the rumen to a minimum bulk volume of 180 ml.

***Determination of rumen fluid pH***

Rumen fluid pH was determined, immediately after sample collection, with a portable pH meter. The pH meter was re-calibrated after each sampling event with standard buffers pH 4.0 and 7.0.

***Sample preparation for VFA analysis***

In Experiments 6.1 - 6.7, the rumen fluid samples were swirled and a 10 ml subsample poured off, placed on ice, and later frozen prior to VFA analysis. In Experiment 7.2, 4 ml of swirled rumen fluid sample was pipetted into 1 ml of metaphosphoric acid, then immediately placed on ice and later frozen prior to VFA analysis.

***Sample preparation for $NH_3$-N analysis***

The rumen fluid was acidified, as soon as possible after collection and following pH determination and VFA sub-sampling, to pH ≤ 3.0 by adding drops of concentrated sulphuric acid. A 10 ml subsample was then poured off, placed on ice and later frozen for $NH_3$-N analysis.

***Sampling for protozoa enumeration***

A double layer of nylon stocking (rather than the "reinforced toe" section) was used to cover the sampling probe for collection of rumen fluid for protozoa enumeration (Experiments 6.1 and 6.3 - 6.7). The bulked rumen fluid was then re-strained through a single layer of nylon stocking. Four ml of the strained, stirred, rumen fluid was then taken via a 10 ml syringe and added to a 16 ml formal saline solution. The formal saline solution consisted of 0.9 % NaCl and 4 % formaldehyde (w/w) in water. The samples were stored at room temperature awaiting analysis.

The enumeration procedure involved mixing the suspension thoroughly prior to pipetting fluid into a counting chamber (Hawksley, Sussex, England) of 0.2 mm depth and divided into 0.2 $mm^2$ grids. The protozoa were counted under a light microscope using 100x magnification. Counting continued until at least 200 cells were recorded, or until two full slides were counted when numbers were low. Identification of species was carried out with reference to Dehority (1993). Species were divided into nine generic groups, including *Isotricha* spp. and *Dasytricha* spp. (both categorised as Holotrichs) and *Entodinium* spp., *Epidinium* spp., *Diplodinium* spp., *Eudiplodinium* spp., *Ostracodinium* spp., *Metadinium* spp. and *Elytroplastron* spp. (collectively categorised as Entodiniomorphs).

***Sampling for CrEDTA fractional outflow rate***

Background rumen fluid samples were collected from each RF steer during the preliminary feeding periods prior to commencement of marker dosing or infusion. These samples were frozen for later use in standard curve preparation for Cr analysis. After injection of CrEDTA into the rumen, eight to nine rumen fluid samples were taken, as described above, over the following 48 h (sampling times given in the relevant chapters). Ten ml subsamples were frozen prior to analysis for Cr.

### 3.4.10 Rumen digesta sampling from RF steers

Rumen digesta samples were taken from RF steers at six to eight times over the 48 h following Yb marker dosing (Experiments 6.1 - 6.7 and 7.2). Rumen digesta samples were taken by removing the cannula bung and taking a handful of digesta from three different sites within the rumen, on each occasion. These samples were frozen until oven drying ($60^0$C) and then analysis for Yb.

### 3.4.11 Nylon bag incubation

Nylon bag incubations for feed degradability studies (Experiments 6.1 - 6.8, 7.2 and 7.3) were conducted according to a modification of the method of Orskov *et al.* (1980). Incubations for indigestible acid detergent fibre (IADF) determination (Experiment 6.9) were conducted as described in section 6.2.4.4.

In Experiments 6.1 - 6.7 and 7.2, the RF steers consumed the test pasture or treatment diet during nylon bag incubations. In Experiments 6.1 - 6.7 the steers consumed the diet *ad libitum*, while in Experiment 7.2 steers were fed the treatment diet at *ca.* 90 % *ad libitum* intake. In Experiments 6.8, 6.9 and 7.3, the RF steers were fed on a standard diet designed to provide adequate CP for microbial activity (*ca.* 16 % CP). The standard diet consisted of *ca.* 1 kg of cottonseed meal and a hay component (fed *ad libitum*) consisting of 3/8 lucerne hay, and 5/8 of either Rhodes grass (*Chloris gayana*) hay (Experiments 6.8 and 6.9) or pangola grass (*Digitaria eriantha*) hay (Experiment 7.3). During the incubation and sampling period the standard diet was fed at *ca.* 90 % of *ad libitum* intake to facilitate nylon bag removal and was fed in two equal portions at *ca.* 07.30 h and 15.30 h. In all nylon bag incubation experiments, the steers underwent a preliminary feeding period of at least 6 d prior to nylon bag insertion.

Dried samples to be incubated were ground through a 3 mm screen (degradability studies) or a 1 mm screen (IADF determination) prior to weighing (*ca.* 5 g DM) into nylon bags. Fresh samples to be incubated were processed as described in section 6.2.4.2. Filled nylon bags were attached to a length of chain (16 x 6 cm links) and then soaked in water for *ca.* 5 - 10 min prior to immersing in the rumen of RF steers. The nylon bags consisted of monofilament polyester and were 24 x 10 cm in dimension (outer bag dimensions prior to tying) with a pore size of 45 µm (Allied

Filter Fabrics). Bag replications and times of removal are described in the relevant chapters.

Upon removal of the bags from the rumen, the excess material was washed off the bags and they were immediately frozen if more times of bag removal followed. Once all bags had been removed from the rumen, the frozen bags were defrosted by placing them in cool water. The bags were then each given a constant number of squeezes by hand under running tap water (ten to twenty-five squeezes depending on the experiment) before being rinsed on a set cycle in an automatic washing machine. Upon removal from the washing machine, the bags were oven dried ($60^0$C for 48 h), cooled in a desiccator and then weighed to determine the proportional DM loss. Dried bag residue was ground through a 1 mm screen prior to chemical analyses.

## 3.5 ANALYTICAL PROCEDURES

### 3.5.1 DM and ash

Dry matter was determined by drying duplicate samples to constant weight in a forced draught oven at $60^0$C - $70^0$C. Ash content was determined by combusting dry samples in an electric muffle furnace (Thermogravimetric analyser TGA-601, LECO Corporation) at $600^0$C for 2 h.

### 3.5.2 Total N

Feed and faecal samples were ground to 1 mm prior to analysis for total N content by a combustion method (Sweeney, 1989) using an ELEMENTAR RapidN analyser (ELEMENTAR ANALYSENSYSTEME, Germany).

### 3.5.3 NDF and ADF

Feed and faecal samples were ground to 1 mm prior to analysis for NDF and ADF using a FIBRETEC 2021 Fibrecap system (FOSS TECATOR). In all cases, the NDF was expressed on an ash-free basis but was uncorrected for residual protein.

### 3.5.4 Neutral detergent insoluble N (NDIN) and acid detergent insoluble N (ADIN)

Nylon bag residue was ground to 1 mm prior to fibre determination using FIBRETEC 2021 Fibrecap system (FOSS TECATOR) followed by N analysis by a combustion method (Sweeney, 1989) using an ELEMENTAR RapidN analyser (ELEMENTAR ANALYSENSYSTEME, Germany).

### 3.5.5 VFA in rumen fluid

The concentrations of VFA present in rumen fluid samples were determined by gas chromatography using a polar capillary column (DB-FFAP). The samples were prepared by precipitating the protein with metaphosphoric acid, adding an internal standard (isocaproic acid) and then diluting the solution to minimise loading on the capillary column. A prepared multi-acid standard was mixed with the protein precipitant/internal standard and used to calibrate the gas chromatograph. Samples were analysed using the internal standardisation method for calibration.

### 3.5.6 $NH_3$-N in rumen fluid

Rumen fluid was shaken and then centrifuged (4000 rpm for 12 min) prior to determination of $NH_3$-N concentration on an Olympus Reply clinical analyser. The method is based on a reaction described by Boller *et al.* (1961).

### 3.5.7 PD in urine

The acidified and buffered urine samples (see section 3.4.7) were thawed and, immediately prior to analysis, filtered through Alltech cellulose nitrate or acetate membrane filters (25 mm, 0.2 μm) and then through a C18 300 mg filter to avoid column damage. The samples were analysed for PD and creatinine using a high performance liquid chromatograph (HPLC) according to a modification of the method outlined by Balcells (1992). A Waters (Division of Millipore) HPLC system controller model 600E equipped with an automatic model 712 Wist sampler and a 484 tunable absorbance detector was used for the analyses. Separation and quantification of PD and creatinine was achieved using two Activon Goldpak C18 10 μm reversed-phase columns (150 x 3.9 mm i.d.) connected in series. Waters Maxima 820 software was used for data optimisation, analysis of chromatograms and calculation of the

results. A sample volume of 20 µl was automatically injected and absorbance measured at 205 nm. Flow rate through the column was 0.8 ml/min. Buffers of 10 % ammonium phosphate (Buffer A) and 10 % ammonium phosphate buffer plus 10 % acetonitrile (Buffer B) were used.

### 3.5.8 Cr and Li in urine, serum and rumen fluid

***Cr and Li in urine and serum***

Urine and serum samples were analysed for Cr and Li concentration using a Finnegan Inductively Coupled Plasma Mass Spectrometer. Samples were first deproteinised by diluting 1 ml of sample with 4 ml of 6 % trichloracetic acid. Further dilution with distilled water was sometimes required before samples were directly aspirated into the instrument. A series of standards of known concentration for each element were used to produce the standard curve.

***Cr and Li in rumen fluid***

Rumen fluid samples were centrifuged (4000 rpm for 10 min) prior to analysis for Cr and Li using a Varian 220FS Atomic Absorption Spectrometer with acetylene flame. A series of standards of known concentration were used to develop the standard curve.

### 3.5.9 Cr, Li and Yb in faeces and digesta

***Analysis by Atomic Absorption Spectrometer***

Faecal and digesta samples collected in Experiments 5.1, 5.2 and 6.1 - 6.7 were analysed for Cr, Li and Yb using a Varian 220FS Atomic Absorption Spectrometer with nitrous oxide/acetylene flame and emission detection. The 1 mm ground samples were prepared for analysis by first ashing the sample ($500^0$C overnight) in a muffle furnace and then digesting the residue with a digest acid containing nitric and hydrochloric acids and potassium chloride. The acid digests were centrifuged (2500 rpm for 10 min) prior to analysis. Quality control checks included:

1) two standards were included as every 10th and 11th sample to monitor any loss of sensitivity during a run,

2) the spectrometer was recalibrated after every 10th sample,
3) a standard curve recovery on a non-related faecal material was conducted in every run to determine the recovery of the method. This recovery figure was used as a correction factor,
4) the solution used for standard curve recovery was analysed on the spectrometer to determine the true concentration,
5) between run variation was monitored by repeating two samples in every run, and
6) the transmission value of the standards analysed in each run was compared to the previous run to monitor any loss of sensitivity of the instrument between runs.

To account for variation in background matrices due to pasture type or individual animal variation, a background sample (taken prior to commencement of marker infusion or dosing) was analysed for each animal for each pasture/diet type. This background reading was subtracted (after adjustment for standard curve recovery) from each sample Cr, Li or Yb concentration relating to that steer for a particular experiment or experimental run.

Relatively large intercepts were obtained for some of the Yb standard curves in Experiments 6.1 - 6.7, causing negative values to be obtained for sample Yb prediction at low concentrations. Thus each Yb standard curve linear regression was tested to see if the intercept was significantly different to zero ($H_0$: intercept = 0) prior to adjusting the standard curve to force it through a zero intercept (equation: $y = bx$). In fourteen out of seventeen standard curves, the intercept was not significantly different to zero ($P > 0.05$). However, in two of the digesta standard curves (relating to Experiment 6.6) and one of the faecal standard curves (relating to Experiment 6.4) the intercepts were significantly different to zero ($P < 0.05$). As inclusion of the intercept resulted in negative predicted Yb concentrations in some cases, there was no option but to force the three standard curves with significant intercepts through zero, also. Despite the significance of the intercepts for the three standard curves, the correlation coefficients for these curves, once forced through zero, were still extremely high ($r > 0.99$).

***Analysis by Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES)***

Digesta samples collected in Experiment 7.2 were analysed for Yb using an ICP-AES. The 1 mm ground samples were prepared for analysis by acid digestion. An acid solution consisting of 5 nitric acid : 1 perchloric acid mixture was added to the samples and allowed to stand overnight. The samples were then digested by heating up to $300^0$C before being diluted with water and analysed on the ICP-AES. An acid blank was run after every ten samples. To account for the influence of background matrices on the ability of the ICP-AES to recover all Yb present, a standard curve recovery was conducted on each type of sample being analysed.

## 3.6 CALCULATIONS

### 3.6.1 *In vivo* diet digestibility

The digestibility of DM, OM and NDF in diets was calculated from collections of feed and faeces in metabolism crates as follows:

$$\text{Digestibility (g/kg)} = \frac{(\text{intake (kg)} - \text{faecal output (kg)})}{\text{intake (kg).}} \times 1000$$

### 3.6.2 MCP flow using the PD technique

The intestinal flow of MCP was calculated using equations proposed by Chen & Gomes (1995). Firstly, the absorption of microbial purines (X) was calculated using measured values for excretion of total PD (sum of allantoin, uric acid, hypoxanthine and xanthine) in urine (Y) and the metabolic body weight (kg) of the animal ($W^{0.75}$):

$$\text{X (mmol/d)} = \frac{(\text{Y (mmol/d)} - (\text{endogenous PD excretion (mmol/kg } W^{0.75}) \times W^{0.75}))}{0.85.}$$

MCP was then calculated by:

$$\text{MCP (g/d)} = 0.727\text{X} \times 6.25.$$

Measured values for endogenous PD excretion in *B. indicus* and *B. taurus* cattle were used to calculate MCP flow in Experiment 4.1, in addition to the value proposed in the equation of Chen & Gomes (1995), (0.385 mmol/kg $W^{0.75}$). The measured value for endogenous PD excretion in *B. indicus* cattle, determined in Experiment 4.1, was used to calculate MCP flow for all *B. indicus* cattle in Experiments 5.1, 6.1 - 6.7, and 7.1.

### 3.6.3 Rumen fluid and particle FOR

Calculation of rumen fluid and particle FOR and volume was based on the assumptions that the fluid and particle markers remained associated with their respective phases, that recovery of markers was complete with no transfer of marker between compartments, and that steady state conditions existed in the rumen.

The FOR of Cr and Yb in rumen fluid and digesta, respectively, were determined by regressing the natural log (*ln*) of Cr and Yb concentration against time. The marker concentration declined over time, according to first order dilution kinetics, as described by the equation:

$$A_t = A_0 e^{-kt},$$

where "$A_t$" and "$A_0$" are the concentrations of marker at times "t" and 0, respectively, and "k" is the rate constant described by the slope of the regression line.

Rumen fluid volume and DM content, and rumen fluid and particle FOR were calculated as follows:

$$\text{Rumen fluid volume (l) or DM content (kg)} = \frac{\text{marker injected (mg)}}{A_0 \text{ (mg/l or mg/kg DM)}},$$

$$\text{FOR (\%/h)} = -k\ (h^{-1}) \times 100.$$

### 3.6.4 Determination of DM and OM degradability parameters from nylon bag incubation

The disappearance of DM or OM (g/kg) was plotted against time using the statistical package, Genstat for Windows, 6th edition (Lawes Agricultural Trust, Rothamsted, U.K.) and the model proposed by Orskov & McDonald (1979) and revised by McDonald (1981). In this model, the disappearance data from incubated samples is fitted by two equations:

$Y_1 = A$ up to time $t_0$,

$Y_2 = a + b\,(1 - e^{-ct})$, from time $t_0$ onwards,

where:

- A = measured washing loss from Time 0 nylon bags (g/kg), (note: $A \neq a$),
- a = rapidly degradable fraction (g/kg),
- b = more slowly degradable fraction (g/kg),
- a + b = potential degradable fraction (g/kg),
- c = degradation rate ($h^{-1}$) of b,
- $t_0$ = lag time (h) before the commencement of degradation of b, calculated from the fitted equation as the value of t when Y = A.

### 3.6.5 Calculation of RDP using NDIN/ADIN and the nylon bag technique

This method was based on the assumptions that NDIN is the primary UDP fraction in feedstuffs, that the ADIN component is representative of the indigestible protein fraction, and that neutral detergent removes microbes (Mass *et al.* 1999; Klopfenstein *et al.* 2001).

The ADIN content in unincubated nylon bag samples was first subtracted from the NDIN content in both unincubated and incubated nylon bag samples. The *ln* of the percentage of NDIN (less ADIN) remaining in the residue was then regressed against incubation time (5, 8, 11, 13 and 16 h for Experiments 6.1 - 6.7, and 5, 8, 11, 14 and 16 h for Experiment 7.3) to give slope "$k_d$". Not all incubation times could be used for each pasture type due to plateauing of the degradation curve, which produced a non-linear regression. More specifically, the 16 h incubation time was excluded for

all mid to high quality pasture types (Experiments 6.1, 6.4 - 6.7) as well as for the tropical grass hay incubated in Experiment 7.3, and the 13 h incubation time was excluded for the high quality tropical legume in Experiment 6.5.

The following equation, described by Broderick (1994) and based on the original work of Orskov & MacDonald (1979), was used to determine the content of UDP in feed samples:

$$\text{UDP (g/kg DM)} = \text{B (g/kg DM)} \times \frac{k_p\ (h^{-1})}{(k_p\ (h^{-1}) + -k_d\ (h^{-1}))} + \text{C (g/kg DM)}$$

where "B" is the UDP potentially degradable (insoluble) pool (NDIN - ADIN, in unincubated feed), "C" is the undegradable pool (ADIN concentration in unincubated feed), "$k_p$" is the rumen particulate matter FOR (calculated according to section 3.6.3), and "$k_d$" is the rate of degradation of "B" (calculated as described above).

The UDP and RDP concentrations as a proportion of total plant CP were then determined according to the following equations:

$$\text{UDP (g/g CP)} = \frac{\text{UDP (g/kg DM)}}{\text{CP (g/kg DM)},}$$

$$\text{RDP (g/g CP)} = 1 - \text{UDP (g/g CP)}.$$

# Chapter 4

# Estimation of endogenous purine derivative excretion in the urine of *B. taurus* and high-content *B. indicus* steers during fasting

## 4.1 INTRODUCTION

The measurement of urinary PD excretion is a non-invasive technique for estimating MCP flow to the small intestines in ruminants. This method is based on the principle that the absorption of microbial protein and microbial nucleic acids are correlated, and that the PD in the urine of ruminants originate largely from the degradation of absorbed microbial nucleic acids. However, in addition to purines originating from microbial flow from the rumen, there is an endogenous fraction in the urine resulting from tissue nucleic acid turnover.

The widely used equation developed by Verbic *et al.* (1990) and detailed by Chen & Gomes (1995) allows the calculation of MCP flow from measured values for excretion of PD in the urine of cattle. In this equation a constant value for the endogenous excretion of PD is assumed (385 μmol/kg $W^{0.75}$), which was developed using *B. taurus* cattle. However, recent studies have shown that pure-bred and high-content *B. indicus* cattle may have lower levels of endogenous PD excretion than values measured for *B. taurus* cattle (Osuji *et al.* 1996; Liang *et al.* 1999; Ojeda & Parra, 1999; Pimpa *et al.* 2001). The comparison of breeds has been confounded, however, by the methodology used to estimate endogenous PD excretion. While the majority of experiments to determine endogenous PD excretion in *B. taurus* cattle have involved the intragastric infusion technique, those with *B. indicus* cattle have utilised the fasting technique (see section 2.5.1). As no experiments have compared *B. indicus* and *B. taurus* species using the same methodology, it cannot be determined as to whether the differences in endogenous PD excretion values are due to the species of cattle, or the methodology used.

Nevertheless, further evidence of a lower endogenous PD excretion in *B. indicus* cattle is provided by the negative values for MCP flow that have been obtained in some instances, when using the endogenous PD value assumed in the equation of Chen & Gomes (1995). In such cases, the assumed endogenous PD component has been greater than the total urinary excretion of PD (Ayala & Hovell, 1997).

As high-content *B. indicus* cattle are widely used throughout northern Australia, and in other tropical and sub-tropical environments, it is essential that the equations used to estimate MCP synthesis be validated for this species. Thus, an experiment was designed to compare the endogenous excretion of PD in the urine of *B. taurus* steers with that of high-content *B. indicus* steers using the fasting method, and to allow comparison of these values with those from the literature which have been obtained using a variety of techniques.

***EXPERIMENT 4.1***

## 4.2 MATERIALS AND METHODS

### 4.2.1 Experimental animals

Five Friesian (*B. taurus*) and five high-grade Brahman (>75 % *B. indicus*) steers *ca.* 16 months of age were used in the experiment. The initial average liveweight of the Friesian steers was 323 ± (SE) 3.8 kg and that of the Brahmans was 329 ± 4.7 kg. All steers were treated with moxidectin (Cydectin, Fort Dodge Australia Pty Ltd) to control internal and external parasites at the commencement of the preliminary feeding period. The dose rate of the drug was according to the manufacturer's recommendations, and this procedure applies throughout the thesis wherever standard drugs were given.

### 4.2.2 Experimental plan and diets

The experiment consisted of a 14-d preliminary feeding period in individual, concrete-floor pens and a 14-d sampling period in metabolism crates. The Brahman and Friesian steers were fed low quality tropical grass hays at a fixed level of intake equivalent to ME maintenance requirements (calculated according to SCA (1990))

during the preliminary feeding period and the first 5 d of the sampling period. Rhodes grass (*Chloris gayana*) hay was fed for the first 8 d of the preliminary feeding period and buffel grass (*Cenchrus ciliaris*) hay was fed for the following 6 d of preliminary feeding and the first 7 d of the sampling period. Over days 6 and 7 of the sampling period, the feed intake of the steers was reduced to 60 % and 30 % of ME maintenance requirements, respectively. The steers were then fasted for the next 7 d. On day 15, the steers were moved from the metabolism crates back into individual pens for surveillance while they were gradually brought back onto a hay diet before being released onto pasture.

### 4.2.3 Experimental procedures

Hay intake for each steer was recorded over the first 7 d of the sampling period. Dry matter content was determined daily for feed residues and every second day for the hay fed. Over d 1 - 5 of the sampling period, daily faecal output was recorded for each steer and subsamples were kept for determination of diet OMD, as described in section 3.4.5. Over d 6 - 14 of the sampling period, daily faecal output was recorded and faecal subsamples were taken in duplicate from each individual steer for DM determination ($60^0$C). Over the 14-d sampling period, total urine output was collected as described in section 3.4.5. Over d 1 - 5 of the sampling period, 10 % of each steer's daily urine was subsampled and bulked into a container for each steer. These containers were stored in a freezer over the 5 d of collection. On day 5, the bulk urines were defrosted, shaken and subsampled for later PD analysis as described in section 3.4.7. Over the final 9 d of urine collection (2 d of reducing intake followed by 7 d of fasting) individual daily urines were kept for each steer for PD analysis. The steers were weighed at the commencement of the preliminary feeding period and at the beginning and end of the sampling period.

### 4.2.4 Analytical procedures

Methods for the determination of PD concentration in urine, and DM, ash, N, NDF and ADF concentration in feed and faeces are described in section 3.5.

### 4.2.5 Calculations

The OMD of the buffel grass hay was calculated as described in section 3.6.1. The intestinal flow of MCP was calculated as described in section 3.6.2.

### 4.2.6 Statistical analyses

The statistical package, Genstat for Windows, 6th edition (Lawes Agricultural Trust, Rothamsted, U.K.), was used to test for significant differences between Friesian and Brahman cattle.

An unpaired t-test was used to compare the two breeds of cattle for variables OMD, PD excretion during the feeding period (d 1 - 5 of the sampling period), and MCP flow and eMCP (calculated using both the established equation of Chen & Gomes (1995) and the measured endogenous values for PD excretion determined in the present study).

To test for significant differences in PD excretion over the fasting period (d 8 - 14), a linear mixed model with fixed effects (time and breed) and random effects (steer x time) was used to model the covariance patterns in the steer x time component. Investigations indicated that the most appropriate model for the analysis was an unstructured covariance model. Wald tests (Genstat Committee, 2000) indicated that significant effects were time ($P < 0.001$), breed ($P < 0.001$) and their interaction ($P = 0.004$). There was a large variation in PD excretion between steers within both breeds on day 8 (the first day of fasting), and the inclusion of this day in the analysis caused the interaction of breed with time. Large PD excretion values for several steers on day 8 indicated that these steers had not yet reached their endogenous PD level reflective of dietary conditions. For this reason, as well as the fact that day 8 was only the first day of true fasting, the PD excretion over d 9 - 14 was considered to best represent the true endogenous PD excretion.

Thus the data was re-tested to determine significant differences in PD excretion between the two breeds over d 9 - 14 of the sampling period. The analysis was the same as that described above for d 8 - 14. Wald tests (Genstat Committee, 2000) of the fixed effects of time, breed and their interaction indicated that the interaction was not significant during the d 9 - 14 period, and therefore it was deleted from the model to leave the main effects, i.e., time and breed. Pair-wise differences between means for each day were tested using a protected least significance difference procedure ($P = 0.05$).

## 4.3 RESULTS

### 4.3.1 Chemical composition and quality of the diet

The buffel grass hay fed during the first 7 d of the sampling period was of low quality with low CP concentration and high fibre content (Table 4.1) and low OMD. There was no significant difference (P = 0.72) between Friesian and Brahman steers in values for OMD (average value 580 ± 4.3 g/kg).

**Table 4.1. Chemical composition (g/kg DM) of the buffel grass hay fed during the sampling period**

| | Buffel grass hay |
|---|---|
| OM | 895 |
| CP | 59.4 |
| NDF | 729 |
| ADF | 441 |

ADF, acid detergent fibre; CP, crude protein; DM, dry matter; NDF, neutral detergent fibre; OM, organic matter.

### 4.3.2 Faecal output over the sampling period

The faecal DM output declined rapidly over days 7 and 8 (2nd day of reduced intake and 1st day of fasting, respectively) and then more slowly over the last 6 d of the 7-d fasting period (Figure 4.1). Faecal output had decreased to an average of 132 ± 28.4 g DM by the 7th day of fasting.

### 4.3.3 Weight loss over the sampling period

Brahmans and Friesians had similar liveweight loss over the 14-d sampling period, with liveweight declining by 44 ± 2.7 kg and 48 ± 3.4 kg, respectively.

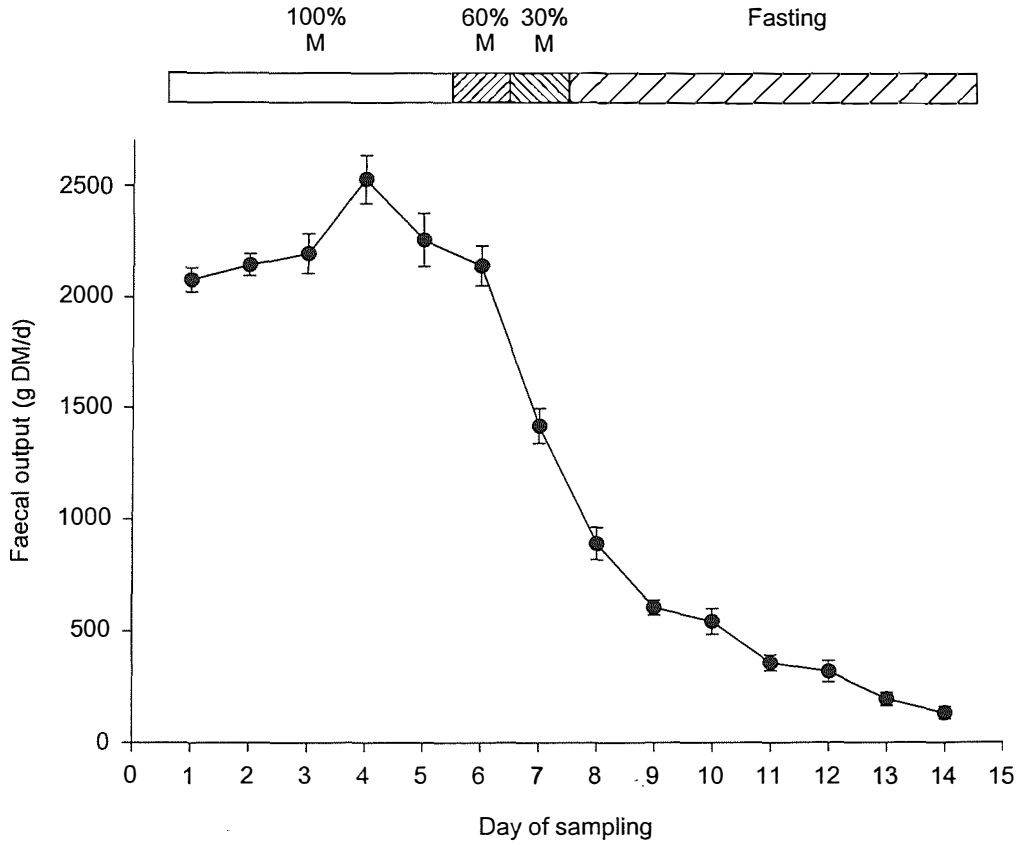


**Figure 4.1. Change in faecal output over the sampling period.** The intake of steers is shown as a percentage of maintenance requirements (M). Values are the mean of all steers from both breeds; error bars represent the standard error of the mean. (DM, dry matter).

### 4.3.4 Endogenous PD excretion

Purine derivative excretion for Brahman and Friesian steers first increased sharply during the period of reducing intake (days 6 and 7), then declined rapidly to low values by day 9, which were sustained for the remainder of the fasting period (Figure 4.2).

As outlined in section 4.2.6, the endogenous PD excretion was calculated as the mean of d 9 - 14 for each steer, and was found to be significantly different between the two breeds ($P < 0.001$). The predicted mean endogenous PD excretion was 189.8 μmol/kg $W^{0.75}$ for Brahman steers and 414.0 μmol/kg $W^{0.75}$ for Friesians (combined SE 37.2). There was no significant difference in PD excretion between each of d 9 - 12 or day 14. However, PD excretion for day 13 was significantly lower ($P < 0.05$) than that for all other days except day 12.

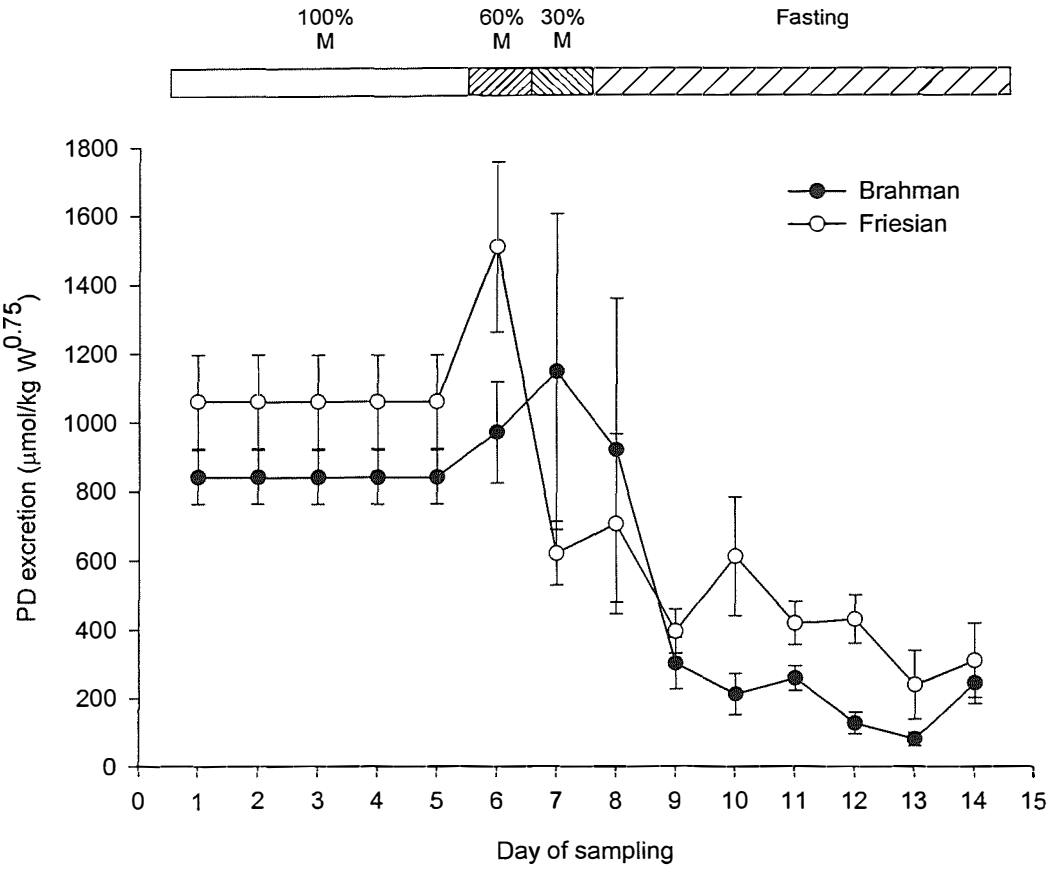


**Figure 4.2. Change in purine derivative (PD) excretion in urine over the sampling period.** The intake of the steers is shown as a percentage of maintenance requirements (M). Values are the mean for each steer group; error bars represent the standard error of the mean. (W, weight).

Although the mean PD excretion during the 5 d of feeding to energy maintenance requirements (d 1 - 5) also appeared greater in Friesian steers than for Brahmans (1059.5 ± 136.04 vs. 840.8 ± 77.52 µmol/kg $W^{0.75}$), these differences were not significant (P = 0.20).

Of the four individual PD measured in the urine, allantoin was the major one present and was largely responsible for the decrease in PD excretion with fasting (Figure 4.3). Negligible amounts of hypoxanthine and xanthine were detected in either Brahman or Friesian urine.

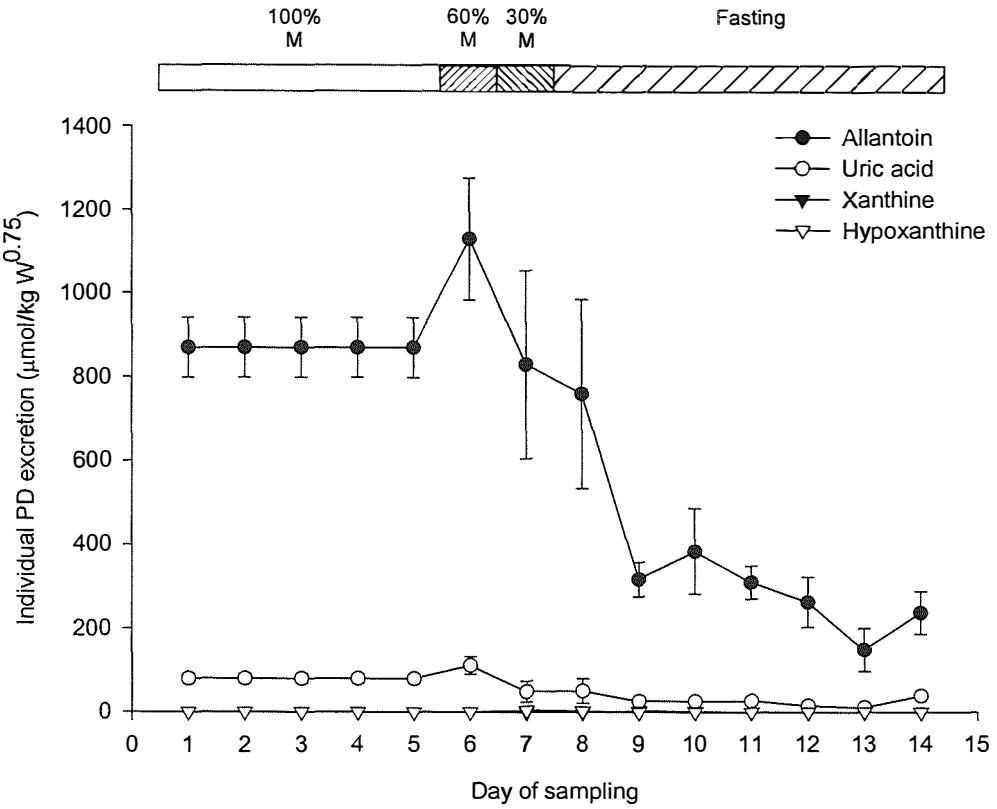


**Figure 4.3. Change in allantoin, uric acid, xanthine and hypoxanthine excretion in urine over the sampling period.** The intake of the steers is shown as a percentage of maintenance requirements (M). Values are the mean of all steers from both both breeds; error bars represent the standard error of the mean. (PD, purine derivatives; W, weight).

### 4.3.5 Impact of the measured endogenous values on MCP flow and eMCP

Table 4.2 summarises the estimates of MCP flow and eMCP calculated using either the endogenous value for PD excretion proposed in the equation of Chen & Gomes (1995), i.e., 385 µmol/kg $W^{0.75}$, or those determined in the present study. While none of the estimates were found to be significantly different between the two breeds ($P > 0.05$, Table 4.2), the numerical difference in estimated MCP flow between cattle breeds was much greater using the published endogenous PD excretion value compared with the values measured in the current experiment (difference of 85 vs. 7 g MCP/d, respectively). This reflected the fact that the values changed little for Friesian steers but by a factor of *ca.* 1.4 for Brahman cattle when the values determined from this study were used instead of the published value.

**Table 4.2. Microbial crude protein (MCP) flow and efficiency of MCP production (eMCP) estimated using Chen & Gomes (1995) endogenous purine derivative (PD) excretion value (385 μmol/kg $W^{0.75}$) or the measured value for each breed.** Values are the mean and standard error (in brackets) for steers within each breed; probability (P) for test of significant difference between breeds is presented

| | Breed | | P |
|---|---|---|---|
| | Friesian | Brahman | |
| **Using Chen & Gomes (1995) endogenous PD value** | | | |
| MCP flow (g CP/d) | 274 (55.3) | 189 (34.4) | 0.23 |
| eMCP (g MCP/kg DOMI) | 98 (20.1) | 78 (12.7) | 0.42 |
| **Using measured endogenous PD values** | | | |
| MCP flow (g CP/d) | 262 (55.3) | 269 (35.2) | 0.91 |
| eMCP (g MCP/kg DOMI) | 94 (20.1) | 112 (12.4) | 0.47 |

CP, crude protein; DOMI, digestible organic matter intake.

## 4.4 DISCUSSION

This experiment provides the first valid comparison of endogenous PD excretion in *B. taurus* and high-content *B. indicus* cattle and indicates a two-fold difference between species in this parameter. The endogenous PD excretion measured here for Friesian steers using the fasting technique (414 μmol/kg $W^{0.75}$) is in the same general order as values obtained by other researchers for *B. taurus* cattle using the intragastric infusion method (456, (Fujihara *et al.* 1987); 514, (Chen *et al.* 1990c); 428 μmol/kg $W^{0.75}$, (Verbic *et al.* 1990)), but slightly below the values obtained using the technique of rumen emptying (560 μmol/kg $W^{0.75}$, (Giesecke *et al.* 1993)) and the extrapolation of infusion/excretion regressions to zero purine input (531 μmol/kg $W^{0.75}$, (Beckers & Thewis, 1994)). The value for Friesian steers measured in the current experiment is also similar to that used in the equation of Chen & Gomes (1995; 385 μmol/kg $W^{0.75}$), which was based on the mean of all measurements made in the laboratory of Verbic *et al.* (1990), with adjustment for 0.22 salvage proportion for PD (see section 2.5.1). However, the endogenous PD excretion measured for *B. taurus* cattle by isotopic labelling of exogenous PB by Orellana Boero *et al.* (2001; 236 μmol/kg $W^{0.75}$), is *ca.* half the values cited above, including

that of the present study. The reason for this divergence is not clear. Unfortunately there are no published values for endogenous PD excretion in *B. taurus* cattle which have been obtained using the fasting technique.

Published values for *B. indicus* content cattle determined by a fasting method similar to that used in the present study, were 172 and 108 (Osuji *et al.* 1996), 275 (Liang *et al.* 1999), and 268, 294 and 269 μmol/kg $W^{0.75}$ (Ojeda & Parra, 1999), and as determined by extrapolating a purine infusion/excretion linear regression to zero purine input, 147 μmol/kg $W^{0.75}$ (Pimpa *et al.* 2001). As in the present experiment, these published values for *B. indicus* content cattle indicate a much lower endogenous PD excretion than that measured for *B. taurus* cattle. Thus, the value measured in the present experiment for high-grade Brahman steers is consistent with the general trend in the literature of lower endogenous PD excretion for *B. indicus* content cattle. It can be concluded that the use of a single value for endogenous PD excretion (385 μmol/kg $W^{0.75}$), for cattle of different species, is inappropriate.

However, an important consideration is the effect that the proportion of *B. indicus* content in crossbred cattle has upon endogenous PD excretion. In various experiments studying *B. indicus* x *B. taurus* cattle, the endogenous PD excretion was found to be in the same range for cattle with lower *B. indicus* content as for purebred *B. indicus* cattle (Table 2.1). This was the case even for those crossbreds with only 1/2 and 3/8 *B. indicus* content (Osuji *et al.* 1996; Ojeda & Parra, 1999). This would appear to indicate that the endogenous PD excretion for *B. indicus* x *B. taurus* cattle is more closely aligned to the *B. indicus* than the *B. taurus* species. Thus it could be inferred that the lower endogenous PD excretion, representative of that found for *B. indicus* cattle, should be used for all crossbred cattle.

The validity of the species comparison in the present experiment is substantiated by their similar values for OMD, liveweight loss, and pattern of both faecal DM output and urinary PD excretion over the sampling period. This indicates that breed or species differences in factors such as diet digestibility or rate of liveweight loss with fasting have not confounded the investigation of differences in endogenous PD excretion as measured by the fasting technique.

It is significant that the fasting technique gave similar results to methods used by other researchers (Fujihara *et al.* 1987; Chen *et al.* 1990c; Verbic *et al.* 1990; Giesecke *et al.* 1993; Beckers & Thewis, 1994; Chen & Gomes, 1995) despite the possibility that nutritional restriction may alter metabolic activity or the rate of degradation of tissue nucleic acids, compared with fully fed animals. There has also been speculation (Osuji *et al.* 1996; Belenguer *et al.* 2002) that the duodenal flow of PB may not be completely discontinued during the period of fasting and thus may contribute to urinary PD excretion, which is assumed to be solely endogenous in origin. While no direct evidence was obtained in this experiment to show that this was not the case, faecal output declined rapidly with continued food restriction to reach negligible values by the 7th day of fasting. This indicates that the majority of residual material in the reticulo-rumen had been voided and therefore would have made negligible contribution to the total urinary PD output.

The use of the endogenous PD excretion values measured in the present experiment, rather than that used in the equation proposed by Chen & Gomes (1995), brings the estimates of MCP flow and eMCP much closer together for the two species of cattle. Using the value of Chen & Gomes (1995) to calculate MCP flow gives the impression that high-content *B. indicus* cattle have a lower (although not significantly in this experiment) MCP flow and eMCP than *B. taurus* cattle consuming the same forage. By contrast, the results of the current experiment suggest that MCP production and eMCP are similar for *B. taurus* and high-content *B. indicus* cattle consuming a low quality tropical forage.

The lower values for endogenous PD excretion in high-content *B. indicus* cattle, as compared to *B. taurus* cattle, could occur due to a lower xanthine oxidase activity in the former. A high activity of xanthine oxidase prevents salvage of a significant proportion of endogenous PD by causing the diversion of hypoxanthine from the salvage cycle to form xanthine, and then uric acid and allantoin, (Chen *et al.* 1990c), neither of which can be incorporated into tissue nucleic acids. High activity of xanthine oxidase has been measured in most *B. taurus* cattle tissues, including plasma (Chen *et al.* 1990c), and has been used to explain the high values for endogenous PD excretion determined for *B. taurus* cattle (385 - 560 μmol/kg $W^{0.75}$) compared to sheep and goats (148 - 202 μmol/kg $W^{0.75}$; Balcells *et al.* 1991; Belenguer *et al.* 2002)

which have low xanthine oxidase in most tissues, and none in the blood (Chen *et al.* 1990c; Belenguer *et al.* 2002).

However, if *B. indicus* cattle do have a lower xanthine oxidase activity than *B. taurus* cattle, higher concentrations of xanthine and hypoxanthine would be expected in the urine, as occurs with sheep and goats. On the contrary, in the present experiment and in others using *B. indicus* content cattle, allantoin has been found to be the major component of total urinary PD, with negligible amounts of xanthine and hypoxanthine, similar to that found for *B. taurus* cattle.

Ojeda & Parra (1999) made measurements of xanthine oxidase activity in *B. indicus* content cattle. These researchers found cattle with 1/2 and 5/8 *B. indicus* content to be similar to *B. taurus* cattle and sheep in having a much higher xanthine oxidase activity in the liver than in intestinal mucosa. However, in contrast to results for *B. taurus* cattle, xanthine oxidase activity was practically absent in the plasma of both *B. indicus* crossbreeds. If the low plasma xanthine oxidase activity does in fact indicate the potential for a significant PD salvage pathway in *B. indicus* cattle, the equation for calculating MCP flow for *B. indicus* content cattle may more closely resemble that developed for sheep and goats, rather than that based on work with *B. taurus* cattle. This deserves further investigation.

Osuji *et al.* (1996) have suggested an alternative hypothesis for the low endogenous PD component in *B. indicus* cattle. These authors postulated that, in relation to their *B. taurus* counterparts, *B. indicus* cattle have evolved the ability to more efficiently utilise and conserve N, through N recycling and by having low endogenous N components, including purine N. This theory implies that a greater proportion of the purine metabolites in the plasma of *B. indicus* cattle are being disposed of via non-renal routes, for example, through recycling to the rumen. However, the validity of this theory is challenged by the results of Ojeda & Parra (1999) and Pimpa *et al.* (2001) who found that the recovery of supplied exogenous PB in the urine was 0.83 for 5/8 *B. indicus* content cattle and 0.85 for Kedah-Kelantan (pure bred *B. indicus*) cattle, respectively, and thus similar to that found for European cattle (0.85 (Verbic *et al.* 1990); 0.86 (Vagnoni *et al.* 1997); and 0.84 (Orellana Boero *et al.* 2001)).

In conclusion, this experiment demonstrated that the endogenous PD excretion in high-content *B. indicus* steers was *ca.* half that for *B. taurus* steers of a similar weight and age, when using the fasting technique. The use of this lower value for endogenous PD excretion will improve the accuracy of estimates of MCP production and eMCP for *B. indicus* content cattle.

# Chapter 5

# The use of intravenously administered CrEDTA and lithium sulphate to estimate urine output from grazing ruminants

## 5.1 INTRODUCTION

The measurement of MCP production in grazing ruminants has been hampered by the lack of simple and accurate methods of measurement. The methods traditionally employed have been based on the use of microbial markers such as RNA, DAPA and $^{35}S$. However, these methods require the use of post-ruminally cannulated animals, methods of achieving prolonged, steady intraruminal infusion of isotopes, and procedures for measuring digesta flow and for separation of microbes. The measurement of urinary excretion of PD is an alternative technique for estimating MCP flow to the small intestines in ruminants (see section 2.5.1). This method has advantages over the more conventional techniques due to its simplicity and deployment of non-invasive sampling techniques. While easily undertaken in experiments using animals housed in metabolism crates, the urinary PD excretion method has not been widely used under field conditions due to the requirement for complete collection of urine.

As reviewed in section 2.5.2 a number of techniques have been proposed for the estimation of total urine output from free-grazing ruminants. In particular, the use of the natural metabolic waste product, creatinine, as internal urine output marker has been investigated. However, Puchala & Kulasek (1992), Faichney *et al.* (1995), Bolam (1998) and Shingfield & Offer (1998) found that the variation in creatinine excretion was too great to allow accuracy in prediction of urine output and thus PD excretion. Furthermore, the assumption that creatinine is excreted at a constant proportion of body weight has been questioned, with Faichney *et al.* (1995) finding creatinine excretion to vary considerably between individuals and with diet type, and Manuel *et al.* (1996) and Bolam (1998) suggesting that creatinine excretion was linearly related to DOMI, N balance and growth rate.

Thus, there is a requirement for an alternative method of estimating urine output to allow accurate use of the PD excretion method in the field. The use of an external marker, which is quantitatively excreted in the urine, would allow estimation of urine output from spot urine samples. As reviewed in section 2.5.2, intravenously administered CrEDTA (chromium complexed with ethylenediamine tetraacetic acid) and lithium sulphate appear promising as urine output markers but have not been previously used for this purpose. The objective of this series of experiments was to develop a method to accurately estimate urine output, and thus total PD excretion and MCP production, in grazing ruminants.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Experiment 5.1. Recovery of Cr in the urine of cattle during continuous jugular infusion

#### *5.2.1.1 Experimental animals and diets*

Three high-grade Brahman (> 75 % *B. indicus*) steers (342 ± (SE) 22 kg) were used in the experiment. The diet consisted of 15 % (w/w, as fed) pangola grass (*Digitaria eriantha*) chaff and 85 % commercial feedlot pellet (Betterblend Beef Expandat Pellet, Betterblend Stockfeeds, Oakey), and was fed at *ca.* 2 % of steer liveweight.

#### *5.2.1.2 Experimental procedures*

The experiment consisted of a 20-d preliminary feeding period in individual, concrete-floor pens followed by an 11-d period in metabolism crates, which included a 2-d adjustment, 7-d infusion and 2-d post-infusion period. The steers were catheterised in the jugular vein and CrEDTA infused as described in section 3.4.1. The catheter tubing was prevented from becoming tangled, broken or chewed upon, by connecting it at intervals to wide-band, soft elastic that was hung from above the crates and attached to the back of the steer, (Plate 2). This allowed the excess tubing, required for the animal to lie down, to be held out of the way while the animal was standing and feeding. The concentration of the infusate solution was *ca.* 255 mg Cr/l. Feed intake was recorded the over 11-d period in the metabolism crates. Dry matter content was determined every 2nd day for the feed offered and daily for feed residues.

Over the 7-d infusion period, total faecal output was recorded daily for each steer and subsamples were kept for Cr analysis and for determination of diet DM digestibility (DMD), as described in section 3.4.5. Total urine output was collected daily over the last 9 d in the metabolism crates as described in section 3.4.5. Urine subsamples for PD analysis were taken from each 24-h urine collection over the 7-d infusion period. Urine subsamples for Cr analysis were taken over the final 9 d in the metabolism crates. Urine was subsampled and processed for PD and Cr analysis as described in section 3.4.7. Steer liveweight was recorded upon exit from the metabolism crates.

#### *5.2.1.3 Analytical procedures*

Methods for determination of PD and Cr concentration in urine, Cr concentration in the faeces, and DM content of the feed and faecal samples are given in section 3.5.

#### *5.2.1.4 Calculations*

The DMD of the diet was calculated as described in section 3.6.1. The recovery of intravenously infused Cr in the urine of each steer was determined as the average of the daily Cr recovery values over the final 6 d of the 7-d infusion period. Day 1 of infusion was not included in the calculation of marker recovery due to unavoidable carryover of urine in the bladder from the pre-infusion period. Daily Cr recovery was calculated as:

$$\text{Cr recovery (\%)} = \frac{\text{Cr excreted in urine over 24 h period (mg)}}{\text{Cr infused over 24 h period (mg).}} \times 100$$

MCP production was estimated from the concentration of PD in the urine and the total urine output as described in section 3.6.2. In addition, a modification of the equations stated in section 3.6.2 was used to estimate MCP production using the daily PD:Cr ratio in the urine and the measured Cr infusion and recovery figures (see below).

The total PD excretion (Y mmol/d) was calculated as:

$$Y = \text{Cr infusion (mg/d) x average Cr recovery \%} \times \frac{\text{PD in daily urine (mmol/l)}}{\text{Cr in daily urine (mg/l).}}$$

#### *5.2.1.5 Statistical analyses*

A paired t-test was conducted to compare the estimates of MCP flow determined from total urine collection versus MCP flow determined from the Cr infusion method. The statistical package, Genstat for Windows, 6th edition (Lawes Agricultural Trust, Rothamsted, U.K.), was used to conduct the analyses.

### 5.2.2 Experiment 5.2. Recovery of Cr and Li in the urine of cattle fed either high or low quality hay, during continuous jugular infusion

#### *5.2.2.1 Experimental animals, design and diets*

Six Charbray (*B. indicus* x *B. taurus*) steers (219 ± 10 kg) were used in the experiment. The steers were used in a crossover design of two treatment diets and two experimental periods. In each period half of the steers were allocated to diet 1 and half to diet 2. The treatment diets were low quality native tropical grass pasture hay (major species *Heteropogon contortus* and *Bothriochloa bladhii*) and high quality lucerne hay (*Medicago sativa*). Steers were allocated to treatment diets by stratified randomisation on the basis of unfasted liveweight.

#### *5.2.2.2 Experimental procedures*

A 14-d preliminary feeding period in individual pens preceded a 9-d period in metabolism crates, which included a 2-d adjustment period and a 7-d infusion and sampling period. The steers were fed *ad libitum* during the preliminary period and then restricted to 90 % of *ad libitum* intake during the marker infusion and sampling period. The steers underwent jugular catheterisation and infusion of CrEDTA and lithium sulphate as described in section 3.4.1 and 5.2.1.2. The infusate solution contained *ca.* 263 mg Cr/l and *ca.* 241 mg Li/l.

Hay intake was recorded over the 9-d period in metabolism crates. Dry matter content was determined every 2nd day for the feed offered and daily for feed residues. Over the 7 d of marker infusion, total urine and faecal output was collected daily as described in section 3.4.5. Faecal subsamples were kept from each 24-h collection for determination of diet OMD as described in section 3.4.5. In addition, spot faecal samples were taken per rectum from each steer, in each period, after 6 d of infusion for Cr and Li analysis. Urine subsamples for Cr and Li analysis were taken from each 24-h urine collection as described in section 3.4.7. The decline in concentration of markers in the urine and serum was monitored up to 5 h and 40 min, respectively, after cessation of infusion. Whole blood samples were taken via jugular catheter after flushing the catheter line with 20 ml saline solution, and were processed as described in section 3.4.8. Steer liveweight was recorded upon entry and exit from the metabolism crates.

#### *5.2.2.3 Analytical procedures*

Methods for the determination of Cr and Li concentration in urine, and DM, ash, N, NDF and ADF concentration in feed and faeces are given in section 3.5.

#### *5.2.2.4 Calculations*

The OMD of the two treatment diets was calculated as described in section 3.6.1. The recovery of intravenously infused Cr and Li in the urine of each steer was determined as described for Cr in section 5.2.1.4.

#### *5.2.2.5 Statistical analyses*

The statistical package, Genstat for Windows, 6th edition (Lawes Agricultural Trust, Rothamsted, U.K.) was used to conduct the analysis. Differences between treatment means were tested by analysis of variance. Where missing values were present in the data, as for Cr and Li recovery, the predicted means from Genstat were used as treatment means and an average SE was calculated from the average of individual ANOVA errors for each treatment. Analysis of Cr and Li recovery over time was conducted by repeated measures of analysis of variance for each period. This analysis adjusts for correlations between measurements over time. Pair-wise differences between days were tested using the protected least significant difference procedure ($P = 0.05$).

### 5.2.3 Experiment 5.3. Concentrations of Cr and Li in serum after, and in rumen fluid during, continuous jugular infusion into steers grazing tropical pastures

#### *5.2.3.1 Experimental animals and diets*

Five to eight RF, high-grade Brahman (> 75 % *B. indicus*) steers were used in this experiment. The RF steers are described in section 3.3. Five RF steers were sampled while grazing an introduced tropical grass pasture (creeping bluegrass (*Bothriochloa insculpta*)) in the mid-wet season as part of Experiment 6.4, Chapter 6. In addition, eight RF steers were sampled while grazing a dry season, native tropical grass pasture (major species *Heteropogon contortus* and *Bothriochloa bladhii*) as part of Experiment 6.3, Chapter 6.

#### *5.2.3.2 Experimental procedures*

The steers were catheterised in the jugular vein and infused with CrEDTA and lithium sulphate over 6 d, while grazing, as described in section 3.4.1. The infusate solution used in Experiment 6.4 contained *ca.* 293 mg Cr/l and 280 mg Li/l; that used in Experiment 6.3 contained *ca.* 278 mg Cr/l and 284 mg Li/l.

After infusion was stopped, the decline in concentration of Cr and Li in blood serum of four steers grazing creeping bluegrass pasture was measured over 2 h. Four whole blood samples were collected from the tail vein of the steers at intervals within this 2-h period. One whole blood sample was taken from the tail vein of the 5th steer *ca.* 48 h after infusion had ceased. The blood samples were processed as described in section 3.4.8.

While steers were grazing dry season native pasture, samples of rumen fluid were taken from each of eight steers on day 2 (*ca.* 08.00 h) and 6 (*ca.* 18.00 h) of the infusion period and the concentration of Cr and Li determined. Rumen fluid was collected as described in section 3.4.9.

#### *5.2.3.3 Analytical procedures*

The methods used for determination of Cr and Li concentration in serum and rumen fluid are described in section 3.5.8.

### 5.2.4 Experiment 5.4. Concentration of Cr in the urine of steers after insertion of $Cr_2O_3$ capsule into the rumen

#### *5.2.4.1 Experimental animals and diets*

Eight RF, high-grade Brahman (> 75 % *B. indicus*) steers (*ca.* 320 ± 4 kg) were used in the experiment. The RF steers are described in section 3.3. The steers grazed an irrigated tropical legume pasture (Dolichos lablab (*Lablab purpureus*)) during the experiment. The test pasture was the same as that used in Experiment 6.5, Chapter 6.

#### *5.2.4.2 Experimental procedures*

The steers grazed the Dolichos lablab pasture for 24 d, including an 11-d preliminary grazing period and a 13-d sampling period. At the beginning of the sampling period, a $Cr_2O_3$ CRD was inserted into the rumen of each of four steers, selected at random. The remaining four steers, without CRDs, acted as a control group. The matrix in the $Cr_2O_3$ capsule (Captec Chrome CRD for cattle, New Zealand) contained 44.5 % Cr (w/w, as presented). Daily during the sampling period, at *ca.* 08.30 h, the linear delivery rate of each CRD was measured (see section 3.4.3) and spot urine samples were collected from all steers, as detailed in section 3.4.6.

#### *5.2.4.3 Analytical procedures*

The method used for determination of Cr concentration in the urine is described in section 3.5.8.

## 5.3 RESULTS

### 5.3.1 Experiment 5.1. Recovery of Cr in the urine of cattle during continuous jugular infusion

#### *5.3.1.1 Digestibility of the diet*

The mixed diet of 15 % (w/w as fed) pangola grass chaff and 85 % commercial feedlot pellets had a DMD of 715 ± 20.8 g/kg when fed to the three steers.

#### *5.3.1.2 Recovery of Cr in the urine, and Cr concentration decline in urine and presence in faeces*

The average Cr recovery for the three steers was 90.0 ± 4.02 %. Cr concentration in the urine declined rapidly after cessation of infusion. Cr output over 24 h on the 1st and 2nd days after cessation of infusion averaged 13.9 ± 2.21 % and 1.1 ± 0.26 %, respectively, of the average for d 2 - 7 of infusion. No Cr was detected in the bulk faecal sample for the 7-d infusion period, for two of the three steers. The third steer was found to have 3.6 µg Cr/g DM in the bulk faeces, which is equivalent to an average Cr output in the faeces of 5.2 mg Cr/d or *ca.* 5.4 % of the average daily 24 h Cr infusion for that steer.

#### *5.3.1.3 Comparison of MCP production estimates from total urine collection vs. Cr infusion methodology*

Microbial protein production estimated from the concentration of PD in the urine and total urine output was 418.8 ± 14.13 g MCP/d whereas that estimated using the Cr infusion technique was 410.0 ± 18.95 g MCP/d. The mean difference between the two estimates was not significant (P = 0.59).

### 5.3.2 Experiment 5.2. Recovery of Cr and Li in the urine of cattle fed either high or low quality hay, during continuous jugular infusion

#### *5.3.2.1 Chemical composition, digestibility and intake of the diets and urine output characteristics*

The treatment hays were at two extremes of quality with lucerne hay having much lower fibre content and greater CP concentration than for native pasture hay. The more favourable chemical composition of the lucerne hay was also associated with greater digestibility, intake and urine output values than for native pasture hay (Tables 5.1 and 5.2).

**Table 5.1. Chemical composition (g/kg DM) of the native pasture and lucerne hays**

| | Native pasture hay | Lucerne hay |
|---|---|---|
| OM | 911 | 918 |
| CP | 43.4 | 193.4 |
| NDF | 662 | 418 |
| ADF | 363 | 327 |

ADF, acid detergent fibre; CP, crude protein; DM, dry matter; NDF, neutral detergent fibre; OM, organic matter.

**Table 5.2. Organic matter digestibility (OMD), digestible organic matter intake (DOMI) and urine output of steers consuming the native pasture and lucerne hays.** Values are mean and standard error (in brackets) across 6 steers; probability (P) for test of significant difference between hays is presented

| | Native pasture hay | Lucerne hay | SE | P |
|---|---|---|---|---|
| OMD (g/kg) | 545 (8.8) | 630 (4.2) | 4.6 | < 0.001 |
| DOMI (kg/100 kg LW)[A] | 0.68 (0.017) | 1.37 (0.066) | 0.048 | < 0.001 |
| Urine output (l/100 kg LW) | 2.48 (0.388) | 4.98 (0.362) | 0.266 | 0.003 |

LW, liveweight.

[A] DOMI values were determined during sampling periods when intakes were restricted to 90 % of the preliminary period *ad libitum* intake.

### *5.3.2.2 Recovery of Cr and Li in the urine*

Recovery of Cr was not significantly different (P = 0.46) between high and low quality forages, with a high average Cr recovery across feeds of 90 % (Table 5.3). However, Li recovery was significantly different when steers were consuming native pasture hay compared to lucerne hay (P < 0.001) and was well below total recovery for both high and low quality forage types. Differences were also found between steers (P = 0.009) and between periods (P < 0.001) for Li recovery.

**Table 5.3. Average recovery (%) of Cr and Li in the urine of steers consuming native pasture and lucerne hays.** Values are the mean and standard error (in brackets) across 5 - 6 steers for individual hays, and 11 steers for overall average across diets. Recovery % was calculated as the average over 6 d of infusion, with the 1st day of infusion excluded. Probability (P) for test of significant difference between hays is presented

| Marker type | Native pasture hay | Lucerne hay | Average across both hay types | P |
|---|---|---|---|---|
| CrEDTA | 89.2 (0.81) | 90.2 (0.88) | 89.7 (0.85) | 0.46 |
| Lithium sulphate | 46.3 (0.40) | 72.6 (0.43) | - | < 0.001 |

Cumulative chromium recovery, averaged over both hay treatments, was relatively constant over the 6 d of measurement (Figure 5.1). Repeated measures of analysis of variance showed some differences in Cr recovery (P = 0.046) between days in period 1 (recovery on days 3 and 4 were lower (P < 0.05) than for most other days) but no between-day differences in period 2 (P = 0.60).

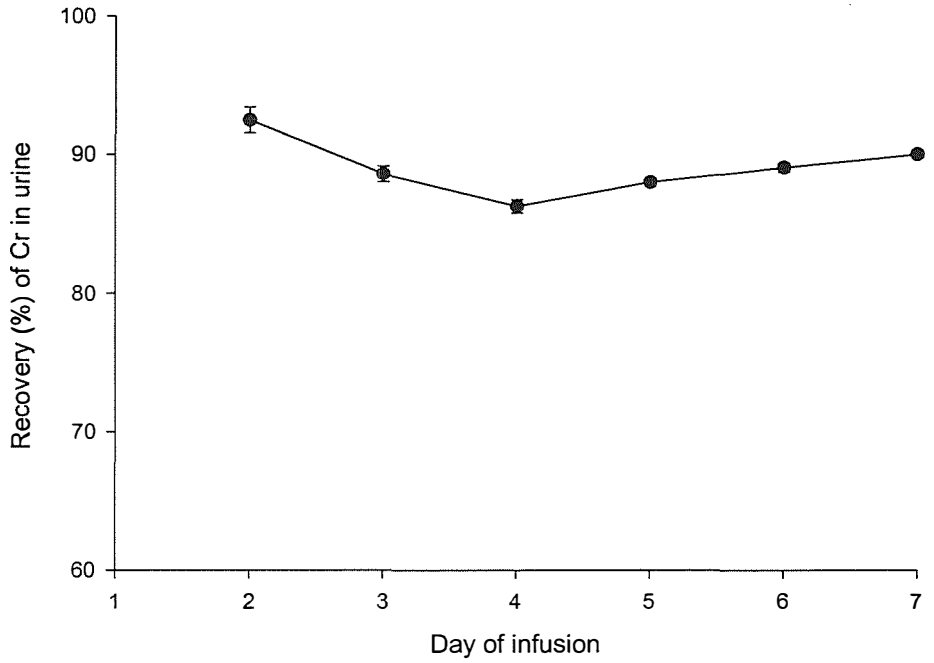


**Figure 5.1. Cumulative Cr recovery in urine of steers, averaged over both hay treatments.** Values are the mean of 11 steers; error bars represent the standard error of the mean.

Daily Li recovery in both periods was found to differ significantly between days (period 1: $P < 0.001$; period 2: $P = 0.034$) with recoveries increasing over time for steers consuming both native pasture and lucerne hays (Figure 5.2).

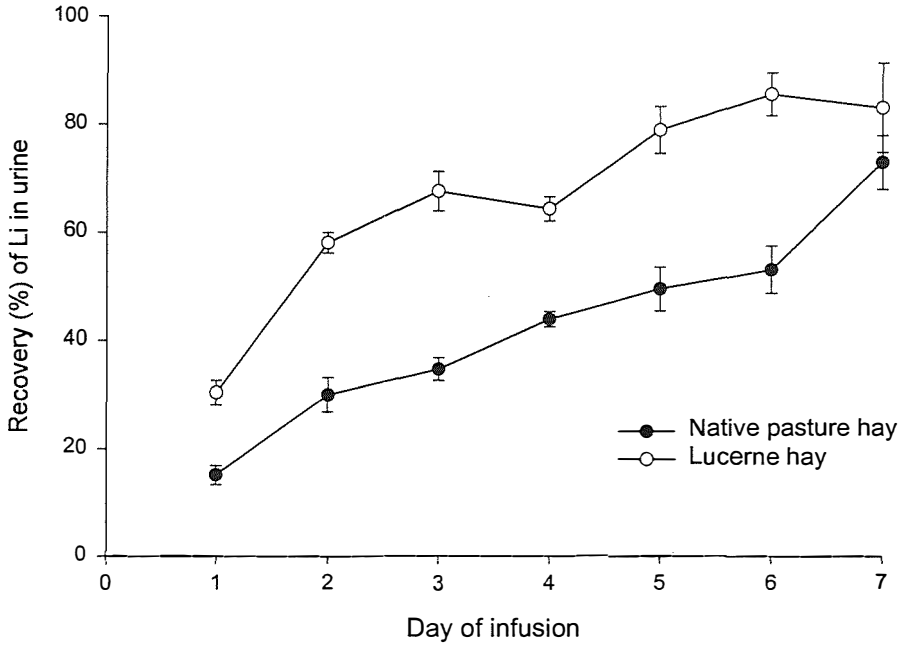


**Figure 5.2. Daily Li recovery in urine for steers consuming native pasture and lucerne hays.** Values represent means for 5 - 6 steers for that day of infusion; error bars represent the standard error of the mean.

#### *5.3.2.3 Concentration of Cr and Li in the faeces*

Minimal concentrations of Cr were detected in the spot faecal samples taken from steers after 6 d of jugular infusion (average 1.4 ± 0.38 μg/g DM). This level of Cr output in the faeces (*ca.* 2.5 mg/d), equates to *ca.* 2.4 % of the average 24-h Cr infusion. Greater concentrations of Li were detected in the same spot faecal samples, being 11.0 ± 2.35 μg/g DM for native pasture hay and 5.3 ± 0.95 μg/g DM for lucerne hay. These levels of Li output (*ca.* 17.0 and 10.5 mg/d) equate to *ca.* 17.3 % and 10.7 % of the average 24-h Li infusion for native pasture and lucerne hay, respectively.

#### *5.3.2.4 Decline in Cr and Li concentrations in urine and serum*

The concentration of Cr in the urine of steers consuming both native pasture and lucerne hays declined rapidly over the 5 h after cessation of infusion, while Li concentration declined less rapidly. It was not possible to determine total Cr and Li output in mg/h from the data collected as the concentrations of Cr and Li were monitored in spot samples, only. In addition, it was not possible to determine FDR due to lack of sufficient data points. This was particularly the case for steers consuming low quality native pasture hay due to the infrequency of urination events, resulting from low urine output, as compared to steers consuming lucerne hay. Representative figures for the decline of Cr and Li concentrations in urine over time, in steers consuming lucerne hay, are displayed (Figures 5.3 and 5.4).

Disappearance of both Cr and Li from the serum was very slow over 5 - 40 min after ceasing infusion, for steers consuming both of the hay treatment diets. While the concentrations of Cr in serum were similar for steers consuming both treatment diets, the concentrations of Li were greater in steers consuming native pasture hay compared to lucerne hay. In addition, serum Li concentrations were lower than serum Cr concentrations in steers consuming both diet types. Between 5 - 40 min after ceasing infusion, serum Li concentration on the native pasture hay diet was *ca.* 47 % of the average Cr concentration across both diets, while serum Li concentration on lucerne hay diet was *ca.* 23 % of the average Cr concentration across both diets. Representative figures are presented for decline in serum Cr and Li concentrations for steers consuming the lucerne hay diet (Figures 5.5 and 5.6).

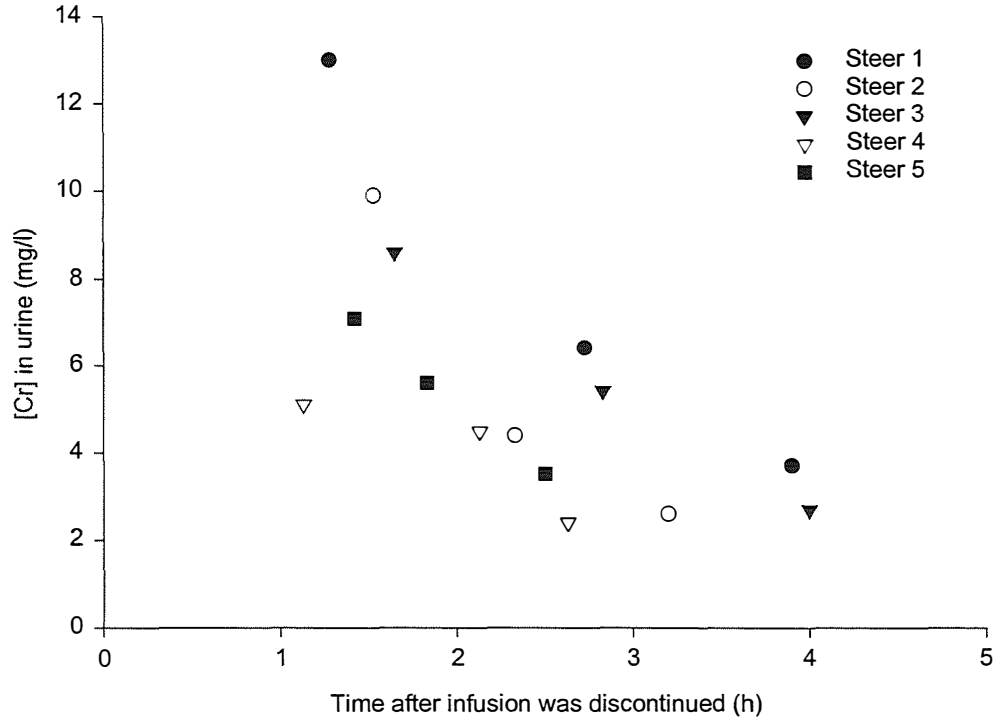


**Figure 5.3. Changes in Cr concentration in urine over time after cessation of infusion in steers consuming lucerne hay.**

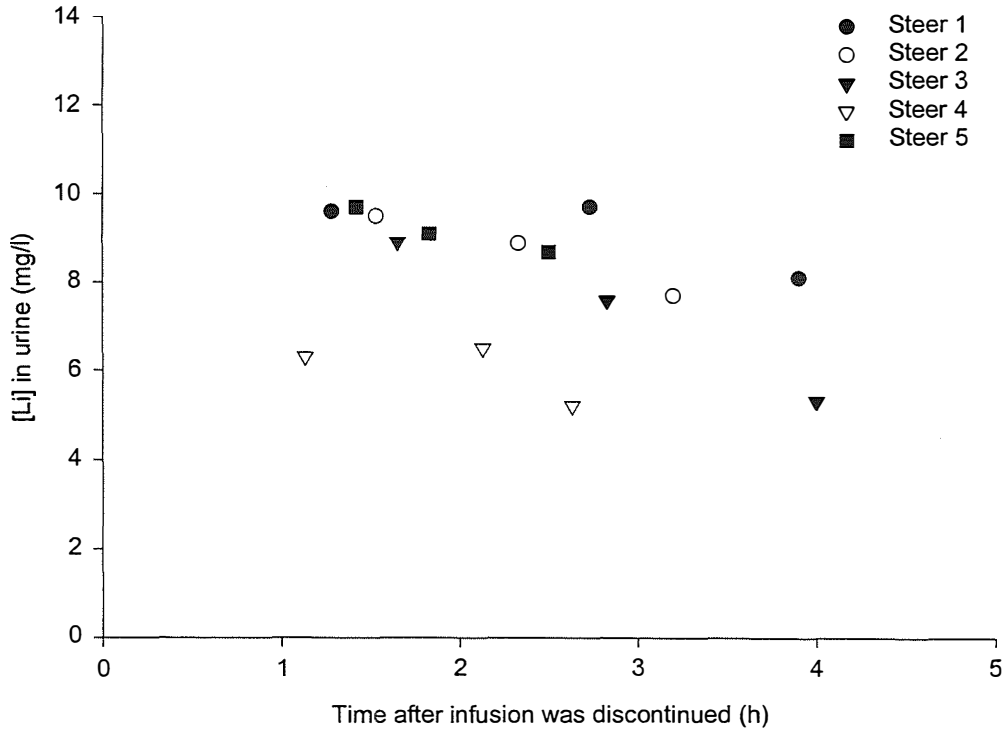


**Figure 5.4. Changes in Li concentration in urine over time after cessation of infusion in steers consuming lucerne hay.**

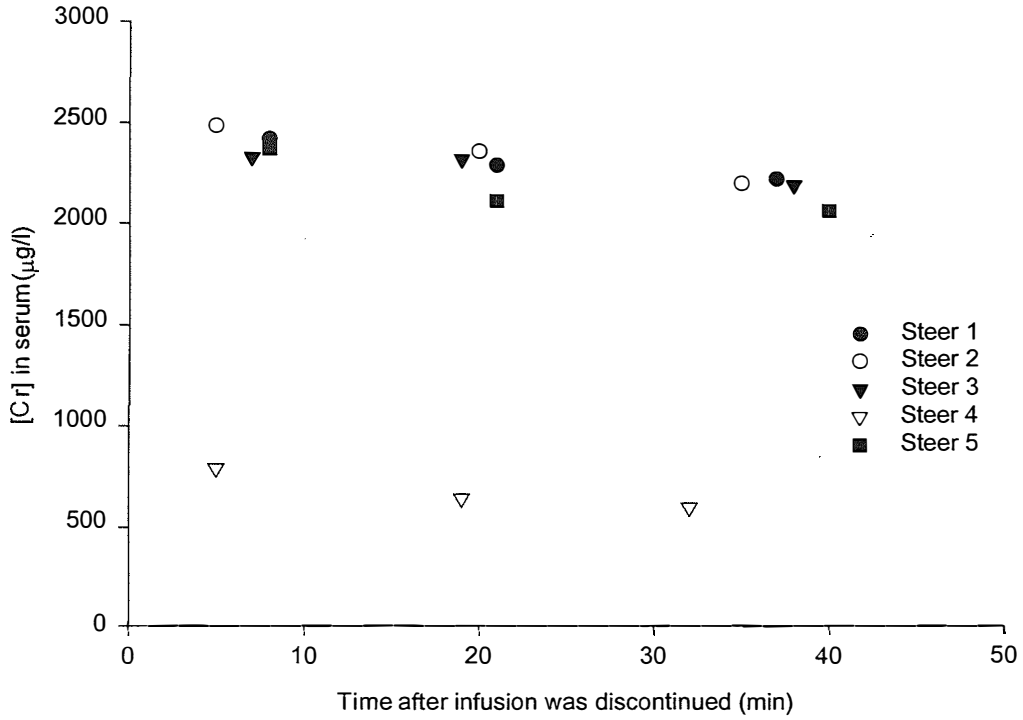


**Figure 5.5. Changes in Cr concentration in serum over time after cessation of infusion in steers consuming lucerne hay.**

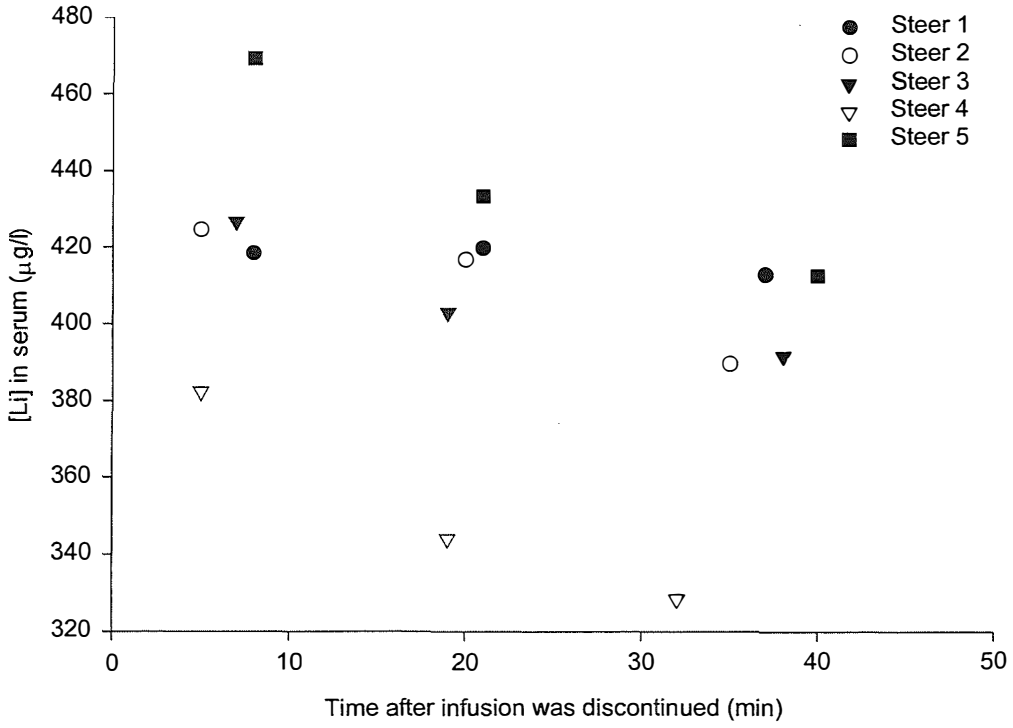


**Figure 5.6. Changes in Li concentration in serum over time after cessation of infusion in steers consuming lucerne hay.**

### 5.3.3 Experiment 5.3. Concentrations of Cr and Li in serum after, and in rumen fluid during, continuous jugular infusion into steers grazing tropical pastures

#### *5.3.3.1 Decline in Cr and Li concentrations in serum*

Disappearance of both Cr and Li from the serum was minimal over 0.02 to 2 h after ceasing infusion (Figures 5.7 and 5.8). The concentration of Li in the serum was similar to that for Cr. A serum sample taken from one of the steers *ca.* 48 h after cessation of infusion, contained Cr and Li in a similar order of magnitude (343 and 146 μg/l, respectively) to that measured 2 h after ceasing infusion.

#### *5.3.3.2 Concentration of Cr and Li in rumen fluid*

No Cr was detected in the rumen fluid of seven out of eight steers on either day 2 or day 6 of continuous jugular infusion. However, trace amounts of Cr were detected in the rumen fluid of one of the steers: 0.07 and 0.18 mg/l, on days 2 and 6 of infusion, respectively. Lithium was detected in the rumen fluid of all eight steers on both days of 2 and 6 of infusion. The Li concentration was higher on day 6 than day 2, with the average across all eight steers being $1.87 \pm 0.079$ and $0.41 \pm 0.025$ mg/l of rumen fluid, respectively.

### 5.3.4 Experiment 5.4. Concentration of Cr in the urine of steers after insertion of $Cr_2O_3$ capsule into the rumen

The average release rate of the $Cr_2O_3$ capsule cores was $1.87 \pm 0.032$ mm/d over the 13 d of incubation in the rumen, equating to a release of *ca.* 1.21 g Cr/d into the rumen. Negligible amounts of Cr were detected in the urine of individual steers either with or without $Cr_2O_3$ capsule insertion and there was no apparent increase in concentration with duration of capsule insertion in the rumen. The average Cr concentration in urine samples, bulked daily for the four treatment steers for d 3 - 13 of capsule insertion, was $0.011 \pm 0.0033$ mg/l.

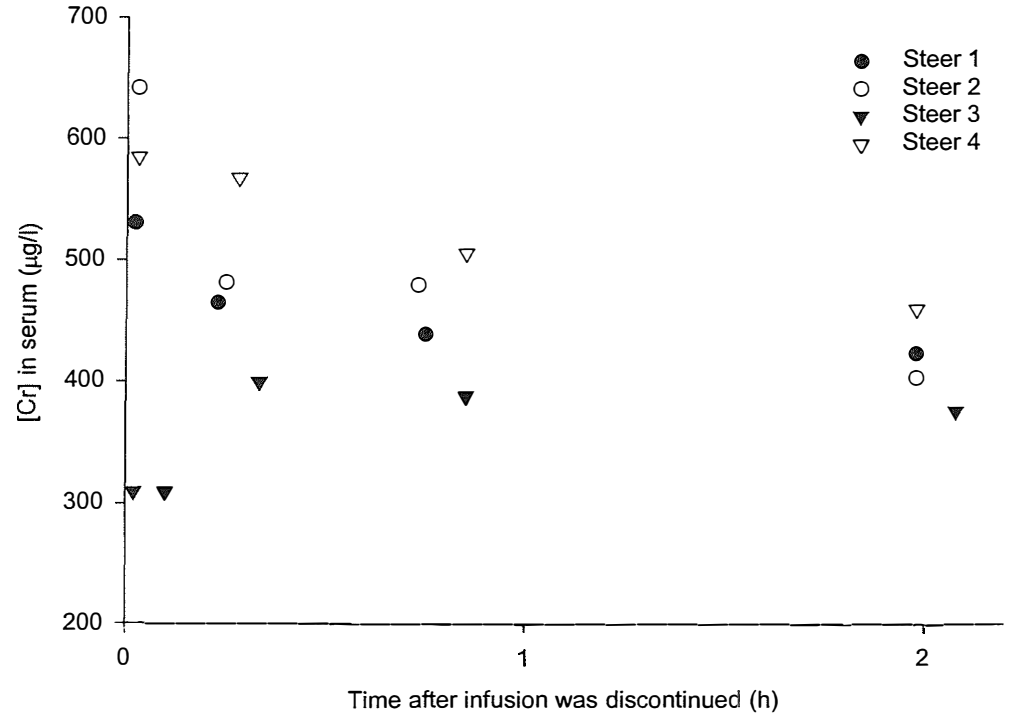


**Figure 5.7. Changes in Cr concentration in serum over time after cessation of infusion in steers grazing creeping bluegrass pasture.**

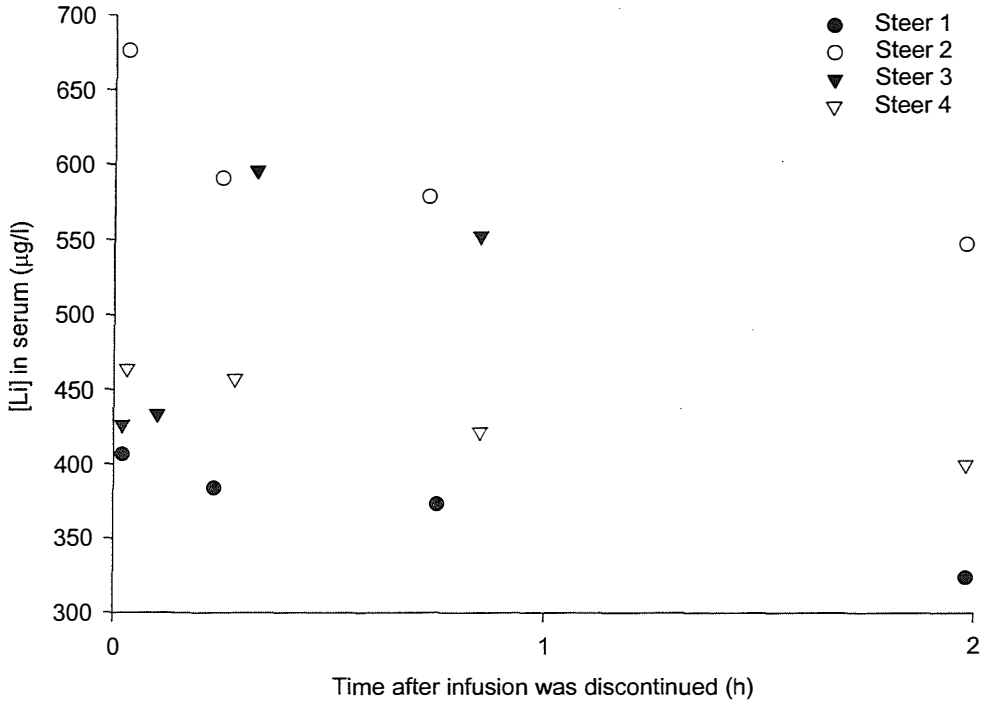


**Figure 5.8. Changes in Li concentration in serum over time after cessation of infusion in steers grazing creeping bluegrass pasture.**

## 5.4 DISCUSSION

### 5.4.1 CrEDTA as a urine output marker in cattle

These results have shown that Cr recovery in the urine of cattle during continuous intravenous infusion of CrEDTA was quantitatively high at 90 %, was relatively constant across days and across animals, and was not affected by diet quality (section 5.3.1.2, Table 5.3, and Figure 5.1). In support, Downes & McDonald (1964) also recorded *ca.* 90 % recovery of Cr in the urine of sheep after a single intravenous injection of $^{51}$Cr, with 86.5 % recovery within 24 h of injection. Combined, these findings indicate that Cr can be confidently used as a marker for estimating urine output, and thereby to estimate MCP production, in grazing ruminants. This was confirmed by the close agreement between estimates of MCP production determined using either total urinary PD output measurement or by the estimate of urine output based on Cr infusion (section 5.3.1.3).

Also in agreement with Downes & McDonald (1964), there was negligible Cr detected in the faeces of steers after jugular Cr infusion, with values being similar to the background concentrations measured pre-dosing (sections 5.3.1.2 and 5.3.2.3). However, these trace amounts in faeces represented up to 2 - 5 % of the daily Cr infusion. This could be a product of the difficulty experienced in accurately determining such low levels of Cr in the faecal samples. When Downes & McDonald (1964) used $^{51}$Cr, and thus had a much greater capacity to detect low levels of Cr in the faeces, they found no radioactivity in faecal samples collected after a single intravenous dose of $^{51}$Cr.

Consistent with the low faecal concentrations, there was, for the most part, negligible Cr in rumen fluid of RF steers, even after 5.5 d of intravenous infusion of CrEDTA (section 5.3.3.2). Downes & McDonald (1964), with sheep, also detected no $^{51}$Cr in rumen fluid samples taken up to 5 h after a single intravenous dose. However, in the current experiment trace amounts of Cr were detected in the rumen fluid of one of eight steers on both days 2 and 6 of infusion. Using the mean rumen fluid volume estimated for that steer in Experiment 6.3, Chapter 6 (41.0 l), the Cr present in the rumen fluid on days 2 and 6 would account for *ca.* 2.4 and 6.1 %, respectively, of the daily infusion for that steer. However, similar to the faecal samples, the

concentrations of Cr in the rumen fluid were near the limits of detection for the analytical technique used, raising some doubt about their accuracy. At the same time, some of the steers, including the steer for which Cr was detected in the rumen fluid, were displaying tendencies to "drink' each other's urine during this experiment on low quality tropical pasture, which may have contributed to the Cr detected in the rumen fluid.

To the author's knowledge, the current experiments and that of Downes & McDonald (1964) are the only research investigating Cr recovery in the urine of ruminant animals after intravenous infusion. It is not possible from these experiments to determine the fate of the 10 % infused Cr, which was not recovered in the urine. As implied above, it is possible that trace amounts of Cr were transferred to the rumen via saliva or returned to the gut post-ruminally. Additionally, in human subjects, Cr in the blood may be concentrated in the liver, spleen, and bone marrow (Furst *et al. ca*. 1998) and it is possible that some of the infused Cr may have a similar fate in ruminants.

Studies of the renal clearance of $^{51}$CrEDTA in sheep (Stacy & Thorburn, 1966) and humans (see review, Fogh-Andersen, 1980) have found CrEDTA to underestimate renal clearance values obtained with inulin by 5 - 15 %. Part of this difference has been explained by the binding of CrEDTA to plasma proteins, with Fogh-Andersen (1980) estimating that there was at least 10 % binding of CrEDTA to human albumin and Stacy & Thorburn (1966) finding that 1.5 - 2 % of $^{51}$CrEDTA in sheep plasma could not be removed by dialysis. This may have similarly contributed to the incomplete recovery of Cr in my experiment.

Evidence was provided in Experiments 5.1 and 5.2 for the rapid decline in concentration of Cr in urine after cessation of infusion to the extent that on the 2nd day after ceasing infusion in Experiment 5.1, Cr output in the urine had declined to *ca*. 1 % of that during the infusion period (section 5.3.1.2 and Figure 5.3). Similarly, Downes & McDonald (1964) found that after a single intravenous dose in sheep, the bulk of excreted Cr (96 % of that recovered in the urine) appeared in the first day's urine.

In contrast to the rapid decline in Cr concentration in urine after ceasing continuous infusion, serum samples taken up to 40 min (Experiment 5.2) and 2 h (Experiment 5.3), after ceasing infusion showed minimal decline (Figures 5.5 and 5.7). This was confirmed by a single sample at *ca.* 48 h after ceasing infusion in Experiment 5.3 which showed serum Cr concentration to be in the same range as that for samples taken within 2 h of ceasing infusion.

A limitation of the serum data in both Experiment 5.2 and 5.3 was the lack of a measurement prior to ceasing infusion. Although only *ca.* 1 - 2 min elapsed prior to the first sampling occasion in Experiment 5.3, and *ca.* 5 min in Experiment 5.2, it is possible that there was initially a rapid decline in Cr concentration prior to taking the first serum sample, with the curve thus approaching plateau for all subsequent measurements.

An alternative explanation is the release of Cr from a slow turnover reservoir pool. As indicated earlier, CrEDTA is known to bind to plasma proteins very strongly, with Stacy & Thorburn (1966) finding 1.5 - 2 % of $^{51}$CrEDTA in sheep plasma to be non-dialysable. Thus it could be inferred this strongly bound Cr would not dissociate under normal conditions in the plasma but would remain attached for the life of the protein to which it was bound. Eventual turnover of the plasma proteins should allow this Cr to be released and then either re-bound to other proteins or excreted in the urine.

The results of the current experiments differ from those of other groups who have measured FDR of CrEDTA from the blood after a single intravenous injection. Downes & McDonald (1964) found $^{51}$Cr to disappear rapidly from the blood circulation of sheep after a single intravenous administration of $^{51}$CrEDTA. These authors resolved the disappearance of specific activity over time (5 h) into two exponential functions with half-times of *ca.* 8 and 83 min, respectively. In experiments using cattle, Norton *et al.* (1979) and King *et al.* (1999) also observed a rapid decline in Cr concentration in the plasma, with King *et al.* (1999) reporting the FDR of CrEDTA from the plasma to be 1.3 - 1.5 %/min. While both research groups fitted a single exponential equation to their data, they both concluded that the dilution curve of $^{51}$CrEDTA would be most accurately described by two exponential functions,

i.e. a two-pool system. However, additional samples would be required during the first 15 min to accurately delineate the fast turnover pool.

Unfortunately, the sampling regime used in the current experiments, precluded the estimation of FDR of Cr from the serum after continuous intravenous infusion. However, the slow decline in concentration of Cr in serum after continuous intravenous infusion as compared to single intravenous injection and the possibility of contributions from a slow turnover reservoir pool, warrant further investigation.

The absence of Cr in the urine after insertion of $Cr_2O_3$ CRD in the rumen over 13 d (section 5.3.4) suggests that there is negligible transfer of Cr, from $Cr_2O_3$, from the rumen to the plasma. Coupled with the negligible transfer of Cr, from CrEDTA, from the plasma to the rumen and faeces as discussed earlier, it appears that the $Cr_2O_3$ CRD could be used as a faecal output marker at the same time that infusion of CrEDTA for urine output estimation is taking place.

### 5.4.2 Lithium sulphate as a urine output marker in cattle

The average Li recovery in urine over 6 d of intravenous infusion into cattle consuming low quality native pasture hay (46 %) was much lower than for lucerne hay (73 %) but similar to that reported by Suharyono (1992), (52 %) over 72 h after a single intravenous injection of LiCl into sheep given oaten chaff. These dietary effects and the discovery that Li recovery increased over days of infusion (Figure 5.2), indicate that Li is not a reliable marker for estimating urine output in ruminant animals.

Considerable amounts of Li were detected in the faeces and rumen fluid of steers that had received continuous intravenous infusion of lithium sulphate, providing evidence of transfer between compartments (sections 5.3.2.3 and 5.3.3.2). The estimated daily excretion of Li in the faeces of steers, by day 6 of continuous jugular infusion, was equivalent to *ca*. 11 and 17 % of the daily Li infusion for steers consuming lucerne and native pasture hay, respectively. Once again, these results are similar to those found by Suharyono (1992) who recovered 17 % of a single intravenous dose of LiCl, in the faeces of sheep consuming oaten chaff. Using measured rumen fluid volumes from Experiment 6.3, Chapter 6 (average: 40.8 l), the quantities of Li detected in the

rumen fluid of steers grazing low quality, dry season native pasture on day 2 and 6 of continuous jugular infusion of lithium sulphate (Experiment 5.3) equated to 13.8 and 63.8 % of the average 24-h Li infusion for day 2 and 6, respectively.

The difference in urinary recovery between steers consuming low and high quality feeds is interesting. The pattern of daily Li recovery in the urine for steers consuming lucerne hay (Figure 5.2) indicates that the recovery of Li in the urine may have reached plateau by day 6 of continuous infusion, at *ca.* 84 % of that infused. In contrast, the daily recovery of Li in the urine of steers consuming low quality native pasture hay was still increasing on day 7 of infusion and was only *ca.* 73 % of the amount infused. A greater urinary output per unit of body weight was recorded for steers consuming lucerne hay as compared to native pasture hay in this experiment (5.0 vs. 2.5 l/100 kg LW), most probably related to the greater N content and intake of the lucerne hay. It may be possible that a greater plasma pool size on lucerne hay, inferred from the greater urinary output, was linked to a greater capacity for excretion of plasma Li.

Considerable evidence has been presented to indicate that Li in plasma can be recycled to the rumen in saliva or returned to the gut post-ruminally (Harrison *et al.* 1963; Ulyatt, 1964; Walker & Hawley, 1965; Suharyono, 1992). It is possible that greater recycling of Li, and thus a greater proportion of Li excretion in the faeces on the native pasture hay diet, was associated with greater recycling of N to the rumen on low quality native pasture hay (43 g CP/kg DM) compared with lucerne hay (193 g CP/kg DM).

As discussed previously, a limitation of the current data set was the lack of sufficient data points to determine FDR, of Cr and Li, in the urine and plasma after ceasing continuous intravenous infusion. However, the data indicates that the decline in Li concentration in the urine was much less rapid than for Cr (Figures 5.3 and 5.4). Similar to the situation for Cr run-down in the serum, the disappearance of Li from serum was very minimal in samples taken over 40 min (Experiment 5.2) and 2 h (Experiment 5.3) after lithium sulphate infusion was discontinued (Figures 5.6 and 5.8). The slow decline in concentration of Li in the urine and plasma may be a

product of the re-entry of Li into the plasma, and eventually into the urine, from other pools within the body, and in particular from the gut.

The lower concentration of Li as compared to Cr in serum of animals intravenously infused with similar concentrations of Cr and Li in the infusate solution (section 5.3.2.4) provides further supporting evidence for the transfer of Li to the gut and other pools within the body. A greater concentration of Li in serum of steers consuming native pasture compared to lucerne hay may be indicative of a greater plasma pool in the steers consuming the higher quality diet.

### 5.4.3 Conclusions

The suitability of CrEDTA as a urine output marker in ruminant animals is indicated by the constant urinary recovery, over time and with varying feed quality, of *ca.* 90 % intravenously infused CrEDTA. This technique has potential for use in field situations for estimating PD excretion, and thus MCP synthesis, from spot urine samples. Due to negligible transfer of Cr between compartments, intraruminal $Cr_2O_3$ capsules could be used to determine faecal output in conjunction with jugular infusion of CrEDTA for urine output estimation. In contrast, intravenously infused lithium sulphate is unsuitable as a urine output marker due to transfer of Li between compartments in the body and variable urinary recovery influenced by feed quality and duration of infusion.

# Chapter 6

# The efficiency of microbial protein production in cattle grazing tropical pastures

## 6.1 INTRODUCTION

Microbial protein provides a significant proportion of the total protein supply to the ruminant (Owens & Zinn, 1988). Low values for eMCP have been recorded for ruminants consuming tropical forages (see section 2.4). Such values have been much lower than the range of values stated in the various feeding systems (130 - 170 g MCP/kg DOMI; ARC, 1984; Madsen, 1985; Ausschuss fur Bedarfsnormen, 1986; INRA, 1988; SCA, 1990; AFRC, 1993; NRC, 1996b).

However, there is a lack of information on the variability in eMCP for tropical grass and legume species, and the effects of seasonal conditions. In addition, there is a dearth of information on eMCP in ruminants consuming fresh tropical forages under grazing conditions. Tropical pastures are characteristically very heterogenous, in terms of both species composition and plant morphology, and vary with land system and soil type. Thus eMCP values obtained in metabolism crates for ruminants consuming chaffed, mature hays may not be representative of those obtained when pasture is consumed under field conditions where ruminants can selectively graze species and plant parts (Stobbs, 1975; Corbett, 1976).

The objectives of this series of experiments were to measure eMCP in cattle grazing a range of tropical pasture types over varying seasonal conditions, and to compare these values with those for the temperate species, ryegrass. Furthermore, measurement of associated pasture parameters, and animal digestion and metabolic factors, was undertaken to identify the factors of most importance in achieving high rates of eMCP.

*EXPERIMENTS 6.1 - 6.9*

## 6.2 MATERIALS AND METHODS

### 6.2.1 General location and period

Experiments 6.1 - 6.7 were conducted as grazing experiments on Brian Pastures Research Station, Gayndah during the 13-month period, January 2000 to January 2001. The research station is located within the sub-humid, sub-tropical 700 - 1000 mm rainfall zone, of Queensland. This area is characterised by summer rainfall (70 % occurs between October and March), which is highly variable in quantity, between years. The average annual rainfall at the research station is 708 mm. The research station is situated in the black speargrass zone of sub-coastal Queensland and the dominant pasture species is black speargrass (*Heteropogon contortus*), which is often associated with forest bluegrass (*Bothriochloa bladhii*) on the alluvial flats.

Associated nylon bag incubations on a standard diet (Experiments 6.8 and 6.9) occurred after the completion of all grazing experiments, in March and September 2001.

### 6.2.2 Experimental animals

Six to eight RF, high-grade Brahman (> 75 % *B. indicus*) steers were used in each of the seven grazing experiments (Experiment 6.1 - 6.7) and the nylon bag incubation studies (Experiments 6.8 and 6.9). At the commencement of experimentation, the steers were *ca.* 15 months of age with an average liveweight of 313 ± (SE) 8 kg. By the final pasture experiment, the steers were *ca.* 27 months of age and 471 ± 11 kg liveweight. When buffalo flies were an irritation to the cattle, ear tags containing 200 g/kg Diazinon (Spike Tags, Novartis Animal Heath, Australia) were used.

Two to four OF, high-grade Brahman (> 75 % *B. indicus*) steers were also used in each of the grazing experiments (Experiments 6.1 - 6.7). They were *ca.* 5 years of age and 599 ± 27 kg liveweight at the commencement of experimentation.

A group of eighteen non-fistulated Brahman (> 75 % *B. indicus*) steers were used in sub-groups of six for determination of liveweight gain (hereafter referred to as LWG steers) in each grazing experiment (Experiments 6.1 - 6.7). These steers were *ca.* 15 months of age and 325 ± 5 kg liveweight at the commencement of experimentation. They were of a similar genotype and age to the RF steers.

### 6.2.3 Experimental plan and diets

***Pasture types and associated soil types and management***

The seven pasture types studied (Experiments 6.1 - 6.7) can be categorised into four main groups, and include:

1) tropical grass pasture ($C_4$) native to the southern speargrass region of Queensland (major species black speargrass (*Heteropogon contortus*) and forest bluegrass (*Bothriochloa bladhii*)) grazed in the (i) early wet season (NPEW, Experiment 6.1), (ii) wet/dry transitional period (NPT, Experiment 6.2) and the (iii) dry season (NPD, Experiment 6.3);
2) introduced tropical grass pasture ($C_4$), creeping bluegrass (*Bothriochloa insculpta* cv. Bisset), (BB, Experiment 6.4), grazed in the mid wet season;
3) introduced tropical legumes ($C_3$), Dolichos lablab (*Lablab purpureus* cv. Highworth), (LL, Experiment 6.5) and butterfly pea (*Clitoria ternatea* cv. Milgarra), (BP, Experiment 6.6); and
4) temperate grass pasture ($C_3$), annual ryegrass (*Lolium multiflorum* cv. Tetila), (RG, Experiment 6.7).

The pastures were studied sequentially, as influenced by seasonal availability and rainfall, commencing on the following dates:

28 January 2000 - NPT (Experiment 6.2),
17 March 2000 - LL (Experiment 6.5),
20 April 2000 - BP (Experiment 6.6),
1 June 2000 - RG (Experiment 6.7),
14 July 2000 - NPD (Experiment 6.3),

24 November 2000 - NPEW (Experiment 6.1),

5 January 2001 - BB (Experiment 6.4).

Experiments 6.1, 6.2 and 6.3 studying NPEW, NPT and NPD, respectively, were conducted on the same paddocks each time. The RF and OF steers were grazed in one paddock and the LWG steers in a second, adjacent paddock, both of which were 4.05 ha. The soil type in this area was a hard setting to weakly self-mulching brown or dark non-cracking and cracking clay (Reid *et al.* 1986). Approximately 1 month prior to commencement of grazing (27 October 2000) for the NPEW study, the native pasture paddocks were burned following 34 mm of rainfall.

A 3.0 ha, 4-year old pasture of creeping bluegrass, grown on soils of moderately to strongly self-mulching brown or dark clays (Reid *et al.* 1986), was used for the BB treatment (Experiment 6.4). The BB paddock was fertilised *ca.* 1 month prior to the start of the experiment (11 December 2000) with 53 kg urea/ha in order to ensure adequate pasture availability.

The BP experiment (Experiment 6.6) was conducted on a recently established pasture (planted 5 January 2000). The soil type in the BP paddock consisted of moderately to strongly self-mulching brown or dark clays (Reid *et al.* 1986). The LL and RG experiments (Experiments 6.5 and 6.7, respectively) were also conducted on recently established pastures on soils of moderately to strongly self-mulching brown or dark clays (Reid *et al.* 1986).

All pastures were dryland (non-irrigated) except for LL and RG, which were irrigated using a travelling irrigator system. Animal numbers and/or paddock areas were varied for each experiment to maintain an appropriate stocking rate for the period of grazing, and for the type and quality of pasture under study. Details of pasture presentation yield and composition are given in the section 6.3.1 of this chapter.

All pastures except for RG were continuously grazed during the experimental period. Grazing of the RG pasture was conducted according to a four-paddock rotational system with an average of 5 d grazing followed by 15 d rest, per paddock. The speed of the rotation was varied according to the pasture growth rate so that pasture was

grazed from *ca.* 20 cm to no lower than 5 cm (minimum of 2.5 cm remaining leaf) before cattle were moved to the next paddock. If the pasture growth rate was excessive, additional stock were brought on to the paddocks being vacated to graze the pasture down to the 5 cm level. On the day the cattle vacated each paddock, fertiliser was applied (76 kg urea/ha) and irrigation was undertaken (12 or 25 mm).

***Experimental plan***

The RF, OF and LWG steers grazed each pasture type for a period of *ca.* 4, 2, and 6 - 11 weeks, respectively. Each experimental period for RF steers consisted of a minimum of 2 weeks of preliminary grazing and marker dosing followed by 2 weeks of sampling and measurements. After a minimum of 6 d preliminary grazing on the treatment paddock (and up to 3 weeks, depending on pasture type), the OF steers were sampled at least twice, often on consecutive days, during the first sampling week for the RF steers. The LWG steers were grazed in a separate, adjacent, paddock to the RF and OF steers. They were grazed concurrently with the RF and OF steer experimental period, but over an additional 4 - 9 weeks (depending on the pasture type). There were two exceptions to this approach. With the BB experiment, LWG steers were not used due to lack of available pasture area. In the RG experiment, there was insufficient available pasture so the LWG experimental period commenced after the RF and OF steer experimental period, but using the same paddocks.

### 6.2.4 Experimental procedures

#### *6.2.4.1 Marker infusion and dosing procedures - Experiments 6.1 - 6.7*

***Jugular infusion of CrEDTA***

Catheterisation of RF steers occurred 3 d prior to commencement of each sampling period, as described in section 3.4.1. Pump connection and infusion procedures are also described in section 3.4.1. As the steers were grazing during the study, the steers were fitted with saddle cloths and packs to hold the infusion equipment. The packs contained the battery-operated, peristaltic infusion pump on the left side and a weight, for balance, on the right side. A protective cover was placed around the neck and held in place by attaching it to both the halter and pack, in order to protect the loose

catheter tubing between exit from the neck-bandage and entry to the pack. The equipment worn by the RF steers during intravenous infusion is shown in Plate 3.

Infusion of CrEDTA commenced on the 2nd day after catheterisation (1 day prior to commencement of the sampling period), and continued for 6 d. The average concentration of the infusate solution for each of the experiments was as follows: Experiment 6.1: 292 mg Cr/l; 6.2: 1086 mg Cr/l; 6.3: 278 mg Cr/l; 6.4: 293 mg Cr/l; 6.5: 511 mg Cr/l; 6.6: 494 mg Cr/l; and 6.7: 518 mg Cr/l. The highest Cr concentration was used for Experiment 6.2, which was the first experiment conducted. As Cr concentration in the urine was well in excess of that required for analytical detection, the concentration was reduced for the next three experiments: 6.5, 6.6 and 6.7, and for the same reason, was reduced again for the final three experiments: 6.3, 6.1 and 6.4. Lithium sulphate was infused in conjunction with CrEDTA for all experiments except Experiments 6.2 (NPT) and 6.5 (LL). However, due to the results obtained in Experiment 5.2, which showed intravenously infused lithium sulphate to be unsuitable as a urine output marker, the results for Li infusion for the Chapter 6 grazing experiments are not included in this thesis.

***Rumen dosing of Yb marker***

The concentration of Yb in the marker doses was varied for each experiment, so that the dose rate would approximate 55 mg Yb/kg estimated DMI of the RF steers. The RF steers were dosed (see section 3.4.2) with one packet of Yb-marked hay, twice-daily at *ca.* 07.30 h and 16.30 h, for the 5 d preceding the start of sampling and for the following 5 d of sampling. On the 6th day of sampling, a final Yb marker dose was inserted into the rumen of each RF steer at *ca.* 09.00 h, following the morning grazing period.

***Rumen dosing of alkanes***

One controlled release alkane capsule (Alkane CRC, Captec, New Zealand) designed for 300 - 650 kg cattle, containing 8 g of n-dotriacontane ($C_{32}$) plus 8 g n-hexatriacontane ($C_{36}$), was inserted into the rumen of each RF steer 7 d prior to commencement of each sampling period. The capsule was removed after a 5-d sampling period (12 d after insertion). The dosing, measurements and sub-sampling procedures are described in section 3.4.3.

***Rumen dosing of CrEDTA***

A single dose of concentrated CrEDTA solution was injected into the rumen of each RF steer at *ca.* 09.00 h, following the morning grazing period, on the 6th day of each RF sampling period. The dose rate and dosing procedure are described in section 3.4.4.

### *6.2.4.2 Samples and measurements - Experiments 6.1 - 6.7*

***RF steer sampling schedule for the first 5 d of the sampling period***

Urine, faecal and rumen fluid samples were collected from RF steers twice-daily, at varying times, over the first 5 d of each RF sampling period. The times of sampling were *ca.* 01.30, 04.00, 06.30, 08.00, 10.30, 13.00, 15.30, 18.00, 20.30, 23.00 h. On any one day, sampling was separated by at least 9 h. Over the 5-d sampling period, ten samples of each type, per RF steer, were collected. On each sampling occasion, a team of four sample collectors approached the steers while they were grazing or resting in the paddock. Voluntary urine and faecal samples were collected at this time as described in section 3.4.6. The steers were then walked to portable panel yards situated in the test paddock. Here, urine and faecal sampling continued from those steers which had not yet given a sample, while rumen fluid sampling, and any other dosing or infusion procedures were conducted as required. The steers were released to pasture as soon as sampling was complete.

***Urine samples from RF steers***

Spot urine samples were collected from the RF steers twice daily as described above and in section 3.4.6. Subsamples were taken and processed prior to PD and Cr analysis as described in section 3.4.7.

***Faeces from RF, OF and LWG steers***

Spot faecal samples (one large handful) were collected from RF, OF and LWG steers and processed as described in section 3.4.6. Faecal samples were taken from the RF steers at the times given above. Only one representative bulk sample (of the ten individual samples for each steer) was analysed for OM, N, Yb and alkanes. Spot faecal samples were taken from each of the OF steers on the days of extrusa sampling. These samples were frozen and then freeze dried for later alkane analysis. In

addition, fresh faecal samples were collected from the ground, for five of the LWG steers during the RG grazing period (Experiment 6.7), and kept chilled until worm egg counts could be performed by the Department of Primary Industries, WORMCHECK laboratory, Yeerongpilly.

***Rumen fluid from RF steers***

Rumen fluid samples were collected from the RF steers twice daily according to the schedule given above, as described in section 3.4.9. The pH of each rumen fluid sample was measured, and subsamples taken for VFA and $NH_3$ analysis, as set out in section 3.4.9. On six of the ten sampling times (04.00, 08.00, 13.00, 15.30, 18.00, and 23.00 h), rumen fluid was kept for each steer for $NH_3$ analysis. One representative bulk sample for each steer (of the ten rumen fluid samples) was analysed for VFA. Two rumen fluid samples per steer (at 06.00 and 20.30 h, before and after the main daily grazing periods, respectively) were kept for protozoa enumeration from all pastures except NPT, as described in section 3.4.9.

After injection of CrEDTA into the rumen at 09.00 h on day 6 of the RF sampling period, rumen fluid samples were collected for Cr analysis from each of the RF steers at times 4, 8, 12, 21, 25, 29, 33, 36 and 48 h after dosing, as described in section 3.4.9.

***Rumen fluid from LWG steers***

Rumen fluid was extracted from two LWG steers (those with the highest and lowest liveweight gain, respectively) during the RG experiment (Experiment 6.7) at *ca.* 13.30 h. A stomach tube and suction pump was used to extract the fluid. To reduce saliva contamination, the first 50 - 200 ml of rumen fluid collected was discarded. The following 200 ml of rumen fluid was strained through nylon stocking. The pH of the sample was reduced to below 3 by adding concentrated sulphuric acid. The samples were immediately placed on ice and later frozen awaiting analysis for $NH_3$-N.

***Rumen digesta from RF steers***

Rumen digesta samples were collected and processed, as described in section 3.4.10, from each of the RF steers over d 6 - 8 of the sampling period at times 4, 8, 12, 25, 36

and 48 h after final Yb marker dosing at 09.00 h on day 6. The 8-h samples were not used for RG pasture due to having very low Yb concentrations.

***Serum urea from RF and LWG steers***

Whole blood samples were taken from each RF steer via the jugular catheter 2 d prior to commencement of the main sampling week, at *ca.* 07.00 - 09.00 h, just prior to commencement of CrEDTA infusion. Blood samples were also taken from the tail vein of two LWG steers (those with the highest and lowest liveweight gain, respectively) during the RG experiment (Experiment 6.7) at *ca.* 13.30 h. All blood samples were processed according to section 3.4.8 and analysed for urea-N concentration.

***Liveweight of LWG, RF and OF steers***

The LWG steers were weighed weekly over each experimental period except for NPT where weights were taken twice-weekly. NPT (Experiment 6.2) was the first experiment conducted, and weighing was reduced to once-weekly for subsequent experiments, as this frequency was deemed adequate. The average liveweight gain presented for each pasture type excluded a 1 - 2 week period of adaptation to each diet. The experimental period was varied according to the time over which each pasture type remained constant in quality and composition (7 - 11 weeks, excluding preliminary grazing periods). Although liveweight measurements were taken for 48 d in the NPEW experiment, results were presented for the first 21 d only, due to a non-linear pattern of liveweight gain after this time which was associated with changes in pasture quality and availability. Between experiments, LWG steers were grazed on native pasture paddocks similar in composition to those studied in Experiments 6.1 - 6.3. Prior to commencement of the NPEW experiment, LWG steers had grazed dry season native pastures followed by *ca.* 1 month grazing on un-burnt native pasture that had received summer rain.

Fasted weights (24 h without food and water) were also determined for the LWG steers at the commencement and completion of each LWG measurement period. However, this procedure was deemed to be a less accurate indicator of weight change due to the effect of feed type and quality on the proportion of gut fill lost during this

relatively short period of fasting. Thus, the liveweight gain results obtained via the fasting method are not included in the results section.

In addition, the liveweight of the RF and OF steers was measured at the commencement and completion of each sampling period. The RF liveweights presented in the results section were generally taken on the day of catheterisation, or as close to the main RF sampling week as possible.

***Extrusa samples from OF steers***

Prior to extrusa sampling, the OF steers were held in yards, off pasture, overnight. At *ca.* 08.00 h the following morning, the oesophageal fistulae were removed and canvas sampling bags attached securely around the neck of each steer. The bags were positioned so that any feed consumed would pass out of the oesophageal opening and into the bag. The steers were then allowed to graze the test pasture for *ca.* 30 min, or until the bags were filled. If the quantity of sample obtained was insufficient, the emptied bags were put back on the animals and the process was repeated. Any samples containing excess saliva were discarded. Extrusa samples were mixed thoroughly, then placed directly on to dry ice and later frozen. Subsamples were freeze-dried prior to analyses for OM, N, NDF, ADF, NDIN and alkane content, and for IVOMD determination and use in nylon bag incubations.

***Pasture sampling***

The test pastures grazed by the RF and OF steers and the LWG steers were sampled at least once during the sampling period between *ca.* 08.00 and 10.30 h in order to estimate pasture presentation yield, DM content, height and species composition. Pasture measurements in the paddocks grazed by the RF and OF steers were made immediately prior to the RF steer sampling period, or during this period in the case of the RG experiment. Only those results for the pastures grazed by the RF and OF steers are presented in this thesis. Additional samples were taken from the pastures grazed by the RF and OF steers for determination of the DM proportions, and for chemical analysis, of the leaf, stem and dead components and for nylon bag incubations of plucked leaf material. Additional plucked leaf samples were also taken from the pasture grazed by the LWG steers during the RG experiment.

*Pasture presentation yield, DM and species composition*

Due to the large area to be sampled, the variability in the sampling area and the large number of species present, the BOTANAL procedure described by Tothill *et al.* (1992) was used to assess yield and species composition in NPEW, NPT and NPD pastures. On each sampling occasion and in each paddock, five trained operators assessed *ca.* twenty-five, 0.25 $m^2$ quadrats for yield and botanical composition. The visual yield estimates were calibrated with ten cut quadrats on each occasion. The Forall computer program (Forall 1.03, CSIRO, Australia) was used to process the data as described by Hargreaves & Kerr (1992).

On BB, LL, BP and RG pastures, yield was estimated from the average of cut quadrats, which were oven dried (60 - 70$^0$C). The size (0.25 $m^2$, 0.5 $m^2$, or 1 $m^2$) and number of quadrats, as well as the sampling design, was varied according to the layout and size of the paddock and the density of pasture species. Determination of botanical composition was unnecessary for BB, LL and RG pastures, as all were monocultures. However, the BP pasture also contained some dry grass species, dry sorghum (*Sorghum bicolour*) stalks and weeds, and the proportion of these was determined on each cut quadrat.

The DM content of the pasture was determined for NPEW, NPT and NPD experiments as the average of quadrats cut for BOTANAL calibration. For all remaining pastures the DM content was determined as the average of all quadrats cut for yield determination.

*Pasture height*

On each pasture sampling occasion, an estimate of pasture height was made by one operator on each quadrat sampled by that person. These estimates were averaged. A ruler was used to make the measurements. For all the pastures except LL, height was estimated as the average of the highest leaves in each quadrat. Due to the significant height and bulk of the LL pasture, height was determined by measuring the highest leaf of the plant closest to the right-hand corner of each quadrat.

*Photographic records*

Photographs were taken on the days of pasture sampling. Three photographs were taken on each occasion, at a constant site for each paddock including 1) a landscape perspective across the paddock, 2) at a $30^0$ angle relative to the ground, and 3) at a $90^0$ angle relative to the ground. Only one representative photograph of each of the pasture types is shown in this thesis. The photographs presented were taken in the paddock grazed by the RF and OF steers and at a $30^0$ angle relative to the ground.

*Leaf, stem and dead components*

On each pasture sampling occasion, additional quadrats or plants were cut in the paddock grazed by the RF and OF steers for determination of green leaf, green stem, and dead material proportions in the pasture. For the grass pastures (NPEW, NPT, NPD, BB and RG), ten representative, randomly selected, quadrats were cut on each occasion (usually on the two diagonals of the paddock) and bulked for later sub-sampling and sorting. For the legume pastures (LL and BP), sampling for leaf, stem and dead composition occurred at the time of cutting quadrats for yield estimates. The plant outside each quadrat, closest to the right-hand corner, was cut for proportional leaf, stem and dead determination. These individual plants were bulked for each pasture type and then thoroughly mixed and progressively halved until a manageable portion remained for sorting into plant components.

For the grass pastures (NPEW, NPT, NPD, BB and RG), the plant components were defined as follows:

1) green leaf material included the leaf blade down to the ligule, where present, or otherwise to the point at which the leaf blade attached to the stem;
2) green stem material included the stem plus leaf sheath;
3) dead material was either leaf or stem consisting of > 50 % dead material.

For the legume pastures (LL and BP) the plant components were defined as follows:

1) green leaf material for LL pasture included each group of three leaflets and their associated rachis, which was pinched off where it joined the petiole. Leaf material for BP pasture included individual leaflets which were pinched off where they joined the rachis;
2) green stem material for both pasture types included all other green material other than that classified above as leaf;
3) dead material was either leaf or stem material consisting of > 50 % dead matter.

The sorted plant components were oven dried (60 - 70$^0$C) to determine their proportion on a DM basis and were retained for alkane analysis.

*Plucked leaf sampling*

On one occasion for each pasture type, during the first RF steer sampling week, representative samples of pasture leaf were plucked by hand from the RF steer test pasture at *ca.* 10.00 - 11.00 h and placed directly on to dry ice to produce a minimum bulk sample of 370 g estimated DM. The leaf material was categorised as defined above with the exception of LL, which included individual leaflets without the rachis. The bulk sample for each pasture was subsampled to produce a minimum of 80 g estimated DM which was cut up with scissors into *ca.* 2 cm segments, initially frozen and then freeze-dried awaiting analysis for content of total non-structural carbohydrates (TNSC), OM, N, NDF, ADF and nitrates (RG pasture only). The remaining portion of the bulk sample was kept frozen and later processed for nylon bag incubation.

In addition, plucked leaf samples were taken from the pasture grazed by LWG steers during the RG experiment on the 2nd day of grazing in that rotation, at *ca.* 17.30 h.

***Nylon bag incubation of plucked leaf***

The DM degradability of plucked leaf in the rumen of RF steers was determined while the steers were grazing the same test pasture as that from which the leaf was plucked. A standard feed was incubated at the same time as the plucked leaf material, to allow

comparison across the pasture types. The standard feed consisted of 3 mm ground native tropical grass pasture hay (major species *Heteropogon contortus* and *Bothriochloa bladhii*) harvested at Brian Pastures Research Station in early February 2000.

On each occasion, the frozen, plucked leaf (described above) was removed from the freezer and processed in a laboratory blender. The equivalent of 5 g DM of blended leaf was then weighed into empty nylon bags and re-frozen until incubation. The equivalent of 5 g DM of standard feed was also weighed into individual nylon bags and stored until incubation. Duplicate bags for each time of removal were incubated in each of three RF steers for plucked leaf samples and each of two different RF steers for the standard feed. The nylon bags were inserted into the rumen of each of the RF steers at *ca*. 09.00 h on the day 8 of the RF sampling period. The bags were removed after 3, 6, 9, 12, 24, 48, 72, and 96 h of incubation. In addition four 0 h bags (not incubated in the rumen) were washed and processed for each pasture. Details of bag specifications, removal and processing are given in section 3.4.11.

#### *6.2.4.3 Nylon bag degradability study of extrusa on a standard diet - Experiment 6.8*

Freeze-dried, 3 mm ground extrusa collected from each of the seven pasture experiments (as described above) was incubated in the rumen to determine degradability parameters after the completion of Experiments 6.1 - 6.7. During nylon bag incubation RF steers were fed on a standard diet as described in section 3.4.11. A representative bulk extrusa sample for each pasture type was incubated in duplicate for each of ten incubation times, in each of three RF steers. Bags were removed after 3, 5, 8, 11, 13, 16, 24, 48, 72, and 96 h of incubation and processed as described in section 3.4.11. In addition, four 0 h bags were washed and processed for each pasture type. After individual bag proportional DM loss was determined, the nylon bag residue was bulked across all steers and duplicates for each incubation time. Each bulk sample was analysed for OM content while the 5, 8, 11, 13 and 16 h samples were analysed for NDIN and ADIN content to allow determination of diet RDP content (see section 3.6.5). A representative, unincubated bulk sample for each pasture type was also analysed for NDIN and ADIN content.

#### *6.2.4.4 Nylon bag incubation of faeces and extrusa for IADF determination - Experiment 6.9*

Nylon bag incubations as part of Experiment 6.9 were conducted after the completion of Experiments 6.1 - 6.7. Faeces from RF steers and extrusa from OF steers (both freeze-dried and 1 mm ground), collected as described above, were incubated in the rumen of RF steers for 7 d in order to use IADF as an internal marker to estimate pasture OMD. During nylon bag incubation RF steers were fed on a standard diet as described in section 3.4.11. A representative bulk extrusa sample from each of the seven experimental pasture types, and a representative bulk faecal sample taken from each RF steer grazing the pastures in Experiments 6.1 - 6.7, was incubated in triplicate in each of three RF steers. All bags were removed together at the end of the 7 d and processed as described in section 3.4.11. After individual proportional DM loss was determined, the nylon bag residues were bulked across all replicates to provide one bulk extrusa residue for each of the seven pasture experiments and one bulk faecal residue for each of the six to eight RF steers for each pasture type (Experiments 6.1 - 6.7). These residues were kept for analysis of OM and ADF content.

Proportional particle loss for 1 mm ground extrusa and faecal samples used in IADF determination, was investigated. A bulk extrusa and faecal sample from each of NPT, LL, BP and RG experiments was tested in triplicate for particle loss from nylon bags. Individual nylon bags were soaked in a 500 ml beaker of water for 5 min and then squeezed by hand twenty-five times under running tap water. The soak and wash water was then filtered through No. 4 Whatman filter paper (particle retention 20 μm) to collect all particles lost from the nylon bags. The filter paper and washed nylon bags were oven dried (60°C for 48 h) and weighed to determine particle DM loss from the bags and total DM loss from bags (soluble and particulate matter loss).

### 6.2.5 Analytical procedures

Methods for determination of DM, ash, total N, NDF, ADF, NDIN, ADIN, rumen fluid VFA, rumen fluid $NH_3$-N, PD, Cr, Li and Yb are given in section 3.5. Additional analyses that have not been described in the general methodology chapter (Chapter 3) are detailed below.

#### *6.2.5.1 Serum urea*

The concentration of serum urea was determined using a test kit manufactured by Thermo Trace Ltd (18 Cleeland Road, South Oakleigh, Victoria 3167). The analysis was performed on an Olympus Reply clinical analyser.

#### *6.2.5.2 Nitrates*

Nitrate concentration was determined on freeze-dried, 1 mm ground plucked leaf samples from the RG pasture during RF steer sampling period (freeze dried sample) and the LWG steer measurement period (oven dried sample). Plant nitrate content was determined colorimetrically by Merckoquant Nitrate test strips (MERCK, Frankfurter Street, 250 64293 Darmstadt, Germany). The precision and reproducibility of the test was improved by comparing standard nitrate solutions (50 mg/l and 100 mg/l) made from analytical grade potassium nitrate ($KNO_3$). The nitrate test strips had been previously validated against a nitrate electrode. Nitrate concentration was reported as a percentage of $KNO_3$ in DM.

#### *6.2.5.3 TNSC*

Total non-structural carbohydrate concentration was determined on a freeze-dried, 1 mm ground plucked leaf sample from each of the pasture experiments (Experiments 6.1 - 6.7) using a modification of the method of Weier *et al.* (1977). A digestion procedure was used to gelatinise and break down starch with α-amylase. This was followed by deproteinisation of the extract with trichloracetic acid solution. After centrifugation (3400 rpm for 10 min), rinsing and re-centrifugation, the extract was filtered through a swinnex-25 millipore filter containing a 0.8 μm cellulose nitrate filter. After this, 0.1 *N* HCl was added to the digested sample filtrate, followed by heating to cause fructan hydrolysis. Ferrocyanide colorimetric analysis was conducted at 420 nm on a UV-1201 Shimadzu Spectrophotometer. Glucose was used as an internal standard. The standards were digested and processed through the fructosan hydrolysis and colorimetric assay in the same way as the crude samples.

### *6.2.5.4 IVOMD*

***Tilley and Terry technique***

IVOMD was determined on freeze-dried, 1 mm ground extrusa samples using a two stage technique (Tilley & Terry, 1963) as modified by Minson & McLeod (1972). Rumen fluid was collected from an RF steer grazing pasture (non-standard diet). The raw IVOMD values were corrected by including standard pasture samples of known *in vivo* digestibility in the assay. Separate standard curves were drawn up to correct raw IVOMD values for tropical grasses ($C_4$), legumes and ryegrass pastures, respectively. The standard curve for adjustment of the tropical grass pasture values included thirteen tropical grass hay standards (*in vivo* OMD range: 498 - 702 g/kg; r = 0.90) and was used to correct samples from NPEW, NPT, NPD and BB experiments. Similarly, a standard curve containing one lucerne and five ryegrass hay standards (*in vivo* OMD range: 581 - 733 g/kg; r = 0.96) was used to correct the LL and BB data. A standard curve containing only the five ryegrass hay standards (*in vivo* OMD range: 581 - 733 g/kg; r = 0.97) was used to correct samples from the RG experiment. It was noted that the corrected IVOMD, for BB, LL and RG extrusa samples, were outside the range of the respective standard curves. The *in vitro* analysis was conducted three times and the average of these results was used as the estimate of OMD for the various diet samples. Very good reproducibility was found between the three analytical runs.

***Pepsin-cellulase technique***

The pepsin-cellulase technique, based on the methods of McLeod & Minson (1978) and McLeod & Minson (1980) was also used to determine IVOMD of RG extrusa. The analysis was conducted at a lab with access to a wide range of *in vivo* standards for temperate ($C_3$) pasture species (FEEDTEST, Agriculture Victoria, Hamilton). Pepsin incubation proceeded first, followed by cellulase (Onozuka 3S) incubation. Six standard forages were used to derive the standard curve (*in vivo* OMD range: 475 - 737 g/kg; r = 0.98). It was noted that the corrected IVOMD for the RG extrusa samples were once again outside the range of the standard curve.

The corrected IVOMD values for RG extrusa samples were very similar for both the Tilley and Terry, and the pepsin-cellulase techniques, differing by only 16 g/kg. Only the mean IVOMD determined using the pepsin-cellulase procedure is presented in the results section.

#### *6.2.5.5 Alkanes*

***Plant and faecal material***

Alkane profiles were determined on 0.5 g, oven dried, 1 mm ground plant, extrusa and faecal samples by direct saponification, with minor variations to the procedure described by Dove (1992), as outlined below. Samples were extracted with n-hexane in place of n-heptane. Quantification of the n-alkanes ($C_{29}$ - $C_{36}$) was performed using a gas chromatograph (Shimadzu GC-17A) fitted with a capillary column (All-Tech EC-1). Standards were mixed ($C_{29}$ - $C_{36}$) commercial n-alkanes and covered a range of concentrations.

***Alkane CRD core segments***

To avoid dilution error associated with analysing the very high concentrations of alkane in the CRD core segments, the following method was used. Alkane CRD core material was ground with mortar and pestle and then added to a paper extraction thimble and extracted with petroleum spirit in a Soxhlet apparatus. From each extract, duplicate samples equal to about 1 % of the extract weight were weighed into glass scintillation vials, and 500 µg of $C_{34}$ internal standard added. Scintillation vial contents were evaporated to dryness and then taken up in 0.8 ml of heptane for gas chromatography.

### 6.2.6 Calculations

Calculation of rumen fluid and particle FOR, rumen fluid volume, *in situ* DM and OM degradability parameters, and diet RDP content was conducted as described in section 3.6. Additional calculations that have not been described in the general methodology chapter (Chapter 3) are detailed below.

#### *6.2.6.1 Estimation of liveweight gain*

The liveweight gain or loss of LWG steers was determined by fitting a linear regression to the weekly/twice-weekly weight measurements for that period over which weight change was linear (following a 1 - 2 week period of preliminary grazing). The average of the individual steer regression slopes was used as the best estimate of liveweight gain. The correlation coefficients (r) of the linear regressions were in most cases greater than 0.90. For the RG experiment, one of the steers was excluded from the average, although apparently in good health, due to losing 0.34 kg/d over the experimental period (linear regression for weight loss: $r = 0.96$) while the remaining seven steers gained weight.

#### *6.2.6.2 Estimation of OMD using odd-chain n-alkanes*

The concentrations of the naturally occurring, odd-chain n-alkanes $C_{35}$, $C_{33}$ and $C_{31}$, in OF extrusa and RF faeces, were used to estimate OMD for each of the seven pasture types (Experiments 6.1 - 6.7). The exact recovery of each of the alkanes in the faeces of cattle grazing the various pasture types, were unknown. Thus, the following recoveries were assumed:

1) 0.95 for $C_{35}$, which was the mean for sheep as reported in the review of Dove & Mayes (1991);
2) 0.87 for $C_{33}$, which was the mean for sheep as reported in the review of Dove & Mayes (1991); and
3) 0.76 for $C_{31}$, which was the average for cattle consuming a range of forage types as found by Sandberg *et al.* (2000).

One mean value for extrusa alkane concentration for each pasture type was assumed to be representative of the alkane concentration in herbage consumed by the RF steers. Individual alkane concentrations in the faeces of each RF steer were used in conjunction with the average extrusa alkane concentration, in the estimation of OMD for each steer. The following calculation was used:

$$\text{OMD (g/kg)} = \left[1 - \frac{\text{alkane in herbage (mg/kg OM)}}{\text{alkane in faeces (mg/kg OM)}}\right] x \quad 1000.$$

### *6.2.6.3 Estimation of OMD using IADF*

IADF was used as a marker to predict digestibility of the extrusa samples from Experiments 6.1 - 6.7 with the equation:

$$\text{OMD (g/kg)} = \left[1 - \frac{\text{IADF in herbage (g/kg OM)}}{\text{IADF in faeces (g/kg OM)}}\right] x \quad 1000.$$

The IADF was the ADF undigested after rumen incubation for 7 d (described in section 6.2.4.4). Due to lack of other data providing relevant IADF recovery figures for the range of pasture types studied, faecal recovery was assumed to be 100 %.

### *6.2.6.4 Estimation of DOMI using either Yb or dosed n-alkane as a faecal output (FO) marker*

Faecal output for individual RF steers was estimated using the external markers Yb and dosed $C_{36}$ n-alkane by the following calculation:

$$\text{FO (kg OM/d)} = \frac{\text{marker dose rate (mg/d)}}{\text{faecal marker concentration (mg/kg OM)}}.$$

Dosed $C_{32}$ n-alkane was not used to estimate FO due to the detection of significant levels in cut pasture components as well as in extrusa and faeces of OF steers (un-dosed).

The Yb dose rate was calculated from the weight of Yb-marked hay that was dosed, and the Yb concentration in representative marker dose samples for each batch. The $C_{36}$ n-alkane dose rate was calculated using average measured linear CRD delivery rates (mm/d) for individual steers (determined as described in section 3.4.3), in combination with the analysed $C_{36}$ content in the CRD core (mg/mm). Captec's

stated $C_{36}$ n-alkane concentrations for each batch were used in the calculations as our analyses, on selected core samples, confirmed the accuracy of Captec's analyses. A faecal recovery of 100 % was assumed for the Yb marker (Siddons *et al.* 1985; Galyean *et al.* 1986) and 95 % for the $C_{36}$ n-alkane (Dove & Mayes, 1991).

Organic matter intake (OMI) of individual RF steers was estimated using individual FO estimates from either dosed Yb or alkane markers in conjunction with an average OMD for each pasture, i.e. IVOMD, predicted from *in vitro* digestibility analysis of extrusa:

$$\text{OMI (kg/d)} = \frac{\text{faecal OM output (kg/d)}}{(1 - \text{IVOMD (g/g)}).}$$

Digestible organic matter intake (DOMI) was determined by:

$$\text{DOMI (kg/d)} = \text{OMI (kg/d)} \times \text{IVOMD (g/g)}.$$

#### *6.2.6.5 Estimation of MCP flow*

Due to the collection of spot urine samples during Experiments 6.1 - 6.7, rather than total urine collection, a modification of the equations stated in section 3.6.2 was used.

The total PD excretion (Y) was calculated by:

$$\text{Y (mmol/d)} = \text{Cr excretion (mg/d)} \times \frac{\text{total PD in spot sample (mmol/l)}}{\text{Cr in spot sample (mg/l).}}$$

Cr excretion was calculated by using the measured infusion rates for each animal on each day, and adjusting for 90 % recovery, as found in Experiments 5.1 and 5.2. Thus, Cr excretion was calculated by:

$$\text{Cr excretion (mg/d)} = \text{Cr infusion (mg/d)} \times 0.90.$$

#### *6.2.6.6 Estimation of diet composition using alkane profiles*

Due to possible contamination of BP extrusa with dry sorghum stalks, which were present in the pasture, an attempt was made to use alkane profiles to verify the botanical composition of the diet selected by OF steers grazing the BP pasture. The alkane profiles in the extrusa and in hand-collected plant components of both butterfly pea and sorghum species were used to estimate the proportion of sorghum in BP extrusa using the software EATWHAT (Dove & Moore, 1995). The program predicted that no sorghum was present in the extrusa samples. However, the sorghum green and dead leaf components contained very low n-alkane concentrations while green sorghum stem contained no detectable n-alkanes. Thus it was deemed that the alkane profile method was not a valid technique to estimate diet composition for the species present in the BP experiment, and the results are not presented in this thesis.

### 6.2.7 Statistical analyses

As Experiments 6.1 - 6.7 were grazing trials it was necessary to conduct the experiments sequentially, in different paddocks and at different times of the year, according to pasture availability and rainfall. Thus, it was not valid to make statistical comparisons between the pasture types studied and this was not the intent of the work. Those statistical analyses that were conducted were carried out using the statistical package, Genstat for Windows, 6th edition (Lawes Agricultural Trust, Rothamsted, U.K.).

#### *DM and OM degradability of material incubated in nylon bags*

Parallel curve analysis was used to compare the exponential pattern of nylon bag DM disappearance for the standard feed incubated in RF steers grazing the test pastures, and to compare the OM disappearance curves for the seven extrusa types incubated in RF steers consuming a standard diet. The experimental unit was the steer. The form of the exponential used was $Y = S + QR^t$, where:

Y = DM or OM disappearance (g/kg),
S = constant parameter,
Q = linear parameter,
R = curvature parameter, and
t = time (h).

The coefficients of the alternative formulation of the exponential curve $Y = a + b (1 - e^{-ct})$, which have more meaning biologically (see section 3.6.4) relate to the coefficients of the fitted curve, as described in Table 6.1. The results of the tests of statistical difference between coefficients are presented in the form of the coefficients of the equation $Y = a + b (1 - e^{-ct})$, with the significance of testing "Q" being applicable to "b" (slowly degradable fraction), the significance of testing "S" being applicable to "a + b" (potential degradable fraction), and the significance of testing "R" being applicable to "c" (degradation rate).

**Table 6.1. Relationship between the coefficients of the exponential curve used in statistical analysis and that used in the description of nylon bag disappearance parameters**

| Coefficient | | Explanation |
|---|---|---|
| **Equation: $Y = S + QR^t$** | **Equation: $Y = a + b (1 - e^{-ct})$** | |
| S | a + b | The upper asymptote of Y, i.e. the maximum as t approaches infinity |
| Q | -b | Linear parameter |
| R | $e^{-ct}$ | Curvature parameter |

The analysis consisted of the following four steps and tested the significance of the change in the model at each step:

1) a common regression was fitted across all pastures types,
2) separate constant parameters (S) were fitted for each pasture with all other parameters in common,
3) separate linear parameters (S and Q) were fitted for each pasture with remaining parameters in common, and
4) all parameters (S, Q and R) were fitted separately for each pasture.

Where there were significant differences ($P < 0.05$) between coefficients, approximate pairwise comparisons between pastures were made using the protected least significant difference procedure ($P = 0.05$).

In the analysis of the DM disappearance of the standard feed in RF steers while grazing the test pastures, the best model was the third one, above, (i.e. separate "S"

and "Q" with common "R"). However, the pairwise comparisons of the "S" and "Q" coefficients are based on the fourth model, as it was not possible to obtain the variance-covariance matrix, and thus the standard errors of the differences between coefficients, for the third model. Therefore, the pairwise comparisons of coefficients for DM disappearance of the standard feed are approximate, only.

## 6.3 RESULTS

### 6.3.1 Rainfall, pasture and diet composition

Rainfall was below average (708 mm long term annual average) for the 13-month period of experimentation (Table 6.2).

**Table 6.2. Monthly rainfall (mm) for Brian Pastures Research Station for the experimental period and the preceding 6 months**

| Month | Year | | |
|---|---|---|---|
| | 1999 | 2000 | 2001 |
| January | | 77.8 | 7.4 |
| February | | 60.8 | |
| March | | 27.4 | |
| April | | 33.4 | |
| May | | 27.0 | |
| June | | 25.9 | |
| July | 83.8 | 5.1 | |
| August | 14.4 | 2.8 | |
| September | 33.4 | 0.0 | |
| October | 107.4 | 171.0 | |
| November | 118.4 | 80.1 | |
| December | 53.4 | 65.4 | |
| **Annual** | **618.4** | **576.7** | |

Plates 4 - 10 show a representative photographic record of each of the seven pasture types studied (Experiments 6.1 - 6.7). The photographs were taken in the paddock grazed by the RF and OF steers at the same time as the pasture measurements for presentation yield, botanical composition and height were made.

**Plate 4.** Native tropical grass pasture in the early wet season (major species *Heteropogon contortus* and *Bothriochloa bladhii*), (NPEW).

**Plate 5.** Native tropical grass pasture in the wet/dry transitional period (major species *Heteropogon contortus* and *Bothriochloa bladhii*), (NPT).

**Plate 6.** Native tropical grass pasture in the dry season (major species *Heteropogon contortus* and *Bothriochloa bladhii*), (NPD).

**Plate 7.** Introduced tropical grass pasture, creeping bluegrass (*Bothriochloa insculpta* cv. Bisset) in the mid wet season (BB).

**Plate 8.** Introduced tropical legume, Dolichos lablab (*Lablab purpureus* cv. Highworth), (LL).

**Plate 9.** Introduced tropical legume, butterfly pea (*Clitoria ternatea* cv. Milgarra), (BP).

**Plate 10.** Temperate grass pasture, annual ryegrass (*Lolium multiflorum* cv. Tetila), (RG).

As demonstrated in Tables 6.3 - 6.6, there was a large range in quality and composition between the seven pasture types studied. In particular, tropical grass pastures (NPEW, NPT, NPD and BB) had much lower CP content and high fibre concentrations than for tropical legumes (LL and BP) and RG. The extremely high CP content of RG pasture was also associated with a high nitrate concentration in plucked leaf samples.

**Table 6.3. General pasture characteristics for the pasture types studied in Experiments 6.1 - 6.7**

| | Pasture type [A] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP[B]** | **RG** |
| Presentation yield (kg DM/ha) | 675 | 2711 | 2803 | 3043 | 6349 | 1277 | 1588 |
| Height (cm) | 12.2 | 18.8 | 25.5 | 11.8 | 90.5 | 38.3 | 26.6 |
| Pasture DM (g/kg) | 385 | 686 | 535[C] | 609[D] | 181 | 412 | 114 |
| Plant components (g/kg DM) | | | | | | | |
| Green leaf | 574 | 295 | 29 | 218 | 381 | 253 | 800 |
| Green stem | 268 | 352 | 86 | 242 | 610 | 515 | 92 |
| Dead material | 159 | 352 | 885 | 540[E] | 9 | 0 | 108 |
| Pods | - | - | - | - | - | 232 | - |

DM, dry matter.

[A] Pastures include: native tropical grass pasture (major species *Heteropogon contortus & Bothriochloa bladhii*) in the early wet season (NPEW), the wet/dry transitional period (NPT) and the dry season (NPD); introduced tropical grass pasture, creeping bluegrass (*Bothriochloa insculpta* cv. Bisset) in the mid wet season (BB); introduced tropical legumes Dolichos lablab (*Lablab purpureus* cv. Highworth), (LL) and butterfly pea (*Clitoria ternatea* cv. Milgarra), (BP); and annual ryegrass (*Lolium multiflorum* cv. Tetila), (RG).

[B] Figures given for butterfly pea species only, which comprised *ca.* 700 g/kg of total pasture DM. Other species present in the pasture included various dry grass species, dry sorghum (*Sorghum bicolor*) stalks and weeds.

[C] DM content was depressed due to 4.2 mm rain overnight prior to morning sampling, and light rain at the time of sampling. DM content measured *ca.* 1 month later, on the pasture grazed by non-fistulated steers used to determine liveweight gain (LWG steers), is probably more characteristic of this pasture type: 872 g/kg DM.

[D] DM content possibly depressed due to 2.5 mm rain overnight, prior to morning sampling.

[E] Dead material included last season's growth that was still connected to the base of the plant.

**Table 6.4. Botanical composition (g/kg DM) of native tropical grass pasture studied in Experiments 6.1 - 6.3**

| | Pasture type[A] | | |
|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** |
| Black speargrass (*Heteropogon contortus*) | 526 | 446 | 477 |
| Forest bluegrass (*Bothriochloa bladhii*) | 342 | 462 | 382 |
| Queensland bluegrass (*Dichanthium sericium*) | 71 | 35 | 50 |
| Other native and introduced grasses | 42 | 46 | 90 |
| Other native and introduced legumes | 14 | 6 | 1 |
| Broad leaved weeds | 4 | 5 | 1 |

DM, dry matter.

[A] Pastures include: native tropical grass pasture (major species *Heteropogon contortus* & *Bothriochloa bladhii*) in the early wet season (NPEW), the wet/dry transitional period (NPT) and the dry season (NPD).

**Table 6.5. Chemical composition of extrusa representative of each of the pasture types in Experiments 6.1 - 6.7.** Values are the mean and standard error (in brackets) for 2 to 4 oesophageal fistulated steers (OF), except for RDP, which was determined on one bulk extrusa sample representative of each pasture type

| | Pasture type[A] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| Number of OF steers | 2 | 4 | 4 | 2 | 4 | 3 | 2 |
| OM (g/kg DM) | 859 | 859 | 865 | 864 | 872 | 876 | 802 |
| | (3.0) | (17.4) | (3.8) | (1.5) | (3.3) | (3.2) | (13.0) |
| CP (g/kg DM) | 79 | 42 | 27 | 59 | 253 | 142 | 354 |
| | (3.4) | (1.3) | (0.8) | (0.0) | (7.8) | (11.5) | (6.3) |
| RDP (g/g CP) | 0.86 | 0.83 | 0.82 | 0.91 | 0.97 | 0.95 | 0.98 |
| NDF (g/kg DM) | 643 | 615 | 657 | 570 | 245 | 393 | 294 |
| | (15.0) | (11.3) | (3.8) | (1.5) | (11.0) | (18.7) | (6.3) |
| ADF (g/kg DM) | 333 | 313 | 378 | 317 | 179 | 271 | 154 |
| | (7.0) | (9.7) | (1.9) | (5.5) | (3.8) | (9.1) | (3.0) |

ADF, acid detergent fibre; CP, crude protein; DM, dry matter; NDF, neutral detergent fibre; OM, organic matter; RDP, rumen degradable protein.

[A] For definition of pasture types, see Table 6.3.

**Table 6.6. Chemical composition (g/kg DM) of plucked leaf representative of each of the pasture types in Experiments 6.1 - 6.7**

| | Pasture type[A] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| OM | 893 | 911 | 865 | 899 | 931 | 909 | 868 |
| CP | 77 | 48 | 24 | 64 | 314 | 210 | 379 |
| $KNO_3$[B] | - | - | - | - | - | - | 62 |
| TNSC[C] | 99 | 106 | 42 | 108 | 129 | 157 | 115 |
| NDF | 600 | 655 | 662 | 603 | 256 | 186 | 289 |
| ADF | 287 | 325 | 402 | 322 | 141 | 153 | 159 |

ADF, acid detergent fibre; CP, crude protein; DM, dry matter; NDF, neutral detergent fibre; OM, organic matter; TNSC, total non-structural carbohydrates.

[A] For definition of pasture types, see Table 6.3.

[B] The morning of RG sampling for plucked leaf was warm and cloud-free.

[C] TNSC expressed as glucose equivalents.

### 6.3.2 Liveweight of steers

The average numbers and liveweight of RF and LWG steers used in Experiments 6.1 - 6.7 are given in Table 6.7. A large range in liveweight gain, of the LWG steers, was evident across the various pasture types, as shown in Table 6.7. The greatest liveweight gain was measured on NPEW while the lowest occurred on NPD, where weight loss of 0.75 kg/d occurred.

**Table 6.7. Liveweight of rumen fistulated (RF) and non-fistulated (LWG) steers, and period of measurement and liveweight gain for LWG steers grazing the pasture types in Experiments 6.1 - 6.7.** Values are the mean and standard error (in brackets)

| | Pasture type[A] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| RF steers | | | | | | | |
| Number used | 8 | 6 | 8 | 8 | 6 | 8 | 6 |
| Average liveweight (kg) | 439 (9.8) | 329 (10.8) | 375 (7.4) | 471 (10.6) | 340 (10.8) | 366 (9.2) | 368 (8.9) |
| LWG Steers | | | | | | | |
| Number used | 6 | 6 | 6 | - | 6 | 5 | 7 |
| Initial liveweight (kg) | 400 (6.0) | 325 (10.7) | 415 (4.3) | - | 333 (11.7) | 356 (10.2) | 378 (1.9) |
| Grazing days | 21 | 57 | 56 | - | 77 | 56 | 57 |
| Liveweight gain (kg/d) | 1.77 (0.093) | 0.50 (0.053) | - 0.75 (0.044) | - | 0.73 (0.059) | 0.33 (0.035) | 0.78 (0.094) |

[A] For definition of pasture types, see Table 6.3.

### 6.3.3 Estimated diet OMD

#### *6.3.3.1 IVOMD*

The IVOMD values determined on OF extrusa, and corrected using *in vivo* standards, are given in Table 6.8. A large range in IVOMD across pasture types was found, with RG having the highest value at 827 g/kg and BP pasture the lowest at 585 g/kg.

**Table 6.8. *In vitro* organic matter digestibility (IVOMD) of extrusa samples representative of each of the pasture types in Experiments 6.1 - 6.7.** Values are the mean and standard error (in brackets) for 2 to 4 oesophageal fistulated steers

| | Pasture type[A] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| IVOMD (g/kg) | 681 | 656 | 608 | 724 | 777 | 585 | 827 |
| | (1.4) | (10.5) | (4.4) | (1.0) | (7.1) | (8.8) | (0.5) |

[A] For definition of pasture types, see Table 6.3.

### *6.3.3.2 Estimated OMD using alkanes and IADF as internal markers*

OMD estimates from use of either odd-chain n-alkanes or IADF as internal markers are presented in Table 6.9 and 6.10, respectively. In general, there was poor agreement of the OMD estimates, predicted from the odd-chain n-alkane and IADF methods, with one another and with the IVOMD estimate.

**Table 6.9. Organic matter digestibility (g/kg) predicted using $C_{35}$, $C_{33}$ and $C_{31}$ n-alkanes as internal markers (Experiments 6.1 - 6.7).** Values are the mean and standard error (in brackets) for 6 to 8 rumen fistulated steers

| | Pasture type[A] | | | | | | |
|---|---|---|---|---|---|---|---|
| **Odd-chain n-alkane** | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| $C_{35}$[B] | 563 | 752 | 682 | 630 | 790 | 544 | 731 |
| | (10.8) | (18.7) | (9.7) | (5.6) | (17.5) | (40.0) | (10.3) |
| $C_{33}$ | 646 | 796 | 689 | 695 | 723 | 627 | 776 |
| | (9.8) | (14.6) | (10.2) | (9.2) | (18.3) | (23.2) | (3.2) |
| $C_{31}$ | 773 | 790 | 718 | 775 | 692 | 740 | 790 |
| | (4.5) | (7.5) | (6.4) | (7.2) | (26.6) | (12.7) | (5.8) |

[A] For definition of pasture types, see Table 6.3.

[B] Concentrations of $C_{35}$ in extrusa samples were very low, at less than 40 mg/kg dry matter (DM) in all cases and less than 10 mg/kg DM for RG and LL pastures.

**Table 6.10. Organic matter digestibility (OMD) predicted using indigestible acid detergent fibre as an internal marker (Experiments 6.1 - 6.7).** Values are the mean and standard error (in brackets) for 6 to 8 rumen fistulated steers

| | Pasture type[A] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| OMD (g/kg) | 532 | 607 | 729 | 496 | 521 | 406 | 783 |
| | (6.8) | (9.3) | (7.5) | (9.2) | (9.1) | (17.2) | (19.8) |

[A] For definition of pasture types, see Table 6.3.

Particle loss from nylon bags used in the IADF method was up to 65 g/kg of initial sample DM for the pasture types investigated, but the difference between extrusa and faecal samples for any one pasture treatment was always less than 35 g/kg DM (Table 6.11). However, the percentage difference in particle loss between extrusa and faecal samples varied widely between pasture treatments, ranging from 8 % for RG samples up to 74 % for BP samples.

**Table 6.11. Particle loss (g/kg of initial DM weight) as an initial wash from nylon bags for 1 mm ground extrusa and faeces representative of 4 pasture treatments (Experiments 6.2, 6.5, 6.6 and 6.7).** Values are the mean and standard error (in brackets) for 3 nylon bags

| | Pasture type[A] | | | |
|---|---|---|---|---|
| | **NPT** | **LL** | **BP** | **RG** |
| Extrusa | 33.5 (0.60) | 28.5 (3.93) | 41.9 (0.86) | 28.8 (4.94) |
| Faeces | 64.6 (15.87) | 61.3 (5.44) | 10.9 (2.83) | 31.2 (3.95) |
| % difference | 48 | 54 | 74 | 8 |

DM, dry matter.

[A] For definition of pasture types, see Table 6.3.

### 6.3.4 Estimated pasture intake

The intake of pasture, DOMI, by the RF steers was estimated using IVOMD (Table 6.8) in conjunction with either dosed $C_{36}$ n-alkane, or Yb, as a faecal output marker. Negligible (< 5 mg/kg DM) concentrations of the dosed $C_{36}$ n-alkane were detected in cut pasture, OF extrusa, or the faeces of un-dosed OF steers. In contrast, significant levels of the dosed $C_{32}$ n-alkane were detected in these components, for all pasture types. Thus, calculations of FO and intake using the n-alkane marker technique were made using the $C_{36}$ dosed n-alkane, only. Measured alkane CRD delivery rates varied considerably over the days of measurement for individual steers (data not presented). As shown in Table 6.12, there was some variation in CRD delivery rates amongst steers consuming a certain pasture type (as evidenced by SE), as well as variation between the different pasture types. With the exception of BB pasture, linear n-alkane delivery rates fell below that stated by the manufacturer, Captec (2.30 mm/d).

**Table 6.12. Linear delivery rate of alkane core from intra-ruminal controlled release device (Experiments 6.1 - 6.7).** Values are the mean and standard error (in brackets) for 6 to 8 rumen fistulated steers

| | Pasture type[A] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| Linear delivery rate (mm/d) | 2.17 (0.040) | 2.10 (0.068) | 1.97 (0.065) | 2.37 (0.032) | 2.29 (0.087) | 2.24 (0.055) | 1.81 (0.107) |

[A] For definition of pasture types, see Table 6.3.

The DOMI estimates made using both the Yb and $C_{36}$ n-alkane markers are presented in Table 6.13. There was a wide spread in DOMI across the seven pasture types, when calculated using either Yb or dosed $C_{36}$ n-alkane as a faecal output marker. The lowest intakes (as a proportion of liveweight) were recorded on NPD and BP pastures, while LL had the highest DOMI.

**Table 6.13. Digestible organic matter intake (kg/100 kg LW) predicted using Yb and $C_{36}$ n-alkane faecal output markers in conjunction with *in vitro* organic matter digestibility (Experiments 6.1 - 6.7).** Values are the mean and standard error (in brackets) for 6 to 8 rumen fistulated steers

| | Pasture type[A] | | | | | | |
|---|---|---|---|---|---|---|---|
| **Marker type** | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| Yb | 1.75 | 1.41 | 1.08 | 1.46 | 1.93 | 0.82 | 1.35 |
| | (0.048) | (0.021) | (0.049) | (0.065) | (0.216) | (0.029) | (0.105) |
| $C_{36}$ n-alkane | 1.51 | 1.39 | 0.82 | 1.48 | 2.00 | 0.85 | 1.18 |
| | (0.057) | (0.045) | (0.019) | (0.048) | (0.117) | (0.046) | (0.087) |

LW, liveweight.

[A] For definition of pasture types, see Table 6.3.

The difference in estimated DOMI between the Yb and the $C_{36}$ n-alkane method varies with pasture type. When DOMI was expressed as kg DOMI/d, there was less than 5 % difference between the $C_{36}$ and Yb estimates for NPT, BB and BP, less than 10 % difference for LL, and less than 15 % difference for NPEW and RG. The largest discrepancy in DOMI estimates between the two methods was recorded for NPD, for which the $C_{36}$ n-alkane estimation was 23 % lower than that estimated using the Yb marker.

### 6.3.5 Estimated MCP production and eMCP

On some occasions it was not possible to collect all ten spot urine samples for each RF steer grazing a particular pasture type. This was due in some cases to the low urine output and infrequent urination, as on NPD, and in other instances due to problems with Cr infusion. There was some variation in the ratios of PD to Cr in the spot urine samples, when expressed as a proportion of the mean of all spot samples for each steer over 24 h, for steers grazing each pasture type. Generally the variation was relatively small, as demonstrated in Figure 6.1, in which data for NPEW is

presented as an example, with ratios ranging from 0.89 to 1.13 of the overall 24-h mean. The variation was greatest for BP pasture where average PD:Cr ratio ranged from 0.49 to 1.57 of the 24-h mean.

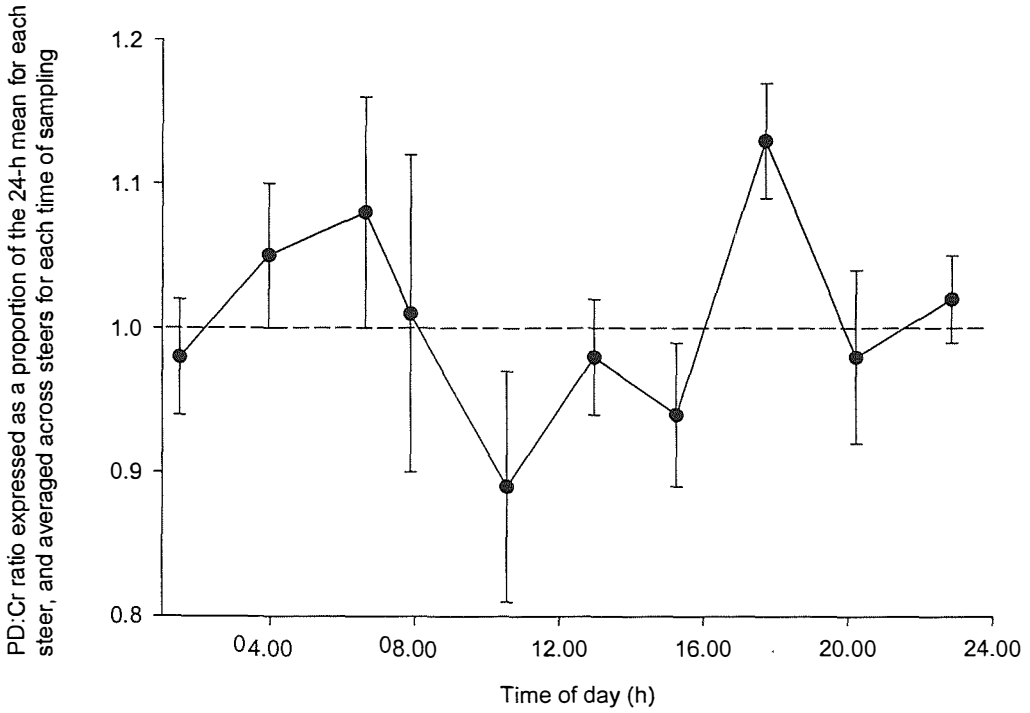


**Figure 6.1. Diurnal variation in purine derivative (PD):Cr ratio in urine spot samples, expressed as a proportion of the 24-h mean for steers while grazing NPEW pasture.** Pasture type defined in Table 6.3. Values represent the mean and standard error for 4 to 8 steers at each sampling time.

The effects of pasture type on MCP flow and eMCP are given in Table 6.14. Efficiency of microbial protein production was calculated using estimates of MCP flow in conjunction with DOMI, estimated using the Yb faecal output marker. While there was some variation in MCP flow within animals on a pasture type, as indicated by SE, this was small relative to the variation between pasture types. Similarly, there was a large range in eMCP across the pasture types. Mature tropical pasture (NPT and NPD) were associated with very low eMCP, while eMCP for early to mid season tropical grasses (NPEW and BB) were almost three-fold higher, but were still less than those values for tropical legumes. The temperate pasture RG was associated with extremely high eMCP values, *ca.* 1.5-fold higher than for the tropical legumes.

**Table 6.14. Effect of pasture type on microbial crude protein (MCP) flow and on efficiency of MCP production (eMCP), (Experiments 6.1 - 6.7).** Values are the mean and standard error (in brackets) for 6 to 8 rumen fistulated steers

| | Pasture type[A] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| MCP flow (g MCP/d) | 653 (41.5) | 120 (12.3) | 141 (11.9) | 616 (29.9) | 840 (50.6) | 430 (33.4) | 1070 (228.5) |
| eMCP (g MCP/kg DOMI) | 85 (4.1) | 26 (2.2) | 35 (3.3) | 90 (3.3) | 135 (14.9) | 144 (11.6) | 209 (33.3) |

DOMI, digestible organic matter intake.

[A] For definition of pasture types, see Table 6.3.

### 6.3.6 Rumen metabolism and digestion parameters

Table 6.15 presents the mean values for rumen fluid pH, total VFA concentration and molar proportions of VFA in rumen fluid for each pasture type. The lowest average rumen fluid pH was measured on LL pasture and the highest on RG pasture, at 6.1 and 6.8, respectively. Total VFA concentration in the rumen fluid was very low on NPEW and BB pastures (< 50 mM), and lower than concentrations measured on mature tropical pasture (NPT and NPD). The greatest VFA concentration was measured on LL pasture at 170 mM. Highest proportions of BCFA were measured on LL, BP and RG pastures (3.8 - 7.1 % of total VFA).

**Table 6.15. pH, and volatile fatty acid (VFA) concentrations and molar proportions in rumen fluid from rumen fistulated (RF) steers grazing 7 pasture types (Experiments 6.1 - 6.7).** Values are the mean and standard error (in brackets) for 6 to 8 steers[A]

| | Pasture type[B] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| Rumen fluid pH | 6.4 (0.03) | 6.6 (0.04) | 6.5 (0.02) | 6.3 (0.05) | 6.1 (0.06) | 6.3 (0.05) | 6.8 (0.04) |
| Total VFA concentration (mM) | 23.0 (1.13) | 80.8 (4.54) | 67.5 (2.26) | 30.5 (1.91) | 169.6 (6.94) | 129.2 (4.09) | 99.6 (2.47) |
| Molar proportions of VFA (% of total) | | | | | | | |
| Acetate | 71.7 | 76.0 | 73.3 | 72.1 | 65.8 | 66.9 | 63.4 |
| Propionate | 17.5 | 12.4 | 14.5 | 19.0 | 17.3 | 17.0 | 16.8 |
| Butyrate | 9.0 | 10.4 | 7.4 | 7.3 | 11.2 | 9.9 | 11.0 |
| Valerate | 0.5 | 0.3 | 3.5 | 0.5 | 1.6 | 2.5 | 1.7 |
| BCFA[C] | 1.3 | 0.9 | 1.4 | 1.1 | 4.1 | 3.8 | 7.1 |

BCFA, branched-chain volatile fatty acids.

[A] Ten spot rumen fluid samples, taken at various times over 5 d, contributed to the mean for each steer, except for NPEW where the mean for 2 of the 8 steers did not include 04.00, 13.00, or 18.00 h samples. Table 6.7 gives details of number of RF steers, except for VFA results for BB pasture, where 7 steers contributed to the mean.

[B] For definition of pasture types, see Table 6.3.

[C] Sum of iso-butyrate and iso-valerate.

Diurnal variation in rumen fluid pH was also monitored (ten spot samples at various times over 5 d). A consistent trend, evident for all pasture types, was a peak in pH at *ca.* 08.00 h, with pH then declining either quickly (for LL and BB pastures), or gradually for others, to baseline levels until about 24.00 h. Diurnal variation was least evident on the native pasture treatments (NPEW, NPT, NPD). One representative figure showing diurnal variation in rumen pH, for LL pasture, is given below (Figure 6.2).

Except for LL and BP treatments, rumen fluid pH was generally maintained above 6.0 during the 24-h period of sampling. Exceptions include one steer in each of NPEW and BB treatments, which both had a pH < 6.0 on one out of ten sampling occasions. On LL pasture, pH fell below 6.0 in five out of six RF steers on at least one, and up to

seven, of the ten sampling occasions. On BP pasture, pH fell below 6.0 in seven out of eight steers on at least one, and up to five, of the ten sampling occasions.

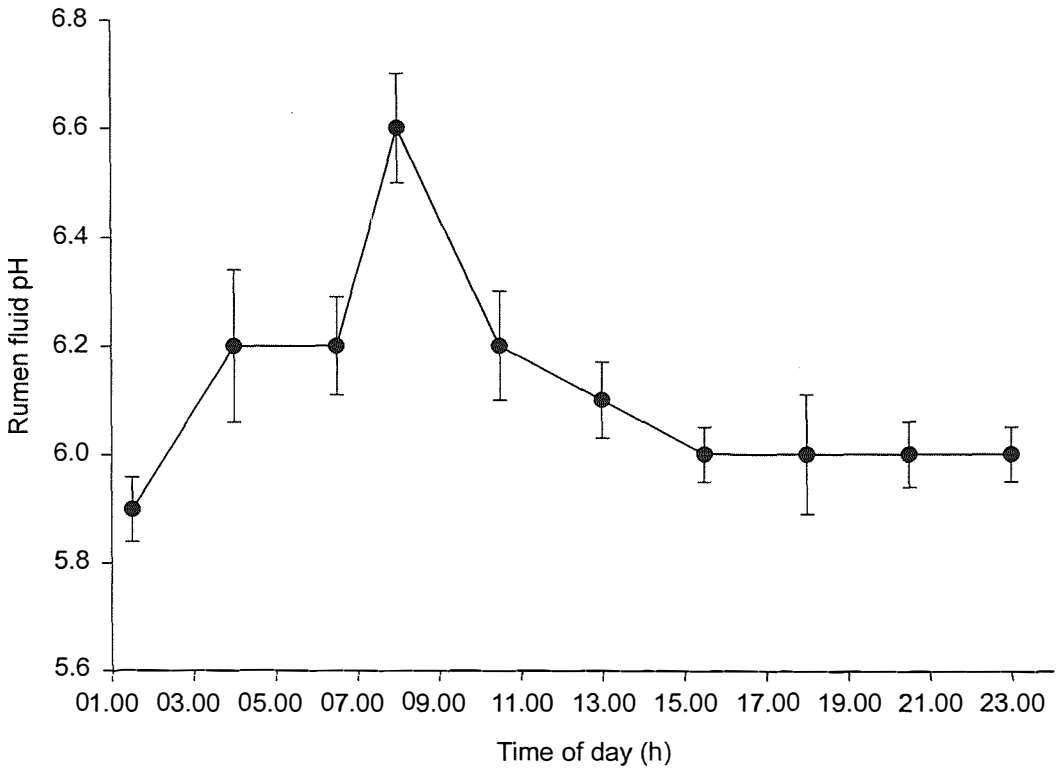


**Figure 6.2. Diurnal variation in the rumen fluid pH of steers grazing LL pasture.** Pasture type defined in Table 6.3. Values represent the mean and standard error for 6 steers. Samples were taken over 5 d.

The numbers and proportions of the main protozoal species found in the rumen fluid of steers grazing each of the seven pasture types are illustrated in Figure 6.3. Protozoal numbers were greatest on LL and least on NPD. There was little difference in protozoal numbers or proportions between sampling times occurring before and after the main grazing periods (06.30 and 20.30 h, respectively), although LL had slightly depressed protozoal numbers after the main grazing period. *Entodinium* spp. were present in greatest proportion on all pasture types, except for BB pasture sampled at 06.30 h. Compared to other pasture types, relatively large proportions of Holotrichs (*Isotricha* and *Dasytricha* genera) were recorded in cattle grazing NPEW, NPD and BB pasture.

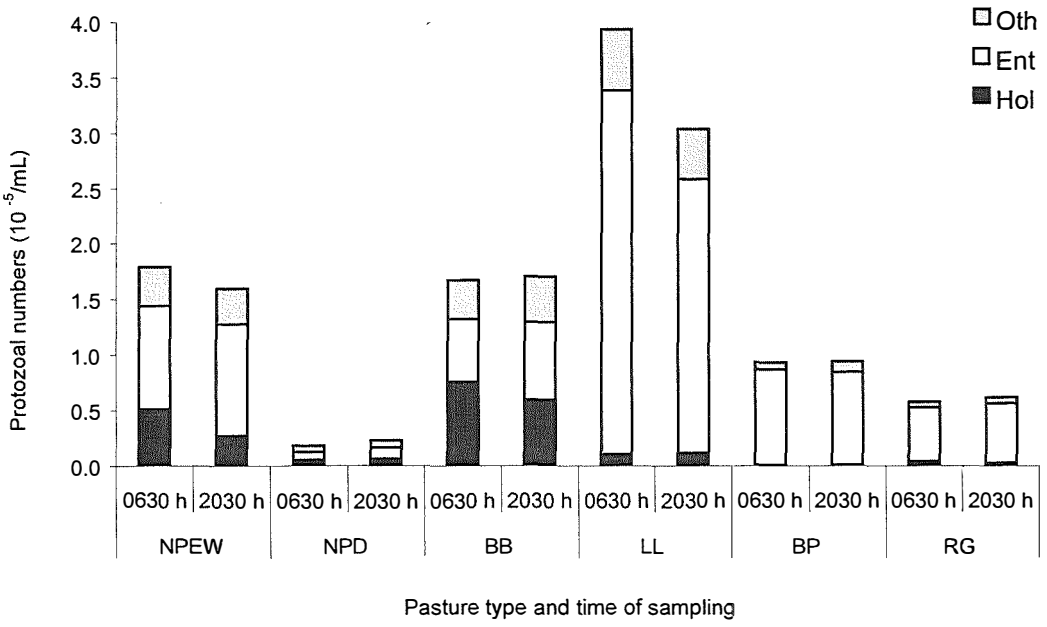


**Figure 6.3. Protozoal numbers in the rumen fluid of steers grazing a range of pastures.** Pasture types defined in Table 6.3. Values represent the mean for 5 to 6 steers. Hol = Holotrichs; Ent = *Entodinium* spp.; Oth = other protozoal species from the Entodiniomorph group (excluding *Entodinium* spp.).

The mean rumen $NH_3$-N and faecal N concentrations over 24 h, and serum urea taken between 07.00 and 09.00 h, for steers grazing the seven pasture types are given in Table 6.16. A large range in N status is evident across the pasture types. Steers grazing the two tropical legumes (LL and BP) and RG had high rumen $NH_3$-N, serum urea, and faecal N concentrations, while those grazing the tropical grass pastures had much lower values. In particular, rumen $NH_3$-N concentrations all averaged much less than 50 mg N/L on tropical grass pastures, including those grazed in the early to mid wet seasons.

**Table 6.16. Concentrations of $NH_3$-N in rumen fluid, urea in blood serum and N in faeces for each of the pastures in Experiments 6.1 - 6.7.** Values are the mean and standard error (in brackets) for 6 to 8 rumen fistulated (RF) steers

| | Pasture type[A] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| $NH_3$-N[B] (mg/l) | 17.0 (1.37) | 8.0 (0.59) | 22.5 (3.74) | 16.7 (1.64) | 378.0 (10.20) | 182.4 (6.52) | 382.3 (7.56) |
| Serum urea[C] (mM) | 1.6 (0.09) | 1.8 (0.27) | 3.0 (0.32) | 2.2 (0.20) | 9.6 (0.32) | 8.7 (0.41) | 9.1 (0.36) |
| Faecal N[D] (g/kg DM) | 15 (0.3) | 10 (0.2) | 10 (0.2) | 14 (0.2) | 29 (0.6) | 18 (0.3) | 33 (1.3) |

DM, dry matter.

[A] For definition of pasture types, see Table 6.3.

[B] Table 6.7 gives details of number of RF steers, except for NPEW and BB where 6 steers contributed to the mean in each case.

[C] The numbers of steers contributing to each mean, for pastures 1 to 7 above, are as follows: 7, 6, 7, 7, 7, 7, and 6.

[D] Table 6.7 gives details of the number of RF steers, except for BB where 7 steers contributed to the mean.

The diurnal variation in $NH_3$-N concentration is illustrated in Figure 6.4. In general, rumen $NH_3$-N concentrations on the high CP content pastures (LL, BP and RG) reached peak or plateau in rumen at *ca.* 13.00 h, while low concentrations were associated with the 08.00 h sampling time. Changes in $NH_3$-N concentration over time for the tropical grass pastures (low CP content) were highly variable across pasture treatment.

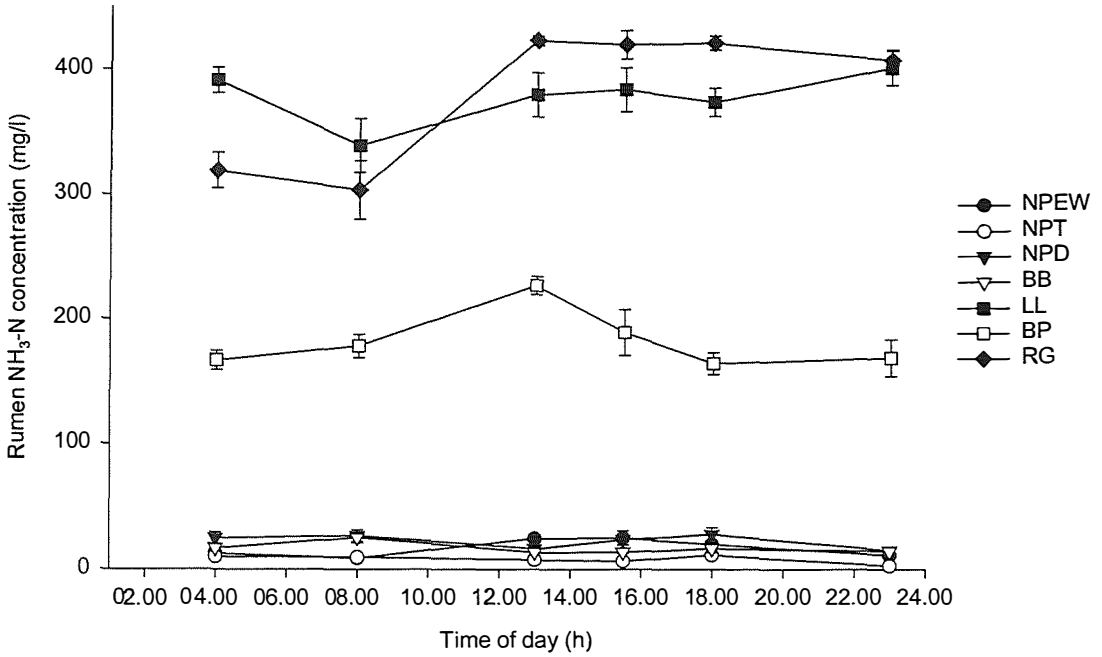


**Figure 6.4. Diurnal changes in the concentration of $NH_3$-N in the rumen fluid of steers grazing a range of pastures.** Pasture types defined in Table 6.3. Values represent the mean and standard error for 6 to 8 steers. Samples were taken over 5 d.

### 6.3.7 Rumen fluid and particle passage rates

Table 6.17 gives the mean rumen fluid and particle FOR, and the estimated rumen fluid volume, for each of the seven pasture types. Rumen DM content could not be estimated due to presence of Yb in the rumen at 0 h, as a result of prior dosing for faecal output estimation. Despite determination of FOR under non-steady state conditions the correlation coefficients for the regressions of *ln* Cr and *ln* Yb concentration against time were extremely high on all pasture types, usually r = 0.99. Early to mid season $C_4$ grasses had fluid and particle FOR in the range of the $C_3$ species (tropical legumes and the RG). However, fluid and particle FOR for mature native $C_4$ grass (NPD) was very low. Rumen fluid volume did not show the same variation as that seen for FOR. A strong relationship between particle FOR and fluid FOR was observed, as illustrated in Figure 6.5.

**Table 6.17. Rumen fluid and particle fractional outflow rate (FOR) and rumen fluid volume for each of the pastures in Experiments 6.1 - 6.7.** Values are the mean and standard error (in brackets) for 6 to 8 rumen fistulated (RF) steers[A]

| | Pasture type[B] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** |
| Fluid FOR (%/h) | 10.8 (0.48) | 7.6 (0.36) | 4.5 (0.33) | 9.5 (0.37) | 11.7 (0.44) | 8.4 (0.70) | 9.3 (0.98) |
| Particle FOR (%/h) | 4.3 (0.28) | 3.0 (0.14) | 2.0 (0.15) | 4.4 (0.24) | 4.4 (0.52) | 2.8 (0.25) | 2.9 (0.27) |
| Fluid volume (l/100 kg LW) | 10.7 (0.96) | 12.4 (0.51) | 11.0 (1.07) | 10.3 (0.40) | 9.6 (0.68) | 11.3 (0.38) | 9.9 (0.29) |

LW, liveweight.

[A] Table 6.7 gives details of number of RF steers except for NPEW where 6 steers contributed to the mean.

[B] For definition of pasture types, see Table 6.3.

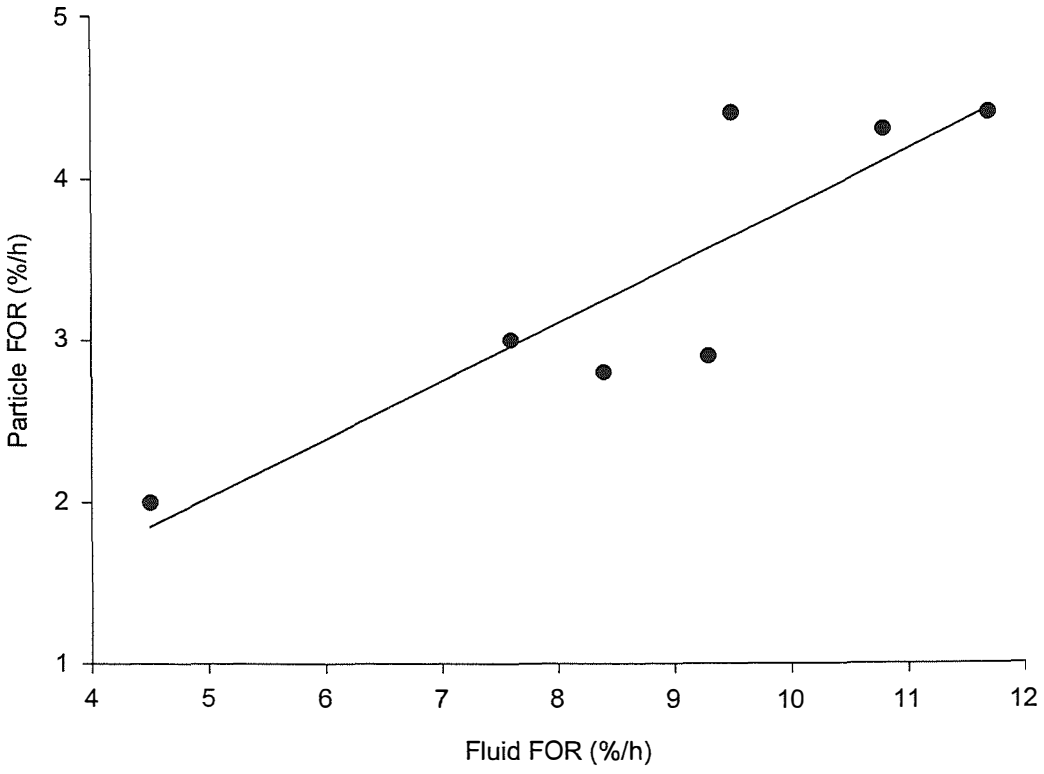


**Figure 6.5. Relationship between fluid and particle fractional outflow rate (FOR) from the rumen in steers grazing a range of pasture types.** Equation: y = 0.358x + 0.242, r = 0.88, RSD = 0.51, P = 0.01.

### 6.3.8 *In situ* degradability

Table 6.18 summarises the DM disappearance parameters for plucked leaf and a standard feed, incubated in nylon bags in the rumen of RF steers grazing the same test pasture as that from which the leaf was plucked. Figures 6.6 and 6.7 illustrate the degradation curves for incubated plucked leaf, and Figure 6.8 illustrates the degradation curves for the standard feed. The graphs display the actual measured data points.

The exponential curve $y = a + b (1 - e^{-ct})$ was a good fit for all data sets except for plucked LL leaf where the fitted curve only accounted for *ca*. 61 % of the variation (RSD 34.8). For all other data sets the percentage variation accounted for by the fitted curve was very high, usually *ca*. 99 %, and always > 90 %.

It was not valid to statistically compare degradation parameters for the incubated plucked leaf. However, it is evident that the potential degradable fraction (a + b) of the incubated plucked leaf was highest for RG, LL and BP, and lowest for the mature tropical grass pasture. Rates of degradation varied across pastures, with NPD plucked leaf having the slowest degradation rate (0.013 /h) and the tropical legumes and RG having fastest degradation rates.

The standard feed was of low quality with chemical composition (g/kg DM) being, OM: 907, CP: 33.1, NDF: 644 and ADF: 348. The degradation parameters for the standard feed were statistically compared. While there was no significant difference between the degradation rates of the standard feed when incubated in steers consuming different pasture types ($P = 0.90$), there were significant differences ($P < 0.001$) between constant and linear parameters (S and Q) of the fitted curve (see section 6.2.7). The potential degradable fraction (a + b) of the standard feed was significantly lower ($P < 0.05$) when incubated in cattle consuming LL pasture than on all other pastures except RG.

**Table 6.18. Dry matter (DM) disappearance parameters for plucked leaf and a standard feed incubated in the rumen of rumen fistulated steers grazing the same test pasture as that from which the leaf was plucked (Experiments 6.1 - 6.7)[†].** Values are the mean and standard error (in brackets) for 3 steers (plucked leaf) and 2 steers (standard feed). Residual standard deviation (RSD) of the fitted equation is presented. Statistical tests only conducted for standard feed (see section 6.2.7); probability (P) for test of significant difference between coefficients is presented where relevant; values in the same row with different superscripts are significantly different ($P < 0.05$)

| | Pasture type[‡] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL[§]** | **BP** | **RG** | **P** |
| **Plucked leaf** | | | | | | | | |
| a (g/kg DM) | 105 (26.1) | 153 (13.8) | 16 (15.6) | 200 (13.7) | -236 N/A | 428 (29.4) | -88 (72.0) | |
| b (g/kg DM) | 704 (24.7) | 615 (14.9) | 714 (104.0) | 599 (13.1) | 1122 N/A | 452 (28.5) | 996 (69.9) | |
| a + b (g/kg DM) | 808 | 769 | 729 | 799 | 886 | 880 | 908 | |
| c ($h^{-1}$) | 0.052 (0.0052) | 0.034 (0.0030) | 0.013 (0.0038) | 0.049 (0.0032) | 0.706 N/A | 0.238 (0.0170) | 0.220 (0.0183) | |
| RSD | 19.9 | 13.2 | 19.2 | 11.1 | 34.8 | 6.9 | 18.6 | |
| **Standard feed** | | | | | | | | |
| a (g/kg DM) | 144 (15.8) | N/A | 126 (15.6) | 147 (6.3) | 144 (18.6) | 147 (7.7) | 152 (28.2) | N/A |
| b (g/kg DM) | $551^{d}$ (18.7) | | $549^{cd}$ (22.2) | $544^{bcd}$ (8.9) | $387^{a}$ (27.3) | $488^{bc}$ (9.4) | $468^{b}$ (53.1) | < 0.001 |
| a + b (g/kg DM) | $696^{b}$ | | $674^{b}$ | $692^{b}$ | $531^{a}$ | $636^{b}$ | $620^{ab}$ | < 0.001 |
| c ($h^{-1}$) | 0.031 (0.0039) | | 0.027 (0.0039) | 0.028 (0.0016) | 0.027 (0.0066) | 0.031 (0.0022) | 0.024 (0.0086) | 0.90 |
| RSD | 21.9 | | 22.7 | 9.2 | 27.1 | 10.9 | 43.9 | |

[†] The loss of DM is described by the equations given by McDonald (1981), where $Y_1 = A$ up to time $t_0$, and $Y_2 = a + b\,(1 - e^{-ct})$, from time $t_0$ onwards (see section 3.6.4). The parameters shown above are defined as: "a" = rapidly degradable fraction; "b" = more slowly degraded fraction; "a + b" = potential degradable fraction; and "c" = degradation rate of "b".

[‡] For definition of pasture types, see Table 6.3.

[§] The exponential curve was a poor fit for LL plucked leaf with only 60.7 % of variance accounted for.

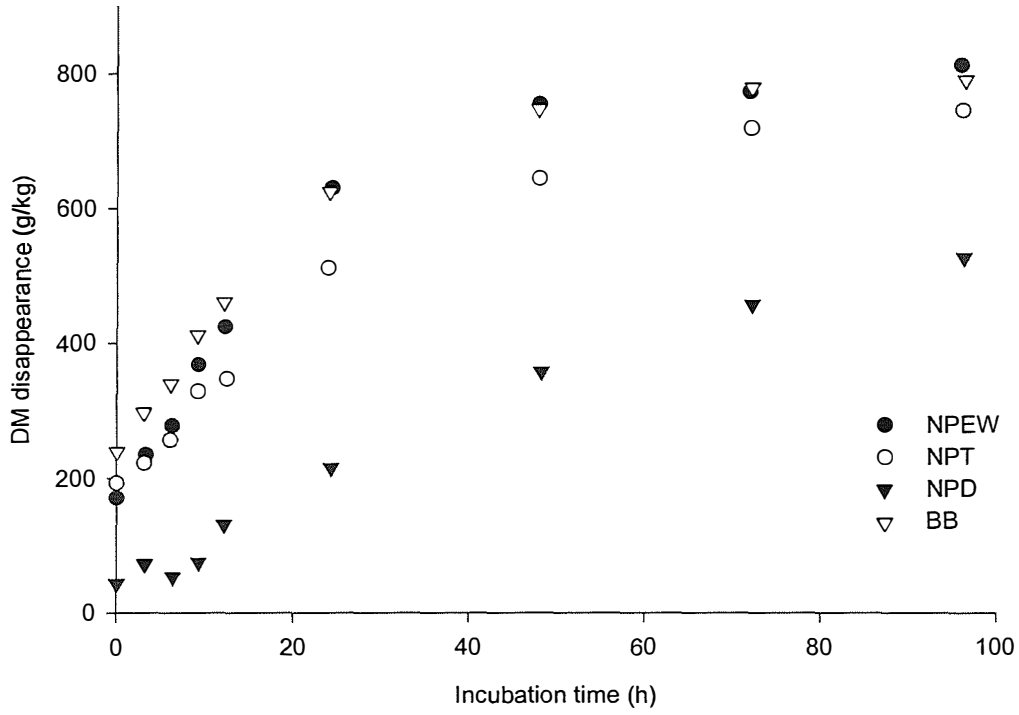


**Figure 6.6. Disappearance of plucked leaf dry matter (DM), (collected in Experiments 6.1 - 6.4) from nylon bags during rumen incubation in steers consuming the same test pasture as that from which the leaf was plucked.** Pasture types defined in Table 6.3. Each point represents the mean for 3 steers.

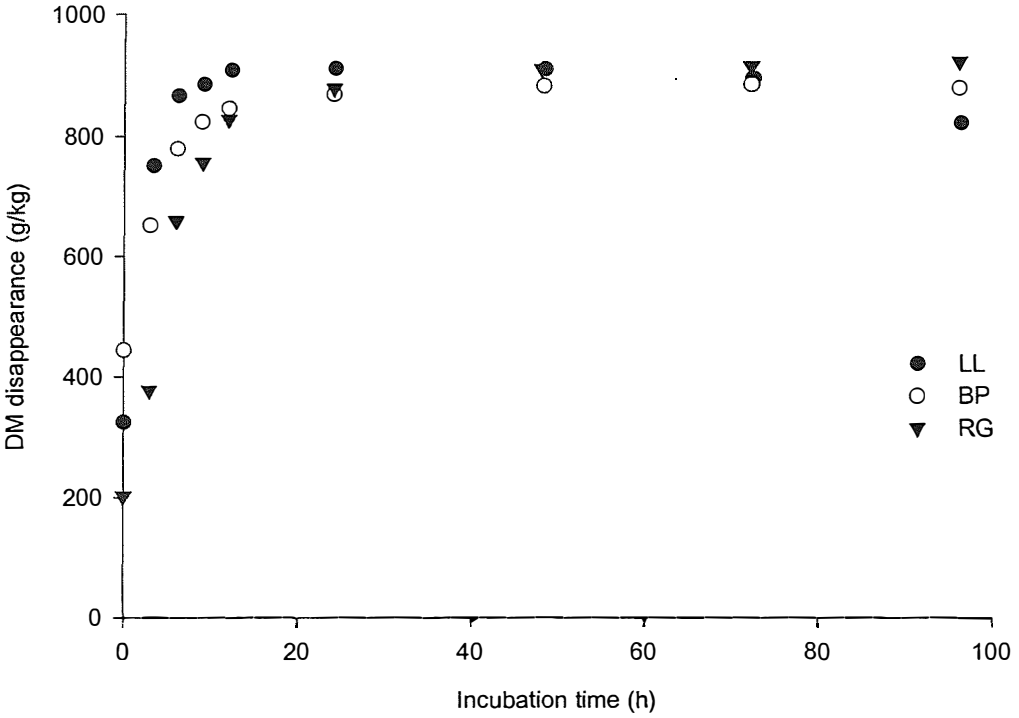


**Figure 6.7. Disappearance of plucked leaf dry matter (DM), (collected in Experiments 6.5 - 6.7) from nylon bags during rumen incubation in steers consuming the same test pasture as that from which the leaf was plucked.** Pasture types defined in Table 6.3. Each point represents the mean for 3 steers.

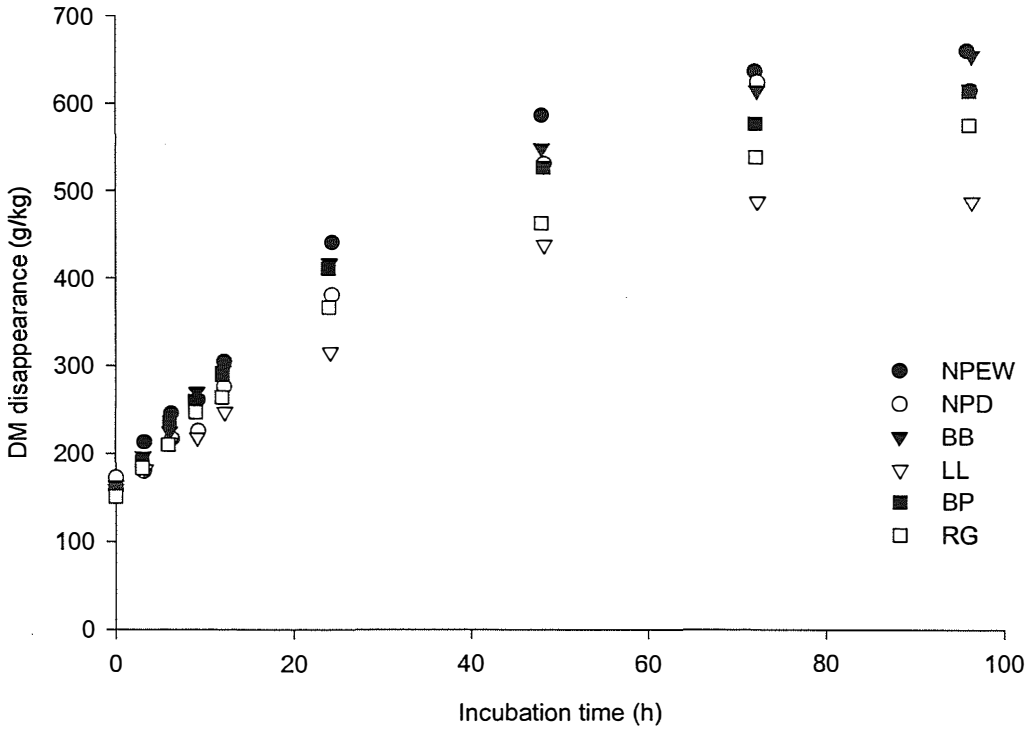


**Figure 6.8. Disappearance of a standard feed dry matter (DM) from nylon bags during rumen incubation in steers grazing 6 pasture types (Experiments 6.1, 6.3 - 6.7).** Pasture types defined in Table 6.3. Each point represents the mean for 2 steers.

Table 6.19 summarises the OM disappearance parameters for 3 mm ground, freeze-dried extrusa, incubated in nylon bags in the rumen of RF steers consuming a standard diet (Experiment 6.8). Figures 6.9 and 6.10 illustrate the degradation curves plotted using measured data points.

The exponential curve $Y = a + b\,(1 - e^{-ct})$ was a good fit for all data sets, with a high percentage of variation accounted for by the fitted curve, i.e. > 99 % for tropical grass pastures and > 94 % for $C_3$ pastures. Statistical tests showed significant differences ($P < 0.001$) in degradation rates (c), slowly degraded fractions (b), and potential degradable fractions (a + b), between the seven pasture types. The potential degradable fraction of RG and LL extrusa was greater ($P < 0.05$) than all other pastures, while BP and NPD extrusa had potential degradable fractions lower ($P < 0.05$) than all other pastures. Degradation rate of LL extrusa was greater ($P < 0.05$) than all other pastures, followed by RG extrusa, which was greater ($P < 0.05$) than all other pastures except LL. NPD had the lowest degradation rate,

which was lower ($P < 0.05$) than for all other pasture types. Degradation rate of BP extrusa was not significantly different to that of early to mid season tropical grasses.

**Table 6.19. Organic matter (OM) disappearance parameters for freeze-dried extrusa collected in Experiments 6.1 - 6.7, in rumen fistulated steers consuming a standard diet (Experiment 6.8)[†].** Values are the mean and standard error (in brackets) for 3 steers. Residual standard deviation (RSD) of the fitted equation is presented. Probability (P) for test of significance between coefficients is presented where relevant (see section 6.2.7); values in the same row with different superscripts are significantly different ($P < 0.05$)

| | Pasture type[‡] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **NPEW** | **NPT** | **NPD** | **BB** | **LL** | **BP** | **RG** | **P** |
| a (g/kg OM) | 34 (12.5) | 104 (12.8) | 61 (7.7) | 176 (14.5) | 52 (92.3) | 353 (19.5) | 100 (56.3) | N/A |
| b (g/kg OM) | $781^{a}$ (12.2) | $719^{b}$ (12.7) | $691^{b}$ (7.5) | $620^{c}$ (14.1) | $821^{ab}$ (89.6) | $374^{d}$ (19.2) | $859^{a}$ (54.0) | < 0.001 |
| a + b (g/kg OM) | $815^{bc}$ | $823^{b}$ | $752^{d}$ | $796^{c}$ | $873^{a}$ | $727^{d}$ | $959^{a}$ | < 0.001 |
| c ($h^{-1}$) | $0.079^{c}$ (0.0024) | $0.060^{d}$ (0.0025) | $0.047^{e}$ (0.0014) | $0.078^{c}$ (0.0034) | $0.280^{a}$ (0.0311) | $0.077^{c}$ (0.0079) | $0.225^{b}$ (0.0167) | < 0.001 |
| RSD | 14.5 | 18.1 | 11.6 | 16.8 | 28.4 | 24.0 | 24.8 | |

[†] The loss of OM is described by the equations given by McDonald (1981), where $Y_1 = A$ up to time $t_0$, and $Y_2 = a + b\,(1 - e^{-ct})$, from time $t_0$ onwards (see section 3.6.4). The parameters shown above are defined as: "a" = rapidly degradable fraction; "b" = more slowly degraded fraction; "a + b" = potential degradable fraction; and "c" = degradation rate of "b".

[‡] For definition of pasture types, see Table 6.3.

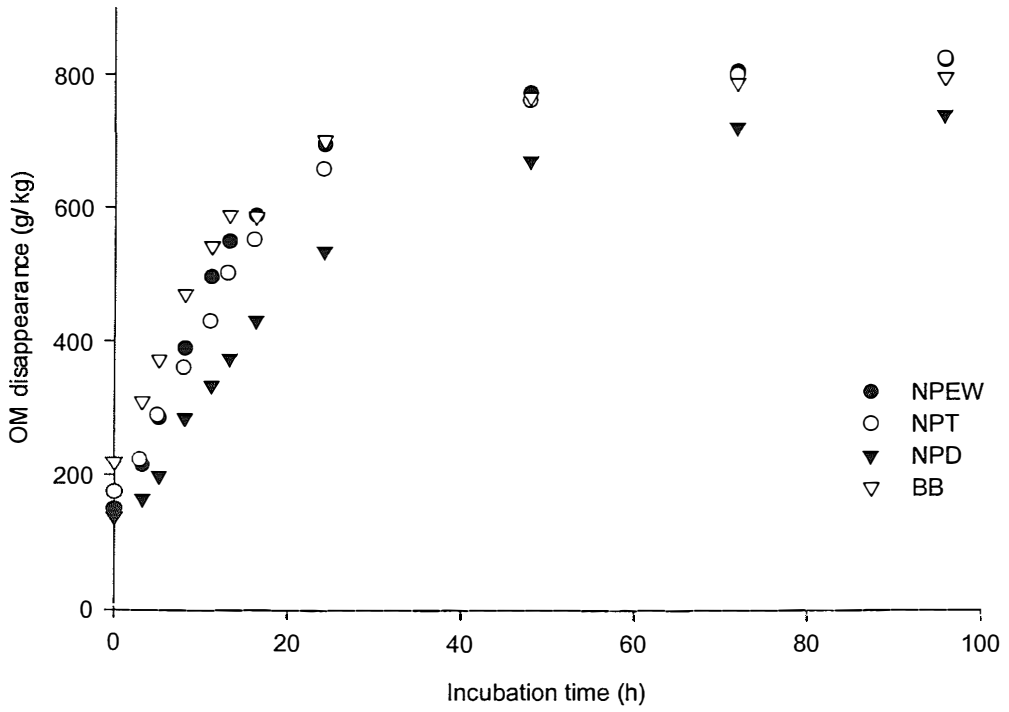


**Figure 6.9. Disappearance of extrusa organic matter (OM), (collected in Experiments 6.1 - 6.4) from nylon bags during rumen incubation in steers consuming a standard diet.** Pasture types defined in Table 6.3. Each point represents the mean for 3 steers.

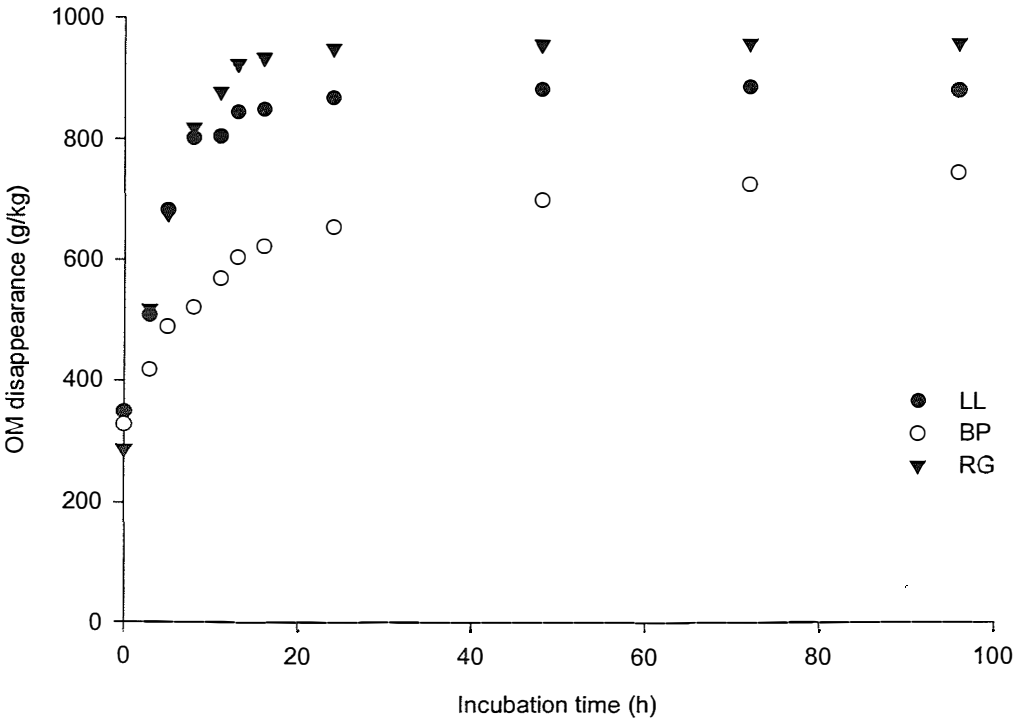


**Figure 6.10. Disappearance of extrusa organic matter (OM), (collected in Experiments 6.5 - 6.7) from nylon bags during rumen incubation in steers consuming a standard diet.** Pasture types defined in Table 6.3. Each point represents the mean for 3 steers.

### 6.3.9 Relationship of various parameters with eMCP

As presented in Figure 6.11, there was a strong positive linear relationship (P = 0.001) between eMCP and RDP/DOMI for all pasture types.

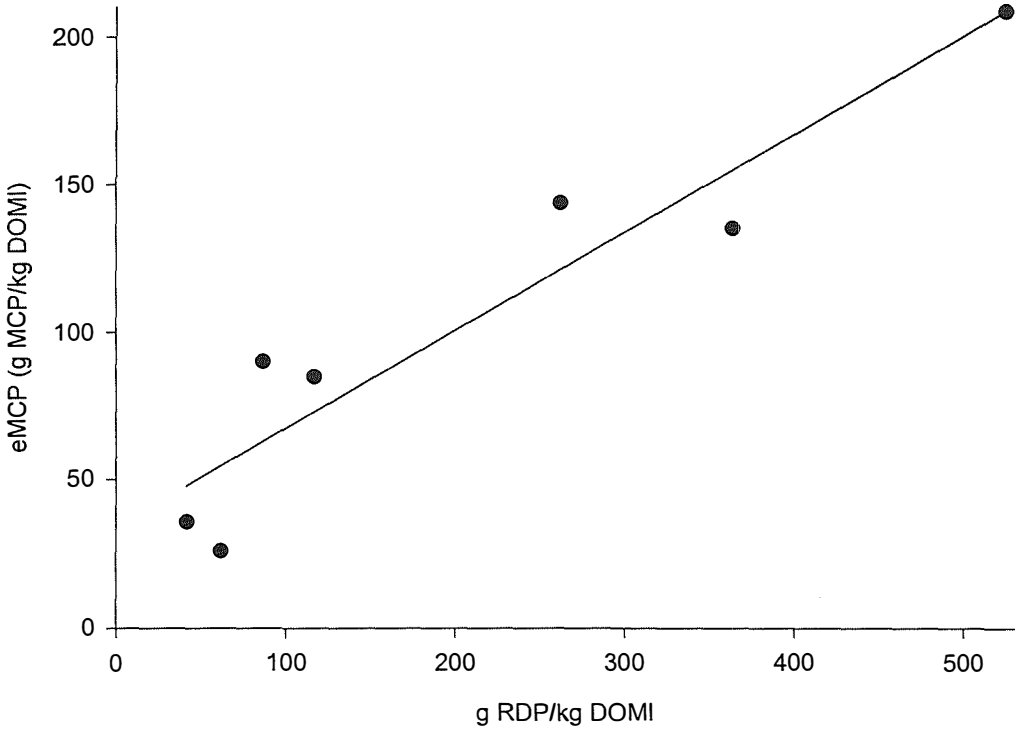


**Figure 6.11. Relationship between efficiency of microbial crude protein production (eMCP) and rumen degradable protein (RDP) supply per unit of digestible organic matter intake (DOMI) in the diet of steers grazing a range of pasture types.** Equation: y = 0.333x + 34.1, r = 0.94, RSD = 23.5, P = 0.001. (MCP, microbial crude protein).

There was no relationship between eMCP and TNSC in plucked pasture leaf, when TNSC was expressed as either g/kg DM or g/kg DOMI (relationships not shown). However, a strong negative linear relationship (P = 0.006) was evident between eMCP and the TNSC/RDP ratio in the diet, across all pasture types (Figure 6.12). It should be noted that the TNSC (expressed as glucose equivalents) was measured in plucked leaf samples while RDP was determined from the disappearance of NDIN from extrusa incubated in nylon bags (see section 3.6.5). As demonstrated in Figure 6.13, there were also strong negative linear relationships between eMCP and both NDF and ADF concentrations in extrusa (P = 0.015 and P = 0.013, respectively). However, no significant relationships with eMCP were evident for DOMI (expressed as proportion of LW), rumen particle FOR or rumen fluid FOR (Figures 6.14 - 6.15).

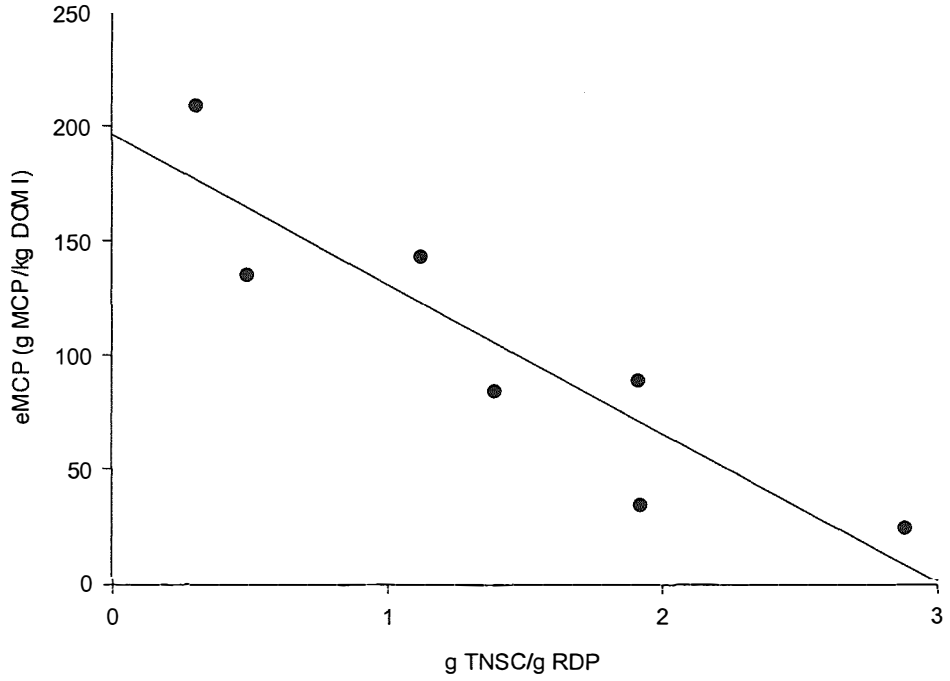


**Figure 6.12. Relationship between efficiency of microbial crude protein production (eMCP) and the ratio of total non-structural carbohydrate (TNSC) to rumen degradable protein (RDP) in the diet of steers grazing a range of pature types.** Equation: y = -64.79x + 196.29, r = 0.90, RSD = 30.6, P = 0.006. (DOMI, digestible organic matter intake; MCP, microbial crude protein).

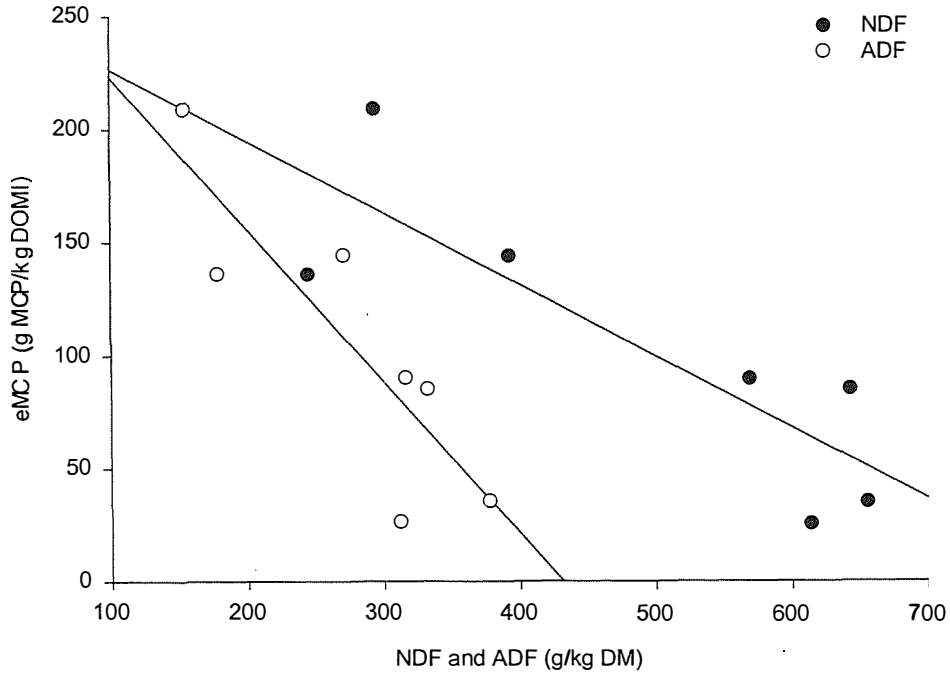


**Figure 6.13. Relationship between efficiency of microbial crude protein production (eMCP) and either neutral detergent fibre (NDF) or acid detergent fibre (ADF) in extrusa from steers grazing a range of pasture types.** NDF equation: y= -3.17x + 258.1, r = 0.85; RSD = 36.8, P = 0.015; ADF equation: y = -6.72x + 289.9, r = 0.86, RSD = 35.9, P = 0.013. (DM, dry matter; DOMI, digestible organic matter intake; MCP, microbial crude protein).

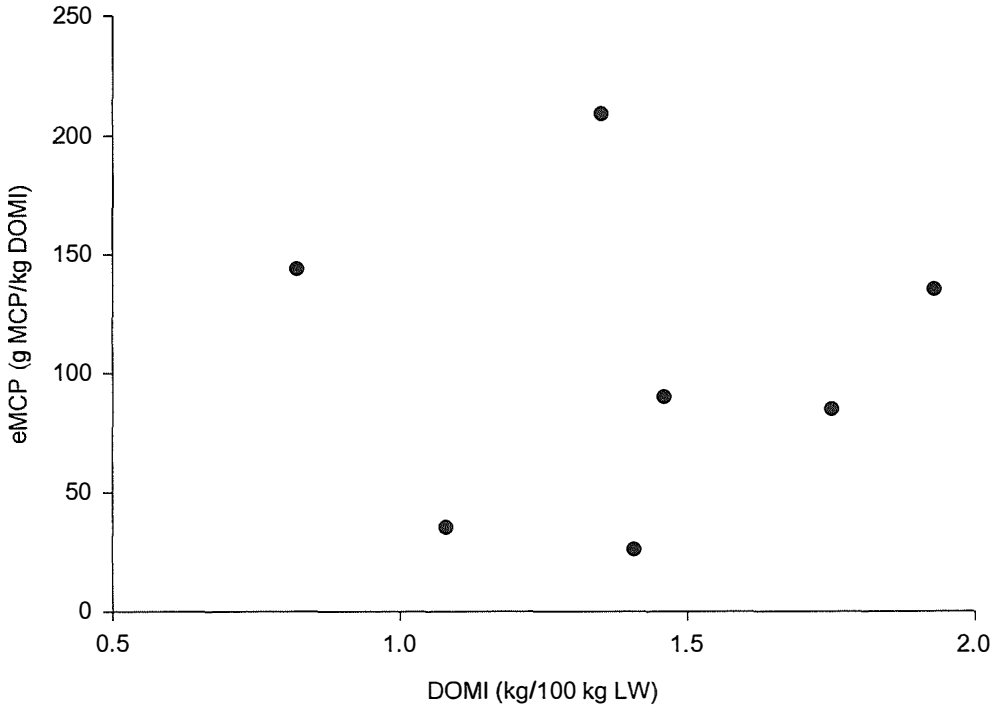


**Figure 6.14. Relationship between efficiency of microbial crude protein production (eMCP) and digestible organic matter intake (DOMI), expressed as a proportion of steer liveweight (LW), for steers grazing a range of pasture types.** Linear regression not significant: P = 0.98. (MCP, microbial crude protein).

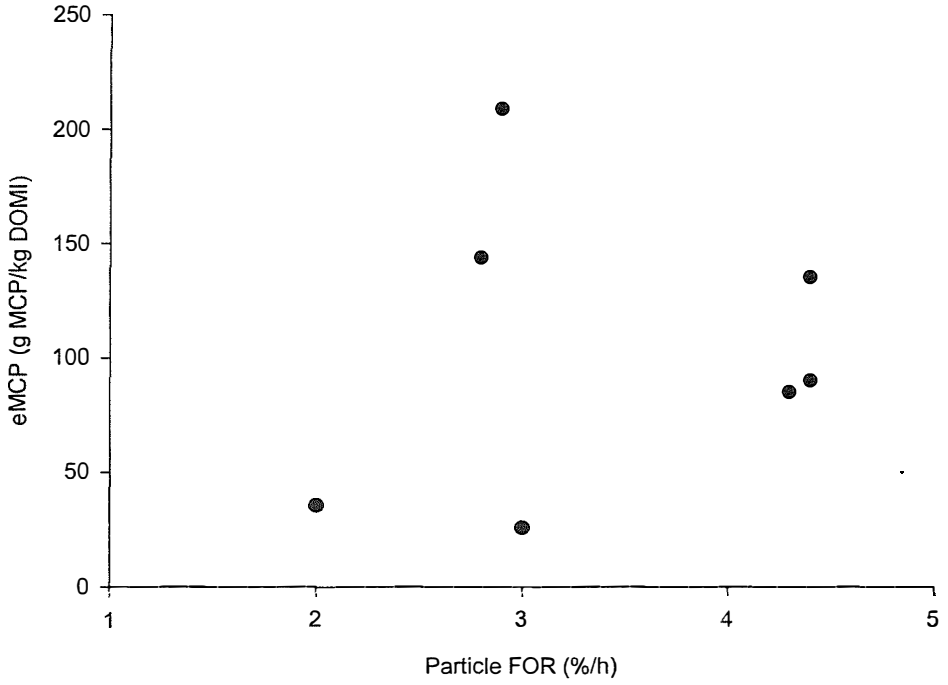


**Figure 6.15. Relationship between efficiency of microbial crude protein production (eMCP) and rumen particle fractional outlfow rate (FOR) in the rumen of steers grazing a range of pasture types.** Linear regression not significant: P = 0.77. (DOMI, digestible organic matter intake; MCP, microbial crude protein).

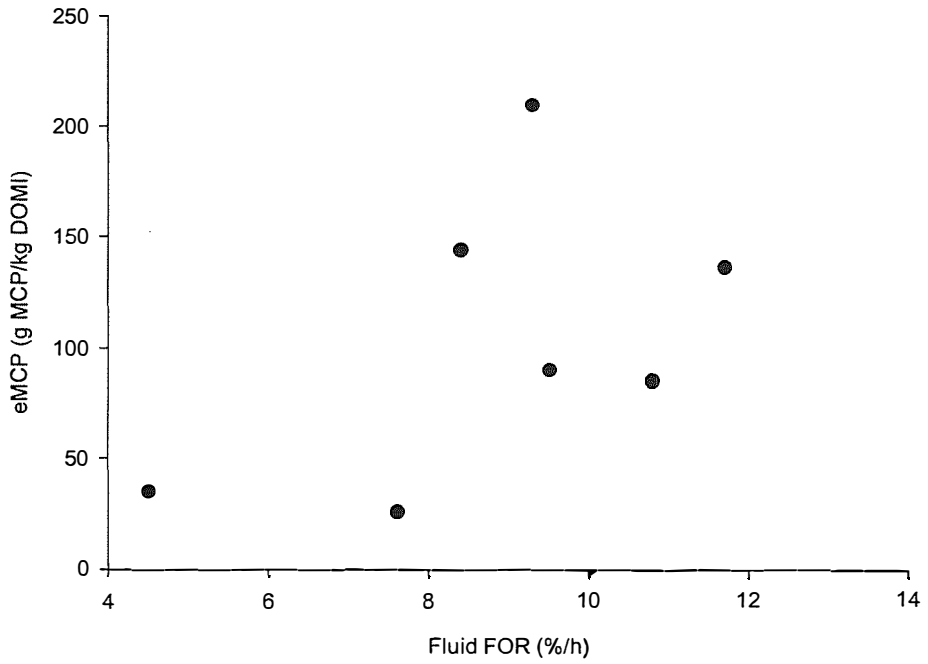


**Figure 6.16. Relationship between efficiency of microbial crude protein production (eMCP) and fluid fractional outflow rate (FOR) in the rumen of steers grazing a range of pasture types.** Linear regression not significant: P = 0.24. (DOMI, digestible organic matter intake; MCP, microbial crude protein).

### 6.3.10 Additional samples taken during the measurement period for LWG steers while grazing RG pasture

Due to lower than expected liveweight gain results for steers grazing the RG pasture, additional samples were taken from both the pasture and the LWG steers during their measurement period. Plucked leaf samples, taken from the LWG pasture at *ca.* 17.30 h after a warm, cloud-free day, had low DM content of 160 g/kg and high CP and $KNO_3$ concentrations of 356 and 40 g/kg DM, respectively.

The highest and the lowest performing LWG steer in the RG experiment had weight gains of 1.15 and -0.34 kg/d, respectively, over the 57-d grazing period. Rumen fluid and serum samples taken from each of these steers during the weight measurement period were found to be similar to one another and thus the mean of the results is presented. The rumen fluid pH and rumen $NH_3$-N concentration for the two LWG steers was high at 7.0 ± 0.65 and 368 ± 73.9 mg/l, respectively. Serum urea was extremely high at 13.3 ± 1.45 mM.

Faecal samples taken from a representative group of the LWG steers showed a low worm egg count (100 eggs per g), which was too low to culture. The faeces were found negative for coccidian infection, and liver and stomach fluke (*Fascioloa* and paramphistomes, respectively).

## 6.4 DISCUSSION

### 6.4.1 Variation in eMCP with pasture type

This was the first series of experiments to estimate eMCP in grazing cattle for a range of tropical pastures relevant to the north Australian beef industry. A large spread in eMCP was found across pasture types and within pasture types across season (Table 6.14). All the tropical grass pastures studied had eMCP that fell well below the extant feeding standards' range of 130 - 170 g MCP/kg DOMI (ARC, 1984; Madsen, 1985; Ausschuss fur Bedarfsnormen, 1986; INRA, 1988; SCA, 1990; AFRC, 1993; NRC, 1996b). Importantly, even tropical grass pastures grazed during the period of highest pasture quality in the early and mid wet seasons (NPEW and BB) had very low eMCP of 85 and 90 g MCP/kg DOMI, respectively. Native grass pasture (NPEW) produced a similar eMCP to introduced tropical grass pasture (BB). The eMCP values for high quality native pasture declined rapidly with increasing maturity, dropping to *ca*. 30 g MCP/kg DOMI by the wet/dry transitional period. Cattle grazing the tropical legumes, Dolichos lablab (LL) and butterfly pea (BP), had eMCP that fell within the extant feeding standards' range, while annual ryegrass pasture (RG) resulted in an eMCP that was much higher than the range, at 209 g MCP/kg DOMI.

However, as discussed in section 2.2, although the various feeding systems give either an average figure or a range for eMCP, the most recent versions recognise that eMCP is not a constant and may vary according to diet type. The Australian feeding standards (SCA, 1990) give efficiency values (g MCP/kg DOMI) for three broad types of diets: 170 for first growths of temperate legumes, grasses and green forages, fresh or dried; 130 for all other fresh and dried forages, and mixed diets; and 95 for silages. The AFRC (1993) recommends the use of an adjustment factor for level of feeding, or rumen outflow rate, while Level 1 of the NRC model (NRC, 1996b) considers pH effects by using an adjustment factor for diets containing less than 40 % forage. Level 2 of the NRC model (NRC, 1996b) includes aspects of the CNCPS,

described by Russell *et al.* (1992), Sniffen *et al.* (1992) and Fox *et al.* (1992), which predicts microbial growth from feed carbohydrate and protein fractions as well as their digestion and passage rates. Furthermore, reported values for eMCP range from 50 to 370 g MCP/kg DOMI (Corbett, 1987; SCA, 1990; Purser & Hogan, 1992) and appear to vary with diet quality, forage type and season of pasture growth. Thus the wide variation in the eMCP values determined in the current experiments is not a new phenomenon.

The high value of eMCP obtained for ryegrass in Experiment 6.7 is close to the theoretical maximum MCP yield of 312 g/kg OMADR, equivalent to *ca.* 200 g MCP/kg DOMI, as proposed by Corbett (1987). However, using the calculation suggested by Corbett (1982), (see section 2.2), to adjust the OMADR/DOMI ratio for pasture digestibility, the theoretical maximum MCP yield can be estimated as *ca.* 230 g MCP/kg DOMI for the highly digestible ryegrass pasture studied in Experiment 6.7. Thus, the eMCP value estimated for ryegrass in Experiment 6.7 appears realistic and gives confidence in the methodology used. Other workers have also estimated high eMCP values for ryegrass, which fall above the extant feeding standards' range of 130 - 170 g MCP/kg DOMI. These include estimates of 186 - 215 g MCP/kg DOMI obtained by Beever *et al.* (1986) using duodenally fistulated cattle consuming freshly cut ryegrass pasture and values of 185 and 191 g MCP/kg DOMI recorded by Prior *et al.* (1998) and King *et al.* (1999) respectively, using the urinary PD excretion method for cattle consuming ryegrass hay. However, lower eMCP values for ryegrass pasture, falling within or below the extant feeding standards' range, have also been measured. These include values for freshly cut forage, of 148 and 169 g MCP/kg DOMI for sheep (Beever *et al.* 1978), and 119 and 141 g MCP/kg DOMI for cattle (Lee *et al.* 2002). These lower eMCP values were associated with lower pasture digestibility and protein concentrations than for the pastures producing the high eMCP estimates cited above. Similar to the estimates for ryegrass pasture, literature values for other temperate grass and legumes species have shown eMCP to commonly fall either within or above the extant feeding standards' range (Hume & Purser, 1974; Corbett & Pickering, 1983; Beever *et al.* 1986; McMeniman *et al.* 1986b).

Feeding studies of tropical grasses either freshly harvested, cut and dried, or fed as hays, have recorded similar low eMCP to those found in Experiments 6.1 to 6.4 (Bolam, 1998; Prior *et al.* 1998; King *et al.* 1999; Mullik, 1999; Shem *et al.* 1999; Nsahlai *et al.* 2000; Marsetyo *et al.* 2002). Similar to the current experiments, all of these research groups have reported eMCP values that fall below the range given in the feeding standards, ie. < 130 g MCP/kg DOMI. However, prior to the current experiments, no measurements had been made to confirm these low values on high quality tropical grasses while cattle were allowed to graze. When ruminants graze pasture, diet selection invariably results in a higher dietary CP content and digestibility than that on offer (Stobbs, 1975; Corbett, 1976). In addition, there are diurnal changes in pasture NSC concentration (Fulkerson & Donaghy, 2001) and diurnal patterns in grazing period and hence in rumen fill and FOR of fluid and particles from the rumen (Thomson *et al.* 1985). All of these factors can influence eMCP and it is for these reasons that researchers have attempted to quantify values for temperate pastures, and for pastures with mixed $C_3$ and $C_4$ species, in grazing animals (Corbett *et al.* 1982; Corbett & Pickering, 1983; McMeniman *et al.* 1986b; Funk *et al.* 1987a; Dove & Milne, 1994). This was also the approach taken in the current experiments with tropical pastures. In the current experiments, eMCP in cattle grazing tropical pastures did not differ markedly from published values for tropical pastures when fed as cut forage or as hays.

There are no reliable literature values for eMCP, measured for pure stands of tropical legumes, with which to compare the values obtained in the current experiments. Kennedy (1982) measured eMCP in cattle consuming hay consisting of green panic and the tropical legume, siratro. The author measured a low eMCP, 72 g MCP/kg DOMI, which was in the same range as the values obtained here for tropical grasses. However, the relative proportions of grass and legume in the hay were not given. It is probable that the eMCP attained in ruminants consuming pastures of mixed $C_3$ and $C_4$ species is dependent on the relative proportions of the $C_3$ and $C_4$ species in the pasture, as well as their maturity. Thus, increasing the proportion of $C_3$ species (whether a temperate pasture species or a tropical legume) in a pasture may be a strategy to increase eMCP up to within the extant feeding standards' range, in addition to the benefits of increasing overall diet digestibility and intake.

Several experiments have estimated eMCP in ruminants grazing pastures containing a mix of tropical grasses, temperate $C_3$ grasses and forbs. In some cases the resulting eMCP has been high (within or above the extant feeding standards' range), as for sheep grazing Mitchell grasslands and mulga/grass association pastures in south-west Queensland (McMeniman *et al.* 1986b). In these experiments, the $C_3$ species formed a significant portion of the diet, from 310 - 880 g/kg of the diet DM on individual experiments. However, in other cases of pastures containing both temperate $C_3$ and $C_4$ species, eMCP has been below the extant feeding standards' range, similar to that for tropical grasses, as in the experiments of Funk *et al.* (1987a) with cattle consuming blue grama rangeland in New Mexico.

In conclusion, the results of the current experiments confirm published values, which indicate that, in general, temperate pastures have greater eMCP than for tropical grass pastures. In addition, these experiments have indicated that tropical legumes are intermediate in eMCP between temperate pasture and tropical grass pasture species. It is probable that inherent dietary attributes associated with these groups of pasture species are the cause of the observed differences in eMCP. These factors are examined and discussed in the subsequent section. Regardless, the large range in eMCP values, across pasture types and with season, indicates the potential to investigate strategies to increase eMCP from the low values confirmed for tropical grass pastures. This would increase protein supply to the animal tissues with the intention of increasing liveweight gain of beef cattle at pasture.

### 6.4.2 Relationship of diet and animal parameters with eMCP

#### *6.4.2.1 Quantity of rumen-available N supplied in the diet*

The principles of reduced eMCP due to low N supply in the rumen are well known, with *in vitro* studies demonstrating that eMCP is reduced due to energetic uncoupling if the quantity of N is limiting (Hespell, 1979). The feeding standards have recognised that if RDP supply is limiting for microbial growth, then eMCP will be less than the potential maximum, and will be equivalent to the RDP supply per unit of energy. In the current experiments, a linear relationship was observed between eMCP and RDP/DOMI for the seven pasture types studied (Figure 6.11). However, this linear relationship, which covered a wide range of values for eMCP (26 -

209 g MCP/kg DOMI), is not biologically sensible, as eMCP would not be expected to increase indefinitely with increasing RDP supply. At some point, the response to RDP supply would be expected to reach plateau due to saturation of the microbial RDP requirement, as recognised by the arbitrary optimum values for eMCP given in the various feeding standards.

As already discussed, eMCP values for the annual ryegrass pasture studied here, similar to literature values for high quality temperate species, fell well above the range given in the feeding standards, while those for tropical grass pastures fell well below this range. It is thus proposed that temperate $C_3$ pasture species follow a separate response relationship that reaches a higher eMCP plateau than for tropical species. When RG pasture was excluded from the relationship of eMCP with RDP/DOMI for the current experiments, an exponential relationship of the form $y = a - b(e^{-cx})$, provided a good fit for the data (P = 0.03), accounting for 84 % of the variance (Figure 6.17).

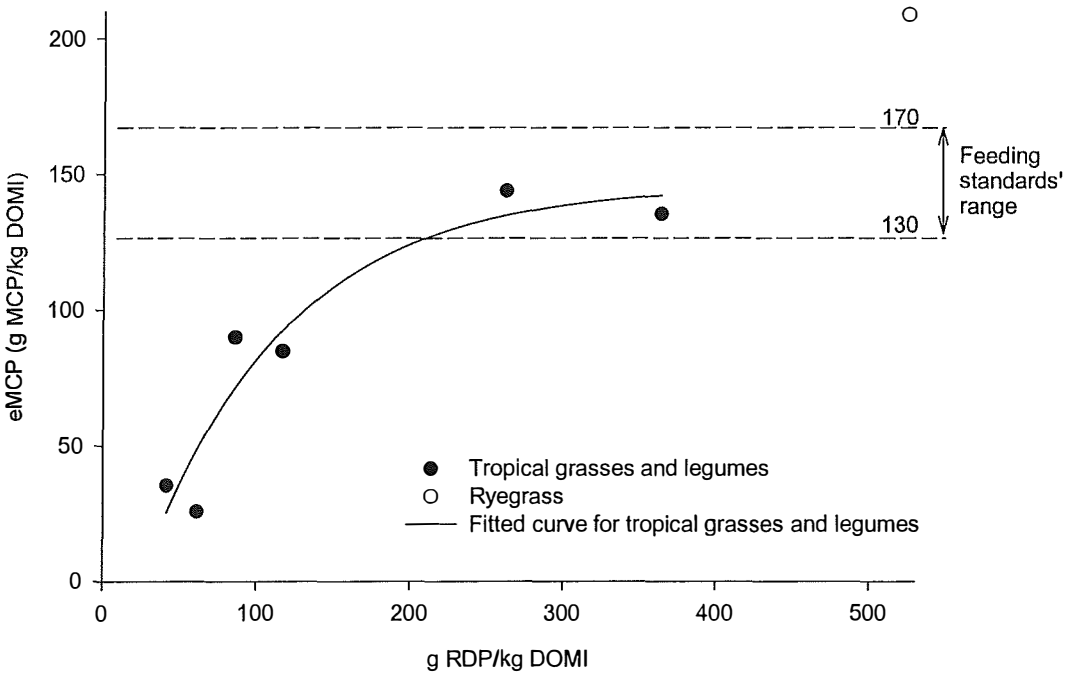


**Figure 6.17. Relationship between efficiency of microbial crude protein production (eMCP) and rumen degradable protein (RDP) supply per unit of digestible organic matter intake (DOMI) in the diet of steers grazing a range of tropical pastures.** Fitted relationship excludes ryegrass. Equation: $y = 145.7 - 189.1(e^{-0.0109x})$, adjusted $r^2 = 0.84$, RSD = 19.5, P = 0.03. Extant feeding standards' range of eMCP indicated. (MCP, microbial crude protein).

This response curve for tropical pastures highlights the fact that the supply of RDP in tropical grasses was well below the level required to produce eMCP in the extant feeding standards' range (i.e. < 130 g RDP/kg DOMI). Furthermore, the curve indicates that once RDP was increased up to within the extant feeding standards' range, as for the tropical legumes, eMCP reached plateau. However, as only one temperate pasture species was studied in this series of experiments, no direct evidence has been provided to support the hypothesis that separate response curves exist for temperate and tropical pastures, and the area warrants further attention. In addition, the variation in eMCP once RDP is adequate also deserves further study, as this may indicate limiting nutrients (e.g. NSC, amino acids or BCFA), or favourable conditions in the rumen, which could be targeted to increase eMCP.

The highest quality tropical grass species studied in the current experiments had RDP supply well below that required to produce eMCP in the extant feeding standards' range (i.e. RDP < 130 g/kg DOMI) and this points to RDP supplementation as the first avenue to be targeted when attempting to increase eMCP in cattle grazing tropical pastures. According to the response curve established above, relatively small quantities of supplemental RDP could be used to achieve marked increases in eMCP when pastures have ratios of RDP/DOMI that align with that part of the response curve where eMCP is increasing rapidly. In addition, the type of rumen-available N supplied in the supplement, i.e. whether as NPN or that derived from true protein, may also affect the eMCP response, as discussed in section 2.3.1.3, and warrants further investigation.

#### *6.4.2.2 Non-structural and structural carbohydrates supplied in the diet*

Although no relationship was evident between eMCP and TNSC supply in the pasture leaf (when TNSC was expressed as either g/kg DM or g/kg DOM), increasing fibre concentrations in the diet were associated with a linear decrease in eMCP (Figure 6.13). As discussed in section 2.3.1.1, the levels of SC and NSC in the feed are expected to influence eMCP through their effects on rates of fermentation, rates of passage and rumen pH, all of which affect microbial maintenance requirements. Higher fibre concentrations in pasture would be expected to lead to slower rates of passage and thus increased maintenance requirements for rumen microbes, which would explain the negative linear relationship between eMCP and fibre concentrations

in the current experiments. Furthermore, it is possible that the proportions of individual microbial species could alter due to changes in feed fibre concentrations, with possible associated changes in inherent maintenance requirements and thus in eMCP.

A review by Chesson *et al.* (1995) discussed the possibility of modifying aspects of the plant cell wall structure by genetic manipulation. In addition, Nelson & Moser (1994) reviewed the potential for modifying leaf:stem ratios and thus decreasing whole plant fibre levels. If such manipulations were successful in increasing total digestibility or rate of digestion of the plant cell wall, or in decreasing total plant fibre levels, it could be speculated that eMCP would also improve. However, as discussed by Satter *et al.* (1999), the factors involved in fibre accumulation, lignification and digestion of plant cell walls are complex and thus difficult to modify, in practice. In addition, the environmental conditions of the tropics cause plants to develop higher SC contents and corresponding lower nutritive value than in temperate environments, regardless of the genetic composition of the plant (Norton, 1982). A further hazard in the modification of tropical plant fibre composition and levels to be more similar to temperate species, is the possibility of inadvertently selecting for plants with lower survivability and yield potential in the tropical environments to which they are adapted (Van Soest, 1994).

Bolam *et al.* (1998) investigated the approach of increasing NSC levels on tropical pasture diets, with the intention of making the overall diet NSC concentrations more similar to that of temperate species. This group increased eMCP on rhodes grass hay, from 62 - 95 g MCP/kg DOMI, up to within the extant feeding standard range with grain or molasses supplementation fed at levels of at least 1.5 % LW. However, eMCP did not increase rapidly when these readily fermentable carbohydrate sources were supplied at low levels (0.5 % LW).

In the current experiments, as TNSC concentrations in plucked pasture leaf increased in relation to RDP concentration in the diet, a strong negative linear relationship with eMCP was observed (Figure 6.12). This finding, in conjunction with the lack of any significant relationship between eMCP and TNSC when expressed per kg DM, or per

kg DOMI, indicates that RDP, rather than TNSC, is the most limiting nutrient for eMCP across the range of pasture types studied.

#### *6.4.2.3 Dilution rate in the rumen*

It should be noted that the dilution rate studies were conducted under non-steady state conditions and thus the values for rumen fluid and particle FOR and rumen fluid volume should be regarded as relative rather than absolute. There was no significant relationship of eMCP with either particle or fluid FOR across the range of pastures studied in the current experiments despite the large range in FOR values across the seven pasture type (4.5 - 11.7 %/h and 2.0 - 4.4 %/h for fluid and particle FOR, respectively), (Figures 6.15 and 6.16). This was unexpected, as a range of both *in vitro* and *in vivo* experiments have shown clear relationships of increasing eMCP with increasing particle and/or fluid FOR (see section 2.3.2). Furthermore, most feeding standards recognise the importance of dilution rates on eMCP, with the AFRC (1993), in particular, predicting eMCP with the use of an adjustment factor for rumen outflow rate.

Continuous culture fermentations of mixed bacterial cultures have generally shown Michaelis-Menton type saturation responses for the effect of fluid dilution rate on eMCP (Stouthamer & Bettenhaussen, 1973; Isaacson *et al.* 1975; Russell & Cook, 1995). However, more recently, Meng *et al.* (1999) found a quadratic model to be the most appropriate for describing the relationship between fluid dilution rate and eMCP for a range of diet-types in a single-effluent continuous-culture system. Isaacson *et al.* (1975) found eMCP to approach maximum values with fluid dilution rates above *ca.* 6 %/h in a continuous culture system. The results of the current experiments conducted *in vivo*, over a range of pasture types, do not corroborate these relationships.

However, much higher fluid FOR than those measured in the current experiments have been reported for cattle consuming temperate pastures, including values up to 17 %/h for cattle consuming freshly cut ryegrass pastures (Beever *et al.* 1986) and 25 %/h for cattle grazing temperate species in the early growing season (McCollum & Galyean, 1985). Thus, the data set obtained from the current experiments lacks values in the very high range of fluid FOR.

An increase in intake has been demonstrated to be an important factor in increasing both liquid and solid pool dilution rates (Estell & Galyean, 1985), and has been associated with increased eMCP in a number of experiments (Chen *et al.* 1992a; Djouvinov & Todorov, 1994). However, in the current experiments, no relationship between eMCP and DOMI (expressed as proportion of LW) was observed (Figure 6.14). This finding is in agreement with the lack of an eMCP response to particle or fluid FOR in the current experiments.

The strong relationship between particle and fluid FOR observed in the current experiments (Figure 6.5) is in line with results of McCollum & Galyean (1985), who observed a similar high correlation of fluid passage rate with particle passage rate ($P < 0.01$; $r = 0.77$) for cattle grazing blue grama rangeland over a number of months. Such a relationship suggests that if any effects of digesta flow rate on eMCP were possible, they would be related to both fluid and particle FOR.

The lack of any relationship between eMCP and dilution rates in the current experiments indicates that factors other than rumen dilution rate were more important in influencing eMCP across the range of pasture types studied, or alternatively, that a complex interaction of factors was occurring. However, it is possible that eMCP may be increased if fluid and particle dilution rates could be increased in ruminants while consuming pasture with low dilution rates and low eMCP characteristics. Such a strategy was found successful by Mullik (1999), who significantly increased rumen fluid dilution rates (from 6.61 to 7.91 %/h) and eMCP (119 to 141 g MCP/kg DOMI) in steers consuming pangola grass hay plus urea supplement, by supplementing with salt supplied at 0.15 % LW. However, as discussed in section 2.3.2, although a number of such strategies have been used to increase dilution rates and eMCP experimentally, none of these have proven suitable for increasing eMCP in practical and commercial situations.

#### *6.4.2.4 Intraruminal recycling*

As discussed by Baker & Dijkstra (1999), protozoa can have a negative effect on eMCP by causing considerable intraruminal recycling of N, as well as through competition for substrates (see section 2.3.3). In the current experiments, eMCP did not increase in association with decreasing protozoal concentrations in the rumen

fluid. In fact, the lowest protozoal numbers were measured in the rumen fluid of steers consuming low quality native pasture (NPD: 0.21 x $10^5$/ml), which produced the lowest eMCP. In addition, the highest numbers were measured on LL pasture (3.5 x $10^5$/ml), which produced high eMCP, in the extant feeding standards' range. However, it is notable that the RG pasture produced low total protozoal numbers (0.60 x $10^5$/ml), second lowest only to dry season native pasture. It is possible that the low total protozoal numbers on RG pasture contributed to the very high eMCP estimated for that pasture type. The lack of any dramatic change in protozoal numbers or proportions of species before and after the main grazing periods (06.30 and 20.30 h, respectively, Figure 6.3) indicates that time of sampling would not have biased the results and they should thus be representative for the pasture types studied.

In addition to total protozoal numbers, the proportion of various species is an important consideration for the impact of protozoa on bacterial predation and eMCP. While *Entodinium* spp. are commonly very small in size, the other species in the Entodiniomorph group, as well as the species from the Holotrich group, have a much greater biological mass (*ca.* ten-fold in some cases, Dehority, 1993) and thus presumably consume greater quantities of bacteria. Examining the results in this light, it is evident that steers grazing RG pasture had low total protozoal biomass in the rumen fluid, as well as low protozoal numbers, due to the minor contribution of Holotrichs and large Entodiniomorph species. In addition, LL pasture resulted in a predominance of *Entodinium* spp. in the rumen fluid (82 % of total numbers), indicating that despite the large total number of protozoa, the effect on intraruminal recycling and substrate competition may be similar to that for NPEW and BB pastures which had greater relative proportions of the larger biomass protozoa from the Holitrich group and the large Entodiniomorph species.

The lack of a clear relationship of protozoal numbers with eMCP for these experiments indicates that protozoa are not the primary factor affecting eMCP across the pasture types studied. However, it is possible that complete defaunation may increase eMCP values for a particular pasture type. In contradiction to the assumption that protozoal numbers may have incremental effects on animal parameters and production, an experiment by McLennan (1992) with sheep consuming molasses-based diets, indicated that simply reducing protozoal numbers did not illicit the same

production responses as complete defaunation of the rumen. It was postulated by the author that this may reflect the ability of individual protozoa to increase in activity, in terms of predation of bacteria, when competition is reduced. Nevertheless, as discussed in section 2.3.3, the opportunity for complete defaunation to be implemented successfully in commercial situations seems limited due to the difficulty in maintaining animals in a defaunated state for any length of time.

#### *6.4.2.5 Rumen pH*

Although a large range was measured for mean rumen fluid pH values across pasture types (6.1 on LL to 6.8 on RG pasture), there was no obvious relationship of pH with eMCP. While a high mean rumen fluid pH on RG pasture was associated with high eMCP, LL and BP had low average pH (6.1 and 6.3, respectively), which were also associated with relatively high eMCP in the extant feeding standard's range.

While some *in vitro* studies have demonstrated that eMCP can decrease significantly at pH values less than 6.5 (Russell & Drombrowski, 1980; Strobel & Russell, 1986; de Veth & Kolver, 2001), others have found that eMCP was either not affected, or increased, with decreasing pH (Hoover *et al.* 1984; Shriver *et al.* 1986; Calsamiglia *et al.* 2002). Furthermore, effects of pH on eMCP *in vivo*, as reviewed by Kolver & de Veth (2002), have been unclear. However, studies both *in vivo* (Mould & Orskov, 1983/84) and *in vitro* (Terry *et al.* 1969; Stewart, 1977) have indicated that the microbial digestion of fibre is severely reduced at pH < 6.2 and is negligible at pH < 6.0.

In the current experiments, five of the seven pasture types studied had mean rumen fluid pH values </= 6.5. These included the two tropical legume pastures (LL and BP) and three of the tropical grass pastures studied (NPEW, NPD and BB). Although eMCP were low (less then the extant feeding standards' range) on the tropical grass pastures, LL and BP produced eMCP in the extant feeding standards' range, thus indicating no negative effect of low pH. It is also of interest that rumen fluid pH fell below 6.0 in most steers grazing LL and BP pasture on a number of the ten sampling times over 24 h (section 6.3.6). These sustained periods of low pH had no obvious negative effects on eMCP for steers grazing these two tropical legume pastures.

As discussed in section 2.3.4, other studies have indicated that the relationship of eMCP with pH *in vivo* may be complicated by interactions with substrate type and rumen dilution rates. Such interactions may explain the lack of any clear relationship between eMCP and pH in the current experiments.

### 6.4.3 Relationships between pasture, animal digestion, metabolic and performance parameters

The range of supporting information obtained in Experiments 6.1 - 6.7, concerning various pasture and animal factors, not only aids in the interpretation of the eMCP results but contributes to the relatively minimal body of current knowledge for the tropical pastures studied, particularly under grazing conditions.

#### *6.4.3.1 Ryegrass pasture*

The IVOMD of RG pasture (827 g/kg) was very high, as expected for its height and stage of maturity, and similar to the *in vivo* measurements of Beever *et al.* (1986), (OMD: 810 - 830 g/kg), for cattle consuming freshly cut perennial ryegrass pasture. Correspondingly, fibre components were low (Table 6.5), in line with results of others such as Beever *et al.* (1986). The high ash content of the extrusa (198 g/kg DM) was *ca.* 48 % greater than for the other pastures studied in the current experiments, which could have been caused by contamination of the pasture with soil during irrigation.

The CP composition of the RG extrusa was extremely high (354 g/kg DM) and was corroborated through analysis of the plucked leaf samples (379 g CP/kg DM). This level of CP was much greater than values stated in the literature for various grazed or cut ryegrass pastures (Beever *et al.* 1978, 1986; Corbett *et al.* 1982; Cruickshank *et al.* 1992; Lee *et al.* 2002) or ryegrass hays (Prior *et al.* 1998; King *et al.* 1999), which range from 100 - 250 g CP/kg DM. The CP concentration in RG pasture was also greater than that stated in the literature for clovers or lucerne (130 - 300 g/kg DM; Hume & Purser, 1974; Corbett *et al.* 1982; Kennedy, 1982; Beever *et al.* 1986). This high CP concentration was most probably caused by the high level of N fertiliser (76 kg urea/ha applied at the end of each grazing period, *ca.* every 20 d), which was intended to achieve the desired pasture growth and stocking rate capacity on the available land area.

In association with the high pasture CP composition, the nitrate content in RG plucked leaf was also extremely high at 62 g $KNO_3$/kg DM. This excessive level was present despite good conditions for plant growth and thus for conversion of plant nitrate to protein (warm, cloud-free weather with adequate irrigation). Plants containing greater than 15 g $KNO_3$/kg DM are commonly identified as being potentially hazardous to ruminant livestock (Dowling & McKenzie, 1993). This toxicity occurs due to conversion of nitrate to nitrite in the rumen by the microbial enzyme, nitrate reductase. Nitrite can then be converted to $NH_3$ by the rumen microbes and used for microbial growth. However, if reducing power is limiting in the rumen, or there is insufficient readily available carbohydrate to allow utilisation of the $NH_3$, nitrite can build up in the rumen (Van Soest, 1994). As discussed by Dowling (1993) and Van Soest (1994), nitrite is toxic to both the rumen organisms, inhibiting cellulose digestion, and to the animal host. Excess nitrite is absorbed into the bloodstream where it oxidises haemoglobin to methaemoglobin, which is unable to transport oxygen. Death can occur from oxygen starvation of body tissues.

However, despite the levels of $KNO_3$ in RG extrusa being *ca.* four times greater than the commonly stated toxic level for ruminants, eMCP was extremely high and apparently unaffected, and no visual signs of ill-health were observed in RF, OF or LWG steers during Experiment 6.7. It is well known that the susceptibility of the animal to nitrite poisoning can be lessened by adaptation of the rumen microbial population to high nitrate levels and by provision of readily available carbohydrate sources in the diet, which increase microbial N requirements (Dowling & McKenzie, 1993; Van Soest, 1994). Although TNSC concentrations were relatively low in plucked ryegrass leaf (115 g of glucose equivalents/kg DM), considerable care was taken to adapt the experimental animals to the ryegrass pasture following prior grazing on dry season native pasture.

Despite no obvious effects on microbial activity or animal health, animal performance appears to have been negatively affected by the high plant CP and/or nitrate concentrations on the RG pasture, with relatively low liveweight gains measured for the LWG steers (average 0.78 kg/d over 57 d; initial steer LW: 378 kg). A range of measurements of both pasture and animal parameters for the LWG steers, including worm burden (section 6.3.10), failed to find any other explanation for the lower than

expected liveweight gains, and values were similar to those determined for the RF steers. However, voluntary intakes estimated for RF steers grazing the RG pasture were very low (1.35 kg DOMI/100 kg LW), and were lower than values estimated for mid to high quality tropical grasses and LL (Table 6.13). It is thus postulated that high blood $NH_3$ and/or urea levels were having a negative feedback effect on animal intake, which would have prevented excess rumen nitrate load and associated development of clinical nitrite toxicity. Similar negative effects of high pasture concentrations of N and nitrate-N on DMI and growth rate of lambs have been demonstrated by de Villiers & van Ryssen (2001).

Additional factors that may have contributed to the low liveweight gain measured on RG pasture include the increased metabolic cost associated with detoxification of $NH_3$ to urea in the liver, as well as a net utilisation of ingested amino acids for this detoxification process (Lobley *et al.* 1995; Greaney *et al.* 1996). Furthermore, the high moisture content of the RG pasture (886 g/kg) may have contributed to the reduced intakes on RG pasture. As reviewed by Van Soest (1994), although the addition of water per se to the rumen has little effect on intake, it has been postulated that water retained in structural components of ingested forage can inhibit intake, in addition to the time required to consume the nutrient dilute feed. Experiments by John & Ulyatt (1987) and Meissner *et al.* (1992) confirmed the importance of forage DM content, finding that animals ate to a constant fresh material mass. Meissner *et al.* (1992) concluded that intake of ryegrass pasture by sheep was only maximised when pasture DM content was at least 180 - 200 g/kg, which is greater than that measured in Experiment 6.7 (114 g DM/kg).

However, despite the strong evidence for reduced animal intake and liveweight gain on RG pasture, the absolute value of the liveweight gain estimate should be considered with some caution as the age, weight and breed of the LWG steers may have influenced the result obtained. Furthermore, the comparison of liveweight gain estimates between the seven pasture types should consider the changing age of the animals, with the seven pasture experiments being conducted over a 13-month period. In addition, the quality of the native pasture, as influenced by season, that was grazed by the LWG steers between experiments may have had a carry-over effect on the subsequent liveweight gain measurements. Despite these factors, the results should

be reliable for the pasture conditions and the animal specifications in each case, with in most cases, the liveweight gain measurements being conducted over a considerable period, of at least 56 d, with good correlation coefficients for liveweight gain over time, in most cases greater than 0.90.

The TNSC concentration measured on RG plucked leaf (115 g/kg DM) was low, being only slightly higher than values measured for high to mid quality tropical grasses and much less than those measured for tropical legumes in the current experiments (Table 6.6). Average daily WSC content measured for perennial ryegrass pastures by Lee *et al.* (2002) and Beever *et al.* (1986) were considerably greater than the TNSC measured in Experiment 6.7, ranging from 145 to 243 g/kg DM. Similar high values for perennial ryegrass were documented over varying stages of regrowth, time of day and sward layer by Delagarde *et al.* (2000). However, similar to the current experiment, biennial ryegrass WSC concentrations measured by Trevaskis *et al.* (2001) were also low, at 72 - 105 g/kg DM. As reviewed by Fulkerson & Donaghy (2001), the addition of N fertiliser initially lowers WSC concentration in grasses by promoting plant growth and this may be an explanation for the low TNSC levels measured in Experiment 6.7. Interestingly, Beever *et al.* (1986) and Lee *et al.* (2002), who fed freshly cut ryegrass to cattle, measured similar low total VFA concentrations (60 - 115 mM) and similar molar VFA proportions to values recorded in the current experiment (Table 6.15), despite their pastures having higher WSC content.

The very high rumen $NH_3$-N (382 mg/l), serum urea (9 mM) and faecal N (33 g/kg DM) concentrations measured in Experiment 6.7 are in keeping with the high pasture CP concentrations. In addition, the relatively low fluid and particle FOR estimated for the RG pasture can be explained by the low pasture intakes. Fluid FOR measured by Beever *et al.* (1986) for cattle consuming freshly cut ryegrass pasture at 24 g DM/kg LW were higher than that measured in the current experiment (9.3 %/h), at 11.5 - 17.0 %/h. However, in the experiments of Beever *et al.* (1986), the pasture CP was not excessive (< 200 g/kg DM) and animal intakes were not depressed.

### *6.4.3.2 Tropical grasses*

As expected, the diets selected by steers grazing native and introduced tropical grasses were much lower in IVOMD, and higher in NDF and ADF concentrations, than that for RG pasture (Table 6.5). The introduced tropical grass pasture, BB, studied in the mid wet season, had slightly higher IVOMD (Table 6.8) and lower fibre concentrations than the native tropical grass pasture studied in the early wet season (NPEW). IVOMD declined with maturity of the native grass pasture, as expected. However, the IVOMD estimates for NPT and NPD (656 and 608 g/kg, respectively) are higher than literature values determined *in vivo* for cattle consuming mature, low quality tropical grass hays, which generally range in OMD from *ca.* 500 - 600 g/kg (Hunter & Siebert, 1980, 1985b; Bolam, 1998; Prior *et al.* 1998; King *et al.* 1999; Mullik, 1999; Marsetyo *et al.* 2002). Nevertheless, it would be expected that under grazing conditions, the steers were able to select a diet higher in quality than that possible with the chaffed hays that are usually fed below *ad libitum* intakes in metabolism crates.

The CP concentrations in the diets selected by steers grazing tropical grass pastures were very low, less than 80 g/kg DM, even for high to mid quality pastures (NPEW and BB). As previously discussed, these levels of CP corresponded to levels of RDP supply/DOMI that are well below that required to achieve eMCP in the extant feeding standards' range (< 130 g RDP/kg DOMI), (Figure 6.17). There are few comparable published values for CP concentrations in the diet selected by cattle grazing tropical pastures. However, similar to the values for NPEW (79 g CP/kg DM), low CP levels were measured by Romero & Murray (1980; 73 g CP/kg DM) in the diet selected by cattle grazing black speargrass based pastures after the start of the wet season. In addition, similar dietary CP values were also estimated by Ash *et al.* (1982; 78 g CP/kg DM) for steers grazing the same paddocks as those used in Experiment 6.1 (NPEW), and similarly, *ca.* 1 month after burning. The CP concentration in the diet of cattle grazing the black speargrass/forest bluegrass pastures of Ash *et al.* (1982) did increase over several months following burning, up to a maximum of 106 g CP/kg DM, before declining again. Although not measured, the corresponding changes in OMD and protein degradability would influence eMCP, and could be predicted from the response curve with RDP/DOMI, such as that given in Figure 6.17. Diet CP concentrations for cattle grazing the introduced tropical grass, buffel grass,

have been documented by McLennan (1997) and while also much lower than values expected for temperate pastures, are slightly higher than values found for tropical grass pastures in the current experiments, with a maximum wet season value of 119 g CP/kg DM.

As soil fertility, rainfall and temperature will influence pasture CP concentrations, some variation is expected for wet season tropical grass pastures. However, it appears from the measurements made in the current experiments, and in those cited above, that dietary CP levels are likely to be low for tropical grass pastures and thus limiting for eMCP. These experiments have been conducted on some of the more fertile soils used for beef cattle production in northern Australia, and thus tropical grass pasture communities found in areas of lower soil fertility would be expected to have even lower CP content and thus RDP supply. Furthermore, the severity of the CP deficiency would be expected to increase rapidly with increasing pasture maturity and the associated decline in CP content, as observed in the current experiments.

The low dietary CP concentrations in tropical grass pastures in the current experiments were associated with low rumen $NH_3$-N, serum urea and faecal N concentrations which were much less than those measured for steers grazing tropical legume and RG pastures (Table 6.16). Rumen $NH_3$-N concentrations for even the higher quality tropical grass pastures (NPEW and BB) were well below the commonly quoted level of 50 mg/l, which is suggested as the minium level for optimal microbial protein synthesis (Satter & Slyter, 1974). *In vivo* studies have shown maximal microbial growth to be attained at $NH_3$-N concentrations ranging from 20 - 240 mg/l (Hume *et al.* 1970; Miller, 1973; Slyter *et al.* 1979; Okorie, 1981). However, the low eMCP values obtained on tropical grass pastures in the current experiments, which were much less than 130 g MCP/kg DOMI, appear to confirm that such low levels of rumen $NH_3$-N (8 - 23 mg/l) are associated with low eMCP.

Pen studies with tropical grass hays of low-N content (28 - 68 g CP/kg DM) have recorded similar low rumen $NH_3$-N concentrations (8.7 - 48 mg/l) to those measured in the current experiments in the grazing situation (Playne & Kennedy, 1976; Hunter & Siebert, 1980, 1985b; Prior *et al.* 1998). Although dietary CP content will have the major influence on average rumen $NH_3$-N concentrations, these values can also be

influenced by passage rate from the rumen, recycling of N to the rumen, and by the rate of $NH_3$ uptake by bacteria, which is in turn related to the rate of carbohydrate fermentation (Hoover, 1986). The interaction of these factors may explain why dietary CP concentrations are not well correlated with rumen $NH_3$-N concentrations for the tropical grass pastures studied in the current experiments, and why slightly lower rumen $NH_3$-N concentrations were obtained for NPEW, BB and NPT pastures than for similar CP content hays documented in the literature (Hunter & Siebert, 1980, 1985b; Prior *et al.* 1998). For example, the relatively high digestibility, and rumen fluid and particle FOR on NPEW and BB pastures may have contributed to lower measured rumen $NH_3$-N concentrations than on similar CP content hays.

Furthermore, rumen $NH_3$-N and serum urea concentrations were greater for steers grazing NPD pasture than for steers grazing NPEW, NPT and BB pastures, which had higher dietary CP concentrations. LWG steers were losing *ca.* 0.75 kg/d on NPD pasture, indicating that considerable tissue catabolism was occurring. It can be postulated that N derived from tissue catabolism elevated plasma urea concentrations, and that plasma urea recycled to the rumen maintained rumen $NH_3$-N concentrations at levels slightly greater than those observed for the higher quality, and higher CP content, tropical grass pastures. This process may also be the explanation for eMCP being maintained at a slightly higher level in steers grazing NPD to that for NPT, despite a lower dietary CP content of 27 g/kg DM on NPD (cf. 42 g/kg DM on NPT).

Total VFA concentrations were also very low (23 - 81 mM) for all tropical grass pastures studied in the current experiments. Similar low values (74.5 and 65.6 mM) have been measured for cattle consuming mature black speargrass hay in pens (Playne & Kennedy, 1976; O'Kelly & Spiers, 1992, respectively). However, O'Kelly & Spiers (1992) measured rumen fluid pH values for Brahman steers consuming black speargrass hay that were greater than those measured in the current experiments for tropical grass pastures (6.9 cf. 6.3 - 6.6). Interestingly, Total VFA concentrations for tropical grass pastures in the current experiments were lower on the highest quality pastures (NPEW and BB), at 23 and 31 mM, respectively (cf. NPT and NPD, at 81 and 68 mM) and thus were not positively correlated with plucked leaf TNSC concentrations or with diet digestibility. Thus, these results do not appear to corroborate the findings of Leng & Brett (1966) who demonstrated that production

rates and concentrations of individual VFA in the rumen of sheep were simply related. However, the higher fluid and particle FOR from the rumen for NPEW and BB pastures (cf. NPT and NPD) may have caused greater washout of VFA from the rumen, resulting in measured concentrations being unrelated to VFA production rates.

Steer DOMI, as a proportion of LW, appeared to be positively associated with diet digestibility and CP content, for the tropical grass pastures studied in the current experiments. Intake of NPEW was very high (1.75 kg DOMI/100 kg LW) and second only to that measured on LL pasture (1.93 kg DOMI/100 kg LW), (Table 6.13). These high intakes were achieved despite a low available pasture DM yield of 675 kg DM/ha for NPEW. The authors of NRC (1987) reviewed data summarised by Rayburn (1986) and concluded that grazed forage intake was maximised when forage availability was approximately 2250 kg DM/ha. In addition, Minson (1990) determined that bite size decreased with a forage mass of less than 2000 kg DM/ha, which was only partially compensated for by an increase in grazing time, resulting in decreased forage intake. However, as discussed by NRC (1996b), the relationship of intake with forage availability can vary with forage type and sward structure.

The pattern of intake by the RF steers, of the native tropical grass pasture at varying stages of maturity, was positively related to the liveweight gain of the intact, LWG steers. However, the extremely high liveweight gain measured over 21 d for intact steers grazing NPEW (1.77 kg/d) was much higher than the weight gains measured on LL or RG pastures (Table 6.7) and was achieved despite low diet CP concentrations (< 80 g/kg DM) and low eMCP (85 g MCP/kg DOMI). These LWG steers had been grazing unsupplemented native pasture over the preceding dry season and were probably losing similar amounts of liveweight to those steers monitored in the NPD experiment (0.75 kg/d over 56-d measurement period). It appears likely that the high growth rates recorded on NPEW were partially caused by compensatory growth effects. Compensatory growth refers to a period of enhanced growth following nutritional restriction and occurs due to reduced maintenance requirements, increased efficiency in growth and fattening, increased protein deposition and an increase in feed intake (Ryan, 1990; Ryan *et al.* 1993a,b). In addition to changes in tissue growth, liveweight changes also reflect changes in gut fill and body water content (McLean *et al.* 1983). It is also recognised that the 21-d period of measurement for

LWG steers on NPEW, which was dictated by pasture condition, was too short for reliable estimates of liveweight gain. Nevertheless, the measurement should represent a reasonable approximation due to prior grazing (*ca.* 1 month) on similar native pasture, exclusion of a 1-week preliminary grazing period from the NPEW data, and the strong correlation coefficients ($r > 0.97$) for individual steer linear regressions for liveweight gain.

It is also relevant to compare the LWG steer liveweight measurements and RF steer faecal N concentrations on the native tropical grass pastures in the current experiments, with those measured by Winks *et al.* (1979) on black speargrass based native pastures in north Queensland. These authors conducted a series of grazing experiments over a 5-year period and found that cattle commenced losing weight when faecal N levels fell below 11 g/kg DM and that liveweight responses to urea-molasses supplements only occurred when faecal N levels fell below 13 g/kg DM. These faecal N levels of Winks *et al.* (1979) have been widely used as critical levels for supplementation across northern Australia, despite the possibility that the results may not be applicable to areas outside the northern speargrass region where the experiments were conducted. The current experiments on black speargrass based pastures in the southern speargrass region did not corroborate the results of Winks *et al.* (1979), with steers grazing NPT and NPD pastures having similar faecal N concentrations of 10 g/kg DM, although steers grazing NPT were gaining weight (0.5 kg/d) while those grazing NPD were losing 0.75 kg/d. Thus, the current experiments indicate that faecal N levels are not a sensitive indicator of liveweight gain. However, as previously discussed, the LWG measurements obtained in the current experiments should be treated with some caution due to the possible influence of cattle age, weight and previous grazing history on the values recorded.

#### *6.4.3.3 Tropical legumes*

Diets selected by steers grazing the tropical legumes, LL and BP, were considerably higher in CP and leaf TNSC, and lower in NDF and ADF concentrations, than diets selected on the highest quality tropical grass pastures (NPEW and BB), (Tables 6.5 and 6.6). The higher dietary CP levels were reflected in higher rumen $NH_3$-N, serum urea and faecal N concentrations (Table 6.16), and in higher diet RDP supply/DOMI and eMCP (Table 6.14 and Figure 6.17) than values measured for steers grazing

tropical grass pastures. The high TNSC concentrations in tropical legume plucked leaf were associated with the highest rumen fluid VFA concentrations and lowest rumen fluid pH of the seven pastures studied (Table 6.15). The low dietary fibre levels, comparative to tropical grasses, were associated with high IVOMD for LL pasture (777 g/kg), but not for BP (585 g/kg), which was lower in IVOMD than even dry season native pasture (NPD), (Table 6.8). The IVOMD determined in Experiment 6.5 for LL extrusa was greater than the average *in vitro* DMD (IVDMD) measured by Hendricksen & Minson (1985) for lablab green leaf (675 g/kg). Unfortunately, there is a lack of other comparative data in the literature for grazed, pure stands of tropical legumes, particularly when fed to cattle.

RF steer DOMI was also very high for LL pasture (1.93 kg/100 kg LW) in association with the high diet IVOMD and green leaf yield (2419 kg DM/ha). However, DOMI for RF steers consuming BP pasture was very low (0.82 kg/100 kg LW) and was associated with the low diet IVOMD and low green leaf yield (323 kg DM/ha). The resulting liveweight gains measured on these pastures reflected the DOMI, with the gain on LL (0.73 kg/d) being more than double that on BP (0.33 kg/d).

As discussed by Jones *et al.* (2000), pasture quality and thus animal performance on tropical legumes is likely to be affected by a range of factors including plant growing conditions, maturity of the pasture, available pasture yield and the proportion of leaf and stem selected. As documented by Jones *et al.* (2000) larger differences in tropical legume fibre and N content are usually observed between leaf and stem components of the one plant than between species, and thus the yield of accessible green leaf would be expected to be an overriding factor determining animal performance. Evidence for the importance of accessible green leaf to animal performance was provided by Hendricksen & Minson (1980) and Hendricksen & Myles (1980), who demonstrated that cattle strongly preferred to consume lablab leaf and consumed little stem before losing weight.

Below average seasonal rainfall was limiting growth on the dryland BP pasture (Table 6.2), and thus pasture yield (1277 kg DM/ha) and availability of green leaf (253 g/kg pasture DM) may have been contributing to the low animal intake as well as to the lower digestibility of the selected diet. This theory, of low availability of

green leaf causing reduced IVOMD and intake on BP pasture, is supported by the greater NDF and ADF concentrations, and lower CP concentrations in BP extrusa compared to plucked leaf (Tables 6.5 and 6.6), indicating that the OF steers were consuming a portion of stem in the diet. In addition, the RF and OF steers were observed to be consuming butterfly pea pods when grazing the BP pasture during the sampling period, as was visually evident in the extrusa, digesta and faecal samples. The digestibility and presence of anti-nutritive factors in the butterfly pea pods is unknown, but may have had some effect, positive or negative, on diet quality.

#### *6.4.3.4 Degradability of plucked pasture leaf and extrusa*

Two aspects were investigated in the nylon bag degradability studies, namely the effect of the pasture itself on disappearance in the rumen, and also the effect of the incubation medium. As extrusa samples could not be processed in time to allow incubation in the RF steers while they were consuming the same basal diet from which the samples came, plucked leaf samples were incubated in RF steers consuming each test pasture. These plucked leaf samples were intended to provide disappearance parameters representative of the highest quality material available on each pasture type, and for rumen conditions representative of the actual pasture from which the samples came.

Although the disappearance parameters for plucked leaf samples could not be statistically compared, it was evident that leaf samples from $C_4$ pasture species had different degradation characteristics to those from $C_3$ pasture species (Table 6.18 and Figures 6.6 and 6.7). LL, BP and RG plucked leaf had similar patterns of disappearance, with very rapid rates of degradation and high potential degradabilities, compared to those for tropical grasses. These aspects are associated with higher IVOMD, lower fibre levels and greater CP concentrations, and thus rumen $NH_3$ levels, for the $C_3$ pasture species as compared to the $C_4$ species (Tables 6.5, 6.8 and 6.16). It was also notable that the NPD plucked leaf had a much slower degradation rate and potential degradability than that measured for the other tropical grasses. This was associated with a lower IVOMD and CP content, and higher fibre concentrations in NPD than in the other tropical grasses and corroborates all other data demonstrating the poor quality of mature, dry season native pasture. Another point of interest is the poor fit of the exponential curve for LL plucked leaf, which appears to

be due to the declining DM disappearance values after 48 h of incubation (Figure 6.7). A possible explanation for the apparent decline in DM disappearance after 48 h would be microbial colonisation within the bags, which may not have been removed with the standard washing procedures.

Rumen incubation of a standard feed (native black speargrass based pasture, intermediate in quality between NPT and NPD) while RF steers were grazing each of the test pastures, gave an indication of the effect of the incubation medium on degradation parameters (Table 6.18 and Figure 6.8). The results indicate that the degradation rate of mid to low quality tropical grass pasture is not affected by incubation medium, and most significantly, not affected by rumen $NH_3$ levels, which varied widely across the seven pasture types studied (Table 6.16). However, the significantly lower potential degradability of the standard feed when incubated in the rumen of steers consuming LL pasture, than on all other pastures except RG, indicates that the proportions of microbe species present in the rumen of steers consuming LL pasture may have been less suited to fermentation of the $C_4$ pasture hay than that present in steers consuming the other pasture types.

The incubation of extrusa from each of the seven pasture types in RF steers consuming a standard diet, allowed comparison of the degradation parameters of each of the pasture diets without confounding effects of incubation environment (Table 6.19 and Figures 6.9 and 6.10). In general, the pattern of degradation, and potential degradable fractions, for extrusa samples incubated on the standard diet were similar to those for plucked leaf incubated in RF steers grazing the test pasture. This, once again, indicated that the nature of the diet itself had the major effect on degradation parameters rather than the incubation medium. However, BP extrusa had much lower degradation rate and potential degradable fraction than BP plucked leaf, which caused the pattern of OM disappearance to more closely resemble that for $C_4$ grasses than for the $C_3$ species, with the potential degradable fraction of BP extrusa not significantly different to that for NPD. These findings corroborate the low IVOMD for BP extrusa and the inference that the steers were selecting a high proportion of stem in the diet.

### 6.4.4 Consideration of *B. indicus* vs. *B. taurus* effects on parameters

MCP flow and eMCP were found to be similar for *B. indicus* and *B. taurus* species when fed low or high quality hays at 70 - 80 % of *ad libitum* intake (Kennedy, 1982), or when fed to energy maintenance requirements in Experiment 4.1 of this thesis. This suggests that the MCP production and eMCP values determined in Experiments 6.1 - 6.7 with high-content *B. indicus* cattle should be representative of both *B. indicus* and *B. taurus* species consuming these pasture types. However, a number of experiments detailed in the literature have shown differences between *B. indicus* and *B. taurus* cattle in rumen digestion parameters, intake and liveweight gain when consuming identical diets.

An experiment comparing Brahman and Hereford steers consuming low and high quality diets (O'Kelly & Spiers, 1992) found the rumen fluid of Brahman cattle to have greater protozoal numbers and bacterial content than for Herefords. These differences in microbial content of the rumen fluid were associated with quantitative differences in the end products of digestion, with greater concentrations of propionic, butyric, isobutyric and isovaleric acids in rumen fluid and greater pH in Brahmans than in Herefords. These breed differences in the end products of digestion, which influence the ME and essential nutrients supplied from the rumen to the body tissues, were postulated to explain the superior ability of *B. indicus* cattle to achieve and maintain a higher body weight than *B. taurus* cattle at the same level of intake. Others have reported a lower maintenance requirement of *B. indicus* cattle compared to *B. taurus* cattle (Frisch & Vercoe, 1977) which would be expected to also result in greater livewight gains for *B. indicus* cattle per unit intake (Frisch & Vercoe, 1969). However, when cattle have been allowed to consume good and medium quality forages *ad libitum*, voluntary intakes have been greater for *B. taurus* cattle (Frisch & Vercoe, 1969, 1977; Hunter & Siebert, 1985b).

Although no significant differences in potential rumen digestibility of DM (Hunter & Siebert, 1985a), or in total tract digestibility of OM, cell wall constituents or N (Frisch & Vercoe, 1969, 1977; Kennedy, 1982; Hunter & Siebert, 1985b; and Experiment 4.1 in this thesis) have been found between purebred, or high-content, *B. indicus* and *B. taurus* species, differences in rates of DM digestion in the rumen have been measured (Hunter & Siebert, 1985a; O'Kelly & Spiers, 1992). These differences in

rumen DM degradation rates have occurred on low-N diets and have been associated with higher rumen-$NH_3$ concentrations in *B. indicus* cattle compared to *B. taurus* (Hunter & Siebert, 1985a). In addition greater rumen fluid dilution rates have been associated with the higher rates of digestion found in *B. indicus* steers consuming low-N hay (Hunter & Siebert, 1985b).

The ability of *B. indicus* cattle to maintain higher rumen ammonia and plasma urea concentrations than *B. taurus* cattle on low-N content diets, has been suggested to be the result of the superior ability of *B. indicus* cattle to recycle endogenous N to the rumen (Hunter & Siebert, 1985b). However, this inference that tropically adapted cattle are better adapted to low dietary protein conditions through better N conservation mechanisms, was contested by Norton *et al.* (1979). These workers found that despite differences between *B. indicus* and *B. taurus* species in urea synthesis and degradation, there was little evidence to suggest that these differences constituted a significant N conservation mechanism as judged by nitrogen balance.

In conclusion, it appears that although values for MCP and eMCP are unaffected by cattle species, caution should be exercised in extrapolating values for other animal parameters measured here for high-content *B. indicus* cattle, to the *B. taurus* species.

### 6.4.5 Methodologies used to estimate eMCP and RDP/DOMI

#### *6.4.5.1 Urinary PD technique and Cr infusion for estimation of MCP production*

As the values for eMCP that were estimated in the current experiments for ryegrass and tropical grass pastures are similar to values published in the literature using a variety of other methods (see section 6.4.1), it appears reasonable to conclude that the PD:Cr ratio technique used to estimate MCP flow here, provided accurate results.

As discussed in section 2.5.1, the urinary PD technique of estimating MCP flow has potential for error in two key areas, which include the assumed ratio of purine-N to total N in the mixed rumen microbial population and the assumed endogenous PD excretion. It was not possible in the current experiments to estimate the purine-N to total N ratio of the microbes flowing to the intestines, thus the ratio assumed in the

equation of Chen & Gomes (1995) was used. However, the use of an endogenous PD excretion value specific for high-content *B. indicus* cattle, which was determined in Experiment 4.1, eliminated considerable error which would have been caused by using the previously assumed value developed for *B. taurus* cattle as stated in the equation of Chen & Gomes (1995). The significance of using the measured endogenous PD excretion value for high-content *B. indicus* cattle is demonstrated by the calculation of negative MN flows for a considerable number of spot urine samples taken on low quality tropical pastures (NPT and NPD) when using the assumed endogenous PD value of Chen & Gomes (1995; 385 µmol/kg $W^{0.75}$). When the lower endogenous PD excretion value for *B. indicus* cattle (190 µmol/kg $W^{0.75}$) was used, no negative values for MCP flow were estimated.

Confidence in the method of continuous intravenous Cr infusion, to allow estimation of urine output and thus total PD excretion in field situations, was developed in the series of experiments conducted in Chapter 5 of this thesis. Although intensive observations and sampling schedules were required to maintain continuous Cr infusion in the field and to collect all designated spot samples, the method was successful in allowing the estimation of total PD excretion in the grazing situation. The diurnal variation in the PD:Cr ratio was sufficiently large so that a sampling regime incorporating regular times within a 24-h period was necessary.

#### *6.4.5.2 Extrusa sampling for pasture quality and IVOMD analysis*

It is assumed that the CP, digestible OM and fibre composition analysed in the extrusa should be representative of that in the diet, as has been previously demonstrated (McManus, 1961; Little, 1972; Saul *et al.* 1986). However, it has been shown (Saul *et al.* 1986) that soluble carbohydrate content cannot be accurately predicted from extrusa, possibly due to soluble material bypassing the fistula, and for this reason, TNSC concentrations were measured on plucked pasture leaf samples only, as was the nitrate composition of the ryegrass pasture. As anticipated, the extrusa samples had a high ash content that was generally greater than that analysed in plucked leaf samples (Tables 6.5 and 6.6). This additional ash content would be expected to be of salivary origin as demonstrated by McManus (1961). It would be expected that the generally lower CP content in extrusa compared to plucked leaf samples would be due to the

inability of the steers to select a diet of 100 % leaf, in most cases, rather than due to leaching of N from extrusa samples (McManus, 1961).

While IVOMD estimates for the four tropical grass pastures (NPEW, NPT, NPD and BB) were high compared to published *in vivo* values for tropical grass pastures, as discussed in section 6.4.3.2, cattle would be expected to select a diet higher in quality under grazing conditions than when fed chaffed hays below *ad libitum* intakes in metabolism studies. Furthermore, the thirteen *in vivo* standards used to form the tropical grass standard curve covered a good range of OMD values and gave high a correlation coefficient for the relationship of *in vivo* / *in vitro* OMD (see section 6.2.5.4). This was also the case for the standard curves developed to adjust the tropical legume and ryegrass pastures. Thus the IVOMD analysis on extrusa samples should have provided accurate digestibility estimates for use in calculation of DOMI and eMCP.

#### *6.4.5.3 Yb method to estimate faecal output and thus OMI*

It is recognised, that error may have come from the assumption of 100 % recovery of Yb in the faeces, on all pasture types. However, the scope of this work did not permit for additional Yb recovery experiments to be conducted for each of the seven pasture types studied in the Experiments 6.1 - 6.7.

As reviewed by Galyean *et al.* (1986), considerable research has been conducted in this area and has shown that Yb is indigestible and as such should be completely recovered in the faeces. A review of the literature by Galyean *et al.* (1986) found that average faecal output estimate from Yb-labelled forage, over a range of experimental conditions, dosing and sampling methods, was 104 % of the total collection values, although Yb-predicted faecal outputs ranged from 86.2 to 144 % of total collection for individual trials. However, other trials, not included in the review of Galyean *et al.* (1986), have confirmed that faecal output estimates determined using Yb marker did not differ significantly from total collection values, for a range of feed types (Siddons *et al.* 1985; Krysl *et al.* 1988). As discussed by Galyean (1986), Yb recoveries < 100 % would be most likely due to marker regurgitation, analytical losses and errors, or failure to establish marker equilibrium. Other errors could be caused if the

frequency of dosing and sampling was insufficient to eliminate diurnal effects of marker excretion. In the current experiments, considerable care was taken to minimise such errors, with dosing and sampling schedules designed to reduce diurnal effects, and attention to detail with analytical processes.

#### *6.4.5.4 The suitability of other methods used to estimate OMD and DOMI under grazing conditions*

Because of the importance of the OMD estimate to the calculation of eMCP, several methods were used, to attempt to improve confidence in the results. However, while the IVOMD estimates appeared reasonable based on literature values for similar pasture types, the estimates made using both the odd-chain n-alkanes and IADF method appeared unreliable.

***Use of odd-chain n-alkanes to estimate OMD***

It is probable that the poor agreement between IVOMD estimates and OMD estimates obtained using the odd-chain alkanes $C_{35}$, $C_{33}$ and $C_{31}$ as internal markers, is due to the use of assumed values for faecal recoveries rather than measured values. A review by Dove & Mayes (1991) found alkane faecal recoveries in cattle to be lower and more variable than for sheep and suggested that more studies were necessary to define and confirm appropriate average recoveries for n-alkanes, similar to that completed for sheep. Later studies with cattle (Herd *et al.* 1996; Dicker *et al.* 1996; Sandberg *et al.* 2000) have confirmed the low and variable nature of recoveries for $C_{31}$ and $C_{33}$, with major differences evident between diets. In addition, Hendricksen *et al.* (2002), in studies with Brahman crossbred steers consuming tropical grass and lucerne hays, found n-alkane faecal recoveries to be highly variable between animals, experiments and diets for each chain-length n-alkane. Furthermore, there was variation in recovery between n-alkanes of different chain-length.

In addition, the $C_{35}$ n-alkane may have caused further inaccuracy in OMD estimation in the present experiments due to the very low concentrations of $C_{35}$ in extrusa samples on all pasture types. Casson *et al.* (1990) suggested that odd chain n-alkane concentrations should be at least 50 mg/kg DM to allow accurate predictions. In the present experiments, all pasture types had $C_{35}$ concentrations less than 40 mg/kg DM, with two of the pastures (LL and RG) having concentrations less than 10 mg/kg DM.

***Use of IADF as an internal marker to estimate OMD***

The poor agreement between IVOMD estimates and IADF-predicted OMD for these experiments were in contradiction of the results of Penning & Johnson (1983) and Dove & Coombe (1992), who found IADF to predict digestibility well for sheep consuming lucerne, ryegrass and clover diets. The current results also challenge those of Tamminga *et al.* (1989) who found IADF to predict OMD well for dairy cattle consuming high concentrate diets.

However, a number of other workers have found variable recoveries for IADF in both sheep and cattle consuming a range of diets, resulting in underestimation of digestibility (Cochran & Adams, 1986; Krysl *et al.* 1988; Sunvold & Cochran, 1991; Huhtanen *et al.* 1994; Ferret *et al.* 1999; Sandberg *et al.* 2000). In addition, Lippke *et al.* (1986) suggested that differential loss of particles from feed and faeces, through the filter crucible during analysis, could bias fibre recovery and thus digestibility estimates.

The possibility of differential particle loss of extrusa and faeces from nylon bags was investigated for four of the seven pastures studied in Experiments 6.1 - 6.7, with particle loss being up to 65 g/kg of initial sample DM for some samples. Furthermore, the percentage difference in particle loss between extrusa and faecal samples varied widely between the pasture treatments, ranging from 8 to 74 % difference (Table 6.11). Thus, it appears that differential particle loss may be a potential source of error and a contributing factor to the poor agreement of IADF-predicted OMD with IVOMD estimates.

***Dosed $C_{32}$ and $C_{36}$ n-alkane as a faecal output marker***

$C_{32}$ n-alkane was found unsuitable as an external faecal output marker in these experiments due to detection of significant levels of this alkane in cut pasture components, and in extrusa and faeces from un-dosed OF steers when grazing all pasture types. This was not the case for $C_{36}$ n-alkane, for which negligible concentrations (< 5 mg/kg DM) were detected in the pasture and in faeces from un-dosed steers. In general, the $C_{36}$ n-alkane marker predicted similar DOMI to the Yb maker (Table 6.13) considering the level of accuracy of these procedures, with predictions differing by less than 5 % for three of the seven pastures studied.

However, the difference in predicted DOMI between the two markers varied with pasture type and the discrepancy was greatest on dry season native pasture (NPD) with the $C_{36}$ n-alkane predicting 23 % less DOMI than that for Yb marker. As recovery experiments were not conducted for $C_{36}$ and Yb markers in these experiments, for each of the pasture types studied, it is impossible to determine which marker was more accurate in predicting DOMI. However, as there are a greater number of experiments validating the recovery of Yb in the faeces (Galyean *et al.* 1986), than those for $C_{36}$ n-alkane (Dove & Mayes, 1991), the predictions made with the Yb marker were used for eMCP estimates.

***Use of the alkane ratio method to estimate intake***

It was possible to make a direct calculation of pasture intake using the ratio of dosed even-chain exogenous n-alkane, $C_{36}$, and adjacent odd-chain n-alkanes in herbage, according to a simplified equation described by Dove & Mayes (1991). However, these calculations were not completed due to the unknown, and possibly variable recoveries for the naturally occurring n-alkanes across the range of pastures investigated. A key assumption of the alkane ratio method to estimate intake, is that the recoveries of adjacent, longer-chain n-alkanes, while not complete, are similar. However Hendricksen, *et al.* (2002) demonstrated that recoveries of odd-chain n-alkanes for tropical forages and lucerne are highly variable and not necessarily similar to those alkanes adjacent in chain length. When these authors used adjacent n-alkanes to estimate intake, agreement with actual intake was often poor.

### *6.4.5.5 Nylon bag incubation and NDIN/ADIN analysis to estimate pasture protein degradability*

An accurate estimation of the RDP component of the pasture diet is essential if MCP production in the rumen, and eMCP, are to be fully understood. Few estimates of forage RDP or UDP are available in the literature, especially for tropical forages. As discussed by Mass *et al.* (1999), although a range of methods exist which allow estimation of RDP and UDP components, the most widely used method has been the nylon bag incubation method described by Broderick (1994). However, this method has been problematic due to the error caused by microbial contamination of incubated samples and the difficulty in correcting for this contamination. The modified NDIN nylon bag incubation method developed by Mass *et al.* (1999) and corroborated by

Klopfenstein *et al.* (2001) has been demonstrated to provide an accurate estimation of forage UDP which is simpler and arguably more precise than previous purine correction methods. For these reasons, the NDIN/ADIN method was used in the current experiments. However, a further criticism in the estimation of RDP and UDP components is the tendency for researchers to use an assumed passage rate to estimate the extent of degradation of protein in the rumen. In the current experiments, measured passage rate data for each pasture type was used in the calculations. The use of this new NDIN/ADIN nylon bag method, in conjunction with measured passage rate data, should thus have provided representative and realistic estimates of RDP for the pasture diets studied in the current experiments.

### 6.4.6 Conclusions

This was the first series of experiments to estimate eMCP, in the grazing situation, in cattle consuming a range of tropical pastures relevant to the north Australian beef industry. A large spread in eMCP was found across pasture types and season. In particular, even high to mid quality tropical grasses were associated with eMCP that fell well below the range of 130 - 170 g MCP/kg DOMI given in the feeding standards. Tropical legumes produced eMCP that fell within the extant feeding standards' range while eMCP for the temperate species, ryegrass, fell well above the range. This wide spread in values indicates that there is indeed considerable scope to manipulate eMCP and thus potential to improve protein supply to cattle grazing tropical pastures. A number of factors, including pasture RDP, TNSC and fibre concentrations, rumen fluid and particle dilution rates, protozoal concentrations and pH were examined for correlation with eMCP. With the exception of a positive linear effect of RDP, and negative linear effects of diet NDF and ADF concentration, none of these factors had any significant relationship with eMCP across the range of pasture types studied. When RG pasture was excluded, a significant exponential relationship, of the form $y = a - b(e^{-cx})$, was found between eMCP and RDP supply in the diet for tropical grass and legume pastures. This response curve highlighted the fact that RDP supply was inadequate to meet requirements for eMCP in the extant feeding standards' range for the tropical grass pastures studied. As RDP supply in the diet was the most influential factor affecting eMCP across the range of pasture types studied, it would be seem that investigation of aspects of quantity and source of RDP supply to cattle grazing tropical pastures would have the most potential to increase

eMCP from the current low levels. The results of these experiments have increased our understanding of eMCP on tropical pastures and thus should allow improved estimates of growth rates and supplementation requirements of beef cattle at pasture.

# Chapter 7

# The effect of rumen degradable protein supply on the efficiency of microbial protein synthesis in cattle consuming tropical forage

## 7.1 INTRODUCTION

Low eMCP values have been documented for ruminants consuming tropical $C_4$ grass pastures, either freshly harvested (Mullik, 1999; Shem *et al.* 1999; Nsahlai *et al.* 2000) or as hays (Bolam, 1998; Prior *et al.* 1998; King *et al.* 1999; Mullik, 1999; Marsetyo *et al.* 2002). These values have all been much lower than the range of 130 - 170 g MCP/kg DOMI given in the various feeding standards (ARC, 1984; Madsen, 1985; Ausschuss fur Bedarfsnormen, 1986; INRA, 1988; SCA, 1990; AFRC, 1993; NRC, 1996b). Such low values were confirmed for cattle grazing tropical grass pastures in Chapter 6 of this thesis, where even mid to high quality tropical grasses were associated with eMCP that fell well below the extant feeding standards' range.

A number of factors have been found to influence, or be correlated with, eMCP both *in vitro* and *in vivo*, and can be grouped under areas of substrate availability, rumen dilution rate, intraruminal recycling and rumen pH (see section 2.3). However, in grazing experiments with tropical pastures and ryegrass described in Chapter 6 of this thesis, the only factors resulting in significant relationships with eMCP were dietary fibre concentrations and RDP supply in the diet. More specifically, negative linear relationships were found between eMCP and diet NDF and ADF concentration, and a positive linear relationship was found between eMCP and diet RDP/DOMI ratio. It was evident that for the tropical grass pastures studied, RDP supply/DOMI in the diet was inadequate to achieve eMCP in the extant feeding standard's range. These results were in accord with the feeding standards, which have recognised that if RDP supply is limiting for microbial growth, then eMCP will be less than the potential maximum, and will be equivalent to the RDP supply per unit of energy.

However, the eMCP response to RDP supply would be expected to reach plateau at some point, due to saturation of the microbial RDP requirement, as recognised by the arbitrary optimum values for eMCP given in the various feeding standards. It was proposed in section 6.4.2.1 that temperate $C_3$ pasture species follow a separate response relationship that reaches a higher eMCP plateau than for tropical species. When the temperate pasture ryegrass was excluded from the data set derived from Chapter 6 experiments, an exponential response relationship was found between eMCP and RDP/DOMI ratio for tropical grass and legume pastures (Figure 6.17). This relationship indicated that eMCP increased rapidly with increasing RDP/DOMI ratio, until plateau was reached at values within the extant feeding standards' range.

Strategies to manipulate or decrease the content of plant fibre fractions are difficult to implement (Norton, 1982; Satter *et al.* 1999) and may simultaneously decrease plant survivability and yield in the tropical environments to which they are adapted (Van Soest, 1994). Thus it would appear that investigation of aspects of RDP supply to cattle grazing tropical pastures would have the most potential to increase eMCP above the reported low levels. In particular, the response curve established in Chapter 6 (Figure 6.17), indicates that relatively small quantities of supplemental RDP should achieve marked increases in eMCP when pastures have ratios of RDP/DOMI that align with that part of the response curve where eMCP is increasing rapidly.

A further possibility is that the type of RDP supplied in a supplement, i.e., whether as NPN or that derived from true protein, will affect the eMCP response, as reviewed in section 2.3.1.3. Forage diets, and in particular tropical grass pastures, are usually low in NSC and high in SC components (Smith, 1973; Norton, 1982), and thus the SC-fermenting bacteria would be expected to be of greatest importance in influencing the rate of substrate breakdown and overall eMCP. While it is has been shown that bacteria fermenting NSC will respond to peptides and amino acids, fibre-digesting bacteria are assumed to primarily use $NH_3$ for MCP synthesis (NRC, 1996b). However, pure culture studies have demonstrated that SC-fermenting bacteria can use peptides and amino acids (Ling & Armstead, 1995; Atasoglu, 1996; Wallace *et al.* 1999) and that the growth of SC-fermenting bacteria is stimulated by these N sources (Cotta & Russell, 1982; Cruz Soto *et al.* 1994). Furthermore, it has been shown that BCFA and other higher volatile fatty acids, which are a product of protein

degradation, are essential factors for the growth of many SC-fermenting rumen bacteria (Hungate *et al.* 1964; Allison, 1965, 1969; Allison & Robinson, 1967; Robinson & Allison, 1969; Bryant, 1973; Kristensen, 1974). Early *in vivo* studies in this area were conducted by Hume (1970a,b), who demonstrated that although eMCP was not increased with dietary BCFA addition compared to urea alone, eMCP was increased by substituting either casein or zein for BCFA and 50 % of urea-N, when sheep were fed a virtually protein-free diet with casein infused abomasally. Although conflicting results from *in vitro* and *in vivo* studies have been reported, there is strong evidence to indicate that rumen degradable, true protein supplements can produce increases in eMCP on both forage and mixed diets (see section 2.3.1.3).

The objective of these experiments was to investigate the effect of quantity and source of RDP supply on eMCP in cattle consuming tropical grass forage. Furthermore, measurement of associated digestion and metabolic factors was undertaken to allow identification of any factors associated with high eMCP.

## 7.2 MATERIALS AND METHODS

### 7.2.1 Experiment 7.1. Determination of eMCP and digestibility in intact steers

#### *7.2.1.1 Experimental animals*

Eight high-grade Brahman (> 75 % *B. indicus*) steers (initial LW 374 ± (SE) 9 kg) were used in the experiment. All steers were treated with moxidectin (Cydectin, Fort Dodge Australia Pty Ltd) to control internal and external parasites, at the commencement of the first preliminary feeding period.

#### *7.2.1.2 Experimental design and diets*

The eight steers were used in a 4 x 4 latin square design, with four treatments and two replicates, giving eight observations per treatment. The steers were fed one of the following dietary treatments:

1) control (low quality, pangola grass (*Digitaria eriantha*) hay), (C);
2) control + urea and ammonium sulphate, to achieve an estimated dietary ratio of 150 g RDP/kg DOMI, (U); (urea 0.627 % w/w, air dry basis; ammonium sulphate 0.090 % w/w, air dry basis);
3) control + casein, to achieve an estimated dietary ratio of 150 g RDP/kg DOMI, (LoCAS); (casein 2.52 % w/w, air dry basis);
4) control + casein, to achieve an estimated dietary ratio of 300 g RDP/kg DOMI, (HiCAS); (casein 12.42 % w/w, air dry basis).

All treatments, including the control, involved the application of water to hay at the rate of 30 % (w/w, air dry basis). Water was sprayed on to the hay with a watering can and then the hay and water were mixed thoroughly. For the U treatment, urea and ammonium sulphate (included to achieve a ratio of N : S in the supplement of 14.3 : 1) were dissolved in the water prior to being sprayed on to the hay and mixed. With the LoCAS and HiCAS treatments, casein (sodium caseinate, Murray Goulburn Co-operative Co Ltd, Australia) was sifted on to the dampened hay (after water application) and then mixed in thoroughly. A mineral block (Olsson's Go-Block, Olsson Industries Pty Ltd, Australia) containing macro and trace minerals, but no N, was made available to all steers during the first 9 d of each preliminary feeding period.

#### *7.2.1.3 Experimental procedures*

A 14-d preliminary period in pens preceded a 7-d measurement period in metabolism crates for each of the four experimental periods. The steers were fed *ad libitum* during the preliminary period and then restricted to 90 % of *ad libitum* during the collection period. All diets were fed once daily at *ca.* 08.00 h.

Feed intake was recorded over the sampling period and a daily subsample of individual feed residue was taken for DM determination and for later chemical

analyses. All feed residue was kept for the HiCAS treatments due to the heterogenous nature of the residue and the difficulty in taking a representative subsample. This was not a problem for the LoCAS diet. The daily residues were later ground (1 mm) and then subsampled to produce one bulk residue for each steer in each experimental period. Routine samples of the basal hay and of casein were taken every 2nd day for DM determination and bulked over the collection period for chemical analysis. A subsample of unchaffed hay representative of the basal diet was taken during each experimental period and sorted into leaf and stem components. The leaf and stem components sorted in the first experimental period were ground (1 mm) prior to chemical analyses.

Total faecal output was collected daily over the 7-d sampling period and subsamples were kept and processed to allow determination of diet digestibility, as described in section 3.4.5. Over each 7-d sampling period in the metabolism crates, total urine output was collected daily as described in section 3.4.5. A 5 % subsample of each steer's daily urine output was bulked into a container for each steer. These containers were stored in a freezer over the 7 d of collection. On day 7, the bulk urines were defrosted and subsampled for PD analysis as described in section 3.4.7. The steers were weighed at the start and finish of each preliminary feeding period.

#### *7.2.1.4 Analytical procedures*

Methods for determining PD in urine, and DM, ash, N, NDF and ADF concentration in feed and faeces are given in section 3.5.

#### *7.2.1.5 Calculations*

DMD, OMD and NDF digestibility were determined for each steer, in each period, as described in section 3.6.1. MCP synthesis was calculated as outlined section 3.6.2.

RDP intake (g/d) was calculated as:

$$\text{RDP intake} = (\text{RDP in hay fed} + \text{RDP in supplement fed}) - (\text{RDP in hay residue} + \text{RDP in supplement residue}).$$

The protein degradability of the basal hay diet was estimated in Experiment 7.3 and used to estimate RDP contribution from the hay portion of the diet. Urea and casein were assumed to be 100 % degraded in the rumen. The proportions of hay and supplement in the feed residues were estimated by substituting known parameters into two algebraic equations:

(1) - residue DM (R) = hay DM (H) + supplement DM (S), and

(2) - R *x* (CP in R) = H *x* (CP in H) + S *x* (CP in S),

and then solving for the unknown parameters, H and S.

#### *7.2.1.6 Statistical analyses*

Differences between treatment means were tested by analysis of variance using the statistical package Genstat for Windows, 6th edition (Lawes Agricultural Trust, Rothamsted, U.K.). Pairwise differences between means were tested using a protected least significance difference procedure (P = 0.05).

### 7.2.2 Experiment 7.2. Determination of rumen digestion and metabolism parameters in rumen fistulated steers

#### *7.2.2.1 Experimental animals*

Four high-grade Brahman (> 75 % *B. indicus*) RF steers (568 ± 28 kg) were used in the experiment. The steers were treated with flumethrin (Bayticol, Bayer Australia Ltd) at intervals during the experiment to control buffalo fly.

#### *7.2.2.2 Experimental design, diets and procedures*

Running concurrently with Experiment 7.1, the four RF steers went through a 4 x 4 latin square design, with identical dietary treatments to those described above, giving four observations per treatment. The RF steers were held in the individual pens for the duration of the experimental period and received a 14-d preliminary feeding period followed by a 6-d sampling period for each of the four runs. On the first 4 d of each preliminary feeding period, the RF steers were allowed to exercise in a group yard with a dirt floor for several hours following the morning feeding. The steers

were fed *ad libitum* during the preliminary periods and then restricted to 90 % of *ad libitum* during collection periods. All diets were fed at *ca.* 08.00 h.

***Intake***

Over the 6-d sampling period, daily diet intake was determined, and hay, casein and residue subsamples were taken and processed as described above for Experiment 7.1.

***Rumen fluid parameters***

Rumen fluid samples were collected, as described in section 3.4.9, from each of the steers at 4-hourly intervals after feeding up to 20 h (0, 4, 8, 12, 16, 20 h) over days 1 and 2 of each sampling period. On each sampling occasion the pH was determined immediately and subsamples were taken for VFA and $NH_3$-N analysis and were processed as described in section 3.4.9. Each of the six individual rumen fluid samples from each steer were analysed for $NH_3$-N analysis, but only one representative bulk subsample of the six sampling times for each steer was analysed for VFA content.

***Rumen fluid and particle outflow rates***

On day 1 of each sampling period, concentrated CrEDTA solution (*ca.* 2.7 g Cr/steer) and Yb-marked hay (*ca.* 1 g Yb/steer) were dosed into the rumen through the cannula immediately prior to daily feeding. The marker manufacture and dosing procedures are described in sections 3.4.1, 3.4.2 and 3.4.4. Rumen fluid and digesta samples were taken over the following three days at 4, 8, 12, 16, 20, 32 and 48 h after dosing, as outlined in sections 3.4.9 and 3.4.10. On each sampling occasion, rumen fluid samples were kept for Cr analysis and digesta samples were kept Yb analysis, for each steer.

**In situ *OM degradation rates***

A representative portion of the basal diet of pangola hay was ground through a 3 mm screen prior to incubation in the rumen of each of the RF steers over d 3 - 7 of the sampling period. The nylon bags, each containing *ca.* 5 g DM of sample, were inserted at *ca.* 08.00 h on day 3 of the sampling period and duplicate bags extracted at 3, 6, 9, 12, 24, 48, 72 and 96 h after insertion. Details of bag insertion, removal and processing are given in section 3.4.11. Individual bag proportional DM loss was

determined and used to determine the rate of DM digestion. Bag residue was bulked across all duplicates and steers within a treatment, for each time of bag removal, and analysed for OM concentration.

***Liveweight***

The steers were weighed at the beginning and end of each preliminary feeding period.

#### *7.2.2.3 Analytical procedures*

Methods for determination of DM, ash, total N, NDF, ADF, rumen VFA, rumen $NH_3$-N, Cr, and Yb concentrations are described in section 3.5.

#### *7.2.2.4 Calculations*

The DOMI of RF steers was calculated by using measured values for OMI and the average OMD for each treatment diet, determined using the intact steers in Experiment 7.1. Rumen fluid and particle FOR, rumen fluid volume and rumen DM content were calculated as outlined in section 3.6.3. Dry matter and OM degradability parameters were calculated as outlined in section 3.6.4.

#### *7.2.2.5 Statistical analyses*

All statistical analyses were conducted using the statistical package Genstat for Windows, 6th edition (Lawes Agricultural Trust, Rothamsted, U.K.). Differences between treatment means were tested by analysis of variance. Where missing values were present in the data, as for particle FOR and rumen DM content, the predicted means from Genstat were used as treatment means and an average standard error was calculated from the average of individual ANOVA errors for each treatment. Pairwise differences between means were tested using a protected least significance difference procedure ($P = 0.05$).

When determining rumen particle FOR and DM content, poor relationships were obtained in some cases for the simple linear regression of *ln* of Yb concentration against time. Thus, the individual regressions were tested to determine whether the slope was significantly different to 0 ($H_0$: slope = 0), and those that were not, were excluded from the calculation of particle FOR. Four out of the sixteen regressions were excluded on this basis.

In order to test for treatment effects on nylon bag DM and OM disappearance parameters, individual exponential curves were fitted to the data for each steer in each period. The form of the exponential used was $Y = S + QR^t$, where:

Y = DM or OM disappearance (g/kg),
S = constant parameter,
Q = linear parameter,
R = curvature parameter, and
t = time (h).

The coefficients (S, Q and R) were extracted from each data set and separately subjected to a latin square analysis of variance. There were no significant differences between the coefficients, and thus a pooled estimate of the curve was used. The mean curve was re-parameterised to the form $Y = a + b (1 - e^{-ct})$ as described in section 3.6.4.

An unpaired t-test was used to test for differences in the overall mean diet CP concentration between the intact and the RF steers in Experiments 7.1 and 7.2, respectively. In addition, a meta-analysis approach was used to enable the effects of experiment, diet and their interaction to be tested. This approach tests whether any difference between diets is consistent for the two experiments. A mixed model was fitted, with experiment, diet and their interaction as fixed effects and the design factors period, steer and their interaction within each experiment as random effects. The residual from each trial was specified separately.

### 7.2.3 Experiment 7.3. Determination of pangola hay RDP content

#### *7.2.3.1 Experimental animals*

Three high-grade Brahman (> 75 % *B. indicus*) RF steers (564 ± 38 kg) were used in the experiment.

#### *7.2.3.2 Experimental diet and procedures*

After completion of Experiment 7.2, RF steers received a 6-d preliminary feeding period followed by a 2-d sampling period in individual pens. The steers were fed on a standard diet as described in section 3.4.11. The same mineral block, as that which was provided in Experiments 7.1 and 7.2, was made available during both the preliminary feeding and the sampling period of this experiment.

Nylon bags containing samples of the basal hay diet (pangola hay; 3 mm ground) were inserted at *ca.* 08.00 h and duplicate bags then removed at 5, 8, 11, 14 and 16 h, as detailed in section 3.4.11. After individual bag proportional DM loss was determined, bag residues were bulked across all duplicates and steers for each removal time. These bulked residues were later analysed for NDIN and ADIN concentrations to allow estimation of the basal diet RDP content.

In addition, rumen fluid samples were taken from each of the three steers to monitor rumen $NH_3$-N concentration (see section 3.4.9), immediately prior to feeding at *ca.* 06.15 h on the day 1 of nylon bag removal.

#### *7.2.3.3 Analytical procedures*

Methods for determination of rumen $NH_3$-N, and NDIN and ADIN concentrations in nylon bag sample residues are given in section 3.5.

#### *7.2.3.4 Calculations*

The RDP content of the pangola hay was calculated as described in section 3.6.5. The measured particle FOR (averaged across the four treatments) from Experiment 7.2 was used in the equation.

## 7.3 RESULTS

### 7.3.1 Chemical composition of the diet

The chemical composition of the basal hay and casein supplement used in the treatment diets is given in Table 7.1. The pangola hay was of low quality, with low CP and high fibre content.

**Table 7.1. Chemical composition (g/kg DM) of the hay and casein used in the treatment diets of Experiments 7.1 and 7.2.** Values for pangola hay and casein are means across Experiments 7.1 and 7.2; values for pangola leaf and stem chemical composition are those from Experiment 7.1, period 1, only

| | Pangola hay | Pangola leaf | Pangola stem | Casein |
|---|---|---|---|---|
| OM | 936 | 927 | 944 | 961 |
| CP | 57.3 | 75.0 | 43.8 | 950.0 |
| NDF | 697 | 700 | 725 | - |
| ADF | 401 | 386 | 443 | - |
| Leaf | 410 | - | - | - |

ADF, acid detergent fibre; CP, crude protein; DM, dry matter; NDF, neutral detergent fibre; OM, organic matter.

Table 7.2 summarises the CP concentration in the diet of both the intact and RF steers (Experiment 7.1 and 7.2, respectively) for each of the four treatments. The CP concentration in the diet of U and LoCAS treatment groups was similar at an average of 80 g/kg DM, and was higher ($P < 0.05$) than that of steers consuming the control diet (58 g/kg DM). The CP concentration in the diet of steers consuming the HiCAS treatment was approximately double that of the U and LoCAS groups, at 160 g/kg DM. No difference ($P = 0.76$) was found between the overall mean diet CP concentration of the intact and RF steer groups. In addition, the interaction between experiment and diet was not significant ($P = 0.55$), meaning that differences between diets were consistent for Experiments 7.1 and 7.2.

**Table 7.2. Crude protein concentration in the diet (g/kg DM) for steers consuming the treatment diets in Experiments 7.1 and 7.2.** Values are the mean and standard error (in brackets) for all steers

| | Treatment[A] | | | |
|---|---|---|---|---|
| **Experiment** | **C** | **U** | **LoCAS** | **HiCAS** |
| 7.1. Intact steers | 58.8 (2.84) | 80.1 (2.68) | 83.1 (2.95) | 162.0 (3.13) |
| 7.2. RF steers | 55.5 (2.00) | 76.4 (1.85) | 79.9 (2.70) | 156.8 (2.69) |
| Mean 7.1 and 7.2[B] | 57.7 (2.00) | 78.9 (1.90) | 82.0 (2.13) | 160.2 (2.42) |

DM, dry matter; DOMI, digestible organic matter intake; RDP, rumen degradable protein; RF, rumen fistulated.

[A] Treatment diets: C = control diet of pangola hay; U = control + urea and ammonium sulphate, to achieve an estimated dietary ratio of 150 g RDP/kg DOMI; LoCAS = control + casein to achieve an estimated dietary ratio of 150 g RDP/kg DOMI; and HiCAS = control + casein to achieve an estimated dietary ratio of 300 g RDP/kg DOMI.

[B] Average and standard error for 12 steers.

The RDP concentration in the pangola hay used in all treatment diets was 0.86 g/g CP. Rumen $NH_3$-N concentrations measured immediately prior to feeding in the rumen of steers used in the nylon bag incubations in Experiment 7.3 were high, at 233 ± 7.5 mg/l. The pangola hay incubated in nylon bags in Experiments 7.2 and 7.3, was similar in composition to the average of that consumed by the experimental animals in Experiments 7.1 and 7.2, viz. OM: 944 g/kg DM, CP: 40.0 g/kg DM, NDF: 691 g/kg DM and ADF: 399 g/kg DM.

### 7.3.2 Microbial protein production

Table 7.3 summarises the dietary ratios of CP and RDP per unit DOMI and the effect of treatment on MCP flow and eMCP in the rumen of the intact steers studied in Experiment 7.1. Protein supply (both CP and RDP) per kg of DOMI was not different between U and LoCAS groups ($P > 0.05$), but these were greater than that for the C group and lower than for the HiCAS group ($P < 0.05$). MCP flow and eMCP were increased with HiCAS feeding relative to all other treatments ($P < 0.05$). However, the U and LoCAS treatments did not increase MCP flow or eMCP relative to the control.

**Table 7.3. Crude protein (CP) and rumen degradable protein (RDP) supply per digestible organic matter intake (DOMI) and effect of treatment on microbial crude protein (MCP) flow and efficiency of MCP production (eMCP).** Values are the mean for steers within each treatment group in Experiment 7.1; SE represents ANOVA estimated standard error; probability (P) for test of significant difference between treatments is presented; values in the same row with different superscripts differ significantly ($P < 0.05$)

| | Treatment[A] | | | | SE | P |
|---|---|---|---|---|---|---|
| | C | U | LoCAS | HiCAS | | |
| g CP/kg DOMI | 127[a] | 167[b] | 169[b] | 307[c] | 2.2 | < 0.001 |
| g RDP/kg DOMI | 109[a] | 150[b] | 153[b] | 293[c] | 2.1 | < 0.001 |
| MCP flow (g MCP/d) | 288[a] | 298[a] | 315[a] | 570[b] | 44.7 | < 0.001 |
| eMCP (g MCP/kg DOMI) | 123[a] | 115[a] | 109[a] | 167[b] | 14.2 | 0.04 |

[A] Treatment diets: C = control diet of pangola hay; U = control + urea and ammonium sulphate, to achieve an estimated dietary ratio of 150 g RDP/kg DOMI; LoCAS = control + casein to achieve an estimated dietary ratio of 150 g RDP/kg DOMI; and HiCAS = control + casein to achieve an estimated dietary ratio of 300 g RDP/kg DOMI.

### 7.3.3 Digestibility, intake and rumen metabolism parameters

Table 7.4 summarises the effect of treatment diet on digestibility and intake parameters. NDF digestibility was not affected by treatment, with a mean digestibility of $599 \pm 5.4$ g/kg DM across all treatments. However, for all other parameters, HiCAS was significantly greater than all other treatments ($P < 0.05$) and LoCAS was greater than C but generally not greater than U ($P < 0.05$). Urea feeding did not increase any digestibility attribute or DOMI relative to the control.

**Table 7.4. Effect of treatment on digestibility and intake parameters.** Values are the mean for steers within each treatment group; SE represents ANOVA estimated standard error for all treatment means; probability (P) for test of significant different difference between treatments is presented; values in the same row with different superscripts differ significantly ($P < 0.05$)

| | Treatment[A] | | | | SE | P |
|---|---|---|---|---|---|---|
| | C | U | LoCAS | HiCAS | | |
| DMD (g/kg)[B] | 489[a] | 502[ab] | 515[b] | 552[c] | 5.4 | < 0.001 |
| OMD (g/kg)[B] | 498[a] | 512[ab] | 525[b] | 563[c] | 5.3 | < 0.001 |
| NDF digestibility (g/kg)[B] | 592 | 603 | 603 | 599 | 5.4 | 0.48 |
| DOMI (kg/100 kg LW)[C] | | | | | | |
| Intact steers | 0.62[a] | 0.67[a] | 0.76[b] | 0.89[c] | 0.020 | < 0.001 |
| RF steers | 0.41[a] | 0.42[ab] | 0.48[b] | 0.56[c] | 0.017 | 0.003 |

DMD, dry matter digestibility; DOMI, digestible organic matter intake; LW, liveweight; NDF, neutral detergent fibre; OMD, organic matter digestibility; RDP, rumen degradable protein; RF, rumen fistulated.

[A] Treatment diets: C = control diet of pangola hay; U = control + urea and ammonium sulphate, to achieve an estimated dietary ratio of 150 g RDP/kg DOMI; LoCAS = control + casein to achieve an estimated dietary ratio of 150 g RDP/kg DOMI; and HiCAS = control + casein to achieve an estimated dietary ratio of 300 g RDP/kg DOMI.

[B] Digestibilities were determined in Experiment 7.1 for intact steers.

[C] DOMI values for both intact and RF steers were determined during sampling periods when intakes were restricted to 90 % of the preliminary period *ad libitum* intake.

The disappearance of pangola hay DM and OM from nylon bags incubated in the rumen of the RF steers was unaffected by treatment, with probabilities for the analyses of S, Q and R coefficients as follows:

(1) S coefficient: $P = 0.57$ (DM disappearance), $P = 0.58$ (OM disappearance);
(2) Q coefficient: $P = 0.66$ (DM disappearance), $P = 0.64$ (OM disappearance);
(3) R coefficient: $P = 0.49$ (DM disappearance), $P = 0.51$ (OM disappearance).

In addition, there were no significant differences between coefficients, for period or steer effects, for DM and OM disappearance curves. The DM and OM disappearance parameters, determined from pooled estimates of the curve representing the four treatment diets, are summarised in Table 7.5.

**Table 7.5. Dry matter (DM) and organic matter (OM) disappearance parameters for pooled estimates of the exponential curve representing all 4 treatments[†].** Values are the mean and standard error (in brackets) for 16 steers; residual standard deviation (RSD) of the fitted equation is presented

| | Factor measured | |
|---|---|---|
| | **DM disappearance** | **OM disappearance** |
| a (g/kg) | 163 (5.0) | 140 (5.0) |
| b (g/kg) | 480 (5.1) | 496 (5.1) |
| a + b (g/kg) | 643 | 636 |
| c ($h^{-1}$) | 0.037 (0.0014) | 0.037 (0.0014) |
| RSD | 4.7 | 4.6 |

[†] The loss of DM and OM is described by the equations given by McDonald (1981), where $Y_1 = A$ up to time $t_0$, and $Y_2 = a + b\,(1 - e^{-ct})$, from time $t_0$ onwards (see section 3.6.4). The parameters shown above are defined as: "a" = rapidly degradable fraction; "b" = more slowly degraded fraction; "a + b" = potential degradable fraction; and "c" = degradation rate of "b".

Rumen $NH_3$-N was lowest on the C diet and highest on HiCAS, while the U and LoCAS treatments were not significantly different from one another (Table 7.6). The diurnal pattern of rumen $NH_3$-N concentration was influenced by treatment (Figure 7.1). The C group maintained a low $NH_3$-N concentration (< 100 mg/l) throughout the 20-h sampling period, with the peak value occurring just before feeding. By contrast, the other treatment groups peaked at 4 h (U and LoCAS) or between 4 and 8 h (HiCAS) after feeding. The U and LoCAS treatment groups followed a similar pattern of change, with $NH_3$-N concentration much lower than HiCAS throughout.

The average rumen pH was not different between treatment diets ($P = 0.09$), averaging $6.8 \pm 0.03$ across all treatments. Total molar concentration of VFA was also unaffected by treatments ($P = 0.99$), averaging $27.6 \pm 2.04$ mM. As summarised in Table 7.6, the LoCAS treatment was associated with an increase in BCFA proportion relative to the C and U treatments ($P < 0.05$), which were not different from one another. There was a further increase in BCFA proportion from LoCAS treatment to the HiCAS treatment ($P < 0.05$) such that the molar proportion for HiCAS was more than four times greater than for the C and U treatments. The trend

for the acetate proportion was in the reverse order, but with differences only between HiCAS and C.

**Table 7.6. Effect of treatment on rumen volatile fatty acid (VFA) proportions and $NH_3$-N concentration.** Values are the mean for steers within each treatment group in Experiment 7.2; SE represents ANOVA estimated standard error for all treatment means; probability (P) for test of significant difference between treatments means is presented values in the same row with different superscripts differ significantly ($P < 0.05$)

| | Treatment[A] | | | | SE | P |
|---|---|---|---|---|---|---|
| | C | U | LoCAS | HiCAS | | |
| $NH_3$-N (mg/l) | $73.4^{a}$ | $134.5^{b}$ | $121.0^{b}$ | $277.0^{c}$ | 8.99 | < 0.001 |
| Molar proportions of VFA (% of total) | | | | | | |
| Acetate | $76.4^{b}$ | $72.1^{ab}$ | $71.9^{ab}$ | $68.9^{a}$ | 1.40 | 0.05 |
| Propionate | 14.1 | 17.1 | 17.1 | 17.2 | 1.57 | 0.49 |
| Butyrate | 7.3 | 9.2 | 8.7 | 8.2 | 1.18 | 0.71 |
| Valerate | 1.3 | 0.6 | 0.6 | 1.2 | 0.45 | 0.60 |
| BCFA[B] | $1.0^{a}$ | $1.0^{a}$ | $1.6^{b}$ | $4.4^{c}$ | 0.16 | < 0.001 |

BCFA, branched-chain fatty acids; DOMI, digestible organic matter intake; RDP, rumen degradable protein.

[A] Treatment diets: C = control diet of pangola hay; U = control + urea and ammonium sulphate, to achieve an estimated dietary ratio of 150 g RDP/kg DOMI; LoCAS = control + casein to achieve an estimated dietary ratio of 150 g RDP/kg DOMI; and HiCAS = control + casein to achieve an estimated dietary ratio of 300 g RDP/kg DOMI.

[B] Sum of iso-butyrate and iso-valerate.

Table 7.7 presents the mean values for rumen fluid and particle FOR, and the estimated rumen fluid volume and DM content for the treatment diets. Rumen fluid FOR was lowest on C and U treatments ($P < 0.05$), but with no difference between U and LoCAS. Furthermore, HiCAS and LoCAS treatment groups were not significantly different in fluid FOR. There was no significant effect of treatment on rumen particle FOR or on rumen fluid volume or DM content.

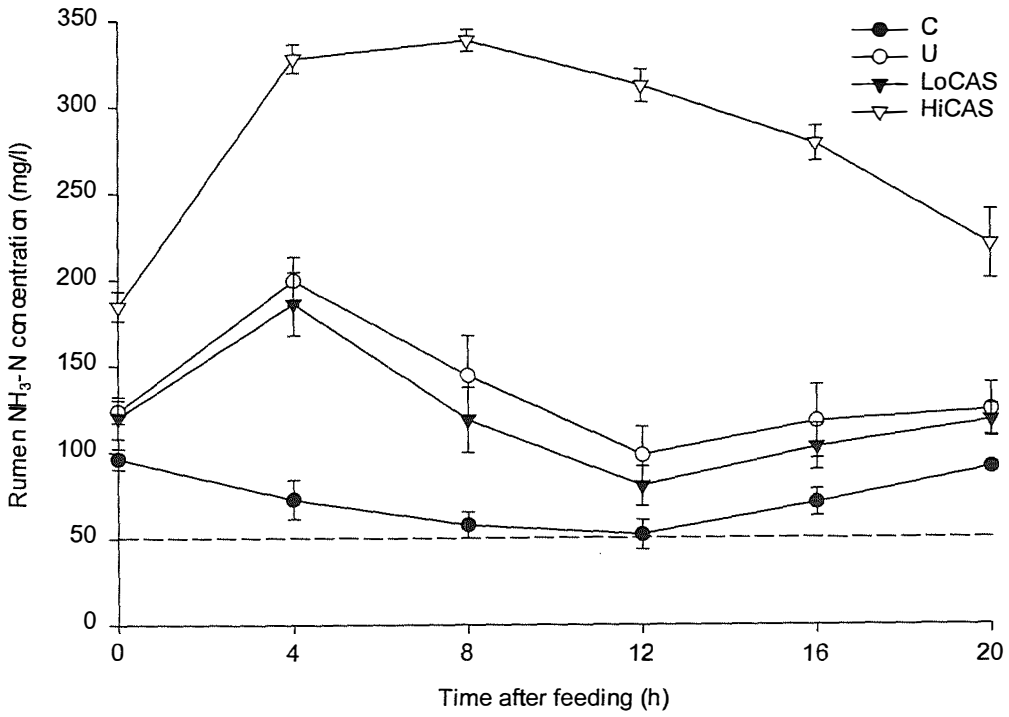


**Figure 7.1. Rumen $NH_3$-N concentrations over time after feeding.** Treatments defined in Table 7.2. Values represent the mean and standard error for 4 steers. Dotted line indicates critical $NH_3$-N level of 50 mg/l (Satter & Slyter, 1974).

**Table 7.7. Effect of treatment on estimated rumen fluid volume and dry matter (DM) content and on fluid and particle fractional outflow rate (FOR).** Values are the mean for steers within each treatment group in Experiment 7.2; SE represents ANOVA estimated standard error for all treatment means; probability (P) for test of significant difference between treatments means is presented; values in the same row with different superscripts differ significantly ($P < 0.05$)

| | Treatment[A] | | | | SE | P |
|---|---|---|---|---|---|---|
| | C | U | LoCAS | HiCAS | | |
| Fluid FOR (%/h) | 4.8[a] | 5.4[ab] | 6.0[bc] | 6.2[c] | 0.19 | 0.01 |
| Particle FOR (%/h) | 2.0 | 2.1 | 1.7 | 3.0 | 0.39 | 0.26 |
| Fluid volume (l) | 57.0 | 52.3 | 57.4 | 52.6 | 3.58 | 0.64 |
| DM content (kg) | 11.0 | 9.8 | 14.1 | 9.9 | 1.25 | 0.22 |

DOMI, digestible organic matter intake; RDP, rumen degradable protein.

[A] Treatment diets: C = control diet of pangola hay; U = control + urea and ammonium sulphate, to achieve an estimated dietary ratio of 150 g RDP/kg DOMI; LoCAS = control + casein to achieve an estimated dietary ratio of 150 g RDP/kg DOMI; and HiCAS = control + casein to achieve an estimated dietary ratio of 300 g RDP/kg DOMI.

## 7.4 DISCUSSION

### 7.4.1 Effect of quantity of RDP supply on eMCP

These experiments demonstrated that eMCP in cattle consuming a low quality tropical grass hay was only increased to within the extant feeding standards' range when RDP was provided at the very high level of 290 g/kg DOMI, whereas lower ratios of 150 g/kg DOMI had no effect (Table 7.3). However, the RDP/DOMI ratio in the basal hay diet was already at a relatively high level for tropical grass pasture, at 109 g RDP/kg DOMI. This ratio was similar to that estimated for the highest quality tropical grass pasture detailed in Chapter 6 of this thesis (117 g RDP/kg DOMI), and much higher than that estimated for mid to low quality tropical grass pastures (61 - 42 g RDP/kg DOMI). When the results from the pangola hay experiments were added to those for the tropical grass and legume pastures studied in Chapter 6, an exponential relationship of the same form as that developed in Figure 6.17 was found to be a good fit (P = 0.001) for the relationship between eMCP and dietary RDP (Figure 7.2).

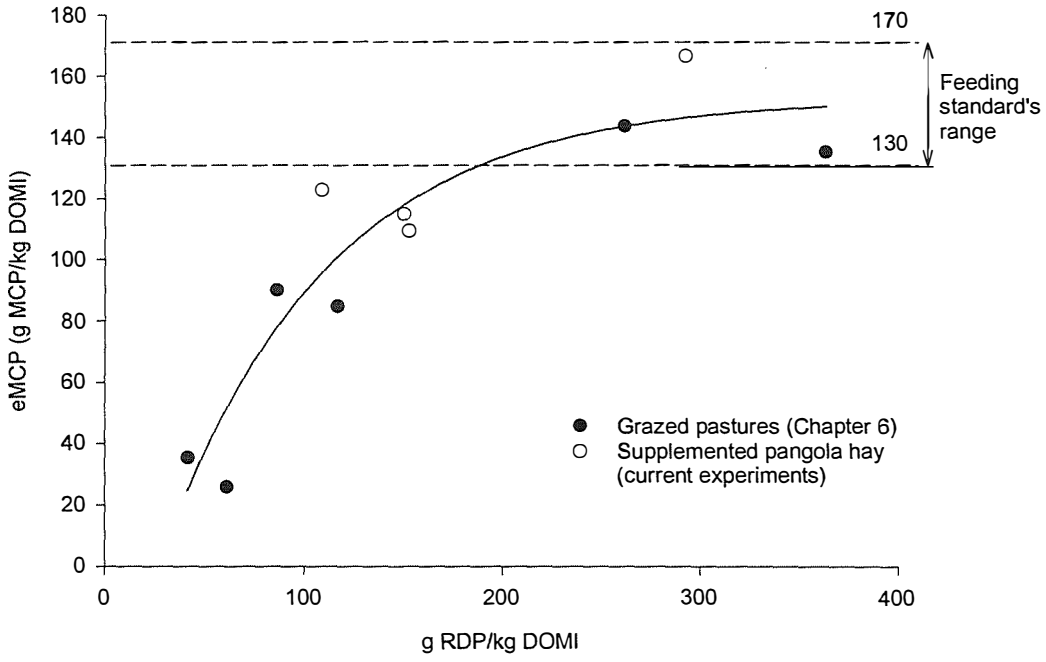


**Figure 7.2. Relationship between efficiency of microbial crude protein production (eMCP) and rumen degradable protein (RDP) supply per unit of digestible organic matter intake (DOMI) in the diet of steers consuming tropical grass and legume forages (Chapter 6) and supplemented pangola grass hay (current experiments).** Equation: $y = 152.9 - 211.2(e^{-0.012x})$, adjusted $r^2$ = 0.82, RSD = 19.4, P = 0.001. Extant feeding standards' range of eMCP indicated. (MCP, microbial crude protein).

It is evident that forage RDP supply in pangola hay was close to the plateau of the response curve detailed in Figure 7.2. This may explain why small increases in the ratio of RDP/DOMI failed to cause significant increases in eMCP, despite the RDP supply in the basal diet being less than 130 g/kg DOMI. The strong relationship found between eMCP and the ratio of RDP/DOMI, when the four new data points from the current experiment were added to those for the tropical pastures studied in Chapter 6, further confirms the controlling influence of RDP supply on eMCP for tropical pastures.

The relatively high ratio of RDP to DOMI in the pangola hay would have been caused by N fertilisation of the pasture during the growing phase, which was carried out to increase forage yield. As a result of the increased pasture growth rate, due to N fertilisation, digestibility of the hay (OMD: 498 g/kg) was low relative to the CP concentration (58 g/kg DM). As N fertilisation is not a common practice in extensive beef cattle production systems from tropical grass pastures in northern Australia, it can be postulated that only young, growing tropical grass pastures would have RDP/DOMI ratios at this relatively high level, similar to that estimated in Chapter 6 for early wet season native pasture. Thus, it would be expected that mid to low quality tropical grass pastures would generally align with that part of the response curve where eMCP is increasing rapidly and where a response to low levels of RDP supplementation would be more probable.

Other research has demonstrated increases in eMCP associated with RDP supplementation of cattle consuming tropical grass forage. Mullik (1999) reported an increase in eMCP (77 to 119 g MCP/kg DOMI) in steers consuming pangola grass hay (CP: 85 g/kg DM; OMD: 490 g/kg) when the basal diet was supplemented with urea at 19 g/kg DM hay. In addition, Bolam (1998) increased eMCP, from 62 g MCP/kg DOMI in cattle consuming Rhodes grass hay (CP: 66 g/kg DM; OMD: 570 g/kg) up to 145 g MCP/kg DOMI by supplementing with cottonseed meal at 2 % of LW, which would have provided a large quantity of additional dietary RDP (RDP content in cottonseed meal: 0.57 g/g CP; NRC, 1996b). However, the cottonseed meal supplement would also have provided other nutrients including energy and true protein pre-cursors, such as BCFA, which may have contributed to the eMCP response obtained by Bolam (1998). The responses to eMCP, obtained by

the above researchers, were associated with lower eMCP in the basal forage than that obtained in the current experiment (123 g MCP/kg DOMI). However, it should be noted that these authors calculated MCP flow and eMCP from urinary PD excretion using a high endogenous PD flow rate determined for *B. taurus* cattle (Chen & Gomes, 1995), which may have been inappropriate for the high-content *B. indicus* cattle used in their experiments (see Chapter 4 of this thesis).

The lack of a response in eMCP to low levels of RDP supplement in the current experiments is also consistent with the average rumen $NH_3$-N concentration of the control steers (73.4 mg/l) which was in excess of the suggested threshold level for optimal MCP synthesis in the rumen (50 mg/l; Satter & Slyter, 1974) throughout the day (Figure 7.1). On this basis, though, the increase in eMCP with the very high level of RDP supplementation (293 g/kg DOMI) cannot be explained in terms of an increase in $NH_3$-N concentration in the rumen. It should be noted that the average rumen $NH_3$-N concentration for the control steers was higher than expected for the CP concentration of the hay (58 g/kg DM), (cf. 16.7 mg $NH_3$-N/l for steers grazing creeping bluegrass pasture in Chapter 6 with a similar CP content (59 g/kg DM)). However, the relatively low digestibility, and low rumen fluid and particle FOR for steers consuming the pangola grass hay may have contributed to higher rumen $NH_3$-N concentrations than on similar CP forages.

Previous *in vivo* studies have defined a range of $NH_3$-N concentrations at which microbial growth was maximised, varying from 20 - 240 mg/l (Hume *et al.* 1970; Miller, 1973; Slyter *et al.* 1979; Okorie, 1981). In the current experiments, maximal eMCP was associated with an average daily $NH_3$-N concentration of 277 mg/l, which is above this range. As reviewed by Durand (1989), such wide variations in optimal $NH_3$-N concentrations can be explained by different microbial requirements for growth and fermentative activities and also by the different pathways of $NH_3$ incorporation, with the major pathways of $NH_3$ assimilation by microbes being influenced by N source and $NH_3$ concentrations. In addition, the type of energy substrate is also known to influence the $NH_3$ requirement, with the optimal level varying with the amount of readily fermentable carbohydrate available (Hoover, 1986). It is possible that the average $NH_3$-N concentration attained on the U and LoCAS treatment diets (128 mg/l) was below the optimal level required to maximise

eMCP on this particular tropical grass hay diet. However this does not explain why eMCP did not increase when $NH_3$-N concentration was increased from 73.5 mg/l on the control diet to 128 mg/l on the U and LoCAS diets.

### 7.4.2 Effect of source of RDP on eMCP

Source of RDP, i.e. urea vs. casein, had no effect on eMCP at low levels of RDP supplementation (150 g/kg DOMI), indicating no advantage of providing RDP in the form of a true protein supplement. The comparison between the two RDP sources (U and LoCAS) was valid, as demonstrated by the similar concentration of RDP per DOMI in the diet of steers consuming both treatment diets (Table 7.3) and the similar average concentrations of $NH_3$-N (Table 7.6) and the diurnal pattern of change (Figure 7.1) in the rumen fluid.

In the current experiments, casein would have provided a source of peptides, amino acids, BCFA and other higher volatile fatty acids to the rumen microbes, as described in section 2.3.1.3. It has been shown that BCFA and other higher volatile fatty acids are essential factors for the growth of many SC-fermenting rumen bacteria (Hungate *et al.* 1964; Allison, 1965, 1969; Allison & Robinson, 1967; Robinson & Allison, 1969; Bryant, 1973; Kristensen, 1974) and that the growth of SC-fermenting bacteria can be stimulated by provision of peptides and amino acids (Cotta & Russell, 1982; Cruz Soto *et al.* 1994).

However, as reviewed in section 2.3.1.3, both *in vitro* and *in vivo* studies have found conflicting results for the effect of rumen degradable, true protein supplements on eMCP. Notably, Cruz Soto *et al.* (1994) found the growth rate of cellulolytic bacteria to be stimulated by peptides and amino acids on a cellobiose but not a cellulose substrate. Similar results were obtained by Chikunya *et al.* (1996) who recorded a response in microbial numbers and yield to a true protein supplement fed to sheep consuming a rapidly degraded sugar beet pulp diet, but not a more slowly degraded grass hay diet. These researchers concluded that on slower fermented fibrous diets, the growth rate of SC-fermenting bacteria is limited by the slow degradation rate of the energy source rather than the availability of peptides and amino acids, when $NH_3$ is in adequate supply. It is evident from a review of the available literature (see section 2.3.1.3), that those diets for which there has been an associated response in

microbial growth to true protein supplements, or to products of protein degradation such as BCFA, have generally contained a substantial component of rapidly degraded carbohydrate.

Tropical grass hays, such as that studied in this Chapter, are usually high in SC compared to NSC content (Smith, 1973; Norton, 1982), and thus the argument presented above may explain the lack of a response found here to a true protein over an NPN source of RDP. Furthermore, it is possible that in the current experiments, intraruminal recycling of protein from the microbes may have been sufficient to provide the required protein precursors for the rumen bacteria. The measured fluid and particle FOR on all treatment diets were low (4.8 - 6.2 %/h for fluid FOR and an average of 2.2 %/h for particle FOR) which would have favoured intraruminal recycling of microbes in the rumen.

Although no associated response in eMCP was observed, mean molar proportions of the BCFA, iso-butyrate and iso-valerate, were increased in the rumen fluid of steers consuming the LoCAS treatment diet compared with the C and U treatments (Table 7.6). However, increasing the intake of casein to the very high level (HiCAS), which did result in an increase in eMCP, was associated with a further significant increase in BCFA concentration. It is possible that the increase in eMCP on the HiCAS diet was related to attainment of a critical level of supply of BCFA for the rumen microbes. Alternatively, the response could have been related to attainment of a critical level of peptides or amino acids in the rumen fluid as the concentrations of these products of protein degradation would also be expected to increase in the rumen fluid with increasing levels of casein supplement. The importance of peptides and amino acids was indicated by the work of Hume (1970a) who found that eMCP in sheep was greater when true protein sources (casein and zein) replaced 50 % of urea-N, compared to a diet where only urea and BCFA were fed.

It could be argued that RDP supply in general, or $NH_3$ concentration in the rumen, is the overriding factor influencing eMCP as is indicated by the response relationship shown in Figure 7.2. However, once the quantity of RDP supply is adequate (i.e. at plateau of the response curve) it could be speculated that further increases in eMCP may occur due to supply of the next limiting nutrient, such as BCFA or other products

of protein degradation, as indicated by the HiCAS treatment in the current experiment.

### 7.4.3 Effect of quantity and source of RDP supply on digestion and metabolism parameters

In these experiments, neither quantity or source of RDP had any effect on rate or extent of digestion of the basal diet of tropical grass hay. *In vivo* NDF digestibility (Table 7.4) and potentially degradable DM and OM fractions from incubation of pangola hay in nylon bags in the rumen (section 7.3.3 and Table 7.5) were similar across all dietary treatments as was the rate of DM and OM disappearance of rumen-incubated pangola hay. Thus, it is evident that the increase in eMCP at the high level of RDP supplementation (290 g/kg DOMI) was not associated with corresponding changes in extent or rate of digestion of the basal diet. It is therefore likely that the increases in DMD, OMD, and total DOMI with casein feeding (Table 7.4) were probably due to substitution of casein for the less digestible hay portion of the diet, rather than to any effect on rate or extent or digestion of the basal hay diet itself.

The results found here are in agreement with those of Hunter & Siebert (1985a) who found that digestion rates of pangola grass and black speargrass hays did not increase once a supplement of rumen degradable N, balanced for S and minerals, increased $NH_3$-N concentrations in the rumen fluid above 60 - 80 mg/l. Later experiments by Boniface *et al.* (1986) and Morrison *et al.* (1988) found even lower concentrations of $NH_3$-N to be optimal for rate of digestion of black speargrass hay, viz. 45 and 25 mg/l, respectively. Furthermore, in Experiments 6.1 - 6.7 of this thesis (Chapter 6) there was no association between rumen $NH_3$-N concentration and rate or extent of digestion of black speargrass-based hay incubated in rumen fistulated steers grazing seven different pasture types with a range of rumen $NH_3$-N concentrations from 8.0 to 382 mg/l.

Some research with continuous or semi-continuous culture systems has, in contrast to the findings of the current experiments, shown increases in the extent of fibre digestion when non-$NH_3$-N sources of RDP replaced $NH_3$ sources (Merry *et al.* 1990; Griswold *et al.* 1996; Carro & Miller, 1999). However, other experiments involving nylon bag incubations (Fujimaki *et al.* 1989; Cruz Soto *et al.* 1994) or continuous

culture systems (Broudiscou *et al.* 1999) have found no effect of non-$NH_3$-N sources on fibre or OM digestibility or degradability parameters. As reviewed in section 2.3.1.3, those experiments reporting a response to non-$NH_3$-N, or true protein supplements, have often utilised mixed diets or diets containing some component of soluble sugars.

The lack of any significant effects of treatment in the current experiments on total VFA concentration, rumen fluid pH, rumen fluid volume, DM content and particle FOR (section 7.3.3 and Table 7.7) are in accord with the lack of effect on rate or extent of digestion of the basal hay diet. However, it should be noted that poor relationships were obtained in some cases for FOR of Yb from the rumen in the current experiments, with four out of sixteen data sets being excluded as discussed in section 7.2.2.5. Thus, the values for rumen particle FOR and DM content should be considered with some caution. Furthermore, as the dilution rate studies were conducted under non-steady state conditions, the values for fluid and particle FOR, rumen fluid volume and DM content should be regarded as relative rather than absolute. Nevertheless, the lack of any strong relationship between eMCP and rumen fluid or particle outflow rates corroborates the results of the pasture experiments in Chapter 6 of this thesis where both rumen fluid and particle FOR lacked any relationship with eMCP. This finding challenges the importance placed on dilution rate by most feeding standards, and in particular, the AFRC (1993), which predicts eMCP with use of an adjustment factor for rumen outflow rate. However, these results do not dispute the possibility that when RDP requirements for the microbes are satisfied, manipulation of factors such as rumen dilution rates may increase eMCP, as was demonstrated by Mullik (1999).

### 7.4.4 Conclusions

These experiments have demonstrated that eMCP in cattle consuming a low quality tropical grass hay could only be increased to within the extant feeding standard's range when RDP was provided at the very high level of 290 g/kg DOMI. Furthermore, source of RDP had no effect on eMCP when supplied at 150 g RDP/kg DOMI. However, the increase in eMCP at the high level of casein feeding could be a response to a critical level of protein precursors, such as BCFA, for the rumen microbes once quantity of RDP supply is adequate.

# Chapter 8

# General Discussion

## 8.1 INTRODUCTION

Low eMCP values, below the range of 130 - 170 g MCP/kg DOMI given in the various feeding standards (130 -170 g MCP/kg DOMI; ARC, 1984; Madsen, 1985; Ausschuss fur Bedarfsnormen, 1986; INRA, 1988; SCA, 1990; AFRC, 1993; NRC, 1996b), have been recorded for tropical forages when fed as chaffed hays in metabolism studies. However, as reviewed in Chapter 2, there is limited information on the variability in eMCP for tropical grass and legume species and there is no data on eMCP for tropical forage consumed under grazing conditions.

The intention of this thesis was to investigate the variation in eMCP in cattle grazing a range of tropical pastures relevant to the north Australian beef industry. The associated study of pasture parameters, and animal digestion and metabolic factors, was undertaken to identify factors of most importance in achieving high rates of eMCP. These studies required the initial development of methodologies to enable estimation of MCP flow under grazing conditions. Final experiments were conducted to examine strategies for increasing eMCP in cattle consuming tropical grass forage. Conclusions from the experiments conducted in this thesis are discussed below in addition to opportunities for future research.

## 8.2 TECHNIQUES TO ESTIMATE MICROBIAL PROTEIN PRODUCTION IN GRAZING CATTLE

Traditional methods of estimating MCP flow have required the measurement of digesta flow in post-ruminally cannulated animals. The tedious and complicated nature of these methods has precluded routine estimation of MCP production under grazing conditions. In this thesis, a method was developed to allow estimation of MCP production in *B. indicus* cattle under grazing conditions. This involved firstly validating the level of endogenous urinary PD excretion, so as to use the technique of urinary PD excretion as an index of MCP flow for *B. indicus* cattle. Secondly, the

suitability of intravenously infused CrEDTA as a urine output marker was established so that measurement of PD and Cr in spot urine samples could allow estimation of daily MCP flow.

The experiment reported in Chapter 4 appears to be the first to directly compare *B. indicus* and *B. taurus* species for endogenous PD excretion. The determination of an endogenous PD excretion value for high-content *B. indicus* cattle, that was approximately half the value for *B. taurus* cattle, was a significant development for the urinary PD method. The use of this lower value for endogenous PD excretion will improve the accuracy of estimates of MCP production, and eMCP, for high-content *B. indicus* cattle. However, a significant number of crossbred cattle with varying levels of *B. indicus* and *B. taurus* content, are used in the Australian beef industry and around the world. Although existing data indicates that cattle with less than 75 % *B. indicus* content have similar low endogenous PD values to purebred *B. indicus* cattle (Osuji *et al.* 1996; Ojeda & Parra, 1999), further research is required to investigate the change in endogenous PD excretion with decreasing levels of *B. indicus* content, and also, the variation across and within breeds. If considerable variation in endogenous PD excretion were found, this would impede widespread application of the method. Another aspect of the urinary PD technique that is in need of further research is the assumed purine-N : total N ratio in the microbes flowing to the intestines. As reviewed in section 2.5.1, this ratio has been found to differ between liquid-associated and solid-attached bacteria and the contribution of these microbial fractions to duodenal flow may vary. This aspect was not investigated in this thesis and has not been routinely measured by others, due to the difficulty in obtaining representative microbial samples. Therefore, these two key areas, of endogenous PD excretion and ratio of purine-N : total N in mixed microbes flowing to the intestines, need to be further investigated and clarified to allow improved confidence in the urinary PD technique.

In a series of experiments reported in Chapter 5 of this thesis, CrEDTA was demonstrated to be an effective urine output marker for ruminant animals due to constant urinary recovery, over time and with varying feed quality, of *ca.* 90 % of intravenously infused Cr. The continuous intravenous infusion of CrEDTA was then used successfully to estimate urine output in steers under grazing conditions in

Experiments 6.1 - 6.7. This technique allowed estimation of daily PD excretion in the urine through a spot sampling regime and thus made possible the estimation of MCP flow under grazing conditions. The diurnal variation in the PD:Cr ratio was sufficiently large so that a sampling regime incorporating regular times with a 24-h period was necessary. Prior to these experiments, no suitable urinary output marker was reported in the literature. The use of the natural metabolic waste product, creatinine, as an internal urine output marker has been investigated. However, Puchala & Kulasek (1992), Faichney *et al.* (1995), (Bolam, 1998) and Shingfield & Offer (1998) found that the variation in creatinine excretion was too great to allow accuracy in prediction of urine output and thus PD excretion. The development of the CrEDTA intravenous infusion methodology for estimating urine output is thus a significant advance in this field and should facilitate any further attempts at determining eMCP under grazing conditions using the urinary PD technique. In addition, the technique would have application for the estimation of daily urinary excretion of a range of other metabolites, such as N, under field conditions.

## 8.3 THE EFFICIENCY OF MICROBIAL PROTEIN PRODUCTION IN CATTLE GRAZING TROPICAL PASTURES

It is recognised that the expression of eMCP as g MCP/kg DOMI is a simplification of actual efficiency of microbial protein production due to ignoring the inability of most rumen bacteria to utilise protein, fat or lipid as an energy source, not accounting for the proportion of digestible OM which is digested in the rumen, and the confounding effects of endogenous OM. However, the use of DOMI as the response variable in this thesis was justified due to the ability to estimate DOMI more easily and accurately under field conditions than rumen digested carbohydrate and due to the range of published values for eMCP, expressed per kg DOMI, available for comparison. Data from this thesis was examined by expressing eMCP as MCP per estimated rumen-digested NDF intake and a positive linear relationship was observed with eMCP expressed per DOMI (not included in this thesis). This indicates that the major conclusions drawn from the use of DOMI as the response variable would be similar to those if rumen-digested NDF intake had been used. However, in future, it might be appropriate to obtain more direct estimates of diet NSC and rumen-digested NDF content to allow expression of eMCP per unit of fermentable carbohydrate.

In the grazing experiments detailed in Chapter 6 of this thesis, the low eMCP values previously published for tropical grass pasture hays (see section 2.4) were confirmed for grazed pastures. The four tropical grass pastures studied in Chapter 6, which covered a range of seasonal conditions from the dry season to the early wet season, all had eMCP values that fell well below the range of 130 - 170 g MCP/kg DOMI given in the feeding standards. The two tropical legumes, Dolichos lablab and butterfly pea, produced eMCP values that fell within the extant feeding standards' range while the temperate species, annual ryegrass fell well above the range, at 209 g MCP/kg DOMI. Comparison of eMCP values determined in this thesis with those published in the literature for similar pasture types (see section 6.4.1) reveals that the thesis values concur with those of others, in finding eMCP for temperate pasture species to fall either within or above the extant feeding standards' range, and tropical grass pastures to fall well below the extant feeding standards' range. However, the lack of comparable data for pure stands of tropical legumes precludes any comparisons with the results obtained in this thesis for lablab and butterfly pea.

Although the various feeding systems give an average figure or range for eMCP (section 2.2), the most recent versions recognise that eMCP is not a constant and may vary according to diet type. In addition, reported values in the literature for eMCP range from 50 to 370 g MCP/kg DOMI (Corbett, 1987; SCA, 1990; Purser & Hogan, 1992). Thus, the wide variation found in this thesis for eMCP values is not a new phenomenon.

The low eMCP values measured in cattle consuming tropical grass pastures have serious implications for protein flow to the animal tissues as they compound the effect of already low MCP flows caused by the generally low digestibility of tropical grass pastures (see section 2.4 and 6.4.3). Therefore, low DOMI, in addition to low MCP produced per kg of DOMI, would result in total daily intestinal protein flow being much reduced compared to that found for temperate species. This issue is discussed further in section 8.6.

Current computer models such as GrazFeed (1993) which is based on the Australian feeding standards (SCA, 1990), NRC1996 which is based on the U.S. feeding standards (NRC, 1996a) and Cambeef (1993), have been shown to predict animal

performance from tropical pastures very poorly (Bolam, 1998). Bolam (1998) concluded that the major cause of the poor predictive ability of these models was their inability to accurately predict animal intake from tropical pastures. However, the use of an assumed eMCP, which is much higher than that actually measured for tropical grass pastures, would also cause error in the model predictions due to over-estimating protein flow to the animal tissues. In this thesis, a large range in eMCP was documented across pasture type, and with stage of maturity. If eMCP could be accurately defined for a particular pasture type and stage of maturity, then these modifications could be incorporated into the existing models to improve their predictive power.

## 8.4 RELATIONSHIP OF DIET AND ANIMAL FACTORS WITH EFFICIENCY OF MICROBIAL PROTEIN PRODUCTION

A number of factors are well documented in the literature as having an effect on eMCP (see section 2.3). These factors can be grouped under four major areas of substrate availability, dilution rate in the rumen, intraruminal recycling and rumen pH. In particular, the AFRC (1993) recognises the importance of dilution rate in recommending the use of an adjustment factor for level of feeding, or rumen outflow rate. Level 1 of the NRC model (NRC, 1996b) considers pH effects by using an adjustment factor for diets containing less than 40 % forage, while Level 2 of the NRC model (NRC, 1996b) includes aspects of the CNCPS, described by Russell *et al.* (1992), Sniffen *et al.* (1992) and Fox *et al.* (1992), which predicts microbial growth from feed carbohydrate and protein fractions as well as their digestion and passage rates.

As part of the grazing experiments conducted in Experiments 6 and the feeding experiments conducted in Experiment 7, a number of factors, including diet RDP, TNSC and fibre concentrations, rumen fluid and particle dilution rates, protozoal concentrations and pH were examined for correlation with eMCP. However, the only factors having significant relationships with eMCP were dietary fibre concentrations and RDP supply in the diet. More specifically, negative linear relationships were found between eMCP and diet NDF and ADF concentration, and a positive linear relationship was found between eMCP and diet RDP/DOMI ratio. While the

manipulation of plant fibre fractions is difficult to implement (Norton, 1982; Satter *et al.* 1999), RDP supplementation, is a more promising strategy for increasing eMCP.

## 8.5 EFFECT OF RUMEN DEGRADABLE PROTEIN SUPPLY ON EFFICIENCY OF MICROBIAL PROTEIN PRODUCTION

It was evident that the tropical grass pastures studied in the grazing experiments in Chapter 6 had insufficient RDP supply/DOMI in the diet to achieve eMCP in the extant feeding standards' range (i.e. RDP supply < 130 g/kg DOMI). This was the case even for the highest quality tropical grass pasture studied in the early wet season. These results are in accord with the feeding standards, which indicate that eMCP is dependent on RDP supply and will be equivalent to RDP supply per unit of energy if RDP is limiting for microbial growth. However, as recognised by the arbitrary optimum values for eMCP given in the various feeding standards, the eMCP response to RDP supply is expected to reach plateau once microbial requirements for RDP are saturated. Such a response is evident in the exponential relationship between eMCP and RDP/DOMI ratio, which was developed for tropical grass and legume forages in Chapter 6 (Figure 6.17) and Chapter 7 (Figure 7.2). This relationship indicated that eMCP increased rapidly with increasing RDP/DOMI ratio, until plateau was reached at values within the extant feeding standards' range.

However, in developing the exponential relationships described above, the value for the temperate pasture species, ryegrass, was excluded from the data sets. It was proposed (section 6.4.2.1) that temperate $C_3$ pasture species follow a separate response relationship that reaches a higher eMCP plateau than for tropical pasture species. This proposal was based on the high value determined for the annual ryegrass pasture studied in Experiment 6.7 (209 g MCP/kg DOMI) which fell well above the range of eMCP given in the feeding standards, similar to that determined for a range of other temperate species documented in the literature (section 6.4.1). However, as only one temperate pasture species was studied as part of the experiments in this thesis, no direct evidence was provided to support the hypothesis that separate response curves exist for temperate and tropical pastures. Thus, a detailed examination of published data for pasture-based diets was conducted in order to further examine the relationship of eMCP with RDP supply/DOMI, and the variation in eMCP at plateau of the response curve. These values are documented in

Table 8.1. In order to compare values on the same basis, data had to be recalculated in some cases to express eMCP in units of g MCP/kg DOMI. In addition, calculations and assumptions had to be made for many data sets to allow conversion to units of g CP/kg DOMI and g RDP/kg DOMI. Assumed values for protein degradability (dg) were based on results obtained as part of this thesis in addition to published values in the feeding standards (SCA, 1990; NRC, 1996b). Any assumed values used in the calculations are detailed in the footnotes of Table 8.1.

**Table 8.1. Comparison of efficiency of microbial crude protein production (eMCP) with diet crude protein (CP) and rumen degradable protein (RDP) supply per digestible organic matter intake (DOMI) for a range of pasture-based diets from the literature.** CP content and digestibility of forage also presented; digestibility values were determined *in vivo* (organic matter digestibility, OMD) or from *in vitro* analyses on extrusa (*in vitro* OMD (IVOMD) or *in vitro* dry matter digestibility (IVDMD))

| Diet | Animal | Estimated parameters | | | Reference |
|---|---|---|---|---|---|
| | | g CP/ kg DOMI | g RDP/ kg DOMI | eMCP (g MCP/ kg DOMI) | |
| **Temperate $C_3$ forages** | | | | | |
| Grazed pasture | Sheep | | | | Corbett *et al.* (1982) and Corbett & Pickering (1983)[A] |
| a) **Phalaris - Dec** *(23.5 % CP (of OM); 74 % OMD)* | | 318 | 277 | 140 | |
| b) **Phalaris - Feb** *(25.1 % CP (of OM); 79 % OMD)* | | 318 | 289 | 151 | |
| c) **Lucerne - Nov** *(21.4 % CP (of OM); 83 % OMD)* | | 258 | 250 | 200 | |
| d) **Lucerne - March** *(23.5 % CP (of OM); 82 % OMD)* | | 287 | 267 | 147 | |
| e) **Native pasture - Jan** *(15.1 % CP (of OM); 66 % OMD)* | | 228 | 189 | 186 | |
| f) **Native pasture - Apr** *(17.7 % CP (of OM); 62 % OMD)* | | 285 | 205 | 127 | |
| Freshly cut forage **Perennial ryegrass** | Sheep | | | | Beever *et al.* (1978)[B] |
| a) **Spring** *(10.0 % CP; 74 % OMD)* | | 151 | 145 | 148 | |
| b) **Autumn, 8 week regrowth** *(13.8 % CP; 70 % OMD)* | | 215 | 206 | 169 | |
| Cut and dried forage **Sub. clover** | Sheep | | | | Hume and Purser (1974)[C] |
| a) **Pre-wilting** *(24.8 % CP; 81 % OMD)* | | 349 | 255 | 159 | |
| b) **Wilting/wilted** *(13.0 % CP; 62 % OMD)* | | 245 | 123 | 123 | |
| c) **Mature** *(15.3 % CP; 65 % OMD)* | | 260 | 120 | 124 | |

| Diet | Animal | Estimated parameters | | | Reference |
|---|---|---|---|---|---|
| | | g CP/ kg DOMI | g RDP/ kg DOMI | eMCP (g MCP/ kg DOMI) | |
| Grazed pasture<br>**Annual ryegrass**<br>*(35.4 % CP; 83 % IVOMD)* | Cattle (high-content *B. indicus*) | 535 | 525 | 209 | This thesis[D] |
| Freshly cut forage<br>**Perennial ryegrass** | Cattle (*B. taurus*) | | | | Beever *et al.* (1986)[E] |
| a) **Early (spring)**<br>*(14.3 % CP; 83 % OMD)* | | 176 | 128 | 215 | |
| b) **Mid (summer)**<br>*(14.2 % CP; 82 % OMD)* | | 185 | 135 | 212 | |
| c) **Late (late summer)**<br>*(17.2 % CP; 81 % OMD)* | | 248 | 193 | 186 | |
| **White clover** | | | | | |
| d) **Early (spring)**<br>*(28.8 % CP; 83 % OMD)* | | 352 | 296 | 196 | |
| e) **Mid (summer)**<br>*(26.9 % CP; 77 % OMD)* | | 411 | 316 | 236 | |
| f) **Late (late summer)**<br>*(29.5 % CP; 81 % OMD)* | | 388 | 291 | 185 | |
| Freshly cut forage<br>**Perennial ryegrass** | Cattle (*B. taurus*) | | | | Lee *et al.* (2002)[F] |
| a) **Intermediate WSC**<br>*(9.9 % CP; 56 % IVDMD)* | | 188 | 179 | 141 | |
| b) **High WSC**<br>*(10.4 % CP; 61 % IVDMD)* | | 181 | 172 | 119 | |
| Hay<br>**Annual ryegrass**<br>*(19.4 % CP; 68 % OMD)* | Cattle (high-content *B. indicus*) | 303 | 287 | 191 | King *et al.* (1999)[GH] |
| Hay<br>**Annual ryegrass**<br>*(12.7 % CP; 63 % OMD)* | Cattle (high-content *B. indicus*) | 216 | 205 | 185 | Prior *et al.* (1998)[GI] |
| Hay<br>**Lucerne**<br>*(16.4 % CP; 65 % OMD)* | Cattle (*B. taurus* & 5/8 *B. indicus* content) | 280 | 238 | 125 | Kennedy (1982)[J] |

| Diet | Animal | Estimated parameters | | | Reference |
|---|---|---|---|---|---|
| | | g CP/ kg DOMI | g RDP/ kg DOMI | eMCP (g MCP/ kg DOMI) | |
| **Tropical $C_3$ legumes** | | | | | |
| Grazed pasture<br>**Dolichos lablab**<br>*(25.3 % CP; 78 % IVOMD)* | Cattle (high-content *B. indicus*) | 374 | 363 | 135 | This thesis[D] |
| Grazed pasture<br>**Butterfly pea**<br>*(14.2 % CP; 58 % IVOMD)* | Cattle (high-content *B. indicus*) | 277 | 262 | 144 | This thesis[D] |
| **Mix of $C_3$ and $C_4$ species** | | | | | |
| Grazed pasture<br>a) **Mitchell grasslands - winter rain**<br>*(9.6 % CP; 64 % IVOMD)* | Sheep | 177 | 136 | 173 | McMeniman *et al.* (1986b) and SCA (1990)[K] |
| b) **Mitchell grasslands - dry season**<br>*(3.7 % CP; 53 % IVOMD)* | | 90 | 52 | 87 | |
| c) **Mulga/grass association - winter rain**<br>*(16.8 % CP; 72 % IVOMD)* | | 266 | 237 | 184 | |
| d) **Mulga/grass association - dry season**<br>*(9.5 % CP; 48 % IVOMD)* | | 222 | 82 | 133 | |
| Grazed pasture<br>**Blue grama rangeland**<br>a) **Early growing season**<br>*(11.6 % CP (of OM); 64 % IVOMD)* | Cattle (*B. indicus* crossbred) | 183 | 155 | 76 | Funk *et al.* (1987a,b)[L] |
| b) **Early summer dormancy** *(8.1 % CP (of OM); 58 % IVOMD)* | | 140 | 119 | 85 | |
| c) **Late summer dormancy**<br>*(7.1 % CP (of OM); 63 % IVOMD)* | | 112 | 93 | 61 | |
| d) **late growing season**<br>*(11.1 % CP (of OM); 65 % IVOMD)* | | 170 | 151 | 77 | |
| Hay<br>**Green panic & siratro**<br>*(7.0 % CP; 54 % OMD)* | Cattle (*B. taurus* & 5/8 *B. indicus* content) | 141 | 113 | 72 | Kennedy (1982)[J] |

| Diet | Animal | Estimated parameters | | | Reference |
|---|---|---|---|---|---|
| | | g CP/ kg DOMI | g RDP/ kg DOMI | eMCP (g MCP/ kg DOMI) | |
| **Tropical $C_4$ grasses** | | | | | |
| Grazed pasture | Cattle | | | | This thesis[D] |
| **Black speargrass and forest bluegrass** | (high-content *B. indicus*) | | | | |
| a) **Early wet season** *(7.9 % CP; 68 % IVOMD)* | | 135 | 117 | 85 | |
| b) **Wet/dry transitional** *(4.2 % CP; 66 % IVOMD)* | | 74 | 61 | 26 | |
| c) **Dry season** *(2.7 % CP; 61 % IVOMD)* | | 51 | 42 | 35 | |
| d) **Creeping bluegrass** *(5.9 % CP; 72 % IVOMD)* | | 95 | 86 | 90 | |
| Freshly harvested **Pangola grass** *(6.3% CP; 69 % OMD)* | Cattle (high-content *B. indicus* content) | 104 | 94 | 72 | Mullik (1999)[GM] |
| Freshly harvested **Napier grass** *(3.9 % CP; 54 % OMD)* | Cattle (*B. indicus* & *B. indicus* crossbred) | 86 | 74 | 90 | Nsahlai, *et al.* (2000)[N] |
| Cut and dried **Napier grass** *(3.9 % CP; 53 % OMD)* | | 91 | 78 | 114 | |
| Freshly harvested **Guatemala grass** *(10.9 % CP; 67 % OMD)* | Cattle (*B. indicus* crossbred) | 179 | 154 | 72 | Shem, *et al.* (1999)[O] |
| **Setaria grass** *(9.0 % CP; 60 % OMD)* | | 163 | 140 | 31 | |
| **Napier grass** *(11.4 % CP; 63 % OMD)* | | 201 | 173 | 53 | |
| **Rhodes grass** *(4.4 % CP; 66 % OMD)* | | 108 | 93 | 75 | |
| Hay **Rhodes grass** *(6.7 % CP; 63 % OMD)* | | 73 | 61 | 50 | |
| Hay **Rhodes grass** *(4.8 % CP; 53 % OMD)* | Cattle (*B. indicus* content - Charbray) | 127 | 102 | 58 | Marsetyo *et al.* (2002)[GP] |

| Diet | Animal | Estimated parameters | | | Reference |
|---|---|---|---|---|---|
| | | g CP/ kg DOMI | g RDP/ kg DOMI | eMCP (g MCP/ kg DOMI) | |
| Hay<br>**Pangola grass**<br>*(5.0 % CP; 51 % OMD)* | Cattle (high-content *B. indicus*) | 99 | 85 | 87 | King *et al.* (1999)[GH] |
| Hay<br>**Pangola grass**<br>+ 200 g molasses/d<br>*(4.8 % CP; 52 % OMD)* | Cattle (*B. indicus* content - Charbray) | 97 | 83 | 44 | Mullik (1999)[GM] |
| Hay<br>**Buffel grass**<br>*(5.9 % CP; 58 % OMD)* | Cattle (high-content *B. indicus* & *B. taurus*) | 114 | 95 | 103 | This thesis[D] |
| Hay<br>a) **Buffel grass - young**<br>*(6.4 % CP; 57 % OMD)* | Cattle (high-content *B. indicus*) | 128 | 108 | 117 | Prior *et al.* (1998)[GI] |
| b) **Buffel grass - mature**<br>*(2.8 % CP; 55 % OMD)* | | 56 | 43 | 85 | |
| c) **Black speargrass**<br>*(5.3 % CP; 60 % OMD)* | | 93 | 80 | 95 | |
| **Tropical $C_4$ grass plus supplement** | | | | | |
| Hay<br>a) **Rhodes grass**<br>*(6.6 % CP; 57 % OMD)* | Cattle (high-content *B. indicus*) | 128 | 107 | 62 | Bolam (1998)[GQ] |
| b) + **CSM** (0.5 % LW) | | 223 | 152 | 90 | |
| c) + **CSM** (1.0 % LW) | | 323 | 205 | 116 | |
| d) + **CSM** (1.5 % LW) | | 427 | 262 | 115 | |
| e) + **CSM** (2.0 % LW) | | 471 | 283 | 145 | |
| Hay<br>a) **Pangola grass**<br>*(8.5 % CP; 49 % OMD)* | Cattle (high-content *B. indicus*) | 188 | 162 | 77 | Mullik (1999)[GR] |
| b) + **urea** | | 301 | 275 | 119 | |
| c) + **urea** + **supplement mix** (0.5% LW) | | 321 | 266 | 110 | |
| d) + **urea** + **supplement mix** (0.5 % LW) + **salt** (0.15 % LW) | | 308 | 258 | 141 | |
| Hay<br>a) **Pangola grass**<br>*(5.7 % CP; 50 % OMD)* | Cattle (high-content *B. indicus*) | 127 | 109 | 123 | This thesis[D] |
| b) + **urea** | | 167 | 150 | 115 | |
| c) + **casein (low)** | | 169 | 153 | 109 | |
| d) + **casein (high)** | | 307 | 293 | 167 | |

| Diet | Animal | Estimated parameters | | | Reference |
|---|---|---|---|---|---|
| | | g CP/ kg DOMI | g RDP/ kg DOMI | eMCP (g MCP/ kg DOMI) | |
| **Purified diets plus RDP supplements** | | | | | |
| Protein-free diet; casein infused abomasally; + **urea** to supply: | Sheep | | | | Hume *et al.* (1970)[S] |
| a) 2.56 g N/d | | 40 | 40 | 81 | |
| b) 4.42 g N/d | | 68 | 68 | 96 | |
| c) 9.20 g N/d | | 135 | 135 | 117 | |
| d) 15.95 g N/d | | 238 | 238 | 116 | |
| Protein-free diet with adequate S; | Sheep | | | | Hume (1970b)[S] |
| a) + 14.86 g N/d from **urea** | | 141 | 141 | 108 | |
| b) + 14.93 g N/d from **urea**, + **BCFA** | | 140 | 140 | 122 | |
| Protein-free diet with adequate S; | Sheep | | | | Hume (1970a)[S] |
| a) + 18.8 g N/d from **urea**, + **BCFA** | | 190 | 190 | 145 | |
| c) + 17.7 g N/d from **casein** and **urea** | | 181 | 181 | 165 | |
| d) + 18.3 g N/d from **gelatin** and **urea** | | 191 | 191 | 151 | |

BCFA, branched-chain fatty acids; CSM, cottonseed meal; dg, degradability of protein in the rumen; DM, dry matter; LW, liveweight; MCP, microbial crude protein; NDIN, neutral detergent insoluble nitrogen; OM, organic matter; PD, purine derivatives; WSC, water soluble carbohydrate.

[A] Phalaris (*Phalaris aquatica*), lucerne (*Medicago sativa*), native pastures (unfertilised grasses indigenous to the Northern Tablelands of New South Wales). MCP flow calculated from flow of markers at the abomasum; S-35 used as marker of MCP; dg determined with allowance for endogenous N component: a) 0.87, b) 0.91, c) 0.97, d) 0.93, e) 0.83, f) 0.72.

[B] Perennial ryegrass (*Lolium perenne*). MCP flow estimated from digesta flow into the duodenum; S-35 used as marker of MCP. Assumption: dg = 0.96.

[C] Subterranean clover (*Trifolium subterraneum* cv. Dinninup). MCP flow calculated from flow of markers into the duodenum; S-35 used as marker of MCP; dg of protein estimated without allowance for endogenous N contribution: a) 0.73, b) 0.50, c) 0.46.

[D] Names of pasture species given in relevant chapter of this thesis. MCP flows predicted from excretion of urinary PD; endogenous PD excretion determined in Chapter 4 for *B. indicus* cattle used in the calculation. dg calculated for each pasture type from the disappearance of NDIN from nylon bags incubated in the rumen in conjunction with measured passage rate data, except for Buffel grass hay where 0.83 dg was assumed.

[E] White clover (*Trifolium repens*). MCP flow estimated from flow of markers into the duodenum; N-15 used as marker of MCP; dg of protein estimated with assumed value for endogenous N contribution: a) 0.73, b) 0.73, c) 0.78, d) 0.84, e) 0.77, f) 0.75.

[F] MCP flows calculated from flow of markers into the duodenum; purine and pyrimidine bases used as markers of MCP. Assumptions: OMD = IVDMD; dg = 0.95.

[G] MCP flows predicted from excretion of urinary PD; endogenous PD excretion assumed in equation of Chen & Gomes (1995) used in the calculation.

[H] Annual ryegrass (*Lolium multiflorum*), pangola grass (*Digitaria eriantha*). Assumptions: ryegrass 0.95 dg, pangola grass 0.86 dg.

[I] Buffel grass (*Cenchrus ciliaris* cv. Biloela), black speargrass (*Heteropogon contortus*). Assumptions: ryegrass 0.95 dg, young buffel 0.85 dg, mature buffel 0.76 dg, black speargrass 0.83 dg.

[J] Green panic (*Panicum maxiumum*), siratro (*Macroptilium purpureum*). MCP flow calculated from flow of markers at the abomasum; sulphur-35 used as marker of MCP. Assumptions: lucerne hay 0.85 dg, pasture hay 0.80 dg.

[K] Mitchell grass pastures (*Astrebla* spp.) and mulga (*Acacia aneura*)/grassland association in south-west Queensland. Up to 55 % of the diet DM on the Mitchell grass pastures after winter rain, and 88 % of the diet DM on Mulga/grassland association pastures after winter rain, consisted of $C_3$ pasture species. The $C_3$ species included forbs, and in the mulga/grassland association, up to 43 % of diet DM of mulga oats (*Monochather paradoxus*) and mulga Mitchell (*Thyridolepis mitchelliana*) grasses. The diet on dry season mulga/grassland pasture also consisted of largely $C_3$ pasture species as well as browse (24 % mulga oats and mulga Mitchell grasses, 7 % forbs, 35 % mulga and 15 % other browse species). The diet on dry season Mitchell grass pasture consisted of *ca.* 100 % $C_4$ grasses. MCP flow calculated from flow of markers at the abomasum; S-35 used as marker of MCP; dg of protein estimated without allowance for endogenous N contribution: a) 0.77, b) 0.58, c) 0.89, d) 0.37. Assumptions: sheep 37.5 kg LW.

[L] MCP flow estimated from flow of markers at the duodenum; nucleic acids used as marker of MCP. Assumptions: dg = a) 0.85, b) 0.85, c) 0.83, d) 0.89.

[M] Assumptions: freshly harvested pangola grass 0.90 dg, pangola hay + 200g molasses 0.86 dg.

[N] Napier grass (*Pennisetum purpureum*). MCP flows predicted from excretion of urinary PD; endogenous PD flows for Zebu and Zebu x Friesian cattle (Osuji *et al.* 1996) were used in the calculation. Assumptions: napier grass fresh and dried 0.86 dg.

[O] Guatemala grass (*Tripsacum fasciculum*), setaria grass (*Setaria splendida*), Rhodes grass (*Chloris gayana*). MCP flows predicted from urinary allantoin excretion; endogenous PD excretion assumed in equation of Chen & Gomes (1995) used in the calculation. Assumptions: Guatemala grass 0.86 dg; setaria grass 0.86 dg, napier grass 0.86 dg, green rhodes grass 0.86 dg, rhodes grass hay 0.83.

[P] Assumptions: rhodes grass 0.80 dg.

[Q] Assumptions: steers 187.4 kg LW; rhodes grass hay 0.83 dg; CSM 0.57 dg.

[R] Supplement mix consisted of a mixture of grains, molasses, urea and CSM. Assumptions: pangola grass hay = 0.86 dg, urea = 1.0 dg, supplement mix = 0.66 dg; dg for higher dilution rate diet d) is similar to diet c).

[S] BCFA consisted of 27.0 % isobutyric, 26.3 % 2-methylbutyric, 29.9 % isovaleric, and 16.8 % n-valeric acid. MCP flows calculated from flow of markers into the omasum; tungstic acid-precipitated N used as MCP marker. Assumptions: all dietary protein is RDP.

The relationship of eMCP with the ratio of RDP/DOMI for data from the literature (see Table 8.1) for temperate and tropical pasture species, including supplemented tropical forage, is given in Figure 8.1. Those data sets relating to mixed tropical and temperate species and purified diets were not included in Figure 8.1. It is evident that there is not a close relationship between eMCP and RDP/DOMI, for the collated data. This is probably reflective of the problems associated with comparing data obtained using a variety of different methods and units to express eMCP in addition to the assumptions made for protein degradability for some data sets. However, the variation in eMCP at a particular level of RDP supply could also reflect the influence of other factors, such as rumen dilution rate, NSC supply or pH, on eMCP.

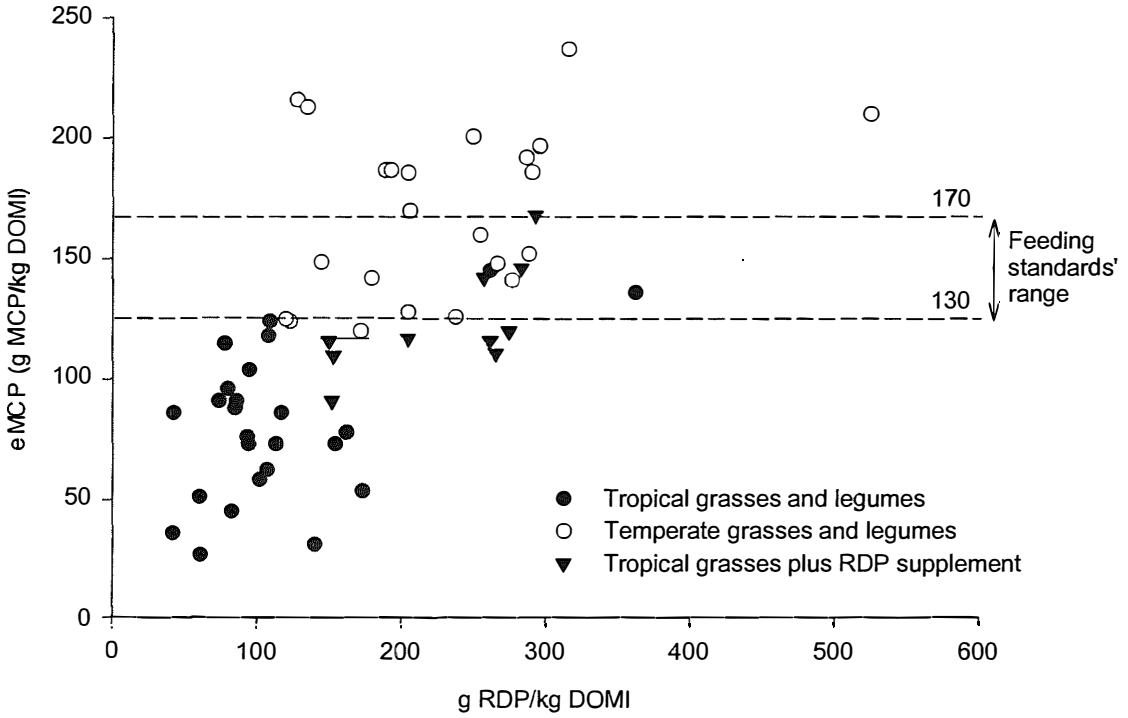


**Figure 8.1. Relationship between efficiency of microbial crude protein production (eMCP) and rumen degradable protein (RDP) supply per unit of digestible organic matter intake (DOMI) in the diet of sheep or cattle consuming temperate or tropical pasture species, or tropical grasses plus RDP supplement.** Values are derived from a number of published experiments as well as this thesis (Table 8.1). Extant feeding standards' range of eMCP indicated. (MCP, microbial crude protein).

Table 8.1 and Figure 8.1 indicate that the estimated ratio of RDP/DOMI in tropical grass forages is generally low, and much less than that required to support eMCP in the extant feeding standards' range. However, tropical legumes and some supplemented tropical grass pastures were associated with eMCP in the extant feeding

standards' range. Furthermore, the data indicates that eMCP for temperate $C_3$ pastures is usually high, in association with non-limiting RDP supply, and that eMCP falls within or above the extant feeding standards' range of 130 - 170 g MCP/kg DOMI. Of additional interest, is the significant variation in eMCP even once RDP supply becomes non-limiting for eMCP in the extant feeding standards' range. This may indicate that once the RDP requirements of the microbes are saturated, further increases in eMCP may occur due to supply of the next limiting nutrient, or due to creating more favourable rumen conditions.

It appears reasonable to conclude from the collated data in Table 8.1 and Figure 8.1, that temperate pasture species do have a higher plateau in eMCP than tropical pasture species, when RDP supply becomes non-limiting. This feature is not recognised in the feeding standards and indicates that factors other than RDP supply alone, are causing the high eMCP in temperate species. However, although a number of factors were studied in this thesis, no clear explanation for the far superior eMCP on temperate pastures was found. Factors such as NSC concentrations, rumen dilution rates and pH have all been implicated previously, as reviewed in section 2.3. In Experiment 6.7, the only factors, other than RDP supply, that appeared to be associated with the high eMCP of ryegrass pasture were high digestibility and low fibre concentrations, comparative to tropical grass and legume species. It is tempting to speculate that the proportions of microbe species in the rumen are altered in relation to these dietary aspects with shifts in general populations of particle-attached and fluid-associated microbes that lead to changes in eMCP. The use of new molecular ecology techniques (Forster *et al.* 2001) to identify changes in the proportions of microbe species in the rumen may facilitate investigation of this issue.

The response relationship developed in Chapters 6 and 7 demonstrates the benefit in using the dietary ratio of RDP/DOMI to assess the adequacy of protein for rumen microbes in cattle consuming tropical pastures, a principle espoused in the feeding standards. If pasture RDP/DOMI ratios are found to align with that part of the response curve where eMCP is increasing rapidly a small amount of RDP supplement should result in a significant improvement in eMCP. The experiments conducted in Chapter 7 indicate that such responses would be expected below *ca.* 120 g RDP/kg DOM, as at this point, the curve begins to plateau.

However, to assess pastures in this way, the rumen-available protein supply must be expressed using the same units as that used for eMCP, i.e. per DOMI. In the past, measures such as CP percentage in pasture DM and rumen $NH_3$-N levels have been used as a guide to the adequacy of dietary protein for microbial growth, with 60 g CP/kg DM (Langlands, 1987) and 50 mg/l (Satter & Slyter, 1974), respectively, being widely accepted critical levels. However, as demonstrated in the experiments of this thesis, and reviewed throughout, these measures are insensitive and are poor predictors of eMCP in practice. The primary constraint to using the measure of g RDP/kg DOMI to estimate eMCP on tropical pastures is the dearth of information pertaining to expected RDP concentrations in tropical pastures. This is primarily due to methodological difficulties as discussed in section 6.4.5.5. The relatively new method of nylon bag incubation and NDIN/ADIN analysis, developed by Mass *et al.* (1999) and corroborated by Klopfenstein *et al.* (2001), should provide a convenient and accurate way of estimating RDP content of forages and thus facilitate improved knowledge of this parameter for tropical pastures.

In addition to RDP supplementation, the inclusion of tropical legume species in tropical grass-based pastures may be a strategy to increase RDP supply/DOMI and thus increase eMCP to within the extant feeding standards' range. Furthermore, as legumes can have greater digestibility and intake characteristics than for tropical grasses (Poppi & McLennan, 1995), overall MCP flow could be increased in addition to net MCP flow. It would seem desirable for further research to be conducted to confirm the relatively high eMCP values obtained in Experiments 6.5 and 6.6 of this thesis for tropical legumes and, in particular, to investigate eMCP on mixed tropical legume and grass pastures.

As well as the quantity of RDP supply, the source of RDP (i.e. whether NPN or true protein) has been found to affect eMCP *in vitro* and *in vivo* (see section 2.3.1.3). However, in experiments described in Chapter 7 of this thesis, source of supplemental RDP (casein vs. urea) was found to have no effect on eMCP when total RDP supply was adequate to support eMCP within the extant feeding standards' range (i.e. 150 g RDP/kg DOMI). However, as discussed in Chapter 7, the increase in eMCP at the very high level of casein feeding (total RDP supply 290 g/kg DOMI) could have

been a response to a critical level of protein precursors, such as BCFA, for the rumen microbes once quantity of RDP supply was adequate.

## 8.6 POTENTIAL TO INCREASE LIVEWEIGHT GAIN THROUGH INCREASING THE EFFICIENCY OF MICROBIAL PROTEIN PRODUCTION

The low eMCP on tropical pastures has major implications for protein flow to the animal tissues. Calculations were conducted to determine the potential effect on cattle liveweight gain, of increasing eMCP on tropical pastures. As discussed above, RDP supplementation is the logical initial strategy to increase eMCP, due to being the first limiting nutrient on tropical pastures. Furthermore, RDP supplements based on urea are convenient and cost-effective and are already widely used by the north Australian beef industry when cattle are consuming dry season pastures. The greatest response in eMCP, to RDP supplementation, would come from targeting pastures on the linear part of the response curve developed in Figure 7.2. Furthermore, those pastures close to the plateau of the response curve in Figure 7.2 were those characteristic of the early to mid wet season, where liveweight gain was already high (e.g. 1.77 kg/d on early wet season native pasture) possibly in part due to compensatory gain effects as discussed in section 6.4.3. Thus, the case of the native tropical grass pasture studied in the wet/dry transitional period (NPT) in Experiment 6.2 of this thesis, was considered due the low eMCP (26 g MCP/kg DOMI) and moderate liveweight gain (0.5 kg/d) associated with this pasture.

The calculations were conducted according to the feeding standards (SCA, 1990) and are outlined in Table 8.2. The effect on liveweight gain of increasing eMCP to either 90 or 120 g MCP/kg DOMI, which equate to increases of 64 or 94 units of eMCP, respectively, was examined. The level of 90 g MCP/kg DOMI was targeted due to this being the maximal eMCP obtained on early to mid wet season tropical grass pastures in the grazing studies conducted as part of Chapter 6. The higher eMCP level represents the point at which the response curve of Figure 7.2 starts to plateau. As demonstrated in Chapter 7, very large amounts of RDP supplementation are required to achieve an increase in eMCP at the plateau of the response curve, and supplementation would be less efficient above this level.

**Table 8.2. Effect of increasing efficiency of microbial protein production (eMCP) above baseline levels on liveweight gain of 325 kg Brahman steers grazing native tropical grass pasture in the wet/dry transition period.** The steer and pasture parameters used in the calculations were those measured in Experiment 6.2: 0.5 kg/d liveweight gain, 26 g MCP/kg DOMI, 660 g/kg diet IVOMD, 1.41 kg DOMI/100 kg LW. Calculations and assumptions were made according to SCA (1990) unless otherwise indicated

| | Increase eMCP from 26 g MCP/kg DOMI to: | |
|---|---|---|
| | **90 g MCP/ kg DOMI** | **120 g MCP/ kg DOMI** |
| **Additional MCP (g/d)** | | |
| DOMI (kg/d) | 4.58 | 4.58 |
| MCP flow at 26 g MCP/kg DOMI (g/d) | 119 | 119 |
| MCP flow at enhanced eMCP (g/d) | 412 | 550 |
| Additional MCP (g/d) | 293 | 431 |
| **Additional ADPLS (g/d)**[A] | | |
| (MCP x 0.8 x 0.7) | 164 | 241 |
| **ADPLS used for growth (g/d)**[B] | 33 | 48 |
| **Protein in EBG gain (g/kg EBG)**[C] | 123 | 123 |
| **Additional EBG (kg/d)** | 0.27 | 0.39 |
| **Additional liveweight gain (kg/d)**[D] | 0.29 | 0.43 |
| **Total liveweight gain (kg/d)** | 0.79 | 0.93 |

ADPLS, apparently digestible protein leaving the stomach; DOMI, digestible organic matter intake; EBG, empty body gain; IVOMD, *in vitro* organic matter digestibility; LW, liveweight; MCP, microbial crude protein; MTP, microbial true protein.

[A] MCP x 0.8 = MTP (assume 20 % nucleic acids); MTP x 0.7 = ADPLS (assume apparent digestibility of MTP = 0.7).

[B] Assume the efficiency of use of ADPLS for growth is 0.2 as indicated by Poppi (1990).

[C] Equation 1.36A (SCA, 1990) used to calculate protein content in gain, which uses an adjustment factor for current liveweight as a fraction of the standard reference weight, and an adjustment for rate of empty body gain or loss.

[D] Assume liveweight gain = EBG/0.92 (SCA, 1990).

The calculations in Table 8.2 indicate that an additional 0.29 or 0.43 kg/d of liveweight gain could be achieved by increasing eMCP, from 26 g MCP/kg DOMI, to 90 or 120 g MCP/kg DOMI, respectively. Such additional levels of liveweight gain

are not insignificant, and could mean the difference in achieving target market weights before the onset of the dry season.

However, an estimation of the amount of liveweight gain expected from the ME intake on the pasture described above, indicates that ME would become limiting for growth at *ca*. 0.75 kg/d (calculations according to ARC (1980)). If this were to occur, the additional benefit in liveweight gain would be limited to 0.25 kg/d. However, as discussed by Poppi (1990), tissue responses to nutrient supply have not been clearly elucidated, due to the impact of the rumen on supply of substrates to the intestines which prevents prediction of the exact proportions and amounts of nutrients absorbed from a particular diet. As described by Black & Griffiths (1975), N retention by the ruminant animal increases linearly in response to increasing protein supply before reaching plateau when the energy supply becomes limiting. However, the exact protein to energy ratio required to reach plateau has not been clearly defined for all situations. In addition, the decreasing efficiency of use of protein for growth as N retention nears plateau (0.2 as indicated by Poppi (1990)), infers that energy could also be made available in this process through catabolism of protein, thus contributing to the ME requirements for additional growth. It should also be noted that the efficiency of use of protein for growth, of 0.2, which was used in the calculations of Table 8.2, is conservative according to the feeding standards such as SCA (1990), which suggest an efficiency of 0.7, and AFRC (1993) which suggest an efficiency of 0.59. The efficiency level of 0.2 was chosen in this instance so as to be representative of the values expected as N retention nears plateau.

It is apparent that the calculations above are only indicative of the possible liveweight response to eMCP and that further research is warranted to determine whether these predictions, of the effect of increased eMCP on liveweight gain, occur in practice. However, it is evident that an additional 0.43 kg/d of liveweight gain could be achieved, for 325 kg steers grazing tropical grass pastures with low RDP/DOMI ratio, by increasing eMCP up to the plateau of the response curve. If such increases could be achieved by supplying an NPN source of RDP, then RDP supplementation should be a simple, cost effective means of increasing growth rates of cattle consuming tropical forage. Such supplementation strategies would mean providing an RDP supplement during the wet/dry transitional period, when tropical grass pastures have

traditionally been thought to be adequate in RDP for MCP production (Winks & Laing, 1972; Winks *et al.* 1979).

## 8.7 CONCLUSIONS

There has been a lack of data documenting the variation in eMCP across a range of tropical grass and legume species, particularly under grazing conditions. Methodological difficulties in measuring MCP flows in the field have been a major impediment. In this thesis, methodology was developed to allow estimation of MCP flow in cattle under grazing conditions. This involved the use of the urinary PD technique, modified for high-content *B. indicus* cattle, in conjunction with intravenously infused CrEDTA as a urine output marker. Efficiencies of MCP synthesis in cattle grazing tropical grass pastures were confirmed as being much lower than the range of 130 - 170 g MCP/kg DOMI given in the various feeding standards, even for early wet season pasture. RDP was identified as the primary factor limiting eMCP in cattle consuming tropical grass forages, with RDP supply being insufficient to meet microbial requirements for eMCP in the extant feeding standards' range on all tropical grass pastures studied (i.e. RDP < 130 g/kg DOMI). The response curve relationship between eMCP and the ratio of RDP/DOMI, determined for tropical grass and legume species in this thesis (Figure 7.2), could be used to estimate eMCP for a given RDP supply in tropical forage. More accurate predictions of eMCP should result in improved estimates of intestinal protein supply, resulting in better predictions of growth rates and supplementation requirements of beef cattle at pasture. This knowledge should assist producers in more consistently meeting market specifications for high quality beef from pasture-based systems in northern Australia.

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