MEAT & LIVESTOCK AUSTRALIA

on farm

More effective supplements for the northern beef industry

Project number NAP3.122
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ISBN 1 74036 597 6

December 2002

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ABSTRACT

A series of pen and metabolism experiments were carried out with young, growing cattle receiving tropical forages and a range of supplement types. The objectives were to establish growth response curves to these supplements and to investigate ways to reduce the substitution effects associated with their feeding. Growth responses to protein meals were higher at low intakes but comparable at higher intakes to those of 'energy sources' such as grains and molasses. Generic response curves to the different supplement types have been incorporated into simple spreadsheets for ration formulation. Of the strategies investigated to reduce substitution, maintaining a higher protein/energy ratio in the supplement successfully reduced substitution at low intakes presumably through correcting an imbalance of nutrients in the rumen. Microbial protein production in the rumen was increased with the inclusion of all supplement types on low quality forage diets, but the largest increases occurred when a true protein source was included. The findings from this project can now be used by producers and their advisers as an aid to objective decision making in the feeding of young, growing cattle on low quality pastures. However, this decision making process will be enhanced through further research to include older, finishing cattle receiving medium quality forages and by inclusion of the response data in a decision support model.

EXECUTIVE SUMMARY

Although supplementary feeding of cattle is used extensively by producers in northern Australia, decision making on feeding strategies is often made difficult by a lack of objective information on the appropriate type and level of intake of supplement to achieve a desired production goal. Furthermore, the cost-effectiveness of supplementary feeding is often compromised when the feeding of supplements is accompanied by a reduction in intake of the pasture, a well-known but little-understood phenomenon called substitution. This project was designed to develop response curves linking cattle growth to intake of different supplement types and to investigate ways of reducing this substitution effect. The stated objectives of the project were as follows:

1 To develop dose response curves relating cattle growth to intake of high lipid, medium protein feed sources (e.g., copra meal and palm kernel expeller (PKE) meal).

2 To determine practical methods of reducing the substitution effect associated with feeding supplements.

3 To establish practical feeding strategies based on aspects 1 and 2 above in association with existing knowledge, and incorporate the information into simple decision support aids with which producers can easily compare supplementation options.

The main strategies investigated in relation to reducing substitution were to alter the site of digestion of the supplement, between the rumen and lower intestines, and to change the protein : energy (P/E) ratio in the diet. A series of three pen feeding trials and three parallel digestion / metabolism studies was carried out to achieve the project objectives, with a response curve design encompassing a range of supplement intakes common to all experiments. A further pen study was added to determine the effects of frequency of feeding a supplement on animal performance. Young growing steers were used throughout.

In pen feeding (70 d) and metabolism experiments, a low quality hay was fed *ad libitum* to yearling steers alone (Control) or with supplements of copra meal (Copra), PKE meal and cottonseed meal (CSM; pens only) at intakes of 0.25, 0.5, 0.75 or 1.0% of liveweight (%W/d; as fed) of the steers. The main findings (all $P<0.05$) were:

- All supplements increased growth rate of steers over that of the unsupplemented Control (0.1 kg/d loss), the response to CSM and Copra being similar but greater than that to PKE, indicating that Copra could be substituted for CSM in commercial supplements despite having lower protein content.
- Hay intake was reduced linearly with increasing supplement intake, the reduction being greater for Copra and PKE than for CSM.
- Copra and PKE did not increase concentration of ammonia-nitrogen (NH_3-N) in the rumen 3 h after feeding in the pen study but CSM caused a steep linear increase in concentration.
- Efficiency of microbial crude protein (MCP) production (EMCP) was low for Control steers (84 g/kg digestible organic matter (DOM)) but increased steadily with increasing intake of Copra and PKE.

The site of digestion studies included a pen feeding trial using a low quality green panic hay and a metabolism experiment using hays of both low quality (as above) and higher quality (ryegrass), fed *ad lib*. The supplement used was sorghum either dry-rolled (low rumen degradability) or expanded (high), with urea included at a low or high (pens only) concentration in the sorghum. Designated supplements intakes were 0 (Control), 0.5, 1.0, 1.5 or 2.0%W/d. The main findings were:

- Control steers gained at 0.25 kg/d and all supplements increased growth rate linearly, with the rate slightly higher for expanded compared with rolled grain; urea concentration had no effect.
- Both supplement types caused a similar level of reduction in hay intake, the reduction being greater for the higher compared to the low quality hay; urea concentration had no effect.
- The higher ruminal digestion of expanded compared to rolled sorghum was indicated by lower NH_3-N concentration in the rumen, greater depression in fibre digestibility and lower rumen pH.

- EMCP was higher for Control steers given high (147) compared with low (82 g/kg DOM) quality forage, but the increase in EMCP with supplement intake was much greater with the low quality hay; grain processing method had no effect on these relationships.

The effect of P/E in the diet was studied using a similar approach to that described above, except that the medium quality forage was immature pangola grass hay. Supplements with low (barley), high (barley/CSM/Copra; 2:1:1; (barley/protein)) and medium (barley/urea; pens only) P/E status were fed at supplements intake levels of 0 (Control), 0.5, 1.0, 1.5 or 2.0%W/d. The main findings were:

- Growth rate was increased over that of the Control (maintenance) with all supplements in the order barley/protein > barley/urea > barley, the former being a quadratic response as the rate of increase in growth rate decreased with increasing supplement intake, and the latter two being linear responses.
- Hay intake was depressed with all supplements, in the opposite order to growth rate increases (above), with barley and barley/urea associated with linear, and barley /protein quadratic, reductions; hay intake only declined with barley/protein feeding when intake exceeded about 0.5%W/d.
- EMCP was higher for steers given medium (115) compared with low (95 g/kg DOM) quality forage, but supplements increased EMCP more for the low compared with medium quality pasture, with barley/protein providing a greater increase than barley on both forage types.

In the final pen feeding experiment, a low quality forage was fed daily *ad lib.*, alone or with supplements of CSM, sorghum/CSM (1:1) and molasses/urea, all at two levels of intake (CSM: 0.25 and 0.5; others: 0.5 and 1.0%W/d) and at two feeding frequencies (daily or twice-weekly). The main findings were:

- Frequency of feeding did not affect steer growth rate with supplements of CSM or molasses/urea but with the sorghum/CSM mix, the growth rate was higher when fed daily compared with twice-weekly.
- The reduction in hay intake (substitution) was greater when molasses/urea was fed than with the other two supplements.

In relation to substitution, the main conclusions were that:

- There was a linear decrease in forage intake with (i) all supplement types fed with medium/high quality forages; and (ii) with 'energy sources' (e.g., grains/ molasses) fed with forages of any quality.
- There tends to be a two-stage effect when protein meals are fed with low quality forages, with no effect or a small increase in hay intake at low intakes (0.3-0.5%W) and a linear decline thereafter.
- Thus changing the P/E ratio in the diet appears to reduce substitution by correcting deficiencies in the rumen but changing the site of digestion of the supplement does not appear to affect substitution.

The research undertaken here represents a major advance in the understanding of the nutrition of cattle grazing tropical pastures in northern Australia, especially as it relates to the use of supplements. Apart from the immediate outcomes for industry (see below), the database created which links supplement and forage intake, growth rate and rumen microbial protein production will be a significant resource in the foreseeable future, especially for modelling and predicting animal performance. The main outputs from the project include:

- A simple spread-sheet based Least–Cost Ration Formulator to assist producers or their advisers to compare supplement options, i.e., protein meals vs. energy sources, for young, growing cattle given a low quality forage diet. This was derived from the series of growth response curves for pen-fed cattle receiving major supplement types.
- A series of intake response curves to varying supplement types and forage qualities which are being used to develop empirical equations relating forage and supplement intake and which can then be used by producers to make decisions on issues such as feed budgeting.
- A simple Ready-Reckoner for estimating urea (or protein) requirements to balance the nutrients in the rumen for optimal microbial protein production.

The main recommendations arising from the outcomes of the current project include:

1 That the response curves relating animal growth, intake and microbial protein production to supplement intake, as generated in this project, be used to modify and improve existing decision support models for predicting cattle performance in the tropics, where the main inputs on pasture/diet quality would be from faecal NIRS outputs.

2 That further strategic experiments be carried out to extend the current response curves and Least-Cost Ration Formulator for young, growing cattle on low quality pasture to older, finishing animals approaching market weight and grazing medium quality pasture.

3 That the possible synergistic effects of protein meals and energy sources fed together be further investigated and quantified.

4 That the data generated in this project be used to augment and improve existing empirical equations predicting forage intake in relation to supplement intake, for feed budgeting purposes.

5 That the data generated in the current project and others be used to develop further 'generic' response curves based on responses to key nutrients of protein and energy (ME), rather than supplement type, so that most cost-effective combinations of different supplement types can be discerned.

6 That research be undertaken to identify the nutrients limiting microbial growth in the rumen of steers grazing low quality tropical pastures.

7 That the requirements for inclusion of a rumen degradable source of nitrogen, e.g., urea, in supplements based on copra and PKE meals be determined and recommendations made to industry.

8 That further investigation into the effects of frequency of feeding supplements on animal performance be undertaken, especially with the 'energy sources'.

9 That the practical feeding information generated in this project be promptly included in the Northern Nutrition EDGE Workshops.

1 BACKGROUND AND GENERAL INTRODUCTION

Supplementary feeding is used extensively by cattle producers in northern Australia for a variety of purposes, including in more recent years as a strategy to increase growth rates of cattle to meet higher-value market specifications. However, choosing an appropriate supplement poses a major challenge to most producers, as was highlighted in a recent quantitative survey of cattle producers, commissioned by MLA as a preliminary step in the development of the MLA EDGE*network* training package, "Nutrition EDGE". A recurring theme of producer feedback was their need for access to more basic information on cattle responses to different supplements under varying pasture type, quality and availability, in order to enhance their decision making capabilities. This response indicated that information about the relative feeding value of different supplements was either not available or was not in a form readily useable by producers and their advisers.

Objective decision making will be considerably advantaged by several factors, viz. (i) availability of good dose response curves relating cattle growth to intake of various types of feed supplements under varying pasture quality; (ii) a better understanding of the effects of supplements on pasture intake and utilisation; and (iii) decision support aids which convert this information into simple, practical and readily accessible form for use by cattle producers.

Considerable information has already been accumulated on responses to some supplement types, but there are others such as the high-lipid protein meals for which comparative data are limited. Examples of these higher lipid sources include copra meal, whole cottonseed and palm kernel expeller (PKE) meal, which are being increasingly used by industry as alternatives to more conventional sources such as cottonseed meal (CSM). Copra meal is a by-product of the extraction of oil from coconuts and palm kernel expeller meal is similarly a by-product of the palm oil industry. These meals have medium levels of protein (16-21%) but relatively high energy content by virtue of the oil remaining after the mechanical extraction process. Where copra meal and cottonseed meal have been compared at a single low level of intake, results indicated that the growth of cattle was similar despite copra meal having only half the protein content of cottonseed meal. This finding needed to be confirmed over a range of intakes. Thus one aspect of the research was to compare copra meal and PKE meal with the industry standard, cottonseed meal, over a range of supplement intakes in order to establish growth response curves for cattle which could be used by producers and their advisers.

Another key element of cost-effective supplementary feeding is the effect of the supplement on pasture intake and utilisation. The objective of supplementary feeding programs associated with more extensive grazing systems is to maximise pasture use, as this is the low cost component of the diet, and provide additional nutrients through the supplement. However, when supplements are fed at higher levels of intake, there tends to be a concomitant reduction in pasture intake by the animal, i.e., a substitution effect, so that the growth response to supplementation is lower than would be expected if pasture intake was not depressed. Previous research (Project DAQ.100, part-funded by MRC) indicated that substitution of supplement for pasture was often a major impediment to achieving the required increases in growth rates of grazing cattle, and that this substitution effect varied with supplement type. Unfortunately, the current feeding standards used by nutritional managers and consultants to predict the effect of supplements on animal performance either fail to account for substitution or, due to the dearth of quantified information on its effects, provide imprecise adjustment in growth estimates.

Under more intensive grazing systems substitution can be used to advantage to allow an increase in stocking rate (SR) when supplementary feeding is used, or alternatively to 'rest' pasture. The economics of feeding can be determined based on these changes. The less intensive the system and the lower the utilisation of the forage the less sensitive the outcome is to substitution for SR effects and the more sensitive it is to individual animal responses. Substitution thus has important implications for cost-effectiveness of feeding and also for sustainable pasture management where a pasture saving contribution of the supplement can be exploited.

The phenomenon of substitution has been well recognised for a long time and there are several very good reviews on the subject (e.g., Horn and McCollum 1987; Dixon and Stockdale 1999; Moore *et al.* 1999). Nevertheless, there is still no real consensus on what is the major causal factor, although there

are several factors known to contribute. Consequently, there were also limited strategies available to managers to manipulate substitution under grazing conditions.

Two strategies were recognised as having the potential for modifying the extent of substitution, viz., (i) manipulating the protein : energy (P/E) ratio in the absorbed end-products of digestion; and (ii) changing the site of digestion of the supplement in the gastrointestinal tract, i.e., in the rumen versus the lower gut, and were thus included for investigation in the present project. The P/E ratio can be manipulated by ration formulation. Changing the site of digestion of nutrients, which influences intake regulation by involving different satiety receptors and also influences the P/E ratio in end-products of digestion, can be achieved by using combinations of commonly available feed sources which vary in extent of digestion in the rumen (e.g., barley, sorghum, molasses), in rumen bypass protein (e.g., cottonseed meal, fish meal, canola meal) and in lipid supply (e.g., whole cottonseed, copra meal). Sorghum is a grain which usually has low digestibility of starch in the rumen but has the potential for increased rumen digestion through more intensive processing, e.g., by steam-flaking or extrusion. It thus has potential as a useful medium to investigate changes in the site of digestion of the supplement.

The main goal of the following studies was to derive the knowledge required to formulate practical supplements aimed at maximising growth with optimal use of the pasture base. This was to be particularly directed at the higher levels of feeding commensurate with production feeding to finish cattle for specific high-value markets. A third aspect of the project derived from an awareness that there was already a considerable body of knowledge on supplementary feeding, but that much of this information was not easily accessible to producers or their consultants or was not packaged in a user-friendly format. The main purpose of this phase of the project was to assemble, collate and rationalise research findings from the current project and from other published sources, and use the information to develop appropriate decision support mechanisms potentially with links to the type of information derived from faecal Near Infrared Reflectance Spectroscopy (NIRS) analyses. There were also obvious links with the proposed MLA-funded NIRS study which was designed to allow an estimation of diet quality based on an NIRS scan of the faeces of the grazing animal. Information on dietary intake and quality can only be translated into practical feeding strategies to modify cattle performance when there is concomitant information on the responses by cattle to nutritional treatments, such as was targeted in the present project.

The stated **objectives** of the project were as follows:

1 To develop dose response curves relating cattle growth to intake of high lipid, medium protein feed sources (e.g., copra meal and PKE meal).

2 To determine practical methods of reducing the substitution effect associated with feeding supplements.

3 To establish practical feeding strategies based on aspects 1 and 2 above in association with existing knowledge, and incorporate the information into simple decision support aids with which producers can easily compare supplementation options.

The research involved a series of pen feeding and associated metabolism studies which are described in detail in the following chapters. These experiments have been designed to provide answers to the questions raised above and to explore various approaches to manipulating substitution. The chapters are written with detailed Materials and Methods and Results sections in publication-ready format, but the Discussion sections are general and truncated and no attempt has been made at this stage to discuss the results fully in relation to published scientific findings. This will follow with the eventual publication of the various chapters.

2A EFFECT OF HIGH LIPID SUPPLEMENTS ON THE INTAKE AND GROWTH OF STEERS GIVEN A LOW QUALITY FORAGE: PEN STUDY

Materials and methods

Animals, treatments and experimental design

The experiment was carried out at "Brian Pastures" Research Station via Gayndah, Qld, between March and June 2001. Commercial Angus/Brahman crossbred weaner steers approximately 10-12 months of age and weighing 220.0 ± 3.37 (± SE) kg liveweight (at trial commencement), were purchased from a grazing property in central Queensland. They were vaccinated against tick fever and bovine ephemeral fever and sprayed on two occasions with Supona® to reduce buffalo fly numbers prior to commencement of the experiment.

The experimental design was a randomised block with three blocks of 14 pens. Treatments consisted of an unsupplemented control group and three supplement types: (i) cottonseed meal (CSM), (ii) copra meal (Copra), and (iii) palm kernel expeller meal (hereafter PKE), fed at each of four levels of daily intake: 0.25, 0.50, 0.75 and 1.0% liveweight (W) on an 'as fed' basis. The Copra and PKE originated from Papua New Guinea and were supplied by Mataranka Grain Pty Ltd, Dayboro Queensland. There were three steers for each supplement treatment, and six for the control, making a total of 42 steers. All steers received a basal diet of green panic (*Panicum maximum*) hay fed *ad libitum*. The treatment plan is shown below.

	Supplement intake (as fed) (%W/d)	Treatment name
Control	0	Control
CSM	0.25	CSM 0.25
	0.5	CSM 0.5
	0.75	CSM 0.75
	1.0	CSM 1.0
Copra	0.25	Cop 0.25
	0.5	Cop 0.5
	0.75	Cop 0.75
	1.0	Cop 1.0
PKE	0.25	PK 0.25
	0.5	PK 0.5
	0.75	PK 0.75
	1.0	PK 1.0

Steers were housed in individual pens. There were 22 large (19.6 m^2) and 20 smaller (12.5 m^2) outside, concrete-floored pens, with half of each on both sides of a central laneway running north/south. A high roof at the front of the pens provided shade over the feed trough and part of the pen area. Pens were

blocked according to their position within the pen complex (i.e., pen size and proximity to feed preparation shed). One block comprised all large pens (southern end), another all small (northern end), and the third central block included 8 large and 6 small pens. Treatments were randomly allocated to pens within blocks subject to the requirement that control and supplemented steers were equally represented on the eastern and western sides of the pen complex.

Procedures

The experiment consisted of a 7 d equilibration period followed by a 70 d experimental period. During the equilibration period the steers were fed green panic hay *ad libitum*, without supplements, in group pens of four steers. The steers were weighed full and fasted (24 h off feed, 16 h off water) at the end of this equilibration period and allocated to treatments by stratified randomisation on the basis of this fasted liveweight (day 0). They were divided into three liveweight classes: heavy, medium and light, with one liveweight class representing each replicate of the supplemented groups and two replicates of the controls, and weight classes randomly allocated to the pen blocks. Steers were randomly allocated to pens within the block structure.

The hay and supplements were fed daily and residues collected once weekly. Hay was chaffed to lengths averaging 5 cm and fed to each steer at a level estimated to provide about 15% in excess of its intake on the previous day, and thus maintain *ad libitum* intake. Supplements were placed in small feed bins located within and at one end of the main trough, and fed separately to the hay. Where supplements were not totally consumed they were also residued once weekly at the same time as the hay or, if residues were excessive, were also residued mid-week. Because of the low palatability of the PKE, it was sieved to remove large hard particles and mixed with molasses included at 10% (w/w, fresh weight) of the meal, i.e., 100 g molasses/kg PKE meal, prior to feeding.

Steers were weighed, unfasted, once weekly, in the morning prior to feeding (8 am). The amount of supplement fed to each steer was adjusted each week on the basis of this most recent liveweight.

Representative samples of the hay and supplements fed, and of the hay and supplement refusals, were collected weekly and dried to constant weight at 60^{o}C to determine dry matter (DM) content and intake. Duplicate samples (undried) of the feed sources were taken daily, bulked over 35 d, ground through a 1 mm screen and retained for chemical analyses.

On day 57 of the experimental period, rumen fluid was collected *per os* from all steers using a stomach tube and vacuum pump. Feeding was staggered so that sampling of each steer occurred 3 h after feeding the hay and supplement in the morning. The total supplement mix was fed at this time. The rumen fluid was strained through muslin and divided into several samples, viz., (i) 15 mL acidified to pH< 3 with concentrated sulphuric acid for determination of the concentration and molar proportions of volatile fatty acids (VFAs); (ii) 4 mL added to equal volume of 0.2N hydrochloric acid to determine ammonia (NH_3-N) concentration; and (iii) 4 mL added to 16 mL of formal saline for later enumeration of protozoa. At the time of rumen sampling, a blood sample was also taken from the tail vein using vacuutainers containing lithium heparin, and the plasma separated by centrifugation and stored frozen awaiting analysis for urea and glucose concentrations. The amount of supplement consumed between feeding and sampling was also determined.

In situ digestibility: In July 2001, six high-content Brahman crossbred rumen-cannulated steers (10 cm i.d. cannula), approximately 30 months of age and weighing 557 kg, were fed a diet consisting of lucerne (*Medicago sativa*; 91.8% OM, 19.3% CP, 41.8% neutral detergent fibre (NDF) and 32.7% acid detergent fibre (ADF)) and green panic hay (88.4% OM, 7.4% CP, 67.5% NDF and 35.7% ADF; 2:1, as fed), plus 1 kg CSM (93.3% OM, 41.6% CP, 22.8% NDF and 18.3% ADF) daily. The hay was fed at 90% of *ad libitum*-established intake and, with the CSM, was provided in two equal meals daily (8 am and 4 pm). Rumen fluid samples were taken from all steers on four occasions, morning and afternoon over two successive days, during the incubation period. Rumen NH_3-N concentrations exceeded 100 mg/L on all occasions. Nylon bags (24 cm x 10 cm; 45 m pore size; Allied Filter Fabrics Pty Ltd) containing *ca.* 5 g (air dry) of test feed were closed at the top using a rubber band and attached with a cable-tie to a steel chain weighing *ca.* 1.7 kg. The test feeds were CSM (93.3% OM, 41.6% CP), copra meal (93.9% OM, 24.7% CP) and PKE meal (96.3% OM, 16.7% CP) which were included in the bags as fed, except for the

PKE which was screened for lumps, as indicated above, but not mixed with molasses. Two bags per test feed were placed in the rumen of each of the three steers for each incubation time (3, 6, 9, 15, 24, 48, 72 h) so that there were six values for DM disappearance for each feed per incubation period. Four bags were also kept to determine 0 h disappearance. The CSM bags were incubated in three steers, and the copra meal and PKE in another three. Just prior to rumen insertion, the bags were immersed in cold water for 5 min. Bags were inserted into the rumen in reverse order and removed at a common time to standardise washing procedures. Upon removal, the bags were submerged in cold water to limit further digestion, rinsed under running water to removed external particulate matter and washed in a domestic washing machine through one wash and one rinse cycle and a final spin cycle (total washing time 20 min). The 0 h bags were handled in the same way. The bags were then dried to constant weight at 55^{o}C over 48 h, cooled in a desiccator and weighed to determine DM content. Bag residues were bulked across replicates and steers (six bags for incubated bags; four for 0 h) and ground through a 1 mm sieve before being analysed for OM and N content. Samples of the test feeds were similarly analysed.

The percentage DM, OM and CP disappearance at each incubation time was calculated from the proportion remaining after rumen incubation. Based on the work of Ørskov and McDonald (1979), it was assumed that, within lag time, $p_0 = A$, the initial washing loss from the bags, and beyond the lag period the degradation rate was described by the following equation:

$$p = a + b\,(1 - e^{-ct})$$

where p is the proportion degraded at time t, a is the intercept representing the immediately soluble component, b is the insoluble but potentially degradable fraction, and c is the degradation rate (/h) of the b fraction. The combined term 'a + b' represents the asymptote of the equation and thus the maximum extent of degradation. The potentially degradable but not soluble component (*PD*) is determined from the equation: $PD = a + b - A$ whilst the effective degradability (*ED*) of the various factors was determined by assuming a fractional outflow rate, k, of 0.02/h in view of the low quality of the forage and likely maintenance only production of the steers, and applying the following equation of Ørskov and McDonald (1979):

$$ED = a + \frac{bc}{c + k}$$

Chemical analysis

Ash: Ash content was determined by combusting dry samples in an electric muffle furnace (Thermogravimetric Analyser TGA-601, LECO Corporation) at 600^{o}C for 2 h.

Total N: Samples were analysed for total N by a combustion method (Sweeney 1989) using an ELEMENTAR RapidN Analyser (ELEMENTAR ANALYSENSYSTEME, Germany).

NDF and ADF: Samples were analysed for NDF and ADF content using a FIBRETEC 2021 Fibrecap system (FOSS TECATOR). The NDF content was expressed on an ash-free basis but was uncorrected for residual protein.

Ammonia-N (NH_3-N) in rumen fluid: NH_3-N concentration was determined using an Olympus Reply Clinical Analyser, based on a reaction described by Boller *et al.* (1961).

Volatile fatty acid (VFA) concentrations and proportions: Volatile fatty acids (VFA) were extracted from rumen fluid samples by acidifying with 1M sulphuric acid followed by vacuum distillation using the general procedure of Daniels *et al.* (1981). The condensates were analysed without further treatment. VFAs were separated by capillary gas chromatography using an Agilent Technologies 6890 gas chromatograph equipped with a flame ionisation detector. A fused silica capillary column, DB-FFAP 30m x 0.53mm with a 1.0μm coating (Agilent Technologies, USA), was used with helium as carrier gas. The column oven temperature program consisted of a thermal gradient from 90°C to190°C at a rate of 10°C/min. VFA were quantified against an external standard mixture comprising VFA from C2 – C7 (Sigma-Aldrich Co, USA).

Fatty acids: Lipids were extracted from samples with chloroform/methanol by the method of Folch *et al.* (1957). Lipid fatty acids were derivitised to their fatty acid methyl esters (FAME) using 14% boron trifluoride-methanol (Van Wijngaarden, 1967). FAME were analysed on an Agilent Technologies 6890 gas chromatograph by capillary gas chromatography using split injection with helium carrier gas and a flame ionization detector. The column used was a DB23 fused silica column, 30m x 0.25mm, with a 0.25µm coating (Agilent Technologies, USA). Column oven temperature was held at 140°C for 5 min and then elevated at 3°C/min to 210°C where it was held until all FAME of interest had been eluted. FAME were identified by comparing their retention times with those of authentic standards (Sigma-Aldrich Co, USA), and were quantified by comparison with the response of an internal standard, heneicosanoic acid.

Statistical analysis

For each response variable, general or generalised linear models were used to test the effects of supplement level and type, to check on the effects of pen size and pen side, and to determine the simplest form of equation to adequately describe the relationship between the variable and supplement intake. In particular the following models were fitted, with a term for replicate included in all models and with statistical significance assessed at the P=0.05 level:

- an analysis of variance model with terms for supplement type and intended supplement intake, to summarise the effects;
- a sequence of models to test the degree of polynomial needed to describe the response to actual supplement intake. Initially a full model (degree 4) was fitted, then the degree sequentially reduced by dropping non-significant terms. For almost all variables the contributions from the higher order terms were not statistically significant, so only linear or quadratic models were used in subsequent analyses. In cases where the overall test indicated a quadratic model but the coefficient for the quadratic term for a supplement was not significant, only a linear term was fitted for that supplement in later models;
- a model to check the significance of pen size and side effects. The significance of these terms was assessed by fitting terms for replicate and linear or quadratic response to actual supplement, then adding terms for pen size and pen side. In almost all cases the pen effects were not statistically significant, so were not included in the later analyses;
- a model to test whether the response relationships for the three supplements were significantly different, and, if so, models to test for differences on a pair-wise basis. In cases where the relationships were not significantly different, common ones were fitted. For the final model the data points and fitted lines were graphed, and residual standard deviations and approximate R^2 values calculated for each supplement plus controls and for the overall model. The assumptions of the analyses and presence of outliers in the data were checked by plotting residuals against fitted values.

For most variables the models were fitted as general linear models with residuals assumed normally distributed. Protozoa counts were assessed by fitting generalised linear models with a Poisson distribution and log link, with the log of the numbers of squares counted as an offset in the linear part of the model. Counts were converted to density (number x 10^{-5}/mL) for the graphs. The proportions of each species in the total protozoa count were also modelled using generalised linear models, with a binomial distribution and logit link.

The statistical package GenStat (2002) was used for all analyses.

Results

The chemical composition of the hay and feed supplements are shown in Table 2.1. The difference in CP content between CSM and the other two supplements were large, with the CP content of PKE not much greater than that of some grains. By contrast, the Copra and PKE meals which were prepared by mechanical extraction of the oil had much higher lipid content than CSM which was solvent extracted. Copra and PKE meals were high in saturated fat content whilst CSM was high in unsaturated fats, especially $C_{18:2}$.

Table 2.1. Nutrient composition and fatty acid composition of hay and supplements

	Hay	CSM	Copra meal	PKE – as received	PKE – as fed [A]
Nutrient composition of diets (% DM)					
OM	89.4	92.9	93.7	96.3	95.6
CP	5.4	42.8	24.2	16.9	16.1
EE	1.8	1.9	7.3	10.7	9.8
Total fatty acids	ND	2.86	5.26	8.41	ND
NDF	67.4	22.8	49.8	65.8	59.0
ADF	38.1	18.3	26.1	40.3	36.9
Ca	0.30	1.14	0.56	0.58	0.54
P	0.30	0.45	0.08	0.24	0.29
Fatty acid composition of supplements (% total fatty acids)					
$C_{8:0}$				1.8	
$C_{10:0}$			3.4	2.8	
$C_{12:0}$			45.4	46.3	
$C_{14:0}$		0.6	20.7	17.0	
$C_{16:0}$		24.7	12.4	9.1	
$C_{16:1}$		0.4			
$C_{18:0}$		3.2	4.3	3.3	
$C_{18:1}$		18.2	10.8	16.7	
$C_{18:2}$		51.7	2.9	2.9	
$C_{18:3}$		0.4			
$C_{20:0}$		0.4			
$C_{22:0}$		0.4			

[A] Prior to feeding the PKE meal was screened to remove large, hard lumps and mixed with 10% molasses (w/w; as fed).

EE – Ether extract; ND – not determined.

The digestion of CP of the three protein meals in nylon bags over time is illustrated in Figure 2.1 and the relevant equations describing the degradation curves are as follows:

CSM: $$p = 20.72 + 73.70 (1 - e^{-0.0741\,t});$$

Copra: $$p = 0.61 + 96.71 (1 - e^{-0.0799\,t});$$

PKE: $$p = -6.92 + 103.08 (1 - e^{-0.1148\,t}).$$

These curves indicate similar rates of protein disappearance for CSM and Copra but a faster rate for PKE. Lag times for Copra and PKE (4.0 and 3.4 h) were considerably longer than for CSM (0.7 h). The potentially degradable components for the CSM, Copra and PKE were 69.9, 70.3 and 69.7% and effective degradabilities at passage rate of 0.02/h were 78.8, 78.9 and 81.9%, respectively.

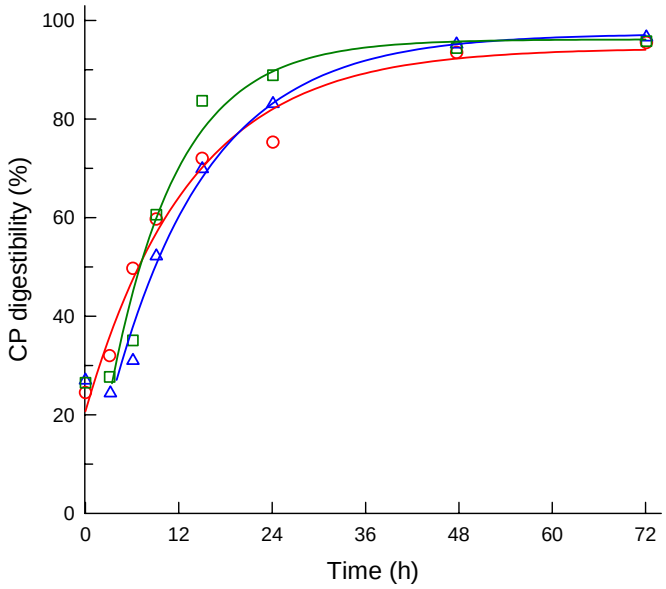

Figure 2.1. Digestibility over time of protein in different meals (CSM, ○; Copra, △; PKE, □) incubated in nylon bags in the rumen of fistulated cattle. Curves are plotted from the end of the lag period.

Unsupplemented steers had slight weight losses (0.1 kg/d) over the trial period. Inclusion of CSM or Copra supplement in the diet resulted in a quadratic increase, and of PKE in a linear increase, in growth rate of steers, with no difference between CSM and Copra but with both different from PKE in response (P<0.01). The response curves are shown in Figure 2.2A and the equations describing them for the PKE and the combined CSM / Copra are outlined below:

CSM / Copra: **Average daily gain (kg) = – 0.103 + 1.527 *SI* – 0.788 *SI*** ^{2}, (P<0.01);

(CSM: R^2 = 0.914; RSD = 0.098); (Copra: R^2 = 0.893; RSD = 0.108);

PKE: **Average daily gain (kg) = – 0.103 + 0.797 *SI***, (R^2 = 0.804; RSD = 0.083; P<0.01);

where SI is supplement DM intake (%W/d). The different R^2 and RSD values given for CSM and Copra treatments in relation to the combined CSM/Copra relationship for average daily gain above indicate the extent to which this combined relationship represents the data for each treatment separately.

Hay DM intake for the Control steers was 1.86 ± 0.043 %W or 4.12 ± 0.120 kg/d and was reduced linearly (P<0.01) with the inclusion of all supplements. The rate of reduction in hay intake was much greater for Copra and PKE than for CSM (P<0.01). There was a trend (P=0.06) for intake to be reduced more with PKE than Copra but the combined equation for the two supplements is shown in Figure 2.2B and described below. Total DM intakes by steers followed the reverse trend with CSM associated with a greater rate of increase (P<0.01) than Copra which in turn tended (P=0.06) to have higher total intake than that for PKE. The corresponding response relationships are shown in Figure 2.2B and described in equation form below:

CSM: **Hay DM intake (%W/d) = 1.863 – 0.309 *SI***, (R^2 = 0.493; RSD = 0.136; P<0.01);

Copra/PKE: **Hay DM intake (%W/d) = 1.863 – 0.852 *SI***, (P<0.01);

(Copra: R^2 = 0.711; RSD = 0.177); (PKE: R^2 = 0.708; RSD = 0.149);

CSM: **Total DM intake (%W/d) = 1.863 + 0.691 *SI***, (R^2 = 0.703; RSD = 0.136; P<0.01);

Copra/PKE: **Total DM intake (%W/d) = 1.863 + 0.148 *SI***, (NS);

(Copra: R^2 = 0; RSD = 0.177); (PKE: R^2 = 0; RSD = 0.149).

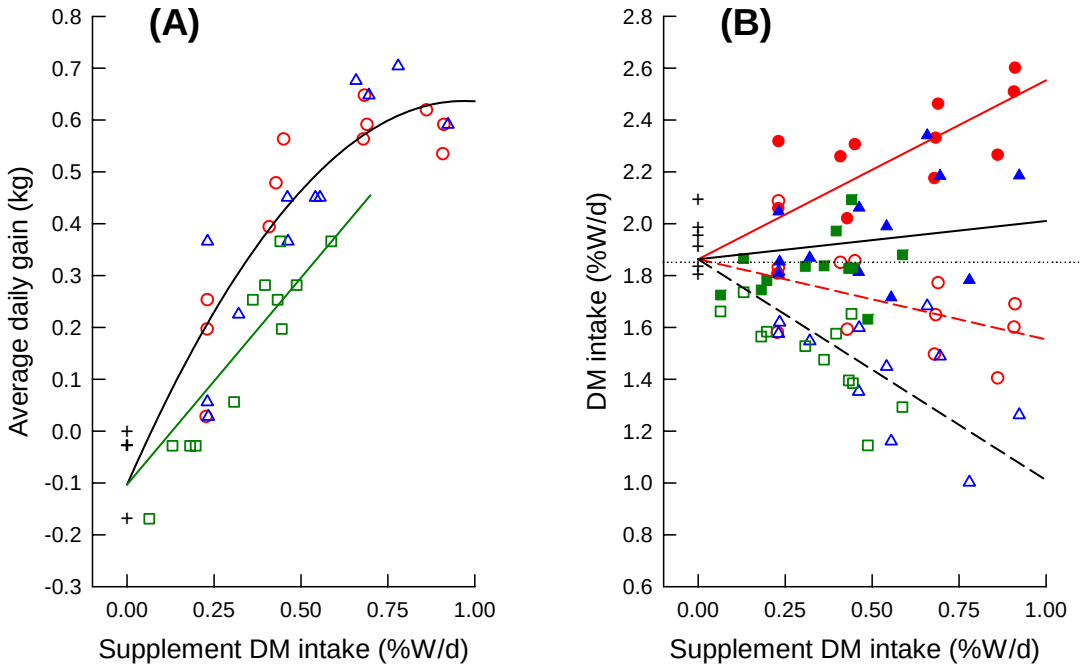

Figure 2.2. Effect of supplements on* (A) *the average daily gain (Control, +; CSM, ○; Copra, △; PKE, □) and* (B) *the intake of hay (dashed lines; Control, +; CSM, ○; Copra, △; PKE, □) and total (solid lines; CSM, ●; Copra, ▲; PKE, ■) DM for steers given a basal diet of low quality hay. A single relationship (black line) is shown for the combined CSM and Copra treatments in Figure A and for combined Copra and PKE treatments (both hay and total) in Figure B. Symbols represent values for individual steers.

The effects of supplement intake in the 3 h prior to rumen and blood sampling on the concentrations of ammonia-N (NH_3-N) in rumen fluid of steers is illustrated in Figure 2.3A. Copra and PKE had no effect on the concentration of NH_3-N in the rumen whilst CSM feeding was associated with a steep linear increase in concentration, a response which differed (P<0.01) from that of the other supplements. Feeding CSM was associated with a quadratic, and Copra with a linear, increase in plasma urea-N concentration whilst PKE had no effect on this metabolite concentration (Figure 2.3B). Differences between all supplements were significant in the order CSM>Copra>PKE (P<0.05). The various response relationships are as follows:

CSM: **NH_3-N concentration (mg/L) = 56.9 + 90.84 *SI-kg***, (R^2 = 0.879; RSD = 31.93; P<0.01);

Copra/PKE: **NH_3-N concentration (mg/L) = 56.9 – 5.29 *SI-kg***, (NS);

(Copra: R^2 = 0; RSD = 16.51; PKE: R^2 = 0; RSD = 18.76);

CSM: **Plasma urea-N (mg/dL) = 7.41 + 11.96 *SI-kg* – 2.13 *SI-kg*** ^{2}, (R^2 = 0.779; RSD = 3.73; P<0.05);

Copra: **Plasma urea-N (mg/dL) = 7.41 + 3.64 *SI-kg*** , (R^2 = 0.626; RSD = 2.01; P<0.01);

PKE: **Plasma urea-N (mg/dL) = 7.41 – 0.05 *SI-kg*** , (R^2 = 0; RSD = 2.23; NS);

where *SI-kg* is supplement DM intake (kg) in the 3 h prior to sampling.

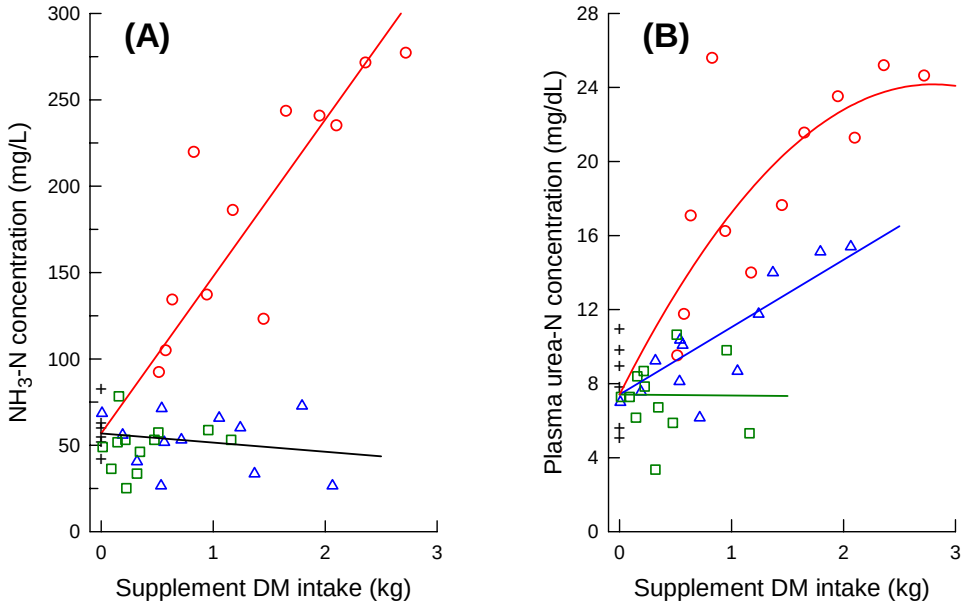

Figure 2.3. Effect of intake of different supplements (Control, +; CSM, ○; Copra, △; PKE, □) in the 3 h before sampling on (A) the concentration of ammonia-nitrogen (NH_3-N) in the rumen, and* (B) the concentration of urea-nitrogen (urea-N) in the blood plasma, *of steers given a basal diet of low quality hay. A single relationship (black line) is shown for the combined Copra and PKE treatments in Figure A. Symbols represent values for individual steers.

Total protozoa numbers averaged 1.43 x 10^5/mL rumen fluid for Control steers at 3 h post-feeding and increased linearly (P<0.05) with CSM additions but the trend (P>0.05) was for a reduction in numbers with Copra or PKE feeding. Differences between CSM and the other supplements were significant (P<0.01). For the Controls, this population comprised, on average (back-transformed values), 16.3% *Isotricha sp.*, 32.2% *Dasytricha ruminantium*, 37.0% *Entodinium* spp., 3.9% *Diplodinium sp.*, and 11.4% other (minor) species (including *Epidinium sp*., *Eudiplodinium sp*., *Ostracodinium sp*., *Metadinium sp*. and *Elytroplastron bubali*. Feeding Copra and PKE resulted in a linear decrease in population density (% total numbers) of *Isotricha sp.* and *Dasytricha ruminantium* (P<0.01) and a linear increase in the proportion of *Entodinium* spp. (P<0.01) but CSM had no effect on the composition of the protozoal population in the rumen. These population trends were different (P<0.01) between CSM and the other supplements, but not between Copra and PKE.

2B EFFECT OF HIGH LIPID SUPPLEMENTS ON THE INTAKE, DIGESTION AND METABOLISM OF STEERS GIVEN A LOW QUALITY FORAGE: METABOLISM STUDY

Materials and methods

Animals, treatments and experimental design

This metabolism experiment was carried out at the University of Queensland's Mt Cotton Research Farm (153°14' East, 27°53' South) on the outskirts of Brisbane between March and May 2001. Ten Brahman crossbred steers approximately 15 months of age and weighing 243 ± 6.5 (± s.d.) kg (unfasted commencement liveweight) were allocated to either of two supplement groups (Copra meal or PKE) by stratified randomisation on the basis of unfasted liveweight. The steers were injected with Ivomec (10 g/L Ivermectin, Merck and Co. Inc. White House Station, New Jersey USA) for the control of internal and external parasites prior to the start of the experiment. After allocation, the steers were subjected to a 15 d pre-experimental adaptation phase during which time they were gradually introduced to their intended supplement type.

The experimental design included two incomplete 5 x 5 Latin squares, one with each supplement type of copra meal (Copra) or PKE meal (PKE), run concurrently. Within each supplement group, chaffed green panic hay was given *ad libitum* together with one of five levels of supplement intake (DM basis), including an unsupplemented control for each supplement group (see below). Each steer was fed, sequentially, three different diets over the three runs of the experiment according to a treatment order and allocation of steers determined randomly at the beginning. Each steer received a different sequence of diets. Thus there were three values for each treatment within experiments; six values for the unsupplemented control across the two experiments. The treatments can be summarised as follows:

	Supplement DM intake (%W/d)	Treatment name
Control	0	Control
Copra	0.25	Cop 0.25
	0.5	Cop 0.5
	0.75	Cop 0.75
	1.0	Cop 1.0
PKE	0.25	PK 0.25
	0.5	PK 0.5
	0.75	PK 0.75
	1.0	PK 1.0

Each of the three measurement periods (runs) consisted of a 14 d preliminary feeding period, the first 12 d in individual yard pens and the remaining 2 d in metabolism cages, followed by a 7 d collection period in metabolism cages. The steers were weighed immediately before entering and after leaving the metabolism cages in order to adjust the supplement intakes to changing steer liveweight. Supplements intakes were determined on a dry matter basis, not on an 'as fed' basis as in the pen experiment, and so were about 10% higher 'as fed' than in the pens. This applied for all following metabolism experiments.

Procedures

The hay was chaffed and given twice daily in equal portions at 0800 h and 1200 h. The amount of hay offered each day was set at 10-15% more than that consumed by the steer on the previous day. The supplements were given once daily at 0730 h and fed separately to the basal diet. Both supplements originated from Papua New Guinea and were supplied by Mataranka Grain Pty Ltd, Dayboro Qld. Fresh drinking water was freely available at all times.

During the collection period, feed intake was measured daily by recording the amount of hay and supplement offered and refused. Sub-samples of the hay and supplements offered to the steers were taken daily and bulked over the collection period. They were subsequently milled through a 1 mm screen and stored for later chemical analysis. A further sub-sample of the supplement was taken daily, and of the hay every 2 d, for DM determination. Hay and supplement refusals were collected once daily, just prior to the morning feeding, weighed, mixed and sub-sampled for DM determination.

Complete faeces and urine were collected separately once daily for the 7 d. The daily faecal collection for each steer was weighed, thoroughly mixed, and a 5% aliquot taken and frozen. At the end of the collection period, these daily samples were thawed, bulked for each steer, mixed and part was dried to constant weight at 60^{o}C to determine DM content whilst another part was freeze-dried and milled through a 1 mm screen prior to chemical analysis for N, OM and NDF. Urine was collected into trays covered with a cloth filter to prevent faecal contamination. The pH of the urine was maintained at just less than 3.0 by adding 10% H_2SO_4 (approximately 150-200 mL/d) to the trays at the start of each daily collection. For each steer, urine collected over a 24 h period was mixed and a 5% aliquot was taken daily and added to a bulk container which was stored in a refrigerator. At the end of each collection period, a 5 mL sub-sample was taken from the bulked samples, diluted to 50 mL with ammonium phosphate stock buffer and frozen awaiting analysis for purine derivative (PD) concentration.

The exogenous purine supply (X, mmol/d) attributable to the microbial population of the rumen was estimated as the total purine excretion (Y, mmol/d) less the endogenous contribution to this total, divided by a recovery factor. Verbic *et al*. (1990) suggested an endogenous purine contribution of 0.385 mmol/kg $W^{0.75}$ and a recovery coefficient of 0.85 for absorbed purines. The calculation thus becomes:

$Y = 0.85\ X + 0.385\ W^{0.75}$.

However, in a more recent study (Bowen, 2003) reported a lower value of endogenous purine excretion (0.190 mmol/kg $W^{0.75}$) for *B. indicus* cattle. As the current experiment used *Bos indicus* cattle this lower value was used in these studies, the equation thus becoming:

$Y = 0.85\ X + 0.190\ W^{0.75}$.

The value of X (endogenous purine supply) was then used in determining estimated microbial N production (EMNP, g/d) through the following equation:

$$\text{EMNP} = \frac{70X}{0.83 \times 0.116 \times 1000}$$

where 0.83 was the assumed digestibility of the microbial protein and 0.116 represented the ratio of purine nitrogen to total microbial nitrogen (Chen *et al*., 1992). A factor of 6.25 was subsequently used to convert the EMNP to microbial crude protein (MCP) production (g/d).

Samples of the hay, supplement, feed refusals and faeces were analysed for OM, N, NDF and ether extract (EE) concentration.

At the end of each collection period, steers were shifted into pens and fed for an additional day on the same diet. Samples of rumen fluid were taken *per os*, using a plastic tube and vacuum pump, 3 h and again 24 h after feeding. The pH of rumen fluid was determined immediately after sampling and 10 mL was collected into 0.2 mL of concentrated H_2SO_4 and stored at –20^{o}C prior to determination of NH_3-N

concentration. Coinciding with the 3 h sampling, blood was collected from the jugular vein of each steer into a heparinised gel tube, mixed gently, centrifuged at 2500 rpm for 15 min and the plasma stored at –20^{o}C awaiting analysis for plasma urea-N concentration.

Chemical analysis

Ash: Ash content was determined by combusting *ca.* 1 g of oven-dried ground sample in a furnace at 550^{o}C for 4.5 h, and OM content was determined as 100 – ash (%).

N: The N concentration in samples was determined using an automatic total N analyser (LECO FP-428). Approximately 0.25 g oven dried material was weighed into a ceramic boat which was placed in a furnace set at 1100^{o}C. A fixed quantity of pure oxygen was added to combust the sample and volatilise all forms of N. The various nitrogen oxides produced (NO, NO_2) were reduced to N (N_2) gas which was then quantitatively determined using a thermal conductivity detector.

NDF: The ash-free NDF content of the samples was determined by the method of Van Soest and Wine (1967) using a fibre extraction unit (ANKOM 220). A ground sample (0.5 g) was weighed into a filter bag. The bag was then placed into the digestion vessel and anhydrous sodium sulfite (0.5 g/50 mL of neutral detergent solution (NDS)) was added followed by the NDS (100 mL/bag) and 4 mL of alpha-amylase. The solution in the vessel was boiled for 1 h. The extraction solution was then drained and the residue was rinsed three times with hot water (4 mL alpha-amylase added to the first two washes) followed by two washes with acetone. The filter bag with residues was air-dried before oven drying at 60^{o}C for 24 h. The filter bag was weighed and ashed in a furnace at 500^{o}C for 4.5 h. The ash content was determined, and ash-free NDF content was calculated.

ADF: Determination of ADF content was the same as that described above for NDF except that acid detergent reagent was used instead of neutral detergent reagent and sodium sulfite was not used.

EE: The EE content was determined using a solvent extraction unit (Soxtec HT6, Tecator, Sweden). Duplicated oven-dried samples were weighed into oven-dried thimbles. Fifty mL of diethyl ether, used as the extraction solvent, was added to a pre-weighed oven-dried extraction cup and the samples were extracted at 100^{o}C for 3 min and then rinsed at 100^{o}C for 30 min. The extract was collected in the collection vessel. This thimble was dried at 60^{o}C for 24 h, weighed and the EE content in the sample calculated by difference from original weight.

NH_3-N: The concentration of NH_3-N in the rumen fluid was determined by a distillation method using a Buchi 321 distillation unit (Buchi Scientific Apparatus, Flawil, Switzerland), and an automatic titrator. The reagents were 2% boric acid (H_3BO_3) solution, a saturated sodium tetraborate solution (>260 g/L), and 0.01M HCL (normality 0.0095). Twenty-five mL of the boric acid solution was measured into a 400 mL receiving flask and placed on the receiver plate under the distillation outlet tube. A 5 mL rumen fluid sample was added to 30 mL of sodium tetraborate in a conical flask and fitted onto the distillation vessel. The distillation proceeded for 4 min until the colour of the receiving flask content had turned from pink to green. The receiving flask was then removed and titrated with HCL (0.01 N) to a pH of 5.0. The titration volume was recorded and the NH_3-N concentration calculated using the following equation:

NH_3-N (mg/L) = TV x 14.01 x N HCL x 200,

where TV is titration volume, 14.01 is molecular weight of nitrogen, N HCL is the normality of HCL and 200 is the conversion factor to express concentration per L of rumen fluid.

Plasma urea-N (PUN): PUN concentration was determined using an enzymatic method (peridochrome® glucose). A spectrophotometer was used to measure the absorbance at wavelength 340 nm and temperature of 27^{o}C. Five urea standard solutions (0.5, 1.0, 1.5, 2.0 and 2.5 g urea/L) were prepared. An 0.02 mL duplicate standard of thawed plasma was mixed with 2 mL reagent (BUN reagent) in a methacrylate cuvette and the absorbance value was read at 30 sec intervals. An initial absorbance was read 30 sec after mixing and the final reading was taken 30 sec after the previous reading. A standard curve was established by plotting the difference of both readings against urea concentration. The urea

concentration in the samples was then calculated using the formula proposed by Tiffany *et al*. (1972) from the regression of the line.

PD: The concentration of PDs in urine was determined using High Performance Liquid Chromatography (HPLC).

Statistical analysis

The effects of supplements were tested and described by fitting general linear models with pen, run, supplement type and supplement level as terms, following a similar procedure to that applied to the data from the pen trials. In particular the following models were fitted, with terms for pen and run included in all models and with statistical significance assessed at the P=0.05 level:

- a sequence of models to test the degree of polynomial needed to describe the response to actual supplement intake. Initially a full model (degree 4) was fitted, then the degree sequentially reduced by dropping non-significant terms. For all variables the contributions from the higher order terms were not statistically significant, so only linear or quadratic models were used in subsequent analyses. In cases where the overall test indicated a quadratic model but the coefficient for the quadratic term for a supplement was not significant, only a linear term was fitted for that supplement in later models;
- a model to test between the Control responses for the two supplement groups of steers being run concurrently. As there were no statistically significant differences (i) the data from the two groups were analysed as a single experiment; and (ii) a single intercept was used in subsequent models to represent the six Control responses;
- a model to test whether the response relationship for the two supplements was significantly different. In cases where the relationships were not significantly different, a common one was fitted. For the final model residual standard deviations and approximate R^2 values were calculated for each supplement plus controls and for the overall model. The assumptions of the analyses and presence of outliers in the data were checked by plotting residuals against fitted values.

The results are presented as equations in the text to describe the fitted response to level of supplement (from the fitted general linear models with terms pen, run and level of supplement). In the figures, fitted values for the various treatments are given with the fitted relationship. The data points represent the fitted mean for the values from three steers, except for the Control treatment where the fitted mean from six steers is used.

The statistical package GenStat (2002) was used for all analyses.

Results

The composition of the dietary components for the various treatments is shown in Table 2.2. The relatively high fat content of the copra meal and PKE meals is consistent with the method of production, viz. mechanical extraction of oils from the parent material.

Table 2.2. Nutrient composition of diet components

	OM	CP	NDF	EE
	(% DM)			
Green panic hay	91.4	5.7	71.0	1.5
Copra meal	94.0	24.2	54.9	7.3
PKE meal	96.8	17.1	67.4	10.3

As stated above, each latin square arrangement (one for each supplement type) included an unsupplemented control. Since there were no significant differences in the various parameters measured between the controls representing the Copra and PKE groups, the mean reported below represents the combined value (n = 6) for unsupplemented animals, in each case. Furthermore, where there were no significant differences between supplement types in the response relationships observed, as was generally the case in this study, a single relationship is described for the combined effects of Copra and PKE intake on the relevant parameter. Separate equations are reported where differences (P<0.05) between supplement types occurred.

Despite some initial problems with palatability of the PKE in particular, most supplements were consumed to the desired levels of intake. The mean hay DM intake for the unsupplemented Control steers was 1.68 ± 0.042% W/d (4.74 ± 0.119 kg/d). For both supplement types, there was a quadratic reduction (P<0.01) in hay DM intake, expressed on a liveweight basis, as intake of supplement increased (Figure 2.4). There was negligible effect of either supplement on hay intake until supplement intake exceeded about 0.2%W/d, after which hay intake decreased sharply. Total DM intake (%W) was quadratically increased (P<0.01) with increasing supplement intake for both supplements (Figure 2.4). Differences between supplement types were not significant for either hay or total intake despite a trend for hay and thus total intake to be reduced more with PKE than with Copra at the highest level of feeding. Total NDF intake (%W/d) also increased (P< 0.01) quadratically with increasing supplement intake and intake of both NDF and DM appeared to plateau at a supplement intake of *ca.* 0.75%W/d. Differences between supplement types in relation to NDF intake were not significant. The equations describing the combined response relationships for the various intake parameters are as follows:

Hay DM intake (%W/d) = 1.68 + 0.447 *SI* – 0.873 *SI* ^{2}, (R^2 = 0.824; RSD = 0.106; P<0.01);

Total DM intake (%W/d) = 1.69 + 1.445 *SI* – 0.958 *SI* ^{2}, (R^2 = 0.867; RSD = 0.099; P<0.01);

Total NDF intake (%W/d) = 1.19 + 0.914 *SI* – 0.529 *SI* ^{2}, (R^2 = 0.785; RSD = 0.097; P<0.01);

where *SI* is supplement intake (%W/d).

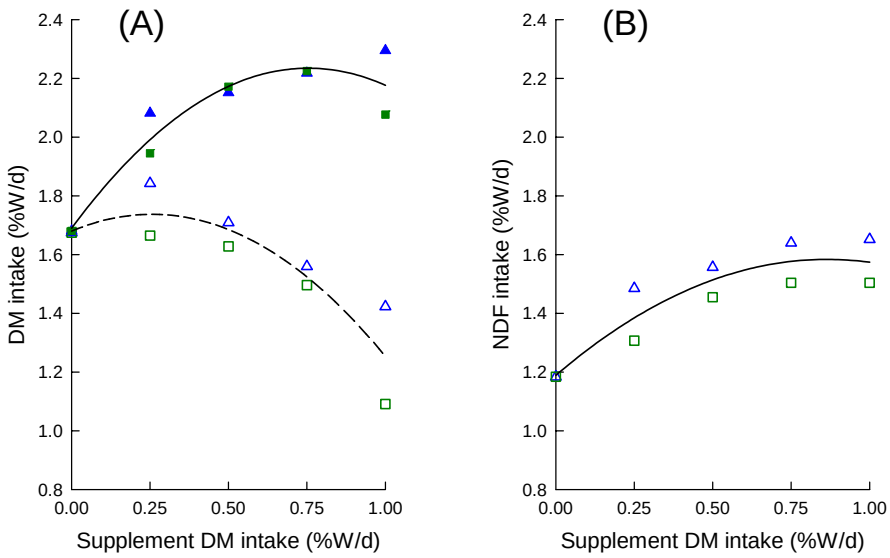

Figure 2.4. Effect of supplements on the intake of* (A) *hay (dashed line; Copra, △; PKE, □) and total (solid line; Copra,▲; PKE, ■) DM or* (B) *total NDF (Copra, △; PKE, □) for steers given a basal diet of low quality hay. Symbols represent the mean of 3 steers except for the Controls (6 steers).

The digestibilities of OM (OMD) and NDF (NDFD) for the Control steers averaged 57.1 ± 0.58 and 63.1 ± 0.72%, respectively. Both OMD and NDFD increased in a quadratic manner (P<0.05) as intake of supplement increased with no difference between supplement types in either case (Figure 2.5). Although there was a trend for higher OMD at high intake of copra meal relative to PKE meal, the response curves were not significantly different (P=0.11). The combined supplement relationships were as follows:

OMD (%) = 57.14 + 16.26 *SI* – 8.29 *SI* ^{2}, (R^2 = 0.867; RSD = 1.456; P<0.01);

NDFD (%) = 63.07 + 15.99 *SI* – 8.93 *SI* ^{2}, (R^2 = 0.773; RSD = 1.819; P<0.05).

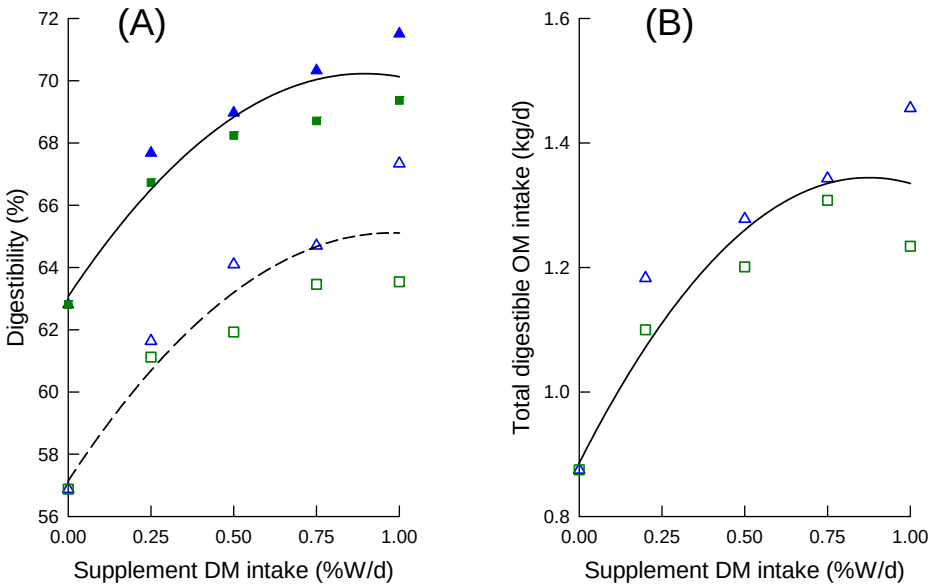

Figure 2.5. Effect of supplements on* (A) *the digestibility of OM (dashed line; Copra, △; PKE, □) and NDF (solid line; Copra, ▲; PKE, ■) and* (B) *the total digestible OM intake (Copra, △; PKE, □), for steers given a basal diet of low quality hay. Symbols represent the mean of three steers except for the Controls (six steers).

The above effects of supplement on total intake and OMD, when combined, translated to a significant quadratic increase in total digestible OM intake (DOMI; %W/d) as supplement intake increased, with no difference between supplement types. Total DOMI seemed to increase up to, and thereafter plateau beyond, a supplement intake level of 0.75%W/d (Figure 2.5B). The relationship is described by the following equation:

Total DOMI (%W/d) = 0.886 + 1.048 *SI* – 0.599 *SI* ^{2}, (R^2 = 0.884; RSD = 0.078; P<0.01).

Inclusion of both supplements in the diet linearly increased lipid intake when expressed as a proportion of total DM intake (DMI), the effect being greater (P<0.01) with the PKE compared with the Copra supplement. These response relationships were as follows:

Copra: **Lipid intake (%DMI) = 1.53 + 2.649 *SI***, (R^2 = 0.994; RSD = 0.075; P<0.01);

PKE: **Lipid intake (%DMI) = 1.53 + 4.144 *SI***, (R^2 = 0.988; RSD = 0.176; P<0.01).

Production of microbial CP (MCP) increased linearly with increasing supplement intake, the rate of increase in production being greater for copra compared with PKE meal (P<0.05; Figure 2.6). The response relationships for the two supplements are as follows:

Copra: **MCP production (g/d) = 220.8 + 430.3 *SI***, (R^2 = 0.963; RSD = 30.33; P<0.01);

PKE: **MCP production (g/d) = 220.8 + 277.1 *SI***, (R^2 = 0.882; RSD = 39.43; P<0.01).

The efficiency of MCP production (EMCP), expressed in terms of g MCP/kg DOMI, increased in a quadratic fashion as Copra intake increased and linearly with increasing intake of PKE, the response relationships being different (P<0.05). EMCP was generally greater for Copra compared with PKE meal across the range of supplement intakes (Figure 2.6B). These relationships are described as follows:

Copra: **EMCP (g MCP/kg DOMI) = 84.03 + 34.0 *SI* + 54.8 *SI*^{2}**, (R^2 = 0.955; RSD = 7.03; P<0.05);

PKE: **EMCP (g MCP/kg DOMI) = 84.03 + 52.71 *SI***, (R^2 = 0.807; RSD = 10.06; P<0.01).

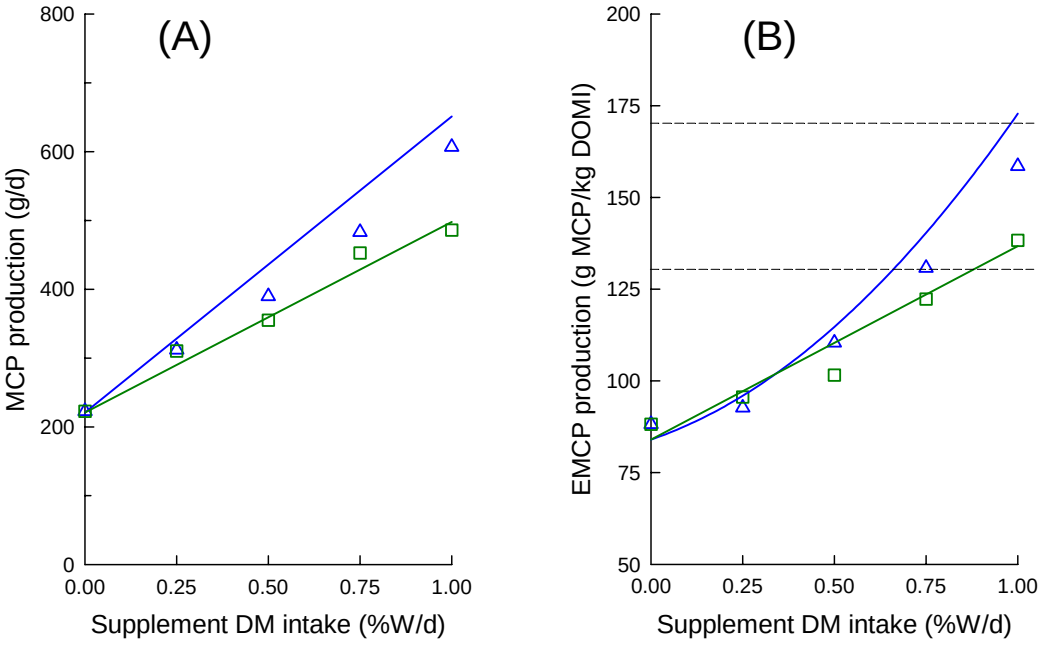

Figure 2.6. Effect of supplements (Copra, △; PKE, □) on* (A) *the production of microbial CP (MCP) and* (B) *the efficiency of production of MCP (EMCP) for steers given a basal diet of low quality hay. Symbols represent the mean of three steers except for the Controls (six steers). The expected range of EMCP, based on the feeding standards, is defined by dashed lines in Figure B.

The concentration of NH_3-N in rumen fluid of the Control steers averaged 37 ± 2.20 and 27 ± 1.43 mg/L at 3 and 24 h after feeding and increased quadratically at both time points with increasing supplement intake, with no difference between Copra and PKE meal. Peak concentrations at the highest level of supplement intake were *ca.* 80 and 55 mg/L at 3 and 24 h, respectively. Equations describing the effects of supplement, combined for supplement type, on NH_3-N concentration at the two times are as follows:

3 h: **NH_3-N concentration (mg/L) = 36.88 + 75.7 *SI* – 36.86 *SI*^{2}**, (R^2 = 0.913; RSD = 5.54; P<0.01);

24 h: **NH_3-N concentration (mg/L) = 27.31 + 65.2 *SI* – 36.62 *SI*^{2}**, (R^2 = 0.935; RSD = 3.61; P<0.01);

Rumen fluid pH averaged 7.0 ± 0.02 and was not affected by supplement type or intake at either 3 h or 24 h after feeding. Urea-N concentration in plasma at 3 h after feeding averaged 4.1 mg/dL for the Controls and increased in a quadratic manner with increasing intake of both supplements. Copra meal was associated with a greater rate of increase (P<0.01) in plasma urea concentration than PKE meal as supplement intake increased, and the relevant equations are as follows:

Copra: **PUN concentration (mg/dL) = 4.10 + 23.09 *SI* – 13.57 *SI*^{2}**, (R^2 = .955; RSD = 7.03; P<0.05);

PKE: **PUN concentration (mg/dL) = 4.10 – 8.93 *SI* + 15.44 *SI*^{2}**, (R^2 = .807; RSD = 10.06; P<0.01).

Discussion: (Chapters 2A & 2B)

General comment on Discussion sections. The Discussions in this manuscript are general observations and conclusions, and are not written as final discussions for publication purposes. They are, in most cases, truncated and not referenced to other published findings. More complete discussions will be provided in the papers written from this research work.

Our interest in evaluating the high-lipid protein meals used in these studies, viz. Copra and PKE, arose for several reasons. Firstly, there was limited practical information about their possible role as alternative protein sources to long-term industry standards such as CSM. Secondly, they represented a 'supplement type' not yet evaluated by our research group in a wider program to determine the effects of different supplement types on intake and growth of cattle given forages of varying quality. Thirdly, because of their varying energy density, by virtue of their different lipid contents, they provided an opportunity to further test the hypothesis that substitution was largely a function of the energy supplied in the supplement. In the pen feeding study CSM was included as a 'positive control' treatment about which we already had considerable information. Furthermore, Copra in particular is often seen as a commercial alternative to CSM yet there has been little research carried out to compare them except sometimes at a single level of intake.

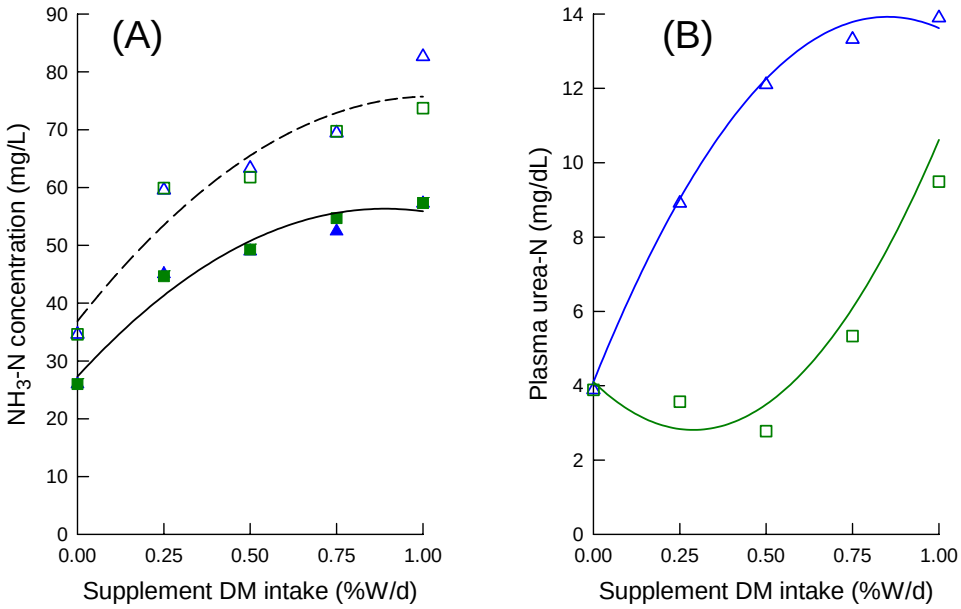

Figure 2.7. Effect of supplements on* (A) *the concentration of ammonia-nitrogen (NH_3-N) in the rumen fluid of steers receiving low quality hay at either 3 h (dashed line; Copra, △; PKE, □) or 24 h (solid line; Copra, ▲; PKE, ■) after feeding, and* (B) *the concentration of urea-N in plasma (same symbols) 3 h after feeding. Symbols represent the mean of three steers except for the Controls (six steers).

Despite the much lower protein content of Copra compared to CSM (24.2 vs. 42.8% DM) growth rates were similar for the two supplements across the full range of supplement intakes. This finding was similar to that reported by some other workers (e.g., B. Gulbransen, pers. comm.) using a single level of supplement intake, but was unexpected here. Given the low quality of the hay, the likely deficit of RDN in the rumen of unsupplemented animals and previous evidence of rapid responses to protein meals (including CSM) fed in even small amounts, a difference between the two protein meals was expected at the lower range of intakes. The results are even more puzzling in view of the low EMCP for steers fed the hay alone diet and the lack of effect of Copra on rumen NH_3-N concentrations in the rumen in the pen feeding study. Whilst total protein supply to the animal was lower for Copra and PKE relative to CSM, these high-lipid supplements did provide more energy per unit weight by virtue of their higher lipid content. Thus it is possible that the higher energy supply from Copra and PKE compensated to some extent for their lower protein supply. This is discussed further below. The lower growth response level

with PKE probably reflects the even lower protein supply by this supplement. PKE can not really be considered a 'protein meal', having only about 17% CP content, and is probably better categorised as a medium-protein 'energy source'. The linear response to PKE in the pen experiment was similar to that recorded with grain sources in other experiments both in this project and in previous ones.

Based on the nylon bag studies of Moss *et al.* (1998), the rumen degradability of protein from CSM, Copra and PKE was 77.1, 36.3 and 70.5%, respectively, when a passage rate of 0.02/h was assumed. This low protein degradability for Copra has been widely accepted for ration formulations. However, the nylon bag studies carried out in association with the current studies indicates little difference between the three supplements in rumen protein degradability (79-82%) which does not easily align with other findings. For instance, as supplement intake was increased in the pen study, rumen NH_3-N concentrations showed a steep increase with increasing CSM additions but no increase at all with Copra or PKE. In the metabolism study there was a gradual increase in NH_3-N concentration over a narrow range with Copra and PKE supplements. The elevation of plasma urea-N concentration with the high lipid supplements in both studies indicates the N was not lost totally to the animal through over-protection of the protein. In Figure 2.3B the urea-N concentrations are regressed against supplement intake (kg). When Copra and CSM responses are compared on a supplement N intake basis (results not shown), there is little difference in slope of the regression. These elevated plasma urea concentrations with Copra in particular could indicate that a large proportion of the high-lipid protein meals were bypassing rumen degradation and that a proportion was entering the blood urea pool after absorption (as amino acids), to be slowly returned to the rumen as urea. However, this conflicts with the nylon bag digestion findings. An alternative explanation is that the protein meals were extensively degraded in the rumen, as the *in situ* work suggests, but that the longer lag times for the high-lipid supplements in nylon bags meant that NH_3-N concentrations in the rumen of steers were still low at sampling time 3 h after feeding, especially in the pen experiment. It is also possible that the NH_3-N was released slowly in the rumen with Copra and PKE and was taken up at the same rate by rumen microbes, preventing an accumulation in the rumen. In the metabolism study, NH_3-N concentrations increased with supplement intake but only at a low rate and concentrations were still elevated at 24 h post-feeding, supporting the contention for slow release of NH_3-N. However, this theory is also at odds with the nylon bag results. This whole issue of rumen degradability of protein needs some clarification and a better method is required to determine this value.

There were very distinct differences between CSM and the other two supplements in their effects on hay intake. The high-lipid supplements resulted in considerably higher rates of substitution than CSM in the pen feeding trial and in both studies there were no differences between Copra and PKE in these effects. Once again this effect was consistent with an inverse relationship between energy supplied by the supplement and that from the hay. Although in the pen study the reduction in hay intake was linear across the range of supplement intakes, a curvilinear effect was recorded with Copra and PKE in the metabolism study such that hay intake was not depressed until supplement intake exceeded about 0.5%W/d. This latter effect suggests a correction of some deficiency in the rumen and RDN is the usual suspect in this regard, but the effects of supplement on rumen NH_3-N concentration do not completely support this contention, as outlined above. In the pen study there was no apparent change in NH_3-N concentration with Copra or PKE feeding at any level of intake whereas in the metabolism study NH_3-N concentration increased gradually with both supplements across the range of intakes. However, the baseline concentration for unsupplemented steers was lower in the metabolism than in the pen study (37 vs. 57 mg/L, at 3 h) and changed across a relatively narrow range (ca. 37 to 75 mg/L) with supplement intakes of up to 1%W/d.

The increases in MCP production, and in the efficiency of production, when increasing amounts of Copra and PKE were fed provide further evidence that the supplements provided key nutrients to the microbes, probably RDN. These supplements were also high in energy content but much of the energy was in the form of lipids and thus not in a fermentable form for microbial use. It was noteworthy that EMCP did not exceed the lower threshold of the range of efficiencies proposed in the feeding standards (130-170 g MCP/kg DOMI) until Copra intake exceeded 0.75%W/d and PKE intake reached 1.0%W/d.

Digestibility of both OM and NDF increased with each increment of Copra but appeared to reach plateau level when intake of PKE reached about 0.75%W/d, although differences between supplements were not significant. Based on the lipid composition of the diet components, the estimated lipid content of the total diet was only 3.0% DM for Copra with intakes of 1%W/d, whilst for PKE it was 4.0% and 5.1% for supplement intakes of 0.75 and 1.0%W/d. Previous research has indicated a threshold for lipid inclusion

in forage-based diets of about 4-5% beyond which fibre digestion is depressed. Our findings are consistent with such an effect at the highest level of PKE inclusion.

Previous research has shown lipid sources high in C_{12} and C_{14} fatty acids to be highly toxic to protozoa and our results tended to support this finding. Whilst total numbers increased with CSM intake, they tended to decline in total, and did so significantly in the case of *Isotricha* sp. and *Dasytricha* sp. numbers, with increasing intake of Copra and PKE which are high in the above saturated fats. The effect may have been greater except that intakes of PKE only reached 0.6%W/d in the pen study. It is possible that some of the increase in MCP production with the high-lipid supplements was attributable to reduced protozoa numbers and thus reduced predation of bacteria. However, at higher levels of inclusion this possible beneficial effect would be countered by the toxic effects of lipid on bacteria in the rumen.

Conclusions

The major practical finding from these experiments was that, under the conditions existing in the pen experiment, Copra could be substituted for CSM without any reduction in cattle growth. Whilst other work has suggested the same from limited treatment comparisons, our experiment included a wide range of intakes of supplement which would be expected to cover any of those likely to be used under commercial grazing situations. The reason for this outcome are still not clear in view of the seemingly low availability of RDN from Copra especially at the lower intake range. The picture is further clouded by incongruence between the nylon bag digestion results and other *in vivo* results from both the pen and metabolism experiments, especially pertaining to rumen and blood metabolite concentrations. A better method of assessing protein degradability of meals *in vitro* is needed. The studies also showed large differences between supplement types in the extent of substitution, with the higher energy supplements associated with greater reduction in hay intake. Whether this is cause and effect is unclear and requires further clarification later.

3A EFFECT OF SITE OF DIGESTION OF THE SUPPLEMENT ON THE INTAKE AND GROWTH OF STEERS GIVEN A LOW QUALITY FORAGE: PEN STUDY

Materials and methods

Animals, treatments and experimental design

This pen feeding experiment was carried out at "Brian Pastures" Research Station between August and October 2001. High-content Brahman crossbred weaner steers approximately 10-12 months of age and weighing 231.6 ± 2.37 kg liveweight (unfasted starting liveweight) were purchased from a commercial property in central Queensland. They were vaccinated against tick fever and bovine ephemeral fever and treated with an anthelmintic (Ivomec) prior to commencement of the experiment.

The experimental design was the same as that described earlier (Chapter 2A) and is summarised below. Green panic hay was fed *ad libitum* alone (control) or with the following supplements: (i) dry-rolled grain sorghum (RG); (ii) expanded grain sorghum, low urea (EGL); or (iii) expanded grain sorghum, high urea (EGH), at various intake rates (as fed basis) as are shown below.

	Supplement intake (as fed) (%W/d)	Treatment name
Control	0	Control
RG	0.5	RG 0.5
	1.0	RG 1.0
	1.5	RG 1.5
	2.0	RG 2.0
EGL	0.5	EG Lo 0.5
	1.0	EG Lo 1.0
	1.5	EG Lo 1.5
	2.0	EG Lo 2.0
EGH	0.5	EG Hi 0.5
	1.0	EG Hi 1.0
	1.5	EG Hi 1.5
	2.0	EG Hi 2.0

All grain supplements included 16 g urea, 3.3 g sulphate of ammonia (GranAm), 20 g bentonite, 20 g molasses and 10 g limestone, per kg sorghum, as fed. For the EGH treatments, urea and sulphate of ammonia was also dissolved and added to the hay at the rate of 10 g urea and 2 g sulphate of ammonia per kg hay, as fed. Rolled and expanded sorghum (variety Buster MR®) were used to represent grains with starch of low and high rumen degradability, respectively. The expanded sorghum was prepared commercially (*Expandat*®; Better Blend Stockfeeds, Oakey Qld) by a process which used heat (high-

temperature, short-time), moisture and pressure to modify starch gelatinisation and thus digestion characteristics, followed by a final crumbling process. Urea inclusions were designed to provide adequate rumen degradable nitrogen (RDN) for microbial protein production at an efficiency of 130 g microbial protein per kg digestible organic matter (DOM; SCA 1990; AFRC 1993) for the expanded grain component alone (EGL) or expanded grain plus hay components of the diet (EGH), assuming organic matter digestibility (OMD) of expanded grain and hay of 78% (starch digestibility in rumen; Huntington 1997) and 50% (McLennan, unpubl. data), respectively. Urea was included in rolled grain at the same rate as for the expanded grain (EGL), despite expected lower RDN requirements for this rolled grain due to its lower digestibility. Sulphate of ammonia was included with urea so that the N:S ratio was 10:1 for these components of the supplement. The three supplement types were each fed at daily rates of (i) 0.5, (ii) 1.0, (iii) 1.5 and (iv) 2.0% of bodyweight (BW; as fed) of the steers.

Procedures

The experiment consisted of a 7 d equilibration period followed by a 70 d experimental period. During the equilibration period the steers were fed green panic hay *ad libitum*, without supplements, in group pens of four steers. The procedures for blocking of pens and for allocation of steers to treatments and pens, were as described earlier (Chapter 2A).

The same procedures were also used for feeding the hay and supplements as have been described earlier (Chapter 2A), except where indicated below. To reduce the risk of acidosis, the amount of grain fed to the steers was gradually increased over the first 9 d of the main experimental period so that stipulated treatment levels of feeding were achieved for the 0.5, 1.0, 1.5 and 2.0%W groups after 2, 5, 7 and 9 d, respectively. The deficit incurred in grain intake relative to stipulated intake during this step-up phase was negated where possible over the next four weeks by feeding marginally in excess of prescribed treatment levels during this period. Supplements were placed in small feed bins and fed separately to the hay. To further reduce the possibility of acidosis, the grain mix was always fed about 30 min after feeding the hay in the morning, and for intakes of 1.5 or 2.0%W, it was fed in two equal meals, the latter being after 2 pm. Where supplements were not totally consumed they were also residued once weekly at the same time as the hay or if refusals were considered excessive, were also residued mid-week. For the EGH treatments, the additional urea/sulphate of ammonia was administered to the hay by making up a concentrated solution, sprinkling it evenly onto the surface of the hay (18 mL/kg hay) and lightly hand-mixing it in.

The procedures used for weighing the steers and for collecting and handling feed and residue feed samples have been described earlier (Chapter 2A).

Between days 60 and 66, faecal samples were collected fresh from the floor of the pen of each steer, within 30 min of excretion, and faecal pH was determined. A representative sample of 10 g fresh faeces was mixed with 40 mL of distilled water, allowed to stand for 5 min, and checked for pH using a portable pH meter. A duplicate sample of the faeces was dried at 60^{o}C in a forced draught oven, milled to 1 mm, and kept for N and starch analysis.

On day 58 of the experimental period, rumen fluid was collected *per os* from all steers using a stomach tube and vacuum pump. Feeding was staggered so that sampling of each steer occurred 3 h after feeding the grain mix. For all treatments, the total daily grain allocation was fed in the morning. The same sampling procedures were used as described earlier (Pen study 1) except that rumen fluid pH was determined immediately and before straining the material. A blood sample was also taken and handled in the manner described above (Chapter 2A). The amount of grain consumed between feeding and rumen sampling was also determined.

In situ digestibility: In December 2001, three high-content Brahman crossbred rumen-cannulated steers (10 cm i.d. cannula), approximately 36 months of age and weighing 600 kg, were fed a diet consisting of green panic (composition as used in feeding trial; see later) and lucerne (composition, as for Chapter 2A) hay (2 : 1, as fed), plus 1 kg CSM and 2 kg rolled sorghum daily. The hay was fed at 90% of *ad libitum*-established intake and, with the CSM and sorghum, was provided in two equal meals daily (8 am and 4 pm). This diet was fed to the steers for 10 d prior to the start of the rumen incubations to acclimatise the animals and their rumen microbial populations to the diets and to the sorghum in particular. Rumen

samples were taken as described in Chapter 2A, and average concentrations of NH_3-N were 114 and 136 mg/L for the morning and afternoon, respectively. A description of the nylon bags, the method of filling and insertion of bags into the rumen and of handling the bags after removal, has been given earlier (Chapter 2A). The test feeds were green panic hay (89.3% OM, 8.2% CP, 73.2% NDF and 36.5% ADF), ryegrass hay (see Chapter 3B; 91.2% OM, 12.9% CP, 61.3% NDF and 31.1% ADF), rolled sorghum (98.5% OM, 11.4% CP and 70.7% starch) and expanded sorghum (97.6% OM, 12.1% CP and 69.9% starch). Both hays were ground through a 3 mm screen but the grains were included in the bags as fed (minus supplements), prior to incubation. In contrast with the previous study, one bag per test feed was placed in the rumen of each of the three steers for each incubation time which were the same as in the previous study except for the addition of a 96 h sample for the two hays. All four test feeds were inserted into each steer. At the end of this incubation run, the procedures were repeated with new bags (replicate 2) so that there were six values for DM disappearance for each feed per incubation period. Three bags were also kept for each test feed per run to determine 0 h disappearance. As previously, the bags were inserted in reverse order. All other procedures were as previously described (Chapter 2A). The test feeds and residues after incubation were analysed as follows: hays, OM and NDF; grains, OM, N and starch.

Chemical analysis

Most methods of analyses have been described earlier (Chapter 2A) and only new analytical methods are detailed below.

Starch: The starch was analysed by conversion to glucose using a two-step enzyme treatment, and colorimetric determination of the glucose with a glucose oxidase/peroxidase reagent. All enzymes and reagents were supplied in kit form (Megazyme, provided by Deltagen, Boronia, Victoria). The enzymatic breakdown of the starch using a heat stable α-amylase and amyloglucosidase is based on the procedure of McCleary *et al*. (1992).

Statistical analysis

The statistical methods were the same as described earlier (Chapter 2A).

Results

The composition of the feed components and the final mixed supplements is shown in Table 3.1. The expansion process only marginally changed the composition of the sorghum relative to dry-rolling.

***Table 3.1. Nutrient composition (% DM) of the feed components and mixed supplements (RG and EG).* A description of the supplements is given in the text**

	OM	CP	EE	NDF	ADF	CF	Starch	Ca	P
Hay	88.3	7.7	ND [A]	67.0	35.0	ND	ND	0.43	0.44
Rolled sorghum	98.4	10.9	3.9	ND	ND	2.3	68.7	0.01	0.32
Expanded sorghum	98.0	11.1	3.1	ND	ND	1.9	70.5	0.11	0.30
RG mix	96.3	15.3	3.4	ND	ND	1.7	68.2	0.41	0.29
EG mix [B]	95.8	15.0	1.4	ND	ND	1.5	66.9	0.47	0.29

[A] ND – not determined.

[B] EG mix used for both EGL and EGH treatments, with additional urea added to the hay.

The fermentability of starch, as determined by incubating samples of grain in buffered rumen fluid at UNE, was much higher for expanded than rolled sorghum when tested 'as fed, without additives' (35 vs. 14%) but not much different when the grains were milled (0.5 mm screen) prior to testing (39 vs. 35%).

Enzyme digestibility, as determined by incubating ground grain samples with starch digesting enzymes for 1 h, was also greater for the expanded compared with rolled sorghum (49 vs. 35%).

The equations describing the digestion of starch of rolled and expanded sorghum from nylon bags incubated in the rumen of steers over 72 h are shown below, and the digestion curves are illustrated in Figure 3.1:

Rolled: **Starch digestibility (%) = 7.890 + 92.647 (1 – e $^{-0.0746\ X}$)** (R^2 = 0.989);

Expanded **Starch digestibility (%) = 32.958 + 69.235 (1 – e $^{-0.0873\ X}$)** (R^2 = 0.980),

where X is time (h) of incubation. The major differences between grains were the much higher immediately soluble component (33.0 vs. 7.9%), and faster rate of digestion of the potentially degradable fraction (0.087 vs. 0.075/h), for the expanded compared with rolled grain. The calculated effective degradability was also higher for expanded than for rolled grain (89.3 vs. 80.9%), assuming a fractional outflow rate of 0.02/h.

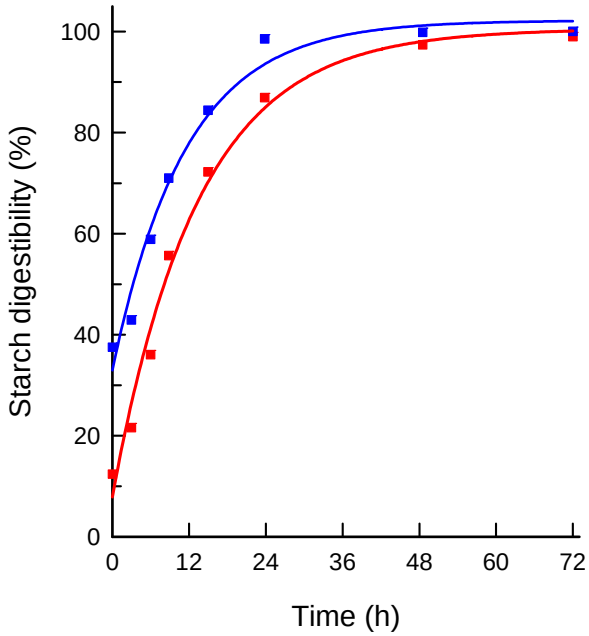

Figure 3.1. Digestibility over time of starch of different grain types (rolled sorghum, ■; expanded sorghum, ■) incubated in nylon bags in the rumen of fistulated cattle.

Unsupplemented steers gained 0.25 ± 0.032 kg/d. Inclusion of supplements in the diet was associated with a linear increase in growth rate by the steers with those receiving expanded sorghum growing at a faster (P<0.05) rate than those on rolled sorghum (see Figure 3.2A). There was no effect of increasing urea intake within the expanded grain treatments. The equations describing the response relationships for RG and the combined EG treatments are as follows:

RG: **Average daily gain (kg) = 0.251 + 0.378 *SI***, (R^2 = 0.874; RSD = 0.092; P<0.01);

EGL / EGH: **Average daily gain (kg) = 0.251 + 0.474 *SI***, (P<0.01);

(EGL: R^2 = 0.854; RSD = 0.122); (EGH: R^2 = 0.889; RSD = 0.103);

where SI is supplement DM intake (%W/d). The different R^2 and RSD values given for EGL and EGH treatments in relation to the combined EGL / EGH relationship above for average daily gain indicate the extent to which this combined relationship represents the data for each treatment separately.

Hay DM intake for the Control steers was 1.89 ± 0.043%W or 4.57 ± 0.117 kg/d and was reduced linearly (P<0.01) with the inclusion of supplements. Conversely, total DM intake was increased linearly with supplement inclusion in the diet (Figure 3.2B). There was no effect of supplement type or of urea inclusion rate in EG treatments on either hay or total DM intakes. Combined response relationships for all supplement types are given below:

All: **Hay DM intake (%W/d) = 1.889 – 0.574 *SI***, (P<0.01);

(RG: R^2 = 0.795; RSD = 0.157); (EGL: R^2 = 0.881; RSD = 0.139); (EGH: R^2 = 0.870; RSD = 0.149);

All: **Total DM intake (%W/d) = 1.889 + 0.426 *SI***, (P<0.01);

(RG: R^2 = 0.785; RSD = 0.157); (EGL: R^2 = 0.728; RSD = 0.139); (EGH: R^2 = 0.749; RSD = 0.149).

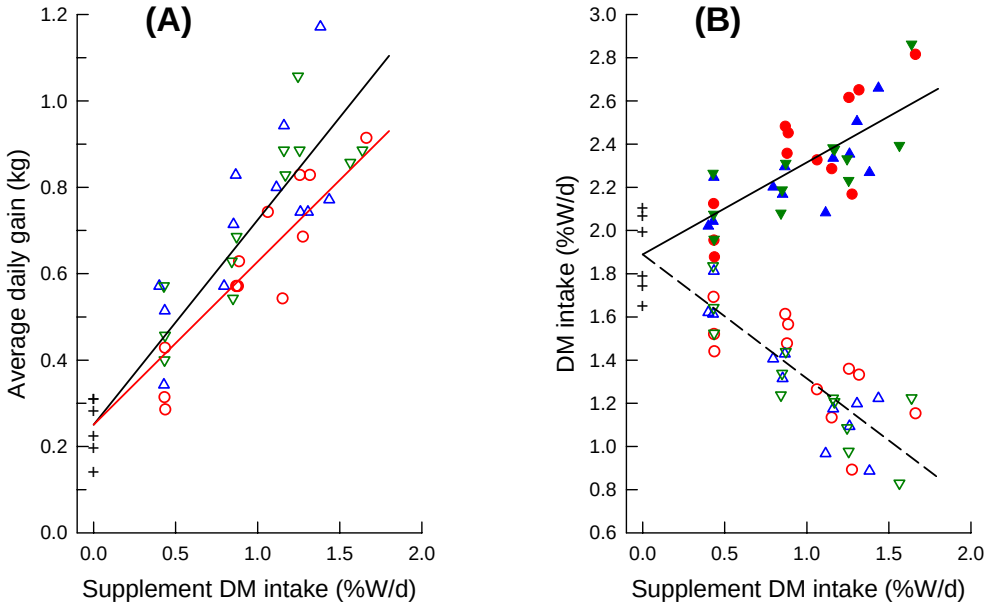

Figure 3.2. Effect of supplements on* (A) *the average daily gain (Control, +; RG, ○; EGL, △; EGH, ▽) and* (B) *the intake of hay (dashed line; Control, +; RG, ○; EGL, △; EGH, ▽) and total (solid line; RG, ●; EGL, ▲; EGH, ♦) DM for steers given a basal diet of low quality hay. A single relationship (black line) is shown for the combined EGL and EGH treatments in Figure A and for all treatments in Figure B. Symbols represent values for individual steers.

The effects of supplement intake in the 3 h prior to rumen sampling on the concentrations of ammonia-N (NH_3-N) and VFAs in rumen fluid of steers are illustrated in Figure 3.3. Rumen NH_3-N concentration increased in a linear manner as intake of supplement increased, this effect being greater (P<0.01) for the RG compared with EG supplements. Concentrations of VFA followed the reverse trend with EG supplements tending (P=0.08) to increase this metabolite concentration more than RG. For both rumen parameters, there was no effect of urea concentration in EG diets. The response relationships are as follows:

RG: **NH_3-N concentration (mg/L) = 74.8 + 51.06 *SI-kg***, (R^2 = 0.860; RSD = 23.12; P<0.01);

EGL / EGH: **NH_3-N concentration (mg/L) = 74.8 + 13.33 *SI-kg***, (P<0.05);

(EGL: R^2 = 0.330; RSD = 23.63; EGH: R^2 = 0; RSD = 53.55);

RG: **VFA concentration (mmol/L) = 65.2 + 2.10 *SI-kg***, (RSD = 10.86; NS);

EGL / EGH: **VFA concentration (mmol/L) = 65.2 + 6.87 *SI-kg***, (P<0.01);

(EGL: R^2 = 0.159; RSD = 20.20; EGH: R^2 = 0.383; RSD = 13.86);

where *SI-kg* is supplement DM intake (kg), in this instance, in the 3 h pre-sampling.

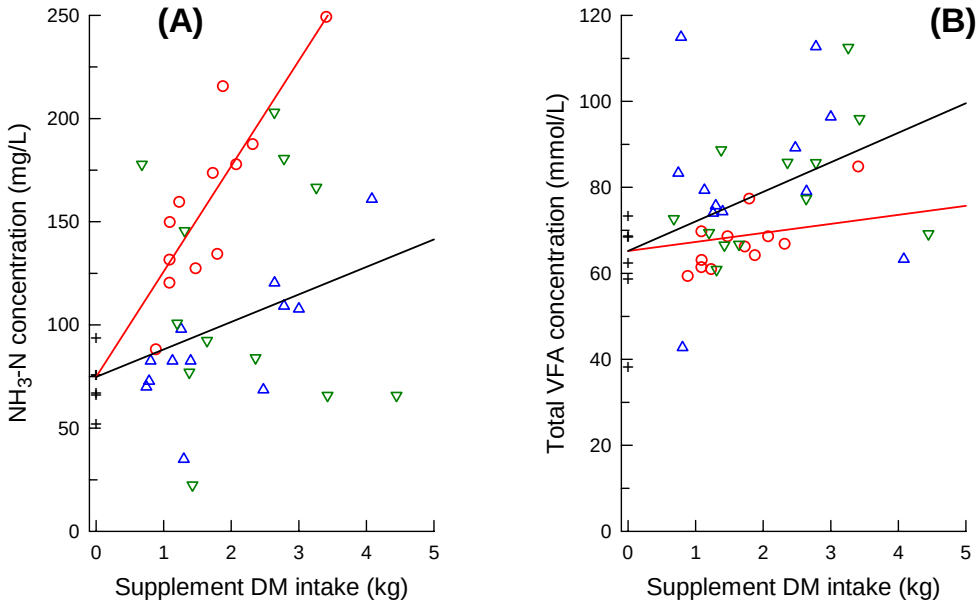

Figure 3.3. Effect of intake of different supplements (Control, +; RG, ○; EGL, △; EGH, ▽) in the 3 h before rumen sampling on the concentration of* (A) *ammonia-nitrogen (NH_3-N) and* (B) *total volatile fatty acids (VFA) in the rumen of steers given a basal diet of low quality hay. A single relationship (black line) is shown for the combined EGL and EGH treatments in Figures A and B. Symbols represent values for individual steers.

Acetate molar proportion declined with increasing supplement intake, with no difference between supplement types. The molar proportion of propionate increased, and that of n-butyrate and minor VFAs (branched-chain and iso-acids) decreased, with increasing intake of EG supplements whilst the opposite trend was observed with RG supplement. These differences between RG and combined EG supplements were all significant (P<0.01) but there was no effect of urea inclusion rate within the EG rations. The response relationships are described by the following equations:

All: **Acetate molar % = 72.85 – 1.107 *SI-kg***, (P<0.01);

(RG: R^2 = 0; RSD = 1.948); (EGL: R^2 = 0.307; RSD = 2.035); (EGH: R^2 = 0.109; RSD = 3.982);

RG: **Propionate molar % = 15.84 – 0.595 *SI-kg***, (RSD = 1.144; NS);

EGL / EGH: **Propionate molar % = 15.84 + 1.643 *SI-kg***, (P<0.01);

(EGL: R^2 = 0.211; RSD = 2.418); (EGH: R^2 = 0.212; RSD = 4.568);

RG: **n-Butyrate molar % = 9.04 + 0.962 *SI-kg***, (R^2 = 42.8; RSD = 1.41; P<0.01);

EGL / EGH: **n-Butyrate molar % = 9.04 – 0.255 *SI-kg***, (NS);

(EGL: R^2 = 0; RSD = 1.134); (EGH: R^2 = 0.027; RSD = 1.289);

RG: **Other VFAs molar % = 2.45 + 0.207 *SI-kg***, (R^2 = 0.331; RSD = 0.351; P<0.05);

EGL / EGH: **Other VFAs molar % = 2.45 – 0.210 *SI-kg***, (P<0.01);

(EGL: R^2 = 0.308; RSD = 0.388); (EGH: R^2 = 0.173; RSD = 0.541).

The acetate/propionate ration was not significantly affected by increasing supplement intake with the RG supplement but decreased markedly with the two EG supplements, with the difference in responses between RG and combined EG supplements being significant (P<0.01). These effects are shown in Figure 3.4 and are described by the following equations:

RG: **Acetate/propionate = 4.68 + 0.119 *SI-kg***, (RSD = 0.402; NS);

EGL / EGH: **Acetate/propionate = 4.68 – 0.417 *SI-kg***, (P<0.01).

(EGL: R^2 = 0.352; RSD = 0.662); (EGH: R^2 = 0.343; RSD = 0.856).

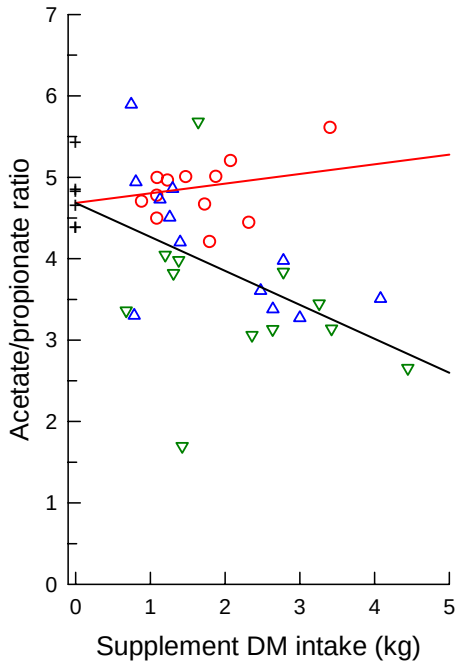

Figure 3.4. Effect of supplements (Control, +; RG, ○; EGL, △; EGH, ▽) on the acetate/propionate ratio in the volatile fatty acids in the rumen fluid of steers given a basal diet of low quality hay. A single relationship (black line) is shown for the combined EGL and EGH treatments. Symbols represent values for individual steers.

Blood parameters were considered in relation to the intake of supplement in the 24 h prior to feeding on the morning of sampling, i.e., on the previous day, in view of the likely longer term effects of supplement on these parameters relative to rumen metabolites. However, there was no difference in the response trends whether supplement intakes were used from this 24 h period or the 3 h period immediately pre-sampling. The urea-N concentration in the plasma of the steers was increased linearly with RG or EGL supplements, with no difference between these supplements, whereas EGH supplement was associated with a quadratic increase in urea-N concentration, the trend being different to that for the other supplements (Figure 3.5). Although there was a trend for glucose concentration in the plasma to increase with increasing supplement intake (Control: 82.8 mg/dL plasma), there was no effect of grain treatment or of urea concentration in EG supplement on this metabolite concentration. The plasma urea response relationships are shown below:

RG/EGL: **Plasma urea-N (mg/dL) = 6.87 + 0.443 *SI-kg***, (P<0.05);

(RG: R^2 = 0.229; RSD = 1.75); (EGL: R^2 = 0.030; RSD = 1.87);

EGH: **Plasma urea-N (mg/dL) = 6.87 + 4.346 *SI-kg* – 0.782 *SI-kg*^{2}**, (R^2 = 0.697; RSD = 1.84; P<0.01);

where *SI-kg* is supplement DM intake (kg), in the above instance being the intake on the previous day up to the time of feeding on the day of sampling (i.e., excluding intake in the 3 h pre-sampling).

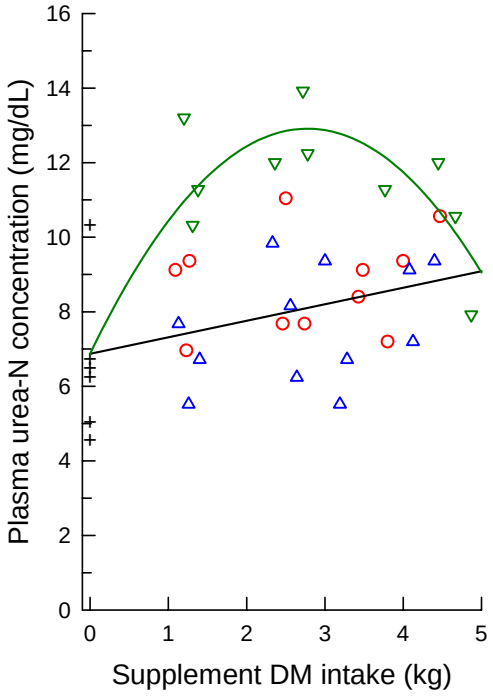

Figure 3.5. Effect of supplements (Control, +; RG, ○; EGL, △; EGH, ▽) on the concentration of urea-nitrogen (urea-N) in the plasma of steers given a basal diet of low quality hay. A single relationship (black line) is shown for the combined RG and EGL treatments. Symbols represent values for individual steers.

Total protozoa numbers averaged 1.67 x 10^5/mL rumen fluid for Control steers at 3 h post-feeding and were increased linearly (P<0.01) by feeding supplement of all types, with no difference between the supplement types. For the Controls, this population comprised, on average (back-transformed values), 19.4% *Isotricha* spp., 37.4% *Dasytricha* spp., 35.9% *Entodinia* spp., 2.6% *Diplodinia* spp., and 6.7% other (minor) species. Across all supplement types, as the supplement intake increased, the proportion of *Entodinia* spp. increased (P<0.01) whilst that of *Isotricha* spp. and *Dasytricha* spp. decreased (P<0.01), with no effects on other species groups. There were no supplement type effects on these species proportions.

Faecal starch content was negligible for unsupplemented steers but was linearly increased by all supplements. The rate of increase in starch content with increasing supplement intake was much greater (P<0.01) for steers receiving RG compared to those given EGL or EGH, which were not different (see Figure 3.6A). Faecal pH averaged 7.1 for unsupplemented steers and was reduced linearly with increasing intake of all supplements, with no between supplement differences. For Control steers, faecal N averaged 1.2% DM and this was increased linearly with increasing intake of all supplements. There was no difference in response to the EGL and EGH supplements which increased faecal N more steeply than the RG supplement across the range of supplement intakes (see Figure 3.6B). The various response trends for the above parameters, relative to intake of supplement (%W/d) in the weeks leading up to sampling, are shown below:

RG: **Faecal starch (% DM) = -0.169 + 19.38 *SI***, (R^2 = 0.890; RSD = 4.079; P<0.01);

EGL / EGH: **Faecal starch (% DM) = -0.169 + 9.89 *SI***, (P<0.01);

(EGL: R^2 = 0.529; RSD = 6.147); (EGH: R^2 = 0.646; RSD = 3.937);

All: **Faecal pH = 7.05 – 1.090 *SI***, (P<0.01);
(RG: R^2 = 0.869; RSD = 0.286); (EGL: R^2 = 0.765; RSD = 0.404); (EGH: R^2 = 0.847; RSD = 0.298);

RG: **Faecal N (% DM) = 1.249 + 0.471 *SI***, (R^2 = 0.838; RSD = 0.131; P<0.01);

EGL / EGH: **Faecal N (% DM) = 1.249 + 0.695 *SI***, (P<0.01);
(EGL: R^2 = 0.727; RSD = 0.243); (EGH: R^2 = 0.875; RSD = 0.189).

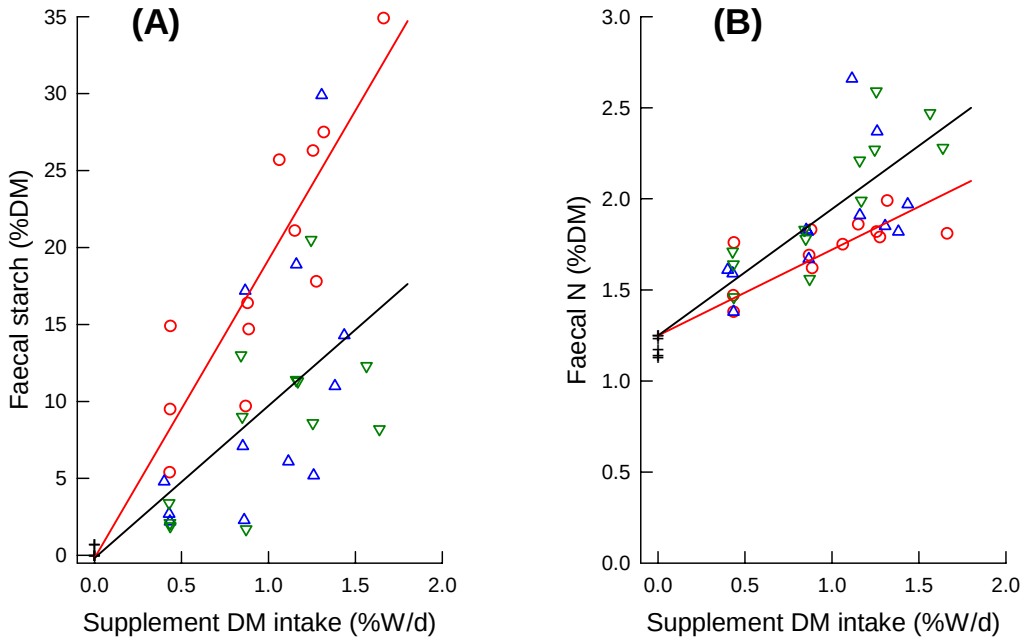

Figure 3.6. Effect of intake of different supplements (Control, +; RG, ○; EGL, △; EGH, ▽) on the concentration of* (A) *starch and* (B) *nitrogen (N) in the faeces of steers given a basal diet of low quality hay. A single relationship (black line) is shown for the combined EGL and EGH treatments in Figures A and B. Symbols represent values for individual steers.

3B EFFECT OF SITE OF DIGESTION OF THE SUPPLEMENT ON THE INTAKE, DIGESTION AND METABOLISM OF STEERS GIVEN EITHER A LOW OR MEDIUM QUALITY FORAGE: METABOLISM STUDY

Materials and methods

Animals, treatments and experimental design

The experiments were carried out at Mt Cotton Research Farm. The trial design involved two incomplete 5 x 5 Latin squares, carried out concurrently, using low quality green panic hay as the basal forage (September to December 2001; LQ) followed by two more similar and concurrent, incomplete 5 x 5 Latin squares using medium quality ryegrass (*Lolium multiflorum cv.* Aristocrat; MQ) hay as the basal ration (January to March 2002). Steers were moved to pasture for three weeks between sets of experiments. The procedures used for each set of experiments were similar to those described for Metabolism study 1, except where indicated below.

Ten Brahman crossbred steers were used throughout the experiments. The steers were initially *ca.* 12 months of age and weighed 223 ± 3.6 (± s.e.) and 280 ± 5.3 kg at the beginning of the first and second pair of experiments, respectively. Two weeks prior to the beginning of the first experiments, the steers underwent a special introductory feeding and education period to accustom them to the experimental diets. They were then allocated to treatment groups by stratified randomisation on the basis of unfasted liveweight. They were re-allocated to treatments for the next experiments with ryegrass. The same two supplement types were used throughout, viz. dry rolled sorghum (RG) or expanded sorghum (EG), as described in Pen study 2. The trial design, as applied to both basal forage types, is summarised below:

	Supplement DM intake (%W/d)	Treatment name
Control	0	Control
RG	0.5	RG 0.5
	1.0	RG 1.0
	1.5	RG 1.5
	2.0	RG 2.0
EG	0.5	EG 0.5
	1.0	EG 1.0
	1.5	EG 1.5
	2.0	EG 2.0

Procedures

Hay was chaffed to lengths of 2-10 cm and fed *ad libitum* once daily. Both supplements were fed at the five levels of supplement intake of nil (Control), 0.5, 1.0, 1.5 and 2.0%W/d, calculated on a DM basis in contrast to the pen experiment in which intakes were similar but on an 'as fed' basis. Supplements were fed twice daily at 0800 h and 1200 h. In order to meet the RDN and sulphur requirements, urea and ammonium sulphate were added and mixed with grain at levels of 1.6% and 0.33% (w/w of grain, as fed), respectively. To balance the Ca:P ratio in the diet, limestone was added to the grain (1% of grain).

Bentonite and molasses were each included at the rate of 2% of grain. The expanded sorghum used here (EG) was equivalent to the low-urea expanded sorghum used in the earlier pen study (Chapter 2A).

Each of the three measurement periods (runs) for each experiment consisted of a 21 d preliminary feeding period in individual yard pens and a 7 d collection period in metabolism cages. During the first 10 d of preliminary feeding, the grain intakes were gradually increased to treatment level to reduce the risk of acidosis. Designated treatment levels were used for the remainder of the preliminary feeding period. Steers were weighed weekly and supplement intakes were modified accordingly.

Procedures used to determine DM intake, faecal DM output, digestibility of nutrients, and urine and PD excretion, have been described in Chapter 2B. In addition to digestibility of DM, OM and NDF, starch digestibility was determined *in vivo* in the first set of experiments with green panic hay. The starch fermentability of supplements was also determined by *in vitro* study at the University of New England, Armidale. Faecal pH was also measured daily between 8 and 9 am on the final day of collections. About 10 g of fresh faeces was taken from each steer, mixed well with deionised water at a ratio 1:2 (w/w) and pH was determined immediately.

On day 3 of each collection period, steers were dosed with chromium-ethylenediamine tetraacetic acid (Cr-EDTA) and ytterbium trichloride hexahydrate ($YbCl_3.6H_2O$) in order to estimate the outflow rate of fluid and particulate matter, respectively, from the rumen. The Cr-EDTA was dosed at a rate sufficient to supply *ca.* 2 g Cr/d, while $YbCl_3.6H_20$ was dosed at *ca.* 1 g Yb/d. The Cr and Yb markers were administered separately. The prescribed amount of $YbCl_3.6H_20$ was measured and mixed with 100 g of the basal hay in a plastic container and then fed to each steer before feeding the main diet. After the steers had completely consumed the hay/Yb mix, the Cr-EDTA dose, which was mixed with 100 g molasses and 50 g of basal hay, was given to each steer. This process of marker administration took between 5-15 min. Samples of each marker were stored at room temperature for later analysis. A sample of faeces was collected from each steer before dosing (0 h, for background value) and at 24, 36, 48, 60, 72, 84 and 96 h after dosing. These were oven dried at 65^{o}C, milled through a 1 mm screen and stored for later analysis for Cr and Yb concentrations. Following Cr and Yb analysis, the natural logarithm of the Cr and Yb concentrations were plotted against time of sampling and fractional outflow rate (FOR) determined as *k*, the slope of the line.

Immediately following the collection period, rumen fluid samples were taken 3 h and 24 h after, and blood samples 3 h after feeding, as described earlier (Chapter 2B).

An *in situ* digestion study was carried out using four feed types, viz., green panic hay, ryegrass hay, rolled grain and expanded grain (grains without additives). This study has been described earlier (Chapter 2A).

Chemical analysis

The analytical procedures for determining DM, OM, N, EE, NDF, NH_3-N and PD concentrations have been described for the earlier metabolism study 1 (Chapter 2B). Starch was analysed as described in Chapter 2A.

Cr and Yb: Faecal and dose samples (Yb) were digested using a solution of 5:1 nitric/perchloric acid and heat. Following digestion, the residues were diluted to known volume using distilled water and marker concentration was determined by analysis using an Inductively Coupled Plasma Atomic Emission Spectrometer. To account for the effects of background matrices on marker recovery, a standard recovery curve was conducted using faecal material spiked with Cr or Yb solution.

Fermentability of starch: This parameter is determined by incubating grain in buffered rumen fluid for 5 h (S. Bird, UNE, personal communication). It thus represents a rate measurement, not an extent measurement (dependent on rumen passage rates), for fermentation of starch in the rumen. Samples of the rolled and expanded sorghum were tested 'as fed' and in the ground (0.5 mm screen) form.

Enzyme digestibility of starch: Enzyme digestibility represents digestion of starch in the intestines and was determined by incubating ground grain with starch digesting enzymes (amylase and amyloglucosidase) for 1 h.

Statistical analysis

The methods of statistical analysis were similar to those described earlier (Chapter 2B). Owing to the limitations of metabolism cage availability, and thus the inability to carry out experiments using the different forage types simultaneously, it was not possible to compare the experiments with different quality hay statistically. Consequently, general trends only are discussed for forage type by supplement interactions. Within sets of experiments for different forage types, the differences between Control (unsupplemented) responses from the RG and EG groups were tested and as there were no statistically significant differences, a single intercept for the control response from the six steers from the two treatment groups was calculated. The data presented in the figures describe the fitted effect (from the general linear models with terms pen, run and level of supplement), not the actual or measured effect.

Results

The nutrient composition of forages and mixed supplements for the experiments with low quality (LQ) and medium quality (MQ) forages is shown in Table 3.2. The higher quality of the ryegrass compared to green panic hay is demonstrated in its higher CP and lower NDF content. Furthermore, the proportions of leaf in the two hays averaged 68 and 57%, respectively. The fermentability of the starch in rolled and expanded sorghum was 35 and 14% when tested in the 'as fed' form (without additives), and 39 and 35%, respectively, when ground before testing. Corresponding values for the enzyme digestibility of starch were 49 and 35%, respectively. There were only small differences in the composition of the RG and EG mixed supplements within experiments, and in the composition of the same supplements across experiments using different forage types.

Table 3.2. Nutrient composition of the hays and mixed supplements. **A description of the supplements is given in the text**

	OM	CP	NDF	EE	Starch
			(% DM)		
Low quality forage experiments					
Green panic hay	90.3	4.8	69.7	0.9	0.5
RG	96.3	14.7	8.6	2.2	69.3
EG	95.9	14.3	8.5	1.7	68.2
Medium quality forage experiments					
Ryegrass hay	91.0	12.3	60.8	1.3	ND
RG	95.1	15.5	8.9	3.9	ND
EG	95.4	15.0	8.2	2.4	ND

ND - not determined.

Within experiments using the same forage type, the Control steers in the RG or EG treatment groups were not different ($P>0.05$) with respect to all the various parameters measured. Consequently, the Control steers were pooled across supplement types ($n = 6$) and values presented below reflect this pooled mean. As described in the earlier metabolism study (Chapter 2B), where there was no effect of supplement type on a particular parameter, a single equation is presented to describe that response relationship for the combined supplements. Separate equations are reported where differences ($P<0.05$) between supplement types occurred.

Total consumption of supplement was achieved in all experiments and at no time did the steers show any signs of ill-health. Hay DM intake averaged 2.02 ± 0.038%W/d and 2.22 ± 0.030%W/d for control steers given LQ or MQ hay, respectively. With increasing intake of supplement, there was a quadratic reduction ($P<0.05$) in hay DM intake by steers on the LQ forage and a linear reduction ($P<0.01$) for steers receiving the MQ forage (Figure 3.7). At the same time, there was a quadratic increase ($P<0.05$) in total DM intake

by steers on the LQ forage, and a linear increase (P<0.01) on the MQ forage (Figure 3.7). Total NDF intake (%W) was reduced quadratically (P<0.05) and linearly (P<0.01) for steers given the LQ and MQ forages, respectively, as supplement intake increased.

Differences between supplement types in relation to the above intake responses were not significant and equations describing these combined supplement effects are as follows:

LQ forage:

All supps:**Hay DM intake (%W/d)= 2.015 + 0.049 *SI* – 0.223 *SI* ^{2}**, (R^2 = 0.941; RSD = 0.0935; P<0.01);

All: **Total DM intake (%W/d) = 2.015 + 1.050 *SI* – 0.223 *SI* ^{2}**, (R^2 = 0.974; RSD = 0.0922; P<0.01);

All: **Total NDF intake (%W/d) = 1.401 + 0.103 *SI* – 0.137 *SI* ^{2}**, (R^2 = 0.908; RSD = 0.0542; P<0.01);

MQ forage:

All: **Hay DM intake (%W/d) = 2.218 – 0.611 *SI***, (R^2 = 0.972; RSD = 0.0918; P<0.01);

All: **Total DM intake (%W/d) = 2.218 + 0.389 *SI***, (R^2 = 0.934; RSD = 0.0919; P<0.01);

All: **Total NDF intake (%W/d) = 1.351 – 0.281 *SI***, (R^2 = 0.956; RSD = 0.0537; P<0.01),

where *SI* is supplement intake (%W/d).

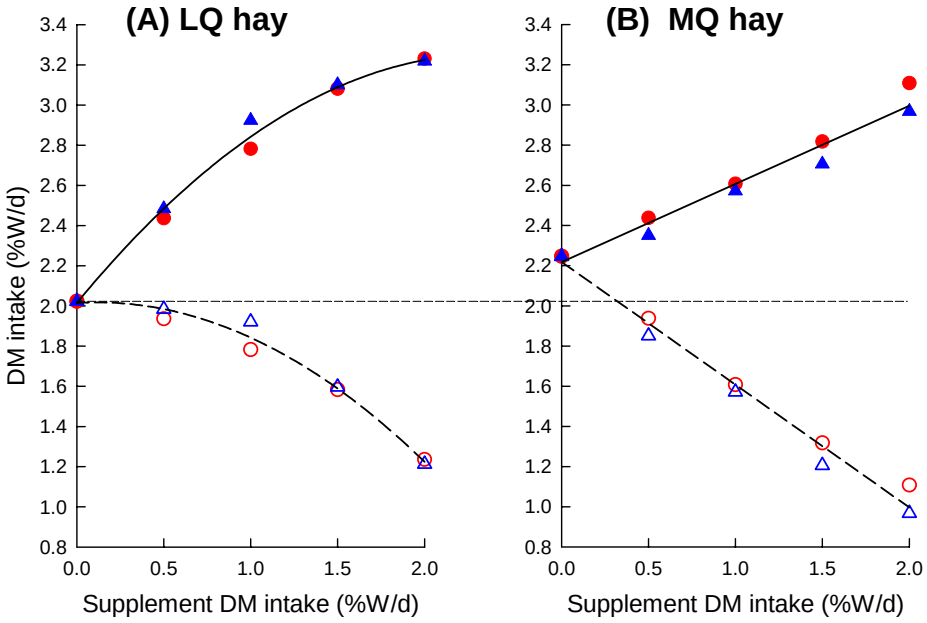

Figure 3.7. Effect of supplements on the intake of hay (dashed line; RG, ○; EG, △) and total (solid line; RG, ●; EG, ▲) DM for steers given a basal diet of (A) low quality hay or (B) medium quality hay. Symbols represent the mean of three steers except for the Controls (six steers).

The OM digestibility (OMD) of the unsupplemented LQ and MQ forages averaged 52.9 ± 0.24 and 62.4 ± 0.39%, and corresponding values for NDF digestibility (NDFD) were 58.4 ± 0.51 and 65.0 ± 0.53%, respectively. On both forage types, the EG supplement was associated with a greater increase in OMD than the RG supplement (P<0.05; Figure 3.8A). By contrast, NDFD showed a greater (P<0.05) rate of decline with the EG compared with the RG supplement on both basal diets (Figure 3.8B). The equations representing these relationships are detailed below:

LQ forage:

RG: **OMD (%) = 52.85 + 1.935 *SI***, (R^2 = 0.948; RSD = 0.3886; P<0.01);

EG: **OMD (%) = 52.85 + 5.951 *SI* – 1.004 *SI*^{2}**, (R^2 = 0.948; RSD = 0.8431; P<0.05);

RG: **NDFD (%) = 58.40 – 1.688 *SI***, (R^2 = 0.402; RSD = 1.759; P<0.05);

EG: **NDFD (%) = 58.40 – 3.475 *SI***, (R^2 = 0.869; RSD = 1.120; P<0.01);

MQ forage:

RG: **OMD (%) = 62.37 + 1.933 *SI***, (R^2 = 0.784; RSD = 0.877; P<0.01);

EG: **OMD (%) = 62.37 + 8.82 *SI* – 3.022 *SI*^{2}**, (R^2 = 0.880; RSD = 1.201; P<0.01);

RG: **NDFD (%) = 64.97 – 1.631 *SI***, (R^2 = 0.399; RSD = 1.732; P<0.05);

EG: **NDFD (%) = 64.97 – 5.866 *SI***, (R^2 = 0.935; RSD = 1.413; P<0.01);

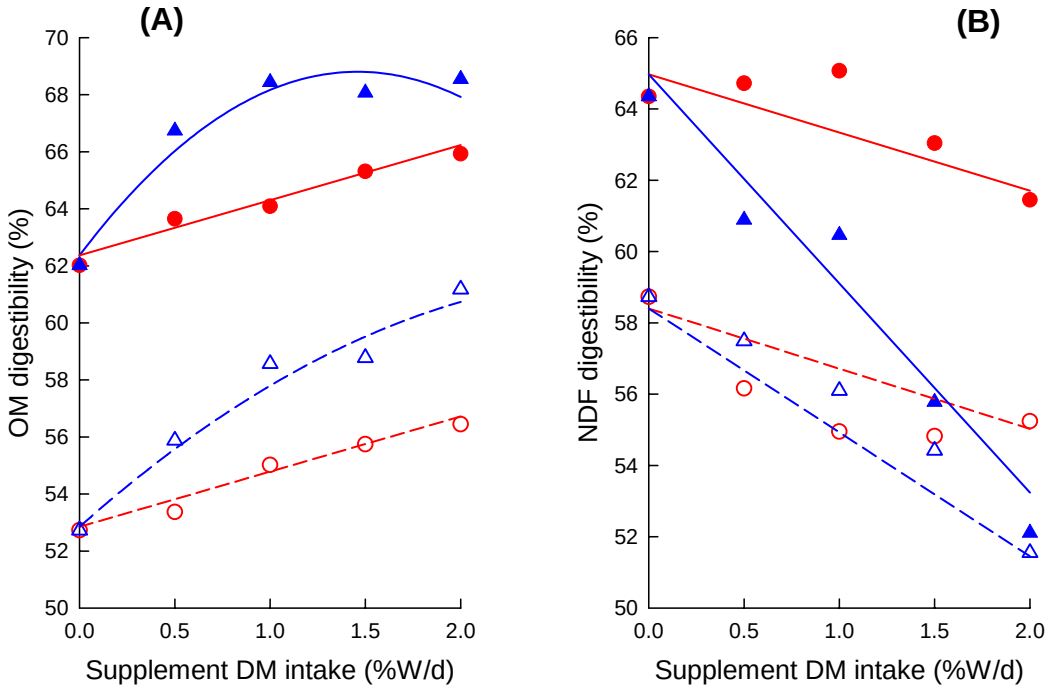

Figure 3.8. Effect of supplements on the digestibility of* (A) *OM and* (B) *NDF for steers given a basal diet of either low quality (dashed line; RG, ○; EG, △) or medium quality (solid line; RG, ●; EG, ▲) hay. Symbols represent the mean of three steers except for the Controls (six steers).

Total digestible OM (DOM) intake by steers increased with supplement intake but was not affected by supplement type on either forage base and the combined supplement response relationships are as follows:

LQ forage:

All: **Total DOM intake (%W/d) = 0.948+ 0.6327 *SI* – 0.1106 *SI*^{2}**, (R^2 = 0.977; RSD = 0.058; P<0.01);

MQ forage:

All: **Total DOM intake (%W/d) = 1.270+ 0.315 *SI***, (R^2 = 0.960; RSD = 0.057; P<0.01).

The estimated production of MCP averaged 190 ± 14.4 and 557 ± 17.6 g/d for steers receiving the LQ and MQ forage without supplements, respectively, and was linearly increased on both forages with

increasing supplement intake, but without differences between supplement types (Figure 3.9A). These response curves for the combined supplements are described as follows:

LQ forage:

All: **MCP production (g/d) = 189.5 + 238.2 *SI***, (R^2 = 0.957; RSD = 42.42; P<0.01);

MQ forage:

All: **MCP production (g/d) = 556.5+ 237.0 *SI*** , (R^2 = 0.938; RSD = 54.26; P<0.01).

The efficiency of MCP production (EMCP) was also increased in a linear fashion with increasing supplement intake and once again there was no effect of supplement type on this parameter (Figure 3.9B). Mean values for EMCP for the LQ and MQ forages without supplement were 82 ± 2.7 and 147 ± 2.1 g MCP/kg DOMI, respectively. The response relationships can be summarised as follows:

LQ forage:

All: **EMCP (g MCP/kg DOMI) = 82.31 + 32.29 *SI***, (R^2 = 0.919; RSD = 8.060; P<0.01);

MQ forage:

All: **EMCP (g MCP/kg DOMI) = 147.0 + 14.81 *SI***, (R^2 = 0.812; RSD = 6.348; P<0.01);

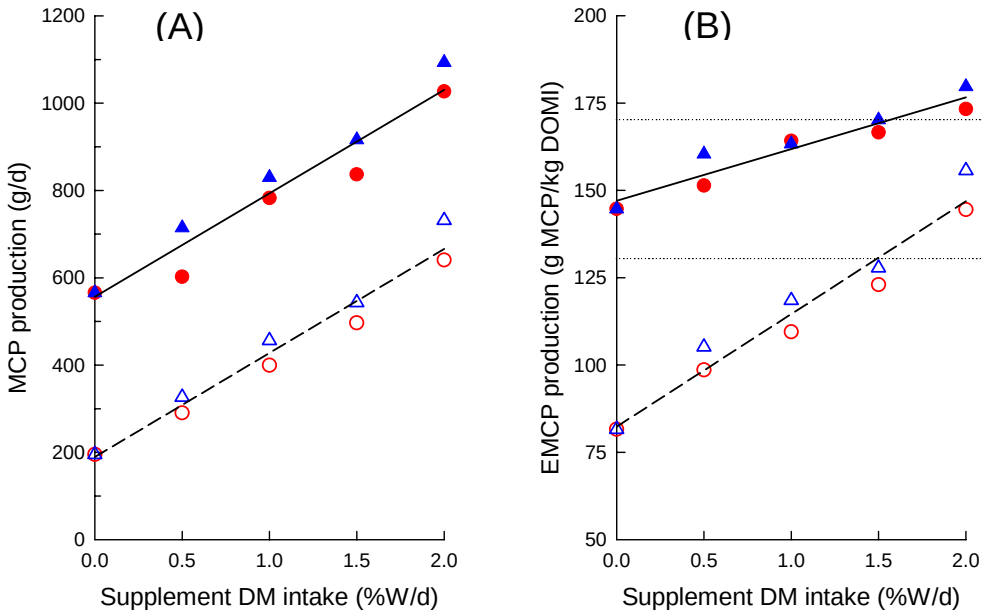

Figure 3.9. Effect of supplements on* (A) *the production of microbial CP (MCP) and* (B) *the efficiency of production of MCP (EMCP) for steers given a basal diet of either low quality (dashed line; RG, ○; EG, △) or medium quality (solid line; RG, ●; EG, ▲) hay. Horizontal lines on section* (B) *indicate the expected range for EMCP from the feeding standards. Symbols represent the mean of three steers except for the Controls (six steers).

Increasing supplement intake was associated with a linear increase in the concentration of ammonia-N (NH_3-N) in the rumen fluid of steers at 3 h post-feeding on both forage types, from a low of 28 ± 1.80 and 84 ± 6.28 mg/L for the LQ and MQ unsupplemented steers, respectively (Figure 3.10A). With the LQ forage the rate of increase in concentration was greater (P<0.01) with the RG compared to the EG supplement but there was no effect of supplement type with the MQ forage. There was also a linear increase in NH_3-N concentration with supplement intake at 24 h post-feeding with both forage types, but the rate of increase was considerably lower and there was no effect of supplement type with either forage (Figure 3.10B). The response relationships are described as follows:

LQ forage: 3 h after feeding

RG: **NH_3-N conc. (mg/L) = 27.8 + 62.56 *SI***, (R^2 = 0.989; RSD = 5.676; P<0.01);

EG: **NH_3-N conc. (mg/L) = 27.8 + 51.46 *SI***, (R^2 = 0.988; RSD = 4.619; P<0.01);

24 h after feeding

All: **NH_3-N conc. (mg/L) = 23.61 + 18.121 *SI***, (R^2 = 0.959; RSD = 3.152; P<0.01);

MQ forage: 3 h after feeding

All: **NH_3-N conc. (mg/L) = 84.1 + 72.84 *SI***, (R^2 = 0.918; RSD = 19.32; P<0.01);

24 h after feeding

All: **NH_3-N conc. (mg/L) = 62.4 + 11.84 *SI***, (R^2 = 0.956; RSD = 2.25; P<0.01).

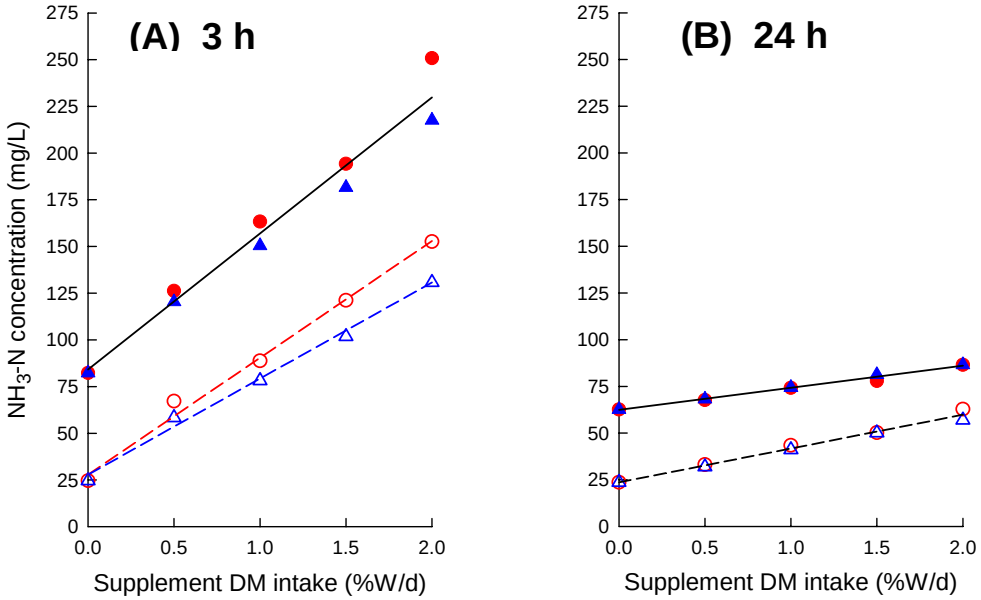

Figure 3.10. Effect of supplements on the concentration of ammonia-nitrogen (NH_3-N) in the rumen fluid of steers receiving either low quality (dashed line; RG, ○; EG, △) or medium quality (solid line; RG, ●; EG, ▲) hay at either (A) 3 h or (B) 24 h after feeding. A single relationship is shown for the combined RG and EG treatments on medium quality hay in Figure* A *(black line) and for both hay types in Figure* B*. Symbols represent the mean of three steers except for the Controls (six steers).

On both forage types, feeding grain reduced rumen fluid pH linearly, from a mean of *ca.* 7.0 for unsupplemented steers, at both 3 h and 24 h after feeding. The reduction in pH was greater (P<0.01) for EG compared with RG steers on LQ diets at 3 h post-feeding but there was no supplement type effect on the MQ forage at 3 h, or on either forage at 24 h (Figure 3.11). The response relationships are as follows:

LQ forage: 3 h after feeding

RG: **Rumen pH = 6.95 – 0.248 *SI***, (R^2 = 0.876; RSD = 0.080; P<0.01);

EG: **Rumen pH = 6.95 – 0.421 *SI***, (R^2 = 0.943; RSD = 0.086; P<0.01).

24 h after feeding

All: **Rumen pH = 6.97 – 0.265 *SI***, (R^2 = 0.646; RSD = 0.165; P<0.01).

MQ forage: 3 h after feeding

All: **Rumen pH = 7.0 – 0.445 *SI***, (R^2 = 0.888; RSD = 0.141; P<0.01);

24 h after feeding

All: **Rumen pH = 7.0 – 0.233 *SI***, (R^2 = 0.830; RSD = 0.094; P<0.01).

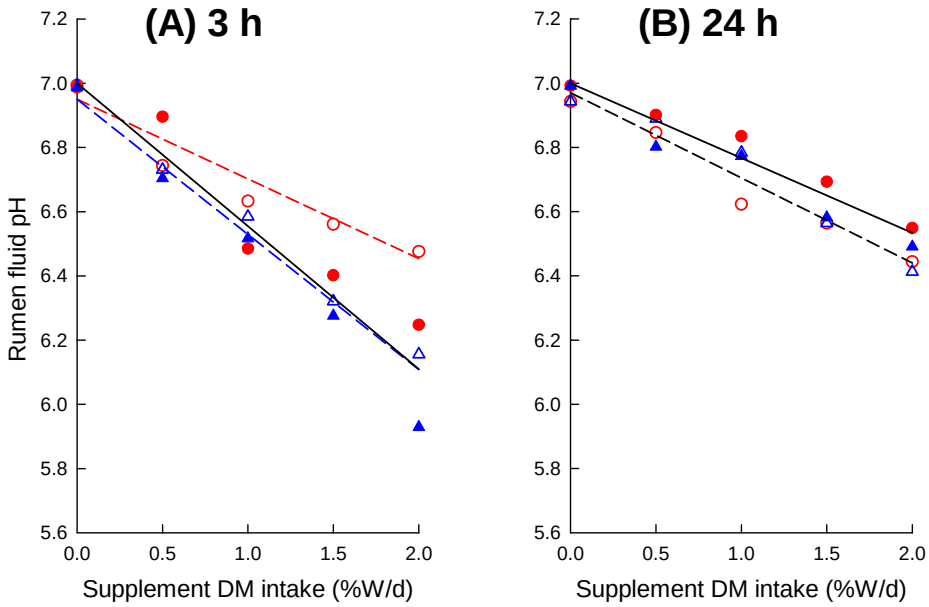

Figure 3.11. Effect of supplements on the pH in the rumen fluid of steers receiving either low quality (dashed lines; RG, ○; EG, △) or medium quality (solid lines; RG, ●; EG, ▲) hay at either (A) 3 h or (B) 24 h after feeding. A single relationship is shown for the combined RG and EG treatments on medium quality hay in Figure A (black line) and for both hay types in Figure B. Symbols represent the mean of three steers except for the Controls (six steers).

Plasma urea-N concentration was increased with increasing supplement intake for steers receiving the LQ forage but there was no effect of supplement type (P=0.06; Figure 3.12). Mean plasma urea-N concentrations for the LQ and MQ forages, without supplement, were 1.9 ± 0.18 and 9.1 ± 0.34 mg/dL, respectively. Supplement intake did not affect plasma urea concentrations on the MQ forage. The response relationship for the LQ forage diets is as follows:

LQ forage:

All: **Plasma urea-N (mg/dL) = 1.906 + 3.809 *SI***, (R^2 = 0.966; RSD = 0.604; P<0.01).

Faecal pH was reduced linearly and quadratically in association with increasing supplement intake on both LQ and MQ forages, respectively, with no effect of supplement type on either forage base. These relationships between supplement intake and faecal pH are shown in Figure 3.13 and are described by the following equations:

LQ forage:

All: **Faecal pH = 7.25 – 0.794 *SI***, (R^2 = 0.872; RSD = 0.256; P<0.01).

MQ forage:

All **Faecal pH = 7.04 – 1.348 *SI* + 0.276 *SI*^{2}**, (R^2 = 0.969; RSD = 0.139; P<0.01);

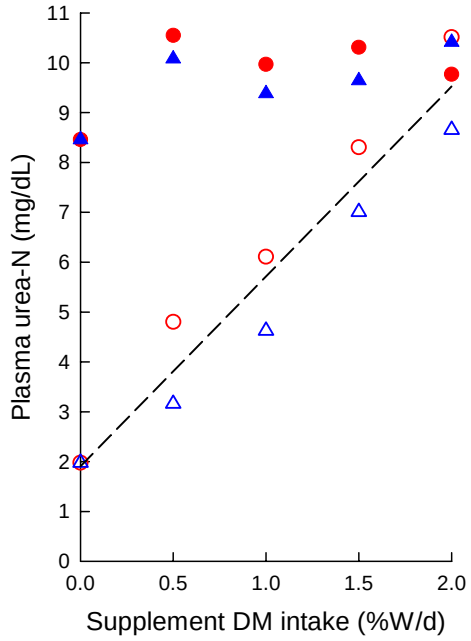

Figure 3.12. Effect of supplements on the concentration of urea-nitrogen (urea-N) in the plasma of steers receiving either low quality (dashed line; RG, ○; EG, △) or medium quality (RG, ●; EG, ▲) hay. Symbols represent the mean of three steers except for the Controls (six steers).

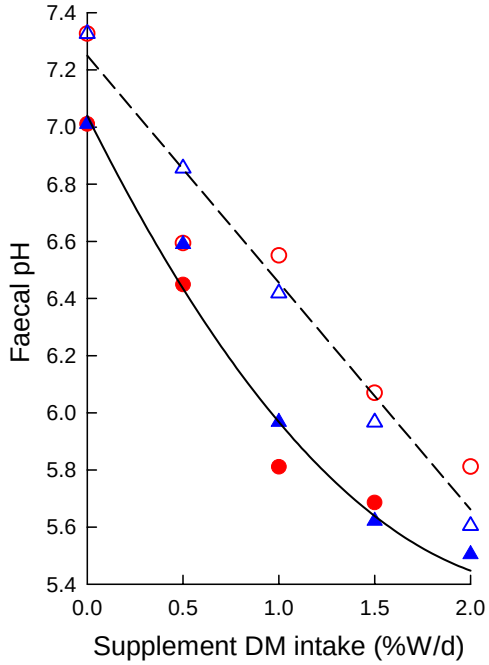

Figure 3.13. Effect of supplements on the faecal pH for steers receiving either low quality (dashed line; RG, ○; EG, △) or medium quality (solid line; RG, ●; EG, ▲) hay. Symbols represent the mean of three steers except for the Controls (six steers).

Passage rates were not determined for the LQ forage. For the MQ forage, the solid phase (particle) passage rates increased linearly with increasing supplement intake, the rate of increase being greater ($P<0.01$) for the RG compared with the EG supplement. Liquid passage rates also increased with

supplement intake, but there were no supplement type differences in these rates. The response relationships are shown below:

MQ forage:

RG:**Particle passage rate (%/h) = 3.65 + 0.498 *SI***, (R^2 = 0.916; RSD = 0.130; P<0.01);

EG:**Particle passage rate (%/h) = 3.65 + 0.148 *SI***, (R^2 = 0.839; RSD = 0.059; P<0.01);

All: **Liquid passage rate (%/h) = 6.50+ 0.251 *SI***, (R^2 = 0.742; RSD = 0.132; P<0.01).

Discussion: (Chapters 3A & 3B)

The primary aim in these studies was to investigate the effect of varying the site of digestion of the supplement on the extent of substitution when hays of varying quality are fed to cattle. Consequently, the choice of sorghum grains processed in different ways represented an attempt to change site of digestion whilst keeping as many other characteristics of the supplements constant. Sorghum is known to have low rumen starch degradability in the unprocessed or coarsely-milled form but also to respond favourably in terms of extent of digestion to more intensive processing, e.g., steam flaking or extrusion. The expansion process was used here partly due to the lack of steam-flaking capability and also because the drier form of expanded grain compared to steam-flaked grain reduced storage problems thereby allowing all of the processing to be done at one time at the beginning of the experiment. Results obtained in both the pen and metabolism studies indicated that the expansion process did increase the extent of starch degradation in the rumen compared with coarse roller milling. This evidence included the lower rumen pH, higher rumen VFA concentrations but lower acetate:propionate ratio, lower rumen NH_3-N concentrations and increased depression of NDFD for expanded sorghum compared with rolled sorghum treatments. These findings are discussed in more detail below. Further support for this contention was provided in the starch fermentability studies carried out at UNE and in the nylon bag studies which showed greater rate of disappearance of starch, and higher effective degradability of starch, from expanded compared with rolled sorghum. However, the corollary that rolled sorghum thus had greater post-ruminal digestion, did not necessarily follow. In fact the lower total OMD and greatly elevated starch content in faeces, associated with RG compared with EG supplement suggests that much of the grain (starch) escaping the rumen was not subsequently available to the animal in the lower parts of the GIT. The enzyme digestion studies also indicate higher digestion of starch from expanded compared to rolled sorghum in the intestines. The results discussed from hereon need to be tempered accordingly.

The higher growth rates for steers fed a low quality hay in pens and given EG compared to RG supplements (0.47 vs 0.38 kg growth/%W supplement intake) occurred independent of any differences between supplement groups in hay or total DM intake. Results from the metabolism study mirrored this trend and thus confirmed no effect of grain processing method, and hence of site of digestion of the grain, on the extent of substitution. In the metabolism study there was an increase in OMD with EG relative to RG supplement, but total DOM intake was not different for the two supplements. Thus the higher growth rate with the EG supplement appears related to an increased efficiency of use of the available DOM. That there was no additional intake or growth response to a higher urea inclusion rate in the EG supplement in the pens suggests that RDP supply was adequate with the lower urea level for the available energy supply.

These increases in OMD with the different supplements occurred despite marked reductions in NDFD with both supplement types. Inclusion of readily fermented carbohydrate with a low quality, high fibre forage is thought to depress fibre digestion through a reduction in numbers of cellulolytic micro-organisms in response to an increasing population of the faster growing amylolytic microbes in the rumen. Thus the negative effects on fibre digestion were more than compensated for by the higher digestibility of the grain sources in the rumen, resulting in increased OMD. The fact that EG was associated with a greater depression in NDFD, but higher OMD compared to RG, reinforces the assertion that it was more completely digested in the rumen. This is further supported by the higher VFA concentrations in the rumen and lower rumen fluid pH recorded with EG relative to RG supplements across the two experiments.

Despite OMD being almost 10 percentage units higher, and intake 0.2%W higher, for unsupplemented steers given MQ compared with LQ hay, the effects of supplement on intake and digestion followed similar general trends for the MQ hay to those described above for the LQ hay. The major difference in terms of intake was in the shape of the curve describing effects of supplement on hay intake. With MQ hay the reduction in hay intake was linear whereas with LQ hay the quadratic nature of the response curve indicated low rates of substitution with low supplement intakes but an increasing substitution rate as supplement intakes increased. As a result, hay intake was actually lower for MQ compared with LQ hay at the highest level of supplement inclusion. This inverse relationship between hay quality and substitution rate has been described previously in the literature (e.g., SCA 1990). The other notable difference was the much greater depression in NDFD associated with EG supplementation of MQ compared with LQ hay, suggesting a greater disruption of cellulolysis on the higher quality hay.

Notwithstanding the apparent differences in the extent of digestion of the two grain sources in the rumen, MCP production was similar for EG and RG supplements across the full range of intakes. This result is difficult to rationalise as higher MCP production would be expected with the EG supplement in view of the greater supply of fermentable energy in the rumen, unless RDP or some other nutrient was limiting. Based on the lack of intake or growth response to adding additional urea in the EG supplement in the pen study, it seems unlikely that RDP was limiting in relation to the available energy supply. Furthermore, the slope of the MCP production response curve to added supplement was almost identical with the higher protein (and OMD) MQ hay compared to LQ hay, albeit that the baseline production was much higher (557 vs. 190 g/d), suggesting that microbial growth was primarily related to energy supply with RDP not limiting. As we have reported in other studies, EMCP with the LQ hay (82 g MCP/kg DOMI) was well outside the range of 130-170 g MCP/kg DOMI proposed in the feeding standards and in this study required supplement intake of 1.5%W/d before this lower threshold was achieved. Once again, both supplements were used with equal efficiency in this regard. By contrast, the MQ hay was associated with relatively high EMCP in the absence of supplement (147 g MCP/kg DOMI) and EMCP was increased more gradually with increasing supplement intake than with the LQ hay. This finding also confirms earlier results.

In both studies where low quality hay was fed, NH_3-N concentration in the rumen of steers increased at a greater rate with the RG compared with EG supplement indicating greater capture of this N source with the EG supplement. Again this is consistent with the greater extent and rate of fermentation of EG than of RG grain in the rumen but seems to contradict the lack of difference in MCP production rates for the two supplements, as discussed above. It was surprising that the Control pen steers had a relatively high rumen NH_3-N concentration of 75 mg/L, considering the low quality of the hay, whereas the metabolism steers had low rumen concentration (28 mg/L). The lower values for the metabolism study may have resulted from the disruption of the steers and their intake pattern in the process of shifting them from the metabolism cages to the pens. Although in the pen study there were no differences between the EGL and EGH treatments in ammonia concentration trends, this probably transpired due to an anomaly of the feeding method with the EGH treatment. Steers on the EGH treatments received urea-N in both the grain component of the diet and also mixed with the hay. However, the concentration of NH_3-N in the rumen was regressed against the intake of the grain-based supplement fed separately from the hay. Although supplement intake was recorded in the 3 h period before sampling, hay intake was not and ammonia concentrations were a product of the urea in both the supplement and the hay. Steers receiving low amounts of supplement probably consumed more hay, with its constituent urea, than those on higher supplement intakes in this 3 h period. The higher plasma urea-N concentrations for the EGH compared to EGL and RG treatment groups does reflect the higher total N intake from this treatment overall.

High grain diets are usually associated with reduced acetate/butyrate and increased propionate proportions in the rumen, relative to high roughage diets, and the EG supplements fed in the pens showed such a trend. This resulted in a reduced acetate:propionate ratio for the EG relative to RG treatments and is again consistent with greater starch fermentation from the former grain type in the rumen.

High concentrations of starch in the faeces of steers indicate incomplete fermentation of the grain and in this study concentrations were much higher in RG compared with EG treatment groups. Starch concentrations as high as 35% DM in faeces with the RG group suggest that not only was there a low degree of starch fermentation in the rumen but much of the undigested starch leaving the rumen was subsequently not utilised in the lower tract. This represents a very significant nutrient wastage and

explains the often-recorded lower performance of cattle on rolled sorghum-based diets compared with those based on more extensively processed sorghum, or on other grains like barley and wheat. Despite these differences in faecal starch content, faecal pH was not different for the different grain treatment groups. This finding suggests that although there was less starch in the large intestines with EG relative to RG, this starch was more fermentable and thus had a similar effect in lowering pH to that of the higher amount of less fermentable starch from RG.

Conclusions

The aim of this group of studies was to determine the effect of changes to the site of digestion of the supplement, between the rumen and intestines, on the extent of substitution when the supplements were fed to steers given low or medium quality hay. The expansion treatment used on the sorghum did markedly change the digestion characteristics of the grain relative to dry rolling. There was clear evidence that the expanded sorghum had higher rate and extent of digestion of starch in the rumen but unfortunately it was not clear that the rolled sorghum, by corollary, had a higher extent of digestion in the intestines. Instead, it is probable that the expanded sorghum also had higher digestion post-ruminally than the rolled sorghum. Nevertheless, it was possible to compare these otherwise similar supplements in terms of their effects on intake, digestion and growth of animals.

Despite these differences in the grains, the rate of substitution was not affected when compared within hay quality types. Substitution rate was higher for the medium compared to the low quality hay, as has been demonstrated previously, but there was no interaction of supplement type and hay quality. These current findings suggest that the effects of supplement on forage intake are not mediated through some metabolic effect in the rumen, e.g., low rumen pH. However, in previous work we have demonstrated greater substitution with barley (a grain with high rumen starch degradation) compared with sorghum. At the same time, substitution was also greater for molasses than for either grain and molasses is not usually associated with low rumen pH. Some other mechanism, perhaps energy content of the supplement, appears a more likely cause of the different rates of substitution in these various experiments. This is discussed further in the General Discussion.

4A EFFECT OF PROTEIN:ENERGY RATIO ON THE INTAKE AND GROWTH OF STEERS GIVEN A LOW QUALITY FORAGE: PEN STUDY

Materials and methods

Animals, treatments and experimental design

This pen feeding experiment was carried out at "Brian Pastures" Research Station between July and October 2002. Commercial Brahman crossbred weaner steers (approximately 5/8 *Bos indicus*; ex Swans Lagoon Research Station) about 12 months of age and weighing 195.0 ± 1.39 kg liveweight (commencement liveweight) were used. They were vaccinated against tick fever and bovine ephemeral fever and treated with an anthelmintic (Ivomec) prior to commencement of the experiment.

The experimental design was the same as that described earlier (Pen study 1) and is summarised below. Green panic hay was fed *ad libitum* alone (Control) or with the following supplements: (i) rolled barley alone (B); (ii) rolled barley/cottonseed meal/copra meal (2:1:1, as fed; BP); or (iii) rolled barley/urea/S (BU), fed at four levels of intake (as fed basis). The treatments are summarised as follows.

	Supplement intake (as fed) (%W/d)	Treatment name
Control	0	Control
Barley	0.5	B 0.5
	1.0	B 1.0
	1.5	B 1.5
	2.0	B 2.0
Barley / protein	0.5	BP 0.5
	1.0	BP 1.0
	1.5	BP 1.5
	2.0	BP 2.0
Barley / urea	0.5	BU 0.5
	1.0	BU 1.0
	1.5	BU 1.5
	2.0	BU 2.0

All grain supplements included 20 g bentonite, 20 g molasses and 10 g limestone, per kg of cracked barley grain, as fed. For the BU treatments, urea and sulphate of ammonia was added at 18.4g and 1.6g respectively per kg of barley grain. These supplements were dissolved in 20 g of molasses and 15 g of water and then added to the grain while mixing. The BP and B rations were formulated to represent supplements with high and low P/E, respectively; the BU treatment was intermediate. In addition, the BP and BU supplements were formulated to have the same ratio of RDP/DOM. These ratios were determined using various assumptions, viz. (i) at the same rate of supplementation, the ratio of hay/supplement was the same for the different supplement types; and (ii) the digestibility or protein

degradability characteristics of the various supplement components were constant across supplement intakes. The calculations also used the following chemical analyses and assumptions: organic matter digestibility (OMD; McLennan, unpubl. data) – hay, 55%; barley, 75%; CSM, 75%; copra meal, 71%; crude protein (CP) content – hay, 7.5%; barley, 12.4%; CSM, 42.8%; copra meal, 24.2%; rumen degradability of protein (dg) – hay, 75%; barley, 65%; CSM, 75%; copra meal, 40% (Moss et al., 1998). Based on these assumptions the P/E ratio and RDP/DOM ratio for the hay were 136 and 102 g CP/kg DOM, respectively. Using assumed intakes, the corresponding values for steers receiving 2% W supplement were estimated to be: B, 158 and 106; BP, 224 and 172; and BU, 182 and 173 g CP/kg DOM, respectively.

Procedures

The experiment consisted of a 7 d equilibration period followed by a 70 d experimental period. During the equilibration period the steers were fed Green Panic hay *ad libitum* plus 0.4 kg cracked barley per head daily, in group pens of 3 steers. The procedures for blocking of pens and for allocation of steers to treatments and pens, were as described earlier (Chapter 2A).

Fresh hay was fed once daily whilst the supplements were fed twice daily, morning (9 am) and afternoon (4 pm), in equal portions and residues were collected once weekly. Hay was chaffed to lengths averaging 9 cm and fed to each steer at a level estimated to be required to provide about 15% in excess of its intake on the previous day, and thus maintain *ad libitum* intake. Supplements were placed in small feed bins and fed separately to the hay. To reduce the possibility of acidosis, the grain mix was fed about 30 min after the hay in the morning. Where supplements were not totally consumed they were also residued once weekly at the same time as the hay or if refusals were excessive, they were also residued mid-week.

To reduce the possibility of acidosis, a process similar to that described earlier (see Chapter 3A) was used for gradually increasing intake of grain in the first two weeks, and of making up the deficit of grain from this step-up period in subsequent (5) weeks. The designed treatment levels of feeding were achieved for the 0.5, 1.0, 1.5 and 2.0%W B and BU groups after 1, 3, 6 and 9 d, respectively, whilst for the BP treatments the corresponding periods were 1, 1, 3 and 6 d, in keeping with the lower grain content of these supplements (50% barley).

The procedures used for weighing the steers and for collecting and handling feed and residue feed samples have been described earlier (Chapter 2A).

Between days 59 and 63, faecal samples were collected fresh from the floor of the pen of each steer in the morning, within 5 min of excretion, and faecal pH was determined. A representative sample of 5 g was mixed with 10 mL of distilled water, allowed to stand for 5 min, and checked for pH using a portable pH meter. Another representative sample was dried at 60^{o}C for 2 d in a forced draught oven, then milled through a 1mm screen and kept for faecal N and starch analysis. From the same sample collections a 200 g (approx.) sample was kept fresh, frozen and stored awaiting NIRS analysis.

On day 58 of the experimental period, rumen fluid was collected *per os* from all steers using a stomach tube and vacuum pump. Feeding of supplements was staggered so that sampling of each steer occurred 3 h after feeding the grain mix. Only half of the total daily supplement allocation was fed, in keeping with the usual procedure of feeding twice daily, but the total allocation of hay was fed at the same time as the supplement. The same sampling procedures were used as described earlier (Chapter 3A). A blood sample was also taken and handled in the manner described above (Chapter 2A). The amount of grain consumed between feeding and rumen sampling was also determined.

In situ digestibility: In May 2002, three high-content Brahman crossbred rumen-cannulated steers (10 cm i.d. cannula), approximately 40 months of age and weighing 560 $\pm$ 47 kg, were fed a diet consisting of pangola (93.9% OM, 6.3% CP, 66.4% NDF and 39.4% ADF) and lucerne (91.2% OM, 19.1% CP, 44.0% NDF and 35.5% ADF) hay (2 : 1, as fed), plus 1 kg CSM (composition as for feeding trial; see later) and 2 kg rolled sorghum (composition as for feeding trial; see later) daily. The hay was fed at 90% of *ad libitum*-established intake and, with the CSM and sorghum, was provided in two equal meals daily (8 am and 4 pm). This diet was fed to the steers for two weeks prior to the start of the rumen incubations to

acclimatise the animals to the diets. Rumen samples were taken as described for Pen study 1 but only in the afternoon, and average concentrations of NH_3-N were 233 mg/L. A description of the nylon bags, the method of filling and insertion of bags into the rumen and of handling the bags after removal, has been given earlier (Chapter 2A). The test feeds were green panic hay (89.0% OM, 6.8% CP, 68.6% NDF and 41.1% ADF), cracked barley (97.9% OM, 13.8% CP, 13.0% NDF and 53.5% starch), CSM (93.3% OM, 41.1% CP, 27.1% NDF and 20.1% ADF) and copra meal (93.7% OM, 24.7% CP, 52.4% NDF and 28.8% ADF). The hay was ground through a 3 mm screen but the barley, CSM and copra meal were included in the bags as fed (minus supplements), prior to incubation. Two bags per test feed were placed in the rumen of each of the three steers for each incubation time which included 3, 6, 9, 15, 24, 48 and 72 h for the supplements but with additional samples at 13 and 96 h for the hay. Thus there were six values for DM disappearance for each feed per incubation period. All four test feeds were inserted into each steer. Three bags were also kept for each test feed per run to determine 0 h disappearance. As previously, the bags were inserted in reverse order. All other procedures were as previously described (Chapter 2A). The test feeds and residues after incubation were analysed as follows: hay, OM and NDF; CSM and copra meal, OM and N; barley, OM, N and starch.

Chemical analysis

The methods of chemical analyses have been described in previous chapters.

Statistical analysis

The methods of statistical analysis have been described earlier.

Results

The composition of the feed components and the final mixed supplements is shown in Table 4.1. The barley used was of quite high quality, containing 14.2% CP. Addition of protein meals increased the final CP (equivalent) content of the supplement more than addition of urea (23.7 vs. 18.8%). The BP supplement had a much lower starch content than B or BU in keeping with the lower contribution of barley (<50%) to the total ration.

***Table 4.1. Nutrient composition (% DM) of the feed components and mixed supplements (B, BP and BU).* A description of the supplements is given in the text**

	OM	CP	EE	NDF	ADF	CF	Starch	Ca	P
Hay	90.0	4.9	- [A]	71.9	41.3	-	-	0.31	0.32
Barley	98.0	14.2	2.5	-	-	3.8	57.9	0.06	0.28
CSM	91.9	47.5	3.5	19.9	11.8	9.1	-	0.23	1.46
Copra meal	93.6	24.0	8.4	56.3	29.8	13.6	-	0.07	0.56
B mix	96.0	13.5	2.4	-	-	3.6	57.6	0.30	0.27
BP mix	94.4	23.7	4.0	-	-	7.0	29.0	0.27	0.58
BU mix	95.6	18.8	2.4	-	-	3.4	55.5	0.38	0.26

[A] Not determined.

Unsupplemented steers gained 0.05 ± 0.028 kg/d. Inclusion of B or BU supplement in the diet was associated with a linear increase in growth rate by the steers with the rate of increase greater for BU compared with B supplement ($P<0.05$). However, steers receiving BP supplement showed a curvilinear growth response to supplement intake, which was greater than for the other two supplements ($P<0.01$; Figure 4.1A) at intakes less than 1.5%W. The equations describing these response relationships are as follows:

B: **Average daily gain (kg) = 0.047 + 0.504*SI***, (R^2 = 0.960; RSD = 0.070; P<0.01);

BU: **Average daily gain (kg) = 0.047 + 0.570 *SI***, (R^2 = 0.958; RSD = 0.085; P<0.01),

BP: **Average daily gain (kg) = 0.047 + 1.121 *SI* – 0.333 *SI*^{2}**, (R^2 = 0.942; RSD = 0.116; P<0.01);

where *SI* is supplement DM intake (%W/d).

Hay DM intake for the Control steers was 1.80 ± 0.046 %W or 3.53 ± 0.112 kg/d and was reduced linearly by the inclusion of B and BU supplements with the rate of depression greater for the B supplement (P<0.05). By contrast, the BP supplement had a curvilinear effect on hay intake, with no reduction or a slight increase initially (*SI*<0.75%W) and then a lower depression in hay intake compared to the other supplements until *SI* exceeded about 1.25%W. These trends were significant (P<0.05) for BP relative to B and BU treatments. As a consequence of these effects on hay intake total DM intake also increased linearly with B and BU, and curvilinearly with BP, supplement additions with differences between all three intake trends (P<0.05). The response relationships are described below and illustrated in Figure 4.1B:

B: **Hay DM intake (%W/d) = 1.797 – 0.520 *SI***, (R^2 = 0.795; RSD = 0.159; P<0.01);

BU: **Hay DM intake (%W/d) = 1.797 – 0.409 *SI***, (R^2 = 0.815; RSD = 0.132; P<0.01),

BP: **Hay DM intake (%W/d) = 1.797 + 0.263 *SI* – 0.483 *SI*^{2}**, (R^2 = 0.862; RSD = 0.155; P<0.01);

B: **Total DM intake (%W/d) = 1.797 + 0.480 *SI***, (R^2 = 0.812; RSD = 0.159; P<0.01);

BU: **Total DM intake (%W/d) = 1.797 + 0.591 *SI***, (R^2 = 0.915; RSD = 0.132; P<0.01),

BP: **Total DM intake (%W/d) = 1.797 + 1.263 *SI* – 0.483 *SI*^{2}**, (R^2 = 0.837; RSD = 0.144; P<0.01).

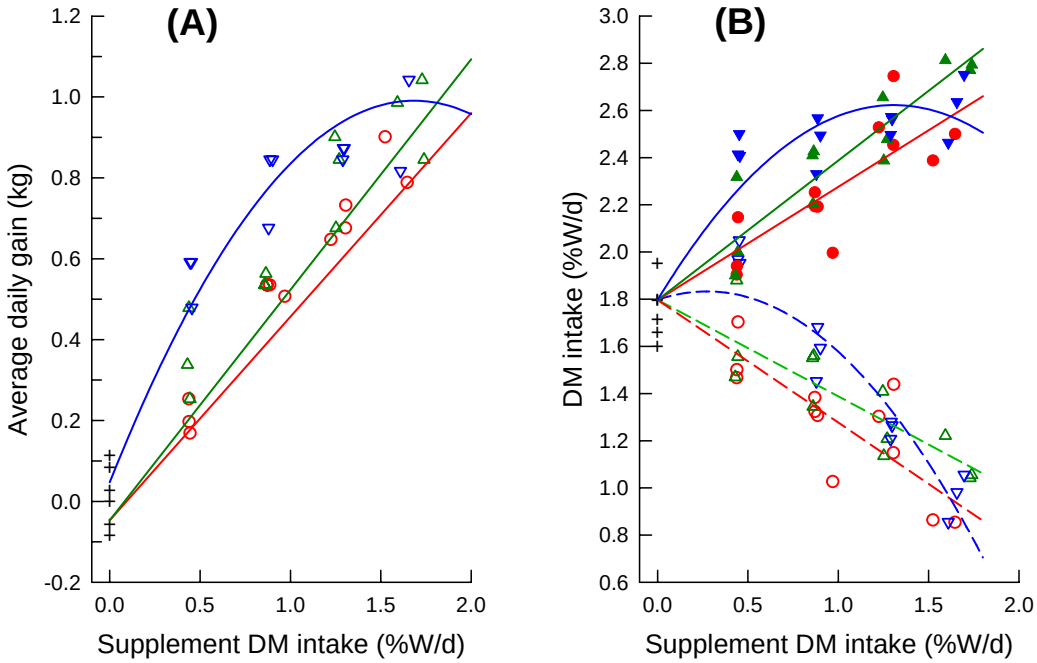

Figure 4.1. Effect of supplements on* (A) *the average daily gain (Control, +; B, ○; BU, △; BP, ▽) and* (B) *the intake of hay (dashed lines; Control, +; B, ○; BU, △; BP, ▽) and total (solid lines; B, ●; BU, ▲; BP, ♦) DM for steers given a basal diet of low quality hay. Symbols represent values for individual steers.

The effects of supplement intake (kg/d) in the 3 h prior to rumen sampling on the concentrations of ammonia-N (NH_3-N) and VFAs in rumen fluid of steers are illustrated in Figure 4.2A. Rumen NH_3-N concentration in rumen fluid averaged 49.7 mg/L for the Controls and increased in a linear manner as intake of all supplements increased, the ranking being BU > BP > B (P<0.01; Figure 4.2A). These

relationships are described below. Concentrations of VFA tended to increase linearly with supplement intake for the B and BU and there were no differences between these groups. A similar trend was apparent for the BP supplement at lower intakes but VFA concentration declined at high intakes, primarily due to low values recorded for two steers (see Figure 4.2B). The relationships for the combined B and BU treatments, and for the BP treatment, were different (P<0.05) and are presented below:

B: **NH_3-N concentration (mg/L) = 49.7 + 9.16 *SI-kg***, (RSD = 25.9; NS);

BU: **NH_3-N concentration (mg/L) = 49.7 + 80.87 *SI-kg***, (R^2 = 0.858; RSD = 30.5; P<0.01);

BP: **NH_3-N concentration (mg/L) = 49.7 + 57.00 *SI-kg***, (R^2 = 0.647; RSD = 34.1; P<0.01);

B / BU: **VFA concentration (mmol/L) = 67.4 + 3.41 *SI-kg***, (P=0.07);

(B: R^2 = 0.084; RSD = 9.89); (BU: R^2 = -0.258; RSD = 6.92);

BP: **VFA concentration (mmol/L) = 67.4 + 23.42 *SI-kg* – 14.1 *SI-kg*^{2}**, (R^2 = 0.377; RSD = 7.86; P<0.01),

where *SI-kg* is supplement DM intake (kg), in the above instances being the intake in the 3 h pre-sampling. The different R^2 and RSD values given for B and BU treatments in relation to the combined B/BU relationship for VFA concentration indicate the extent to which this relationship represents the data for each treatment separately.

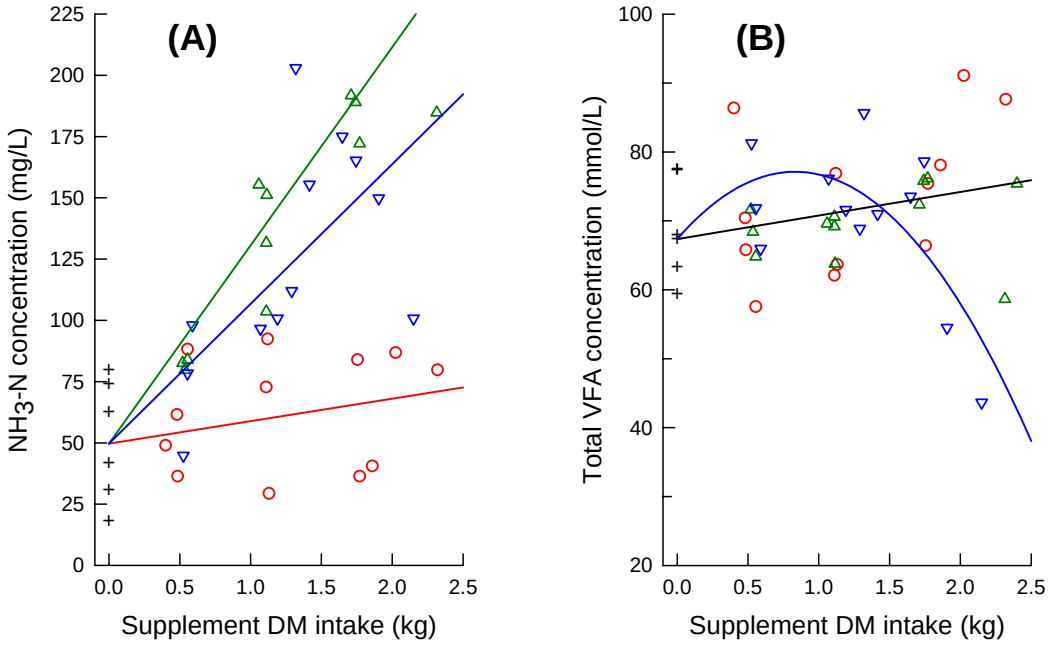

Figure 4.2. Effect of intake of different supplements (Control, +; B, ○; BU, △; BP, ▽) in the 3 h before rumen sampling on the concentration of* (A) *ammonia-nitrogen (NH_3-N) and* (B) *total volatile fatty acids (VFA) in the rumen of steers given a basal diet of low quality hay. A single relationship (black line) is shown for the combined B and BU treatments in Figure B. Symbols represent values for individual steers.

Supplement type did not affect the proportions of acetate, butyrate or minor acids in the VFA mix in the rumen. The proportion of acetate declined whilst that of butyrate and minor (other) VFAs increased as supplement intake increased. The relationships between supplement intake and the molar proportions of these various VFAs for the combined treatments are shown below:

All: **Acetate molar % = 73.55 – 2.984 *SI-kg***, (R^2 = 0.547; RSD = 2.04; P<0.01);

All: **n-Butyrate molar % = 9.95 + 1.493 *SI-kg***, (R^2 = 0.398; RSD = 1.38; P<0.01);

All: **Other VFAs molar % = 1.78 + 0.721 *SI-kg***, (R^2 = 0.636; RSD = 0.41; P<0.01).

Propionate molar proportion changed quadratically with B or BU supplement such that it was increased with increasing supplement intake (Figure 4.3), but with differences (P<0.05) between the supplement responses. The BP supplement had no effect on this VFA proportion. The response trends are as follows:

B: **Propionate molar % = 15.82 – 5.07 *SI-kg* + 3.04 *SI-kg*^{2}**, (R^2 = 0.711; RSD = 1.18; P<0.01);

BU: **Propionate molar % = 15.82 – 2.29 *SI-kg* + 1.18 *SI-kg*^{2}**, (R^2 = 0.192; RSD = 1.35; P<0.05).

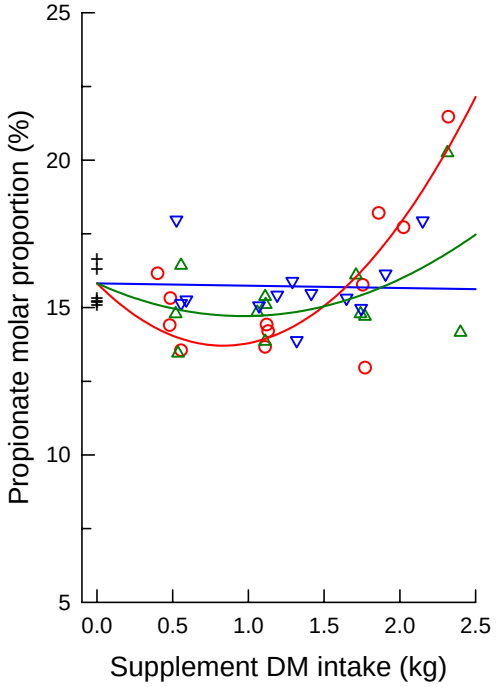

Figure 4.3. Effect of supplements (Control, +; B, ○; BU, △; BP, ▽) on the molar proportion of propionate in the rumen fluid of steers given a basal diet of low quality hay. Symbols represent values for individual steers.

There was no consistent effect of supplement type on the ratio of acetate to propionate, but the trend was for a decrease in this ratio with increasing supplement intake.

As in Chapter 2A, blood parameters were considered in relation to the intake of supplement in the 24 h prior to feeding on the morning of sampling, i.e., on the previous day, in view of the likely longer term effects of supplement on these parameters relative to rumen metabolites. The urea-N concentration in the plasma of the unsupplemented steers averaged 4.0 mg/dL and was increased linearly with increasing intakes of all supplements (see Figure 4.4). The rate of increase in urea-N concentration was greater for BP compared with BU, and for BU compared with B supplement (all P<0.01). The response trends are described as follows:

B: **Plasma urea-N (mg/dL) = 3.96 + 0.518 *SI-kg***, (R^2 = -0.288; RSD = 2.22; NS);

BU: **Plasma urea-N (mg/dL) = 3.96 + 2.360 *SI-kg***, (R^2 = 0.743; RSD = 2.27; P<0.01);

BP: **Plasma urea-N (mg/dL) = 3.96 + 3.514 *SI-kg***, (R^2 = 0.840; RSD = 2.77; P<0.01),

where *SI-kg* is supplement DM intake (kg), in the above instance the intake in the 24 h prior to feeding on the day of sampling.

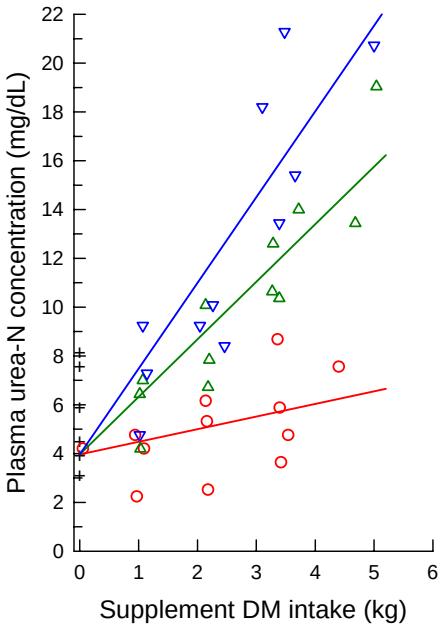

Figure 4.4. Effect of supplements (Control, +; B, ○; BU, △; BP, ▽) on the concentration of urea-N in the plasma of steers given a basal diet of low quality hay. Symbols represent values for individual steers.

Faecal starch content was negligible for unsupplemented steers (0.4% DM) but was increased by all supplements. Both B and BU were associated with a similar increasing starch composition in the faeces and a single quadratic relationship is shown below for these treatments (Figure 4.5A). The BP supplement gave a lower, linear increase in faecal starch composition which was different to the other supplements ($P<0.05$). Faecal pH averaged 7.4 for unsupplemented steers and was reduced linearly with increasing intake of all supplements, with no between supplement differences. For Control steers, faecal N averaged 1% DM and this was increased with increasing intake of all supplements. There was no difference in response to the B and BU supplements which increased Faecal N quadratically (Figure 4.5B), but both were different to the BP supplement which was associated with a steeper linear increase in faecal N content. The various response trends for the above parameters, relative to intake of supplement (%W) in the weeks leading up to sampling, are shown below:

B / BU: **Faecal starch (% DM) = 0.401 + 0.96 *SI* + 1.591 *SI*^{2}**, ($P<0.05$);

(B: $R^2 = 0.668$; RSD = 1.66); (BU: $R^2 = 0.855$; RSD = 0.95);

BP: **Faecal starch (% DM) = 0.401 + 1.696 *SI***, ($R^2 = 0.891$; RSD = 0.42; $P<0.01$);

All: **Faecal pH = 7.37 – 0.567 *SI***, ($R^2 = 0.406$; RSD = 0.394; $P<0.01$);

B / BU: **Faecal N (% DM) = 0.980 + 0.367 *SI* + 0.1645 *SI*^{2}**, ($P<0.01$);

(B: $R^2 = 0.933$; RSD = 0.103); (BU: $R^2 = 0.951$; RSD = 0.106);

BP: **Faecal N (% DM) = 0.980 + 0.843 *SI***, ($R^2 = 0.979$; RSD = 0.089; $P<0.01$);

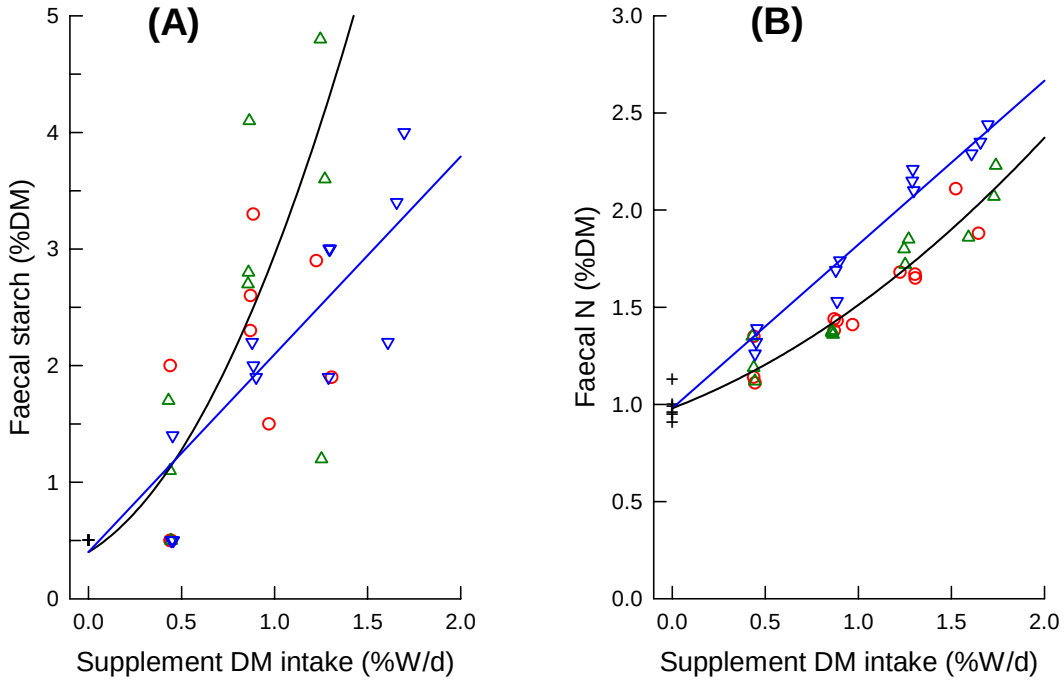

Figure 4.5. Effect of intake of different supplements (Control, +; B, ○; BU, △; BP, ▽) on the concentration of* (A) *starch and* (B) *nitrogen (N) in the faeces of steers given a basal diet of low quality hay. A single relationship (black line) is shown for the combined B and BU treatments in Figures A and B. Symbols represent values for individual steers.

Total protozoa numbers averaged 1.36 x 10^5/mL rumen fluid for Control steers and were increased linearly (P<0.01) by feeding supplement of all types, with no difference between the supplement types. For the Controls, this population comprised, on average (back-transformed values), 11.5% *Isotricha sp.*, 39.1% *Dasytricha ruminantium*, 37.9% *Entodinium* spp., 1.7% *Diplodinium sp.*, and 12.3% other (minor) species. In general, the proportion of *Entodinium* spp. increased (P<0.01) whilst all other species groups decreased (P<0.05) as the supplement intake increased, regardless of supplement type. The only treatment differences were for *Isotricha sp.* where the proportion decreased at a greater rate (P<0.05) with the BU supplement than for B and BP, with no difference between B and BP.

4B EFFECT OF PROTEIN:ENERGY RATIO ON THE INTAKE, DIGESTION AND METABOLISM OF STEERS GIVEN EITHER A LOW OR MEDIUM QUALITY FORAGE: METABOLISM STUDY

Materials and methods

Animals, treatments and experimental design

The experiments were carried out at Mt Cotton Research Farm. The experimental design was essentially the same as that described in Chapter 3B. Ten Brahman crossbred steers were used throughout. They were *ca.* 12 months old at the commencement and weighed 202 ± 5.69 (s.e.) kg and 235 ± 5.74 kg the beginning of the first and second pair of experiments, respectively. Prior to the commencement of the experiments, the steers underwent a special introductory feeding and education period to accustom them to the experimental diets and experimental housing. In addition, all steers were treated with Ivomec (1 mL per 10 kg of liveweight) to reduce internal and external parasite burdens at the beginning of this adaptation period.

As previously described (Chapter 3B), the trial design involved two incomplete 5 x 5 Latin squares, carried out concurrently, using low quality green panic hay as the basal forage (April to June 2002) followed by two more similar and concurrent, incomplete 5 x 5 Latin squares using medium quality pangola grass hay (July to September 2002) as the basal ration. The pangola grass was fertilized with urea and cut at a young stage of growth to obtain as high a quality of this tropical grass hay as possible. Upon the completion of the green panic experiments, the steers grazed pasture for three weeks before being re-allocated to new treatments for the pangola experiments.

The supplements fed were similar to two of those used in the pen study (Chapter 4A), viz., B (barley only; low P/E) and BP (barley/copra/CSM; 2: 1: 1; high P/E), and were fed at the same levels of intake except that intakes were calculated on a DM basis here and on an 'as fed' basis in the pen experiment. A summary of treatments used with each forage type is as follows:

	Supplement DM intake (%W/d)	Treatment name
Control	0	Control
Barley	0.5	B 0.5
	1.0	B 1.0
	1.5	B 1.5
	2.0	B 2.0
Barley /protein	0.5	BP 0.5
	1.0	BP 1.0
	1.5	BP 1.5
	2.0	BP 2.0

Procedures

The procedures used for each set of experiments were similar to those described for earlier metabolism studies (see Chapter 2B), except where indicated below. Each of the three measurement periods (runs) for each experiment consisted of a 14 d preliminary feeding period in individual yard pens and a 7 d collection period in metabolism cages. During the first 6 d of preliminary feeding, the grain intakes were gradually increased to treatment level to reduce the risk of acidosis. Designated treatment levels were used for the remainder of the preliminary feeding period. As previously described, steers were weighed weekly and supplement intakes were modified accordingly.

Procedures used to determine DM intake, faecal DM output, digestibility of nutrients, and urine and PD excretion, have been described earlier (Chapter 2B). No estimate of starch fermentability was made in the current study. Fractional outflow rates were not measured on the steers receiving the green panic hay but were estimated using the Cr and Yb markers for the pangola hay experiments, as described in Metabolism study 2. Immediately following the collection period, rumen fluid samples were taken 3 h and 24 h after, and blood samples 3 h after feeding, as described earlier (Chapter 3B).

The estimated degradability of CP of barley grain, copra meal and CSM were determined using the *in sacco* technique. The procedures used have been described in Pen study 3. The digestibility estimates were as follows: hay, OM and NDF; CSM and copra meal, OM and N; barley, OM, N and starch. No measurement of protein degradability for the hays was made due to the problems encountered with microbial colonisation of the feed material and its impact on N content of residues in the bag.

Chemical analyses

The methods of chemical analysis have been described previously (Chapters 2B and 3B).

Statistical analysis

The method of data analysis has been described earlier (Chapters 2B and 3B). As two different hays were used, methods relating to the analysis of data from this experiment were similar to those used in the previous metabolism experiment (Chapter 3B).

Results

The nutrient composition of the hay and supplement components, and of the mixed supplements, are shown in Table 4.2. The pangola hay (MQ forage) had twice the CP content, and slightly lower NDF content, than the green panic hay (LQ forage). Adding the copra meal and CSM to the barley almost doubled the CP content of the supplement but also increased the NDF content by virtue of the high NDF content of the copra meal.

The steers consumed all of the supplement offered and showed no signs of any ill-health throughout the experiments. Hay DM intake averaged 1.67 $\pm$ 0.033%W/d and 2.07$\pm$ 0.032%W/d for control steers given LQ or MQ hay, respectively. As supplement intake increased, there was a quadratic reduction in hay DM intake and a quadratic increase in total DM intake by steers on the LQ forage, with no difference between supplement types (Figure 4.6A). Both supplements linearly reduced hay DM intake and linearly increased total DM intake on the MQ forage (Figure 4.6B), with the B supplement associated with a greater reduction ($P<0.05$) in hay intake and lower increase ($P<0.05$) in total intake compared with the BP supplement. Total NDF intake (%W) was reduced quadratically and linearly for steers given the LQ and MQ forages, respectively, as supplement intake increased. The rate of reduction in total NDF intake was greater for B compared with BP on both the LQ ($P<0.05$) and MQ ($P<0.01$) forages. The equations describing the above response relationships are shown below:

Table 4.2. Nutrient composition of the hays, supplement components and mixed supplements (B and BP). **A description of the supplements is given in the text**

	OM	CP	NDF	EE
		(% DM)		
Low quality forage experiments				
Green panic hay	90.1	5.7	67.2	1.7
CSM [A]	93.3	43.5	28.5	2.5
Copra meal [A]	93.4	23.4	52.2	9.2
B mix	95.4	12.5	22.8	2.1
BP mix	93.0	23.4	28.6	3.6
Medium quality forage experiments				
Pangola grass hay	93.1	11.2	62.1	1.8
B mix	96.3	13.4	20.7	2.1
BP mix	93.2	24.3	27.3	3.9

[A] Same CSM and copra meal used in both sets of experiments

LQ forage:

All: **Hay DM intake (%W/d) = 1.673 – 0.059 *SI* – 0.208*SI*^{2}**, (R^2 = 0.968; RSD = 0.084; P<0.01);

All: **Total DM intake (%W/d) = 1.673 + 0.942 *SI* – 0.208 *SI*^{2}**, (R^2 = 0.974; RSD = 0.084; P<0.01);

B: **Total NDF intake (%W/d) = 1.119 + 0.2424 *SI* – 0.172 *SI*^{2}**, (R^2 = 0.847; RSD = 0.055; P<0.01);

BP: **Total NDF intake (%W/d) = 1.119 + 0.2176 *SI* – 0.115 *SI*^{2}**, (R^2 = 0.608; RSD = 0.056; P<0.01);

MQ forage:

B: **Hay DM intake (%W/d) = 2.067 – 0.626 *SI***, (R^2 = 0.977; RSD = 0.087; P<0.01);

BP: **Hay DM intake (%W/d) = 2.067 – 0.541 *SI***, (R^2 = 0.968; RSD = 0.108; P<0.01);

B: **Total DM intake (%W/d) = 2.067 + 0.374 *SI***, (R^2 = 0.939; RSD = 0.087; P<0.01);

BP: **Total DM intake (%W/d) = 2.067 + 0.459 *SI***, (R^2 = 0.938; RSD = 0.108; P<0.01);

B: **Total NDF intake (%W/d) = 1.266 – 0.184 *SI***, (R^2 = 0.909; RSD = 0.053; P<0.01);

BP: **Total NDF intake (%W/d) = 1.266 – 0.058 *SI***, (R^2 = 0.412; RSD = 0.064; P<0.05),

where *SI* is supplement intake (%W/d).

The OMD of the unsupplemented LQ and MQ forages by steers averaged 54.4 ± 0.50 and 63.7 ± 0.38%, and corresponding values for NDF digestibility (NDFD) were 57.9 ± 0.42 and 65.2 ± 0.27%, respectively. Provision of supplement increased OMD on the LQ forage base, with no supplement type effect (Figure 4.7A) whilst on the MQ forage, the BP mix was associated with a greater (P<0.01) increase in OMD than the B mix, especially at higher intakes (Figure 4.7A). NDFD was reduced in a linear fashion with both supplements on the LQ forage, the effect being greater (P<0.01) for B compared with BP supplement. There was a linear increase in NDFD with the BP supplement on the MQ forage but a linear reduction with the B supplement (Figure 4.7B), these effect being significantly different (P<0.01). The equations representing these relationships are detailed below:

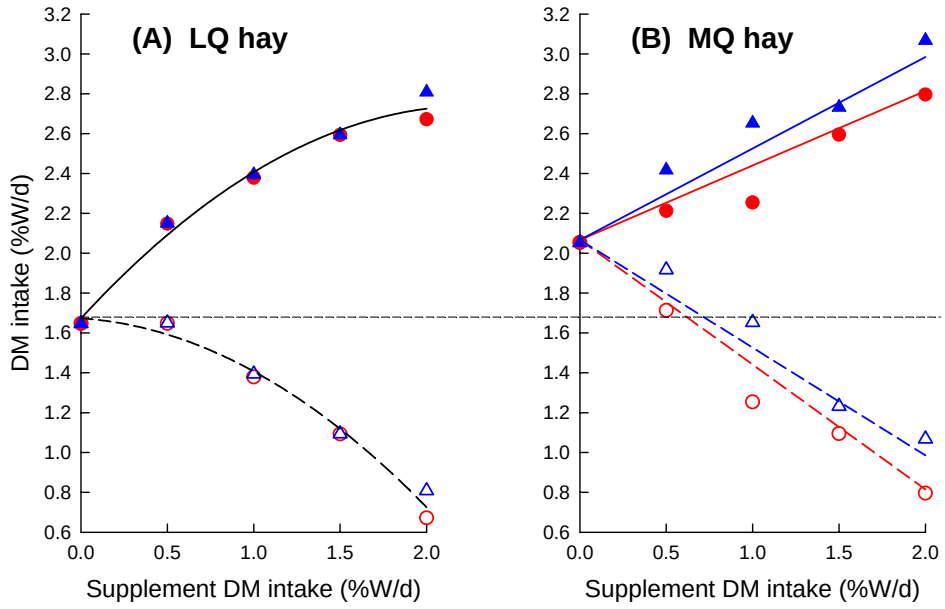

Figure 4.6. Effect of supplements on the intake of hay (dashed line; B, ○; BP, △) and total (solid line; B, ●; BP, ▲) DM for steers given a basal diet of (A) low quality hay or (B) medium quality hay. For both hay and total DM intakes, a single relationship is shown for combined B and BP treatments in Figure A. Symbols represent the mean of three steers except for the Controls (six steers).

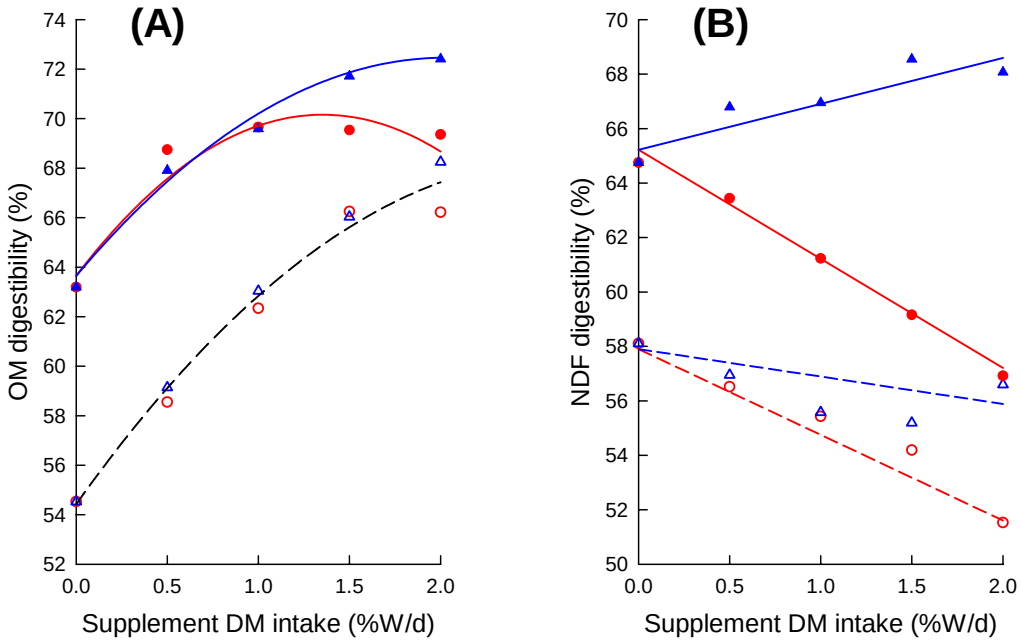

Figure 4.7. Effect of supplements on the digestibility of (A) OM and (B) NDF for steers given a basal diet of either low quality (dashed line; B, ○; BP, △) or medium quality (solid line; B, ●; BP, ▲) hay. A single relationship is shown for the combined B and BP treatments for low quality hay in Figure A. Symbols represent the mean of three steers except for the Controls (six steers).

LQ forage:

All: **OMD (%) = 54.42 + 10.34 *SI* – 1.917 *SI*^{2}**, (R^2 = 0.958; RSD = 1.302; P<0.01);

B: **NDFD (%) = 57.90 – 3.149 *SI***, (R^2 = 0.880; RSD = 0.992; P<0.01);

BP: **NDFD (%) = 57.90 – 1.008 *SI***, (R^2 = 0.282; RSD = 1.467; P=0.05);

MQ forage:

B: **OMD (%) = 63.65 + 9.62 *SI* – 3.554 *SI*^{2}**, (R^2 = 0.894; RSD = 1.118; P<0.01);

BP: **OMD (%) = 63.65 + 8.70 *SI* – 2.147 *SI*^{2}**, (R^2 = 0.973; RSD = 0.748; P<0.01);

B: **NDFD (%) = 65.22 – 4.005 *SI***, (R^2 = 0.960; RSD = 0.746; P<0.01);

BP: **NDFD (%) = 65.22 + 1.688 *SI***, (R^2 = 0.756; RSD = 0.875; P<0.01).

Total digestible OM (DOM) intake by steers increased with supplement intake but was not affected by supplement type on the LQ forage base. By contrast, on the MQ forage, supplements were associated with linear increases in total DOM intake, with the rate of increase greater for BP compared with B (P<0.05). The supplement response relationships are as follows:

LQ forage:

All: **Total DOM intake (%W/d) = 0.814 + 0.699 *SI* – 0.1251 *SI*^{2}**, (R^2 = 0.984; RSD = 0.055; P<0.01);

MQ forage:

B: **Total DOM intake (%W/d) = 1.249 + 0.318 *SI***, (R^2 = 0.973; RSD = 0.048; P<0.01);

BP: **Total DOM intake (%W/d) = 1.249 + 0.397 *SI***, (R^2 = 0.954; RSD = 0.080; P<0.01).

The estimated production of MCP averaged 186 ± 11.6 and 373 ± 12.8 g/d for steers receiving the LQ and MQ forage without supplements, respectively, and was linearly increased on both forages with increasing supplement intake. On both forage types MCP production was increased more (P<0.01) with BP than with B supplement over the range of supplement intakes (Figure 4.8A). The response curves are described as follows:

LQ forage:

B: **MCP production (g/d) = 186.1 + 181.1 *SI***, (R^2 = 0.975; RSD = 24.72; P<0.01);

BP: **MCP production (g/d) = 186.1 + 279.9 *SI***, (R^2 = 0.974; RSD = 42.02; P<0.01);

MQ forage:

B: **MCP production (g/d) = 372.8 + 168.8 *SI***, (R^2 = 0.964; RSD = 29.64; P<0.01);

BP: **MCP production (g/d) = 372.8 + 285.5 *SI***, (R^2 = 0.969; RSD = 46.56; P<0.01).

The BP mix was also associated with a greater rate of increase in the efficiency of MCP production (EMCP) compared with the B mix on both the LQ and MQ forages (P<0.01; Figure 4.8B). Mean values for EMCP for the LQ and MQ forages without supplement were 95 ± 2.4 and 115 ± 1.5 g MCP/kg DOMI, respectively. For all except the B supplement on LQ forage, EMCP exceeded the lower threshold of the feeding standard range of 130 g/kg DOMI with relatively low supplement intake. The response relationships can be summarised as follows:

LQ forage:

B: **EMCP (g MCP/kg DOMI) = 94.90 + 22.28 *SI***, (R^2 = 0.920; RSD = 5.61; P<0.01);

BP: **EMCP (g MCP/kg DOMI) = 94.90 + 75.13 *SI* – 14.97 *SI*^{2}**, (R^2 = 0.975; RSD = 7.27; P<0.01);

MQ forage:

B: **EMCP (g MCP/kg DOMI) = 115.1 + 15.72 *SI***, (R^2 = 0.962; RSD = 2.84; P<0.01);

BP: **EMCP (g MCP/kg DOMI) = 115.1 + 30.22 *SI***, (R^2 = 0.955; RSD = 5.98; P<0.01).

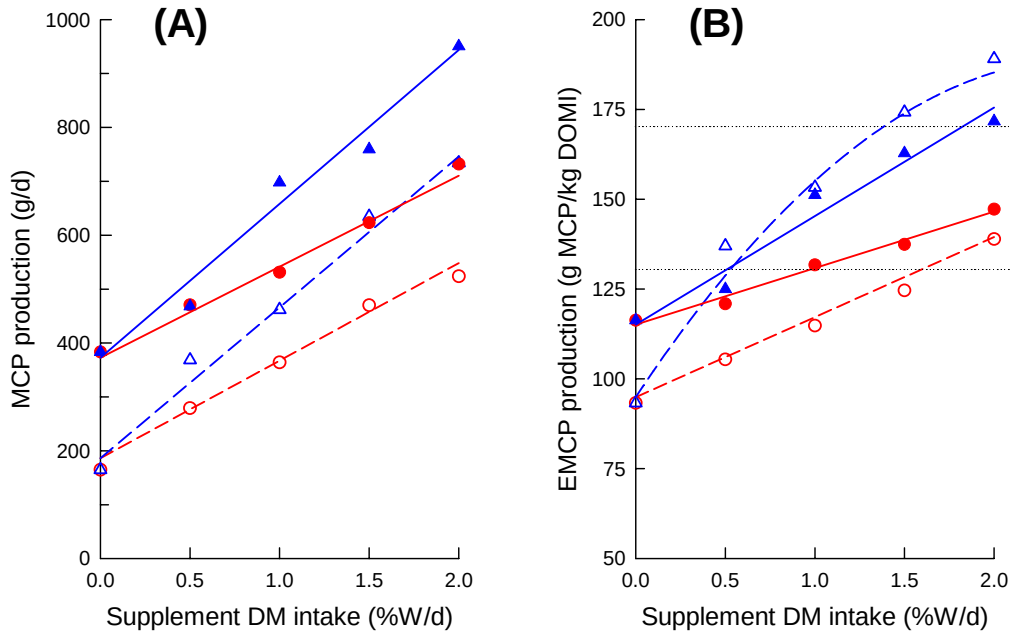

Figure 4.8. Effect of supplements on* (A) *the production of microbial CP (MCP) and* (B) *the efficiency of production of MCP (EMCP) for steers given a basal diet of either low quality (dashed line; B, ○; BP, △) or medium quality (solid line; B, ●; BP, ▲) hay. Horizontal lines on section (B) indicate the expected range for EMCP from the feeding standards. Symbols represent the mean of three steers except for the Controls (six steers).

Increasing supplement intake was associated with a linear increase in the concentration of ammonia-N (NH_3-N) in the rumen fluid of steers at 3 h post-feeding on both forage types, from a low of 47 ± 1.79 and 113 ± 2.12 mg/L for the LQ and MQ unsupplemented steers, respectively (Figure 4.9A). With both forage types the rate of increase in concentration was greater (P<0.01) with the BP compared to the B supplement. There was also a linear increase in NH_3-N concentration with supplement intake at 24 h post-feeding with both forage types (Figure 4.9B), with the rate of increase generally lower than at 3 h, and once again the response was greater (P<0.01) for BP compared with B supplement on both forage types. The response relationships are described as follows:

LQ forage: 3 h after feeding

B: **NH_3-N conc. (mg/L) = 47.1 + 30.67 *SI***, (R^2 = 0.981; RSD = 3.66; P<0.01);

BP: **NH_3-N conc. (mg/L) = 47.1 + 67.70 *SI***, (R^2 = 0.989; RSD = 6.53; P<0.01);

24 h after feeding

B: **NH_3-N conc. (mg/L) = 37.0 + 19.26 *SI***, (R^2 = 0.959; RSD = 3.39; P<0.01);

BP: **NH_3-N conc. (mg/L) = 37.0 + 38.87 *SI***, (R^2 = 0.968; RSD = 6.43; P<0.01);

MQ forage: 3 h after feeding

B: **NH_3-N conc. (mg/L) = 113.0 + 16.83 *SI***, (R^2 = 0.881; RSD = 5.65; P<0.01);

BP: **NH_3-N conc. (mg/L) = 113.0 + 52.45 *SI***, (R^2 = 0.978; RSD = 7.16; P<0.01);

24 h after feeding

B: **NH_3-N conc. (mg/L) = 65.3 + 7.22 *SI***, (R^2 = 0.861; RSD = 2.64; P<0.01);

BP: **NH_3-N conc. (mg/L) = 65.3 + 29.10 *SI***, (R^2 = 0.965; RSD = 5.09; P<0.01).

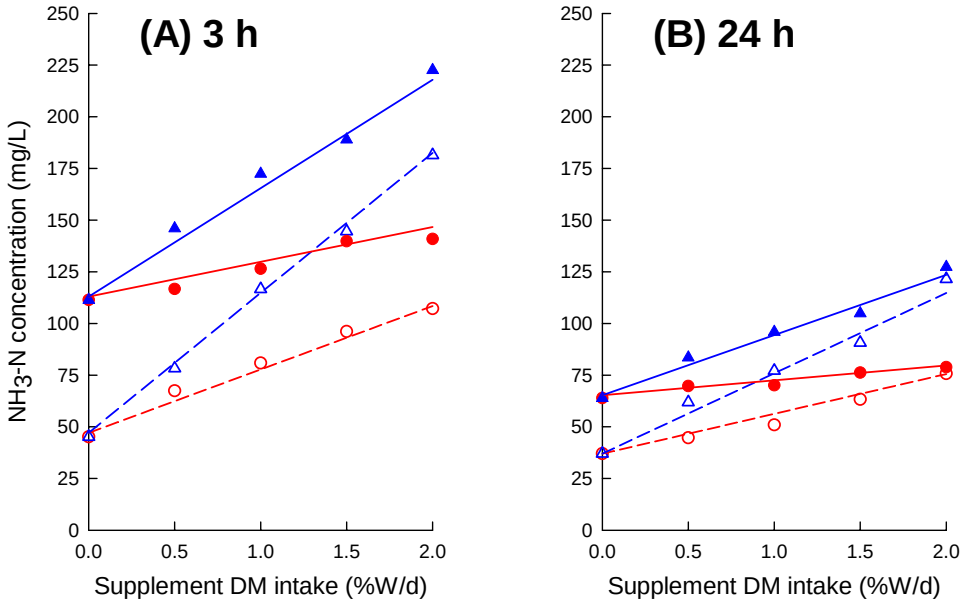

Figure 4.9. Effect of supplements on the concentration of ammonia-nitrogen (NH_3-N) in the rumen fluid of steers receiving either low quality (dashed line; B, ○; BP, △) or medium quality (solid line; B, ●; BP, ▲) hay at either (A) 3 h or (B) 24 h after feeding. Symbols represent the mean of three steers except for the Controls (six steers).

On both forage types, rumen fluid pH 3 h after feeding was reduced linearly, from a mean of *ca.* 7.0 for unsupplemented steers, with increasing supplement intake. The rate of reduction in pH with supplement intake was greater (P<0.01) with B compared to BP supplement on both forage types (Figure 4.10A). When measured 24 h after feeding, rumen fluid pH was also reduced with increasing supplement intake on the LQ forage, but without any difference in effect attributable to supplement type (Figure 4.10B). There was no effect of supplement on rumen fluid pH with the MQ forage at 24 h. The relevant relationships between supplement intake and rumen pH were as follows:

LQ forage: 3 h after feeding

B: **Rumen pH = 7.00 – 0.409 *SI***, (R^2 = 0.906; RSD = 0.112; P<0.01);

BP: **Rumen pH = 7.00 – 0.103 *SI***, (R^2 = 0.463; RSD = 0.101; P<0.05).

24 h after feeding

All: **Rumen pH = 6.93 – 0.073 *SI***, (R^2 = 0.400; RSD = 0.079; P<0.01).

MQ forage: 3 h after feeding

B: **Rumen pH = 6.95 – 0.367 *SI***, (R^2 = 0.934; RSD = 0.089; P<0.01);

BP: **Rumen pH = 6.95 – 0.063 *SI***, (R^2 = 0.222; RSD = 0.108; NS).

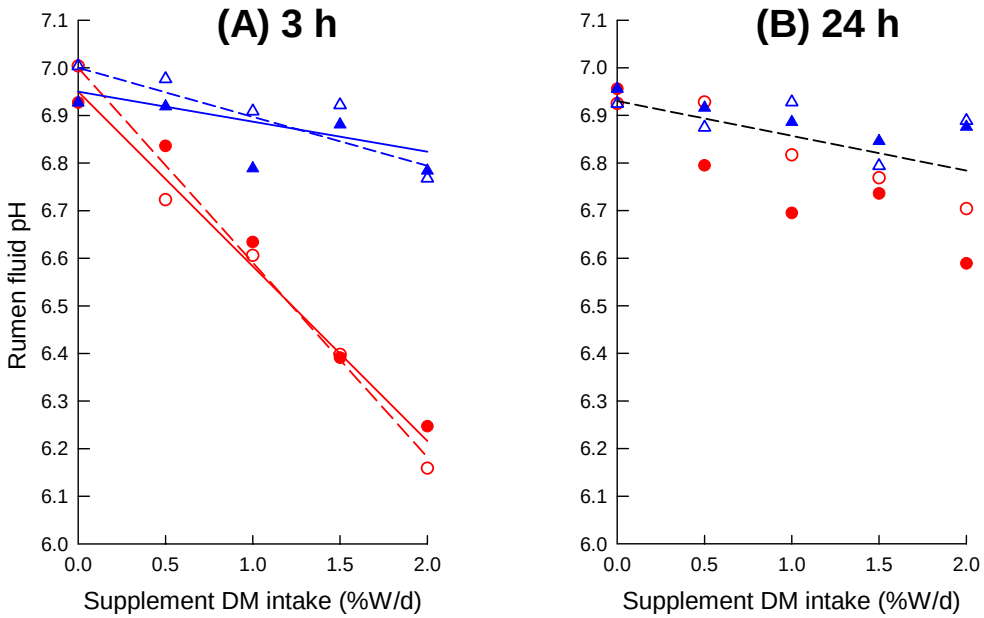

Figure 4.10. Effect of supplements on the pH in the rumen fluid of steers receiving either low quality (dashed line; B, ○; BP, △) or medium quality (solid line; B, ●; BP, ▲) hay at either (A) 3 h or (B) 24 h after feeding. A single relationship is shown for combined B and BP treatments for low quality hay in Figure B but no relationship is shown there for medium quality hay groups. Symbols represent the mean of three steers except for the Controls (six steers).

Plasma urea-N concentration averaged 7.4 ± 0.31 and 6.9 ± 0.27 mg/dL for Control steers receiving the LQ and MQ forages, respectively. The BP supplement was associated with a quadratic increase in urea-N concentration on both the LQ and MQ forages whilst the B supplement had no effect or resulted in a slight linear increase in urea-N concentration with the LQ and MQ forages, respectively (Figure 4.11). Differences between supplement types were significant (P<0.01) on both forages. The response relationship for the various supplements and forage types are as follows:

LQ forage:

BP: **Plasma urea-N (mg/dL) = 7.43 + 6.98 *SI* – 1.564 *SI*^{2}**, (R^2 = 0.925; RSD = 1.100; P<0.05);

MQ forage:

B: **Plasma urea-N (mg/dL) = 6.92 + 0.676 *SI***, (R^2 = 0.358; RSD = 0.827; P<0.05);

BP: **Plasma urea-N (mg/dL) = 6.92 + 7.322 *SI* – 1.200 *SI*^{2}**, (R^2 = 0.982; RSD = 0.650; P<0.05).

Faecal pH was reduced linearly in association with increasing supplement intake on both LQ and MQ forages. The rate of reduction in pH was greater (P<0.05) for B compared with BP on LQ forage but although the same trend was apparent on the MQ forage, the difference between supplements did not achieve significance. These relationships between supplement intake and faecal pH are shown in Figure 4.12 and are described by the following equations:

LQ forage:

B: **Faecal pH = 7.19 – 0.639 *SI***, (R^2 = 0.948; RSD = 0.128; P<0.01);

BP: **Faecal pH = 7.19 – 0.345 *SI***, (R^2 = 0.782; RSD = 0.167; P<0.01);.

MQ forage:

All **Faecal pH = 6.98 – 0.431 *SI***, (R^2 = 0.848; RSD = 0.167; P<0.01).

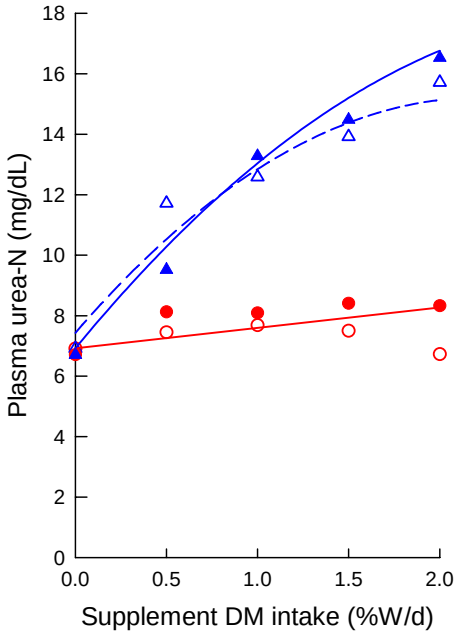

Figure 4.11. Effect of supplements on the concentration of urea-N in the plasma of steers receiving either low quality (dashed lines; B, ○; BP, △) or medium quality (solid lines; B, ●; BP, ▲) hay. No relationship is shown for the B treatment on low quality hay. Symbols represent the mean of three steers except for the Controls (six steers).

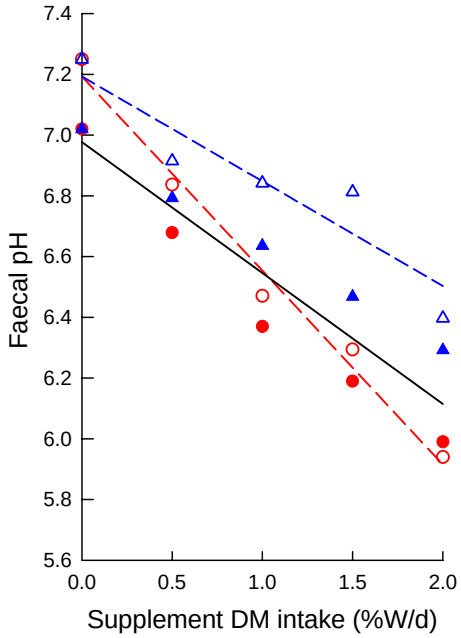

Figure 4.12. Effect of supplements on the pH in the faeces of steers receiving either low quality (dashed lines; B, ○; BP, △) or medium quality (solid line; B, ●; BP, ▲) hay. A single relationship is shown (black line) for combined B and BP treatments on medium quality hay. Symbols represent the mean of three steers except for the Controls (six steers).

Discussion: (Chapters 4A & 4B)

As outlined earlier, the aim of these experiments was to determine the effect of varying the P/E ratio in the absorbed products of digestion, on the intake of hay by steers and thus on the extent of substitution. Supplements were designed to create diets of distinctly different P/E characteristics. Barley grain was used without protein addition (treatment B) to provide a low P/E diet by virtue of its high fermentable energy, but low rumen degradable (RDP) and undegraded dietary protein (UDP), content. Conversely, the BP supplement provided additional RDP largely in the form of CSM to stimulate MCP production and also UDP particularly from the copra meal, which is reported to have a low protein degradability in the rumen. The third dietary treatment was BU which was designed to provide a similar RDP/DOM ratio to the BP supplement. Development of these diets relied on the use of various assumptions in relation to protein degradabilities and digestion coefficients for the various supplements. In addition, results from previous experiments were used to estimate the likely ratio of hay to supplement.

The table below shows the estimated P/E and ratio of RDP/DOM for the pen feeding study using recorded intakes of hay and supplement. The P/E value used here refers to the calculated ratio of CP supplied to the duodenum per kg DOM of ingested feed and as such is only an estimate of the P/E of absorbed nutrients available to the tissues for metabolism. Notwithstanding this, it is obvious that there was a clear distinction between the B and BP diets in terms of P/E, with the BU treatment intermediate, and that the diets were therefore appropriate for testing the stated hypothesis. The aim of equilibrating the BU and BP diets in terms of RDP/DOM ratio was achieved at the lower supplement intakes, but estimated values were lower for BU at higher intakes perhaps due to higher than anticipated hay intakes of the BP groups. According to these estimates, RDP/DOM was less than the lower threshold value (130 g RDP/kg DOM) in the feeding standards (e.g., SCA 1990) for optimum MCP production, at all levels of feeding for the B ration, but only at supplement intakes less than 1%W for the BU and BP rations.

Table 4.3. Estimated protein / energy ratio (P/E; g CP supply at duodenum/kg digestible organic matter) and the ratio of rumen degradable protein / DOM (RDP/DOM; g/kg) in diets of steers fed various supplements in the pen experiment

	Supplement intake (%W)				
	0	**0.5**	**1.0**	**1.5**	**2.0**
Barley					
P/E ratio	90	121	142	157	168
RDP/DOMI	68	88	101	111	118
Barley / CSM / Copra					
P/E ratio	-	158	196	214	236
RDP/DOMI		109	138	167	201
Barley / Urea					
P/E ratio	-	139	170	174	178
RDP/DOMI		106	131	148	161

Assumed protein dg: Hay – 75%; Barley – 67%; CSM – 75%; Copra meal – 40%.

Despite the absence of any added protein in the grain, steers in the B group showed a linear growth response to increasing intake of supplement and at 2%W/d intake approached a growth rate of about 0.9 kg/d. Control steers were only maintaining liveweight. This response reflected the steep increase in total DM intake, and thus of ME intake by virtue also of increasing OM digestibility, when B supplement was fed with the low quality hay. In the pen study the intake response was linear, and in the metabolism study, quadratic. These total intake responses occurred despite steep reductions in intake of hay in both studies as supplement intake increased. The combined intake and growth responses suggest that the barley alone was providing nutrients deficient or imbalanced in the rumen. Barley is extensively

fermented in the rumen and thus obviously provided the rumen microbes with a ready supply of fermentable carbohydrate which may have been expected to exacerbate an imbalance between RDP and DOM supply. However, it was also relatively high in protein content and thereby would have contributed RDP for microbial growth. Based on the assumptions used above (Table 4.3), inclusion of B alone in the ration gradually increased the RDP/DOM ratio but it never achieved the lower threshold value of 130 g RDP/kg DOM for optimal efficiency of MCP production. These predictions closely parallel actual eMCP values reported in the metabolism study where the threshold efficiency was only achieved with the highest increment of barley feeding. This is discussed further below.

Adding urea to the barley supplement (BU treatment) resulted in a further small increase in total DM intake and in growth rate in the pen study, indicating a further response to supply of RDP in the rumen. Urea inclusion also slightly reduced the rate of decline in hay intake when barley was fed to steers indicating a likely pivotal role of the NPN in balancing rumen nutrients. This treatment was not represented in the metabolism study so no information is available on actual eMCP.

By far the greatest effect on intake and liveweight gain was realised when protein meals were included with barley in the supplement (BP treatments). In the pen study the trend was for a slight increase, or at worst no effect, in hay intake to the first increment of BP feeding whereas hay intake declined linearly over the full range of supplement intakes for the B and BU supplements. This result suggests that the BP supplement was initially providing nutrients limiting in the rumen for microbial growth and EMCP responded rapidly to this supplement in the metabolism study, surpassing the 130 g MCP/kg DOM threshold value at about the 0.5%W/d intake level. However, if the intake response was mainly a function of the added RDN, a similar response should have occurred with the BU supplement. Alternatively, the protein meals may have supplied preformed amino acids or co-factors for microbial growth in the rumen. It is more likely that the intake response was a function of both increased supply of RDN and other nutrients in the rumen correcting an inherent imbalance, and also of additional amino acids of UDP and MCP origin reaching the small intestines for absorption. Egan (1965), Kempton and Leng (1979) and others have demonstrated an intake response on low quality forages to additional supply of amino acids post-ruminally. The curvilinear growth response pattern recorded here is consistent with those reported in our earlier pen feeding experiments, e.g., Chapter 1, present document and Project DAQ.100.

It is difficult to rationalise the lack of difference in hay intake effects between B and BP for the low quality hay in the metabolism study in view of the effects described above for the pen study. The hay, barley and protein meals were from the same sources and the cattle were similar. In the metabolism study, intakes were measured over a much shorter period (7 d) and whilst animals were confined to metabolism cages, but other similar studies have shown intake responses under similar circumstances. No other explanation can be offered at this stage.

The low and medium quality hays used in the metabolism study differed substantially in CP content (5.7 vs. 11.2%) and thereby also in intake for unsupplemented steers (1.67 vs. 2.07%W/d). Surprisingly, there was a difference in intake effect between the B and BP supplements with the medium quality hay, but not with the low quality forage. With the former, hay intake was depressed at a slightly lesser rate with BP than with B supplement, i.e., less substitution with BP. This differential response on MQ but not LQ hay again suggests an effect not solely originating in the rumen. The other feature of these results was the demonstration that substitution was considerably greater on the medium compared with the low quality hay. With the highest supplement intake levels, hay and total intakes were similar for the two hays, at least with the B supplement, despite the much higher intake of the medium quality hay unsupplemented. This direct relationship between forage quality and the degree of substitution has been well established previously (e.g., SCA 1990).

With the LQ hay, NDFD decreased with both supplements although more so with B compared with BP. A reduction in fibre digestion is common when a high starch source is used to supplement a high fibre diet and is thought to be associated with a reduction in cellulolytic and an increase in amylolytic activity in the rumen. This is particularly so when rumen pH is depressed, especially below 6.0. Nevertheless, OMD was increased by both supplements across the full range of supplementation reflecting the replacement of the lowly digestible hay with the more digestible grain or grain/protein meal combination. Both OMD and NDFD were considerably higher for the MQ compared to the LQ hay in the absence of supplement, and the supplement response trends were also quite different. NDFD was incrementally depressed by the B supplement but increased through the full range of intakes with the BP supplement on the MQ hay.

This disparity in results could have occurred for one or more of several reasons, viz. (i) the lower starch content of the BP (only 50% barley) compared with the B supplement had a lesser effect on lowering rumen pH and therefore on reducing cellulolysis; (ii) the protein meals in the BP supplement provided a highly digestible form of NDF; or (iii) the protein meals provided essential precursors for cellulolytic microbes thereby maintaining favourable conditions for cellulolytic activity in the rumen. Whatever the cause, the flow-on effect of higher NDFD was an increase in OMD for the BP supplemented steers across the full intake range whereas with the B supplement OMD was only increased with the first increment of feeding (0.5%W/d). Thus OMD trends attributable to supplements represent the balance between changes in fibre digestion and differences in the OM digestibility of the supplement and the basal forage it replaces, at any given supplement intake.

MCP production in the rumen of steers was greater with BP compared with B supplement on both hay types in the metabolism study, due primarily to higher EMCP production, as total DOM intake was not significantly different for the two treatments. Values of EMCP for unsupplemented steers on both hay types were in the 'expected' range (LQ: 95; MQ: 115 g MCP/kg DOM) based on previous studies by our group. Intakes of B ranging from 1.0 and 2.0%W/d were required to achieve EMCP levels within the Feeding Standards range of 130-170 g MCP/kg DOM, whereas only 0.5%W/d of BP supplement was required on both hay types. These higher efficiencies with the BP supplement suggest that the protein meals were providing key limiting nutrients for microbial growth in the rumen, as suggested earlier. Although NH_3-N concentration in the rumen was low (at 3 h post-feeding) for unsupplemented steers on LQ hay (47 mg/L), even low intakes of B or BP increased this concentration to well beyond the theoretical lower threshold for optimum microbial growth on forage diets (50 mg/L), albeit that this threshold is likely to increase with increasing supply of readily fermentable carbohydrate provided by grain. Even unsupplemented steers on MQ hay exceeded (113 mg/L) this threshold value. Admittedly, these concentrations at 3 h post-feeding probably approached peak diurnal values and much lower concentrations would have prevailed for much of the day, as indicated by those recorded at 24 h post-feeding. However, even at 24 h the MQ unsupplemented steers had mean NH_3-N concentrations of 65 mg/L and it seems unlikely that the linear increase in EMCP production was a function solely of increasing ammonia concentration in the rumen. It is more likely that the effect was also attributable to provision of other growth factors for the rumen microbes, like amino acids, peptides and growth precursors such as branched-chain fatty acids, provided by the protein supplied by protein meals and to a lesser extent by the barley.

Ammonia concentrations followed similar trends in the pen study. Higher concentrations for the BU compared with BP treatments at 3 h probably reflect the more rapid release of ammonia from urea compared to true protein sources but it is likely that the BP supplement sustained these high concentrations for longer due to slower ammonia release rates. In fact, plasma urea concentrations showed reverse trends with higher concentration for BP than for BU. This may reflect the more prolonged release of ammonia in the rumen and also release and capture of urea post-ruminally from UDP sources.

The reduction in faecal pH with increasing supplement intake in the metabolism study indicates increasing microbial fermentation of undigested starch in the large intestine. Faecal starch concentrations were lower for BP than for B or BU in the pen study, in keeping with the lower grain content (50%) of the BP ration, but the maximum starch concentration was only about 5% indicating relatively high utilisation rates for the barley grain by the animal. The higher faecal N concentration for BP compared with other treatments in the pen study probably reflects incomplete utilisation of the protein from the meals fed.

Conclusions

The extent of substitution was influenced by supplement formulation in the current (pen) studies with the high P/E supplement associated with a lower rate of depression in hay intake at low rates of inclusion. However, attributing cause and effect is more difficult and some evidence indicates an effect at the rumen level in maintaining higher hay intakes. For instance, the demonstrated higher efficiencies of MCP production with the BP compared with B supplement suggest that the protein meal was supplying key limiting nutrients to the rumen microbes, as has been discussed earlier. The greater intakes with BP compared with BU suggest that NPN was not solely the nutrient in deficit. Identifying these key nutrients will present the next major challenge. Increases in P/E could also have a feed-back effect on stimulating

intake as has been shown with amino acids infused post-ruminally. As supplement intake increased, the differences between supplements of different P/E status decreased indicating that similar mechanisms were involved, e.g., the interaction of ME from the supplement and that from the hay components of the diet. This is discussed further in the General Discussion. The net effect of these changes in intake was that steers receiving BP demonstrated a curvilinear increase in growth rate and those with B and BU a lower, linear response, these trends being similar to those reported earlier for 'energy sources' with and without added true protein.

5 EFFECT OF FREQUENCY OF FEEDING SUPPLEMENTS ON THE INTAKE, DIGESTION AND GROWTH OF STEERS GIVEN A LOW QUALITY FORAGE: PEN STUDY

Materials and methods

Animals, treatments and experimental design

This pen feeding experiment was carried out at "Brian Pastures" from September to November 2003. Commercial Brahman crossbred weaner steers (approx. 5/8 *Bos indicus*; ex Swans Lagoon Research Station) about 12 months of age and weighing 166.2 ± 1.41 kg liveweight (commencement liveweight) were used. They were vaccinated against tick fever and bovine ephemeral fever and with 5-in-1 vaccine and treated with an anthelmintic (Ivomec® Pour-On) to reduce any parasitic burdens. The steers were pre-fed a molasses-based supplement including Rumensin® to control coccidiosis during pre-trial grazing.

The experimental design was a randomised block with three blocks of 14 pens, and with the blocking, pen layout and allocation of treatments to pens as described earlier (Chapter 2A). Treatments consisted of an unsupplemented control group and three supplement types: (i) cottonseed meal (CSM), (ii) sorghum and cottonseed meal, in equal amounts (SC), and (iii) molasses (M), fed at each of two levels of mean daily intake and two frequencies. The average daily amounts fed were 0.25 and 0.50% liveweight for CSM and 0.5 and 1.0% liveweight for SC and M, with the rates calculated on an 'as fed' basis. Each supplement was fed either daily (7x) or twice a week (2x). There were three steers for each supplement treatment, and six for the control, making a total of 42 steers. All steers received a basal diet of green panic (*Panicum maximum*) hay fed *ad libitum*. The treatment plan is summarised below:

	Supplement intake (as fed) (%W/d)	Frequency of feeding (times per week)	Treatment name
Control	0	7	Control
CSM	0.25	7	CSM-Lo 7
		2	CSM-Lo 2
	0.5	7	CSM-Hi 7
		2	CSM-Hi 2
Sorghum/CSM	0.5	7	SC-Lo 7
		2	SC-Lo 2
	1.0	7	SC-Hi 7
		2	SC-Hi 2
Molasses/urea	0.5	7	M-Lo 7
		2	M-Lo 2
	1.0	7	M-Hi 7
		2	M-Hi 2

For the SC supplement, CSM was mixed (1:1, as fed) with a grain mixture comprising cracked grain sorghum plus (per 100 kg sorghum, as fed) 2 kg molasses, 2 kg bentonite, 1 kg limestone, 500 g urea, 100 g sulphate of ammonia (GranAm) and 100 g Rumensin100®. The molasses supplement comprised molasses plus (per 100 kg molasses, as fed) 3 kg urea and 1 kg sodium di-hydrogen phosphate, the latter to correct deficiencies in sodium and phosphorus in molasses. No water was added to this mixture. The proportion of urea in both the SC and M supplements was that calculated to provide sufficient RDN for complete fermentation of the supplement (not hay) component of the diet.

Procedures

The experiment consisted of a 7 d equilibration period followed by a 70 d experimental period. During the equilibration period the steers were fed Green Panic hay *ad libitum* plus 1 kg/head/d of cracked sorghum (no additives) in group pens of three steers. The procedures for blocking of pens and for allocation of steers to treatments and pens, were as described earlier (Chapter 2A) except that steers were not fasted and were allocated on full weights at the end of the equilibration period.

The same procedures were also used for feeding the hay and supplements as have been described earlier (Chapter 2A), except where indicated below. The CSM and SC supplements were fed, as previously described, from small feeders placed at one end of the main feed trough containing the hay, whilst the M mix was placed in a feed trough hanging from a side fence. All supplements were fed separate from the hay. To reduce the risk of acidosis from the SC rations, the amount of grain fed to the steers was increased in two steps over the first 4 d of the main experimental period for the higher intake group (1.0%W). The deficit in grain intake relative to stipulated intake during this step-up phase was negated over the next week by feeding higher than prescribed treatment levels during this period. To further minimise the risk of acidosis, the SC supplement was fed twice daily in equal amounts at 9 am and 3 pm; other supplements were fed once daily in the morning. The DM content of the molasses supplement fed and residued was determined by weighing 5 g of the mixture into a dried, weighed crucible, adding and mixing in *ca.* 5 mL water, placing into the crucible a piece of dried, weighed filter paper to soak up the solution, and drying in an oven at 105^{o}C for 48 h.

Intakes (as fed basis) of hay and supplement were determined on a daily basis during week 6. The hay was fed daily, according to normal practice, but residue hay was collected, weighed and returned to the trough each day. The supplements were fed in the usual way but again residues were weighed daily and returned.

Total faecal DM output for each steer was determined during week 8. All faeces on the floor (concrete) of each pen was collected each morning prior to feeding, weighed, thoroughly mixed and a representative 10% sub-sample taken and frozen. At the end of the week the daily samples were thawed, bulked for each steer, mixed in a mechanical mixer and sub-sampled. These samples were dried to constant weight at 60^{o}C, weighed to determine DM content, milled through a 1 mm screen and kept for subsequent analysis for OM, N and NDF content. The digestibilities of DM, OM and NDF were calculated. On two separate occasions during this collection period, at 24 and 72 h after feeding the twice-weekly supplement, small sub-samples of the faeces were used for pH determination. A 5 g sample was mixed with 5 mL distilled water and pH was read after 30 sec.

On day 58 of the experimental period, rumen fluid was collected *per os* from all steers using a stomach tube and vacuum pump. Feeding for the 7-times weekly groups was staggered so that sampling of each steer occurred 3 h after feeding the hay and supplement in the morning; the twice weekly feeding groups were fed on the previous day (day 57), effectively ca. 27 h before sampling. For all treatments, the total daily supplement allocation was fed in the morning. The same sampling procedures were used as described earlier (Chapter 2A) except that rumen fluid pH was determined immediately and before straining the material. A blood sample was also taken and handled in the manner described earlier (Chapter 2A). The amount of supplement consumed between feeding and rumen sampling was also determined. A further blood sample was taken from each steer, as is described above, before feeding on the morning of day 60 which coincided with 72 h after the twice-weekly supplement groups were fed (day 57) and 24 h after the 7-times weekly group were fed.

Chemical analysis

The methods of chemical analyses have been described in previous chapters.

Statistical analysis

The methods of statistical analysis were very similar to those used for the high lipid pen trial (Chapter 2A), but modified to account for the factorial structure (3 types by 2 frequencies) in the supplementation treatments and for the reduced number of levels of feeding for each combination. Specifically, because there were only three nominal levels of feeding (0, low and high) for each supplementation treatment, only linear and quadratic terms were considered in the tests for the degree of the polynomial for the model relating response to level of supplement. In addition, differences in response relationships were initially tested considering supplement type and frequency main effects and type by frequency interaction. Where the interaction and the frequency main effect were not significant, as was the case for most variables, these terms were ignored and further analyses considered only the supplement type; that is the same methods as in the other pen trials were then used to determine the simplest form of equations to adequately describe the relationships. Where the interaction was significant, the simplest form was determined from those for the six treatments by initially combining over frequency level within type where differences were not statistically significant, then over the resultant equations based on pair-wise tests. Only the plasma and rumen variables showed strong frequency effects, and as these were attributed to the time between feeding of supplement and sampling, the data for the two frequencies were analysed separately in these cases.

Results

The composition of the feed components and of the final mixed supplements is shown in Table 5.1. The hay was relatively low in quality according to its CP content. Protein content of the mixed supplements varied between 20 to 44% DM.

***Table 5.1. Nutrient composition (% DM) of the feed components and mixed supplements (SC and M).* A description of the supplements is given in the text**

	OM	CP	EE	NDF	ADF	CF	Starch	Ca	P
Hay	89.9	6.0	1.5	69.8	39.8			0.26	0.27
CSM	93.0	44.4	2.2	26.4	20.4			0.20	1.26
Sorghum	98.7	10.6	3.7	10.5	6.6	2.4		0.05	0.21
Molasses	82.3	6.2						1.08	0.11
SC mix	94.5	28.0	2.7	18.3	13.8	8.8		0.33	0.70
M mix	84.2	20.2						0.84	0.44

The weekly patterns of intake of supplement and hay for the twice-weekly fed groups are shown in Figure 5.1. Intake patterns were relatively constant across days for the groups fed daily and are not shown in this figure. All of the CSM supplement fed twice weekly was consumed on the day it was offered, as was the SC supplement fed at the lower intake level (0.5%W/d). There was a small carryover of the SC and M supplements fed at 1.0 and 0.5%W/d, respectively, to the next day whilst the M mix fed at 1.0%W/d on the Friday (4 d mix) was consumed over the next 4 d. Hay intake was relatively uniform between days for the control group and for both CSM groups. However, there was a marked reduction in hay intake on the day of feeding for the SC supplement groups, especially at the higher intake level, and intakes increased over about the next 3 d before the steers were fed again.

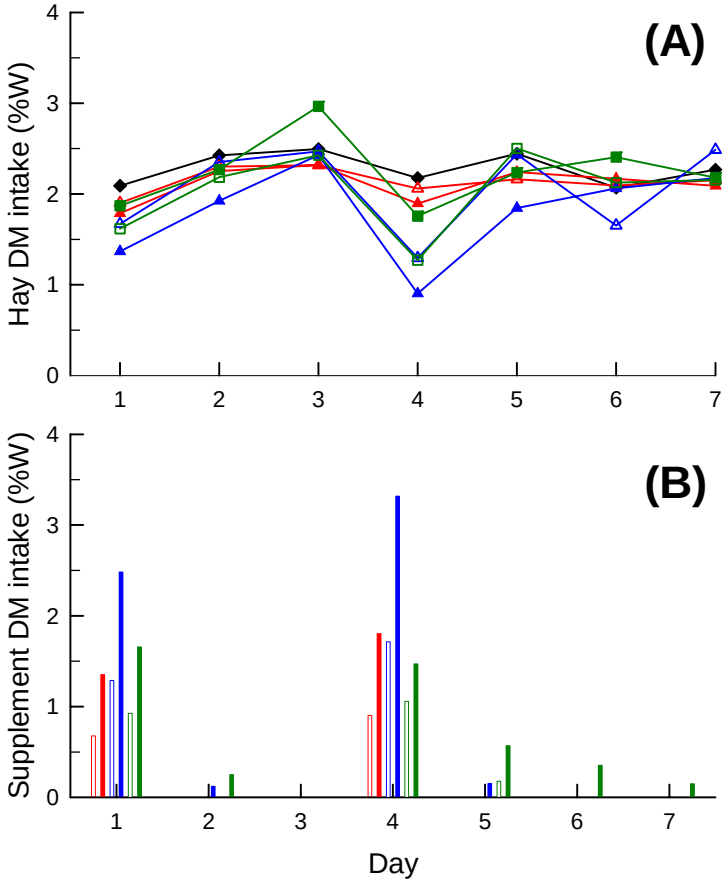

Figure 5.1. Daily intakes of* (A) *hay (Control, ●; CSM: 0.25%W/d - △, 0.5 - ▲; SC: 0.5 - △, 1.0 - ▲; M: 0.25 - □, 0.5 - ■) and* (B) *supplement (CSM: 0.25 - □, 0.5 - ■; SC: 0.5 - □, 1.0 - ■; M: 0.25 - □, 0.5 - ■) DM for steers in week 7 of the experiment. Data points represent the mean for three steers in each treatment.

Unsupplemented steers had an average growth rate of 0.22 ± 0.030 kg/d. All supplements increased growth rates although the effect of M supplement was small and non-significant (P=0.07). Inclusion of CSM or SC supplements in the diet resulted in quadratic increases in growth rate which were much greater than those for equivalent intakes of M (P<0.01; see Figure 5.2A). There were no effects of frequency of feeding on growth except for the SC supplement, where steers fed daily had higher growth rates than their counterparts fed twice weekly (P<0.05). Growth responses were similar for both CSM groups and the SC-2x group. Response relationships for the combined CSM and SC-2x treatments, for the SC-7x treatment and for both M treatments, are shown below and illustrated in Figure 5.2A:

CSM all/ SC-2x: **Average daily gain (kg) = 0.222 + 1.520 *SI* – 0.781 *SI*^{2}**, (P<0.01);

(CSM: R^2 = 0.869; RSD = 0.099); (SC 2x: R^2 = 0.967; RSD = 0.076);

SC-7x: **Average daily gain (kg) = 0.222 + 2.236 *SI* – 1.397 *SI*^{2}**, (R^2 = 0.963;RSD = 0.099; P<0.01);

M: **Average daily gain (kg) = 0.222 + 0.155 *SI***, (R^2 = 0.145; RSD = 0.085; NS);

where *SI* is supplement DM intake (%W/d). The different R^2 and RSD values given for CSM and SC treatments in relation to the combined CSM/SC relationship for ADG indicate the extent to which this relationship represents the data for each treatment separately.

Hay DM intake for the Control steers was 2.35 ± 0.051 %W or 4.21 ± 0.104 kg/d and was decreased linearly by inclusion of all supplements (see Figure 5.2B). This trend was for a greater depression in hay intake with the M supplement compared with the other two (CSM: P=0.07; SC: P=0.09), with no difference in hay intake between CSM and SC treatments. Total DM intakes followed the reverse trends

with the combined CSM and SC treatments tending to increase total intake more than M (probabilities as shown above). There was no effect of frequency of feeding on either hay or total DM intake. The response relationships are described below for the combined CSM and CS treatments, and for M treatment, and are illustrated in Figure 5.2B:

CSM / SC: **Hay DM intake (%W/d) = 2.350 – 0.412 *SI***, (P<0.01);
(CSM: R^2 = 0; RSD = 0.202); (SC: R^2 = 0.349; RSD = 0.189);

M: **Hay DM intake (%W/d) = 2.350 – 0.680 *SI***, (R^2 = 0.311; RSD = 0.217; P<0.01);

CSM / SC: **Total DM intake (%W/d) = 2.350 + 0.588 *SI***, (P<0.01);
(CSM: R^2 = 0.317; RSD = 0.202); (SC: R^2 = 0.659; RSD = 0.189);

M: **Total DM intake (%W/d) = 2.350 + 0.320 *SI***, (R^2 = 0.188; RSD = 0.217; P<0.05).

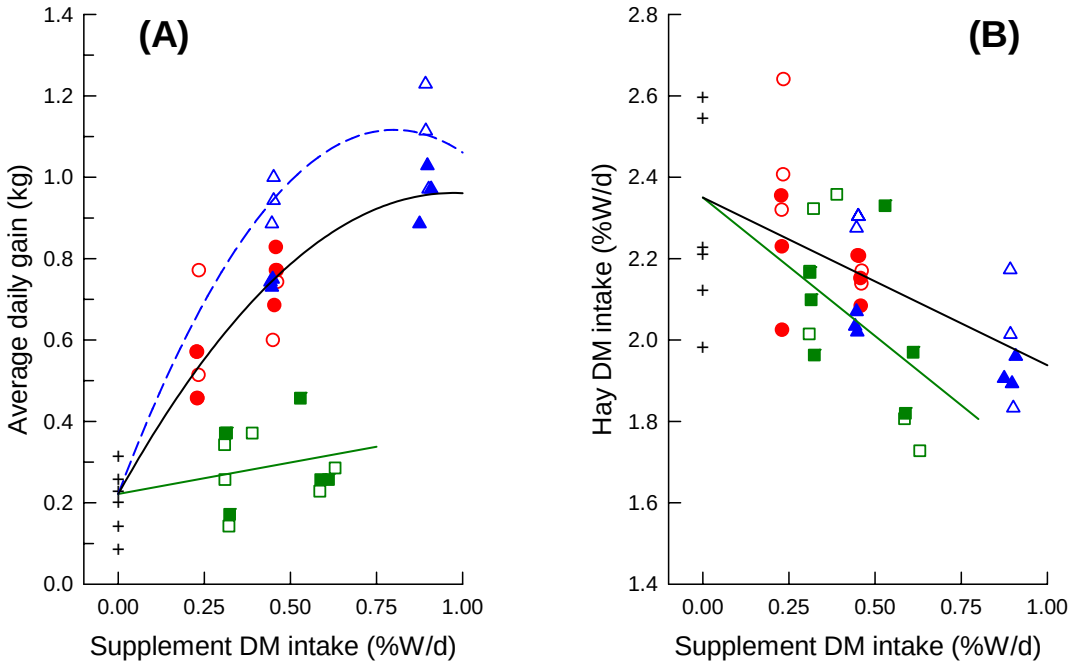

Figure 5.2. Effect of supplements fed either seven (7x) or two (2x) times weekly (Control, +; CSM: 7x - ○, 2x - ●; SC: 7x - △, 2x - ▲; M: 7x - □, 2x - ■) on (A) the average daily gain and (B) the intake of hay DM, for steers given a basal diet of low quality hay. In Figure A, a combined relationship is shown for both CSM groups and the SC-2x group (black solid line) and also for the two molasses groups (green solid line), with a separate relationship for the SC-7x group (blue dashed line). In Figure B, a combined relationship is shown for all of the CSM and SC groups (black solid line) and for the two molasses groups (green solid line). Symbols represent values for individual steers.

The digestibility of OM (OMD) was increased by all supplements (P<0.01) but there were no supplement type or frequency of feeding main effects or interactions on OMD. There were differences between supplement type for NDF digestibility (NDFD), however, with the M treatments associated with a greater depression in NDFD than the SC treatment (P<0.05) and a similar trend for the CSM treatment (P=0.08), but with no difference between CSM and SC treatments. There were no frequency of feeding effects on digestibility of OM or NDF. These digestibility trends are illustrated in Figure 5.3 and the relationships are described below:

All: **OMD (%) = 57.54 + 3.64 *SI-2***, (P<0.01);

(CSM: R^2 = 0; RSD = 2.065); (SC: R^2 = 0.164; RSD = 2.367); (M: R^2 = 0.230; RSD = 2.029);

CSM / SC: **NDFD (%) = 63.15 – 8.74 *SI-2***, (P<0.01);

(CSM: R^2 = 0.065; RSD = 2.818); (SC: R^2 = 0.562; RSD = 2.422);

M: **NDFD (%) = 63.15 – 13.53 *SI-2***, (R^2 = 0.602; RSD = 2.765; P<0.01);

where *SC-2* was the intake of supplement (%W/d) in week 8, the week digestibility was estimated.

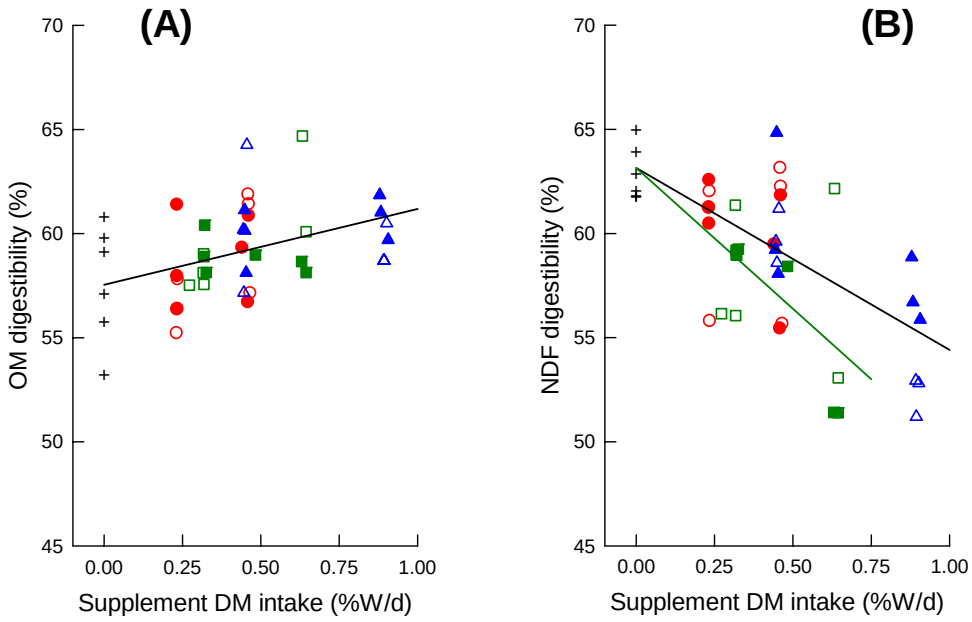

Figure 5.3. Effect of supplements fed either two (2x) or seven (7x) times weekly (Control, +; CSM: 7x - ○, 2x - ●; SC: 7x - △, 2x - ▲; M: 7x - □, 2x - ■) on the digestibility of (A) organic matter (OMD) and (B) neutral detergent fibre (NDFD) by steers given a low quality hay. In Figure A, a single relationship is given for all groups. In Figure B, a single relationship is given for the combined CSM and SC groups (black solid line) and for the M groups (blue solid line). Symbols represent values for individual steers.

Faecal pH was linearly reduced (P<0.01) by the SC supplement but there was no effect of feeding frequency (see Figure 5.4A). Neither the CSM or M supplements had any effect on faecal pH. At any given supplement intake, faecal N concentration was higher for CSM and SC treatments than for M (P<0.01) and there was a trend for CSM to be higher than SC in this attribute (P=0.06). These trends are shown in Figure 5.4B. Once again there was no effect of frequency of feeding on this parameter. The response relationships are described below:

SC: **Faecal pH = 7.75 – 0.791 *SI-2***, (R^2 = 0.414; RSD = 0.379; P<0.01);

CSM / SC: **Faecal N (% DM) = 1.309 + 0.745 *SI-2***, (P<0.01);

(CSM: R^2 = 0.821; RSD = 0.081); (SC: R^2 = 0.958; RSD = 0.064);

M: **Faecal N (% DM) = 1.309 + 0.495 *SI-2***, (R^2 = 0.724; RSD = 0.089; P<0.01).

Because of the timing of the rumen sampling, there is no valid comparison of frequency of feeding effects for the various supplement type treatments. By rumen sampling the steers on the Wednesday morning, those steers on the daily feeding regime were sampled 3 h after feeding the supplement whilst those on the twice-weekly regime were sampled about 24 h after feeding, albeit after receiving the equivalent of three times their average daily supplement intake at that time. In most cases the supplement was completely consumed on the day of feeding for these twice-weekly groups and they had no intake of supplement on the morning of sampling. Thus comparisons of rumen parameters between groups fed at the different frequencies on the basis of intake in the 3 h before sampling, or alternatively in the 24 h before sampling, were not meaningful especially as NH_3-N concentration is highly sensitive to effects of N intake in the short-term and effects are short-lived. As a result, the concentrations of these rumen metabolites are compared only within frequency treatments, i.e., for the 7x and 2x treatments separately.

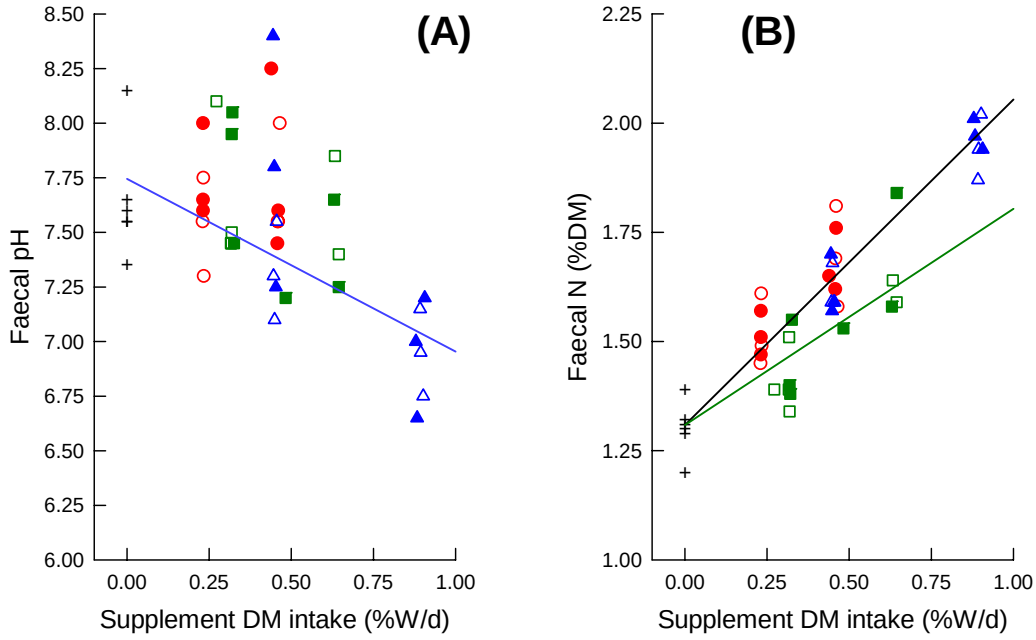

Figure 5.4. Effect of supplements fed either two (2x) or seven (7x) times weekly (Control, +; CSM: 7x - ○, 2x - ●; SC: 7x - △, 2x - ▲; M: 7x - □, 2x - ■) on (A) the pH and (B) the concentration of N, in the faeces of steers given a low quality hay. The relationship for SC only in shown in Figure A and a single relationship (black line) is shown for the combined CSM and SC treatments in Figure B. Symbols represent values for individual steers.

The concentration of NH_3-N in the rumen fluid of unsupplemented steers was relatively high at 107.3 ± 19.92 mg/L considering the low quality of the hay. For the steers fed daily, NH_3-N concentration increased much more steeply with the M supplement than for the other two (see Figure 5.5A) when considered in relation to the intake of supplement in the 3 h prior to sampling. However, differences in the response relationships were significant (P<0.01) for M relative to the SC treatment only. In the case of the twice-weekly fed groups, NH_3-N concentration in the rumen was increased linearly and to a similar extent (P>0.05) by the CSM and SC supplements relative to the intake of supplement in the previous 24 h, but there was no relationship for the M groups (Figure 5.5B). The response relationships are described below:

Fed daily (7x):

CSM / SC: **NH_3-N concentration (mg/L) = 107.3 + 69.3 *SI-kg (3)***, (P<0.01);

(CSM: R^2 = 0.542; RSD = 46.50); (SC: R^2 = 0.379; RSD = 90.14);

M: **NH_3-N concentration (mg/L) = 107.3 + 240.3 *SI-kg (3)***, (R^2 = 0.908; RSD = 41.6; P<0.01);

where *Si-kg (3)* is the supplement DM intake (kg) in the 3 h before sampling.

Fed twice-weekly (2x):

CSM / SC: **NH_3-N concentration (mg/L) = 87.64 + 18.42 *SI-kg (24)***, (P<0.01);

(CSM: R^2 = 0.628; RSD = 26.47); (SC: R^2 = 0.677; RSD = 34.63);

where *Si-kg (24)* is the supplement DM intake (kg) in the 24 h before sampling.

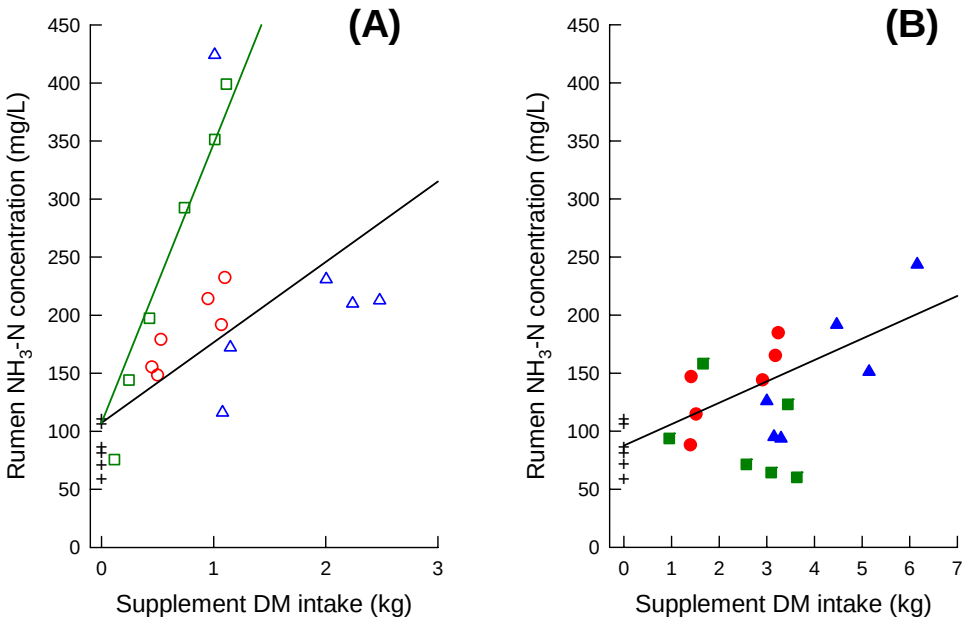

Figure 5.5. Effect of intake of supplement in either (A) the 3 h pre-sampling for steers fed seven times (7x) weekly (Control, +; CSM: 7x - ○; SC: 7x - △; M: 7x - □), or (B) in the 27 h prior to sampling for steers fed twice (2x) weekly (Control, +; CSM: 2x - ●; SC: 2x - ▲; M: 2x - ■), on the concentration of ammonia-nitrogen (NH_3-N) in the rumen fluid of these steers receiving a low quality hay. In Figures A and B, a combined relationship is shown for the CSM and SC treatments (black solid line). In Figure B, no relationship is shown for the M treatment. Symbols represent values for individual steers.

For the reasons given above, the concentrations of urea-N in plasma have also been treated separately for the daily and twice-weekly fed groups. Although plasma urea-N concentration appears less sensitive to short-term supplement intake than NH_3-N concentration in the rumen, the effects still exist and the different supplement intake patterns had a greater effect on plasma concentration of urea-N than supplement type in some cases. The effects of supplement on the concentration of urea-N in plasma, as determined at the Wednesday sampling, are shown in Figure 5.6. For the daily fed groups, this represents the concentration at 3 h after feeding commenced on Wednesday morning and it was regressed against intake of supplement in that 3 h period (Figure 5.6A). For the twice-weekly fed groups, it represented the concentration about 27 h after feeding on the Tuesday morning and it was regressed against intake in the period leading up to sampling, i.e., approximately 27 h (Figure 5.6B). In most cases there was negligible supplement consumed by these twice-weekly fed groups in the 3 h before sampling. For both the daily and twice-weekly groups, urea-N concentration was increased linearly with increasing intakes of all supplements, the rate of increase being greater for CSM compared with SC and M (P<0.01), with no difference between the latter two supplements. The response trends are described as follows:

Fed daily (7x):

CSM: **Plasma urea-N (mg/dL) = 5.778 + 8.70 *SI-kg (3)***, (R^2 = 0.753; RSD = 2.60; P<0.01);

SC / M: **Plasma urea-N (mg/dL) = 5.778 + 3.86 *SI-kg (3)***, (P<0.01);

(SC: R^2 = 0.723; RSD = 2.74); (M: R^2 = 0.377; RSD = 2.53);

where *Si-kg (3)* is the supplement DM intake (kg) in the 3 h before sampling.

Fed twice-weekly (2x):

CSM: **Plasma urea-N (mg/dL) = 5.224 + 5.09 *SI-kg (27)***, (R^2 = 0.891; RSD = 2.64; P<0.01);

SC / M: **Plasma urea-N (mg/dL) = 5.224 + 1.906 *SI-kg (27)***, (P<0.01);

(SC: R^2 = 0.801; RSD = 2.78); (M: R^2 = 0.219; RSD = 2.99);

where *Si-kg (27)* is the supplement DM intake (kg) in the 27 h before sampling.

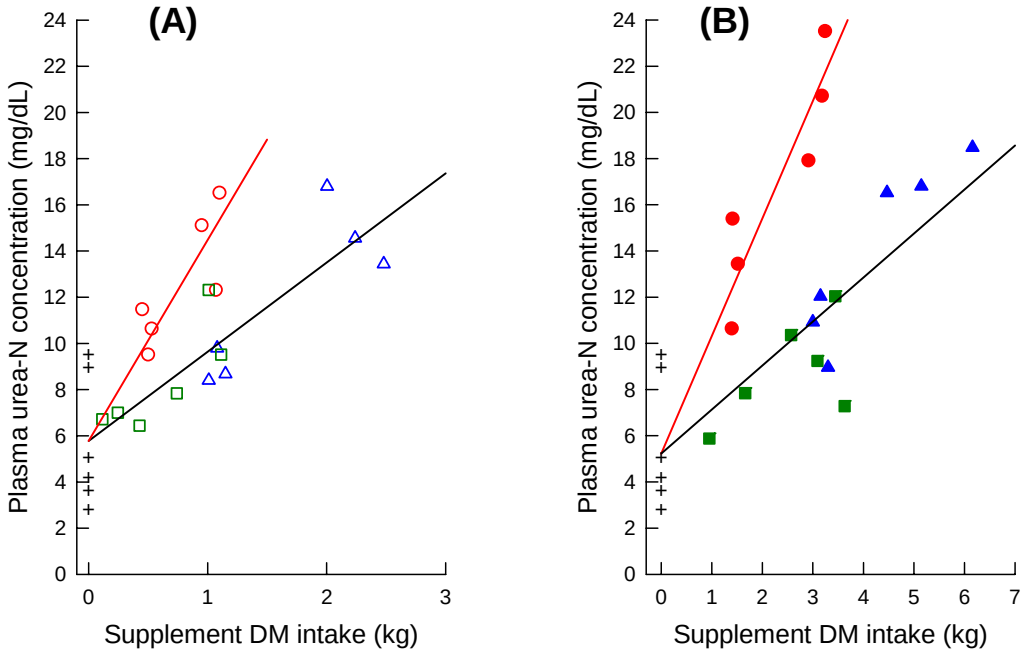

Figure 5.6. Effect of supplements fed either (A) seven (Control, +; CSM: ○; SC: △; M: □) or (B) two times weekly (Control, +; CSM: ●; SC: ▲; M: ■), on the concentration of urea-N in the plasma of steers at (A) 3 h after, and (B) 27 h after feeding (both sampled Wednesday). Symbols represent values for individual steers. In both Figures A and B, a single relationship is given for the combined SC and M groups (black solid line).

The concentrations of urea-N in plasma were also compared at two times, which corresponded for the twice-weekly fed groups to *ca.* 27 (Wednesday) and 72 h (Friday) after feeding the supplement (Tuesday morning) and for the daily-fed groups to 3 h and *ca.* 24 h (sampled before feeding) after feeding. Thus where the data are presented as means in Figure 5.7, the time of sampling refers to the times after feeding for the twice-weekly fed groups, not those for the daily-fed groups. Trends only are discussed here as the statistical analysis within treatment (frequency of feeding) groups have been detailed earlier. At the first sampling time, within supplement type, plasma urea concentrations were higher for steers receiving the higher supplement intake level and tended also to be higher for groups fed twice-weekly compared with their daily-fed counterparts. All supplemented groups had higher urea-N concentrations than the Control. The trend was for plasma urea-N concentration at this sampling time to increase in order of M (lowest), SC and CSM. At the second sampling time, concentrations of plasma urea-N for supplemented steers fed twice weekly were only equal or marginally greater than that for the Control group. Steers fed either CSM or SC daily had slightly higher plasma urea concentrations than their twice-weekly fed counterparts at this sampling time, which represented the pre-feeding concentration for the daily-fed groups. Values for the daily-fed M groups were only equivalent to the Control levels at this time.

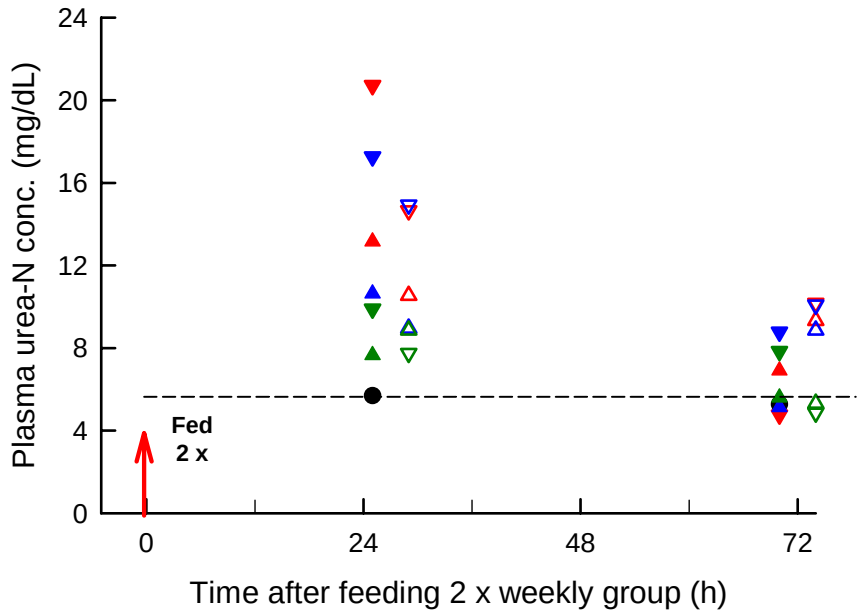

Figure 5.7. Effect of different supplements fed at either low (L) or high (H) intake level and either two (2x) or seven (7x) times weekly (Control, ●; CSM: 7x L - △, 7x H - ▽, 2x L - ▲, 2x H - ▼ ; SC: 7x L - △, 7x H - ▽, 2x L - ▲, 2x H - ▼; M: 7x L - △, 7x H - ▽, 2x L - ▲, 2x H - ▼) on the concentration of urea-N in the plasma of steers at 27 (Wednesday) and 72 h (Friday) after feeding the 2x groups (Tuesday). Symbols represent the mean values for each sub-group (n=3). Data for 7x and 2x groups are slightly off-set about the 27 and 72 h sampling time for clarity of presentation.

Discussion: (Chapter 5)

The results of this experiment have important practical implications. Supplements are usually fed less frequently than daily under commercial grazing conditions but there has been limited information available on the effects of infrequent intake of supplement on the growth rate and pasture intake of cattle. However, our finding that feeding CSM only twice weekly did not deleteriously affect intake or growth rate of steers compared with feeding daily supports the limited information already available on this type of supplement, albeit that unlike our experiment most studies only included a single level of supplement intake. The mechanism for this effect is unknown but probably involves the sustained elevation of plasma urea concentrations with infrequent feeding of the protein meals. Although in the present experiment plasma urea concentrations were elevated 24 h after feeding but had declined to baseline values for unsupplemented steers by 72 h, in other studies we have carried out the plasma urea concentrations have still been elevated up to 48 h after feeding. If plasma urea is the main mechanism involved here, the effects on growth of the animals will be a function of the amount of protein meal fed, the period over which it is consumed and the duration for which animals are without supplement.

There are some indications from our experiment that the 'energy sources' cannot similarly be fed infrequently without sacrificing some animal production. The one supplement showing an effect of feeding frequency on growth rate was the SC supplement where feeding daily produced a higher level of response than less frequent feeding. This supplement did comprise 50% cottonseed meal and so might be expected to perform similarly to the CSM treatments but plasma urea-N concentration was not elevated to the same extent after 24 h as with CSM and was similarly at baseline level after 72 h. Safety issues associated with feeding high amounts of grain infrequently precluded inclusion of a sorghum only treatment. Infrequent feeding of the SC supplement also caused reductions in hay intake for one or two days after feeding (Figure 5.1), especially at the higher supplementation level, but these did not translate into statistically significant reductions in overall hay intake. It might be expected that fibrolytic activity would be more compromised by feeding high amounts of supplement in a single meal, with adverse

consequences for overall fibre digestion and intake. However, in our study there was no effect of frequency of feeding on NDFD although there was a tendency for greater depression in NDFD when the SC supplement was fed daily compared with twice-weekly (Figure 5.3B). This may indicate that regular (e.g., daily) inclusion of a readily fermentable carbohydrate in the diet has a greater negative effect on fibre digestion than the same amount of supplement fed in fewer meals. This observation bears further examination and validation.

The lack of any similar effect of feeding frequency with the M supplement may be attributable to several factors, viz. the low overall DM intake of the molasses (maximum 0.7%W/d) and the fact that even with twice-weekly feeding, the steers often took more than one day to consume the full amount of supplement provided. Thus differences in intake pattern between the two frequency of feeding treatments were less distinct than for the SC. Low intakes of molasses have been a regular problem in our experiments where the molasses is fed in association with a forage which is provided *ad libitum*. It is significant though that molasses was associated with a greater reduction in NDFD and of hay intake than the other two supplements. This is consistent with previous findings from our own research and suggests that the soluble sugars in molasses are reducing numbers and activity of fibre-digesting microbes more than the starch from the SC supplement. This is probably mainly due to the low degradability of starch from sorghum in the rumen.

Perhaps the other most notable result from the current experiment was that feeding the SC supplement (daily) increased growth rate at a faster rate than did the CSM supplement. Thus the same amount of a 50% sorghum/50% cottonseed meal supplement (SC) stimulated growth more than 100% cottonseed meal (CSM) which was providing more CP. The reason for this finding is unclear. It is doubtful that the energy content of the supplements was very different as the feeding tables indicate CSM (11.3 MJ/kg) has only slightly lower M/D than sorghum (12.4 MJ/kg) and our work and that of others suggests these values for sorghum are often overestimated in view of the low starch degradability in the rumen and total tract. Furthermore, hay intake and total DM intake were not different for the two supplements so ME intake should also have been similar at similar supplement intakes. This is confirmed by the similar effects of the various two supplements on OMD. The practical implications of these findings are that, after a certain threshold in protein inclusion is reached, further production increments can be achieved with the less expensive option of an energy source like grain or molasses. Determining this threshold is the challenge.

One of the important benefits of including protein meals in the supplement is the maintenance of NH_3-N concentrations in the rumen at optimum or greater levels for longer than occurs with a NPN source. In the present experiment, steers fed M supplement (which included urea) daily had very high NH_3-N concentrations in the rumen 3 h after feeding whereas those fed M twice-weekly had low concentrations 24 h after being given access to the supplement. This confirms that NH_3-N concentrations peak soon after urea ingestion but also decline rapidly in the absence of further intake. By comparison, the steers receiving either CSM or SC had lower concentrations at 3 h (daily-fed) but higher concentrations at 24 h after feeding for the twice-weekly fed groups. These higher concentrations at 24 h for the SC and CSM treatments probably reflect both slower breakdown of the protein to ammonia and also possibly some recycling of urea back to the rumen. Plasma urea concentrations with these supplements increased steeply and in direct proportion to the amount of cottonseed meal fed whereas only small increases in concentration were observed with the M supplement.

Conclusions

This experiment has confirmed that intake and growth rate of steers is unlikely to be disadvantaged if the feeding of protein meals like cottonseed meal is changed from daily to twice-weekly. It is possible that the frequency of feeding this supplement type can be reduced even further, but factors like level of intake and effects on plasma urea concentration may determine this. On the contrary, there is evidence that feeding the ‘energy sources’ like grains or molasses less frequently than daily could have a negative effect on growth rate and this needs to be explored further as it has important practical implications.

6 GENERAL DISCUSSION

Growth response curves

The results of the foregoing studies have contributed to the growing bank of information available on the responses to supplements by growing steers receiving, in the main, low quality tropical forages. In so doing, they have added to the body of information already available from our previous grazing, pen feeding and metabolism studies carried out under MLA Project DAQ.100. This database represents a valuable resource for use in formulating supplementation strategies for cattle in northern Australia, but its value extends to all tropical and sub-tropical regions of the world. The response curve approach used throughout lends itself well to incorporation into decision support systems aimed at identifying most cost-effective supplementation strategies for any desired production target.

The major response relationships established in Project DAQ.100 and in the current project are shown in Figure 6.1. Different relationships are shown for the protein meal supplements and for the 'energy sources', the latter including the grains and molasses supplements. From these different relationships, a generic (average) response curve has been derived to represent each of the different major supplement types (Figure 6.1B). They illustrate the linear nature of the relationship for energy sources and the curvilinear nature of the protein meal curves. Whilst there is some variability associated with these curves, especially for the protein meals, they represent a useful starting point for predicting growth of cattle given these supplement types.

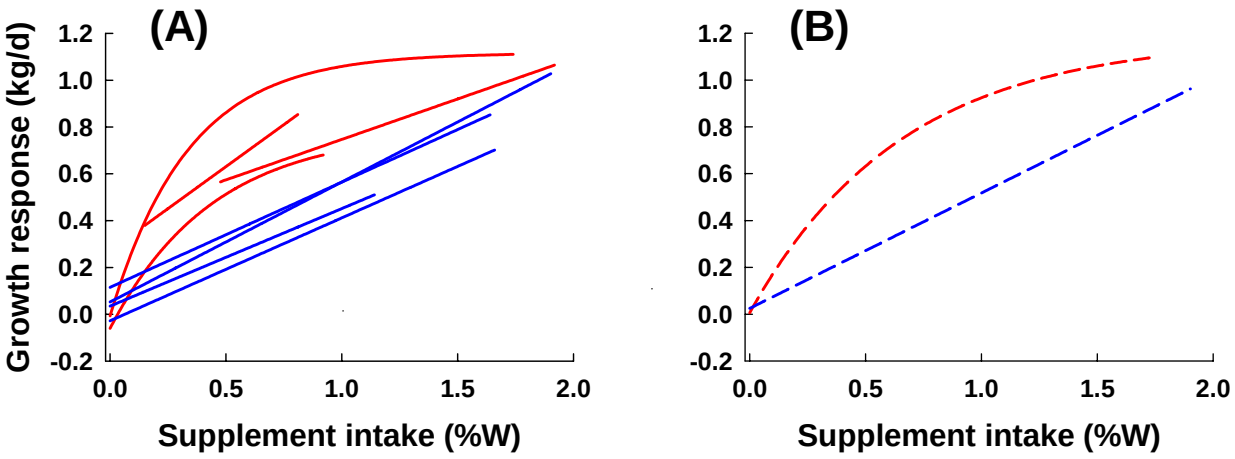

Figure 6.1. Effect of intake of supplements of protein meal (red lines) and 'energy sources' (blue lines) on the growth response by steers given a low quality forage. Relationships shown in Figure A represent responses recorded in different experiments and those in Figure B represent the relationships representative of the combined experiments.

It is not surprising that there is some variability between experiments but the reasons for this between-experiment variability are not clear. Even where the same supplement, CSM, has been fed there have been some differences in the response pattern. In all of the experiments included in Figure 6.1, the forages used had similar characteristics, viz., they were low quality tropical hays, usually <1.2% N and OMD of 52-57%. Either Rhodes grass or green panic hay was used. Differences in the cattle used may explain some of the variability, for instance their breed, previous history and extent of compensatory growth. Alternatively, some feature of the hay not described by the chemical analysis, e.g., leaf : stem ratio, may have contributed to the difference. Understanding this variability in response is important but at this stage the generic response curve provided in Figure 6.1B provides a useful 'average' response for the various supplement types which can be used in ration formulation.

The question most asked by producers will be the converse of that answered in the generic curves shown in Figure 6.1B, viz., for a desired growth response, what amount of supplement is required. To take this a

step further, the question becomes – what is the least costly alternative to achieving this growth response. Thus response curves shown in Figure 6.1B need to be realigned (with growth response now on the x-axis) and costs allocated to the various supplements so that the supplements can be compared on the basis of cost per unit of growth response. As costs are different for every beef enterprise according to factors such as proximity to supply, and are continually changing, some flexibility needs to be built into these response curves to accommodate these variations in costs. As one of the outcomes of the current project, a simple Excel spreadsheet Least-Cost Ration Formulator has been developed to assist producers and their advisers in making informed, objective decisions on (i) whether to feed supplements at all, and (ii) if so, what supplement to feed based on relative costs per day (c/d) and costs per kg (c/kg) additional response. The latter comparison allows producers to compare costs of producing the additional liveweight against the likely return from the extra gain. It should be stressed that at this stage the spreadsheets apply primarily to the young, growing animal on low quality tropical grass pasture and should not be extrapolated to finishing (fattening) cattle or animals on medium to higher quality pasture. It is also important to recognise that the response relationships were derived largely from pen-fed animals with no allowance made for the activity of walking etc., so the spreadsheet is a guide only but a valuable one just the same. The output of this spreadsheet is shown in Appendix 1.

Most of the discussion to this point has centred around the responses to individual supplement types, i.e., protein meals and energy sources. However, the final supplement that best fits the requirements for growth response and cost-effectiveness will probably be a mixed supplement incorporating both major types. This would incorporate the higher responses to protein meals at low intakes and the lower cost of an energy source relative to protein meal at high intake, where the growth responses to the two types of supplement are comparable. It would be envisaged then that the progression with increasing supplement intake would go from protein meal alone, to mixed protein meal/energy source to energy source alone. The transition between these stages would vary with the relative costs of the component supplements.

Initially it was envisaged that the combined supplement would give a response somewhere between that of the protein meal and energy source fed alone. However, the results of the frequency of feeding experiment suggest otherwise. In that study the response to a mixed sorghum/CSM supplement was greater than that to CSM alone at any supplement DM intake. This is illustrated in Figure 6.2 where the growth response to sorghum/CSM is compared with that to CSM alone, with either supplement DM intake or CSM DM intake on the X-axis. It is unlikely that this finding was due to the higher ME intake from the supplement as the energy density of rolled sorghum has been shown to be quite low (8-9 MJ/kg DM) due to the high excretion of starch in the faeces. Thus it is likely that the energy density of CSM is greater than that of sorghum/CSM combined. This finding suggests that there may be some synergistic effect of feeding a starch and true protein source together, perhaps at the rumen level where the microbes benefit from availability of both nutrients. This bears further investigation as any substitution of grain (or molasses) for protein meal, without sacrificing animal growth, would represent a significant cost saving.

Substitution

Recent projects have similarly now contributed to a large database on the effects of supplements on the intake of the basal forage. In the current project the major emphasis has still been on the lower quality forage but the metabolism studies were designed to provide additional information on medium to higher quality forages. An attempt is made here to identify some of the key principles operating in the interaction of supplement and basal forage intake, i.e., those influencing substitution.

The first point to be made is that all supplements, regardless of type or nutrient composition, will reduce forage intake when their intake is increased sufficiently. Secondly, the extent to which they affect forage intake varies widely. This was well demonstrated initially in the early pens studies (DAQ.100) comparing barley, sorghum, molasses and cottonseed meal. As an example, barley was associated with a much greater substitution rate than sorghum but this was not related to effects on animal growth, where barley was superior. The current project delivered similar results. Despite the very basic nature of this observation, it is often overlooked when the likely effects of supplement on forage are considered in the context of a predictive exercise. Our findings have provided clear demonstration of the differences between supplements under feeding conditions encountered in northern Australia and, because of the response curve approach taken, the information can be directed to modelling likely effects on voluntary intake. Some possible reasons for these differences in substitution are discussed below.

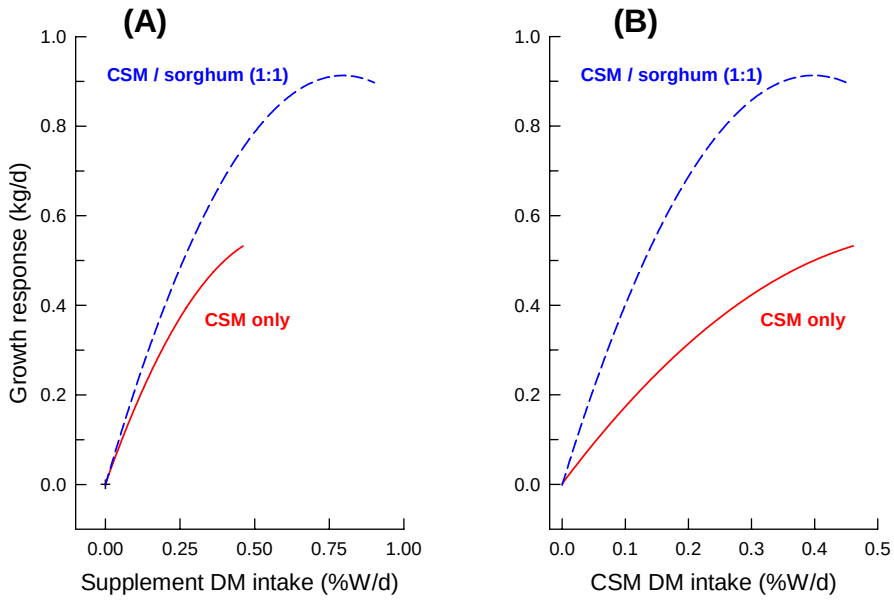

Figure 6.2. Effect of (A) supplement DM intake or (B) cottonseed meal (CSM) DM intake on the growth response by steers to supplements of CSM alone (red line) or sorghum/CSM (1:1; w/w, as fed; blue dashed line). The CSM relationships represent the combined response for steers fed daily and twice-weekly as there was no effect of feeding frequency; the sorghum/CSM relationship is for those fed daily only.

The influence of forage quality (e.g., CP content and digestibility) on substitution has been well established previously, e.g., SCA (1990) and was confirmed in the metabolism studies conducted here. As a general observation, the rate of substitution increased with the quality of the forage base. Thus although forage intake predictably increased with forage quality in the absence of supplement, the depression in forage intake when supplements were fed also increased with forage quality. Consequently, at higher levels of supplementation, differences in total intake between high and low quality forages were often negligible.

In our experiments, there were two main response patterns relating forage and supplement intake. The first (Type 1 response) described a linear reduction in forage intake through the whole range of supplement inclusion rates and applied to (i) all supplements, regardless of type, fed with medium to high quality forages and (ii) all 'energy source' supplements irrespective of the quality of the basal forage. A single value for the substitution rate could then be ascribed to the supplement in these circumstances, where substitution rate is the unit reduction in forage intake per unit intake of supplement or the slope of the regression line relating forage and supplement intake. The second response type (Type 2 response) was a quadratic relationship whereby there was low or no reduction or even possibly a small increase in forage intake when supplement intake was low (say up to 0.3 – 0.5%W/d) and then a steeper, relatively linear reduction in forage consumption as intake of supplement increased. This response type was restricted to the lower quality forages and protein meal supplements. It is proposed that the latter response type represents a two-stage effect of supplement on forage intake with the transition between stages occurring at about 0.3 – 0.5%W/d of supplement intake. This response curve precludes estimation of a single value for substitution rate although a value could be estimated for the second phase of the response curve where the curve approaches linearity. These initial low levels of substitution seem to occur with forages low in RDP (relative to DOM) and where the supplement is correcting this N deficiency in the rumen. Beyond this point of balancing nutrients in the rumen, substitution effects seem to occur in the same way as with the higher quality forages.

These intake responses to supplement have been explored by other research groups including Moore *et al.* (1999). In a modelling exercise, these researchers established empirical multiple regression equations

to estimate the effects of supplement on voluntary forage intake. They found that where supplements increased forage intake the ratio of total digestible nutrients (TDN) to CP (TDN:CP), an indicator of the amount of N relative to available energy, in the forage was > 7, i.e., a deficit of N relative to energy. Forage TDN can be assumed to be equivalent to digestible OM. Thus in our studies the green panic hay used generally had a TDN:CP ratio of between 10 and 11, indicating a N deficiency relative to available energy and also suggesting a likely increase in forage intake with provision of a N supplement. The green panic hay used in the site of digestion pen study (Chapter 3A) had a lower estimated TDN:CP ratio of only 7. If general trends in our experiments are considered, the instances of no depression (Chapters 2A, 2B and 5) or slight increases in hay intake (Chapter 4A and 4B), at low supplement intake, all seemed to occur with forages having a TDN:CP ration in excess of 7 and where the supplement was a high-protein source, thus supporting the conclusions of Moore *et al.* (1999).

These workers also deduced that supplements decreased intake when (i) the forage TDN:CP ratio was < 7, (ii) forage OM intake fed alone was > 1.75%W, or (iii) supplement TDN intake was > 0.7%W. With both the medium to higher quality hays used in our experiments (TDN:CP = 5.1 – 5.7), hay intake was depressed over the full range of supplement intakes (Chapters 3B and 4B). Furthermore, even on the N deficient hays and with high-protein supplement fed, hay intake was depressed when supplement intake exceeded between 0.25 and 0.5%W/d which translate with these supplements to a TDN intake of about 0.14 – 0.37%W. Thus the depression in forage intake occurred well within the threshold suggested by Moore and co-workers. On the third aspect of forage (OM) intake fed alone, in our experiments most of the low quality hays had intakes in the range 1.5 – 1.8%W/d with one high value at 2.1%W/d (Chapter 5). The medium quality hays used had corresponding intakes of 1.9 – 2.0 %W/d. Our results generally agree with the proposal of Moore and colleagues in that there was no increase in forage intake with any supplement type on the medium to higher quality hays, but the findings of the experiment reported in Chapter 5 (unsupplemented forage OM intake 2.1%W/d) tend to be at variance in that there appeared little reduction in hay intake until supplement intake exceeded about 0.25%W/d, despite the statistical analysis suggesting an overall linear decline in hay intake with increasing supplement intake. In another series of experiments in which CSM was fed with a range of forage types ranging from very low (mature Mitchell grass) to high (ryegrass) quality (J. Gibbs, pers. comm..) it was found that intake of hay was not increased with supplement when DM intake of the hay fed alone exceeded 1.8%W/d, equivalent to about 1.6%W/d on an OM basis. Thus in the main there is general support for the conclusions of Moore *et al.* (1999).

The equations derived by Moore *et al.* (1999) have been applied to the data sets derived in the current and some previous projects and the results are shown in Figure 6.3. For this exercise, the results have been separated for the 'energy sources' (Figure 6.3A) and the protein meals supplements (Figure 6.3B). In general there was good agreement between the recorded forage OM intakes and those predicted using the published equations for the energy sources, but not for the protein meals. In the latter case, small changes in recorded intake were sometimes associated with very large increases in predicted intake, and *vice versa*. The reason for these sometimes large deviations from the predicted response are not clear but probably relate to the fact that the intakes of protein meals used in the current studies often go well beyond those of the data base used in deriving the equations. Thus if the equations are predicting a stimulus or minimal effect of protein meal on forage intake because the data base operated within these narrow intake boundaries, it would not be surprising for poor predictions when much higher intakes are used. Whatever the reason, predictions for this supplement type need further development. We are collaborating with Moore (Dr J.E. Moore, Oklahoma State University, Stillwater OK) and colleagues in this endeavour and making our data available for inclusion in a wider data base.

In their paper, Moore and his colleagues do not attempt to explain the reasons for the forage intake responses to supplement inclusion in the diet but others have advanced intake regulation models in an endeavour to explain these effects. The intake relationships described in experiments from this and the previous project are to a large extent consistent with the conceptual model of intake regulation proposed by Weston (1996). He suggested a model of regulation based on the opposing signals to the brain of (i) the amount of digesta in the rumen (rumen load) and (ii) the animal's energy deficit, this deficit being the difference between the capacity of the animal to use energy and the useful energy intake (NE or DOM). The capacity of the animal to use energy is a function of the breed, physiological state etc. of the animal and is determined by its ability to dispose of this energy. Weston proposed a direct relationship between the energy deficit and the rumen digesta load, within the boundaries set by the point of zero energy deficit (forage providing sufficient energy to meet the capacity of the animal to dispose of energy) and the upper

physiological load limit (see Figure 6.4). Consequently, when energy-rich concentrates are fed, the energy deficit is reduced and rumen load is also reduced. This reduction in rumen load is associated with a proportional reduction in forage intake, as the two have been shown to be proportional for a given forage type. Thus in the context of the current results, the greater the energy density of the supplement (DOM or M/D), the greater the decrease in energy deficit for unit intake of supplement and the greater the depression that could be expected in forage intake. This explains the generally greater substitution effects with more digestible supplements, e.g., a unit intake of barley would have caused a greater reduction in energy deficit than the less digestible sorghum and thus a greater reduction in rumen load and hay intake.

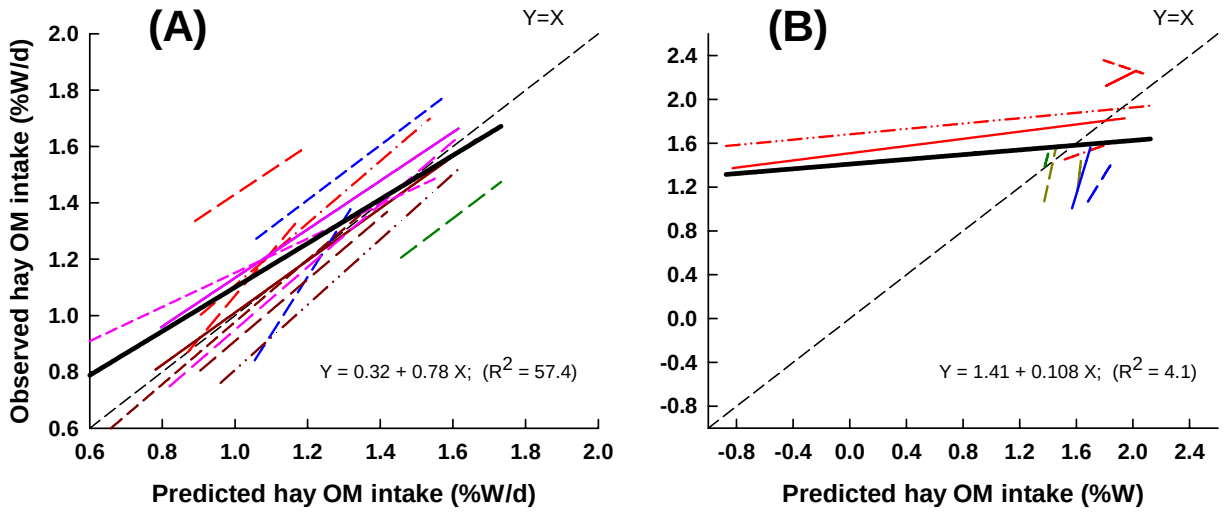

Figure 6.3. Regression of observed forage OM intake on that predicted with the empirical multiple regression equation of Moore* et al. *(1999) for forages supplemented with (A) energy sources or (B) protein meals. Separate lines relate to separate supplements within various experiments. The bold black lines represent the total data set in each figure and the equations for these lines are shown.

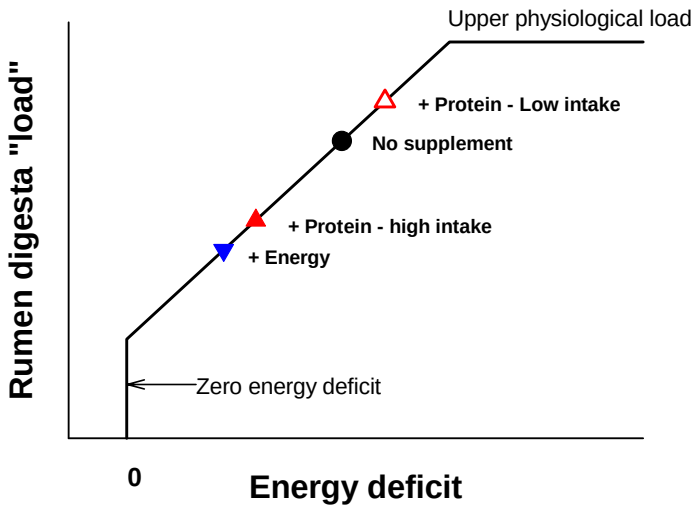

Figure 6.4. Conceptual relationship between the magnitude of the rumen digesta 'load' and energy deficit for forage diets, after Weston (1996). Plots represent hypothetical values for animals with no supplement and receiving either 'energy' supplement or small or large amounts of protein meal which at low intake corrects a N deficiency in the rumen.

The situation with a deficiency in the rumen, e.g., protein, may be quite different in that this deficiency impairs the capacity of the animal to dispose of energy. Thus when a protein supplement is fed at low intake sufficient to overcome the N deficit in the rumen, the energy deficit may increase due to the animal's greater capacity to dispose of energy and the greater difference between this capacity and NE intake. As a consequence, 'load' would increase (relative to unsupplemented animals) and be associated with an increase in forage intake. As intake of the protein meal is further increased beyond the point of correcting the N deficit, the energy deficit decreases in line with the increasing energy supplied by the supplement and rumen digesta load is also reduced in the same way as for 'energy source' supplements. Hence substitution occurs similarly for energy sources and protein meals at higher intakes. This model of Weston's is consistent with our findings of a linear reduction in forage intake when energy sources are fed with forages of all qualities, or when either type of supplement is fed on higher quality forages, and also with the quadratic effects described here for protein meals fed with low quality (low CP) forages.

Despite the wide variation in supplement and forage intakes with different types and levels of intake of supplement, the relationship between estimated energy intake and energy retention (substitute liveweight gain) followed the expected direction in keeping with the general laws of thermodynamics. As an example, where barley, barley/urea and barley/protein meal were fed in the pen study described in Chapter 4A, different forage and total intake and steer growth rate patterns were recorded (see Figure 4.1) but the relationships between energy retention and intake were similar across supplement type (see Figure 6.5), confirming that animal growth was largely a function of energy intake and the major difference between supplements was in the way they affected forage and thus total intake. The small variations in individual supplement relationships shown probably reflect errors in estimating the M/D of the different supplements. It should be noted that such relationships generally apply to changing intakes of a diet of constant energy density (M/D) but in our experiments the M/D would have increased with increasing supplement intake. Nevertheless, it shows that the general principles apply.

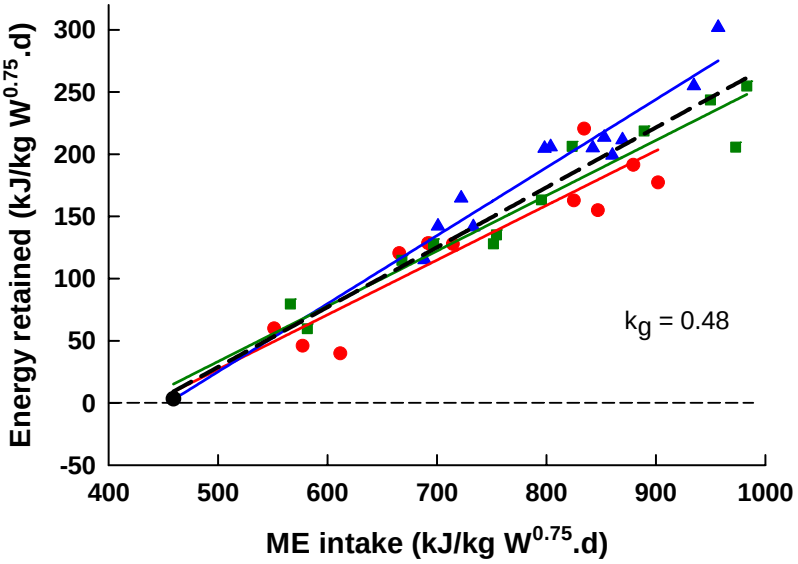

Figure 6.5. Regression of estimated energy retention on estimated ME intake for steers receiving a low quality green panic hay and various supplements (Control, ●; Barley, ●; Barley/urea, ■; Barley/protein, ▲). Symbols represent values for individual steers. The combined relationship for all treatments (R^2 = 0.882) is shown as a black dashed line.

Microbial protein production

As has been found in our previous studies, both MCP production and the efficiency of MCP production (EMCP) were low for steers receiving low quality forages alone. Values for EMCP were well below those proposed in the various feeding standards (130 – 170 g MCP/kg DOM; SCA, 1990; AFRC, 1992; NRC, 2000), ranging from 82 to 95 whereas, in contrast, the corresponding value for the medium quality

pangola hay was 115 and 147 g MCP/kg DOM for the ryegrass. The major factor affecting MCP production is RDN supply which is a function of CP content and degradability of the CP. Thus there is considerable scope for improvement in the EMCP on these low quality forages. Both MCP production and EMCP responded to increasing intake of supplement of all types with these lower quality forages but there were some apparent differences between supplement types. EMCP did increase with barley fed alone in the experiment described in Chapter 4B but the response was much steeper when an additional N source was included in the grain-based diet, i.e., protein meal (Chapter 4B) or urea (Chapter 3B). The gradual increase in EMCP when barley was fed alone reflects the fact that barley was quite high in CP content for a grain source and that a considerable amount of this N was available to the microbes in the rumen. Nevertheless, the diet was still unbalanced in terms of RDP:DOM (<130 g RDN/kg DOM) and the animals responded when additional protein was supplied in the diet. Although not compared in the one experiment, the response to additional N provided as true protein (Chapter 4B) appeared greater than when provided as urea (Chapter 3B). This confirms some preliminary results from our research team (M.K. Bowen, pers. comm.) and suggests that the true protein is providing nutrients or co-factors other than NH_3-N. These could be preformed amino acids and peptides but nutrients such as branched-chain fatty acids are also implicated as these are provided as degradation products of proteins in the rumen and are known to be essential nutrients for microbial growth. More work is required to confirm this finding and, if there are other factors involved, identify them with the aim of providing these nutrients, in association with non-protein N, in catalytic amounts to stimulate microbial growth without the high cost of true protein sources.

The first priority though seems to be to provide a balanced amount of RDN relative to energy supply and one of the outputs of this project is the development of a simple Ready-Reckoner to determine appropriate levels of inclusion of RDN. This spreadsheet is designed primarily to ensure sufficient RDN is provided in the supplement to supply the RDN required by microbes in the rumen to utilise not only the energy supplied in the supplement but also that in the forage. A target of 130 g RDP/kg DOM is generally used but this can be adjusted. This represents an upper level for requirements as no allowance is made for N recycling to the rumen which could be substantial on low quality forages. The output of the spreadsheet is shown in Appendix 2. Optimising microbial protein production is a major priority for low quality forages encountered in tropical regions throughout much of the year and represents the most cost-effective supplementation strategy available.

7 ACHIEVEMENT OF OBJECTIVES, INDUSTRY IMPACT AND RECOMMENDATIONS

Achievement of objectives

The stated **objectives** of the project were as follows:

1 To develop dose response curves relating cattle growth to intake of high lipid, medium protein feed sources (e.g., copra meal and PKE meal).

2 To determine practical methods of reducing the substitution effect associated with feeding supplements.

3 To establish practical feeding strategies based on aspects 1 and 2 above in association with existing knowledge, and incorporate the information into simple decision support aids with which producers can easily compare supplementation options.

Objective 1: The dose response curves to various high lipid, medium protein feed sources, viz. copra meal and PKE meal, were developed and the results are reported in Chapters 2A and 2B. Various aspects of the effects of this supplement type on the digestion and metabolism of the animal were also investigated.

Objective 2: As discussed in Chapter 6 in the section on Substitution, the main practical method of reducing substitution that was determined in these studies involved formulating supplements to ensure an adequate P/E ratio in supplement and also in the total diet. It was shown that on low quality forage-based diets, there was a two stage effect of supplements on intake of forage with the major opportunities to manipulate substitution occurring in the first phase, i.e., at low supplement intake. Optimising protein inclusion in the diet in this phase to ensure no nutritional limitations on microbial growth in the rumen, reduced the substitution effect. A Ready-Reckoner (see Appendix 2) has been developed to facilitate simple calculation of the N inclusion rates in the supplement. Another strategy for reducing substitution effects, viz., changing the site of digestion of the supplement, was investigated but did not prove effective in our studies.

Objective 3: The Least-Cost Ration Formulator (see Appendix 1) developed from the results of this and previous projects (e.g., DAQ.100) provides industry with a simple decision support tool for use in comparing supplement options for growing cattle grazing low quality tropical forages.

Project outputs and industry impact

The main tangible outputs from the project are as follows:

1 An extensive data base relating cattle growth responses to supplement intake for young growing cattle given low quality tropical forage and a range of supplement types commonly used by the beef industry in northern Australia.

2 An extensive data base relating forage and total intake to supplement intake (i.e., substitution effects) for young growing cattle given low and medium quality forages and a range of supplement types.

3 An extensive data base relating MCP production and EMCP to supplement intake for young growing cattle given low and medium quality forages and a range of supplement types.

4 A simple Ready-Reckoner to estimate urea (or protein) requirements to balance the nutrients in the rumen for optimal microbial protein production.

5 A simple spreadsheet-based Least-Cost Ration Formulator for use by producers or their advisers to compare supplementation options for increasing growth rate of young growing cattle given a low quality tropical forage.

The data bases described in 1, 2 and 3 above represent an extension of existing data bases developed previously by our research team. They also represent the most extensive data bases, published or otherwise, available anywhere in the world for cattle grazing tropical forages. The dose response approach used in this and previous studies make the information extremely valuable for modelling purposes and it is likely the data will form the basis of modelling exercises in the foreseeable future. The key elements of any decision support tools will be relationships between supplement intake and the intake of the basal forage, the liveweight performance of the animals and the amount of microbial protein and also total protein available for absorption by the animal. This project has provided information on all three aspects. A key priority will be to incorporate the data into a simple decision support model for use with cattle grazing extensive tropical pastures. Thus there is immediate benefit to industry from the use of the Least-Cost Ration Formulator for comparing supplementation options (see above) and likely on-going benefits emanating from the use of the established databases. The response curves derived here should replace those proposed by Cheffins (1996) in the DPI/MRC publication "Nutritional and Managerial Opportunities for Meeting Beef Markets". A collaboration has already been established with Dr John Moore, Oklahoma State University, Stillwater OK, to incorporate the data from the current project into his existing data base to develop improved empirical equations for determining the effects of supplements on forage intake.

Recommendations

Based on the outputs of the current project the following recommendations are made for further R, D & E:

1 That the data generated by the current project, and previous ones, be incorporated into a decision support model to facilitate the prediction of growth rate of cattle grazing tropical pastures.

   This decision support model should provide the link between the diet quality information provided by the faecal NIRS analysis (primarily CP and digestibility) and the growth response curves, the intake response curves and the microbial protein production trends developed in these projects. It is proposed that the data be used to consolidate or modify existing decision support models rather than create a new one.

2 That further strategic experiments be set up to provide similar growth response curves to those established in the current project, for older, finishing cattle and for medium quality basal forages.

   Rather than repeating all of the work completed with the younger growing cattle, the aim would be to use representative supplements from the 'energy source' (e.g., barley/urea) and protein meals (eg., CSM) in a few strategic experiments using hays of low and medium quality and cattle within 100 kg of finishing weight for the domestic or export market. This would provide a similar spreadsheet output to that illustrated in Appendix 1 and identify variations in response associated with the different physiological state of the animals.

3 That the data generated in the current projects be used to develop further 'generic' response curves based on responses to key nutrients of protein (perhaps metabolisable protein) and energy (ME) rather than supplement type.

   The generic response curves developed in the current project differentiate between protein meals and 'energy sources', yet it is recognised that protein meals provide energy as well as protein to the animal and energy sources lead to increased protein supply through MCP production. Furthermore, it is obvious that the most cost-effective supplement will be a combination of both supplement types, with high protein required at low intakes and the cheapest form of energy required at higher intakes of supplement. Development of the proposed generic response curves will allow producers and their advisers to formulate cost-effective supplements based on these responses and the availability and cost of supplements locally. Some experiments to validate these 'predictions' would be warranted.

4 That further investigation into the effects of possible synergistic effects of combining protein meals and energy sources on animal performance be undertaken.

The synergistic effects indicated in the frequency of feeding trial, and illustrated in Figure 6.2, have important practical implications in terms of reducing the cost of feeding (relative to protein meals alone) without compromising animal performance. An experiment using graduated changes in the proportion of the different supplement types is envisaged so that the appropriate response curve can be incorporated into the Least-Cost Ration Formulator.

5 That the data generated in these projects be used to develop predictive equations for the effects of supplement intake on forage intake.

It is proposed that the data be used to validate and, where appropriate, modify existing equations developed by Moore *et al.* (1999) in a collaborative exercise. The data should also be used in existing decision support models (see 1 above) to predict effects of supplements on forage intake and ultimately on animal growth.

6 That research be directed towards identifying key nutrients (other than RDN) limiting microbial growth and the EMCP in the rumen of cattle grazing tropical pastures, and in particular the reasons behind the different responses to RDN supplied as non-protein nitrogen (NPN) or true protein.

Low EMCP remains a major limitation to cattle production on low quality tropical forages, especially when compared with the temperate pasture situation. Results from the current project and others within our research group have reinforced earlier findings that NPN and true protein are not interchangeable as N sources to the rumen microbes but the reasons for this are not clear. It is apparent that growth factors other than RDN contribute to the EMCP and identification of these factors may lead to more cost-effective strategies for increasing cattle production on low quality pastures.

7 That better methods of determining the degradability of protein in supplements are developed for use in determining the nutrient supply to the animal.

The nylon bag method used for estimating degradability of protein in concentrates has serious limitations, as indicated by the assays used to compare CSM with copra meal and PKE meal. A system of incubating samples in nylon bags in a culture of rumen microbes within a fermenter and measuring ammonia concentration changes may provide a useful assay method. Similar systems have been used previously but could be refined for greater simplicity and repeatability.

8 That the need be determined for an RDN source to be included with copra meal and PKE meal supplements in view of their suspected low protein degradability in the rumen.

Despite the findings from the nylon bag studies conducted in the current project, the degradability of protein in copra meal and PKE meal is considered to be low and inclusion of an RDN source such as urea is often recommended. Results of the pen experiment conducted in the current project question the need for such inclusion as growth responses were similar for copra meal and CSM which reputably has higher rumen protein degradability. Urea inclusion increases the risks of toxicity and may be largely ineffective if the supplement is consumed on an irregular basis. Nevertheless, non-inclusion of urea may reduce the effectiveness of the supplement. This issue requires clarification and follows on from recommendation 6 above.

9 That further investigation into the effects of frequency of feeding supplements on animal performance be undertaken, especially with the ‘energy sources’.

The results presented here indicate that infrequent feeding of an ‘energy source’ such as grain or molasses, but not protein meal, may reduce growth rate of cattle compared with daily access to the same amount of supplement. Unfortunately these results were confounded by the low overall intakes of molasses/urea recorded and by the inclusion of protein meal in the sorghum supplement. In view of its practical relevance, this issue deserves further attention and clarification. Limited further experiments are suggested, perhaps using restricted intake of forage to increase supplement intake.

10 That the information generated from this project and previous ones be incorporated into the Northern Nutrition EDGE workshops as soon as possible.

11 That the factors pertaining to the forage base which influence the response to supplement be identified and quantified.

Within supplement types there is considerable variability between the growth and intake responses by animals. The reasons for this variability are not clear but may relate to physical factors such as the proportion of leaf and stem, rather than differences in chemical analysis. An understanding of these factors would assist in the practical recommendations of feeding strategies under different grazing conditions.

ACKNOWLEDGMENTS

This research was carried out with joint funding from MLA and the Beef Quality CRC and their financial support over several years is gratefully acknowledged.

The copra meal and palm kernel expeller meals used in the various experiments were provided by Mataranka Grains Pty Ltd, Dayboro Qld and their support is very much appreciated. The assistance of the manager, Mr John Mullaly and staff at DPI's Brian Pastures Research Station and of the managers, Mr Jim Hale and Mr Andrew Gibbon, and staff of UQ's Mt Cotton Research Farm, is also very gratefully acknowledged. The chemical analyses were carried out in laboratories at DPI's Animal Research Institute, Yeerongpilly and at UQ's Dept of Land and Food Science, St Lucia and the generous contributions and expertise of Mr Peter Martin and Mr Adam Pytko (DPI) and Mr Mick Nielsen (UQ) and their support staff are very much appreciated. The guidance of a Nutrition Steering Committee comprising representatives from other scientific groups and producer members was highly valued. Finally, the project team gratefully acknowledge the support of the Agency for Food and Fibre Science (Beef) especially Mr Jim Walkley (Program Leader) and also that of Mr Shane Blakeley, MLA Project Coordinator.

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APPENDICES

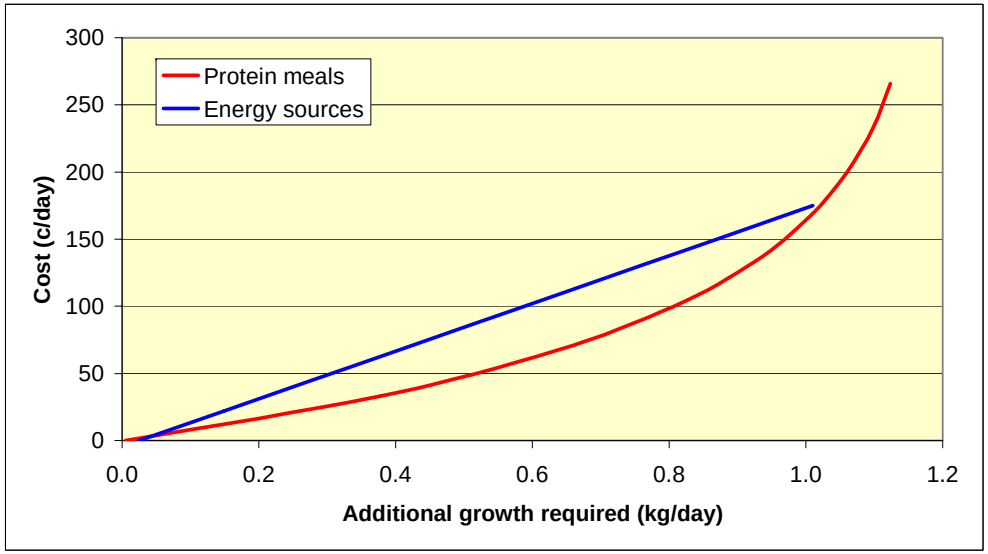
Appendix 1. Least –cost Ration Formulator

Least-cost rations

Protein meal and "energy supplements

Steer LW (kg)		350
Addn growth reqd. (kg/day)		0.5
Supplement costs ($/tonne)	Protein meal	380
	Energy source	250
	Urea	500

	Protein meal	Energy source
Cost of supp. (c / kg)	38.0	25.0
Intake supp. reqd (kg/d)	1.25	3.37
Cost (c / d)	47.6	84.4
Cost (c / kg extra gain)	95.3	168.7



These response relationships apply under the following conditions:

1. **Cattle are in growth, not finishing phase**
2. **Pasture not limiting in supply (>2 tonne/ha)**
3. **Pasture quality low - <6% CP**
   <50% DMD
4. **Growth rate of unsupplemented steers <0.2 kg/day**

Appendix 2. Urea requirements Ready-Reckoner

Urea requirements

	Feed source	Feed composition DM (%)	OMD (%)	CP (%) DM basis	Est. M/D (MJ/kg)	dg (%)	eMCP (g/kg DOM)	Intake (kg, as fed)
Feed 1	GP hay	90	55	7.5	7.0	75	130	3.00
Feed 2	Barley	90	75	12.4	10.2	67		1.50
Feed 3	Cottonseed meal	90	75	42.8	10.2	75		0.00
Feed 4	Copra meal	90	71	24.2	9.6	70		0.00
Feed 5								0.00
	Urea/S	95						0.000

Data can be inserted in yellow-shaded cells.

Note: Take care if adding urea to supplement. High intakes of urea over short duration can be toxic.

Requirements (g, as fed):

Urea	**25.4**
Sulfate of ammonia	**5.3**

P/E	RDP/DOMI
148	**106**