PROTEIN FROM PASTURE

by

PAUL EDWARD DONNELLY

A thesis

submitted in partial fulfilment

of the requirements for the Degree

of

Doctor of Philosophy

in the

Department of Biological Sciences

at the

University of Waikato

1980

ABSTRACT

Ryegrass, white clover, ryegrass/white clover and lucerne herbages were used in an industrial pilot-scale study of the effects of regrowth period and season on protein extraction, separation and recovery. Herbage was harvested, adjusted to 10% dry matter, pulped and (belt) pressed. Protein in the expressed juice was thermally coagulated and centrifugally separated to produce wet leaf protein concentrate (LPC) which was spray dried. Nitrogen recovery ratios ranged from 15 to 27%, and decreased with increasing herbage maturity. Largest decreases were for ryegrass as the herbage flowered. Summer 1977 ratios were lucerne 22%, white clover 19%, ryegrass/white clover 18% and ryegrass 17%. The higher recovery ratio for lucerne was due to a higher extractability. The grazing rotation intervals used in seasonal dairying on ryegrass/white clover pastures in New Zealand would be satisfactory for protein extraction. Estimated recoverable protein yields for irrigated and non-irrigated pasture, and lucerne, were 979, 675 and 660 kg/ha/yr, respectively. For integrated dairying plus protein extraction, total (animal plus plant) protein yields were estimated to be 1479, 1051 and 980 kg/ha/yr, for the above respective circumstances.

LPC contained from 32 to 55% crude protein which was higher for the legumes than ryegrass. Crude fat was higher in ryegrass (9%) than white clover (5%) and lucerne (7%) LPC. Amino acid content was not consistently affected by regrowth period. LPC histidine and lysine contents (g/16g N) and *in vivo* protein true digestibilities were: ryegrass 2.4, 5.7, 70%; white clover 2.7, 6.0, 73%; ryegrass/white clover 1.9, 5.4, 70%; lucerne 2.6, 6.1, 82%. Relative Nutritive Values (RNV) of the protein (rat growth assay, lactalbumin as reference) were: ryegrass 0.53, white clover 0.47, ryegrass/white clover 0.45 and lucerne

0.63. With supplemental methionine, the respective RNV were 0.79, 0.88, 0.82 and 0.89. Neither season (autumn v. spring) nor herbage age (4, 6 or 8 weeks regrowth) affected protein quality. In a second experiment LPC were prepared with and without juice treatment with metabisulphite. Protein true digestibility, available lysine (g/16g N) and the percentage true protein in the chloroplastic fraction were: ryegrass 73%, 4.8, 73%; white clover 80%, 5.7, 56%; ryegrass/white clover 74%, 5.1, 73% and lucerne 80%, 5.4, 49%. It is published that the chloroplastic fraction is less digestible and contains less lysine and histidine and more fat, than the cytoplasmic fraction. The lower digestibility of ryegrass and ryegrass/white clover than legume protein is due to a higher proportion of the protein being in the chloroplastic fraction for the former two herbages.

Metabisulphite treatment increased chemically available lysine (2%, $P<0.05$) and methionine (7%, $P<0.05$) and also increased RNV from 0.59 to 0.73. RNV further increased to 0.84 with supplemental methionine. Metabisulphite did not improve the availability of the second limiting amino acid(s) for growth. Nutritionally available methionine and cystine, without and with metabisulphite treatment, were respectively, methionine 1.57 g/16g N (81% availability) and 1.64 (85% availability) and cystine 0.26 (27% availability) and 0.70 (73% availability). The biggest improvement in cystine availability (10% to 65%) due to metabisulphite was for ryegrass/white clover, the only herbage for which metabisulphite also improved protein digestibility (by 4 units). Cystine is more prone to nutritional damage during processing than methionine.

ACKNOWLEDGEMENTS

In presenting this thesis I thank the Ministry of Agriculture and Fisheries for permission to pursue this degree while in her employment and for the use of resources and facilities.

My thanks are especially due to Drs. P. V. Rattray and A. S. Edmonds for their guidance and encouragement, and assistance with the preparation of this thesis. I am also grateful to Mr. R. M. McDonald and staff for operation of the protein extraction laboratory; to Mr. M. G. Ashby and staff for supervision of the nutrition studies and field experiments; to Mr. R. P. Newth and staff for the execution of chemical analyses; to Dr. N. R. Cox for advice on and assistance with statistical matters; to staff of the Meat Industry Research Institute (Ruakura) and the Poultry Research Centre (Massey University) for amino acid analyses and to staff of the Ruakura Wine Research Group for the sulphur dioxide analyses. Miss Beverley Binns is thanked for her excellent typing.

Finally, I take pleasure in expressing my gratitude to my wife Jill, for her support and encouragement throughout this period of endeavour.

Paul Donnelly.

CONTENTS

Page

DEFINITION OF TERMS AND ABBREVIATIONS

Terms

Juice.	The cell sap, including added water, pressed from the herbage.
Pressed herbage.	The residue remaining after the juice has been extracted from the herbage by pressing.
Deproteinised juice (DPJ).	The fraction of the extracted juice that remains after coagulation and separation of the leaf protein concentrate.
Leaf protein concentrate (LPC).	The protein-rich fraction separated from the coagulated leaf juice. Leaf protein concentrate may be wet or dry.
Extraction ratio.	The proportion of the herbage obtained in a juice fraction as a percentage by weight; expressed on the basis of dry matter or N (crude protein - Nx6.25).
Separation ratio.	The proportion of the juice which is obtained as leaf protein concentrate on a percentage by weight; expressed on the basis of dry matter or N.
Recovery ratio.	The proportion of the original herbage obtained as leaf protein concentrate on a percentage by weight. It is the product of the extraction and separation ratios and is expressed on the basis of dry matter or N.

Abbreviations

AU	autumn.

b	regression slope coefficient.
CAA	crystalline amino acids.
df	degrees of freedom.
DM	dry matter.
FDNB	flourodinitrobenzene.
L	lucerne.
LC	linear contrast.
M(Met.)	methionine.
MBS	metabisulphite.
MFP	metabolic faecal protein.
M+C	methionine plus cystine.
MS	mean square.
N	nitrogen.
PTD	protein true digestibility.
r	correlation coefficient.
RE	rat experiment.
RG	ryegrass.
RG/WC	ryegrass/white clover.
RNV	Relative Nutritive Value.
RSS	Residual sum of squares.
SD	standard deviation.
Se	standard error.
SP	spring.
WC	white clover.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

In the past research on protein extraction has been carried out for a variety of reasons. Thus in England during the 1940's the prime objective was the production of protein products for human consumption in case of wartime blockade. Subsequently, the production of food for less developed countries was (Pirie, 1971a), and still is (Singh, 1971; Kalamanathan & Devadas, 1971), important. In these cases near-exhaustive extraction of the forage was usually sought to maximise yields of protein. It is well recognised that ruminants are inefficient converters of protein in herbage to saleable products. This is due in part to the excess of protein in herbage relative to requirements. The desire to improve this efficiency of recovery was in substantial part responsible for the research on 'on-farm' fractionation which would yield a plant protein product and a pressed crop for ruminant use. Such research has been carried out in the U.S.A. (Stahmann, 1975), in Ireland (Maguire, 1977), in Scotland (Houseman & Connell, 1976), in Australia (McKenzie, 1977) and in New Zealand (Vartha & Allison, 1973; Ostrowski, 1976). A further incentive to research on protein extraction has been the desire to improve the economy of green crop drying. Goodall (cited by Tilley & Raymond, 1957) suggested that this could be achieved by mechanical dejuicing prior to drying. This concept has been intensively researched in California (U.S.A.) (Kohler, 1975), and also in Hungary (Hollo & Koch, 1971) and England (Connell & Foxell, 1976). A commercial venture employing dewatering before drying the forage crop and producing a leaf protein concentrate also has been in operation in France since 1976 (Truchetto, 1977).

In reviewing past work in 1970, Hutton commented "In much

of this previous work, considerable emphasis has been given to extraction procedures suitable for application at the village level in developing regions.....there is also a present need to examine the practicalities of establishing larger operations in association with grassland-based livestock industries". New Zealand has approximately 5.5M hectares of flat to rolling country estimated to annually produce 2700 kg of protein per hectare (Hutton, 1972). The efficiency of recovery of this protein is only 10 to 15% under dairying and is even less under beef farming (Everitt, 1972). Assuming that 40% of the protein in pasture could be extracted and recovered and that 300 kg/ha of milk protein could be produced by cows fed the pasture after extraction, Hutton estimates total protein yields could be 1380 kg/ha. This is substantially higher than potential (580 kg/ha) and current average (245 kg/ha) yields of protein under dairying.

Following Hutton's analysis of potential yields a wide-ranging programme of research was instigated at the Ruakura Agriculture Research Centre to examine the technical and economic feasibility of developing integrated protein extraction-livestock grazing systems. The general structure envisaged was that the herbage would be harvested and extracted on the farm. The protein-containing juice would be transported to a centralised factory for processing and the extracted herbage consumed on the farm by livestock as either a substitute for, or supplement to, grazed herbage (Hutton, 1970).

The factors studied in the research programme were:

1. The effects of agronomic variables on protein extraction, separation and recovery ratios.
2. The feeding value to ruminants of the pressed herbage.

3. The development of equipment capable of harvesting, pulping and pressing herbage on the farm.
4. Improvements in the technology of processing grass juice to improve protein recovery, maintain or improve protein quality and to minimise energy inputs.
5. Investigation of biological sources of variation in protein quality.
6. Measurement of extractable protein yields, pasture and animal production and soil fertility factors in an integrated system of protein extraction and ruminant grazing.
7. Examination of the economics of an integrated protein extraction - ruminant grazing operation.

Research projects falling into categories 1 and 5 and to a lesser degree 4 above, were under the author's direction and the results obtained form the basis of this thesis which is presented in two parts. In Part 1 the effects of some agronomic variables on protein extraction and recovery is examined and in Part 2 results on protein quality are reported. Each of these two parts has its own literature review.

PART 1

THE EXTRACTION, SEPARATION AND RECOVERY OF PROTEIN FROM HERBAGE.

CHAPTER 1. REVIEW OF LITERATURE

Extraction, Separation and Storage

1.1.1 Extraction Equipment

Juice extraction involves firstly cell rupture and then pressure application to express the sap (Pirie, 1966). An assortment of equipment was first used to pulp (macerate) the crop after it had been harvested (cf. Tilley & Raymond, 1957). In some instances this was carried out by rotating beaters or knives and in others by the friction of rollers or expellers. At Rothamsted equipment was developed to separately perform the functions of maceration and pressing (Pirie, 1966). This consisted of a pulper which is similar in principal to a hammer mill (Davys & Pirie, 1969). Herbage enters a cylinder at one end and is then pulverised by radially disposed beaters on a revolving rotor; the pulp is then discharged at the other end. Edwards *et al.*, (1978) reported improved yields at pressing when lucerne is pulped compared with chopping. The press developed at Rothamsted to function with this pulper is a belt type which squeezes the pulped herbage between a belt and a perforated roller (Davys & Pirie, 1965). The juice passes through the perforated roller. A small-scale portable beam press has also been developed at Rothamsted (Davys *et al.*, 1969). Although the pulper is still used prior to pressing, screw presses are usually now used for juice expression (Truchetto, 1977; Kohler *et al.*, 1978; Pirie, 1977).

For small laboratory scale work an extraction technique which has been used is mincing the herbage through a household meat mincer and squeezing the pulp by hand in a muslin-type cloth (Crook & Holden, 1948; Byers, 1961).

1.1.2 Juice Processing and Protein Recovery

Expressed juice contains enzymes which will hydrolyse the protein (Singh, 1962; de Fremery *et al.*, 1972). This hydrolysis results in loss of recoverable true protein and hence juice should be processed immediately after pressing to minimise losses (Pirie, 1971c). However, a beneficial effect of delay after pressing may be the destruction, by ribonuclease, of nucleic acids which can have anti-nutritional properties (Singh, 1960).

The following methods are used to coagulate the juice true protein: heating, pH change, fermentation (which results in acidification), solvent washing and electrolyte addition (cf. Pirie, 1971c; Ostrowski, 1976). These methods give different crude protein yields and of the two most commonly used, heat and tricarboxylic acid addition, the latter usually, (Byers, 1971a; Pirie, 1971c), although not consistently (Deveraj *et al.*, 1970) gives higher protein yields and lower protein quality (Subba Rau & Singh, 1970). Of the above methods, heating yields the most readily filtered coagulum and is best achieved by steam injection of the juice (Pirie, 1971c).

For small laboratory-scale work, recoverable protein is usually estimated by centrifuging the coagulated juice and then Kjeldahl analysis of the sediment (e.g. Byers, 1971a). On a slightly larger scale, filtration can be used in place of centrifugation (Pirie, 1971c). The most successful large pilot industrial scale system has been developed in California where heat coagulated

juice from lucerne has been separated and dewatered to 50% dry matter content using a decanter-type centrifuge (the steam coagulated juice usually contains about 10% dry matter) (Kohler *et al.*,1978). This system is now in commercial operation (Truchetto, 1977). Filter presses (Morrison & Pirie, 1961) and filter-belt presses (Foxell, 1977) have been tried for separating coagulated juice but with limited success.

On semi-industrial and laboratory scales it is possible to separate green 'chloroplastic' and whitish 'cytoplasmic' protein fractions using two stage heating (Edwards *et al.*,1975a). This involves heating the juice to approximately 50°C and centrifuging out the green fraction and then centrifuging the supernatant again, after heating to 90°C, to obtain the white fraction.

The extent of mechanical dewatering of the coagulated juice at separation can influence the yield and composition of the protein concentrate because the aqueous phase of the juice contains soluble constituents, some of which are nitrogenous compounds (Pirie, 1971c; Kohler *et al.*,1978).

1.1.3 Storage of the Leaf Protein Concentrate (LPC)

Although moist leaf protein has been successfully stored by means other than drying (canning; formic-, acetic-, lactic- or mineral acid addition; salt addition; Tilley & Raymond, 1965; Subba Rao *et al.*,1967; Pirie, 1971d; Arkcoll, 1973) the presence of at least 50% moisture in the stored product makes it bulky. Drying to less than 10% moisture has also been studied and provided the temperature of the product does not exceed 80°C during the process, protein quality will be maintained (Woodham, 1971). Pilot-industrial scale tests have successfully used the following processes: spray (Hartmann, Akeson & Stahmann, 1967;

Hollo & Koch, 1971), rotary drum and fluidized bed (Kohler *et al.*,1978). The latter system is used commercially (Truchetto, 1977). Organic solvent washing has also been used for drying - on a partial basis in combination with heating (Duckworth & Woodham, 1961), and also in association with the extraction of pigments to remove the green colour (Bray *et al.*,1978).

Results from studies on the storage stability of lipids in LPC are equivocal. Lea & Parr (1961), Shah (1968), Arkcoll (1973) and Buchanan (1969a), observed oxidation to proceed, while Hudson & Karis (1976), Hudson & Warwick (1977) and Ross & Wallace (1978), observed the lipids in LPC to be stable to oxidation. Betschart & Kinsella (1975) directly measured the fatty acids in LPC from soya bean foliage and observed losses of linolenic acid during storage which was associated with decreased protein *in vitro* digestibility (see also Buchanan 1969a,b).

If LPC is stored in the presence of oxygen losses of carotenoids and chlorophyll occur (Arkcoll, 1973; Witt *et al.*, 1972; Ross & Wallace, 1978). Such losses proceed more slowly in the dark (Arkcoll, 1973) and can be reduced by the presence of antioxidants and oils (Witt *et al.*,1972). When oxygen is totally excluded losses are virtually eliminated. Under all storage conditions, losses are reduced by holding at near zero degrees celsius.

Agronomic Factors Affecting Protein Yields

The factors which can influence protein yields may be conveniently grouped into three categories through their effects on: 1. ease of protein extraction, 2. separation of true protein from the coagulated juice and 3. plant growth. These categories

are used in this review. Extraction, separation and recovery ratios are defined on page 33.

1.1.4 Factors Affecting Ease of Protein Extraction

The major agronomic factors which have been shown to influence the extractability of protein are herbage species or varieties (Crook & Holden, 1948; Byers, 1961; Byers & Sturrock, 1965; Lexander *et al.*,1970; Deveraj *et al.*,1970; Arkcoll & Festenstein, 1971; Maguire & Brooks, 1973; Deshmukh *et al.*,1974; Matai *et al.*, 1976; Mungikar *et al.*,1976; Heath & King, 1977); season, leaf age or maturity (Byers & Sturrock, 1965; Arkcoll & Festenstein, 1971; Allison & Vartha, 1973; Maguire & Brooks, 1973; Vartha & Allison, 1973; Dev *et al.*,1974; Deshmukh *et al.*,1974; Gore *et al.*, 1974; Jones & Houseman, 1975; Matai *et al.*,1976; Heath & King, 1977; McKenzie, 1977, Vartha & Brusse, 1977); and soil fertility factors (Byers & Sturrock, 1965; Arkcoll & Festenstein, 1971, Allison & Vartha, 1973; Vartha & Allison, 1973; Jones & Houseman, 1975; Matai *et al.*,1976; Vartha & Brusse, 1977; McKenzie, 1977). Within plant factors which have been recognised as having major affects on extractability, and which are influenced by the above, are moisture, protein and fibre contents. The moisture serves as a flushing medium to carry the protein constituents out when pressure is applied. Thus higher moisture content improves extractability. Similarly a higher herbage protein content results in higher protein availability for extraction as was illustrated clearly by Crook & Holden (1948) for tobacco. This relationship however is not strong between species (Byers & Sturrock, 1965). A further point here is that as plants approach flowering and vegetative growth slows, total herbage N and the ratio of true protein N to total N decrease (Arkcoll & Festenstein, 1971), reducing the yield of true protein

for any given total N extraction. Also in association with advancing maturity in many species, particularly members of Graminaceae, there is a tendency for herbage fibre content and strength to increase. This reduces N extractability by reducing the effectiveness of pulping, resisting compression at pressing and filtering out the chloroplastic protein (Arkcoll & Festenstein, 1971; Heath & King, 1977). These authors discuss the individual effects of soil phosphorus, potassium and nitrogen on the extractability of herbage nitrogen. Overall, good soil moisture and plant nutrition lead to low fibre to juice ratio and a high protein content both of which favour ease of extraction.

Recently Heath & King (1977), using regression analysis on results from four herbage species taken over several regrowth ages, showed crop dry matter content to account for 90% of the variation in the extraction ratio. The inclusion of herbage fibre, cellulose, hemicellulose, lignin, water soluble carbohydrates, juice pH and protein N as further independent variables, failed to account for any more of the variation in the dependent variable than crop dry matter alone. This was probably because most of these variables are correlated with environmental factors as reviewed above.

Heath & King (1977) have also discussed possible effects of some herbage chemical factors. Protein extractability was reduced in herbages with low pH juice, e.g. dock, rhubarb and soursob. This coagulates the protein in the herbage at pulping (Arkcoll & Festenstein, 1971; McKenzie, 1977). Natural phenols, tannins and reducing sugars can complex with proteins. Arkcoll & Festenstein (1971) attributed low extractability in silver leaf *Desmodium*

and strawberry to the presence of tannins. The addition of phenols to pulped crop reduced the N extraction ratio (Heath & King, 1977). Highly mucilagenous plant sap (e.g. from sweet potato, comfrey, bracken, elm, hybrid sorghum) makes difficult the separation of juice from fibre at pressing (Arkcoll & Festenstein, 1971; Deshmuk *et al.*, 1974; McKenzie, 1977).

In summary, herbages containing high moisture and protein proportions and low fibre levels favour easy extraction, e.g. grazing legumes, especially lucerne.

1.1.5 Factors Affecting Protein Separation

After juice expression, it is necessary to coagulate the true protein (by one of several means - see section 1.1.2) to facilitate its separation from other juice constituents and also to reduce its moisture content.

The proportion of the total N present in the juice as true protein N and the proportion of this true protein N which can be coagulated and separated, will affect the separation ratio (LPC/juice). True protein N to total N in the juice differs between species (Lexander *et al.*, 1970; Byers, 1971a), and decreases with increasing crop maturity (Pleshkov & Fowden, 1959; Arkcoll & Festenstein, 1971; Jones & Houseman, 1975; Mungikar *et al.*, 1976). Soil nutritional factors may also lead to variations in the ratio of protein N to total N in juice (Pleshkov & Fowden, 1959; Lexander *et al.*, 1970; Arkcoll & Festenstein, 1971).

Brief mention was made earlier of the efficiency of coagulation of plant juice protein by various laboratory scale methods (section 1.1.2). However, there has been no investigation of the effects of agronomic factors on protein coagulation and/or

separation using industrial equipment. Byers (1971a), however, has found that the sedimentation characteristics of protein differ for three species processed at several ages on a laboratory scale. Whether or not these differences would occur with industrial equipment is unknown.

1.1.6 Factors Affecting Plant Growth

1.1.6.1 True Protein Accumulation and Age at Harvest

Heath & King (1977) present data showing that for any single growth phase, the maximum yield of true protein is reached earlier than the maximum yield of dry matter. Initially the leaf laminae accumulate protein N faster than the stem and sheath but this rate eventually declines. In contrast, the slower rate of accumulation by the stem and sheath is maintained over a longer period. Thus the laminae determine to a large extent the high initial rate of accumulation of whole crop true protein which is predicted by total N accumulation. Arkcoll & Festenstein (1971) discuss the changing balance between protein synthesis and degradation with age. The proportion of total N constituted by non-protein N in extracted juice for wheat increased from 11% prior to ear emergence to 26% after ear emergence. Since the rate of proteolysis was relatively constant over the period studied, the rise in NPN appeared to derive from a decline in the rate of protein synthesis. This is also seen in the results of Jones & Houseman (1975) with ryegrass. Arkcoll & Festenstein (1971) state that most protein is obtained by harvesting just before vegetative growth ends and floral growth starts (e.g. rye, wheat). However some species continue to grow vegetatively after this stage (e.g. fodder radish,

fat hen), and the optimum age is extended over a longer period.

1.1.6.2 Plant Regrowth Ability and Cutting Interval.

The capacity for regrowth is important as it is associated with delay in floral development and ensures succulent tissue for subsequent extraction (Arkcoll, 1971). Cereals generally do not regrow vigorously but the maximum yield of wheat, barley and rye can be increased by harvesting when they are about 30cm tall which, relative to cutting at later stages, promotes better regrowth. Cutting at this height coincides with maximum true protein accumulation and hence favours high protein yields. It also enables initiation of a new growth period (Heath & King, 1977). In contrast to cereal crops, the legumes, red clover and lucerne, have vigorous regrowth capacity as also do the grasses.

There have been numerous studies of various regrowth intervals/cutting frequencies with different species:

Lucerne: In a monsoon climate cutting intervals of 30 days or less gave better yields than an interval of 36 days (Dev *et al.*, 1974) whereas in a temperate climate, although cutting at 4 or 5 weeks regrowth, or at early flowering, differentially affected dry matter yields, there were no differences in extracted protein yields (Allison & Vartha, 1973). Pirie (1971b) reported unpublished results of Vartha & Jones from the same research station showing that a five week cutting interval gave higher protein yields than intervals of three or seven weeks.

Westerwolds ryegrass: Higher protein yields were obtained with a six than three or twelve week cutting intervals in the

same temperate climate (Vartha & Allison, 1973).

Perennial ryegrass/white clover pasture: A twelve week interval gave lower extracted protein yields than four or six weeks (Vartha & Brusse, 1977).

Hybrid Napier grass: In a monsoon climate, this herbage did not give consistently different yields between years with cutting frequencies of five, eight or eleven and twelve times per year (Gore *et al.*, 1974).

Other results for the effect of cutting interval/age at harvest, on extractable protein yields for various crops are given by Deshmukh *et al.*, (1974), Matai *et al.*, (1976), Bagchi & Matai (1976) for the Indian monsoon climate, and by Byers & Sturrock (1965) and Arkcoll & Festenstein (1971) for south-east England.

1.1.6.3 Fertilizer Effects

Numerous experiments have shown that protein yields from non-legume species respond positively to N fertilizer in the Indian monsoon climate (Deshmukh *et al.*, 1974; Gore *et al.*, 1974; Matai *et al.*, 1976; Mungikar *et al.*, 1976), in south-east England (Byers & Sturrock, 1965; Arkcoll & Festenstein, 1971) and in temperate Australia and New Zealand (McKenzie, 1977; Vartha & Allison, 1973; Vartha & Brusse, 1977). In Australia the application of N fertilizer to ryegrass/white clover pasture in spring suppressed the clover content and in turn the extractable protein yield (McKenzie, 1977). This was not the case in New Zealand however, where N yields from ryegrass/white clover pasture responded to fertilizer N in spring, summer and autumn in one year but only in spring the following year (Vartha &

Brusse, 1977). In wheat and ryegrass protein N yields responded up to higher levels of fertilizer N than did yields of dry matter (Arkcoll & Festenstein, 1971; Heath & King, 1977). In the case of wheat there was no advantage of split over single application. Arkcoll & Festenstein (1971) refer to the effect of season (year) on growth to illustrate possible fertilizer N by climate interactions. Higher dressings of fertilizer N can result in lower true protein N to total N ratios in juice from ryegrass (Jones & Houseman, 1975), whereas conversely, a nitrogen deficiency will raise the ratio of true protein N to total N in barley (Pleshkov & Fowden, 1959).

The efficiency with which extra fertilizer N was recovered as extracted protein has been reported by Byers & Sturrock (1965) and Mungikar *et al.* (1976). In the former case this efficiency ranged from zero to 26% and in the latter case highest efficiencies were in the range 40-60%.

Soil N is not considered to be the first limiting plant nutrient for legume growth. Results show that when compared with non-legumes high yields are possible with zero or small applications of N to legumes (Table 1 p. 18). Several authors have drawn attention to this economy (Arkcoll & Festenstein, 1971; Allison & Vartha, 1973).

Most experiments with legumes have included fertilizer phosphate and/or potash with all treatments (Byers & Sturrock, 1965; Arkcoll & Festenstein, 1971; Allison & Vartha, 1973). However, several studies have recorded the effects of fertilizer phosphorus and potassium and, farm yard manure, on protein yields from legumes (Allison & Vartha, 1973; Dev *et al.*, 1974; Bagchi & Matai, 1976). In the experiment of Allison & Vartha

lucerne gave improved growth with dung and urine return by stock even when liberal dressings of potash and fertilizer were made.

In summary then, where nitrophilous species are grown, the application of fertilizer N is likely to give large protein yield responses (Arkcoll & Festenstein, 1971; Heath & King, 1977). This derives from its positive effects on protein growth and plant succulence, and associated with the latter, improved ease of extractability. Other soil nutrients are also important, especially for legumes.

1.1.6.4 Drilling and Seeding Conditions

Numerous authors have studied the protein yield responses to increased seeding rates (Arkcoll & Festenstein, 1971; Arkcoll, 1971; Bagchi & Matai, 1976; Matai *et al.*,1976). In general higher seed rates result in improved early vegetative growth and hence protein yields but responses are species dependent. Dev *et al.* (1974) examined several row spacings for lucerne. Yields of extractable protein were lower at the widest spacing.

Simazine can theoretically increase protein synthesis by stimulating nitrate reductase activity but failed to increase protein yields when applied to wheat and field beans (Arkcoll & Festenstein, 1971). Dev *et al.* (1974) did obtain a protein and dry matter increase with lucerne given 35 g/ha of simazine; at higher rates simazine was toxic.

1.1.6.5 Climatic and Seasonal Factors

Climate can significantly influence yields. For instance in the warm monsoon Indian climate Dev *et al.* (1974) recorded

extractable protein yields of 3 500 kg/ha with lucerne. At Rothamsted (S.E. England) however, the best yield with lucerne has been 1 106 kg/ha (Arkcoll & Festenstein, 1971). This difference derives largely from generally more vigorous and all-year-round growth in India where a total of 15 harvests were made and total dry matter production was 24 532 kg/ha. At Rothamsted however the growing season is of only five to six months duration and only three cuts were made.

Annual and seasonal variations in temperature and rainfall will effect plant growth and yields. In a monsoon zone, climatic variation affected protein yields from tithonia more than nitrogenous fertiliser application (Mungikar *et al.*,1976). At Rothamsted a very warm spring in one year lifted the three-year average protein yield for wheat from 600 kg/ha to 930 kg/ha (Arkcoll & Festenstein, 1971). Also, when wheat and fodder radish were grown sequentially in four successive years extracted protein yields were 1 244, 1 448, 2 014, and 1 133 kg/ha. The increase in extracted protein resulting from the removal of summer moisture stress by irrigation is illustrated in the results of Arkcoll & Festenstein (1971) for kale and in the results of Vartha and Jones (cf. Pirie 1971b) for lucerne In the latter case irrigation improved dry matter yields by 38% and extracted protein yields by 56%. The improvement in protein yield resulted from increases in herbage dry matter yield, herbage N content and increased N extractability. In the case of kale however irrigation did not improve dry matter yields but did increase herbage protein content and protein extraction rate.

1.1.7 Extractable Protein Yields: Cropping and Perennial Grass/Legume systems.

Table 1 presents results selected from those recorded in England, India, Australia, and New Zealand. The tabulated data were chosen because they are the highest yields in the locality for either crops or perennial grasses/legumes. Where available sequential year results are included.

Lucerne was the only herbage grown in all four localities and a comparison of the results for it suggests that climate is a very important factor affecting yield. In the monsoon climate of India the yield (2 650-3 100 kg/ha) was substantially higher than in Britain and Australia (1 000-1 100 kg/ha) and also somewhat higher than in New Zealand (1 500-2 340 kg/ha). At Rothamsted the best cropping system (wheat and fodder radish) gave a four year average yield of 1460 kg/ha. The best individual year result (2 014 kg/ha) was only slightly better than the one year's result for cocksfoot (1 670 kg/ha) obtained in a comparatively dry year. The result for wheat and fodder radish for this same year was 1133 kg/ha. Heath & King (1977) argue that perennial grasses such as cocksfoot and tall fescue form a better forage basis for protein extraction than do annual crops which have higher costs associated with ground preparation and seed, and are more vulnerable to climatic variation.

Many grasses/crops other than lucerne have been tested for extractable protein yields in India (cf. Mungikar *et al.*,1976; Matai *et al.*,1976; Bagchi & Matai, 1976; Deshmukh *et al.*,1974; Gore *et al.*,1974). Apart from lucerne the best results were obtained with hybrid Napier grass and cowpea. The former is

TABLE 1: RESULTS SELECTED FROM PUBLISHED DATA ON YIELDS OF EXTRACTED PROTEIN

Source	Crop	Fertiliser N (kg/ha)	Year	Yield of protein (kg/ha/yr)	Location
Arkcoll & Festenstein (1971)	Wheat and fodder radish	528	1966	1244	South-east England
	Wheat and fodder radish	528	1967	1448	
	Wheat and fodder radish	528	1968	2014	
	Wheat and fodder radish	528	1969	1133	
	Red clover (irrigated)	0	1967	1247	
	Red clover	0	1968	934	
	Red clover	0	1969	525	
	Lucerne	0	1968	1009	
	Lucerne	0	1969	1106	
	Cocksfoot	528	1969	1670	
Vartha & Jones (cited by Pirie, 1971)	Lucerne (irrigated)	0	NA	2340	South Island
	Lucerne	0	NA	1500	New Zealand
Allison & Vartha (1973)	Lucerne (irrigated)	0	NA	1860 - 2260	
Vartha & Allison (1973)	Westerwolds ryegrass	68	1971	945	
		360	1972	1210	
Vartha & Brusse (1977)	Perennial ryegrass/white clover (irrigated)	0,90 180	NA	689- 761	
Dev *et al.* (1974)	Lucerne (irrigated)	0	NA	1500	Aurangabad, India
	Lucerne (irrigated)	125 (+P+K)	NA	3100	
Mungikar *et al.* (1976)	Lucerne	0,125 250,500	NA	2800	
Gore *et al.* (1974)	Hybrid Napier grass	280	NA	2000	
Deshmukh *et al.* (1974)	Cowpea	60	year 1	1444	
		60	year 2	1976	
McKenzie (1977)	White clover (irrigated)	0	NA	1180 - 1460	Victoria, Australia
	Lucerne (irrigated)	0	NA	1140	

relatively fibrous and despite relatively low protein extractability (21-38%), the yield was 2 000 kg extracted protein/ha. A similar result was recorded for cowpea in year two but to achieve these yields this crop had to be newly sown three times per year.

Unfortunately the New Zealand results for lucerne, Westerwolds ryegrass and perennial ryegrass/white clover were not all obtained in the same year(s) and therefore cannot be strictly compared. However, the yields from lucerne, particularly where irrigated, are substantially higher than from the other herbages and those from six months growth of Westerwolds ryegrass are higher than from irrigated perennial ryegrass/white clover pasture. Thus lucerne appears to be the crop most suited to protein extraction in this environment, due to its high summer production and relatively long growing season (Vartha & Brusse, 1977).

McKenzie (1977) investigated extractable protein yields from a range of pastures and crops in Victoria, Australia. The highest yields were recorded for white clover and lucerne. The N extractability for perennial ryegrass was much lower than for these legumes and contributed to low yields for perennial ryegrass/white clover pastures.

In summary then, lucerne is the universally suited crop for protein extraction. The ecology of lucerne is reviewed by Leach (1978) and Hunt & Peadon (1975) for grazing and harvesting systems, respectively.

1.1.8 New Zealand Pastures

Despite the suitability of lucerne for protein extraction, the area of land planted in it is relatively small (0.2 M ha;

Anon., 1975/6) in relation to the total land area of contour suitable for protein extraction (approx. 5.5 M ha; Hutton, 1972). Most land in this latter category supports perennial ryegrass/white clover pastures. Thus if protein extraction is to be extensively applied to existing pastures in New Zealand the suitability of ryegrass/white clover for the process has to be examined. Data on ryegrass/white clover and lucerne crude protein levels is reviewed below.

Results for year-round trends in crude protein levels of pasture in the North Island (Johns, 1955; Hutton, 1961; Hutton *et al.*, 1967; McNaught & Doroffaef, 1968; Ross *et al.*, 1978; Saunders, W.H.M. & Metson, A.J. unpub. data; Bryant, A.M. & Hutton, J.B. unpub. data), have been collated and monthly means are presented in Fig. 1. Also plotted in Fig. 1 are monthly means for lucerne. These were calculated from 15 sets of year-round data comprised of results collected over several years from six different sites (farms) located on the Central North Island Volcanic Ash derived soils (N. Percival, unpub. data). A fall in pasture crude protein content commences in September and reaches a trough in November. Further decreases in January and February may be offset by relieving moisture stress with irrigation. For the whole of the spring-summer period, lucerne has a higher protein content than ryegrass/white clover pasture. The irrigated pasture had a higher protein content than non-irrigated lucerne.

The seasonal trend in the crude protein content of pasture will be determined by the botanical composition of the herbage and the protein content of the individual species. Thus in Fig. 2 it is seen that white clover is higher in crude protein than

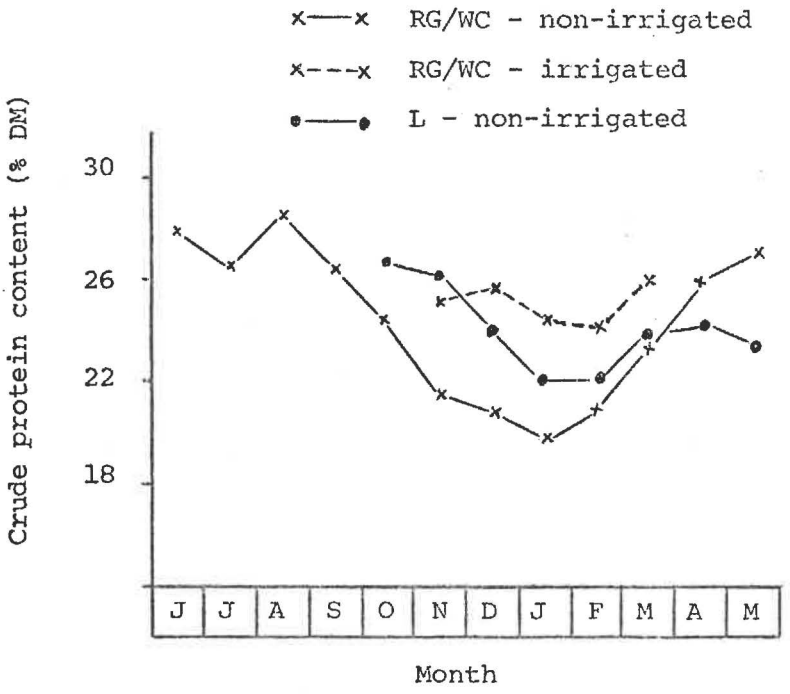

FIGURE 1: Ryegrass/white clover (RG/WC) and lucerne (L) herbage crude protein levels.

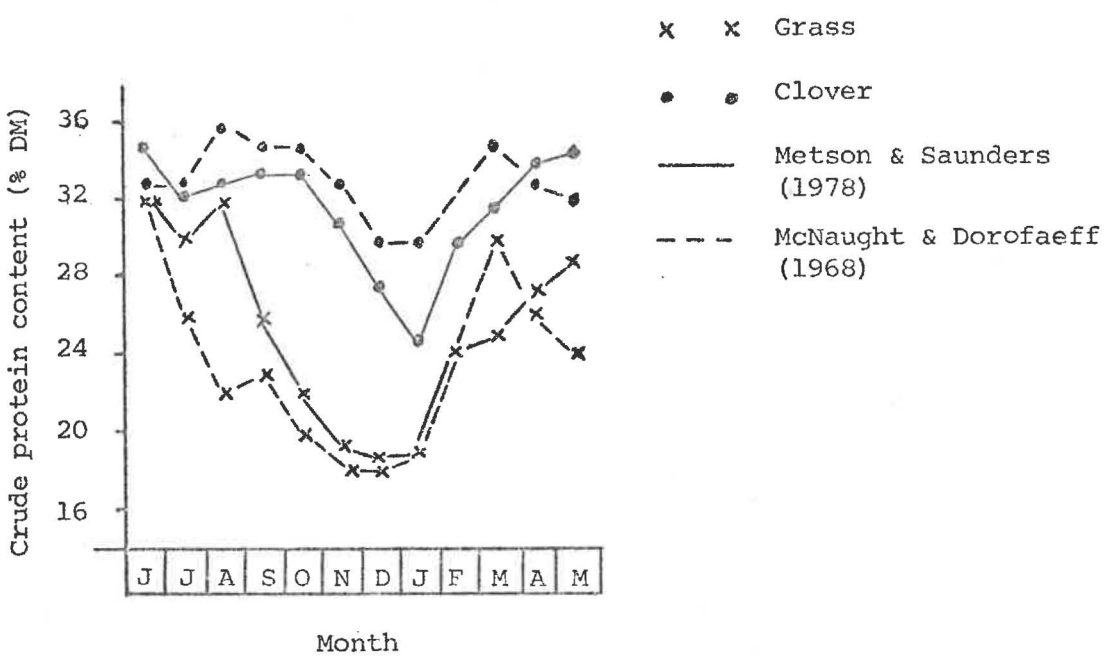

FIGURE 2: Seasonal changes in the crude protein content of ryegrass and white clover species.

ryegrass for all seasons of the year. The decrease in percentage crude protein over the spring-summer period is also much greater for ryegrass than clover.

A further factor to be considered in relation to pasture crude protein content is the amount of non-protein nitrogen present. Ross *et al.* (1978) and Metson & Saunders (1978) have shown the non-protein N content of herbage to rise in the spring and autumn; the latter especially after a drought. Non-protein N is also higher in ryegrass than clover herbage (Johns, 1955) and is also known to rise significantly with luxury dressings of fertilizer N (cf. Ross *et al.*, 1978; Metson & Saunders, 1978). Non-protein N can constitute 4-6 percentage crude protein units under normal growing conditions and up to 8 units with heavy fertilizer N application. It can thus account for 15-25% of total N in the spring and autumn and will probably be an even higher proportion in the extracted juice because of its high solubility and higher extractability than true protein N.

The actual botanical composition of ryegrass/white clover based pastures is determined by a host of factors. These will not be reviewed here but may be summarised as, climatic (Daly, 1973), edaphic (Daly, 1973; Brougham *et al.*, 1978), grazing management (Harris, 1978; Brougham *et al.*, 1978) and probably also pests and diseases.

Radcliffe and associates (Radcliffe, 1974, 1975a,b, 1976; Radcliffe & Sinclair, 1975; Baars, 1976; Rickard & Radcliffe, 1976; Round-Turner *et al.*, 1976) have published results for the contribution of grasses and clovers to total sward production in 'rate of growth' experiments throughout New Zealand. On low-land sites the contribution of white clover varied from as low

as 12-13% in the Gisborne and Dargaville localities, to 18-28% in Southland, Nelson, Hawkes Bay, Manawatu, dryland Canterbury (with and without irrigation), up to 38% on the west coast of the South Island. At 50% of the sites the white clover contribution to total seasonal production was lower and higher respectively, for the spring and summer seasons than the year-round means cited above. Botanical dissection results from the Waikato show the white clover content of swards to vary from 14 to 17% in the spring up to 25-28% in the summer (Bryant & Parker, 1971; Jagusch *et al.*,1978). These results indicate that at least two thirds and usually three quarters of the variation in pasture crude protein content is determined by the grass component of the sward in New Zealand. Thus the spring-early summer decline in crude protein illustrated in Fig. 1 is determined largely by the ryegrass component. Grasses other than ryegrass will contribute to this seasonal trend in a manner similar to ryegrass itself because of the similar change in crude protein content with maturity shown by a range of grass species (Minson *et al.*, 1960; 1964).

1.1.9 Conclusions

Equipment and processes have been successfully developed for the extraction, separation and drying of leaf protein. Species have been screened to assess their suitability for extraction and detailed work on the effects of agronomic variables on protein yields has been recorded. This work has been extended to the selection and testing of forage and cropping systems to maximise extractable protein yields for a limited range of circumstances. Lucerne is the crop most universally suited for protein extraction. However in New Zealand the land area in its culture

is small relative to ryegrass/white clover pastures for which little research has been done to assess suitability for protein extraction. This is especially so with respect to integration of protein extraction with existing pastoral animal production.

PART 1

CHAPTER 2. EXPERIMENTAL

1.2.1 INTRODUCTION

The experiments reviewed earlier were carried out almost exclusively with the objective of maximising protein yields. Thus cropping sequences and herbage cutting systems were not carried out with a view to their integration into existing ruminant grazing systems. The work of Allison and colleagues and McKenzie is a partial exception to this. In particular, ryegrass/white clover pastures have received scant attention and even then, grazing/harvesting intervals have not resembled rotation intervals employed in seasonal dairy farming in New Zealand. Furthermore, the extraction procedures used in most previous work were chosen with the objective of maximising extracted protein yields. The effects of such relatively intensive extraction on either the feeding value of pressed forage for ruminants or ensilage derived from pressed material, is unexplored.

The objectives of the experiments in Part 1 were:

1. measurement of the effects of regrowth interval and season on the extraction, separation and recovery of protein from ryegrass/white clover dairy pasture.
2. to make the measurements as under 1. on botanically pure swards of ryegrass, white clover and lucerne.
3. to take the results for protein recovery ratios and together with published data for herbage dry matter

production and crude protein content, to estimate potential recoverable protein yields.

As far as possible, herbage regrowth intervals were chosen to span those used commercially in dairy cattle grazing systems to assess their suitability for pasture management under protein extraction. Extraction and processing was carried out on a pilot industrial scale. There were two particular advantages of this. Firstly, the results obtained would approximate those at a full industrial scale, and secondly, it enabled the production of sufficient quantity of LPC for biological quality evaluation. Pulping and pressing procedures were selected to give a moderate (nominal 30%) rate of protein extraction. This was for three reasons. Firstly to ensure that for most circumstances, at least 16% crude protein remained in the pressed herbage to meet the protein requirements of lactating cows (NRC, 1971). Secondly, because of power and weight constraints, equipment being developed for on-farm extraction was aiming for moderate extraction performance (R.A. Mills pers. com.). Finally, moderate extraction would remove less soluble nutrients from the pressed herbage compared with more exhaustive extraction and hence would leave a product of higher nutritive value (Ulyatt, 1973).

1.2.2 MATERIALS AND METHODS

1.2.2.1 Site Description

The field plots were situated on Hamilton clay loam at the Ruakura Agricultural Research Centre, Hamilton, and the experiments were carried out between September, 1976, and December, 1977. All

of the plots had been regularly grazed by dairy stock and had received at least 35 kg/ha or phosphorus and 70 kg/ha of potassium as 30% potassic superphosphate in the previous twelve months.

1.2.2.2 Herbage Treatments

Four herbage treatments were examined. These were perennial ryegrass (RG) *(Lolium perenne)*, white clover (WC) *(Trifolium repens)*, mixed ryegrass/white clover pasture (RG/WC) and lucerne (L) *(Medicago sativa* - var. Wairau). The 'pure' stands of ryegrass and white clover were generated by taking paddocks dominant in these respective species and spraying with selective herbicides (see Appendix 1). Weed growth in the lucerne stand was controlled with selective sprays applied during the winter.

1.2.2.3 Regrowth Period Treatments

Regrowth period is the time lapse between topping and subsequent harvest for processing. Study was made of the effects of varying regrowth intervals on protein extraction and recovery characteristics for each of the herbage types outlined above. These measurements were made in late spring 1976 (for RG, WC, and RG/WC), summer 1977 (RG, WC, RG/WC, L), autumn 1977 (RG, RG/WC, L) and spring 1977 (RG/WC, L). The regrowth periods varied from four to twelve weeks. Each herbage plot was replicated. Sub-plot size varied from 100 m^2 to 600 m^2; the larger sized plots being associated with the shorter regrowth periods when there was less harvestable dry matter.

1.2.2.4 Plot Management

Fertilizer: During the experiments the RG, WC and RG/WC stands each received 25.5 and 21 kg/ha of phosphorus and potassium respectively in the autumn and 21 and 42 kg/ha of phosphorus and

potassium respectively in the spring. The lucerne stand received 20 and 96 kg/ha of phosphorus and potassium respectively in each of the spring and autumn seasons; trace applications of boron were also made. In addition, the RG and RG/WC plots received, respectively, 40 and 20 kgN/ha (as urea) in each of the summer, autumn and spring seasons of 1977. These N applications were made to help ensure good growth in the absence of continuous grazing (M. B. O'Connor pers. com.). Fertilizer application usually took place prior to topping at the commencement of each seasons regrowth treatments. In some instances however, due to the absence of rain, urea application was held over into the first week of regrowth.

Grazing: In the time interval between experimental harvest and commencement of the subsequent seasons treatments the plots were grazed with cattle.

Irrigation: The RG, WC and RG/WC swards were spray irrigated in the summer and autumn of 1977 when the soil moisture deficit was calculated to exceed 17.5 mm in January and February, and 24 mm in March. The amount of water applied was calculated to reduce the deficit to 8 to 10 mm in January and February and 12 to 14 mm in March.

Topping and Harvesting: At the nominated time in each season (see Appendix 2) the plots were topped with a flail harvester (Greens Distributing Ltd, Papakura) to a stubble height of 3-5 cm. The two replicates were topped one week apart and likewise after the nominal regrowth period were harvested one week apart. Duplicate one was always harvested between 0750 - 0825 hours and duplicate two between 1025 - 1100 hours. Details

are discussed in Appendix 3.

1.2.2.5 Harvesting and Processing

At harvest the herbage was cut to 3-5 cm stubble height with the same forage harvester used for topping. The harvested material was transported to the laboratory in a forage trailer. Within half an hour of harvest, processing had commenced.

A diagram of the processing system is shown in Fig. 3. The herbage feeding system consisted of a variable speed conveyor and levelling reel; feed rate was set at 100 kg dry matter/hour. Water was metered onto the herbage to reduce its dry matter content to a constant 10% (an infra-red quick determination of herbage moisture content was used to set the water addition rate). Pulping was done by a rotary pulper (of International Biological Programme design) which fed the pulped material directly onto the moving 200 mm wide belt of the press. The rate of travel of the belt was 11 cm/second and the static tension was 100 Newtons. The pulp was then squeezed between the belt and a slotted drum through which the juice passed. The fine fibre present in the juice was removed by a rotary filter with a 0.125 mm screen. The sludge from the filter was collected and added back to the grass stream as it went into the pulper during processing. Juice protein was coagulated by in-line steam injection to raise the temperature to 85-95°C. This was followed by cooling to 30°C. A self-desludging centrifuge (Alfa Laval BRPX209) separated the protein concentrate from the deproteinised juice (DPJ). The wet protein concentrate was then chilled to 6°C and held for a period of 0.5 to 3.0 hours prior to drying (to approximately 95% dry matter) by a spray process (Niro Production Minor Spray Drier; nominal capacity 20 kg/hour

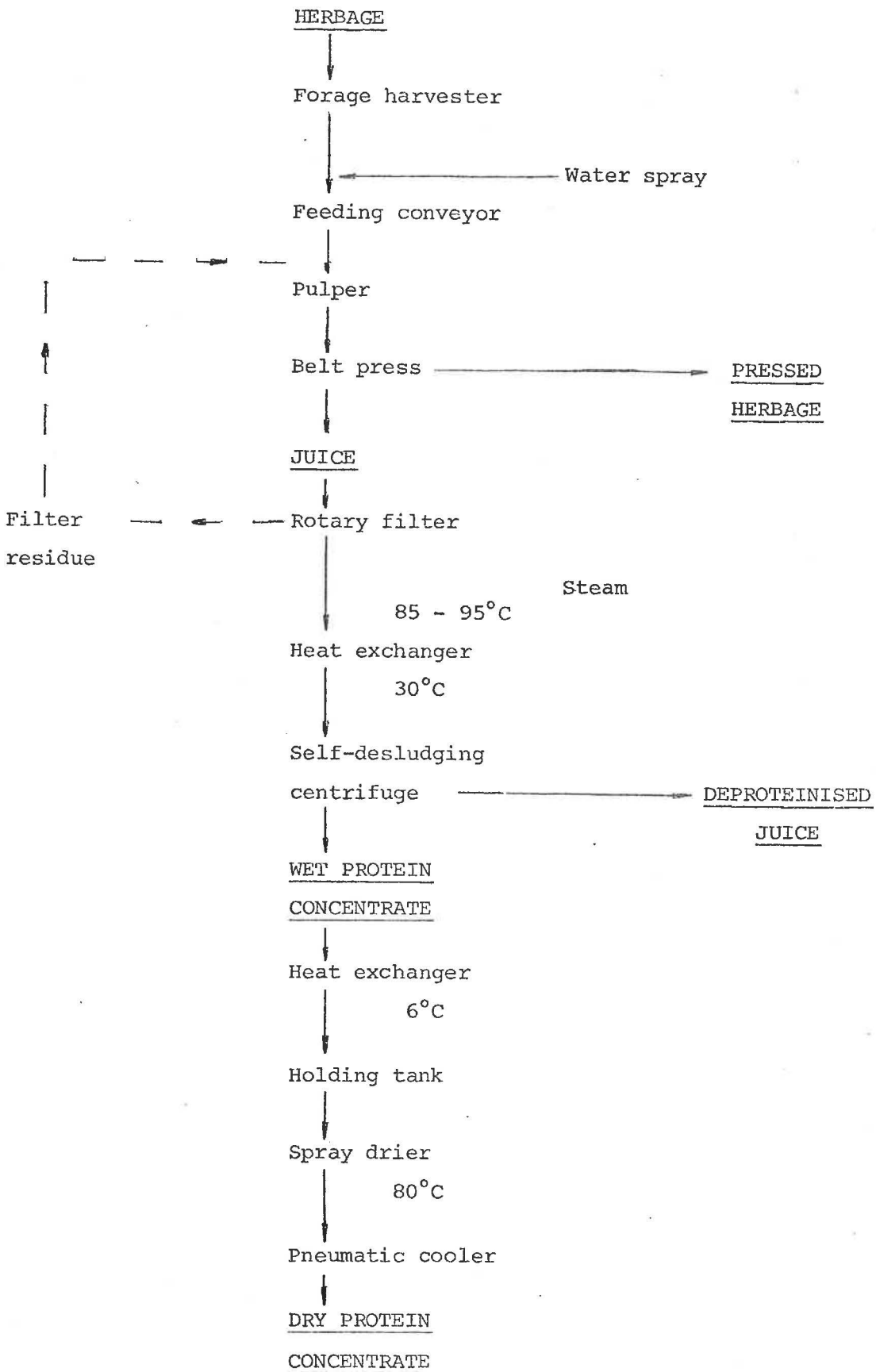

FIGURE 3: Extraction and processing system.

evaporation). The drier inlet and outlet temperatures were 200 and 80°C respectively, but the temperature of the product did not exceed 80°C. Immediately after drying the product was cooled to near ambient temperature with a pneumatic cooler and then stored under nitrogen at -15 °C in heat sealed gas impermeable plastic laminate bags.

Two hundred to four hundred kilograms of fresh herbage was processed on each occasion resulting in 2 to 4 kg of dry LPC.

Centrifuge Operation: Because of variation between treatments in the characteristics of both the juice and wet LPC, the centrifuge operating controls could not be held constant across all treatments. The quantity of wet LPC present in the centrifuge bowl was primarily determined by juice throughput, discharge interval, bowl open time at discharge and the dry matter (solids) concentration in the juice. The former three factors were centrifuge operating variables. The centrifuge was 'tuned' at a nominal throughput (10 l/min) for each treatment duplicate to give maximum dry matter content of the discharged wet LPC consistent with adequate clearance of sediment from the bowl and clarity of the deproteinised juice. Despite this 'tuning' the wet LPC discharged from the centrifuge varied in moisture content. The effects of this variation in the amount of deproteinised juice in the LPC on yields of dry matter and N at separation was corrected for (section 1.2.2.9).

The practise of adding water to the herbage to achieve 10% dry matter was adopted because preliminary work (Foster, R. F., unpub. data) had shown the extraction ratio to be affected by crop moisture in the range 10 to 20%. Thus uncontrollable field variables such as dew fall, rain and sunshine could affect grass

moisture and hence extraction results.

1.2.2.6 Processing Records

For mass balance calculations, weights of the herbage processed, pressed herbage, wet LPC and dry LPC were determined. The volume of juice and DPJ was determined for each by two methods - readings from volumetric tanks and flow meters; the average from both observations was used in calculations. The volume of water sprayed onto the herbage at pulping was monitored by flow meter. At the completion of centrifuging for each treatment, the centrifuge was washed out, and washings were collected for dry matter analysis. The dry weight was added to the weight of LPC for the calculation of yields.

1.2.2.7 Materials Sampling

Botanical dissection: twelve randomly selected samples, cut to 4 cm from the ground with hand shears immediately prior to the harvesting of each plot, were pooled, mixed and a representative sub-sample dissected into the categories shown in Table 2 (page 36). At the time of sampling the plots, subjective observations were made on the stage of reproductive growth of the herbage (i.e. early vegetative, late vegetative, early inflorescence, inflorescence). Representative samples of, herbage, pressed herbage, residual filter sludge, juice, wet LPC, DPJ, centrifuge washings and dry LPC were taken for dry matter analysis. After drying, the herbage and pressed herbage were ground and sub-sampled for laboratory analysis.

1.2.2.8 Analytical Procedures

Dry matter content was determined by drying at 100°C (herbage, pressed herbage, juice, DPJ and wet and dry LPC).

Organic matter content of the above samples was determined by ashing at 600°C for 12 hr. The N content of herbage, pressed herbage, juice, DPJ (spring 1976, summer 1977 only) and LPC was determined by Kjeldahl digestion followed by steam distillation and titration (Kjel-Foss Automatic 16210; A/S N. Foss Electric, Denmark). For digestion, sample size varied from 0.5 to 1.0 gm DM to which was added 15 gm of potassium sulphate, 0.75 g of mercuric oxide, 10 ml of 32% hydrogen peroxide and 15 ml of concentrated sulphuric acid. The digestion temperature was 400°C. Crude protein is calculated as N x 6.25. All analyses were duplicated.

1.2.2.9 Calculation of Results

The extraction ratio is calculated as the weight percent of the original herbage appearing in the juice. The separation ratio is the weight percent of the juice which appears in the leaf protein concentrate and the recovery ratio is the product of the above two, defining the weight percent of the original herbage present in the LPC. All of the above are on dry matter or nitrogen (crude protein) bases. Total balances for dry matter were calculated at the pressing and separation stages.

The wet leaf protein concentrate as discharged from the centrifuge contains soluble and insoluble phases (Pirie, 1971c; Kohler *et al.*, 1978), the latter representing the 'true' LPC. The aqueous phase is similar in composition to the DPJ. The N content (DM basis) of the aqueous phase is lower than the insoluble phase. As the degree of dewatering increases the yield of LPC decreases due to DM loss in the DPJ, but the N content of the LPC rises. The centrifuge used in this study discharged LPC containing 10 to 20% dry matter. However, machinery is now available

which will discharge sediment at up to 50% dry matter (Kohler *et al.*, 1978; McDonald & Donnelly, 1978). It seems that reduction of the moisture content of LPC to 40% could be regularly achieved under routine commercial operation (Vinconneau, H. pers. com.; Kohler *et al.*, 1978) and therefore the results for yields of LPC in this study were corrected to 40% dry matter.

1.2.2.10 Experimental Data

A large volume of experimental results were accumulated during the course of this work and only collated results are recorded in this thesis. Copies of the unprocessed data are held by the author and on computer file at the Ruakura Agricultural Research Centre.

1.2.2.11 Statistical Methods

The significance of changes within herbage treatments in association with regrowth period was tested by linear contrast regression analysis. The effect of herbages within seasons was tested by analysis of variance.

The following symbols are used to indicate levels of significance:

$P<0.001$ = ***; $P<0.01$ = **; $P<0.05$ = *; $P<0.10$ = +

1.2.3 RESULTS

1.2.3.1 Regrowth Period Treatments

The dates of topping coinciding with the commencement of regrowth in each season are shown in Appendix 2. The weeks of regrowth after which harvest took place are shown in Table 3 which summarises herbage crude protein levels.

In spring '76 harvesting took place weekly at four through to eight weeks for RG and RG/WC. For WC there was insufficient herbage until after the fifth week. The lucerne stand did not become available until after this first spring season. In summer '77 all herbage treatments were harvested weekly after four to eight weeks regrowth. Autumn growth was slower and commencement of harvest was delayed until the fifth and sixth week of growth for RG and RG/WC respectively. In the latter case harvesting was carried out at two-weekly intervals and extended through to 12 weeks. No WC could be harvested because of slow growth. Only RG/WC and L herbages were examined in spring '77; for the former harvesting was weekly from four to ten weeks regrowth and for L it was from five to nine weeks.

1.2.3.2. Botanical Composition of Swards

The results (Table 2) are presented as means for each season under the appropriate herbage main effect. In some instances composition changed in association with regrowth period. In spring '76 this was so for RG with its ryegrass content decreasing from an average of 87% in weeks 4, 5, and 6, to 66% in week 8. There was a corresponding increase in other grasses, mainly *Paspalum* species. In summer '77, changes occurred for all of RG, WC and RG/WC. For RG there was a decrease in ryegrass content from 78%

TABLE 2: MEAN (±SD) BOTANICAL COMPOSITION OF THE HERBAGE TREATMENTS (g/100g DM)

Component species	Herbage treatments: Ryegrass			White clover		Ryegrass/White clover				Lucerne	
	Ryegrass	Other grass	Remainder	White clover	Remainder	Ryegrass	Other grass	White clover	Remainder	Lucerne	Remainder
Season											
Spring '76	81 ± 5	16 ± 5	3 ± 1	91 ± 2	9 ± 3	37 ± 5	10 ± 2	26 ± 4	28 ± 4	-	-
Summer '77	64 ± 7	24 ± 7	12 ± 2	78 ± 5	21 ± 5	26 ± 5	20 ± 3	35 ± 2	19 ± 4	97 ± 1	3 ± 1
Autumn '77	91 ± 2	3 ± 1	6 ± 2	-	-	48 ± 5	5 ± 1	30 ± 3	29 ± 5	98 ± 1	2 ± 1
Spring '77	-	-	-	-	-	44 ± 3	20 ± 2	31 ± 2	4 ± 1	83 ± 4	17 ± 6

(weeks 4 and 5) to 48% (weeks 7 and 8) accompanied by an increase in the proportion of other grasses (from 12 to 34%). For WC there was a decrease in the proportion of white clover from 90% (weeks 4 and 5) to 70% (weeks 7 and 8). For RG/WC the changes occurred in the proportions of ryegrass (37% in weeks 4 and 5 down to 13% in weeks 7 and 8), other grasses (10% in weeks 4 and 5 up to 26% in weeks 7 and 8) and the 'remainder' (16% in weeks 4 and 5 up to 24% in weeks 7 and 8).

1.2.3.3 Stage of Growth of Swards

In spring '76 all herbage treatments were at the late vegetative-early inflorescence stage in the fourth and fifth weeks with a high proportion in flower by the seventh week.

In summer '77 the ryegrass plants were at the late vegetative-early inflorescence stage by weeks four and five of regrowth, when a small proportion of white clover plants (<12%) and 10% of the lucerne was flowering.

In autumn '77 the ryegrass, white clover and lucerne herbage all remained in the vegetative stage.

In spring '77 the *Poa* sp. present in the RG/WC treatment were flowering by the fourth week of growth, but ryegrass and clover plants did not flower until weeks seven and eight. Approximately 10% of the lucerne plants were flowering by the seventh week.

1.2.3.4 Herbage Crude Protein Content

There were significant species differences in crude protein levels in all seasons (Table 3). White clover contained more protein than RG/WC which in turn was higher than RG. Lucerne levels were similar to RG/WC in summer '77, lower in autumn '77

TABLE 3: HERBAGE CRUDE PROTEIN CONTENT (g/100 g DM)

Season	Herbage	Regrowth period (weeks)[a]								SD[b]	LC ± Se[c]	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	16.7	13.6	12.3	10.1	9.4	-	-	-	0.5	-1.8 ± 0.1	***[d]
	WC	-	27.0	25.1	23.0	22.1	-	-	-	1.3	-1.7 ± 0.4	*
	RG/WC	21.9	19.2	15.7	15.2	13.8	-	-	-	1.2	-2.0 ± 0.3	***
Summer '77	RG	18.7	16.5	17.2	15.7	15.8	-	-	-	1.0	-0.6 ± 0.2	*
	WC	25.1	22.4	25.4	23.1	20.9	-	-	-	3.7	-0.8 ± 0.8	NS
	RG/WC	22.4	22.7	22.8	21.7	20.3	-	-	-	0.9	-0.5 ± 0.2	+
	L	22.5	21.1	20.0	18.5	18.9	-	-	-	2.1	-1.0 ± 0.5	+
Autumn '77	RG	-	24.6	22.4	20.8	19.3	19.3	19.8	-	2.4	-3.4 ± 1.4	+
	RG/WC	-	-	28.1	-	23.8	-	25.2	24.1	1.0	-0.5 ± 0.2	*
	L	22.9	20.6	22.1	23.0	-	-	-	-	2.5	0.2 ± 0.8	NS
Spring '77	RG/WC	21.8	20.6	18.5	16.8	14.9	14.1	12.6	-	1.2	-1.6 ± 0.2	***
	L	-	26.1	24.6	21.5	21.1	20.5	-	-	0.8	-1.5 ± 0.2	***

a: regrowth period = weeks of growth from topping to harvesting

b: SD = standard deviation of a mean

c: LC ± Se = linear contrast ± standard error

d: Significant quadratic term in regression of 0.3 ± 0.1 ($P<0.05$)

There was a significant effect due to herbage type in spring '76 ($P<0.001$), summer '77 ($P<0.001$), autumn '77 ($P<0.05$) and spring '77 ($P<0.01$)

and higher in spring '77. In general the effect of regrowth period, as shown by linear contrast coefficients, was larger in the spring and autumn (for RG only) than in the summer. In spring '76 the rate of decline with increasing regrowth was similar for all herbages. For RG a quadratic term indicated a diminishing rate of decline. Although in summer '77 all linear contrasts indicated declining herbage crude protein content with increasing maturity, only for RG was this significant ($P<0.05$). RG and RG/WC crude protein levels fell with increasing regrowth in the autumn ($P<0.10$ and $P<0.05$, respectively), but those of lucerne did not. Decline in crude protein levels with maturity was highly significant in spring '77 and the rate similar, for both RG/WC and L.

1.2.3.5 Extraction Ratios for Nitrogen and Dry Matter

Overall, extraction ratios for N (crude protein) (Table 4) ranged from 22% (RG spring '76, week 8) to 37% (L spring '77, week 7). The ratios for RG were lower than for all other compared herbages while ratios for RG/WC and WC tended to be similar. Higher ratios were observed for lucerne than RG/WC and WC in seasons in which they were compared.

Declining extraction ratios with increasing herbage maturity occurred for RG in spring '76 and summer '77 and for L in summer '77. Similar trends approached significance for RG/WC in each of the two spring seasons. For RG, due to the relatively low results in weeks 5 and 6 an increasing extraction ratio with increasing regrowth also approached significance in the autumn season. In spring '77 the ratio for RG/WC fluctuated between 25 and 29% with a slight positive association with maturity. In the

TABLE 4: NITROGEN EXTRACTION RATIOS (g N in juice/100g N in herbage)

Season	Herbage	Regrowth period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	31.1	27.7	24.1	25.4	22.2	-	-	-	2.1	-2.0 ± 0.5	**
	WC	-	28.8	29.7	28.4	28.3	-	-	-	2.2	-0.3 ± 0.7	NS
	RG/WC	30.5	29.0	30.2	29.1	23.2	-	-	-	2.7	-1.4 ± 0.6	+
Summer '77	RG	25.5	27.8	26.2	23.9	20.7	-	-	-	1.9	-1.3 ± 0.4	*
	WC	28.1	28.8	25.4	31.6	25.6	-	-	-	3.1	-0.2 ± 0.7	NS
	RG/WC	28.2	27.9	26.7	27.3	25.9	-	-	-	2.1	-0.5 ± 0.5	NS
	L	35.9	34.0	33.9	32.5	29.1	-	-	-	1.3	-1.5 ± 0.3	**
Autumn '77	RG	-	24.3	27.5	29.9	28.4	28.8	29.2	-	1.9	0.8 ± 0.3	+
	RG/WC	-	-	31.3	-	29.7	-	27.4	27.5	2.5	-0.7 ± 0.4	NS
	L	37.1	31.9	33.0	34.4	-	-	-	-	3.2	-0.7 ± 1.0	NS
Spring '77	RG/WC	25.1	26.1	26.4	29.2	27.5	26.3	28.4	-	1.6	0.4 ± 0.2	+
	L	-	29.6	34.0	36.7	30.1	29.6	-	-	2.0	-0.4 ± 0.4	NS[a]

a: Significant quadratic contrast of -1.4 ± 0.4 ($P<0.05$)

There was a significant effect due to herbage type in summer '77 ($P<0.001$), autumn '77 ($P<0.01$) and spring '77 ($P<0.01$).

case of lucerne this trend was quadratic - increasing from 29 to 37% and decreasing again to 30%. Lucerne material did not separate very satisfactorily in the press at week 5 and there were large residues from the rotary filter, which would have contributed to the lower result for that week..

Dry matter extraction ratios are tabulated in Appendix 4. Results fell within the range 12 to 23% and were higher for WC than RG/WC which were higher than those for RG. Lucerne gave higher ratios than RG/WC. Regrowth period effects were generally as for N extraction ratios.

1.2.3.6 Juice Dry Matter and Crude Protein Content

The DM content of the expressed juice ranged from 2.2% to 7.8% (Appendix 5). Juice from the legumes had higher DM content than that from the ryegrass herbage. With the exception of WC in summer '77, juice DM content decreased with increasing regrowth.

The crude protein content of juice (Table 5) was higher for the two legume species than ryegrass in spring and summer '77. RG/WC tended to be similar to WC in summer '77 but intermediate to RG and WC in spring '76. Lucerne juice crude protein content was higher than that of RG/WC in the 1977 spring and summer seasons. For the autumn season all results were similar.

For juice obtained in the first season from RG and RG/WC crude protein content decreased with increasing regrowth; for RG the quadratic term was significant. In summer '77 there was a decline in crude protein with maturity for all herbage types, however this decline approached significance for RG/WC and L only. There was no significant association between regrowth period and

TABLE 5: JUICE CRUDE PROTEIN CONTENT (g/100 g DM)

Season	Herbage	Regrowth period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	26.0	20.1	17.5	18.8	17.5	-	-	-	0.9	-1.8 ± 0.2	***[a]
	WC	-	33.1	33.5	32.6	32.3	-	-	-	1.9	-0.3 ± 0.6	NS
	RG/WC	32.6	30.3	25.8	25.7	24.0	-	-	-	1.7	-2.2 ± 0.4	**
Summer '77	RG	29.6	29.6	30.4	28.4	28.3	-	-	-	2.4	-0.4 ± 0.5	NS
	WC	34.4	31.6	33.3	36.9	27.6	-	-	-	6.2	-0.8 ± 1.4	NS
	RG/WC	34.5	31.8	33.5	31.1	30.9	-	-	-	1.4	-0.8 ± 0.3	+
	L	37.4	35.6	33.7	34.2	33.7	-	-	-	1.8	-0.9 ± 0.4	+
Autumn '77	RG	-	34.5	32.1	29.8	27.3	29.4	32.7	-	4.6	-0.5 ± 0.8	NS
	RG/WC	-	-	35.4	-	30.1	-	33.4	33.5	1.5	-0.1 ± 0.2	NS
	L	35.4	27.7	30.9	32.9	-	-	-	-	5.5	-0.4 ± 1.7	NS
Spring '77	RG/WC	32.7	30.7	28.4	26.7	25.3	23.3	24.5	-	2.0	-1.5 ± 0.7	***
	L	-	36.7	36.9	34.7	35.5	34.9	-	-	1.4	-0.5 ± 0.3	NS

a: Significant quadratic contrast of 0.9 ± 0.2 ($P<0.05$)

There was a significant effect due to herbage type in spring '76 ($P<0.001$), summer '77 ($P<0.01$) and spring '77 ($P<0.001$).

juice crude protein for any of the herbages in autumn '77 while in spring '77 only RG/WC juice showed a decline in crude protein content.

1.2.3.7 The Crude Protein Content of the Pressed Herbage

These results follow the same general pattern as the parent herbage (Table 6) with the decline in crude protein in association with increasing maturity being marked for all herbages examined in the spring seasons. For RG only, a similar trend was found for the summer and to a lesser extent the autumn seasons.

1.2.3.8 The Separation Ratios for Dry Matter and Nitrogen

The corrected separation ratios for dry matter and N are presented in Tables 7 and 8 respectively; uncorrected results are in Appendices 6 and 7 respectively. Over all observations, the wet LPC (i.e. after separation) averaged 13% dry matter, detailed results are presented in Appendix 8. Correction reduced the average dry matter separation ratio from 48% to 42% and the N separation ratio from 71 to 68%. Patterns of response were similar for the actual and corrected results but only the latter are discussed.

Dry Matter Separation Ratios: The dry matter separation ratios (Table 7) were lower for RG than WC in the first two seasons and RG/WC was intermediate between these. The relativity of results between L and RG/WC was inconsistent with L higher in autumn and spring 1977 and lower in summer. The separation ratios for RG are low in weeks 7 and 8 of the summer season. This was due to very low ratios for one of each duplicate (30.5 and 26.6% vs 46.5 and 56.0% for the other two duplicates).

TABLE 6: THE CRUDE PROTEIN CONTENT (g/100 g DM) OF THE PRESSED HERBAGE

Season	Herbage	Regrowth period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	13.8	12.0	10.4	8.5	8.2	-	-	-	0.5	-1.5 ± 0.1	***
	WC	-	22.8	22.1	19.6	19.2	-	-	-	1.3	-1.3 ± 0.4	*
	RG/WC	18.7	16.7	14.1	13.3	12.6	-	-	-	1.1	-1.6 ± 0.2	**
Summer '77	RG	16.9	14.4	14.6	14.2	13.8	-	-	-	0.8	-0.6 ± 0.2	*
	WC	22.6	21.1	22.6	21.9	19.2	-	-	-	3.0	-0.6 ± 0.7	NS
	RG/WC	19.7	20.1	20.6	19.0	18.1	-	-	-	1.1	-0.4 ± 0.3	NS
	L	18.1	16.3	15.2	15.4	16.2	-	-	-	1.4	-0.5 ± 0.3	NS
Autumn '77	RG	-	21.4	19.5	17.5	17.4	17.3	17.8	-	1.9	-0.7 ± 0.3	+
	RG/WC	-	-	21.2	-	21.4	-	22.9	22.0	3.2	0.2 ± 0.5	NS
	L	19.7	18.6	20.2	20.9	-	-	-	-	1.7	0.5 ± 0.5	NS
Spring '77	RG/WC	19.9	18.9	16.9	15.2	13.2	12.5	11.2	-	0.8	-1.5 ± 0.1	***
	L	-	22.1	21.0	18.5	17.4	17.5	-	-	0.5	-1.3 ± 0.1	***[a]

a: Significant quadratic contrast of 0.3 ± 0.1 ($P<0.05$)

There was a significant effect due to herbage type in spring '76 ($P<0.001$), summer '77 ($P<0.001$), autumn '77 ($p<0.01$) and spring '77 ($P<0.05$).

TABLE 7: DRY MATTER SEPARATION RATIOS (g DM in LPC/100 g DM in juice)

(Results corrected to 40% dry matter in the wet LPC)

Season	Herbage	Regrowth period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	37.4	30.0	30.5	31.9	28.2	-	-	-	3.6	-1.6 ± 0.8	+
	WC	-	48.2	47.9	45.6	39.9	-	-	-	4.7	-2.7 ± 0.7	*
	RG/WC	43.3	36.8	34.5	31.0	31.5	-	-	-	4.0	-2.9 ± 0.4	**[b]
Summer '77	RG	43.0	45.0	46.0	35.0[a]	39.0[a]	-	-	-	10.6	-1.9 ± 2.4	NS
	WC	55.6	55.6	54.9	49.4	53.0	-	-	-	5.7	-1.1 ± 1.3	NS
	RG/WC	53.0	48.1	46.5	40.8	40.9	-	-	-	5.2	-3.1 ± 1.2	*
	L	47.3	44.5	43.5	39.2	36.7	-	-	-	3.6	-2.7 ± 0.8	*
Autumn '77	RG	-	48.6	43.4	37.9	35.6	37.0	37.0	-	4.5	-2.3 ± 0.8	*
	RG/WC	-	-	48.8	-	42.5	-	44.7	42.0	1.3	-0.9 ± 0.2	*
	L	58.4	50.6	54.4	49.3	-	-	-	-	7.4	-2.3 ± 2.3	NS
Spring '77	RG/WC	40.0	43.2	37.1	34.6	31.9	26.7	25.7	-	3.0	-2.9 ± 0.4	***
	L	-	45.4	40.8	39.2	39.3	41.2	-	-	0.9	-1.0 ± 0.2	**[c]

a: Low separation ratio for one of the duplicates of each of these two means (see text).

b: Significant quadratic term of 0.9 ± 0.4 ($P<0.10$)

c: Significant quadratic term of 1.0 ± 0.2 ($P<0.01$)

There was a significant effect due to herbage type in spring '76 ($P<0.05$), summer '77 ($P<0.01$), autumn '77 ($P<0.01$) and spring '77 ($P<0.05$).

Low centrifuge separation efficiency was also indicated by the colour of the deproteinised juice which was opaque green instead of the usual translucent pale brown. This incomplete separation did not result from failure of the steam injection equipment as indicated by juice temperature being lifted to within the range 85-95°C. The cause of this separation failure is not clear. The linear contrast coefficients show that dry matter separation ratio declined with increasing regrowth period for all herbages in all seasons, but some lacked significance. For RG in summer '77 this was because of poor separation in weeks 7 and 8. In two instances the relationships were quadratic (RG/WC spring '76; L summer '77).

Nitrogen Separation Ratios: The N separation ratios were similar for RG and WC in spring '76 but higher for WC in summer '77. The RG/WC ratio was lower than for either of the above two herbages in the first season but intermediate in the second season. Individual RG duplicates for weeks 7 and 8 of summer '77 were 31.5 and 55.7%, and 27.4 and 70.8%. In the summer and spring '77 seasons ratios were similar for RG/WC and L, but the autumn ratios were higher for RG/WC. RG on average was the lowest in the autumn due to a strong decrease with time.

Coefficients relating N separation ratio to regrowth period were all negative with one exception (L, autumn '77). Only in two cases however, was this association strong enough to be significant at $P<0.05$ or greater (RG autumn '77; RG/WC spring '77) and in one case (WC summer '77) the relationship was quadratic, being at its highest in week six.

1.2.3.9 Recovery Ratios for Dry Matter and Nitrogen

The mean DM recovery ratio for all observations was 8.3%.

TABLE 8: NITROGEN SEPARATION RATIOS (g N in LPC/100g N in juice)

(Results corrected to 40% DM in wet LPC)

Season	Herbage	Regrowth period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	71.8	74.7	78.5	73.2	66.7	-	-	-	7.1	-1.2 ± 1.6	NS
	WC	-	77.0	79.1	74.3	66.9	-	-	-	8.9	-3.5 ± 1.4	+
	RG/WC	71.9	61.6	63.9	63.0	60.4	-	-	-	7.7	-2.2 ± 0.8	+
Summer '77	RG	68.0	62.0	59.0	41.0[a]	47.0[a]	-	-	-	15.3	-6.3 ± 3.4	NS
	WC	69.4	71.2	78.5	61.8	63.9	-	-	-	4.0	-2.0 ± 0.9	+[b]
	RG/WC	70.2	72.9	69.0	62.1	58.4	-	-	-	7.6	-3.4 ± 1.7	NS
	L	75.1	64.9	70.1	65.4	61.6	-	-	-	9.8	-2.6 ± 2.2	NS
Autumn '77	RG	-	77.2	68.7	62.8	65.1	63.9	56.3	-	4.4	-3.3 ± 0.7	**
	RG/WC	-	-	78.5	-	78.8	-	76.2	74.4	5.1	-0.8 ± 0.8	NS
	L	69.3	68.3	74.1	69.9	-	-	-	-	6.7	0.8 ± 2.1	NS
Spring '77	RG/WC	71.8	75.3	72.3	72.1	67.2	62.8	56.5	-	3.3	-2.7 ± 0.4	***
	L	-	75.7	65.9	69.9	67.5	66.8	-	-	3.0	-1.6 ± 0.7	+

a: Low separation ratio for one of the duplicates of each of these two means (see text)

b: Significant quadratic contrast of -1.7 ± 0.7 (P<0.10)

There was a significant effect due to herbage type in spring '76 (P<0.05), summer '77 (P<0.05) and autumn '77 (P<0.05).

Thus for a given 100 kg of DM pulped and successively processed, on average, 8.3 kg of LPC was obtained. Results are presented in Table 9. There was a significant effect due to herbage type in all seasons with the legumes generally giving the higher results. The linear contrast coefficients indicate decreasing recovery with increasing regrowth for all treatments; only two coefficients failed to achieve significance.

For Nitrogen the overall average recovery ratio was 20% (Table 10). The effect of regrowth period can be clearly seen in Figures 4 a - d. The coefficients relating regrowth period to N recovery ratio were similar for all herbages in spring '76. Though WC appeared highest this was not significant. In summer '77 the decrease in recovery ratio with maturity was significant for all herbages except WC. Average ratios were highest and lowest for L and RG respectively. RG/WC was the only herbage for which recovery declined significantly in the autumn season. Again the highest and lowest ratios were observed for L and RG respectively, however RG/WC gave similar results to L in its earlier stages of regrowth. In spring '77, the association between N recovery ratio and maturity was quadratic for both RG/WC and L. For L, this was only at the 10% level of probability and derived from a very high result in week 7. On average lucerne gave a higher ratio than RG/WC.

1.2.3.10 Mass Balances

The recoveries of whole matter and dry matter immediately after the pressing and centrifuging stages are presented in Table 11. Results are meaned by herbage treatments for each season. Whole matter recovery at the press averaged 96% for all seasons. This was lower than the overall recovery for dry matter

TABLE 9: DRY MATTER RECOVERY RATIOS (g DM in LPC/100g DM in herbage)[a]

Season	Herbage	Regrowth period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	7.5	5.6	5.2	4.3	3.4	-	-	-	0.4	-1.0 ± 0.1	***
	WC	-	11.3	10.7	9.1	7.7	-	-	-	1.3	-1.2 ± 0.2	**
	RG/WC	8.9	6.7	6.3	5.3	4.2	-	-	-	0.8	-1.1 ± 0.1	***
Summer '77	RG	6.9	7.0	6.8	4.7	4.6	-	-	-	1.6	-0.7 ± 0.4	NS
	WC	11.4	11.3	10.8	9.7	10.3	-	-	-	0.8	-0.4 ± 0.2	*
	RG/WC	9.7	9.5	8.4	7.8	7.0	-	-	-	0.5	-0.7 ± 0.1	**
	L	10.2	9.0	8.7	6.9	6.0	-	-	-	0.8	-1.1 ± 0.2	**
Autumn '77	RG	-	8.4	8.3	7.9	7.2	7.0	6.6	-	0.4	-0.4 ± 0.1	**
	RG/WC	-	-	12.1	-	10.2	-	9.2	8.3	0.5	-0.6 ± 0.1	**
	L	14.1	12.0	12.8	11.7	-	-	-	-	1.1	-0.6 ± 0.3	NS
Spring '77	RG/WC	6.6	7.5	6.4	6.4	5.2	4.2	3.8	-	0.4	-0.6 ± 0.1	***[b]
	L	-	9.5	9.2	8.9	7.0	7.1	-	-	0.3	-0.7 ± 0.1	***

a: Calculated as the product of the extraction ratio and the separation ratio at 40% DM.

b: Significant quadratic term of -0.1 ± 0.03 ($P<0.01$)

There was a significant effect due to herbage type in spring '76 ($P<0.01$), summer '77 ($P<0.001$), autumn '77 ($P<0.001$), spring '77 ($P<0.001$).

TABLE 10: NITROGEN RECOVERY RATIOS (g N in LPC/100g N in herbage)[a]

Season	Herbage	Rest period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	22.4	20.6	18.9	18.6	14.8	-	-	-	1.6	-1.7 ± 0.4	**
	WC	-	22.1	23.5	21.0	18.9	-	-	-	3.0	-1.2 ± 0.5	+
	RG/WC	21.9	17.9	19.3	16.1	14.1	-	-	-	2.2	-1.7 ± 0.2	***
Summer '77	RG	17.3	17.3	15.3	10.0	9.3	-	-	-	2.8	-2.3 ± 0.6	*
	WC	19.6	20.5	19.9	19.5	16.4	-	-	-	2.6	-0.8 ± 0.6	NS
	RG/WC	19.7	20.2	18.3	16.9	15.1	-	-	-	0.9	-1.3 ± 0.2	**
	L	26.8	22.1	23.7	21.3	17.9	-	-	-	2.9	-1.9 ± 0.7	*
Autumn '77	RG	-	18.8	18.8	18.8	18.5	18.4	16.5	-	1.5	-0.4 ± 0.3	NS
	RG/WC	-	-	24.5	-	23.4	-	20.7	20.5	1.7	-0.7 ± 0.3	*
	L	25.8	21.8	24.5	24.1	-	-	-	-	3.8	-0.2 ± 1.2	NS
Spring '77	RG/WC	18.0	19.6	19.1	21.0	18.5	16.5	16.2	-	1.0	-0.4 ± 0.1	*[b]
	L	-	22.4	22.4	25.7	20.3	19.7	-	-	1.6	-0.8 ± 0.3	+[c]

a: Calculated as the product of the extraction ratio and separation ratio at 40% DM.

b: Significant quadratic contrast of -0.3 ± 0.1 ($P<0.05$)

c: Significant quadratic contrast of -0.7 ± 0.3 ($P<0.10$)

There was a significant effect due to herbage type in summer '77 ($P<0.01$), autumn '77 ($P<0.001$) and spring '77 ($P<0.05$).

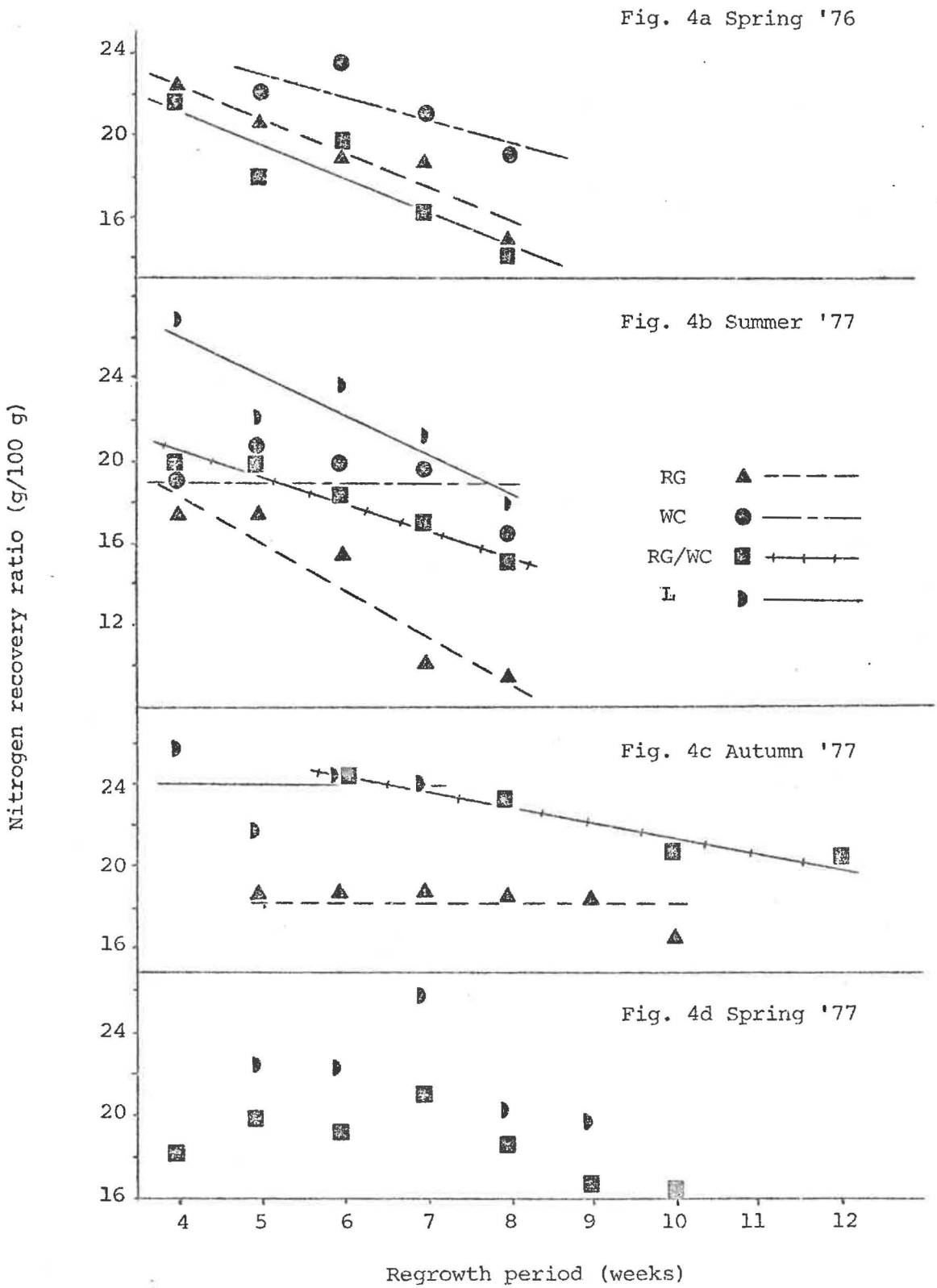

FIGURE 4: Relationships between nitrogen recovery ratio and regrowth period.

TABLE 11: RECOVERIES (±SD) OF WHOLE MATTER AND DRY MATTER AFTER PRESSING AND AFTER CENTRIFUGING (g/100g)

Season	Species	whole matter after pressing[a]	dry matter after pressing[b]	whole matter after centrifuging[c]	dry matter after centrifuging[d]
Spring '76	RG	97 ± 3	102 ± 4	105 ± 4	99 ± 4
	WC	95 ± 4	96 ± 3	105 ± 4	100 ± 8
	RG/WC	96 ± 4	99 ± 3	106 ± 2	91 ± 8
Summer '77	RG	98 ± 3	102 ± 3	110 ± 3	98 ± 5
	WC	99 ± 3	99 ± 4	107 ± 4	95 ± 6
	RG/WC	100 ± 1	102 ± 4	112 ± 4	100 ± 4
	L	96 ± 3	104 ± 4	108 ± 3	97 ± 8
Autumn '77	RG	97 ± 2	100 ± 2	111 ± 2	100 ± 3
	RG/WC	97 ± 3	98 ± 4	110 ± 3	99 ± 3
	L	94 ± 2	96 ± 3	115 ± 6	96 ± 10
Spring '77	RG/WC	96 ± 2	96 ± 3	108 ± 6	97 ± 4
	L	93 ± 2	96 ± 2	107 ± 8	98 ± 4

a: Calculated as weight of herbage plus water added divided by weight of juice plus filter residue plus pressed herbage.

b: As for [a] but on a dry matter basis.

c: Calculated as weight of wet LPC plus DPJ divided by weight of juice processed.

d: As for [c] but on a dry basis and including the weight of centrifuge washings in the numerator.

(99%) because of moisture loss off the conveyor and vapour loss at pulping. Whole matter recovery after centrifuging exceeded 100% because of water addition in the form of steam at coagulation. Dry matter recovery at the centrifuge was on average 97%. The very low result for RG/WC in spring '76 derived from low recoveries of dry matter in DPJ for two replicates.

1.2.4 DISCUSSION

The results are discussed under three major headings; extraction ratios, separation ratios and recovery ratios. Other results which relate tc these major headings are discussed as appropriate. Finally, the recovery ratios are used in conjunction with estimated protein yields to estimate recoverable protein yields.

1.2.4.1 Extraction Ratios

The effect of herbage type

Since all the extractions were carried out at similar crop moisture content and machine settings, differences in extraction ratio between herbage treatments must have been determined by herbage factors: primarily fibre and protein contents (see review). Thus lucerne and white clover had higher protein contents than ryegrass in spring 1976 and summer 1977 and had higher extraction ratios. In the autumn however, ryegrass had a similar protein content to lucerne, but had a lower extraction ratio. Similarly in summer 1977, despite a higher overall protein content, white clover yielded a much lower extraction ratio than lucerne. This suggests herbage protein content itself is not the strongest determinant of extraction ratio. Heath & King (1977) observed a lower extraction ratio for ryegrass than lucerne and attributed

this to the former's higher fibre content. However, herbage protein content decreases and fibre content increases in association with advancing maturity especially in grasses. It is therefore not possible to clearly differentiate the effects of these two herbage components on extraction ratio.

The effect of regrowth period

For ryegrass, the crude protein extraction ratio decreased with increasing plant maturity in each of the first two seasons and was associated with a decline in herbage crude protein content. There would also have been an increase in herbage fibre content (Bailey, 1973) and hence greater resistance to pulping and pressing. Also, in the spring and summer seasons, the increase in the proportion of other herbage species, particularly *Paspalum* sp., which have a higher fibre content (Bailey, 1973) and lower extractability than ryegrass (Ostrowski, H. T., unpub. results), may have further contributed to the decreasing extraction ratio. In autumn 1977, although herbage crude protein declined from 25% to 20%, there was no decline in extraction ratio. This contrasting result is probably due to the herbage remaining in the vegetative stage throughout. Bailey (1973) comments that when grasses are maintained in a vegetative state by management techniques, fibre content does not rise.

Dry matter extraction ratios generally showed a response to increasing maturity similar to that observed for crude protein. Reasons for the rather low result for both of these measures at the first harvest in the autumn season are not clear, but may be related to herbage succulence (see later). In the case of crude protein extraction ratio, these low results were largely responsible for the overall increase with advancing growth.

Pure white clover herbage was processed in the first two seasons only. Crude protein extraction ratio did not decline with advancing regrowth in either season, despite a marked decline in herbage crude protein content in spring 1976 and a lesser decline in summer 1977. White clover herbage shows little change in fibre content as it ages (Bailey, 1973) and this is probably why increasing maturity did not affect the extraction ratio.

The ryegrass/white clover herbage comprised 46 to 64% grasses and 26 to 35% white clover (Table 2). Bearing in mind the crude protein levels displayed by the pure swards of ryegrass and white clover the herbage crude protein levels (Table 3), were as expected from these botanical composition results. The high autumn levels (24-28%) are in agreement with previous observations (see review). Crude protein content declined in association with increasing maturity in all seasons. In contrast, there were only small and non-significant decreases in crude protein extraction ratio with increasing maturity in the first three seasons. In the final season the linear contrast showed an increase with increasing herbage maturity. For the first two seasons then, and in contrast to the results for the ryegrass treatment, the effect of the grass component of the sward was not sufficiently large to result in a depression in the extraction of crude protein with increasing maturity. This is possibly explained by the substantially higher crude protein content of the clover than the grass herbage and hence despite an approximately 2:1 grass/clover dry matter ratio an approximately 1:1 protein ratio occurred in the herbage. The protein in the clover would also have had a higher extractability than that in the grasses. Furthermore, at dissection 20-30% of

the components of the ryegrass/white clover herbage were categorised as 'remainder'. This group was comprised largely of weeds. The effect of maturity on the ease or resistance of the weed species to extraction is unknown. In the autumn, despite regrowth extending over 84 days, extractability did not decrease with increasing age. This result is generally in agreement with results for the other herbages and is very likely a consequence of herbage remaining in the vegetative state. In contrast however, and rather unexpectedly, nitrogen extractability increased as regrowth progressed in spring 1977. Reasons for this are not clear. However Weiringa (1978) has also observed that young succulent ryegrass and mixed pasture herbages give lower extraction ratios than more mature herbage. Thus relative to older herbage, young lush material may, on pulping, produce a sap with a higher viscosity rating making separation from the fibrous fraction more difficult. Arkcoll (1971) also has observed that some species extract badly and in these cases he described the sap as mucilaginous. Spring regrowth commenced earlier in 1977 than 1976 (Appendix 2). Thus after the same growth interval, and as judged from later flowering, the ryegrass and clover herbage was at an earlier vegetative stage in 1977 than 1976. This may have been associated with the difference between years in the change in extraction ratio with advancing growth.

For the lucerne herbage, crude protein content decreased with advancing maturity in the summer and spring but not autumn seasons. The extractability of crude protein decreased in parallel in summer but not autumn and in spring 1977 peaked at week 7. Lucerne herbage was at one tenth bloom by the fourth week of regrowth in the summer season. Advancing maturity thereafter with decreasing and increasing herbage protein and fibre

(Bailey, 1973) contents, respectively, would have been responsible for the decreasing extraction ratios. In the autumn, growth rate was low and the herbage remained vegetative. Thus extraction ratios remained relatively unchanged with increasing age. In spring 1977, the lucerne herbage did not reach 10% flowering until the seventh week of regrowth when extraction of dry matter and crude protein were highest. The lower ratios at earlier regrowth may be associated with immaturity and succulence of the herbage as discussed for ryegrass and ryegrass/white clover.

Regression analysis was used to study the relationship between herbage protein content and protein extraction ratio on a within herbage basis (Table 12). The results indicate that herbage protein level was not strongly and consistently related to extraction ratio for any of the herbages. At most, only 67% of the variation in extraction ratio was accounted for by crude protein content.

TABLE 12: CORRELATION COEFFICIENTS BETWEEN CRUDE PROTEIN EXTRACTION RATIO AND HERBAGE CRUDE PROTEIN CONTENT

	Herbage			
Season	Ryegrass	White clover	Ryegrass/ White clover	Lucerne
Spring '76	0.82**	-0.03	0.62*	-
Summer '77	0.26	0.20	0.74*	0.48
Autumn '77	-0.70*	-	0.17	0.78**
Spring '77	-	-	0.58	-0.13

Relationship of crude protein extraction ratio to dry matter extraction ratio

There was a positive correlation between these two variables (Table 13), however, only in a few cases was this relationship

sufficiently strong to account for 70% or more of the variation in crude protein extraction ratio.

TABLE 13: CORRELATION COEFFICIENTS BETWEEN CRUDE PROTEIN EXTRACTION RATIO AND DRY MATTER EXTRACTION RATIO

Season	Herbage			
	Ryegrass	White clover	Ryegrass/ White clover	Lucerne
Spring '76	0.84**	0.68*	0.89**	-
Summer '77	0.80**	0.67*	0.88**	0.88**
Autumn '77	0.70*	-	0.80*	0.44
Spring '77	-	-	0.23	0.84**

It would thus not be possible to predict with reasonable accuracy the yield of crude protein from results of dry matter extraction.

Juice dry matter content

On average, juice dry matter content (App. 5) reflected the ease of extraction, being higher for white clover than ryegrass with ryegrass/white clover generally intermediate. Similarly, juice from lucerne averaged higher dry matter content than ryegrass/white clover juice in autumn and spring 1977. However in summer 1977,despite extraction being higher for lucerne, juice dry matter content was higher for ryegrass/white clover. Correlation coefficients between juice dry matter content and dry matter extraction ratio were greater than 0.80 in several instances (Table 14), however, any one herbage did not yield high coefficients for all seasons. Correlations between juice dry matter content and protein extraction ratio were in all cases lower than those in

Table 14 and are not presented.

TABLE 14: CORRELATION COEFFICIENTS BETWEEN JUICE DRY MATTER CONTENT AND DRY MATTER EXTRACTION RATIO

Season	Herbage			
	Ryegrass	White clover	Ryegrass/ White clover	Lucerne
Spring '76	0.80**	0.66*	0.90**	-
Summer '77	0.85**	0	0.28	0.77**
Autumn '77	0.20	-	0.82*	0.22
Spring '77	-	-	0.38	0.63*

The dry matter concentration in juice has implications for juice transport and processing. If on-farm extraction was developed with juice transport to a central factory for further processing, then juice with a higher dry matter content would have lower transport costs per unit dry matter than more dilute material. Other things being equal, further savings associated with higher juice dry matter content would be:

(a) lower storage volume per unit weight of dry matter.

(b) lower heat input for coagulation per unit dry matter.

(c) lower juice volume requiring centrifugal separation for any given quantity of dry matter.

(d) a lower volume of deproteinised juice requiring treatment/ disposal for any given weight of leaf protein concentrate produced.

All of these factors lead to lower capital and plant running costs.

1.2.4.2. Separation Ratios

At separation, coagulated juice is separated into wet LPC and deproteinised juice. The major commercial objective would be to minimise the moisture content of the wet LPC consistent with acceptable throughputs and losses of insolubilised solids in the deproteinised juice. The centrifuge used dewatered coagulated juice up to 10 to 20% dry matter content (Appendix 8). Separation yields were corrected to 40% dry matter; this was to standardise them, and give yield data comparable to that which would be obtained with a decanter centrifuge (section 1.2.2.9). The actual and corrected average separation ratios for N were 71 and 68%, respectively; on the basis of dry matter these respective ratios were 48 and 42%. The corrected averages are similar to the separation ratios calculated from the data of Kohler *et al.* (1978) for lucerne juice processed through a decanter centrifuge (73% for N and 45% for DM).

The effect of herbage type

There was a significant effect of herbage type on dry matter separation ratios in all seasons (Table 7). N separation ratios were similarly affected in all but the spring 1977 season (Table 8). Herbage factors which might have caused these effects are: juice N and protein N contents, the susceptibility of the latter to coagulation and centrifugal separation (see section 1.1.5) and juice dry matter content. Examination of the influence of juice N and dry matter contents is made later.

Within season between herbage differences in average DM and N separation ratios were not consistent. For example DM separation ratios were higher for white clover than ryegrass in the

first two seasons and although N separation ratios were higher for white clover in summer 1977, both species gave similar ratios in spring 1976. This variation is not explained by juice N and DM contents which were higher for white clover in both seasons. Similarly, when comparing lucerne and ryegrass/white clover results from the summer season, average DM separation ratios were higher for ryegrass/white clover but the ratios for N were similar for the two herbages. In this season average juice DM and N contents were similar for the two. These between herbage differences for DM and N separation ratios may be partly due to the calculation of N ratios on a total N and not a true protein N basis, since for any given wet LPC total N content, the concentration of N in the aqueous phase will have been higher for those herbages with lower protein N content.

The effect of regrowth period

Increasing herbage maturity resulted in decreasing DM separation ratios for all herbages in all seasons (Table 7). In not all cases, however, were the linear contrasts significant. As expected there was also a general tendency, significant in some cases, for N separation ratios to decrease with increasing maturity (Table 8). These effects of maturity of herbage were more clearly expressed in the DM than the N separation ratios.

In three instances the relationship of separation ratio to regrowth period was curvilinear. Two of these for DM (RG/WC, spring 1976; L, spring 1977) showed very small deviations from linearity, while curvilinearity for white clover in summer 1977 was derived largely from an unusually high result for week 6.

Reasons for this are not clear. Poor separation efficiency occurred for one ryegrass duplicate in each of weeks 7 and 8 in summer 1977. Vartha & Allison (1973) recorded separation difficulties with steam precipitated ryegrass juice using low speed centrifugation through a cloth filter. These authors suggested there may be problems separating LPC from coagulated juice obtained from mature ryegrass herbage. However, in the present experiment there were no problems separating the other duplicate for each of the two treatment weeks in question and also separation was successfully carried out on coagulated juice extracted from ryegrass harvested after 9 and 10 weeks of growth in the autumn season.

Relationship of separation efficiency to juice composition

To determine if variations in separation efficiency were associated with juice DM and N contents, correlation coefficients were calculated between these and each of DM and N separation ratios. For each of juice DM and N contents, correlations were higher with DM separation ratio than N separation ratio. Only the coefficients for DM separation ratio are given here (Table 15). They are moderately high for the relationship with juice dry matter content on all seasons except autumn 1977. However, those with juice N content are highly variable between herbage types and seasons. Multiple regression analysis was also carried out relating DM separation ratio to both juice DM and N contents. However, for each herbage type (within seasons) the correlation coefficients were generally no higher than the highest coefficient from the single regressions with each of juice DM and N content as independent variables. These results suggest that juice DM

and N contents are not strongly related to DM separation efficiency. However, juice DM content at least should be

TABLE 15: CORRELATION COEFFICIENTS BETWEEN DM SEPARATION RATIO[a] AND JUICE DM CONTENT AND JUICE N CONTENT

Season	Independent variable	Rye-grass	White clover	Ryegrass/ White clover	Lucerne
Spring '76	Juice DM%	0.68**	0.71*	0.74*	-
	Juice N%	0.73**	0.17	0.88**	-
Summer '77	Juice DM%	0.56	0.53	0.67*	0.48
	Juice N%	-0.50	-0.20	0.46	0.22
Autumn '77	Juice DM%	0.18	-	0.13	0.40
	Juice N%	0.82**	-	0.52	0.20
Spring '77	Juice DM%	-	-	0.73**	0.68*
	Juice N%	-	-	0.91**	0.51

a: DM separation ration at 40% solids in wet LPC.

examined in further studies of biological factors affecting separation performance.

1.2.4.3. Recovery Ratios

The recovery ratio is the proportion of the protein in the original herbage obtained in the LPC.

The effect of herbage type

Recovery ratios of DM and N were generally highest for the legume herbages further confirming their superiority, especially lucerne, for extraction and separation processing (see review).

The effect of regrowth period

The effects of advancing herbage maturity on this ratio are generally as expected from the effects on the extraction and separation ratios. Since both of these latter ratios, for both DM and N, showed a general tendency to decrease with increasing regrowth their summed effects in the recovery ratio showed a clearer response, and the number of linear contrasts achieving significance was higher. Over all herbages and seasons, the rate of decrease in separation ratio with advancing herbage maturity was smaller for DM than N (average linear contrast = 0.8 v 1.1; Tables 9 & 10). However, the effects of maturity were more clearly shown for DM than N (10 v 7 linear contrasts significant at $P<0.05$ or greater).

Relationships between recovery ratios and herbage crude protein content

Correlation coefficients were calculated between each of DM and N recovery ratios and herbage crude protein content. The coefficients were generally higher for DM recovery ratio than N recovery ratio. For DM they were highest in the two spring seasons (1976 - RG 0.96, WC 0.79, RG/WC 0.96; 1977 - RG/WC 0.86; L 0.69) when there was a highly significant decline in each of herbage crude protein content and DM recovery ratio with advancing regrowth for all herbage types. The correlation coefficients were smaller in the summer and autumn seasons, possibly because of the less marked affect of maturity on herbage crude protein content and DM recovery ratio in these seasons relative to the spring seasons.

1.2.4.4 Integration with Grazing Management

The objectives of this work were partly to investigate

aspects of the integration of protein extraction from pasture herbage with rotational grazing management as practised on seasonal dairy farms in New Zealand. Thus extraction and separation ratios were measured on ryegrass/white clover based pasture, and its major species components, after a range of intervals of growth appropriate to the spring, summer and autumn-winter seasons of the year.

In reviewing the results consideration is given to:

i. the recovery ratios achieved in relation to rotation intervals used in industry.

ii. the crude protein content of the pressed herbage in relation to dairy cow feed requirements.

These factors are discussed for each of the seasons of study.

Spring

Grazing intervals fall within 14 to 28 days on seasonal dairy farms (A.M. Bryant pers. com.). In this experiment insufficient dry matter had accumulated for satisfactory harvest after 14 and 21 days of growth in both the 1976 and 1977 years and the first harvest was made after 28 days. Hence for an integrated system, grazing/harvest intervals of not less than about one month would be necessary. In practise, harvest intervals would be influenced by pasture growth rates, pasture density, the area of pasture available on a daily basis and the minimum quantity of herbage required for extraction. Extraction ratios were satisfactory for the ryegrass/white clover herbage in both years although lower in 1977 at the earlier regrowth intervals. In 1977 the experiments were started earlier in the season and carried on for a longer period (10 weeks growth) to obtain data

appropriate to forage conservation by ensiling. Extraction ratios remained relatively high through to the tenth week although separation ratios declined and hence so also did recovery ratios. The latter, however, were still satisfactory at the tenth week.

The crude protein content of the pressed herbage fell below the minimum of 16% for lactating cows (NRC, 1971) after six and seven weeks growth respectively in the 1976 and 1977 years. On this criteria alone then, and where the pressed herbage was to be fed unsupplemented to lactating cows, such regrowth periods approximate the longest tenable rotation intervals. However, the botanical composition of the sward is a factor which could modify this since pressed ryegrass and white clover had, respectively, lower and higher crude protein contents than the nominal limit of 16%. The effect of locality on sward white clover content was reviewed in section 1.1.8. This same approximate minimum limit for crude protein in the pressed herbage (16%) would apply also to pressed herbage intended for ensiling if the resulting material was to be fed to lactating cows. However, if this feed was to be offered unsupplemented to non-lactating cows or other 'dry' stock then the nominal crude protein minimum could reduce to 11% for non-lactating cows or 13% for growing replacement stock (NRC, 1971). In this case respective regrowth periods of 10 and 8 weeks would be maximum.

Summer

The herbage treatments other than lucerne were irrigated in summer to ensure adequate growth. Under these conditions N extraction rates for ryegrass/white clover herbage were 26 to 30% over the four through eight weeks regrowth. However, separation

efficiencies declined and so also did recovery ratios from 20% (week 4) to 15% (week 8). The crude protein content of the pressed herbage remained well above 16% over the four through eight week regrowth intervals and hence would not influence chosen grazing intervals.

Under conditions where irrigation was not practised pasture growth and protein content would be reduced if rainfall was so low that soil moisture deficit limited growth (see next section).

Autumn-Winter

On seasonal dairy farms available pasture is rationed, along with conserved supplements, over the late autumn-winter period. This enables accumulated autumn growth to be carried forward and fed over later autumn-winter months when pasture growth is minimal and usually below daily feed requirements. This rationing sometimes results in intervals between grazing extending to 100 or more days. However, in the autumn season, harvesting and processing of the ryegrass and ryegrass/white clover extended over 70 and 84 days, respectively. Although N extraction ratios declined slightly over this period they were adequate (27 - 29%). Likewise there was a small decline in separation efficiency over the same period and hence recovery ratios fell from 24% (week 6) to 20% (week 12). Recovery of protein from autumn saved pasture grown over 84 days at least appears to be practical.

The crude protein content of both the ryegrass/white clover and ryegrass herbages remained above 21% and 17%, respectively, after extraction. Protein content of the pressed herbage would thus not influence selected pasture harvesting intervals under seasonal dairying.

1.2.4.5 Estimation of Protein Yields

Pasture and lucerne growth

Growth data was taken from long-term studies in the central Waikato, for ryegrass/white clover, and the Volcanic Plateau, for lucerne, of the North Island. This enabled calculation of means with standard deviations based on variability between years.

The Waikato pasture growth data are presented in Figure 5. Irrigation both increased total annual growth by 34% and reduced the between year standard deviation considerably in the summer period (i.e. from 84% to 9% in February). Total annual protein production was estimated using the monthly means of herbage crude protein level (with and without irrigation, respectively, from Fig. 1), and of DM growth rates per ha (Fig. 5). Estimated annual crude protein production was 4533 $\pm$ 666 and 3207 $\pm$ 982 kg/ha, respectively, for irrigated and non-irrigated sites.

Lucerne growth records for the Volcanic Plateau (Wairakei Research Station) were taken over the period 1965-6 to 1971-2 (J. A. Douglas pers. com.). Data for 1968-9 were incomplete and are excluded. Mean annual production was 11449 $\pm$ 1292 kg DM/ha. Using the mean crude protein level for lucerne from Fig. 1 (24%), estimated crude protein yield was 2748 $\pm$ 310 kg/ha.

Recoverable protein yields

For ryegrass/white clover pasture, the protein recovery ratios used for the spring, summer and autumn-winter seasons were respectively 22, 20 and 24%. For lucerne a standard recovery ratio of 24% was used. The standard deviations quoted derive only from the between year variation in pasture growth (Table 16).

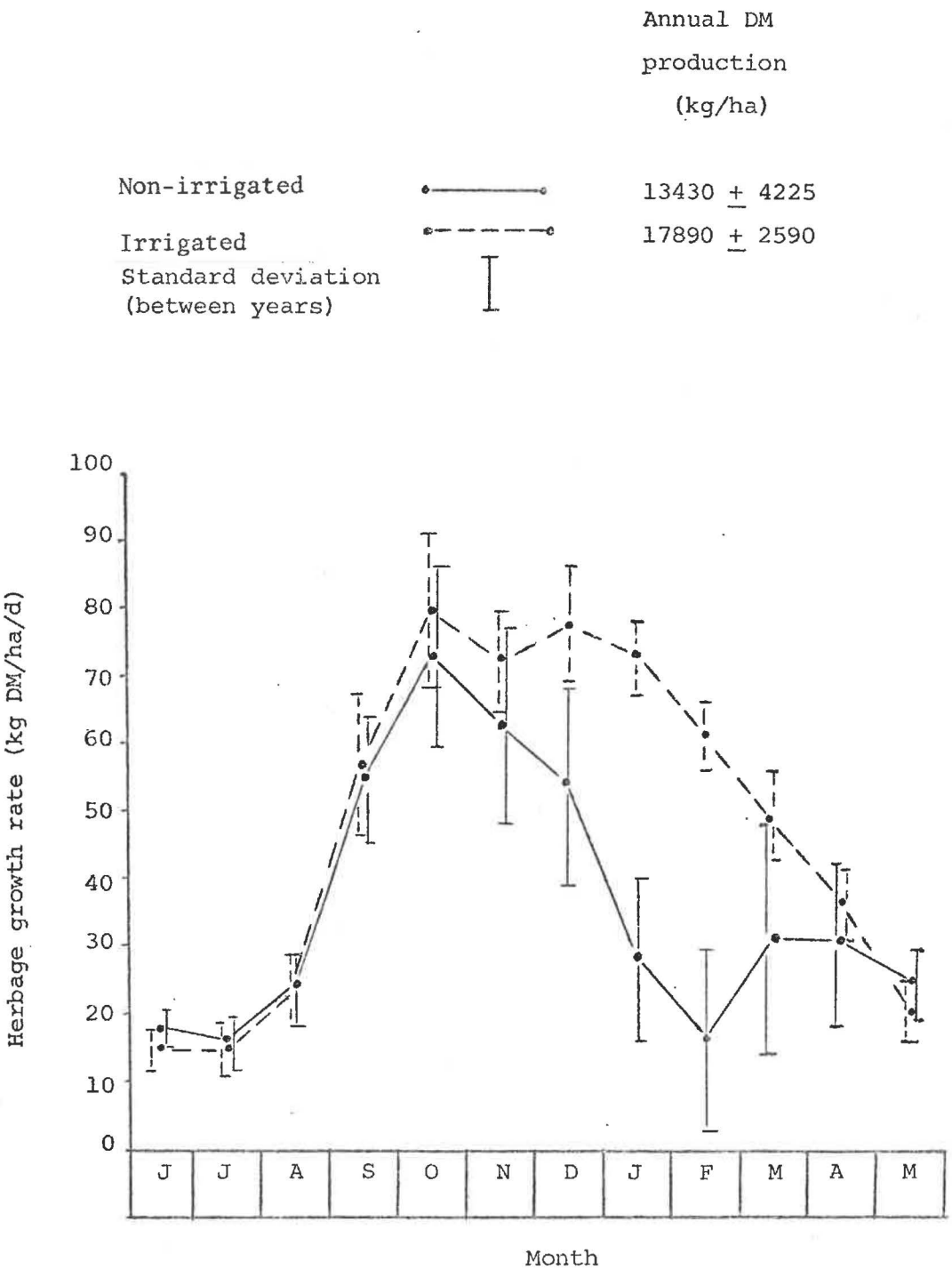

FIGURE 5: Ryegrass/white clover dry matter production in the Waikato (W. Weeda, pers. com. Means from 10 years, 1953/4 - 1963/4; Hamilton clay loam soil type).

TABLE 16: ESTIMATED RECOVERABLE CRUDE PROTEIN YIELDS (kg/ha)

	Irrigated	Non-irrigated
Ryegrass/white clover (Waikato, Hamilton clay loam soil)	979 ± 145	675 ± 204
Lucerne (Wairakei, Atiamuri sand derived from pumice)	-	660 ± 74

In the Waikato, irrigation lifted yields by 50% and also halved the coefficient of variation. In terms of commercial exploitation irrigation would also reduce the catchment area for a given factory throughput and would reduce the variability in yields between years over the summer period.

Although lucerne dry matter yields on the Volcanic Plateau are slightly lower than those for non-irrigated ryegrass/white clover pasture in the Waikato, estimated protein yields are similar for the two; however the standard deviation is substantially lower for lucerne.

These estimated yields are similar to those recorded by Vartha and Allison in the South Island for ryegrass/white clover pasture (Table 2). For lucerne, the current estimates are lower than the yields obtained by these authors both with and without irrigation. This is because firstly, of higher lucerne dry matter yields in Canterbury and secondly, Vartha and Allison used a beam press to give crude protein extraction ratios of 40 to 50%.

For seasonal dairying, Hutton (1972) quotes a pasture dry matter harvesting efficiency of 90% and a dry matter to milk

protein conversion efficiency of 25%. Based on the dry matter production data reviewed earlier, milk protein yields from a solely dairying operation would approximate 480, 640 and 410 kg/ha from non-irrigated and irrigated Waikato pasture and Wairakei lucerne, respectively. The recoverable LPC-protein yields (Table 16) are approximately 50% higher than these. If an integrated dairying-protein extraction system was developed for each of the above circumstances and allowing for a 30% reduction in milk protein production (due to 20% dry matter extraction and 90% efficiency of utilisation of pressed herbage by livestock) then the total(animal + plant) saleable protein yields would approximate 1051, 1479 and 980 kg/ha, respectively, for the above circumstances. These calculations have been made assuming the 25:1 dry matter to milk protein conversion ratio holds for pressed herbage also. However recent results suggest this may be an over estimate (Trigg, T. E. and Bryant, A. M. unpub. results). Despite inaccuracies in this conversion ratio, which can only influence one half of the estimated protein yields, the above estimates suggest that the successful integration of protein extraction technology with seasonal dairy farming in New Zealand could significantly increase the production of saleable protein.

1.2.5 CONCLUSIONS

1. The extractability of protein was higher for white clover and lucerne than ryegrass for which extractability decreased with advancing maturity. Compared with more mature herbage, young succulent material in the early stages of regrowth sometimes gave lower extraction yields.
2. The percentage recovery of protein was higher for lucerne and white clover than ryegrass and ryegrass/white clover, and

decreased with advancing herbage regrowth. Coagulated ryegrass juice gave low separation yields on two occasions.

3. Grazing rotation intervals used in seasonal dairying would be suitable harvest intervals for protein extraction which could also be combined with fodder conservation.

4. With a nominal 30% crude protein extraction ratio, estimates of recoverable protein range from 660 to 980 kg/ha/year. For an integrated protein extraction dairying system, the combined yields of protein as milk and LPC could be at least double the milk protein yields of a solely dairying operation.

PART 2

THE EVALUATION OF PROTEIN QUALITY

CHAPTER 1. REVIEW OF LITERATURE

In this review, published data on the gross chemical composition of LPC is briefly summarised. This is followed by a review of the results from amino acid analyses and protein nutritional evaluation by growth and digestibility procedures. Reference also is made to toxic/harmful elements shown to be present in LPC.

2.1.1 The Gross Chemical Composition of LPC

Protein.

Pirie (1966) quotes the protein content of LPC to range from 56-69% on a dry basis. However, Deveraj *et al.* (1970) reported a wider range of 29% to 54% which could be reduced by washing the herbage prior to extraction to remove surface soil and hence contamination of the LPC.

The true protein in extracted juice is comprised principally of the cytoplasmic and chloroplastic fractions. The former is soluble, whitish in colour, and derives from the cell sap; the latter is insoluble, green in colour, and derives from the chloroplast structural elements in the cell. Lexander *et al.* (1970) and Byers (1971a) have shown that the proportion of each of the true proteins in plant juice can vary between species.

Lipid.

The lipid content of LPC varies from 4 to 12%, when determined by ether extraction, and from 20 to 28%, when determined by hot chloroform-methanol extraction (Pirie, 1966; Buchanan,

1969b; Byers, 1971a; Oelshlegel *et al.*, 1969; Hudson & Karis, 1973; Gastineau, 1975; de Fremery *et al.*, 1975). The lipid material is associated largely with the chloroplastic protein fraction (Gastineau, 1975; Byers, 1971a; Edwards *et al.*, 1975a). Byers (1971a) showed the chloroplastic fraction to contain 28-35% lipid and the cytoplasmic fraction to contain 5-11% lipid. In excess of half the total fatty acids in LPC may be unsaturated (Lima *et al.*, 1965; Buchanan, 1969b; Hudson & Karis, 1973, 1976).

Ash.

In the study of Deveraj *et al.* cited above, the ash content of LPC ranged from 4.6% (dhaincha) to 37.0% (carrot). The ash content of carrot LPC was reduced to 11.5% by washing the vegetation prior to pulping. Leaf protein concentrate prepared from lucerne in a commercial operation has been reported to contain 18% ash (Truchetto, 1977). Byers (1971a) and Edwards *et al.* (1975) have reported that the ash content of the cytoplasmic fraction is lower than that of the chloroplastic fraction.

Carbohydrate.

Festenstein (1976), for a variety of herbages, recorded total carbohydrate contents ranging from 5 to 10%; the component sugars being mainly galactose, arabinose and xylose.

2.1.2 Protein Quality

The nutritive value of a protein is directly related to its ability to furnish dietary essential amino acids (Woodham, 1976). Thus amino acid analysis is usually carried out in any programme of protein quality evaluation. The results obtained can be used to grade quality by an index or scoring system such as the

'chemical score' (Block & Mitchell, 1946), in which case the most limiting essential amino acid is the ranking control. For such scoring systems the 'ideal' pattern of requirement against which the pattern of the test protein is compared is important. An evaluation of such reference patterns was given by Kaba & Pellet (1975). For various reasons amino acid scoring systems do not actually give the same quality results as do growth assays (Woodham, 1976; Kaba & Pellet, 1975). A factor which could account for these differences is the nutritional availability of the amino acids, in particular, of that one which is first limiting to protein quality. Variations in the nutritional availability of amino acids are largely, although not entirely, determined by the digestibility of the source protein (Erbersdobler, 1976; Miller, 1976). Hence digestibility measurements are a further useful tool in protein quality evaluation.

In this review, published results on amino acid composition and biological tests of quality and true digestibility will be discussed with special attention given to biological and processing factors resulting in quality variation.

2.1.2.1 Amino Acid Composition of Leaf Protein Concentrate The effect of biological factors.

Research prior to the 1960's was inconclusive in determining the effect of plant species, leaf age and fertiliser treatment on the amino acid content of leaf protein (Davies & Evans, 1952; Byers, 1971b). Much of this early work was done without the use of the more modern and precise analytical techniques, and compared with more recent data, was probably less precise (Wilson & Tilley, 1965; Byers, 1971b). For this reason only data from 1965 onwards

are reviewed here. Distinction is made between assays on whole leaf protein and leaf protein concentrate because in the latter case the degree to which the coagulated protein is dewatered or even washed (sometimes two to three times) will influence the quantity of soluble non-protein N remaining in the analysed product. Recently Eppendorfer (1977) showed that as N fertiliser application to ryegrass, white clover and lucerne plants increased, herbage nitrate increased, but lysine, cystine plus methionine and amino acid N as a proportion of total N, all decreased. This review is confined therefore to results obtained for leaf protein concentrate.

Byers (1971b) has reviewed the work of Chibnall *et al.* (1963), Gerloff *et al.* (1965), Wilson & Tilley (1965) and Byers (1971a) and concluded that, with the exception of lysine and methionine, the amino acid composition of LPC from different species is similar, and that this composition is unaffected by physiological age or state of the plant or by fertilizer treatment. LPC from grasses and cereals may contain higher levels of methionine than LPC from legumes. The amino acid composition of LPC has also been determined by Oelschlegel *et al.* (1969), Sentheshanmuganathan & Durand (1969), Byers (1970), Eggum (1970) and Tao *et al.* (1972). The results are in agreement with the general review interpretation of Byers (1971b) with the most variation between species being in lysine and methionine contents.

Byers (1971a) has measured the amino acid composition of the cytoplasmic and chloroplastic protein fractions for three species (barley, lupin, and chinese cabbage). The means for individual amino acids calculated across the three species for each of the cytoplasmic and chloroplastic fractions and whole LPC

are presented in Table 17. The ratio cytoplasmic over chloroplastic is also given. Deviations of approximately 20 units or greater from 100 are quite large and suggest the chloroplastic fraction to be higher in serine, glycine, isoleucine, leucine and phenylalanine and the cytoplasmic fraction to be higher in tyrosine, lysine and histidine. Bickoff *et al.* (1975a) have also reported the amino acid composition of cytoplastic and chloroplastic preparations derived from lucerne juice. For some of the essential amino acids the trends observed by Byers are confirmed with less isoleucine and leucine in the cytoplasmic fraction and more lysine, cystine, tryptophan and threonine in the cytoplasmic fraction. The above differences between the two protein fractions in the relative contents of lysine, phenylalanine, leucine and isoleucine have been found also for a further species (*Cnidoscolus chayamansa* - Nagy *et al.* (1978)).

In summary, lysine appears to exhibit most variation between species. This may be due to differences in the amount of true protein in the cytoplasmic and chloroplastic fractions.

The effect of processing factors.

Byers (1970) examined the effects of 'fast' and very 'slow' processing. The slowly processed material contained 10% less lysine and more methionine sulphoxide than the 'fast' preparation (30% v 18%).

Protein in the expressed juice can be flocculated by several different methods. Some of these have been shown to differentially effect lysine and methionine content of the resulting LPC (Byers, 1971a; Tao *et al.*, 1972; Nanda *et al.*, 1977). Heat coagulation usually resulted in lower lysine and methionine contents than acid or organic solvent precipitation.

TABLE 17: MEAN AMINO ACID COMPOSITION OF PROTEIN FRACTIONS FROM THREE SPECIES

(Calculated from Byers 1971b)

	Whole LPC	Chloroplastic fraction	Cytoplasmic fraction	Ratio cytoplasmic/ chloroplastic x100
Amino Acid (g/100g recovered amino acids)				
Aspartic acid	9.9	9.9	9.9	100
Threonine	5.1	4.9	5.3	108
Serine	4.5	5.1	4.1	80
Glutamic Acid	11.7	11.2	12.1	108
Proline	4.7	5.0	4.6	92
Glycine	5.6	6.2	5.3	85
Alanine	6.3	6.6	6.1	92
Valine	6.2	6.0	6.3	105
Methionine	2.0	2.1	2.1	100
Isoleucine	4.8	5.3	4.5	85
Leucine	9.4	10.5	8.8	84
Tyrosine	4.6	4.3	5.2	121
Phenylalanine	6.2	7.0	5.8	83
Lysine	6.8	5.2	7.2	138
Histidine	2.4	1.9	2.7	142
Arginine	6.6	6.2	6.8	110

McKenzie (1978) compared the effects of drying temperature on FDNB available lysine in LPC from white clover. The drying treatments, with available lysine results in brackets, were: freeze drying (5.68 g/16 gN), 85°C for 30 minutes (5.11), 85°C for 60 minutes (4.74), 100°C for 30 minutes (4.84), 100°C for 60 minutes (4.56) and 121°C for 60 minutes (4.37). The availability of methionine was 75% for the freeze dried material and 40% for the material dried at 121°C for 60 minutes.

The factors causing oxidation of methionine during processing are not clearly known, but Byers (1971b) and Pirie (1975) have suggested conjugation with quinones, heat induced oxidation and lipid protection of thiol groups. Regardless of the cause of the oxidation of methionine (and possibly cystine) in LPC, the extent to which this proceeds is reduced by the treatment of juice with the reducing agent sodium metabisulphite (Bickoff *et al.*, 1975b).

Amino acid composition in relation to nutritional requirements.

Bickoff *et al.* (1975a) have compared their essential amino acid results for whole LPC and for the chloroplastic (PRO-XAN II) and cytoplasmic (PRO-XAN I) fractions, with published requirements for the rat. The chemical scores suggested total sulphur amino acids and lysine to be about equal first limiting for all fractions. The scores were highest for the cytoplasmic protein, indicating better overall balance. However, growth studies showed conclusively that sulphur amino acids were first limiting. This suggests that the sulphur amino acids are not wholly nutritionally available. Further chemical score calculations by Bickoff *et al.*, against the FAO (1973) human reference pattern, suggests that both whole LPC and the cytoplasmic fraction will adequately satisfy requirements for

all essential amino acids.

2.1.2.2 Protein Utilisation and True Digestibility

Published data on protein quality evaluation are reviewed under the following sub-titles:

1. the effect(s) of processing conditions
2. studies of leaf maturity and species
3. amino acid supplementation studies
4. mixtures of LPC with other protein sources
5. nutritional problems with LPC
6. miscellaneous studies

The effect(s) of processing conditions.

In a detailed review of the literature on animal evaluation of leaf protein concentrates, Woodham (1971) pointed out that despite the expectation of a high nutritive value because of good amino acid composition, the results from early quality tests were usually disappointing. This was attributed largely to improper processing, usually drying at temperatures high enough to be damaging (Duckworth & Woodham 1961), but possibly also to extraction and/or processing methods, the presence of toxic element(s) and the choice of source herbage. Duckworth & Woodham (1961) established specifically that the temperature of the preparation should not exceed 80°C if drying damage was to be avoided. Higher temperatures could be tolerated provided the moisture level in the LPC was high enough for evaporation to be taking place - under these conditions the latent heat of vaporisation was keeping the temperature of the LPC below that which was damaging. These authors also showed that protein quality remained high if the preparation was acetone dried followed by mild heating

in vacuo, if the moisture of the wet LPC was absorbed by the dry compounded diet or if the preparation was freeze dried. These results showing high temperature damage have since been confirmed by Henry & Ford (1965), Buchanan (1969a), Subba Rau & Singh (1970), Bickoff *et al.* (1975b) and McKenzie (1976, 1978).

Turning now to the initial stages of the extraction process, some studies have been made of the effects of chemical and other treatments on protein quality. Thus when lucerne herbage was heated to 60°C before pulping there was a small improvement compared with the unheated control (Woodham *et al.*, 1974). This improvement may have been due to selective in-leaf coagulation of the chloroplastic fraction and hence selective extraction of the cytoplasmic fraction which is of higher biological value. This group also studied the effect of adding a detergent (Tween) at pulping but there was no effect on quality (see also Fafunso & Byers, 1977).

Adjustment of the pH at pulping has also been examined (Woodham *et al.*, 1974; Nanda *et al.*, 1977). In the former case natural pH was adjusted to the range 7.5 to 8.5 and affected a quality improvement in LPC from lucerne but not from cocksfoot. Nanda *et al.*, used acid (pH 4) coagulation of the protein after extraction from *Lablab atropurpureus* at each of pH 8, 9, 10 and 11. Protein true digestibilities were respectively 69, 71, 74 and 76. Biological values increased in a similar manner, though not significantly. Pierpoint (1979) suggested that extraction at higher pH may lead to more complete dissolution of protein-tannin complexes.

The effect on protein quality of adding water to lucerne herbage at pulping (range 1 l to 4 l/kg leaves) was studied by

Woodham *et al.* (1974). Pirie (1975) had suggested the resulting juice dilution would reduce tendencies towards the tanning of protein by polyphenols. However, dilution had no effect on quality.

There have been numerous suggestions that phenolic compounds in extracted juice could be oxidised to quinones and then react with proteins (Pirie, 1975; Synge, 1975; Pierpoint, 1971, 1979), and result in decreased digestibility of protein (Horigome & Kandatsu, 1966, 1968) and reduced availability of lysine (Pirie, 1975; Allison, 1971). They may also enhance the oxidation of methionine and because of their affinity for -SH groups they would almost certainly react with cystine (Pierpoint, 1979). Inhibition of the oxidation of sulphur amino acids by interactions with polyphenols, was then the objective, which prompted studies of the effects of the reducing agent, sodium metabisulphite, on protein quality (Woodham *et al.*, 1974; Bickoff *et al.*, 1975a). The latter authors added metabisulphite at pulping prior to the separation of a white (cytoplasmic fraction) LPC. This resulted in improved methionine and cystine content and protein utilisation for growth. There was no effect on true digestibilities of N which were already over 95%. Woodham *et al.*, observed sulphite treatment to improve the protein quality for cocksfoot and lucerne. When after sulphite addition, the juice was adjusted to acid (pH 3.5) or alkaline (pH 9.0) conditions, quality was highest under the acid conditions.

It was observed at Rothamsted in a study of the effects of speed of processing on quality, that compared with a rapidly processed sample a very slowly processed one contained 10% less lysine and 12% more methionine sulphoxide (Byers, 1970). The

expected effects of this on nutritional quality were confirmed by Woodham *et al.* (1974) who observed that almost without exception, 'fast' processing gives a product measurably superior than 'slow' processing for lucerne, cocksfoot and wheat.

There have been several studies of coagulation method and conditions on nutritional value. Thus when compared with the control 80°C coagulation temperature, one of 100° did not effect protein utilisation for lucerne and cocksfoot (Woodham *et al.*, 1974). After extraction at pH 11, Nanda *et al.* (1977) compared coagulation by acid (pH 4) and by heat (steam) with the pH at 5. For the heat precipitated material the true digestibility of protein and its utilisation for growth were lower. This conflicts with the results of Subba Rau & Singh (1970) who measured a higher protein utilisation efficiency for heat than acid coagulated LPC.

Booth *et al.* (1972) measured the effects of time of holding the juice at the coagulation temperature (85°C). Digestibility decreased 10 units over the first hour and a further 10 units over the next 19 hours. Recently McDonald and Donnelly (1978) studied the effects of four coagulation temperatures (80, 86, 92, 98°C) on centrifuge performance and protein quality. Treatment did not effect available lysine, available methionine or protein quality by rat growth assay.

The original discovery that LPC contained saponins which were deleterious to chick growth and that such saponins could be washed out of the LPC (Cowlishaw *et al.*, 1956), was the primary reason for recommended routine washing of the wet LPC curd (Morrison & Pirie, 1961). More recently, washing has not resulted in consistent improvement in protein quality (Subba Rau *et al.*, 1972; Woodham *et al.*, 1974; Bickoff *et al.*, 1975b; McKenzie, 1976).

However, there may be interactions with species (Woodham *et al.*, 1974; McKenzie, 1976) and there may also be an increase in the protein, amino acid and metabolizable energy content of LPC (Bickoff *et al.*, 1975b).

The chloroplastic and cytoplasmic protein fractions in expressed juice may be separated and prepared as white or green LPC. Numerous studies have been carried out on the nutritive value of these two fractions (Davies & Evans, 1952; Henry & Ford, 1965; Subba Rau *et al.*, 1969; Bickoff *et al.*, 1975a). True digestibility of protein has ranged from 54 to 75% for the chloroplastic fraction and from 75 to 99% for the cytoplasmic fraction. Similarly protein utilisation for growth has been higher for the cytoplasmic than the chloroplastic fraction.

Studies of leaf maturity and species.

Davies & Evans (1952) observed that as the age of the plant increased there is a decrease in both biological value and true digestibility of the protein. In contrast, Henry & Ford (1965) observed both the above parameters to increase as herbage aged and attributed this to decreased extractability of the chloroplastic relative to the cytoplasmic fraction as the leaf matures and overall protein extractability decreases. Thus extracted protein from mature herbage was purported to contain a higher fraction of the cytoplasmic material.

Early results suggested that LPC prepared from different species showed little variation in quality (Davies & Evans, 1952; Duckworth & Woodham, 1961). However subsequent results have indicated large differences can exist between LPC prepared from different species (Henry, 1963; Henry & Ford, 1965; Woodham, 1965; Subba Rau *et al.*, 1972; Tao *et al.*, 1972). In the results

of Henry & Ford biological values ranged from 38 to 84 but the range in digestibilities of protein was much smaller (71 - 90%). In contrast, Subba Rau *et al.*, who examined LPC from 19 different species, found variation in biological values was much smaller than variation in protein true digestibilities which ranged from 40% (carrot) to 87% (lucerne). These authors suggested the following factors, occurring either singly or in combination, adversely affected the quality of leaf proteins: low N, high ash, high soluble solids, high phenolic: N ratios and/or low organic sulphur: N ratios. First limiting amino acid analysis by chemical score procedures failed to explain the quality differences. This is in keeping with the small range in biological values. Woodham (1965) noted that different batches of LPC prepared from the same species could vary in quality. It was also a general feature in the above studies that LPC prepared from clover was of lower than average quality.

Amino acid supplementation studies.

It is without question that methionine/cystine are first limiting to protein utilisation for LPC which has been properly prepared (Larson & Halverson, 1962; Henry & Ford, 1965; Shurpaleker *et al.*, 1965; Reddy [cited by Woodham, 1971]; Hove *et al.*, 1974; Clifford *et al.*, 1975; Bickoff *et al.*, 1975a, b; Ohshima & Oouchi, 1976). In several (Reddy, loc. cit.; Bickoff *et al.*, 1975a, b; Ohshima & Oouchi, 1976) though not all cases (Cowlishaw *et al.*, 1956; Shurpalekar *et al.*, 1969; Myer & Cheeke, 1975), lysine has been shown to be second limiting. In most instances where lysine was first limiting (Cowlishaw *et al.*, 1956; Myer & Cheeke, 1975), the LPC was probably heat damaged during preparation. Two attempts have been made to determine the third limiting amino

acid. In one case (Reddy loc. cit.) it was leucine (lucerne LPC) and in the other (Ohshima & Oouchi, 1976) threonine (clover LPC). Cytoplasmic or white fraction LPC has shown similar order of limiting amino acids as whole LPC (i.e. methionine first and lysine second; Clifford *et al.*, 1975).

Mixtures of LPC and other protein sources.

As pointed out by Woodham (1971), LPC would not ordinarily be fed as a complete diet in itself, but would be fed along with other protein-containing foods and its true value must be assessed in the light of its ability to complement these associated materials. Evaluation of mixed diets has been carried out with rats (Duckworth & Woodham, 1961; Shurpalekar *et al.*, 1969; Garcha *et al.*, 1971; Oke [cited by Woodham, 1971]; Subba Rau & Singh, 1971; Hove *et al.*, 1974; Myer & Cheeke, 1975; Goel *et al.*, 1977), with growing chickens (Hughes & Eyles, 1953a; Duckworth & Woodham, 1961; Woodham, 1965; Kuzmicky *et al.*, 1972; Kuzmicky & Kohler, 1977; McKenzie, 1976, 1978; Johns, 1978), with laying hens (Cowlishaw & Eyles, 1956; Hughes & Eyles, 1953b; Morris, 1977; Kuzmicky *et al.*, 1978; McKenzie, 1978; Morimoto, H. unpub. report), with pigs (Barber *et al.*, 1958; Duckworth *et al.*, 1961; Myer *et al.*, 1975; Carr & Pearson, 1976), and with rabbits (Cheeke, 1974). These studies have adequately proved the complementarity of LPC with cereals. The experiments with rats were largely aimed at evaluating diets formulated on the basis of human nutrition. Very small supplements of LPC gave large increases in efficiency of use of total protein and where LPC provided 50% of the protein in a wheat based diet, this combination eliminated deficiencies of isoleucine, threonine, valine and lysine and left only a minor cystine deficiency relative to the FAO human reference pattern

(Subba Tau & Singh, 1971). In general the chick growth evaluation of LPC-supplemented rations gave good results also. The meticulous approach of Kuzmicky & Kohler (1977), of first defining the metaboliseable energy content of LPC and then formulating test rations in accord with this, resulted in showing LPC to have feeding value equivalent to soy bean meal. Where diets have contained from one to 20% LPC, egg production by layers has been satisfactory, although at the higher dietary LPC levels, the deposition of pigments in eggs was observed. Although acceptable, the growth rates by baby pigs and rabbits decreased as the content of LPC in the rations increased. However for older pigs, LPC proved a satisfactory replacement for fish and soy bean meals.

Nutritional problems with LPC.

Mention has been made of the need to avoid heat damage when drying LPC and of the possible deposition of pigments in eggs when layers are fed diets containing 18 to 20% LPC. Other problems which have been reported are: diarrhoea in chicks fed diets containing 54% LPC (Kuzmicky *et al.*, 1972), the presence of growth-depressing saponins in LPC prepared from lucerne (Cowlishaw *et al.*, 1954), and photosensitivity in pigs and rats when fed lucerne LPC (Lohrey *et al.*, 1974, Carr & Pearson, 1976). The diarrhoea observed by Kuzmicky *et al.*, was attributed to excess mineral intake and the problem was not seen in a subsequent experiment when the ration content of LPC was lower (up to 20%) and mineral supplementation rates were reduced. Chick growth depression due to the presence of saponins in LPC prepared from lucerne was overcome by supplementing the diet with cholesterol and by washing the LPC with hot water (Cowlishaw *et al.*, 1956). Recent experiments

have shown no response to cholesterol supplementation of lucerne LPC (Subba Rau & Singh, 1970; Kuzmicky *et al.*, 1972). In these cases the varieties of lucerne may have had low herbage levels of saponins. Livingston *et al.* (1979) have recently given results for the distribution of saponins in the fractions of the leaf protein extraction process for both high and low saponin lucerne herbage. For high (1.70%) and low (0.14%) saponin lucerne herbages, the content of saponin in the dried LPC was respectively 1.9 and 0.1%.

The oedematous skin reactions associated with photosensitisation have been observed in some of the studies involving children (cf. Singh, 1971) as well as those with pigs and rats. The effect is thought to be due to pheophorbide a, a breakdown product of chlorophyll. When plant juice is heated to 70 to 80°C for coagulation, the enzyme chlorophyllase removes the phytyl side chain from chlorophyl to form chlorophyllides, which when acidified (as in the stomach), lose their magnesium to become pheophorbides (Arkcoll & Holden, 1973; Holden, 1967). The enzyme chlorophyllase is present at higher levels in lucerne than grass herbage and is active at temperatures in the range 45 to 80°C but not at 100°C. The problem can be avoided by coagulating the protein in juice at a temperature higher than 90°C.

Miscellaneous studies.

Hove *et al.* (1974) made a long term study of rats fed diets containing up to 48% LPC. Compared with the casein-fed controls, rats fed LPC grew more slowly, had lower body fat content at slaughter (8.0% v 15.6%) but had similar reproductive performance.

2.1.3 Conclusions

This review shows leaf protein can have high nutritional value. This is especially so when LPC is used as a supplement to cereal based diets and the deficiencies of certain amino acids in the various protein sources are largely balanced out. Although only few studies have been made of high level and long term feeding of LPC no serious anti-nutritional factors have been observed. The major factor potentially damaging to protein quality during processing is high temperature at drying. Variation exists between herbage species in protein quality, protein true digestibility and to a lesser degree, amino acid composition. The causes of this variation has not been determined.

PART 2

Experimental Programme

The gross chemical composition of the leaf protein concentrates produced in the experimental programme reported in Part 1 of this thesis was determined. The results are reported and discussed in Chapter 2. Chapter 3 records the results from an investigation of the effects of herbage type, age-at-harvest and season-of-harvest on amino acid composition and protein quality. Study was subsequently made of the causes of between herbage differences in protein quality, and of the effects on protein quality of treatment of the juice with a reducing agent. The results are recorded in Chapter 4. Chapter 5 records the effects of the above treatments on the nutritional availability of the sulphur amino acids. Finally, Chapter 6 presents a general discussion.

PART 2

CHAPTER 2. THE GROSS CHEMICAL COMPOSITION OF LEAF PROTEIN CONCENTRATE

2.2.1 MATERIALS AND METHODS

2.2.1.1 Treatments and Production of LPC

These were as set out in Part 1 (section 1.2.2).

2.2.1.2 Chemical Analyses

The methods for dry matter, organic matter and N determinations were as described in section 1.2.2 while crude fat content was determined by diethyl ether extraction for 24 hours using

Soxhlet apparatus. All analyses were duplicated and the results are expressed on a dry basis.

2.2.1.3 Statistical Methods

Measurement of the significance of the effects of treatment on composition used the methods set out in section 1.2.2.

2.2.2 RESULTS

Moisture content.

The moisture content of the dried LPC fell within the range 3-5%.

Organic matter content (Table 18).

Overall the organic matter content of LPC averaged 84%. In the first two seasons the mean result for RG was higher than that for WC with RG/WC higher than both of these. The results for WC in summer '77 and lucerne in autumn '77 are low. Only in two instances were there significant associations between organic matter content and regrowth period. These were for RG/WC in spring '76 ($LC = -0.3 \pm 0.1$; $P<0.05$) and for RG in summer '77 ($LC = -2.9 \pm 1.0$; $P<0.05$).

Crude protein content.

The crude protein content of LPC was within the range 36 to 55% (Table 19). On average LPC from WC contained more protein than that from RG in spring '76 and summer '77, the average result for RG/WC was between these for spring '76 only. LPC from lucerne contained more protein than that from RG/WC in the summer and spring '77 seasons but not in the autumn season. The results for WC in summer '77 and lucerne in autumn '77 are lower than expected. Associated with this are high standard deviations.

There were significant decreases in crude protein content in association with increasing regrowth period for RG in all three seasons in which this herbage was examined and also for RG/WC in spring '76.

TABLE 18: THE ORGANIC MATTER CONTENT OF LEAF PROTEIN CONCENTRATE (means adjusted to the 6th week of regrowth).

Season	Herbage	Organic matter (g/100g DM)
Spring '76	RG	85.4 ± 1.5[a]
	WC	81.5 ± 1.7
	RG/WC	89.0 ± 0.5
Summer '77	RG	79.3 ± 2.1
	WC	74.0 ± 4.8
	RG/WC	82.4 ± 1.2
	L	84.7 ± 2.3
Autumn '77	RG	87.5 ± 0.6
	RG/WC	89.5 ± 0.4
	L	72.8 ± 4.5
Spring '77	RG/WC	95.2 ± 0.7
	L	91.8 ± 0.3

[a] standard deviation.

There was a significant effect due to herbage type in spring '76 ($P<0.001$), summer '77 ($P<0.05$) and autumn '77 ($P<0.001$).

The overall average protein content of LPC, after correction to 40% dry matter at separation, was 50%, and was 4 units higher than the unadjusted mean (Detailed results are tabulated in Appendix 9).

TABLE 19: THE CRUDE PROTEIN CONTENT (g/100g DM) OF LEAF PROTEIN CONCENTRATE

Season	Herbage	Regrowth period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	44.1	43.0	38.2	38.0	37.7	-	-	-	3.0	-1.8 ± 0.7	*
	WC	-	47.9	50.3	49.5	49.5	-	-	-	5.0	0.4 ± 0.8	NS
	RG/WC	50.0	47.1	46.9	46.8	42.8	-	-	-	0.7	-1.4 ± 0.2	***
Summer '77	RG	45.6	39.2	37.5	33.0	31.9	-	-	-	4.3	-3.4 ± 1.0	*
	WC	43.7	38.7	44.8	44.1	32.5	-	-	-	9.9	-1.7 ± 2.2	NS
	RG/WC	42.3	44.1	46.4	44.0	42.0	-	-	-	1.8	-0.1 ± 0.4	NS[a]
	L	55.2	48.9	50.9	53.6	53.9	-	-	-	3.7	0.2 ± 0.8	NS
Autumn '77	RG	-	50.1	46.3	45.3	44.5	45.4	45.1	-	1.3	-0.8 ± 0.2	*
	RG/WC	-	-	52.0	-	49.2	-	51.1	51.7	1.0	0.1 ± 0.2	NS
	L	40.7	36.2	39.7	44.9	-	-	-	-	8.9	1.6 ± 2.8	NS
Spring '77	RG/WC	52.2	48.2	49.8	49.7	48.4	48.9	48.9	-	1.5	-0.4 ± 0.2	NS
	L	-	55.3	53.8	54.7	53.7	53.2	-	-	1.6	-0.4 ± 0.4	NS

a: Significant quadratic term of -0.9 ± 0.3 ($P<0.05$)

There was a significant effect due to herbage type in all four seasons ($P<0.001$)

Crude fat content (Table 20).

Diethyl ether extraction gave an overall mean estimation for crude fat of 9.1%. In the first two seasons a lower result was recorded for WC than RG; RG/WC was intermediate. Lucerne contained markedly less crude fat than RG/WC in autumn and spring '77 but similar amounts in summer '77. The linear contrast relating percentage crude fat to rest period was significant only for lucerne in summer '77 (0.8 ± 0.2; $P<0.01$) and for RG in autumn '77 (-1.2 ± 0.4; $P<0.05$).

TABLE 20: THE CRUDE FAT CONTENT OF LEAF PROTEIN CONCENTRATE

(means adjusted to the 6th week of regrowth)

Season	Herbage	Crude fat content (g/100g DM)
Spring '76	RG	9.5 ± 1.1
	WC	7.2 ± 0.5
	RG/WC	8.7 ± 1.0
Summer '77	RG	9.2 ± 1.1
	WC	5.0 ± 0.5
	RG/WC	7.9 ± 0.5
	L	7.2 ± 0.3
Autumn '77	RG	16.5 ± 1.0
	RG/WC	10.6 ± 1.0
	L	6.9 ± 1.0
Spring '77	RG/WC	12.6 ± 0.9
	L	9.7 ± 0.6

There was a significant effect due to herbage type in all four seasons ($P<0.05$).

2.2.3 DISCUSSION

LPC ash content.

The overall crude ash content (100 - organic matter %) of 16% is similar to the figure of 18% reported for LPC prepared from lucerne by a commercial process (Truchetto, 1977). Both figures are higher than results usually reported for LPC prepared from lucerne on a small laboratory scale and where the wet LPC is washed prior to drying (Kuzmicky & Kohler, 1977). In the latter case, where the wet LPC was drained, pressed, or dilute acid washed, prior to drying, the ash contents were 13.2, 11.3, and 7.8%, respectively. Since in the current experiment LPC was dewatered at separation to only 10-20% dry matter, this could partially account for the relatively high ash content. A further contributing factor may have been the machine used for harvesting the herbage. The flails which cut the herbage also create an air up-draught to aid herbage delivery to the forage wagon and this tends to carry ash-containing dust with the herbage (Mills, R. A. & Donnelly, P. E. unpub. results). This phenomenon probably also explains why the legume LPC generally contained more ash than the RG and RG/WC LPC, since the legume swards were less dense.

Crude protein content.

Legume LPC tended to have higher crude protein content than that from RG. This was so when comparing the adjusted sixth week result for RG with those for WC in spring '76 and with those for WC and L in summer '77 (Table 21, DM basis). However RG was higher than L in autumn '77, and RG/WC higher than WC in summer '77. Expression on an organic matter basis lifted the overall mean crude protein content from 46 to 54% (Table 21). The increase

being larger for LPC of lower organic matter content and this generally widened the differences between the legumes and RG.

TABLE 21: THE SEASONAL MEAN CRUDE PROTEIN CONTENT OF LPC (on dry matter (DM) and organic matter (OM) bases - adjusted to the 6th week of growth)

Season	Herbage	CP/DM (g/100g)	CP/OM (g/100g)
Spring '76	RG	40.2	47.1
	WC	49.3	60.5
	RG/WC	46.9	52.4
Summer '77	RG	37.4	47.1
	WC	40.8	54.6
	RG/WC	43.8	52.8
	L	52.5	61.8
Autumn '77	RG	44.9	53.9
	RG/WC	51.0	57.0
	L	40.4	55.1
Spring '77	RG/WC	49.4	53.0
	L	54.1	59.0

There was a significant effect due to herbage type in all four seasons ($P<0.01$).

It also reversed the relativity of results for RG and L in the autumn season and for WC and RG/WC in the summer season. The expression of crude protein content on an organic matter basis thus shows the legumes to be consistently higher than RG.

The relationship between herbage and LPC crude protein content (within season basis) was significant ($P<0.05$, $r = >0.63$) in seven cases (Table 22). Only for RG was there a significant decline in the crude protein content of each of LPC and herbage in all seasons. This herbage also showed the most consistent association

between LPC and herbage crude protein content for all seasons. For the remaining herbages, although the correlations were high for some seasons, they are generally not indicative of a consistent and strong association between herbage and LPC crude protein content.

TABLE 22: CORRELATION COEFFICIENTS BETWEEN LPC CRUDE PROTEIN CONTENT AND SOURCE HERBAGE CRUDE PROTEIN CONTENT (LPC crude protein content on organic matter basis)

	HERBAGE			
Season	RG	WC	RG/WC	L
Spring '76	0.72[a]	0.35	0.84	-
Summer '77	0.87[a]	0.88	0.58[a]	0.37
Autumn '77	0.85[a]	-	0.56	0.87
Spring '77	-	-	0.76[a]	0.40

a: indicates those treatments for which there was a significant linear contrast indicating an effect of regrowth period on the crude protein content of each of the source herbage and the LPC.

LPC crude fat content.

LPC prepared from WC contained less crude fat than that from RG in the first two seasons (Table 20). Similarly, in the seasons in which both were compared (summer and autumn), LPC from L contained less extract than that from RG and, with the exception of summer '77, this was also the case for L versus RG/WC. These results suggest then, that LPC prepared from legumes contains less crude fat than LPC prepared from ryegrass. In material reviewed earlier it was noted that the chloroplastic fraction contains more lipid than the cytoplasmic fraction. If then, relative to legumes, ryegrass protein contained a higher ratio of chloroplastic to

cytoplasmic material, this could explain the above results.

Significant changes in the crude fat content of LPC in association with regrowth period occurred in only two instances. Byers (1971a) also observed that the fat content of LPC was not influenced by age at harvest. These results indicate then, that although the lipid content of the leaf tissue decreases with increasing maturity (Hawke, 1973), such decreases are not generally reflected in the LPC.

2.2.4 CONCLUSIONS

1. The ash content of LPC on average was high (16%). This could probably be reduced in a commercial system by dewatering the wet LPC to above 20% DM (i.e. 40 - 50% DM) before drying and/or by the use of forage harvesting machinery not reliant upon air-assisted delivery from the cutting head.
2. For ryegrass, the crude protein content of LPC increases with the protein content of the herbage.
3. Crude fat is higher in ryegrass LPC than in legume LPC.

PART 2

CHAPTER 3. THE EFFECT OF HERBAGE TYPE, REGROWTH PERIOD AND SEASON ON PROTEIN QUALITY

2.3.1 INTRODUCTION

Previous studies on the effects of herbage age at harvest on the quality of protein in LPC have been equivocal (section 2.1). More recent amino acid analyses suggest there is no effect of herbage age on composition. It does however appear that the digestibility of leaf protein and the content of some amino acids can vary according to source herbage species. Pirie (1975) questions the validity of experimental procedures under which the digestibility observations were made and claims that "... the supposed differences between species (must be shown to be) greater than differences between preparations made from the same species harvested at different ages".

From the evidence available it was not possible to conclude that the age of herbage at harvest had any effect on protein quality. Furthermore there have been no studies of the effects of season of harvest on protein quality. The effects of these two factors on the protein quality of LPC were therefore examined. It seems probable that protein quality may vary between herbage species. It was therefore necessary to measure the quality of LPC from ryegrass/white clover herbage and its component species and to make comparisons with preparations from lucerne.

Selected LPC produced in the programme described in Part 1 were assayed. The measurements made were amino acid composition, the quality of the protein by rat growth assay and the *in vivo* true digestibility of protein using rats.

2.3.2 MATERIALS AND METHODS

2.3.2.1 Treatments

The effect of herbage source on amino acid content.

Total amino acid composition and lysine availability were determined in LPC prepared from ryegrass, white clover and ryegrass/white clover herbages harvested after four, six and eight weeks regrowth in the spring '76 season. Subsequently, LPC from lucerne in the summer '77 season was analysed.

The effect of herbage type on protein quality and digestibility.

LPC prepared from all four herbage types harvested after four weeks growth (summer '77) were assayed by rat growth and *in vivo* digestibility (Rat Experiment [RE] 1). Samples from these same LPC were fed in a second experiment (RE 2) along with methionine supplements.

The effect of herbage age at harvest (regrowth period) on protein quality.

The rat growth test was applied to LPC prepared from RG/WC and L herbage harvested after four, six and eight weeks regrowth. Each LPC was tested without and with methionine supplementation (RE 3 for RG/WC and RE 4 for L).

The effect of season of harvest on protein quality.

The growth assay was applied to LPC prepared from RG/WC and L herbage harvested after six weeks growth in the autumn and spring '77 seasons. Only methionine supplemented LPC were compared (RE 5).

2.3.2.2 Rat Growth Assay

At the time of commencement of this study a National Academy

of Sciences - National Research Council (NRC) Committee of the U.S.A. were revising the recommended methods of protein quality assessment. Although not published, the decision had been made to recommend a slope-ratio growth assay in place of the previously recommended (AOAC, 1965) protein efficiency ratio technique (Hegsted, D. M. pers. com.). The major deficiency of the latter assay procedure is that differences in results are not proportional to actual differences in protein quality (NRC, In press). Thus a protein which has a protein efficiency ratio of one-half that of another protein cannot be assumed to have a nutritional quality of 50% of that of the other protein. Multi-point slope ratio assays, an example of which is that developed by Hegsted and associates (Hegsted *et al.*, 1968), do not suffer from this serious deficiency. This procedure is now officially recommended by the NRC (NRC, In press) and was therefore chosen as the growth measure of protein quality in this work.

Experimental procedures were as follows. Male weanling 21-day old rats were fasted overnight and then allocated to dietary treatments which were balanced for litter and rat weight. An initial slaughter group, comprising 8-12 rats, was then asphyxiated. Their stomach and intestines were emptied and the whole rat stored in a plastic bag at -15°C until further processed. After 20 days of feeding the experimental rats were again fasted overnight, and treated similarly to the initial slaughter group. Determination of body water content involved chopping the cadaver into 5 mm segments and drying (105°C) to constant weight. Weighing of the rats took place after the initial fast, after the final fast, after asphyxiation, after emptying the stomach and intestines, and also

after chopping and drying. Body water accumulation during growth parallels very closely protein accumulation (NRC, In press) and is used as the index of growth. This was measured by predicting initial body water content of the experimental animals using fasted weight - water content relationships from the initial slaughter group and subtracting this from final body water content.

Diet formulation was generally as recommended by Hegsted *et al.* (1968). Each diet contained 3.7% salt mix, formulated to meet the NRC (1972) recommended mineral requirements, 1% vitamin mix, formulated to meet the AOAC (1965) recommended requirements, 1% cellulose, 8% corn oil, a quantity of protein concentrate to supply approximately 3, 6 or 9% dietary crude protein and corn starch to 100%. Where diets were supplemented with methionine this was added at 2 g/100g protein. Each formulated diet was thoroughly mixed and sampled for chemical analyses prior to feeding. For each experiment a blank or protein-free diet was fed to a representative group of five or six rats. Each test protein was fed to three groups of five or six rats at three different levels (as above). Lactalbumin (New Zealand Co-operative Dairy Co. Ltd.) was fed as a standard or reference protein in each experiment.

The rats were individually housed in suspended racks. Food was replenished as necessary - usually once weekly, and deionised water was available. Food was offered in small tared jars located in larger tared jars. Spilled food collected mainly in the larger jars and also beneath the cages on blotting paper. The total uneaten material was subtracted from food offered to estimate intake.

The efficiency of use of protein for growth was estimated as

the coefficient(b) from the linear regression ($Y = a + bX$) of body water gain(Y) on protein intake(X). Relative Nutritive Value(RNV) is the ratio of the regression coefficients for the test protein and the standard (lactalbumin). This is discussed further in Appendix 10.

2.3.2.3 Protein True Digestibility (PTD)

At the completion of each experiment the spilled food and faeces, which collected under the cages, were separated. The faeces were then freeze dried, weighed and milled.

Protein true digestibility can be estimated by factorial calculation or by regression analysis where a range of protein intakes is fed (Njaa, 1963). The factorial procedure is as follows:

$$PTD\% = \frac{Y - (Z - X)}{Y} \quad 100$$

where Y = food protein intake

Z = faecal protein output

X = metabolic faecal protein (MFP)

MFP is of endogenous origin and can be assumed to be directly proportional to dry matter intake (Njaa, 1963). It was calculated using the ratio of faecal protein to dry matter intake observed for the group fed the protein-free diet.

To obtain the estimate of PTD by regression analysis, faecal protein was related to protein intake and the coefficient of relationship, which defined indigestibility, was subtracted from 1.

These two methods of estimating PTD are compared in Appendix 11. The results given in the text were calculated by the regression method.

2.3.2.4 Analytical Methods

Dry matter, organic matter and N contents were measured on LPC and, rat feeds and faeces, by the methods given earlier (section 1.2.2.).

The amino acid analyses on LPC were carried out on pooled samples representative of treatment duplicates. Sub-samples (250 mg) were weighed out in duplicate into tubes and 250 ml of 6 N HCl was added. N_2 was bubbled through the sample-acid mixture for one hour prior to hydrolysis which was then carried out for 20 hr at 120°C under N_2. The hydrolysates were cooled, filtered and made up to 250 ml. A 5 ml aliquot was taken to dryness under reduced pressure and then redissolved in citrate buffer. Norleucine standard was added to a 1 ml aliquot which was then resolved on a Technicon Amino Acid analyser. The standard elution gradient recommended for hydrolysates was used. The results were corrected for decomposition during hydrolysis using data from curves prepared after 20, 48 and 72 hr hydrolyses times for a like sample of LPC. Tryptophan was determined on separate samples by the procedure described by Matheson (1974). Chemically available lysine was determined by the FDNB 'difference' procedure as described by Couch (1975). Results are presented as g of amino acid per 16 g Kjeldahl N.

All analyses were carried out in duplicate.

2.3.2.5 Statistical Methods

Amino acid composition.

Analysis of variance was used to test for differences between herbages and regrowth week. Variance was partitioned into that due to herbage source (3 degrees of freedom), regrowth weeks (linear and quadratic - 1 df each), herbage by regrowth interaction

(6 df) and error (12 df). Variation between analytical duplicates was partitioned into the error term. Herbage effects were tested against herbage by regrowth interaction instead of against the 'error' term; i.e. a herbage effect had to be consistent at the three stages of regrowth before it was considered real. The effect due to regrowth was tested against the same interaction term. The significance of the interaction was tested against the error variance. Although the lucerne LPC was derived from a different season, its results were included in the analysis.

Statistical treatment of the rat growth and protein true digestibility results is given in Appendices 10 and 11 respectively, where various methods of analysis are discussed.

2.3.3 RESULTS

2.3.3.1 Amino Acid Composition

The results for analytical duplicates are presented in Appendix 12, Tables 1 to 4.

The total amino acids at analysis, expressed as the sum of the ratio g/16g N for all amino acids including tryptophan, was WC 94.4, RG 90.5, RG/WC 88.2 and L 90.8. The total for WC was higher ($P<0.05$; average Se of difference = 1.7) than all others. The totals do not include the result for ammonia and cysteine was not detected. The presence of non-amino acid N other than ammonia in LPC may also have contributed to the above totals being less than 100.

Although there were species by regrowth interactions for some herbage types, there were no significant changes in amino acid concentrations in association with regrowth period (by linear contrast analysis, $P<0.05$) that were consistent for all herbage

types. Regrowth period means are presented in Appendix 12, Table 5.

The essential amino acids for which significant herbage by regrowth interactions were found are as follows:

- available lysine increased with regrowth in RG and WC ($P<0.05$).
- lysine availability, increased and decreased with regrowth in RG and L, respectively ($P<0.01$).
- tyrosine increased with regrowth in WC ($P<0.01$).
- phenylalanine decreased with regrowth in RG/WC ($P<0.01$).

The only non-essential amino acid to show a significant ($P<0.01$) herbage by regrowth interaction was glutamic acid in lucerne LPC, which increased from 9.5 (week 4) to 9.6 (week 6) to 9.8 (week 8) g/16g N.

Table 23 presents the average amino acid content of each herbage type. In Table 24 differences between herbage types in essential amino acid content are given. The essential amino acids which showed some significant differences between herbage types were histidine, lysine and arginine. Histidine was lower in RG/WC than all other herbage types, and was also lower in RG than WC. Lysine, available lysine and lysine availability are lower for RG/WC than L, WC ($P<0.05$, not shown in Table 23) and the algebraic RG-WC mean. However, for lysine the difference between RG/WC and RG+WC/2 was not significant. For arginine, the result for RG/WC was lower than those of RG, WC and the RG+WC mean ($P<0.001$).

Only two of the non-essential amino acids showed between herbage type differences that were significant. Thus aspartic acid was lower in LPC from RG than LPC from each of WC (1.3 ± 0.2;

P<0.001), L (1.4 ± 0.2; P<0.001) and RG/WC (0.7 ± 0.2; P<0.01). It was also lower in RG/WC than L (0.7 ± 0.2; P<0.01) and WC (0.6 ± 0.2; P<0.05). Proline content was lower in LPC from L than that from RG (0.4 ± 0.1; P<0.01), WC (0.6 ± 0.1; P<0.001) and RG/WC (0.5 ± 0.1; P<0.001).

TABLE 23: THE AMINO ACID CONTENT OF LPC FROM FOUR HERBAGE TYPES

(Results meaned for 4, 6 and 8 weeks regrowth)

	Herbage type				Average SD of means[a]
	RG	WC	RG/WC	L	
Amino acid (g/16g N)					
Histidine	2.4	2.7	1.9	2.6	0.2
Arginine	6.5	6.5	5.7	6.1	0.2
Aspartic acid	7.7	9.0	8.4	9.2	0.2
Threonine	4.8	5.4	4.8	5.0	0.3
Serine	4.8	5.1	5.1	4.9	0.4
Glutamic acid	10.2	10.4	10.4	9.7	0.5
Glycine	5.1	5.1	4.8	4.8	0.3
Alanine	6.0	5.5	5.5	5.4	0.4
Valine	5.2	5.8	5.2	5.4	0.3
Methionine	2.4	2.1	2.3	2.1	0.2
Isoleucine	4.6	4.7	4.6	4.6	0.3
Leucine	8.0	8.6	7.8	8.4	0.3
Tyrosine	4.4	4.8	4.1	4.7	0.3
Phenylalanine	5.8	5.7	5.3	5.8	0.3
Proline	4.3	4.4	4.3	3.8	0.2
Tryptophan	2.5	2.5	2.5	2.5	0.1
Lysine	5.7	6.0	5.4	6.1	0.3
Available lysine	5.1	5.4	4.6	5.6	0.4
Lysine availability %	89.3	90.2	85.5	91.5	1.7

a: calculated including variation associated with regrowth week and analytical duplicates.

TABLE 24: DIFFERENCES BETWEEN HERBAGES IN ESSENTIAL AMINO ACID CONTENT OF LPC.

Difference	RG-WC	RG/WC-(RG+WC)/2[a]	L-RG/WC	Avg SE of differences
Amino acid (g/16g N)				
Histidine	-0.3*	-0.7***	0.7***	±0.1
Arginine	0.1	-0.8***	0.4	±0.2
Threonine	-0.6	-0.2	0.1	±0.2
Valine	-0.6	-0.2	0.1	±0.2
Methionine	0.2	0.1	-0.2	±0.1
Isoleucine	-0.1	-0.1	0.1	±0.2
Leucine	-0.6	-0.5	0.6	±0.3
Tyrosine	-0.3	-0.5	0.6	±0.2
Phenylalanine	0.1	-0.5	0.5	±0.3
Tryptophan	0	0	0	±0.1
Lysine	-0.3	-0.5	0.7*	±0.2
Available lysine	-0.3	-0.7*	0.9*	±0.3
Lysine availability %	-0.8	-4.2*	6.0*	±1.5

a: (RG + WC)/2 = algebraic mean of individual RG and WC results.

2.3.3.2 Growth and Digestibility Assays

The effect of herbage source.

Food intakes, protein intakes and body water gains from RE One and Two are summarised in Appendix 13 Tables 1 and 2, respectively. The regression coefficients and Relative Nutritive Value (RNV) estimates from the analysis of these data are presented in Table 25. The significance of differences between protein sources in quality was estimated using a t test. This was applied to the regression coefficients rather than the RNV ratios because the

latter were more highly correlated owing to having a common denominator within experiments.

Relative to lactalbumin, unsupplemented LPC has a biological value of 0.45 to 0.63 (RE One). The regression coefficient is larger for L than RG, WC and RG/WC ($P<0.01$) (see Appendix 10, Table 3). Similarly the coefficient for RG is higher than that for WC and for RG/WC ($P<0.05$). When supplemented with methionine, Relative Nutritive Values increased to 0.79 to 0.89 (RE Two). In this case the regression coefficients are of similar value for L and WC, and both are higher ($P<0.05$) than that for RG. That for L is also higher ($P<0.05$) than that for RG/WC (Appendix 10, Table 3).

Protein true digestibility.

Average group faecal dry matter and protein production are tabulated in Appendix 13, Tables 3 and 4 for RE One and Two, respectively. As discussed in Appendix 11, the estimates of PTD obtained by regression analysis are used comparatively (Table 25). It was considered that the best estimate of PTD for each LPC was the average of results from both experiments. These averages were, RG 69.8 $\pm$ 0.8, WC 73.3 $\pm$ 0.8, RG/WC 70.0 $\pm$ 0.7 and L 81.8 $\pm$ 0.8. The mean for L is significantly ($P<0.001$) higher than all others and that for WC is higher ($P<0.01$) than that for RG or RG/WC.

The effect of regrowth period on protein quality.

The intakes and growth data for RE Three and Four are tabulated in Appendix 13, Tables 5 and 6, respectively. The regression coefficients and RNV estimates from the analysis of these data are given in Table 26.

None of the herbages showed any change in protein quality

TABLE 25: THE EFFECT OF HERBAGE TYPE ON PROTEIN QUALITY

Protein Source	RE One (LPC only fed)			RE Two (LPC + met. fed)		
	b[1] ± Se	RNV ± Se	PTD ± Se	b ± Se	RNV ± Se	PTD ± Se
Lactalbumin	2.53 ± 0.03	1.00	92.7 ± 0.5	2.69 ± 0.06	1.00	93.3 ± 0.9
LPC - RG	1.33 ± 0.05	0.53 ± 0.02	71.4 ± 0.7	2.14 ± 0.06	0.79 ± 0.02	68.3 ± 0.8
- WC	1.19 ± 0.05	0.47 ± 0.02	73.2 ± 0.7	2.36 ± 0.05	0.88 ± 0.02	73.3 ± 0.8
- RG/WC	1.13 ± 0.06	0.45 ± 0.02	69.1 ± 0.5	2.21 ± 0.06	0.82 ± 0.02	70.8 ± 0.8
- L	1.59 ± 0.05	0.63 ± 0.02	80.9 ± 0.7	2.38 ± 0.06	0.89 ± 0.02	82.6 ± 0.9
Regression intercept	-6.7			-8.3		
Regression RSD	1.4			2.2		

1: b = coefficient from regression of body-water gain on protein intake - see Appendix 10 for explanation.

in association with age at harvest. This was so when fed both with and without methionine supplement.

Protein quality was similar for LPC from L and RG/WC when fed with methionine (average RNV = 0.86 v 0.81) but was 20% higher for lucerne (average RNV = 0.61 v 0.51) when fed without methionine. The effect of methionine supplementation was to increase RNV by an average of 40 (L) to 60 (RG/WC) %.

The effect of season of harvest on protein quality.

The intakes and growth data from this experiment (RE Five) are tabulated in Appendix 13, Table 7. The regression coefficients and RNV estimates derived from the analysis of these data are given in Table 27. All LPC were fed with methionine supplement.

The regression coefficients did not differ significantly between seasons for either herbage source. Similar RNV estimates were obtained for LPC prepared from each of L and RG/WC herbage.

2.3.4 DISCUSSION

Amino acid composition.

In general the results recorded here confirm the conclusion of Byers (1971a), that unfractionated extracts prepared from different species have a generally similar composition and that this is not affected by the physiological age of the plant. However, the current results do present some exceptions. The nutritionally essential amino acids lysine, histidine and arginine showed between herbage differences. For histidine the relative order of results was WC>L>RG>RG/WC. A similar relative order existed for total and available lysine as follows WC, L>RG>RG/WC. This shows clearly then, that the protein in LPC prepared from ryegrass and mixed ryegrass/white clover based herbage contained lesser proportions of these two amino acids than LPC prepared from the legumes.

TABLE 26: THE EFFECT OF REGROWTH PERIOD ON PROTEIN QUALITY.

Protein Source	b ± Se	RNV ± Se
RE Three		
Lactalbumin	2.39 ± 0.05	1.00
LPC - RG/WC - 4[1]	1.18 ± 0.08	0.49 ± 0.03
- 6	1.22 ± 0.08	0.51 ± 0.03
- 8	1.24 ± 0.08	0.52 ± 0.03
LPC - RG/WC - 4 + M[2]	1.94 ± 0.06	0.81 ± 0.02
- 6 + M	1.88 ± 0.06	0.79 ± 0.02
- 8 + M	1.89 ± 0.06	0.79 ± 0.02
Regression intercept	- 7.9	
Regression RSD	1.72	
RE Four		
Lactalbumin	2.54 ± 0.07	1.00
LPC - L - 4	1.57 ± 0.08	0.62 ± 0.03
- 6	1.62 ± 0.08	0.64 ± 0.03
- 8	1.50 ± 0.08	0.59 ± 0.03
LPC - L - 4 + M	2.23 ± 0.08	0.88 ± 0.03
- 6 + M	2.07 ± 0.08	0.82 ± 0.03
- 8 + M	2.26 ± 0.08	0.89 ± 0.03
Regression intercept	- 7.3	
Regression RSD	2.3	

1: indicates week of regrowth at which harvested; all LPC from summer '77 season.

2: M indicates methionine supplementation.

TABLE 27: THE EFFECT OF SEASON ON PROTEIN QUALITY

Protein Source	b ± Se	RNV ± Se
	RE Five	
Lactalbumin	2.30 ± 0.04	1.00
LPC - RG/WC		
AU - 6 + M[a]	2.00 ± 0.06	0.87 ± 0.02
SP - 6 + M[a]	2.02 ± 0.05	0.88 ± 0.02
LPC - L		
AU - 6 + M	1.99 ± 0.06	0.86 ± 0.02
SP - 6 + M	1.83 ± 0.06	0.80 ± 0.03
Regression intercept	- 7.7	
Regression RSD	1.88	

a: AU and SP indicate autumn and spring seasons of harvest, respectively; 6 - indicates week of regrowth at harvest and M indicates methionine supplementation.

Since Byers (1971a, b) has shown that compared with the chloroplastic protein fraction, the cytoplasmic fraction has higher lysine and histidine contents, the above results suggest that compared with juice from ryegrass and ryegrass/white clover, juice from the two legumes contains a higher proportion of cytoplasmic protein than chloroplastic protein. The arginine content of LPC was lower for RG/WC than each of RG, WC and the algebraic RG-WC mean. This is not explained by the protein composition factors suggested to account for the lysine and histidine variation. However, as well as arginine, RG/WC was lower than the algebraic RG-WC mean in histidine, total and available lysine, and lysine availability. This may possibly be due to botanical composition factors as the RG/WC herbage contained 21% weeds which may have contributed substantially lower proportions of these amino acids and/or, possibly have been responsible for reducing their chemical availability at hydrolysis.

The two non-essential amino acids showing consistent herbage differences were aspartic acid and proline. In the case of the former, the results were higher for L and WC than RG and RG/WC. Explanation for this would not in the first instance, appear to lie in differing cytoplasmic:chloroplastic true protein ratios as suggested for histidine and lysine since Byers (1971a) showed the two protein fractions to have a similar content of this amino acid (Table 17).

Available lysine, lysine availability, tyrosine and phenylalanine showed significant herbage by regrowth period interactions for some herbage types. A possible explanation for all but one of these changes with maturity, is that put forward by Henry & Ford (1965) to explain increases in biological value and true

digestibility as herbage aged. They suggested there was decreased extractability of the chloroplastic compared with the cytoplasmic fraction as herbage aged and this was reflected in LPC composition and quality. In the current experiment, increases in the age of herbage at 4, 6 and 8 weekly harvests was accompanied by increases in available lysine in RG and WC, increased lysine availability for RG, increased tyrosine content in WC (tyrosine is higher in the cytoplasmic than the chloroplastic fraction - see Table 17) and decreased phenylalanine in RG/WC (phenylalanine is lower in cytoplasmic than chloroplastic material). The one result conflicting with this tentative interpretation is that for lucerne, where lysine availability decreased with increasing herbage maturity. Despite Henry & Ford's suggestion however, Byers (1971a) claims that the ratio of cytoplasmic to chloroplastic protein is unaffected by leaf age. A further factor in support of Byers' evidence is that if the argument of Henry & Ford is correct, it could reasonably be expected that histidine would show the same pattern of response as lysine.

The effect of herbage source on protein quality.

The coefficient from the regression of body water gain on protein intake provides a measure of the incremental efficiency of utilisation of dietary protein. In RE One these coefficients indicated that protein in the LPC from lucerne had the highest quality. The lowest quality was recorded for WC and RG/WC together and the result for RG was approximately intermediate between these two and lucerne. Since in RE Two protein quality improved with methionine supplementation, confirming that methionine plus cystine

are first limiting to protein quality, the ranking between unsupplemented LPC sources is also an indication of the between herbage ranking in available methionine plus cystine levels. The RNV ratios indicate LPC protein quality to be 45 to 63% of that for lactalbumin. When methionine was supplemented these ratios increased to 79 to 89% of the value for lactalbumin. Under these conditions quality was highest and similar for WC and L. The results for RG and RG/WC were also similar.

Amino acid availability is largely determined by protein true digestibility (Erbersdobler, 1976). Thus the results for available lysine and PTD could be expected to show a similar ranking between herbages. This was generally the case and the coefficient of correlation between the two was moderately high (0.79*).

These results confirm published findings, that there are differences in the quality of LPC due to species (section 2.1.2.2). The current results, giving unsupplemented clover LPC a lower value than ryegrass LPC are in agreement with the results of Henry (1963), Henry & Ford (1965) and Woodham (1965). The variations in protein true digestibility in these reports did not however show the same pattern as recorded here. Tao *et al.* (1972) also observed protein utilisation of unsupplemented LPC to be higher for lucerne than WC. They also observed a true digestibility of protein for lucerne LPC similar to that observed here (83%), but for WC obtained a higher value (81 v 73%).

It is not possible to determine from the data collected in this experiment the cause(s) of the between herbage differences in protein true digestibility. However, it is suggested that variation between species in the proportion of the true protein

in the juice from the cytoplasmic and chloroplastic fractions may be responsible. The cytoplasmic fraction has a much higher digestibility than the chloroplastic fraction (75 - 99% v 54 - 75%, respectively - see section 2.1.2.2), and, as discussed above, the cytoplasmic fraction has higher total and available lysine and histidine contents. The results then for these amino acids as well as those previously reported for crude fat content of LPC, concur with this suggestion.

The effect of regrowth period on protein quality.

Age of herbage at harvest spanned the period four through eight weeks regrowth for both the ryegrass/white clover and lucerne herbages. Protein utilisation, which as measured encompassed effects on both digestibility and biological value, was not effected by maturity for either herbage treatment. This was so when the LPC was evaluated both without and with methionine supplementation indicating no effect of herbage maturity on the nutritional availability of either the first or the second limiting amino acid(s). These results contrast with two previous attempts to assess the effect(s) of herbage age on protein quality. Davies & Evans (1952) observed true digestibility and biological value of the protein to decrease as age of the herbage increased and Henry & Ford (1965) observed both of the above to increase as herbage aged.

The effect of season of harvest on protein quality.

LPC prepared from ryegrass/white clover and lucerne herbage harvested at the sixth week of regrowth in spring and autumn 1977 were compared. Only methionine supplemented material was examined. There was no effect of season on protein utilisation for either

herbage. However, unlike the results from RE Two, protein utilisation for methionine-supplemented LPC was similar for RG/WC and lucerne materials.

2.3.5 CONCLUSIONS

1. Compared with LPC prepared from ryegrass and ryegrass/white clover herbage, that prepared from lucerne and white clover showed either a clear or marginal tendency towards higher total and available lysine and histidine contents.

2. Rat growth protein utilisation for LPC prepared from ryegrass, white clover, ryegrass/white clover and lucerne herbages was about 50% of that for lactalbumin. Results were highest for lucerne and lowest for white clover and ryegrass/white clover.

3. When the above LPC were supplemented with methionine, protein utilisation improved to 80 to 90% of that for lactalbumin. Utilisation was highest and similar for white clover and lucerne and lowest and similar for ryegrass and ryegrass/white clover.

4. The true digestibility of protein was highest for LPC from lucerne (82%), lowest for LPC from ryegrass and ryegrass/white clover (70%) and intermediate for LPC from white clover (73%).

5. There was no effect of season (autumn v. spring) or age of herbage at harvest (4, 6, or 8 weeks regrowth) on the quality of protein in LPC.

6. Despite standardised procedures for harvesting, processing and drying the LPC, and for assessing protein quality, undefined factors still appeared to cause variation in methionine-supplemented protein quality.

PART 2

CHAPTER 4. THE EFFECT OF HERBAGE TYPE AND REDUCING AGENT ON PROTEIN QUALITY

2.4.1 INTRODUCTION

In the previous experiment it was shown that for LPC prepared from ryegrass, the protein had a lower true digestibility than for LPC prepared from lucerne or white clover. It was suggested that this difference might be associated with differing cytoplasmic: chloroplastic ratios in the protein; the chloroplastic ratio being higher in the species with the lower digestibility. Other evidence in support of this hypothesis was higher lysine and histidine contents in the LPC prepared from white clover and lucerne compared with that from ryegrass, and conversely, higher crude fat in LPC from ryegrass than those from the legumes. An objective of this experiment then was to measure the proportion of the true protein in the expressed juice that derived from the chloroplastic and cytoplasmic fractions for the four herbage types, and to measure the true digestibility of the protein in the resulting LPC.

It has been suggested that polyphenols and their derivatives could reduce the quality of leaf proteins (Pirie, 1975; Synge, 1975; Pierpoint, 1971, 1979). *In vitro* studies have confirmed this (Horigome & Kandatsu, 1966, 1968; Allison, 1971). Studies have been made of the effects on protein quality of adding the reducing agent sodium metabisulphite to grass juice to inhibit polyphenol-protein interactions (section 2.1.2.2). Quality improvements were recorded, but as yet have only been identified as increases in the nutritional availability of the first limiting amino acids. Another objective of this experiment was to test if

metabisulphite treatment would improve protein quality in LPC from the four herbages and to investigate if such improvements were mediated via effects on the availability of amino acids and/or the digestibility of the protein. Effects of metabisulphite treatment on extraction, separation and recovery performance were also measured.

2.4.2 MATERIALS AND METHODS

2.4.2.1 Treatments

Leaf protein concentrates were prepared from pure stands of ryegrass, white clover and lucerne, and from a stand of ryegrass/white clover pasture. The method of preparation was as described in section 1.2.2 with two exceptions. Firstly, the forage harvester used earlier which had 'flat-on' flails (these created an air up-draught) was replaced by one with 'edge-on' flails (Taarup DC 1500) and which considerably reduced up-draught and therefore soil and dust contamination (Mills, R. A. & Donnelly, P. E. unpub. results). The second modification was that the belt press was replaced by a screw press (Mills *et al.*, 1980) which gave similar extraction performance.

The experiments were carried out in the late summer-autumn of 1978 and each herbage type was harvested in the fifth week of regrowth. Harvesting was carried out at 0800 hr and processing commenced within one hour of arrival at the laboratory. Each herbage treatment was replicated four times and replicates of individual herbage treatments were harvested over four consecutive days. Upon arrival at the laboratory each load of herbage (400 - 800 kg) was divided into two representative equal portions, which were processed one after the other. A solution of sodium metabisulphite (MBS) was sprayed onto one portion as it was delivered

from the conveyor to the pulper. The rate of MBS addition was calculated to result in 1000 ppm of sulphur dioxide in the expressed juice.

Measurements were made of the quality of the resulting dried LPC, of the protein composition in the expressed juice and of the extraction, separation and recovery ratios. The latter calculations were carried out as indicated previously (section 1.2.2.9).

2.4.2.2 Relative Nutritive Value Assays and Digestibility Measurements

Leaf protein concentrate from L and RG/WC, each prepared without and with MBS treatment, were compared in RE Six. Similar comparisons were made in RE Seven, but the LPC were from RG, WC and RG/WC. In RE Eight LPC from RG and WC, each prepared without and with MBS, were compared when fed without and with methionine supplementation (2 g/16g N x 6.25) at feeding. Similar comparisons were made in RE Nine but the LPC were from RG/WC and L.

Protein true digestibility measurements were made in RE Six and Seven only. As stated previously, LPC treatments were replicated four times, however in the rat experiments, LPC samples assayed were homogeneous pooled sub-samples representative of each of the four replicates of each treatment.

2.4.2.3 Analytical Methods

Representative samples of each of herbage, juice and wet and dry LPC were analysed for the following:

herbage - dry matter and N contents and botanical composition.

juice - dry matter, organic matter, N, protein N, chloroplastic protein N and sulphur dioxide contents.

wet LPC - dry matter, N and protein N contents.

dry LPC - dry matter, N, chemically available lysine and methionine, and polyphenol content.

For the above materials, sampling was by procedures defined in section 1.2.2. Rat food and faeces were sampled as in section 2.3.2 and were analysed for dry matter and N. Dry matter, organic matter, N and botanical composition determinations were carried out as described in section 1.3.2.

Protein N in juice was estimated by Kjeldahl N analysis of the sediment obtained after centrifuging (1600 x g for 15 min) a 5 ml juice sample which had been diluted with 45 ml distilled water and then tricarboxylic:silicotungstic (50:50) acid added to a final concentration of 5%. The supernatant was discarded and the sediment quantitatively transferred to the Kjeldahl flask.

Chloroplastic protein N was estimated by Kjeldahl analysis of the sediment obtained after centrifuging (40 000 x g for 30 minutes) a 5 ml juice sample to which 45 ml distilled water had been added. The supernatant was discarded and the sediment quantitatively transferred to a Kjeldahl flask. The cytoplasmic protein fraction was estimated by subtraction of the chloroplastic protein N result from the protein N result.

Sulphur dioxide content of the MBS treated juice was measured by an alkalimetric procedure (Rankin & Pocock, 1970).

Protein N content of the wet LPC was determined as follows: A 5 g aliquot of LPC was diluted with 40 ml distilled water and centrifuged (3000 x g) for 5 minutes. The supernatant was discarded and the sediment quantitatively transferred to a Kjeldahl flask for N analysis.

Chemically available lysine in LPC was measured by the

'direct' procedure using FDNB (Booth, 1971).

Chemically available methionine in LPC was measured by the procedure described by McKenzie (1977). Ethylthiocyanate was used as the internal standard.

Polyphenols were extracted from LPC with boiling ethanol under reflux after petroleum spirit extraction to remove pigments (Beck, 1964; Glencross *et al.*, 1972). The extract was taken to dryness and dissolved in acetone. Titanium tetrachloride in concentrated HCl was added to a sample of the above and the maximum optical density read from a spectral scan in the region 400 to 500nm (Eskin *et al.*, 1978). The results are quoted in chlorogenic acid equivalents.

For all chemical analyses, except that for polyphenols, assays were carried out in duplicate on respective samples from individual replications through the process laboratory. Single polyphenol determinations were carried out on individual replicates.

2.4.2.4 Statistical Methods

Analysis of variance was used to assess the significance of treatment effects assuming a split-plot design. Statistical methods for the RNV and digestibility assays have been given previously.

2.4.3 RESULTS

2.4.3.1 Botanical Composition of the Swards

The RG herbage averaged 77% (SD±9) ryegrass and 6% of other grasses; the remainder was dead material. The WC herbage averaged 92 ± 4% white clover, 6% grasses and weeds and 3% dead matter. The mixed RG/WC herbage averaged 53 ± 7% ryegrass, 20 ± 3% white clover, 5% other grasses, 18% weeds and 5% dead matter. The lucerne herbage comprised 92 ± 7% lucerne and the remainder was grasses,

weeds and dead matter.

2.4.3.2 Composition of Herbage and Juice (Table 28)

There was a significant effect of herbage type on herbage crude protein level, due mainly to the comparatively low result for lucerne. When expressed on a dry matter basis, the organic matter content of juice was significantly affected by both herbage and MBS treatment. The effect of the latter was to lower organic matter content. The N content of juice was strongly influenced by both herbage and MBS treatment. Because however one of the four herbage treatments (L) was unaffected by MBS treatment, there was a significant herbage by MBS interaction. Taking the means for the non-MBS treated plots only, juice from RG had the highest N content, WC and RG/WC were intermediate, with lucerne the lowest.

As for N, juice protein N was significantly affected by herbage and MBS treatment. The latter effect was consistent across all herbages. For the non-MBS means, the between herbage pattern differed from that for total N, with RG and WC being higher than RG/WC and L. When however protein N was expressed as a ratio of N, then lucerne yielded the highest result followed by WC, RG/WC, and RG in descending order. This herbage effect was significant, as also was the MBS effect which resulted in the true protein N proportion of total N being, on average, 6.5 percentage units lower.

Results for the chloroplastic protein N content of juice were strongly affected by herbage treatment with RG yielding the highest and lucerne the lowest results. The effect of MBS was inconsistent between herbages. When chloroplastic protein N was expressed as a ratio to juice protein N, there was a similar

TABLE 28: THE EFFECT OF HERBAGE AND METABISULPHITE ON THE COMPOSITION OF JUICE

Herbage type	RG		WC		RG/WC		L		Herbage		MBS		Herbage x MBS
MBS[a] treatment	-	+	-	+	-	+	-	+	effect	Se(d)[d]	effect	Se(d)[e]	effect
Herbage crude protein (DM basis)	25.6	25.8	28.3	27.6	26.2	26.3	21.8	22.3	***	0.7	NS	0.5	NS
Juice organic matter% (DM)	78.0	74:5	85.4	82.9	75.8	74.3	86.1	81.4	***	1.4	**	0.8	NS
Juice N% (DM)	6.7	6.1	6.1	5.8	5.8	5.3	5.2	5.2	***	0.1	***	0.1	*
Juice PN% (DM)[b]	4.8	4.1	4.8	4.4	4.4	3.5	4.5	4.0	*	0.2	**	0.1	NS
Juice PN/N%	71.8	66.2	79.0	77.0	75.0	66.4	85.9	76.3	**	3.3	*	2.7	NS
Juice chlor. PN% (DM)[c]	3.6	3.7	2.7	2.7	3.2	2.4	2.2	1.8	***	0.2	+	0.1	NS
Juice chlor. PN/PN%	73.3	91.8	56.1	60.3	73.2	67.0	49.2	46.7	***	3.1	NS	3.2	+
Juice SO_2 content ppm		902		850		988		1308					

a: MBS - indicates metabisulphite treatment

b: PN = protein nitrogen

c: chlor. PN = chloroplastic protein nitrogen

d: Se(d) - average standard error of differences, means of 8 observations

e: Se(d) - average standard error of differences, means of 16 observations

highly significant herbage effect with RG again the highest and L the lowest. The effect of MBS treatment on this index was small for all but the RG herbage treatment. In this case the high figure of 92% resulted largely from the low juice protein N content. Subtraction of these percentages from 100 gives an estimate of the proportion of the total protein N contributed by the cytoplasmic fraction. For the herbage control treatments, these results are RG 26.7, WC 43.9, RG/WC 26.8 and L 50.8%.

Metabisulphite treatment closely achieved or exceeded the objective of 1000 ppm sulphur dioxide in the juice in only two cases. However, since 200 ppm has been shown to be effective in improving leaf protein quality (Bickoff *et al.*, 1975a), even the two lower levels should have been adequate.

2.4.3.3 Extraction, Separation and Recovery Ratios (Table 29)

Extraction ratios.

Dry matter and N extraction ratios were significantly affected by herbage source only. N extraction was highest and lowest for L and RG, respectively.

Separation ratios.

Separation ratios for DM, although affected to a small degree ($P<0.10$) by herbage type due to the low result for L, were consistently influenced ($P<0.05$) by MBS. In contrast, N separation ratio was significantly affected by herbage type but not by MBS treatment. Adjustment of the separation ratios to 40% solids in the wet LPC did not change either the between herbage ranking of yields or the size of the MBS effects. As expected, separation of both DM and N decreased because of this (on average 46 to 41%, 70 to 68%, respectively). The detailed results are not presented.

TABLE 29: THE EFFECT OF HERBAGE AND METABISULPHITE ON PROCESS YIELDS

Herbage type		RG		WC		RG/WC		L		Herbage		MBS		Herbage x MBS
MBS treatment		-	+	-	+	-	+	-	+	effect	Se(d)	effect	Se(d)	effect
Extraction ratio (%)	- DM[a]	12.3	14.0	19.9	20.1	17.4	18.9	19.6	19.8	***	0.5	NS	0.5	NS
	- N	20.3	20.8	26.8	26.4	24.2	23.9	29.5	29.2	***	1.1	NS	1.1	NS
Separation ratio (%)	- DM	48.5	45.1	49.3	46.5	47.9	44.5	44.0	42.4	+	1.7	*	1.0	NS
	- N	66.9	66.4	75.2	71.3	69.5	71.1	73.3	69.0	*	2.0	NS	1.2	NS
Recovery ratio (%)	- DM	6.0	6.3	9.8	9.4	8.4	8.4	8.5	8.4	***	0.2	NS	0.3	NS
	- N	13.6	13.8	20.2	18.8	16.8	17.0	23.0	20.2	***	1.2	NS	0.9	NS

a: DM - dry matter basis

Recovery ratios.

Recovery ratios for both DM and N were significantly affected by herbage but not MBS treatment. Although the recovery of DM was highest for WC the recovery of N was highest for L.

2.4.3.4 The Composition of LPC (Table 30)

The dry matter content of the wet LPC differed between herbage treatments and was marginally affected by MBS.

As was the case for juice, both herbage and MBS treatment, affected the organic matter and N content of LPC. The effect of MBS was to lower organic matter and although a decrease in N content was also the usual response to MBS, the trend was inconsistent (for RG/WC), and resulted in a significant interaction. As for total N, there were significant herbage and MBS effects on protein N in the LPC. Although not significant, the MBS effect showed an interaction for RG/WC, where the response was opposite to that for the other herbage types. On average, protein N constituted 92% of total N in the LPC. Highest ratios were for WC and L and the lowest for RG/WC. The effect of MBS treatment was inconsistent.

The herbage effect on chemically available lysine and methionine was highly significant, and although the MBS effect was small (average increases of 2% for lysine and 7% for methionine), it was consistent and significant. The biggest improvement in available methionine was for RG (18%).

Extractable polyphenol content (as chlorogenic acid) was similar in LPC from all herbage types and was increased by MBS treatment.

2.4.3.5 Rat Evaluation of Quality

The food and protein intake and growth data for RE Six, Seven,

TABLE 30: THE EFFECT OF HERBAGE AND METABISULPHITE ON THE COMPOSITION OF LEAF PROTEIN CONCENTRATE

Herbage type	RG		WC		RG/WC		L		Herbage		MBS		Herbage x MBS
MBS treatment	-	+	-	+	-	+	-	+	effect	Se(d)	effect	Se(d)	effect
Dry matter in wet LPC (%)	13.0	13.0	15.1	15.3	12.8	14.4	15.6	16.0	**	0.7	+	0.3	NS
Organic matter % (DM)	93.0	92.6	95.5	95.2	93.4	92.8	95.1	94.6	***	0.4	*	0.1	NS
N % (DM)	9.3	9.0	9.3	8.8	8.4	8.5	8.7	8.4	***	0.1	**	0.1	*
Protein N % (DM)	8.6	8.3	9.0	8.3	7.2	7.9	8.2	8.0	**	0.2	**	0.1	NS
Protein N/N %	92.7	92.4	96.4	94.3	85.7	92.5	94.1	94.4	*	1.9	NS	1.2	+
C. avail. lys. (g/16g N)[a]	4.77	4.91	5.73	5.79	5.11	5.28	5.43	5.53	***	0.1	*	0.04	NS
C. avail. met. (g/16g N)[b]	1.72	1.94	1.52	1.60	1.83	1.88	1.82	1.94	***	0.05	*	0.04	NS
Chlorogenic acid (g/100g DM)	1.36	1.78	1.46	1.87	1.33	1.64	1.90	1.96	NS	0.80	*	0.30	NS

a: C. avail. lys. = chemically available lysine

b: C. avail. met. = chemically available methionine

Eight and Nine are tabulated in Appendix 13, Tables 8, 10, 12 and 13, respectively. The faecal dry matter and protein production data from RE Six and Seven, from which PTD was calculated by regression analysis, are tabulated in Appendix 13, Tables 9 and 11, respectively. The results from the statistical analysis of the data from the above experiments, to obtain the RNV and PTD estimates, are presented in Appendix 14, Table 1, 2, 3 and 4 for RE Six, Seven, Eight and Nine, respectively. A summary of these results is presented in the text in Table 31.

Relative Nutritive Values were similar for LPC prepared without MBS for all herbage types (RE Six and Seven) and were increased (at least $P<0.05$) by MBS treatment. The largest and smallest responses to this treatment were for RG/WC (32%) and WC (10%), respectively. The improvements for RG and L were approximately 20%.

Estimated protein true digestibility for LPC ranged from 72.5% to 80.8%. It was higher for L than RG/WC (5.4 ± 1.1; $P<0.001$; RE Six), and higher for WC than RG (8.3 ± 1.1; $P<0.001$; RE Seven) and RG/WC (6.6 ± 1.1; $P<0.001$; RE Seven). MBS treatment improved the digestibility of protein for RG/WC by 4.5 units ($P<0.001$) in RE Six. To confirm this result and also to get a direct comparison with LPC from RG and WC, the RG/WC material was fed in the next experiment also. The improvement in digestibility due to MBS treatment was confirmed (4.3 ± 1.1%; $P<0.001$). MBS treatment did not significantly affect PTD for the other herbage types.

Methionine supplementation at feeding lifted the RNV of LPC made both without and with MBS treatment from 0.59 to 0.84 (on average) and from 0.72 to 0.84, respectively. The response to

TABLE 31: THE EFFECT OF HERBAGE AND METABISULPHITE ON PROTEIN QUALITY

Rat Experiment	Six		Seven		Eight	Nine
	RNV	PTD%	RNV	PTD%	RNV	RNV
Lactalbumin	1.00	90.5	1.00	92.5	1.00	1.00
LPC - RG			0.60	72.5		
- RG.MBS			0.71	74.4	0.79	
- RG + M[a]					0.82	
- RG.MBS + M					0.84	
- WC			0.57	80.8		
- WC.MBS			0.63	78.7	0.68	
- WC + M					0.84	
- WC.MBS + M					0.86	
- RG/WC	0.62	73.1	0.57	74.2		
- RG/WC.MBS	0.82	77.7	0.75	78.5		0.75
- RG/WC + M						0.81
- RG/WC.MBS + M						0.80
- L	0.61	78.5				
- L.MBS	0.75	80.6				0.66
- L + M						0.82
- L.MBS + M						0.86
Average SE	0.04	1.3	0.02	0.9	0.02	0.03

a: M - indicates supplementation with methionine at feeding.

methionine for MBS treated material was largest for WC ($P<0.001$) and L ($P<0.001$), respective increases in RNV being 26 and 30%, and smallest for RG ($P<0.10$) and RG/WC (NS), for which the increases in RNV were 6 and 7%, respectively. However, the response to methionine for MBS treated LPC from lucerne may not be as large as indicated in RE Nine, since in RE Six the RNV for the same material (when fed without methionine) was higher (0.75) than in RE Nine (0.66). It was only in RE Nine that the response to methionine was directly measured however.

Sulphite treatment lifted the response at methionine supplementation by an average of only 0.02 RNV units (RE Eight and Nine) and for no herbage type was the margin significant.

2.4.4 DISCUSSION

These results confirm the hypothesis that the differences between ryegrass and the legume species in lysine content and the digestibility of protein in LPC are associated with, and probably derive from, variations in the proportion of the true protein from the cytoplasmic and chloroplastic fractions. The variation between species in the composition of protein was in the direction expected with the chloroplastic fraction being highest in RG (73%) and substantially lower in WC (56%) and L (49%). Conversely, the cytoplasmic true protein fraction was higher in L (51%) and WC (44%) than in RG (27%). These results are generally in agreement with those from the bloat studies of McArthur & Miltimore (1969), who showed that the soluble protein content of juice was higher in extracts from clover and lucerne compared with extracts from ryegrass. The correlation between the chloroplastic protein N:protein N ratio and available lysine was -0.75*. In this respect, the lysine and histidine content of LPC declined as the digestibility

of protein declined on a between herbage basis (r = 0.98* and 0.97*, respectively) in the data of Subba Rau *et al.* (1972), and suggests, for their study also, that the observed variation between species in protein digestibility was associated with the protein composition variables examined here.

The ranking between RG and the two legumes in true digestibility and available lysine results is similar in this and the previous experiment. However, the digestibility of protein in WC was higher in this than in the earlier experiment (80 v 73%). Also, available lysine was higher for RG/WC than RG in the current experiment (5.1 v 4.8 g/16g N), whereas the reverse was the case previously (4.6 v 5.1 g/16g N). These discrepancies are unexplained, although for RG/WC, botanical composition factors could be involved.

In an earlier experiment a higher RNV was recorded for L than for RG, WC, and RG/WC when the protein concentrates were fed unsupplemented (Table 25). This was not the case here, where similar RNV were recorded for all herbages (Table 31). Since available sulphur amino acids are first limiting to protein quality, the current similarity of RNV results is not supported by the available methionine results which do differ between herbages (Table 30). However, the available methionine assay does not measure available total sulphur amino acids; it also does not measure methionine sulphoxide (Ellinger & Duncan, 1976) which may be nutritionally available (Gjoen & Njaa, 1977). A further difference between the earlier and the current experiment is in the ranking between herbages in RNV obtained with methionine supplementation. Thus whereas previously results (Table 25) were higher for the legumes (L 0.89, WC 0.88) than RG (0.79) and RG/WC (0.82), in the current experiment the results obtained under these feeding conditions were similar

for all herbages (Table 31). This similarity is unexpected in view of the higher true digestibility of protein for WC and L compared with RG and RG/WC which should have conferred on the former two species a higher efficiency of protein utilisation. To this extent the rankings from the previous experiment appear the more logical. Since the non-protein N in LPC could have a different amino acid balance and availability compared with the true protein fraction, measurements were made to define each of these in the LPC prior to drying. However, with one exception (RG/WC control) the ratios for protein N/N are similar for all herbage types and do not show a pattern of variation which could explain the discrepancy between results for methionine-supplemented RNV and protein true digestibility. However, this interpretation ignores the possibility that the amino acid composition of the non-protein N fraction could vary between herbages. This aspect is discussed further in the next chapter.

Metabisulphite treatment.

This treatment lowered the organic matter, the N and the protein N content of both juice and LPC. The mass effect(s) of these changes were not, however, big enough to effect the extraction ratios of dry matter and N or the separation ratio for N. The separation ratio for dry matter was, however, consistently reduced for all herbage types suggesting that the presence of MBS in juice may reduce centrifuge efficiency. Edwards *et al.* (1975b) have also observed MBS to negatively effect the processing characteristics of coagulated juice.

Metabisulphite treatment resulted in a consistent but small (2%) increase in chemically available lysine (Table 30). Sulphite is known to inhibit enzymatic and non-enzymatic sugar-amino acid

'browning' reactions (Synge, 1977) and also to inhibit the conversion, by polyphenoloxidase, of polyphenols to the highly reactive quinone species which can bind with lysine (Pierpoint, 1971). The improvement in available lysine could have resulted from one or both of these effects. The improvement in chemically available methionine in association with sulphite treatment was of a larger margin (average 7%) than that for lysine (Table 30). Snow *et al.* (1976) have demonstrated that aqueous MBS will reduce methionine sulphoxide to methionine and Pierpoint (1979) claims that peptide bound methionine does not conjugate with phenolic compounds. It seems probable then that the increases in available methionine were due to inhibition by sulphite of methionine oxidation. Of the 'pure' species, the enhancement in available methionine was greatest for RG, intermediate for L and smallest for WC. For RG/WC it was small and similar to WC. These improvements (MBS minus control) do not correlate strongly with the actual control results. Thus although the control result is greater for RG than WC, and similarly the improvement is greater for RG (13%) than WC (5%), this pattern does not follow for RG/WC and L which both have higher control results than RG and both have lower percentage improvements due to MBS. Explanation therefore, for the between herbage ranking in the size of the sulphite induced improvement in available methionine, does not seem to lie with the magnitude of the control results.

It was not the intention in the polyphenol assay to measure total polyphenol content of the LPC since this would have required enzyme hydrolysis of the protein (Lahiry & Satterlee, 1975). Polyphenols which are loosely held by hydrogen bonding can be eluted with organic solvents; however those which are covalently

bound are not so easily removed (Khanna *et al.*, 1968). Thus any affect of MBS treatment on the results as obtained here might indicate an effect on the intensity of binding of the polyphenols. The results showed an increase in extractable polyphenols due to MBS treatment suggesting that this treatment may have resulted in more phenolic material remaining in a 'loosely' bound condition and less proceeding to a more 'tightly' bound condition. If this was the case, such effects were not accompanied by a consistent change in the digestibility of the protein as might be expected from a decrease in the quantity of protein 'tightly' bound to polyphenols. However, this apparent decrease in 'tightly' bound material may be related to the increase in available lysine content resulting from MBS treatment rather than an increase in available methionine.

Protein utilisation for growth was significantly improved by MBS addition at pulping. The between herbage ranking of RNV results for MBS treated material generally paralleled the ranking for available methionine results. This contrasts with the situation for the control LPC where, although available methionine content differed between herbage types, the RNV results showed no variation. This aspect is discussed further in the next chapter.

Also associated with MBS treatment was an improvement in the digestibility of the RG/WC protein by four units (Table 31). This was shown in two independent assays. Since like improvements were not recorded for the 'pure' species treatments the reason for this may lie in the weed component which comprised 18% of the sward dry matter. This effect of sulphite treatment requires further investigation, firstly to see if it can be repeated and secondly

to identify the factors responsible.

The response in RNV to MBS treatment with methionine supplementation was negligible for all herbage types. Provided then that the level of supplementation with methionine lifted the total nutritionally available sulphur amino acids to an excess of requirements, this suggests MBS treatment did not affect the nutritional availability of the second limiting amino acid(s).

2.4.5 CONCLUSIONS

1. Protein in LPC prepared from ryegrass has a lower digestibility than protein in LPC prepared from white clover or lucerne. This was associated with a higher proportion of the juice true protein being from the chloroplastic fraction for ryegrass compared with the two legumes. The chloroplastic fraction is of inherently lower digestibility than the cytoplasmic fraction.

2. Treatment of the juice with metabisulphite caused a small increase in chemically available lysine in LPC prepared from all four herbages.

3. This same treatment improved both chemically available methionine and protein utilisation for growth. The improvement was largest for ryegrass and smallest for white clover.

4. When LPC was fed with methionine supplement, protein utilisation for growth did not differ between the four herbage types. This occurred despite protein true digestibility being higher for the legumes than ryegrass and ryegrass/white clover.

PART 2

CHAPTER 5. THE NUTRITIONAL AVAILABILITY OF METHIONINE AND CYSTINE IN LPC

2.5.1 INTRODUCTION

In Chapter 3 it was shown that LPC prepared from lucerne had a higher RNV than LPC from each of ryegrass, white clover and ryegrass/white clover. Because methionine plus cystine are first limiting to protein quality, this suggested that the nutritionally available levels of these amino acids were higher in the LPC from lucerne compared with the others. In a subsequent experiment however, LPC prepared from each of the above four herbages yielded similar RNV (Chapter 4). When the juice from which these LPC were made was treated with metabisulphite, there was an overall improvement in RNV of 20%. An improvement in the levels of chemically available methionine also resulted. Changes in the availability of cystine were not measured.

The objectives of the current experiments were firstly, to measure the nutritionally available levels of methionine and cystine in LPC and secondly, to measure the effects of herbage type and metabisulphite treatment on their availability.

Nutritional availability was measured by preparing and feeding a crystalline amino acid formulation in a semi-synthetic diet nutritionally adequate in all respects. The amino acid under test was excluded from the formulation to enable (a) its inclusion at graded levels to produce a dose-response reference curve or (b) the inclusion of given amounts of LPC to give growth responses within the range recorded for the reference curve and so enable comparison of the growth responses to estimate the nutritionally available amino acid content of the LPC. Determination, by

chemical analysis, of the total methionine and cystine content of LPC was also carried out to enable calculation of the percentage of these amino acids that was nutritionally available.

2.5.2 MATERIALS AND METHODS

2.5.2.1 Treatments

The leaf protein concentrates assayed were those prepared from ryegrass, white clover, ryegrass/white clover and lucerne herbages, each with and without MBS treatment, as described in Chapter 4.

2.5.2.2 Availability Assay Procedure

The procedures used are based largely on those used by Netke & Scott (1974) and Sasse & Baker (1973) and the methionine/cystine supplementation levels are based on the results of Sowers *et al.* (1972). The diets were formulated as follows:

12% crystalline amino acids (Table 32).
8% corn oil
1% cellulose
1% vitamin mix
3.7% mineral mix
plus x% crystalline amino acid and/or y% of LPC and wheat starch to 100%.

The corn oil and cellulose, and the composition of the vitamin and mineral mixes were as described in Chapter 3. All amino acids were 'analytical' grade and the L isomers were used.

Utilisation of the crystalline amino acid formulation for growth.

In RE Ten the utilisation for growth of the crystalline amino acid (CAA) formulation was tested against lactalbumin in the

standard RNV assay. Each of the CAA mixture and lactalbumin were included in diets at nominal protein levels of 2.5, 5.0 and 7.5%. Four rats were included at each protein level and a diet devoid of protein was also fed.

TABLE 32: COMPOSITION OF CRYSTALLINE AMINO ACID FORMULATION

Amino acid	Content (%)
Arginine	5.90
Histidine	2.22
Isoleucine	4.60
Leucine	8.08
Lysine	9.00
Phenylalanine	5.56
Tyrosine	4.43
Threonine	4.94
Tryptophan	3.12
Valine	5.29
Alanine	5.45
Asparagine	3.20
Aspartic acid	8.79
Glutamic acid	10.04
Glycine	4.83
Proline	4.07
Serine	4.98
Methionine	6.00

Measurement of available methionine plus cystine (M+C).

In RE Eleven the objective was to determine the range of dietary methionine levels over which the dose-response function was approximately linear. The CAA mixture was formulated as in Table 32 but excluding methionine which was added to individual diets at 0.10, 0.17, 0.24, 0.31, 0.38 and 0.45% of the air dry diet.

The difference between the actual methionine addition rate and that nominated in Table 32 was made up with glycine. A sample of LPC (L, prepared without MBS) containing 52.4% crude protein was also added to individual diets devoid of methionine at 7, 13, 19 and 25% to enable estimation of the dietary content which also would give linear dose-response function. Five weanling rats (male and female) were individually fed each diet. The procedures for feeding the rats and estimating food intake were as in the RNV assay. The criterion for growth was empty body weight gain; the procedures used were similar to those used in the RNV assay to measure body water gains, except body water content was not measured (Sowers *et al.* 1972). For the initial slaughter group six to eight rats were used and the experimental period was three weeks. However, results were also collated after a two week period to test the use of a shorter experimental period. In RE Twelve the eight LPC were assayed for available M+C. Dietary levels of methionine were 0.13, 0.19 and 0.25% and of LPC were 14, 20 and 26% (air dry basis). Six rats were included on each diet for the reference curve and four on each diet for the test proteins. All other assay procedures were as above except that the experimental period was reduced to two weeks.

Measurement of available methionine by growth assay and available cystine by difference.

The procedures for this assay were essentially as in the assay for M+C, except all diets were supplemented with a basal level of cystine (0.35% of the diet) and the methionine supplementation rates for the reference curve were lower. This level of cystine was estimated to equal or exceed 64% of the total dietary crystalline cystine plus methionine, and hence ensured the growth response

was to methionine only (Sowers *et al.*, 1972).

RE Thirteen was a preliminary experiment to examine the procedure and to define the supplementation rates of methionine and LPC which would give linear dose-response, i.e., 0.050, 0.076, 0.102, 0.128, 0.154 and 0.180% methionine and 8, 11, 14 and 17% LPC. The LPC had been prepared from MBS treated juice. This experiment was followed by RE Fourteen in which the available methionine levels were assayed in all the LPC designated under Treatments. Methionine and LPC feeding rates are presented in section 2.5.3.3 (Table 37).

Nutritionally available cystine was estimated by subtracting the result for available methionine from that for available M+C.

2.5.2.3 Analytical Procedures

Determinations of dry matter and N content were carried out in duplicate as described previously. Total sulphur amino acid content of LPC was determined by ion exchange chromatography after performic acid oxidation and acid hydrolysis of the samples. Performic acid was prepared by adding 1 ml of 30% hydrogen peroxide to 9 ml of 88% formic acid. After standing at room temperature for one hour the mixture was cooled in an ice bath and 5 ml was then added to 10 mg of LPC. The mixture was held in an ice bath for 16 hr and then 0.75 ml of hydrogen bromide was added and mixed before being concentrated to dryness at 40°C. The bromine which distilled over was absorbed with 1 N sodium hydroxide. For hydrolysis, 6 N HCL was added (1000:1 acid:sample ratio) and the mixture heated at 130°C for 20 hr under nitrogen reflux. After cooling, the hydrolysate was filtered, evaporated to dryness under reduced pressure and then made up to volume. The internal standard, norleucine, was added to samples immediately prior to injection.

The amino acid analyser was a Beckman 120C and the recommended procedures were used for resolution. Cysteic acid and methionine sulphone results were converted to cystine and methionine according to their respective molecular weights and were corrected for a standard 94% recovery. Single analyses were carried out on samples representative of the four replicates for each of the herbage by metabisulphite treatments.

2.5.2.4 Statistical Procedures

Regression analysis was used to describe the dose-response relationships for each of the CAA standard curve and the test proteins. The resulting regression coefficients were used to estimate, by the slope:ratio technique, the available levels of the amino acids in the test proteins (Netke & Scott, 1974).

2.5.3 RESULTS

2.5.3.1 Utilisation of the Crystalline Amino Acid Formulation for Growth

The regressions of body weight gain (g,Y) on protein intake (g,X) for each of the CAA mixture and lactalbumin were:

LACT. $Y = 2.51(\pm 0.13)X - 9.2\ (\pm 1.0)$; $r = 0.98$, RSD = 2.7

CAA $Y = 2.68(\pm 0.09)X - 9.9\ (\pm 0.6)$; $r = 0.99$, RSD = 1.7

The regressions do not differ significantly and indicate the crystalline formulation was utilised at least as efficiently as lactalbumin for growth. It was therefore considered a satisfactory formulation to use in the availability assays.

2.5.3.2 Available Methionine plus Cystine

RE Eleven.

Results for the intake of food, methionine and protein, and

TABLE 33: INTAKE AND GROWTH RESULTS (±SD) FROM RAT EXPERIMENT ELEVEN.

Reference diets	Food intake[a] (g/3 wk)	Methionine intake (mg/3 wk)	Body weight gain (g/3 wk)
0.10% met.	79 ± 11	79 ± 11	-5.0 ± 1.5
0.17% met.	113 ± 20	192 ± 33	6.7 ± 4.4
0.24% met.	154 ± 22	371 ± 52	27.1 ± 6.4
0.31% met.	170 ± 17	527 ± 52	36.0 ± 3.2
0.38% met.	184 ± 13	699 ± 50	45.1 ± 4.1
0.45% met.	188 ± 13	847 ± 59	50.8 ± 6.1
LPC diets		Protein intake (g/3 wk)	
7% LPC	82 ± 9	3.0 ± 0.3	-4.6 ± 2.6
13% LPC	104 ± 14	7.1 ± 1.0	5.4 ± 4.5
19% LPC	128 ± 12	12.8 ± 1.2	16.3 ± 3.3
25% LPC	150 ± 20	19.7 ± 2.6	27.6 ± 3.6

a: dry matter basis

the gains of body weight, are presented in Table 33. Treatment group mean data points are plotted in Figure 6. Regression analysis of the dose-response relationships for the reference curve and the test protein yielded a significant ($P<0.05$) quadratic term for each. For the LPC group, the quadratic term was no longer significant if the results for the group fed the lowest protein level were omitted from the analysis. For the methionine group, omission of the results for the three highest methionine levels from the analysis produced a regression in which the quadratic

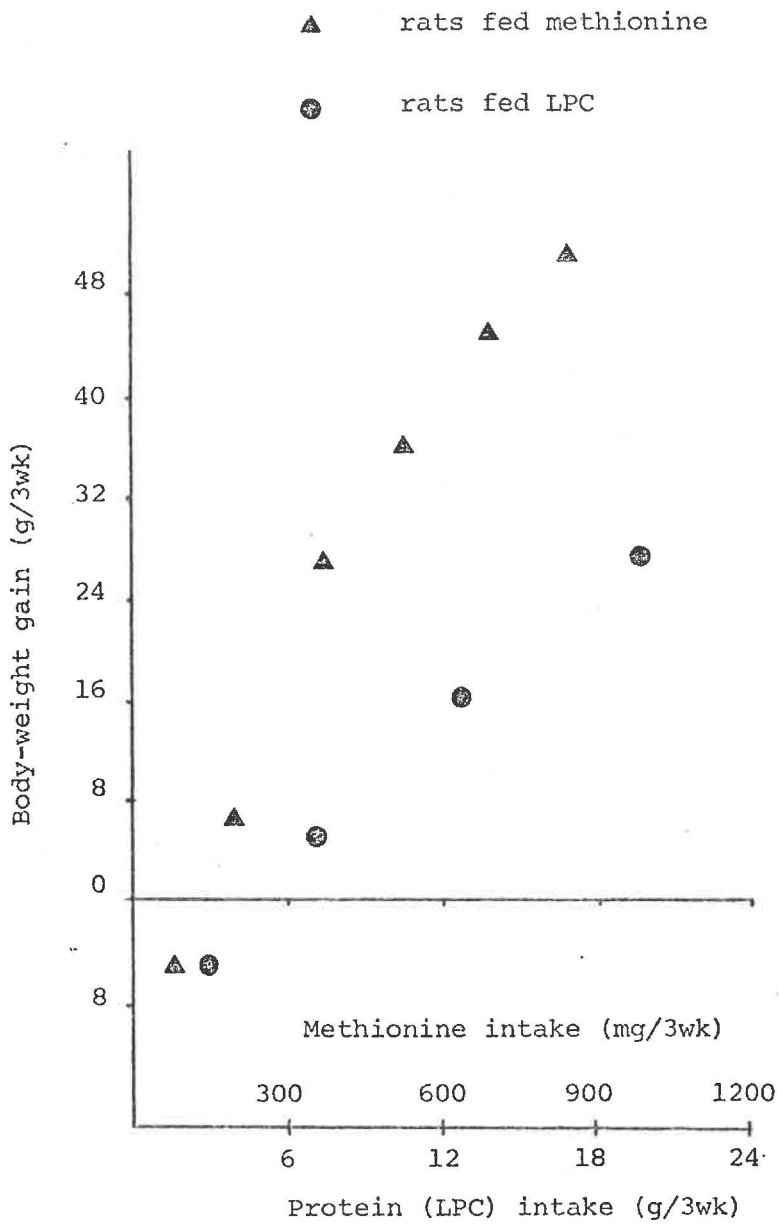

FIGURE 6: The relationships between growth and methionine and LPC-protein intakes in RE Eleven.

term was not significant. The bivariate regression of body weight gain (g/3wk,Y) on methionine intake (mg/3wk,X_1) and protein intake (g/3wk,X_2) was calculated, using data from the three highest LPC groups and the three lowest methionine groups. The resulting equation was:

$$Y = 0.101(\pm 0.005)X_1 + 2.050(\pm 0.098)X_2 - 11.2; \quad R^2 = 0.95$$

This regression gave an estimate for available M+C of 2.03 g/16g N. When the growth and intake data were analysed after completion of only two weeks of the experimental period the results were as above, and it was concluded that a two week period was satisfactory.

The dietary methionine inclusion rates chosen for RE Twelve were 0.13, 0.19 and 0.25% and the LPC inclusion rates were 14, 20 and 26%.

RE Twelve.

Results for the intakes of food, methionine and protein and the gains of body weight are presented in Table 34. Group means from the dose-response relationships for the groups fed LPC are plotted in Figure 7. For all control LPC the relationships are linear. However, for the MBS treated LPC there is curvilinearity ($P<0.05$) at the highest dietary level. The 26% inclusion rate was therefore excessive and resulted because LPC produced without MBS was used in RE Eleven to select the inclusion rates. In the bivariate regression analysis, results from the diets containing 26% of MBS treated LPC were excluded. The results from this analysis and the calculation of available M+C are presented in Table 35. When comparing the regression coefficients for LPC produced without MBS, the result for RG/WC is significantly ($P<0.05$) lower than the other three herbages. Metabisulphite treatment resulted in an average 33% increase ($P<0.001$) in available M+C

TABLE 34: INTAKE AND GROWTH RESULTS (±SD) FROM RAT EXPERIMENT TWELVE

Reference diets		Food intake (g/2 wk)	Methionine intake (mg/2 wk)	Body weight gain (g/2 wk)
0.13% met.		71.4 ± 5.9	92.8 ± 7.6	2.0 ± 2.0
0.19% met.		93.1 ± 11.3	176.9 ± 21.5	13.2 ± 1.1
0.25% met.		99.7 ± 12.0	249.4 ± 30.0	20.4 ± 5.1
LPC diets			Protein intake (g/2 wk)	
RG	14%	70.0 ± 7.2	5.4 ± 0.6	3.8 ± 2.1
	20%	82.5 ± 9.9	9.0 ± 1.1	12.0 ± 3.6
	26%	107.3 ± 9.5	15.3 ± 1.4	27.7 ± 4.7
RG.MBS	14%	80.0 ± 7.2	6.0 ± 0.5	9.3 ± 2.7
	20%	107.5 ± 13.8	11.6 ± 1.5	26.8 ± 6.8
	26%	104.3 ± 7.3	14.6 ± 1.0	29.5 ± 5.7
WC	14%	68.7 ± 14.0	5.4 ± 1.1	3.1 ± 3.6
	20%	84.0 ± 12.2	9.4 ± 1.4	13.0 ± 4.2
	26%	94.8 ± 21.5	13.7 ± 3.1	22.2 ± 6.5
WC.MBS	14%	89.8 ± 8.1	6.8 ± 0.6	10.9 ± 0.6
	20%	101.7 ± 7.1	11.1 ± 0.8	23.4 ± 2.9
	26%	108.2 ± 4.5	15.3 ± 0.6	30.2 ± 5.0
RG/WC	14%	63.2 ± 8.0	4.7 ± 0.6	0.6 ± 2.4
	20%	84.4 ± 8.5	8.9 ± 0.9	10.0 ± 2.3
	26%	94.6 ± 8.7	13.0 ± 1.2	17.2 ± 3.3
RG/WC .MBS	14%	91.7 ± 6.6	6.8 ± 0.5	11.0 ± 2.0
	20%	114.9 ± 4.8	12.1 ± 0.5	27.1 ± 1.1
	26%	115.7 ± 8.3	15.8 ± 1.1	32.1 ± 2.7
L	14%	69.7 ± 7.3	5.1 ± 0.5	4.6 ± 1.9
	20%	83.0 ± 15.3	8.6 ± 1.6	12.5 ± 3.3
	26%	88.3 ± 10.7	12.0 ± 1.4	18.3 ± 4.4
L.MBS	14%	88.5 ± 13.2	6.4 ± 1.0	11.0 ± 5.0
	20%	91.5 ± 1.0	9.4 ± 1.0	19.4 ± 0.5
	26%	104.8 ± 3.8	14.0 ± 0.5	29.8 ± 3.5

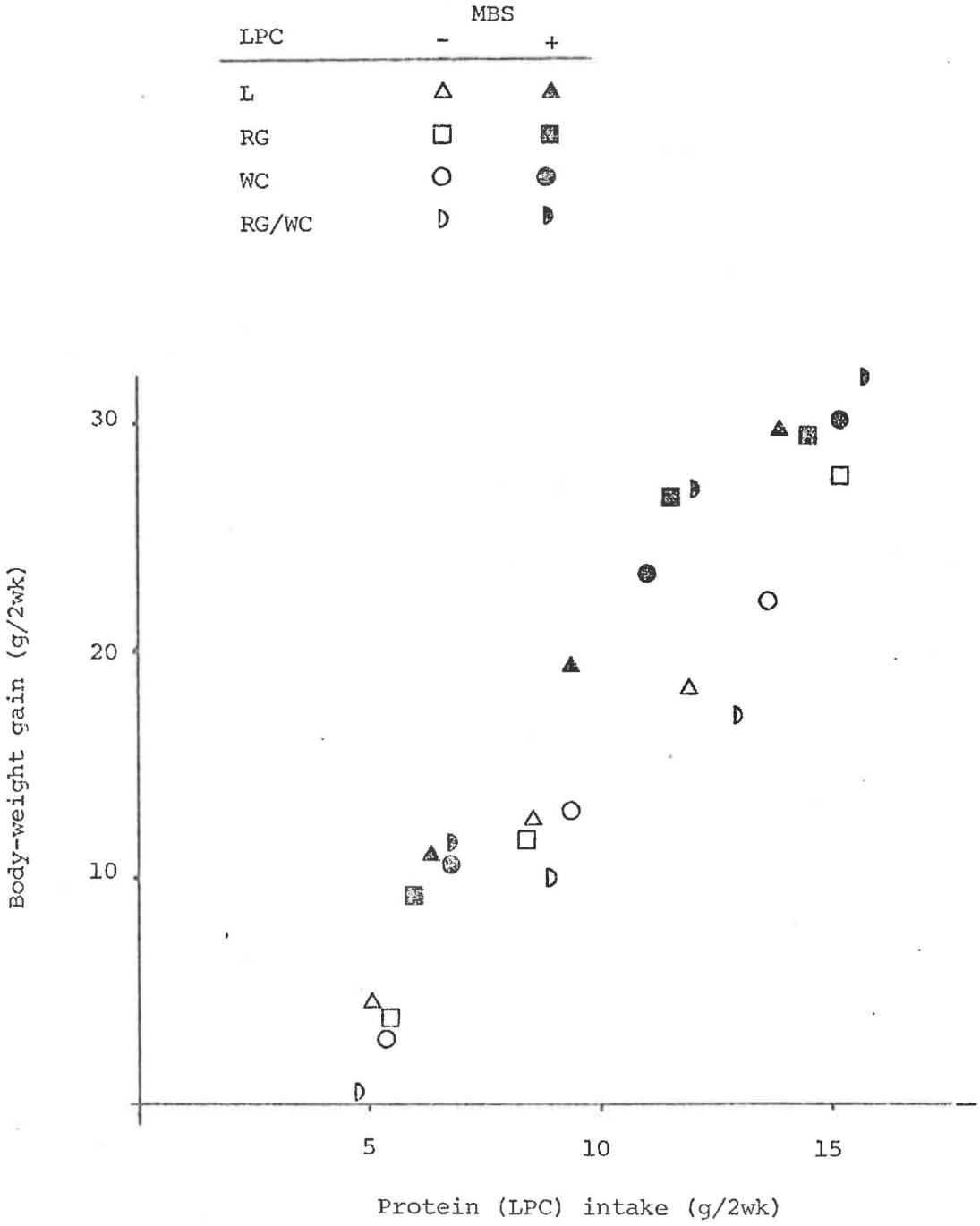

FIGURE 7: The relationships between growth and LPC-protein intake in RE Twelve.

TABLE 35: PARAMETERS FROM THE REGRESSION ANALYSIS OF RESULTS FROM RAT EXPERIMENT TWELVE[a]

Diet	Coefficients[b]		Ratio[c]
	b_1	b_2	b_2/b_1
Methionine	1.18		
LPC - RG		2.35	1.99
- RG.MBS		3.06	2.59
- WC		2.25	1.90
- WC.MBS		2.88	2.43
- RG/WC		2.01	1.70
- RG/WC.MBS		2.93	2.48
- L		2.34	1.98
- L.MBS		3.03	2.56
Regression intercept	-8.6		
Average SE of coefficients		0.08	
Average SE of differences		0.10	

a: Regression = $Y = b_1 X_1 + b_2 X_2 + C$, where Y = body weight gain (g/2 wk), X_1 = met. intake (mg/2 wk x 10^{-1}), X_2 = protein intake from LPC (g/2 wk).

b: b_1, b_2 - coefficients from above regression.

c: Ratio - this ratio is the estimate of available M+C (g/16g N).

but none of the differences between herbages were significant.

2.5.3.3 Available Methionine

RE Thirteen.

The results for food, methionine and protein intakes, and growth, are presented in Table 36. Group means for the dose-response relationships are presented in Figure 8. There was no evidence of curvilinearity in either the reference curve relationship or the test LPC relationship. The dietary methionine inclusion rates chosen for RE Fourteen were 0.10, 0.12, 0.14 and 0.16% and the LPC inclusion rates were approximately 11, 13 and 15%.

RE Fourteen.

Results for food, methionine and protein intakes and growth are presented in Table 37. The growth of the rats fed the reference diets were much lower than those from the comparable diets in RE Thirteen. The cause of this is not clear. The reference results could not therefore be used. The LPC fed in RE Thirteen (L.MBS) was also fed in RE Fourteen. The dose-responses for this LPC in each of these two experiments were compared, to see if the results from the reference curve from the preceding experiment could safely be used in the current experiment. The relationships ($Y = a + bX$) between body weight gain (g/2 wk, Y) and protein intake from LPC (g/2 wk, X) were:

RE Thirteen $Y = 4.34(\pm 0.25)X - 9.3(\pm 1.5)$; $r = 0.97$, RSD = ± 2.1

RE Fourteen $Y = 4.42(\pm 0.53)X - 11.1(\pm 2.3)$; $r = 0.93$, RSD = ± 2.4

These relationships do not differ significantly and show the growth response of the animals to unit methionine intake was the same in both experiments. Therefore, when analysing statistically the intake and growth data from RE Fourteen, the results from the following

TABLE 36: INTAKE AND GROWTH RESULTS (±SD) FROM RAT EXPERIMENT THIRTEEN

Reference diets	Food intake (g/2 wk)	Methionine intake (mg/2 wk)	Body weight gain (g/2 wk)
0.050% met.	48.1 ± 8.8	24.1 ± 4.4	-2.5 ± 1.2
0.076% met.	57.4 ± 10.3	43.6 ± 7.8	0.5 ± 3.4
0.102% met.	63.7 ± 15.8	65.0 ± 16.1	6.9 ± 5.3
0.128% met.	88.3 ± 15.2	113.0 ± 19.5	19.3 ± 4.5
0.154% met.	94.6 ± 6.7	145.7 ± 1.0	26.1 ± 3.8
0.180% met.	110.2 ± 9.4	198.3 ± 1.7	37.1 ± 5.6
LPC diets		Protein intake (g/2 wk)	
8% LPC[a]	48.0 ± 5.8	2.0 ± 0.2	-0.6 ± 1.6
11% LPC	58.6 ± 8.9	3.3 ± 0.5	4.9 ± 2.2
14% LPC	69.5 ± 12.6	5.0 ± 0.9	12.7 ± 4.1
17% LPC	93.0 ± 7.3	8.2 ± 0.6	26.1 ± 3.8

a: LPC = L.MBS as included in RE Twelve and Fourteen.

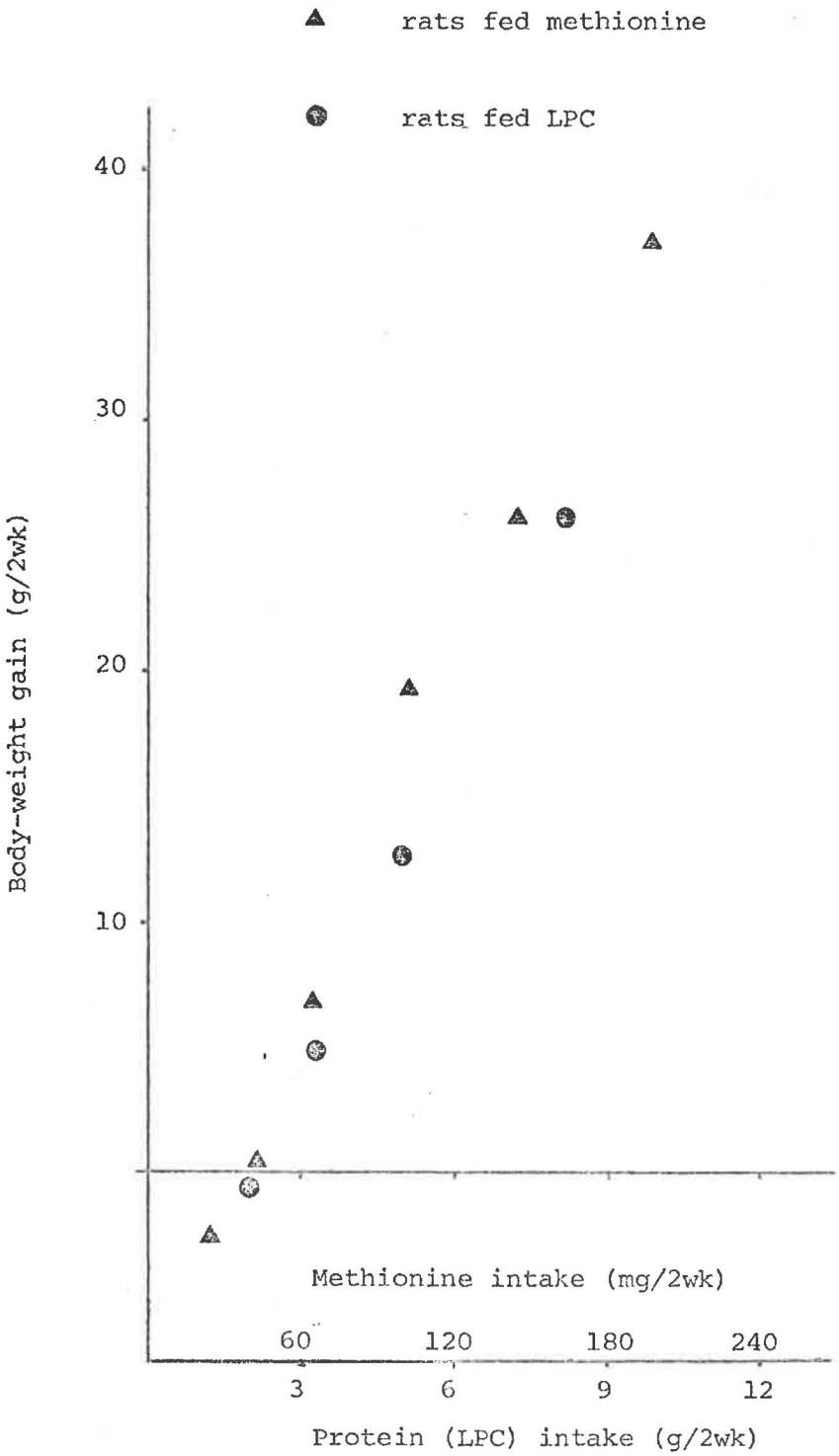

FIGURE 8: The relationships between growth and methionine and LPC-protein intakes in RE Thirteen.

TABLE 37: INTAKE AND GROWTH RESULTS (±SD) FROM RAT EXPERIMENT FOURTEEN

Reference diets		Food intake (g/2 wk)	Methionine intake (mg/2 wk)	Body weight gain (g/2 wk)
0.10% met.		64.1 ± 10.3	64.1 ± 10.0	1.3 ± 4.2
0.12% met.		79.4 ± 15.5	95.3 ± 18.0	9.1 ± 5.0
0.14% met.		79.2 ± 12.7	110.9 ± 18.0	11.9 ± 4.7
0.16% met.		75.7 ± 14.9	121.0 ± 24.0	8.3 ± 7.4
LPC diets			Protein intake (g/2 wk)	
RG	11.2%	72.4 ± 12.8	4.4 ± 0.8	7.3 ± 1.9
	13.3%	78.4 ± 10.2	5.7 ± 0.7	12.6 ± 4.4
	15.4%	92.2 ± 16.7	7.8 ± 1.4	20.6 ± 7.7
RG.MBS	10.1%	62.1 ± 5.5	3.4 ± 0.3	2.7 ± 2.0
	12.0%	69.6 ± 5.7	4.5 ± 0.4	8.6 ± 2.0
	13.9%	74.3 ± 8.1	5.6 ± 0.6	12.1 ± 3.2
WC	12.4%	64.3 ± 11.9	4.4 ± 0.8	6.1 ± 3.2
	14.8%	68.2 ± 5.4	5.6 ± 0.4	10.8 ± 3.0
	17.1%	82.1 ± 13.3	7.8 ± 1.3	18.6 ± 6.2
WC.MBS	12.1%	61.9 ± 8.3	4.1 ± 0.5	6.2 ± 2.8
	14.4%	75.9 ± 12.3	5.9 ± 1.0	14.0 ± 2.9
	16.7%	82.1 ± 10.0	7.4 ± 0.9	19.5 ± 2.9
RG/WC	10.9%	61.9 ± 6.1	3.6 ± 0.3	4.4 ± 2.2
	13.0%	72.1 ± 10.3	4.9 ± 0.7	9.7 ± 3.3
	15.0%	77.2 ± 18.8	6.1 ± 1.5	13.8 ± 5.1
RG/WC .MBS	10.6%	61.5 ± 9.9	3.4 ± 0.6	3.8 ± 2.4
	12.6%	74.2 ± 5.6	4.9 ± 0.4	10.8 ± 2.4
	14.6%	85.5 ± 4.2	6.6 ± 0.3	17.3 ± 2.2
L	11.1%	61.2 ± 5.3	3.5 ± 0.3	4.5 ± 2.0
	13.2%	70.8 ± 3.6	4.9 ± 0.2	10.7 ± 2.9
	15.3%	81.8 ± 11.1	6.5 ± 0.9	16.0 ± 1.9
L.MBS	10.6%	64.2 ± 7.6	3.5 ± 0.4	4.2 ± 2.4
	12.6%	70.8 ± 8.9	4.6 ± 0.6	8.6 ± 2.2
	14.6%	82.4 ± 11.2	6.2 ± 0.8	16.7 ± 4.3

four diets in RE Thirteen (0.102, 0.128, 0.154 and 0.180% methionine) were substituted for the reference diet results in Table 37. Table 38 presents the results from the regression analysis and summarised in Table 39 are the results for available M+C, available methionine and available cystine. Overall, the available methionine levels in the control LPC averaged 1.58 g/16g N. The result for WC was lower than those for L and RG ($P<0.05$). The average improvement associated with MBS treatment was small (4%, $P<0.10$). The largest increase was for WC (9%, $P<0.05$). In contrast, MBS treatment increased available cystine levels by an average 175% ($P<0.01$), the biggest increase being for RG/WC (550%), which in the absence of MBS treatment was very low.

2.5.3.4 Total Methionine and Total Cystine

There was no evidence of an effect of MBS treatment on the total methionine and cystine content of LPC and the results are given here as herbage means (Table 40). Both methionine and cystine contents were lowest in LPC from WC.

Based on these results and the nutritionally available methionine and cystine results in Table 39, percentage availabilities were calculated for these two amino acids and are also given in Table 40. For methionine, availability averaged 81% and was marginally increased ($P<0.10$) to 85% by metabisulphite treatment. For cystine the percentage availability was increased ($P<0.01$) by MBS treatment from 27% (on average) to 73%. In the absence of MBS treatment availability was lowest for LPC prepared from RG/WC.

2.5.4 DISCUSSION

The average nutritional availability of methionine plus cystine

TABLE 38: PARAMETERS FROM THE REGRESSION ANALYSIS OF THE RESULTS FROM RAT EXPERIMENT FOURTEEN[a]

Diet	Coefficients b_1	Coefficients b_2	Ratio[b] b_2/b_1
Methionine	2.40		
LPC - RG		3.84	1.60
- RG.MBS		3.83	1.60
- WC		3.56	1.48
- WC.MBS		3.86	1.61
- RG/WC		3.80	1.58
- RG/WC.MBS		4.00	1.67
- L		3.93	1.64
- L.MBS		4.06	1.69
Regression intercept	-9.2		
Average SE of coefficients		0.17	
Average SE of differences		0.16	

a: Bivariate regression as in Table 35.

b: Ratio - this ratio is the estimate of available methionine (g/16g N).

TABLE 39: NUTRITIONALLY AVAILABLE METHIONINE PLUS CYSTINE, METHIONINE, AND CYSTINE IN LPC

	LPC			
	RG	WC	RG/WC	L
Avail. M+C (g/16g N)				
- MBS	1.99	1.90	1.70	1.98
+ MBS	2.59	2.43	2.48	2.57
Avail. methionine				
- MBS	1.60	1.48	1.58	1.64
+ MBS	1.60	1.61	1.67	1.69
Avail. cystine[a]				
- MBS	0.39(0.31)[b]	0.42(0.34)	0.12(0.10)	0.34(0.27)
+ MBS	0.99(0.79)	0.82(0.66)	0.81(0.65)	0.88(0.70)

a: Avail. cystine - available cystine calculated as available M+C less available methionine.

b: All results are in terms of methionine and the figures in brackets are converted to cystine equivalents on a weight for weight basis assuming methionine has 80% of the metabolic activity of cystine (Baker, 1977).

TABLE 40: TOTAL METHIONINE AND CYSTINE IN LPC AND THEIR NUTRITIONAL AVAILABILITY.

	LPC			
	RG	WC	RG/WC	L
Total methionine (g/16g N)	1.89	1.79	2.05	2.04
Total cystine	0.99	0.86	1.00	1.01
MBS treatment	Methionine availability %			
-	85	83	77	80
+	85	90	81	83
	Cystine availability %			
-	31	40	10	27
+	80	77	65	69

(expressed as methionine) for all herbages was 60%, and was increased to 80% by treatment with the reducing agent. Although the effect of this treatment on the availability of methionine was small, its effect on cystine was large, resulting in availability increasing, on average, from 27% to 73%. The results concur with the suggestion by Pierpoint (1979), that quinones are more likely to conjugate with cystine than methionine and to this extent it appears that MBS may have inhibited such reactions. In spite of the large effect of MBS on cystine availability, this was not accompanied by a consistent effect on protein digestibility (Table 31). The one exception to this was LPC from RG/WC for which MBS treatment lifted digestibility (4 units) and coincidently for which cystine availability was very low at only 10% in the absence of MBS treatment.

The nutritional availability of methionine ranged from 77 to 85% for control LPC; and was similar to the range of results recorded for protein true digestibility (Table 31). This, along with the very small improvement in methionine availability with MBS treatment, suggests that under good processing conditions methionine is not rendered any more unavailable than might be expected from the results for protein true digestibility. Byers (1970) recorded that even 'rapidly' processed LPC contained 18% methionine sulphoxide. As mentioned earlier however, this compound may be nutritionally available.

In the previous chapter results for available methionine determined by a chemical assay were presented. This assay indicated a larger effect of MBS treatment on available methionine than did the growth assay (7 v. 4%). Also, the results by the chemical assay are consistently higher than those by the growth

procedure, differences averaging 12% and 15% for LPC prepared without and with MBS treatment, respectively. Furthermore, although both assays gave a similar between herbage ranking in available methionine for control LPC, this was not the case for the treated LPC and, in consequence, the correlation between the two sets of results was low (0.58). Some of this variation between results from the two procedures may be due to the fact that the chemical assay does not measure methionine sulphoxide. However, the systematic difference between the chemical and the growth results would not have derived from this as the chemical results are the higher.

Percentage availability for chemically determined methionine averaged 89% for control LPC and 95% for MBS treated LPC. Both these and the growth determined availabilities (77-90%), do not differ markedly from the results of microbiological assays (90%, Byers, 1971b).

Relative Nutritive Value results obtained without methionine supplementation should reflect the available first limiting amino acids. Thus the ranking between herbages by this assay should agree with the ranking by the available M+C assay. For LPC produced without MBS, RNV results for all herbages fell within the range 0.57 to 0.62. Similarly the range in available M+C was small between RG, L and WC. However, the result for RG/WC was 10-12% lower ($P<0.05$), and was not paralleled by a lower RNV result.

The RNV results from RE Seven for MBS treated LPC from RG, WC and RG/WC were 0.71, 0.63 and 0.75 respectively. The result for WC is significantly ($P<0.001$) lower. In contrast, the available M+C assay indicated no significant differences between these three treatments (results for L.MBS are excluded from this comparison

because of inconsistencies between RE Six and Nine). A factor which could have contributed to this inconsistent ranking between the two types of assay is the metabolic demand for methyl donors in the conjugation/detoxification of polyphenols. Although there are methyl donors other than methionine (e.g. choline), both methionine and choline have been shown to improve the growth of rats fed sunflower seed containing 1.3% chlorogenic acid (Delic *et al.*, 1976). Such use of methionine would reduce its availability for growth. However, the results from the polyphenol assay (Table 30) do not support this suggestion.

Bickoff *et al.* (1975) observed MBS treatment to increase the total measurable methionine and cystine levels in LPC. A similar effect was not observed here. The results obtained generally fall within the range expected for methionine (2.0 - 2.4 g/16g N) and cystine (0.9 - 1.25 g/16g N) (Byers, 1971b; Bickoff *et al.*, 1975). The lower result for WC would need further investigation to determine if the difference is real.

In Chapter 4 a similar RNV result was reported for LPC from all herbage types when supplemented with methionine. This was despite a higher true digestibility of protein for L and WC compared with RG and RG/WC. As pointed out earlier this contrasts with the results in Chapter 3 where, for LPC prepared in a different experiment, higher methionine-supplemented RNV were recorded for WC and L than RG and RG/WC. Although a similar methionine supplementation rate at feeding was used in all experiments, the possibility was considered that this supplementation rate was not high enough to ensure nutritionally available M+C was not still limiting growth. Estimates of the M+C requirement of the growing rat range from 3.9 to 5.2 g/100g protein (NRC, 1972; Rama Rao *et al.*, 1964; Stockland

et al., 1973; Ngwira & Beames, 1978). The supplementation rate used, when added to the available M+C results in Table 39, gives total available M+C ranging from 3.7 to 4.6 g/100g protein. These figures fall within the range of estimated requirements and suggest that the supplementation rate was high enough to ensure that M+C were not limiting growth. An examination of the effects of MBS treatment on RNV determined in the presence of supplemental methionine confirms this. Thus although MBS treatment increased available M+C by 33%, it did not improve RNV determined in the presence of methionine, hence indicating that on the absence of MBS treatment, the supplementation rate was adequate. More detailed studies will be required, involving in particular, measurement of the second limiting amino acid(s) and their nutritional availability, to determine why the LPC with higher digestibility of protein did not yield higher RNV results when supplemented with methionine.

2.6.5 CONCLUSIONS

1. The nutritional availability of methionine in untreated LPC was as expected from the results for the true digestibility of protein (viz. 70 - 80%). For cystine, however, the nutritional availability was very low, ranging from 10 to 40%.

2. Treatment of the extracted juice with metabisulphite during preparation of the LPC marginally increased nutritionally available methionine, but did, however, increase available cystine levels by an average 175%. Percentage availability was increased threefold.

3. For available methionine, there were systematic and random

differences between results from a chemical assay and a growth assay.

4. Of the four herbage types examined, the availability of cystine was lowest in LPC from ryegrass/white clover and was substantially increased by treatment with metabisulphite, possibly because it was accompanied by an increase in the true digestibility of the protein.

PARTS 1 and 2

CHAPTER 6: GENERAL DISCUSSION

This thesis is concerned with two aspects of protein extraction. Firstly, with the application of extraction and processing technology to grazed herbage under dairying, and secondly, with the quality of protein extracted from pasture and lucerne herbages. In the former case, emphasis has been directed towards herbage and regrowth factors in relation to recoverable protein yields. Experiments were made with four herbage types over four seasons, (spring 1976, summer, autumn and spring 1977), and extraction and processing were carried out on an industrial pilot scale. With respect to protein quality, measurements were made of the effects of herbage type, herbage maturity, season and chemical treatment. The assay criteria used were amino acid analysis, protein utilisation for growth by rats, *in vivo* protein true digestibility and amino acid availability by both chemical and bio-assay techniques.

For dairy-type ryegrass/white clover pasture, protein recovery ratios varied from 22% down to 15%. For the first three seasons (spring 1976, summer and autumn 1977), recovery declined with increasing regrowth, and in the fourth season of study (spring 1977), the relationship of recovery with regrowth was curvilinear, being at its highest in week 7 (21%) and lowest in weeks 4 (18%) and 10 (16%). Recovery ratio is the product of extraction and separation ratios. Although extraction and separation ratios both showed a tendency, not often significant, to decline with increasing herbage maturity, their product, recovery ratio, more clearly displayed the decline with maturity. The curvilinear relationship for recovery in spring 1977 was due to a similar trend in extraction yields.

Assessment of the contribution of the individual ryegrass and white clover components of the mixed sward, to the results for the total, is partly confounded by the ingression of other species into the two pure swards as they matured, and also by the presence of other species, mainly weeds, in the mixed sward. However, the pure ryegrass and white clover herbages showed diverse extraction yield responses to increasing herbage maturity in the first two seasons. Thus for ryegrass, but not white clover, extraction yields declined with increasing herbage maturity. For ryegrass this would probably be due to the combined effects of decreasing herbage protein content, increasing herbage fibre content and increasing sward paspalum content. Despite this decline in extraction yield with increasing herbage maturity for ryegrass, these two pure herbage treatments gave similar extraction, separation and recovery yields in the early stages of regrowth (i.e. when herbage was vegetative) in these two first seasons of study. In the autumn season, the separation yields for ryegrass were low and declined with increasing herbage maturity. This might have been due to an increasing proportion of non-protein nitrogen in the herbage in this season (section 1.1.8). In conclusion, wide variations in the ryegrass and white clover content of mixed swards are unlikely to diversely effect processing yields from herbage harvested in the vegetative stage of growth. However, in spring and summer, increases in sward ryegrass content are likely to be associated with decreasing extraction yields if the herbage is flowering. Similarly, as sward ryegrass content increases, separation yields would decrease in the autumn where the herbage was harvested after extended periods of regrowth.

Protein recovery ratios were higher for lucerne than ryegrass/white clover in the summer and spring seasons. They were also higher,

on average in the autumn season, although when compared at the sixth week of regrowth there was no difference (see Figure 4). The differences between ryegrass/white clover and lucerne in nitrogen separation yields were negligible for all but the autumn season when they were higher for ryegrass/white clover (77 v. 70%). Hence the higher recovery ratios for lucerne in the spring and summer derived from higher extraction ratios which were on average 33 and 27% (summer), 34 and 29% (autumn) and 32 and 27% (spring) for lucerne and ryegrass/white clover, respectively. The causes of these differences are not clear but do not seem to be related to whole plant protein content, within (Table 12) or between, herbages. Crude protein content was higher in ryegrass/white clover than lucerne in the summer and autumn seasons, but lower in the spring season. Variation between species in the distribution of protein and fibre between the aerial parts of the plant (particularly leaves versus stems: Bailey, 1973; Allison & Vartha, 1973) and the interaction of this with pulping and pressing, may contribute to the differences between lucerne and ryegrass and ryegrass/white clover, in ease of extractability.

It was shown in Part 2 that, relative to ryegrass and ryegrass/white clover, lucerne and white clover yielded a protein containing higher proportions of cytoplasmic than chloroplastic material. It is not known if these two protein fractions differentially affect separation performance, although Byers (1971a) has shown that the sedimentation characteristics of uncoagulated chloroplastic material can vary with species and herbage age. There was no consistent pattern between herbages in separation yields of nitrogen or dry matter, which suggested that this variation in juice protein composition influenced centrifuge performance.

Estimated extractable protein yields ranged from 660 (lucerne) to 979 (irrigated pasture) kg/ha/year. These yields are substantially higher than those achieved by the highest protein yielding pastoral animal production system (section 1.2.4.5). However, the integration of dairying and protein extraction could increase total yields further, into the range 980 to 1480 kg/ha. These estimated protein yields are based on a moderate protein extraction rate. Although plant protein yields could probably be higher with a higher extraction rate, the current estimates, and experimental programme, were based on the hypothetical integration of protein extraction and dairying with the nutritional protein requirements of the cow and the extraction capabilities of equipment for on-farm use, determining nominal extraction rates. An important advantage of an integrated dairying-protein extraction system, would be that the leaf protein product would be largely in addition to existing livestock protein production. For the latter, the technology of production, processing and marketing is well developed and only for the protein extraction component of the operation would new technology be required.

Milk protein yields of 480 and 640 kg/ha are equivalent to nitrogen yields of 75 and 100 kg/ha; for the combined system, estimated recoverable nitrogen yields from pasture are 208 (unirrigated) and 232 (irrigated) kg/ha/year. These recoveries approximate the average soil nitrogen fixation rate of 184 (range 85 to 342) kg nitrogen/ha/year for lowland pastures in New Zealand (Hoglund *et al.*, 1979). For a solely dairying system without irrigation, nitrogen losses from the system as saleable protein are equivalent to 40% (range 20 to 90%) of annual mean nitrogen fixation. Since artificial nitrogen fertilizer inputs are not necessary to sustain pasture and dairy production, it would seem that these fixation rates and losses,

are in satisfactory balance. The mean fixation rate of 184 kg nitrogen/ha/year is much lower than earlier published figures (410-650 kg nitrogen/ha/year; Hoglund *et al.*, 1979), which were apparently derived when sward white clover content was much higher, and soil organic nitrogen content much lower, than is presently the case. The consequences on soil nitrogen economy under grazing, of introducing a system (protein extraction plus dairying) which could remove in excess of 100% of the nitrogen annually fixed into the sward-soil complex, are unknown. Two possible effects could be that soil organic nitrogen reserves would decline and clovers would introduce more atmospheric nitrogen into the soil complex. This might also result in increased sward white clover content. These, and other aspects of soil fertility, pasture composition and production, require long term study in protein extraction-grazing experiments, in which plant and animal protein yields are also examined.

Previous research on leaf protein quality has shown differences between species in protein utilisation for growth and protein true digestibility (section 2.1.2.2). The causes of this variation were not clearly demonstrated. Differences between herbages in the digestibility of protein were found in the present study also (Tables 24 and 31). These were associated with variation between herbages in the proportion of the protein from the cytoplasmic and chloroplastic fractions which themselves differ markedly in digestibility (section 2.1.2.2). The percentage of the protein present as the chloroplastic fraction was: lucerne 49%, white clover 56%, ryegrass 73% and ryegrass/white clover 73%. The correlation coefficient between the chloroplastic protein : true protein ratio and protein true digestibility was -0.79*. Other evidence confirming these protein composition differences between herbages was the higher (available) lysine and

histidine contents in LPC from the legumes than ryegrass, and conversely, the higher fat content in LPC from ryegrass than the legumes. Published data (section 2.1) indicates lysine and histidine contents to be higher, and fat content to be lower, in the cytoplasmic than the chloroplastic fraction. The correlation between available lysine content and protein true digestibility for all observations was 0.82***.

In conclusion then, in this study and probably also in that of Subba Rau *et al.* (1972), the differences between species in protein true digestibility were a result of variation between species in juice chloroplastic and cytoplastic protein content.

Neither season of the year (autumn v. spring) nor age of herbage at harvest (4, 6 or 8 weeks regrowth), affected the quality of protein in LPC. Although published evidence on these effects is equivocal (section 2.1.2.2), with respect to herbage maturity, the current results are in agreement with the observation of Byers (1971a), that the ratio of chloroplastic to cytoplastic protein in the juice is unaffected by herbage age.

Initially protein utilisation for growth varied between herbages when fed to test animals both with and without supplemental methionine. These differences were not verified for newly-prepared LPC in subsequent experiments. It was not determined if this variation between experiments was caused by genuine variation in protein quality and/or from variation in the rat growth assay. Evidence that the latter may have contributed was found in Rat Experiments Six and Nine (App. 14, Tables 1 and 4). In experiment Six there was no significant difference in quality between ryegrass/white clover (RNV=0.82) and lucerne (0.75) for metabisulphite-treated LPC. However, in experiment Nine, where these two LPC were fed again, quality did differ significantly

($P<0.01$, RG/WC 0.75 v. L 0.66). However, Woodham (1965) observed that different batches of LPC prepared from the same species could vary in quality and in this study the digestibility of protein in LPC from white clover differed between two preparations (73 v. 80%). This aspect of protein quality requires further investigation.

Treatment of the extracted juice with metabisulphite improved protein utilisation for growth by 24% (RNV increased from 0.59 to 0.73). However, even with this treatment, available sulphur amino acids were still first limiting for protein utilisation, and supplementation with methionine further increased RNV by 15% to 0.84. Metabisulphite treatment clearly improved the nutritional availability of the sulphur amino acids. Of the 20% increase, two units derived from improvements in methionine, and 18 units from improvements in cystine. Cystine is thus the amino acid most prone to nutritional damage during processing. After treatment with metabisulphite, the nutritional availability of methionine and cystine are as expected from the results for protein digestibility.

Metabisulphite treatment also caused a small (2%, 0.12 g/16g N) but significant increase in chemically available lysine. This increase was not reflected in an improvement in protein utilisation for growth in the presence of methionine, possibly because lysine was not second limiting for protein utilisation although published data suggests this usually is the case (section 2.1.2.2). The above improvement in available lysine is small relative to the largest between herbage differences (1.0 g/16g N - RG/WC v. L, Table 22 and RG v. WC, Table 30), which were reflected in results on protein utilisation for growth in earlier but not later experiments.

Metabisulphite treatment improved the digestibility of protein in ryegrass/white clover LPC by four units (Table 31). The largest

improvement in cystine availability with this treatment was also recorded for this herbage (Table 40). However this is not reflected in the polyphenol results (Table 30), where the increase due to chemical treatment is no larger for ryegrass/white clover than the other herbages. Also, the four herbages did not differ in polyphenol content. Phenolic compounds have been shown to decrease the *in vitro* digestibility of protein (Horigome & Kandatsu, 1966, 1968). Since metabisulphite prevents the conversion of polyphenols to the highly reactive o-quinone species (Pierpoint, 1971), this improvement in digestibility was probably mediated through inhibition of protein tanning. Reasons why it should be specific to ryegrass/white clover and not the other three herbages are not clear, but herbage botanical composition factors may be involved since 18% of the herbage was classified as weeds. A further factor which could have been involved is the pH of the extracted juice. Woodham *et al.* (1975) observed sulphite induced improvements to be highest under acid conditions. In contrast, Nanda *et al.* (1977) observed protein digestibility to increase as pH at extraction was increased and Pierpoint (1979) suggests higher pH may result in better dissolution of protein-tannin conjugates. Investigations involving examination of herbage botanical composition factors, varying juice pH at extraction and metabisulphite treatment, may further assist with elucidating the factors responsible for metabisulphite improving digestibility, and assist with developing procedures for improving protein quality.

Protein true digestibility was in the moderately high range of 70 to 80%. Two possible ways of increasing this for whole LPC are discussed above. Another means of getting a leaf protein of higher digestibility is by separately preparing the cytoplasmic fraction. However, this coincidently results in the production of a chloroplastic protein concentrate of much lower digestibility. Buchanan

(1969b) has shown that extraction of LPC with chloroform:methanol can improve the *in vitro* digestibility of protein. Preliminary studies (Donnelly, P. E. unpub. results) have confirmed this finding, and *in vitro* true digestibility increased by seven units for each of ryegrass and lucerne LPC after solvent extraction. Such a procedure could be part of a process to decolourise leaf protein and improve human acceptability (Bray *et al.*, 1978). There are thus several procedures which might be developed for incorporation into juice processing systems to improve leaf protein quality.

APPENDIX 1: Herbicide sprays, and their application rates

Herbicide sprays were sometimes applied prior to commencement of regrowth treatment periods to reduce the proportion of unwanted species in the existing ryegrass, white clover and lucerne stands.

The sprays used were:

Nortron (3 kg active ingredient (a.i.) / ha; ICI NZ Ltd), MCPA (1.5 kg a.i./ha; Frank M. Winstone Ltd) and Dockontrol (2 kg a.i./ha; Ivan Watkins-Dow Ltd) on the ryegrass stand.

MCPB (1 kg a.i./ha, Frank M. Winstone Ltd) and Kerb 50-W (2 kg a.i./ha; Rohm and Haas NZ Ltd) on the white clover stand.

Asulox (2 kg a.i./ha, May and Baker Ltd), Kerb 50-W (2 kg a.i./ha) and Sinbar (1 kg a.i./ha; Neill Cropper and Co. Ltd) on the lucerne stand. In this latter case the Asulox and Kerb 50 W were applied only in the late spring of 1976 and Sinbar in the winter of 1977.

APPENDIX 2: Dates of 'topping' and commencement of regrowth for each herbage type and season

Season / Herbage	Spring 1976	Summer 1977	Autumn 1977	Spring 1977
Ryegrass	11,18/10	3,10/1	14,21/3	-[a]
White clover	12,19/10	4,11/1	-[a]	-[a]
Ryegrass/ White clover	28/10,14/11	5,12/1	16,23/3	5,13/9
Lucerne	-[a]	6,13/1	10,17/3	17,24/10

a: no measurements made

APPENDIX 3: Experimental design

When designing the experiment it was only possible to extract and process two treatment plots in any one day; the harvest times being set at 0800 and 1050 hrs. The decision to 'top' and harvest regrowth period treatments one week apart was arbitrarily made in an attempt to minimise the effects of, for example, weather conditions at the time of harvest, on the results for a given regrowth period. Information was obtained from another experiment (P.E. Donnelly unpub.) that suggested both of the above procedures would not prejudice results. In this experiment 'time of day' and 'week' effects (along with some other factors) were studied in a factorial experiment. The results for 'week' and 'time of day' are set out below.

	Week		Time of day		
	1	2	0800	1050h	Se(d)
Grass DM %	11.56	13.07	12.02	12.61	0.63
Grass N % (DM basis)	3.59	3.62	3.58	3.63	0.06
Juice DM %	4.06	4.06	4.03	4.10	0.18
Juice N %	4.64	4.63	4.63	4.64	0.10
Extraction ratio % (DM)	22.6	23.1	22.8	22.9	0.7
Extraction ratio % (N)	29.2	29.6	29.1	29.4	1.1

The herbage was ryegrass/white clover pasture. Experimental procedures were as in the text for the current experiment. Each mean is comprised of eight observations. Weeks 1 and 2 were topped and harvested one week apart. Only grass dry matter content differed ($P < 0.05$) between 'weeks'; this would not have affected extraction ratios because of wetting the herbage to 90% moisture at pulping. The results suggest the procedures under discussion and used in the experiment reported in Part I were satisfactory.

APPENDIX 4: Dry matter extraction ratios (g juice DM/100g herbage DM)

Season	Herbage	Regrowth period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	20.2	18.8	17.0	13.7	11.9	-	-	-	1.7	-2.2 ± 0.4	**
	WC	-	23.6	22.3	20.0	19.3	-	-	-	1.6	-1.5 ± 0.5	*
	RG/WC	20.5	18.3	18.4	17.1	13.4	-	-	-	3.4	-1.5 ± 0.4	**
Summer '77	RG	16.0	15.5	14.8	13.3	11.7	-	-	-	1.0	-1.1 ± 0.2	**
	WC	20.6	20.3	19.6	19.9	19.4	-	-	-	2.6	-0.2 ± 0.6	NS
	RG/WC	18.4	19.9	18.2	19.1	17.0	-	-	-	1.7	-0.4 ± 0.4	NS
	L	21.6	20.1	20.0	17.6	16.4	-	-	-	1.3	-1.3 ± 0.3	**
Autumn '77	RG	-	17.5	19.2	20.9	20.1	18.9	17.9	-	1.5	0	NS
	RG/WC	-	-	24.8	-	23.6	-	20.6	19.8	0.8	-0.9 ± 0.1	**
	L	24.2	23.7	23.6	24.1	-	-	-	-	2.4	0	NS
Spring '77	RG/WC	16.6	17.5	17.3	18.4	16.2	15.9	14.7	-	1.2	-0.4 ± 0.2	+[a]
	L	-	21.1	22.6	22.8	17.9	17.3	-	-	0.9	-1.2 ± 0.2	**[b]

a: Significant quadratic contrast of -0.2 ± 0.09 ($P<0.10$)

b: Significant quadratic contrast of -0.7 ± 0.2 ($P<0.05$)

There was a significant effect due to herbage type in spring '76 ($P<0.10$), summer '77 ($P<0.001$), autumn '77 ($P<0.01$), spring '77 ($P<0.01$)

APPENDIX 5: Juice dry matter content (g/100g)

Season	Herbage	Rest period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	4.7	4.0	3.4	3.5	2.3	-	-	-	0.2	-0.5 ± 0.05	***
	WC	-	6.4	5.5	4.4	3.9	-	-	-	0.6	-0.9 ± 0.2	*
	RG/WC	4.2	3.6	3.1	3.0	2.5	-	-	-	0.6	-0.4 ± 0.1	*
Summer '77	RG	3.3	3.3	2.6	2.6	2.2	-	-	-	0.4	-0.3 ± 0.09	*
	WC	7.2	7.2	5.9	4.3	6.3	-	-	-	1.6	-0.5 ± 0.4	NS
	RG/WC	5.6	5.2	3.6	3.8	3.2	-	-	-	0.7	-0.6 ± 0.2	*
	L	4.6	4.6	4.0	3.3	3.2	-	-	-	0.5	-0.4 ± 0.1	*
Autumn '77	RG	-	3.7	3.5	3.2	3.6	3.2	3.0	-	0.3	-0.1 ± 0.05	+
	RG/WC	-	-	4.5	-	4.7	-	3.8	4.0	0.2	-0.1 ± 0.03	*
	L	6.5	5.8	4.9	4.5	-	-	-	-	0.8	-0.7 ± 0.3	+
Spring '77	RG/WC	4.6	4.7	4.3	3.6	3.1	3.4	3.1	-	0.4	-0.3 ± 0.05	***
	L	-	7.8	6.1	4.8	3.5	3.5	-	-	0.6	-1.1 ± 0.1	***

There was a significant effect due to herbage type in spring '76 ($P<0.05$), summer '77 ($P<0.001$), and autumn '77 ($P<0.001$).

APPENDIX 6: Dry matter separation ratios (g DM in LPC/g DM in juice) (uncorrected)

Season	Herbage	Rest period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	44.6	35.9	37.4	37.7	32.6	-	-	-	4.8	-2.2 ± 1.1	+
	WC	-	55.4	55.3	51.2	45.7	-	-	-	3.1	-3.3 ± 1.0	*
	RG/WC	48.6	42.7	37.7	35.0	36.4	-	-	-	5.1	-3.2 ± 0.6	**[b]
Summer '77	RG	48.0	49.0	50.0	39.0[a]	41.0[a]	-	-	-	10.8	-2.4 ± 2.4	NS
	WC	62.3	61.7	60.7	55.4	57.3	-	-	-	5.5	-1.6 ± 1.2	NS
	RG/WC	61.1	56.0	51.5	45.1	44.9	-	-	-	6.2	-4.3 ± 1.4	*
	L	52.2	49.6	47.7	43.0	40.1	-	-	-	2.8	-3.1 ± 0.6	**
Autumn '77	RG	-	54.1	49.8	43.4	42.4	44.1	44.4	-	4.5	-1.9 ± 0.8	*
	RG/WC	-	-	55.5	-	49.9	-	51.6	50.7	1.2	-0.6 ± 0.2	*[c]
	L	65.8	56.6	59.7	54.5	-	-	-	-	8.3	-3.1 ± 2.6	NS
Spring '77	RG/WC	46.5	49.5	42.4	39.9	36.2	30.7	29.2	-	3.2	-3.4 ± 0.4	***
	L	-	53.1	47.6	47.0	47.5	45.5	-	-	1.7	-1.5 ± 0.4	**

a: Low separation ratio for one of the replicates of each of these two means (see text)

b: Significant quadratic term of 1.2 ± 0.5 ($P<0.10$)

c: Significant quadratic term of 1.2 ± 0.4 ($P<0.05$)

There was a significant effect due to herbage type in spring '76 ($P<0.01$), summer '77 ($P<0.01$), autumn '77 ($P<0.01$) and spring '77 ($P<0.05$).

APPENDIX 7: Nitrogen separation ratios (g N in LPC/100g N in Juice)

(uncorrected)

Season	Herbage	Rest period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	74.8	76.8	80.4	75.3	68.7	-	-	-	7.1	-1.4 ± 1.6	NS
	WC	-	81.0	81.9	76.5	69.9	-	-	-	4.1	-3.8 ± 1.3	*
	RG/WC	75.0	65.5	66.0	59.3	63.1	-	-	-	5.0	-3.0 ± 1.1	*
Summer '77	RG	71.0	65.0	61.0	45.0	49.0	-	-	-	15.3	-6.3 ± 3.4	NS
	WC	75.3	76.1	81.7	66.0	68.0	-	-	-	2.3	-2.5 ± 0.5	**[a]
	RG/WC	75.7	76.6	72.0	65.0	61.1	-	-	-	7.4	-4.1 ± 1.6	+
	L	77.8	77.8	72.5	67.7	63.8	-	-	-	8.8	-2.9 ± 2.0	NS
Autumn '77	RG	-	79.6	72.2	66.2	68.9	67.9	61.5	-	3.8	-2.9 ± 0.6	**
	RG/WC	-	-	81.5	-	81.7	-	79.1	78.2	4.3	-0.6 ± 0.6	NS
	L	75.2	73.2	76.7	73.0	-	-	-	-	6.9	-0.3 ± 2.2	NS
Spring '77	RG/WC	74.2	77.6	74.2	74.2	68.9	64.5	58.3	-	3.2	-2.8 ± 0.4	***[b]
	L	-	80.0	69.5	74.0	72.0	69.5	-	-	3.6	-1.9 ± 0.8	+

a: Significant quadratic term of -1.4 ± 0.4 (P<0.01)

b: Significant quadratic term of -0.8 ± 0.2 (P<0.05)

There was a significant effect due to herbage type in spring '76 (P<0.05), summer '77 (P<0.001) and autumn '77 (P<0.01).

APPENDIX 8: Dry matter content (g/100 g) of the wet leaf protein concentrate

Season	Herbage	Rest period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	12.9	12.4	10.0	11.8	9.5	-	-	-	1.1	-0.7 ± 0.2	*
	WC	-	14.9	14.1	15.4	13.3	-	-	-	0.7	-0.4 ± 0.2	NS
	RG/WC	13.6	11.0	12.0	10.5	9.2	-	-	-	1.2	-0.9 ± 0.3	*
Summer '77	RG	12.5	13.6	12.6	10.6	13.1	-	-	-	1.8	-0.2 ± 0.4	NS
	WC	15.3	16.6	15.7	14.2	19.0	-	-	-	1.5	0.5 ± 0.3	NS
	RG/WC	12.9	12.3	13.3	14.0	13.8	-	-	-	0.7	0.4 ± 0.2	+
	L	15.1	14.9	15.3	14.0	14.3	-	-	-	1.5	-0.2 ± 0.3	NS
Autumn '77	RG	-	12.8	11.0	10.7	10.1	9.6	8.5	-	1.0	-0.8 ± 0.2	**
	RG/WC	-	-	12.5	-	12.5	-	11.5	9.9	0.8	-0.4 ± 0.1	*
	L	14.8	14.5	15.4	14.6	-	-	-	-	3.3	0	NS
Spring '77	RG/WC	12.8	13.6	14.1	12.2	12.6	11.8	12.3	-	1.2	-0.2 ± 0.2	NS
	L	-	17.7	15.7	12.4	9.6	13.2	-	-	1.2	-1.5 ± 0.3	**[a]

a: Significant quadratic contrast of 0.8 ± 0.3 ($P<0.05$)

There was a significant effect due to herbage type in spring '76 ($P<0.05$), summer '77 ($P<0.01$) and autumn '77 ($P<0.001$)

APPENDIX 9: The crude protein content (g/100g DM) of LPC adjusted to 40% dry matter in the wet LPC

Season	Herbage	Rest period (weeks)								SD	LC ± Se	Sig.
		4	5	6	7	8	9	10	12			
Spring '76	RG	50.5	49.8	45.6	43.6	42.0	-	-	-	3.1	-2.3 ± 0.7	*
	WC	-	52.4	56.0	54.0	54.4	-	-	-	6.1	0.4 ± 1.0	NS
	RG/WC	53.7	51.4	49.6	51.1	47.2	-	-	-	1.4	-1.3 ± 0.2	***
Summer '77	RG	48.6	41.0	38.9	33.4	32.2	-	-	-	5.1	-4.0 ± 1.1	*
	WC	46.0	40.0	48.0	46.0	33.0	-	-	-	11.6	-1.9 ± 2.6	NS
	RG/WC	45.3	48.6	49.3	46.5	44.0	-	-	-	1.4	-0.5 ± 0.3	NS[a]
	L	58.8	51.6	54.0	56.7	56.7	-	-	-	4.6	0.1 ± 1.0	NS
Autumn '77	RG	-	54.1	50.5	49.3	49.9	50.8	49.7	-	1.7	-0.6 ± 0.3	+
	RG/WC	-	-	57.1	-	55.6	-	56.8	59.1	0.9	0.4 ± 0.1	*[b]
	L	43.0	38.0	42.0	48.0	-	-	-	-	11.1	2.0 ± 3.5	NS
Spring '77	RG/WC	58.8	53.6	55.4	55.7	53.5	54.9	53.5	-	1.4	-0.5 ± 0.2	*
	L	-	61.4	59.6	61.9	60.8	56.5	-	-	2.1	-0.9 ± 0.5	NS

a: Significant quadratic term of -1.1 ± 0.3 (P<0.01)

b: Significant quadratic term of 1.0 ± 0.3 (P<0.05)

There was a significant effect due to herbage type in spring '76 (P<0.01), summer '77 (P<0.001), autumn '77 (P<0.001) and spring '77 (P<0.01).

APPENDIX 10: Statistical analysis of the rat growth results.

Introduction

Hegsted and Worcester (1967) recommended that the dose response data be analysed with concurrent regression lines (model (iii) below). Subsequently the National Research Council - National Academy Sciences committee recommended that individual regressions (model (i) below) be fitted to the data for individual test proteins excluding protein-free group results (NRC, In press). To determine the most appropriate regression model for this study the results for body water gain and protein intake from the first two rat experiments was fitted to each of four mathematical models:

$$(i) \qquad Y_{ij} = c_i + b_i X_{ij}$$

$$(ii) \qquad Y_{ij} = c_i + b X_{ij}$$

$$(iii) \qquad Y_{ij} = c + b_i X_{ij}$$

$$(iv) \qquad Y_{ij} = c + b X_{ij}$$

where Y_{ij} = body water gain and i = protein source

X_{ij} = protein intake j = individual observation

The models were compared using the regression residual sums of squares (RSS). Model (iv) - model (i) tested the appropriateness of individual regressions over a common regression for all protein sources. Model (iii) - Model (i) tested the appropriateness of different intercepts over a common intercept given different coefficients and model (ii) - model (i) tested different coefficients given different intercepts.

The above regression analyses were first carried out on the results excluding the data from the protein-free group which has been shown to inflate intercept values (NRC, In press).

Results

(a) Rat Experiment One.

The regression RSS (Table 1, App. 10) indicated that the data were best described with individual coefficients for different protein sources, but that there was no advantage in having different intercepts given different coefficients. Group mean results and the lines described by regression model (i), excluding the protein-free results, are presented in Figure 1 (App. 10). The body-water loss by the protein-free group is shown and is similar to the intercept values for all test proteins. Hence inclusion of the results from this group in the regression analysis would not distort intercept values.

Regression model (iii) has two major advantages over model (i). Firstly, a common intercept is fitted and thus all variation between test proteins in growth efficiency is channelled into one parameter (the regression coefficient). Secondly, as can be seen in Table 2 (App. 10) the standard errors for the regression coefficients are larger for model (i) without protein-free results, than for model (iii). (This was the case for model (i) with protein-free results also). The importance of this is seen in Table 3 (App. 10) where differences between regression coefficients for protein sources are tested. Model (iii) (and model (i) when calculated with protein-free results) gives a higher number of significant differences than model (i).

(b) Rat Experiment Two.

The leaf protein concentrates were fed with supplemental methionine and the range in protein quality was much smaller compared with RE One (see Figure 2, App. 10). There was a significant reduction in the RSS from fitting individual regressions

(model (i)), compared with a common regression (model (iv)), for all data (Table 4, App. 10). The RSS did not differ significantly between models (i), (ii) and (iii). However, of models (ii) and (iii), the latter, which fits a common intercept and individual coefficients for protein sources, yielded the lowest RSS. The standard errors of the regression coefficients are larger for model (i) (calculated both with and without protein-free data) than model (iii). This is reflected in the tests of differences between regression coefficients (Table 3, App. 10). For model (i) excluding protein-free data, no differences were significant. One difference attained significance when this model included the protein-free results. For model (iii) however, four differences attained significance.

Conclusions

Regression model (iii) was chosen in preference to model (i) for analysing the data and assessing protein quality differences in experiments reported in this thesis. This was because it channelled all of the between protein source differences in protein quality into one parameter (the regression coefficient), and had sufficiently low standard errors to facilitate better discrimination between proteins.

APPENDIX 10, TABLE 1: Residual sums of squares (RSS) from regression analyses of the results from Rat Experiment One, (calculated excluding results from the protein-free group).

Regression model	RSS	DF	MS	F
(i) $Y_{ij} = c_i + b_i X_{ij}$	217.378	96	2.264	
(ii) $Y_{ij} = c_i + b X_{ij}$	909.869	101	9.009	
(iii) $Y_{ij} = c + b_i X_{ij}$	228.328	101	2.261	
(iv) $Y_{ij} = c + b X_{ij}$	3029.559	106	28.581	
Due to different regressions (iv-i)	2812.181	10	281.22	124.2
Due to different coefficients (ii-i)	692.491	5	138.498	61.17
Due to different intercepts (iii-i)	10.95	5	2.19	0.97

APPENDIX 10, TABLE 2: Regression models (i) and (iii) and protein values for Rat Experiment One.

Regression model	(i)			(iii)	
	b ± Se	C	RPV[1] ± Se	b ± Se	RNV[2] ± Se
Calculated *without* protein-free group results					
Lact.	2.57 ± 0.10	-7.4	1.00	2.52 ± 0.04	1.00
LPC-RG	1.28 ± 0.06	-6.2	0.50 ± 0.03	1.32 ± 0.06	0.52 ± 0.02
-WC	1.16 ± 0.07	-6.4	0.45 ± 0.06	1.18 ± 0.05	0.47 ± 0.02
-L	1.41 ± 0.07	-4.9	0.55 ± 0.07	1.58 ± 0.05	0.63 ± 0.02
-RG/WC	1.12 ± 0.12	-6.6	0.44 ± 0.05	1.12 ± 0.06	0.44 ± 0.02
				C = -6.6	
Calculated *with* protein-free group results					
			RNV ± Se		
Lact.	2.55 ± 0.06	-7.1	1.00	2.53 ± 0.03	1.00
LPC-RG	1.33 ± 0.04	-6.7	0.52 ± 0.02	1.33 ± 0.05	0.53 ± 0.02
-WC	1.20 ± 0.04	-6.8	0.47 ± 0.02	1.19 ± 0.05	0.47 ± 0.02
-L	1.56 ± 0.04	-6.4	0.61 ± 0.02	1.59 ± 0.05	0.63 ± 0.02
-RG/WC	1.15 ± 0.06	-6.9	0.45 ± 0.04	1.13 ± 0.06	0.45 ± 0.02
				C = -6.7	

1: RPV = Relative Protein Value; calculated as the ratio regression coefficient for test protein to regression coefficient for lactalbumin, where regression model (i) was calculated *without* protein-free data.

2: RNV = Relative Nutritive Value; calculated as for RPV where regression is model (iii) or model (i) calculated *with* protein-free results.

APPENDIX 10, TABLE 3: Differences (± Se) between protein sources in regression coefficients.

Regression model	(i)				(iii)			
Protein-free group data in regression	No		Yes		No		Yes	
RE ONE								
L-RG[a]	0.13	NS	0.23	***	0.26	***	0.26	***
L-WC	0.25	**	0.36	***	0.40	***	0.40	***
L-RG/WC	0.29	**	0.41	***	0.46	***	0.46	***
RG-WC	0.12	NS	0.13	*	0.14	*	0.14	*
RG-RG/WC	0.16	*	0.18	**	0.20	**	0.20	***
WC-RG/WC	0.04	NS	0.05	NS	0.06	NS	0.06	NS
Average Se	±0.07		±0.05		±0.05		±0.05	
RE TWO (LPC fed with methionine)								
L-RG[a]	0.12	NS	0.19	*	0.25	***	0.24	**
L-WC	0.18	NS	0.05	NS	0.02	NS	0.02	NS
L-RG/WC	0.25	NS	0.17	NS	0.18	**	0.17	*
RG-WC	0.06	NS	-0.14	NS	-0.22	**	-0.22	**
WC-RG/WC	0.07	NS	0.12	NS	0.15	*	0.15	*
RG-RG/WC	0.13	NS	-0.02	NS	0.07	NS	-0.07	NS
Average Se	±0.11		±0.08		±0.06		±0.06	

a: All LPC from summer '77 week 4 of regrowth.

APPENDIX 10, TABLE 4: Residual sums of squares (RSS) from regression analyses of the results from Rat Experiment Two.

Regression model	RSS	DF	MS	F
(i) $Y_{ij} = c_i + b_i X_{ij}$	406.202	96	4.231	
(ii) $Y_{ij} = c_i + b X_{ij}$	446.861	101	4.424	
(iii) $Y_{ij} = c + b_i X_{ij}$	430.708	101	4.264	
(iv) $Y_{ij} = c + b X_{ij}$	861.605	106	8.128	
Due to different regressions (iv-i)	455.403	10	45.540	10.76
Due to different coefficients (ii-i)	40.659	5	8.132	1.92
Due to different intercepts (iii-i)	24.506	5	4.90	1.16

APPENDIX 10, TABLE 5: Regression models (i) and (iii) and protein values for Rat Experiment Two.

Regression model	(i)			(iii)	
Calculated *without* protein-free results					
	b ± Se	C	RPV ± Se	b ± Se	RNV ± Se
Lact.	2.68 ± 0.13	-8.1	1.00	2.74 ± 0.06	1.00
LPC-RG	2.38 ± 0.09	-11.3	0.89 ± 0.05	2.19 ± 0.06	0.80 ± 0.02
-WC	2.32 ± 0.08	-7.6	0.86 ± 0.05	2.41 ± 0.05	0.88 ± 0.02
-L	2.50 ± 0.15	-9.8	0.93 ± 0.07	2.44 ± 0.06	0.89 ± 0.02
-RG/WC	2.25 ± 0.10	-8.9	0.84 ± 0.06	2.26 ± 0.06	0.82 ± 0.02
				C = -8.9	
Calculated *with* protein-free results					
			RNV ± Se		
Lact.	2.59 ± 0.09	-7.1	1.00	2.69 ± 0.06	1.00
LPC-RG	2.12 ± 0.08	-8.0	0.82 ± 0.04	2.14 ± 0.06	0.79 ± 0.02
-WC	2.26 ± 0.06	-6.9	0.87 ± 0.04	2.36 ± 0.05	0.88 ± 0.02
-L	2.31 ± 0.09	-7.3	0.89 ± 0.05	2.38 ± 0.06	0.89 ± 0.02
-RG/WC	2.14 ± 0.08	-7.3	0.83 ± 0.04	2.21 ± 0.06	0.82 ± 0.02
				C = -8.3	

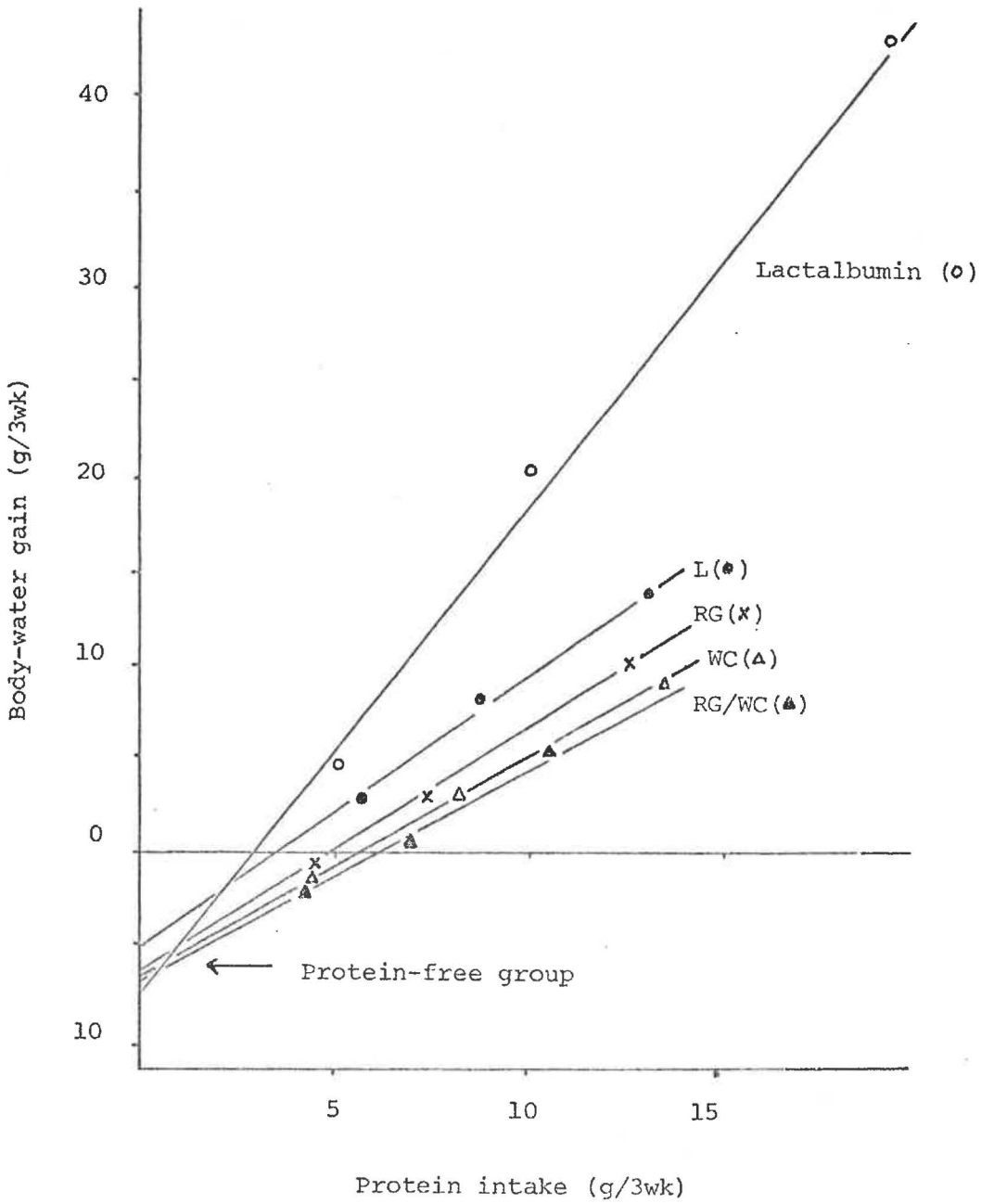

FIGURE 1: (App. 10). The relationships between body-water gain and protein intake from RE One.

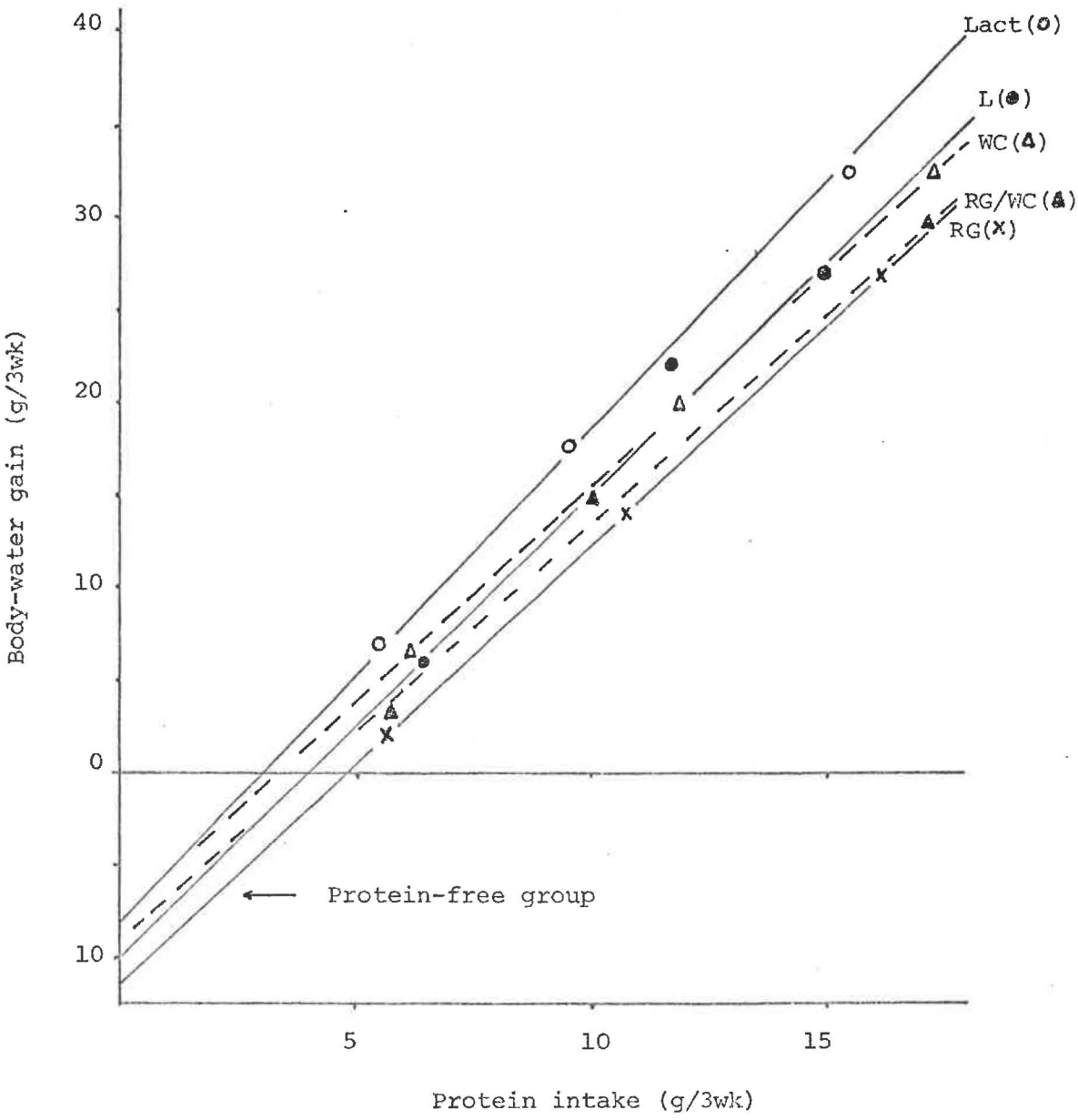

FIGURE 2: (App. 10). The relationships between body-water gain and protein intake from RE Two.

APPENDIX 11: Protein true digestibility.

Introduction

The true digestibility of a protein is assumed to be independent of the protein content of the diet, of the food intake and of the body weight of the experimental animal (Njaa, 1963).

True digestibilities calculated by the factorial method (F) are set out in Table 1 (App. 11) for RE One and Two. In RE One for some proteins there was a significant coefficient ($P<0.05$) relating PTD to protein intake. This was not the case in RE Two. For RE One then, the F method of estimating PTD is not independent of protein intake.

In calculating true digestibility by regression (R) the relationships between faecal protein and protein intake were analysed using regression models (i) and (iii) as given in Appendix 10. The results are presented in Table 3 (App. 11). For RE Two the RSS did not differ between the two models. However, for RE One there was a significant ($P<0.05$) reduction in the RSS with fitting individual intercepts (i.e. model (i)). This was largely due to the zero intercept value for lucerne (Table 3, App. 11). However, it is assumed when calculating the true digestibility of a protein, that endogenous faecal protein losses (estimates of which are given by the regression intercept value) do *not* equal zero and that the rate of loss is not influenced by protein source (Njaa, 1963). Hence the results from regression model (iii), ascribing a common intercept value to all protein sources, are used in subsequent discussion and interpretation.

The F true digestibility estimates are, on average, higher than the R estimates by 3.6 percentage units in RE One and 2.0 units in RE Two. This was consistent for all protein sources and

could have been because the estimate of endogenous faecal protein per unit dry matter intake obtained from the protein-free group was higher than actual endogenous faecal protein losses in the protein-fed groups. This contention is supported by the model (iii) regression intercepts (Table 3), which convert to 0.27 and 0.34 g/100g dry matter intake for RE One and Two, respectively. However, for the protein-free groups, the estimates were 0.53 (RE One) and 0.49 (RE Two) g/100g dry matter intake. These latter estimates ascribe a lower fraction of faecal protein to dietary origin and therefore result in higher true digestibility estimates. Njaa (1963) observed a similar bias when estimating the true digestibility of protein in herring meal. The results determined by regression analysis are considered better estimates as their determination did not directly involve the extreme nutritional insult of feeding a protein-free diet.

Despite the overall difference between F and R results, the between protein source differences within F and R results are consistent (Table 2, App. 11) with one exception only (WC-RG, RE One). This suggests results obtained by either method are satisfactory for comparing proteins. However, for the reason mentioned above, the R results are considered the best estimates and are used to compare herbage types.

APPENDIX 11, TABLE 1: Protein true digestibility results estimated by two procedures[a]

	Lactalbumin	LPC			
		RG	WC	RG/WC	L
RE One					
True digest. F% (means ± Se)	97.1±0.8	75.2±0.8	75.3±1.1	72.0±0.9	85.3±1.1
True digest. R% (1-b ± Se)	92.7±0.5	71.4±0.7	73.2±0.7	69.1±0.5	80.9±0.7
RE Two (LPC + methionine)					
True digest. F% (means ± Se)	95.6±1.8	70.0±2.0	76.3±1.1	72.5±1.3	84.4±1.3
True digest. R% (1-b ± Se)	93.3±0.9	68.3±0.8	73.3±0.8	70.8±0.8	82.8±0.9

a: for RE One and Two group, mean data for faecal dry matter production, faecal protein content, faecal protein production and true digestibilities F are tabulated in Appendix 13, Tables 3 and 4.

APPENDIX 11, TABLE 2: Differences between herbage sources in LPC protein true digestibility (PTD)

	Rat Experiment One		Rat Experiment Two	
	PTD-F	PTD-R	PTD-F	PTD-R
Difference				
L-RG	10.1 ± 0.8***	9.5 ± 0.7***	14.4 ± 1.2***	14.2 ± 0.8***
L-WC	10.0 ± 0.8***	7.6 ± 0.7***	8.1 ± 1.2***	9.3 ± 0.8***
L-RG/WC	13.3 ± 0.8***	11.8 ± 0.8***	11.9 ± 1.2***	11.8 ± 0.8***
WC-RG	0.1 ± 0.8 NS	1.9 ± 0.7*	6.3 ± 1.2***	5.0 ± 0.8***
WC-RG/WC	3.2 ± 0.8**	4.2 ± 0.8***	3.8 ± 1.2**	2.5 ± 0.8**
RG/WC-RG	-3.2 ± 0.8**	-2.3 ± 0.8*	2.4 ± 1.2+	2.5 ± 0.8**

APPENDIX 11, TABLE 3: Parameters from the regressions of faecal protein (g/3 wk) on protein intake (g/3 wk)

	Rat Experiment One				Rat Experiment Two (LPC + Met.)			
	Model (iii)	Model (i)			Model (iii)	Model (i)		
	b±Se	b±Se	C	r	b±Se	b±Se	C	r
Lactalbumin	0.073 ± 0.005 (92.7)[a]	0.059 ± 0.005 (94.1)	0.58	0.95	0.067 ± 0.009 (93.3)	0.059 ± 0.02 (94.1)	0.68	0.60
LPC-RG	0.286 ± 0.007 (71.4)	0.303 ± 0.008 (69.7)	0.21	0.99	0.317 ± 0.008 (68.3)	0.313 ± 0.019 (68.7)	0.63	0.97
-WC	0.268 ± 0.007 (73.2)	0.252 ± 0.014 (74.8)	0.54	0.98	0.267 ± 0.008 (73.3)	0.282 ± 0.014 (71.8)	0.38	0.98
-RG/WC	0.309 ± 0.005 (69.1)	0.313 ± 0.014 (68.7)	0.34	0.98	0.292 ± 0.008 (70.8)	0.286 ± 0.013 (71.4)	0.66	0.98
-L	0.191 ± 0.007 (80.9)	0.227 ± 0.018 (77.3)	0	0.95	0.174 ± 0.009 (82.6)	0.171 ± 0.018 (82.9)	0.63	0.92
Intercept value	0.370				0.583			
RSD	0.195				0.295			

a: percentage digestibility calculated as (1-b)x100.

APPENDIX 12: The amino acid composition of LPC.

TABLE 1: Ryegrass (spring '76)

Regrowth week	4		6		8	
Analytical duplicate	a	b	a	b	a	b
Amino acid (g/16g N)						
Lys.	5.4	5.4	5.8	5.2	6.2	6.4
His.	2.1	2.4	2.6	2.3	2.5	2.7
Arg.	6.5	6.3	6.6	6.4	6.5	6.9
Asp.	7.7	7.7	7.8	7.3	8.0	7.7
Thr.	4.7	4.8	4.7	4.4	4.9	5.5
Ser.	4.6	4.5	4.6	4.3	5.4	5.7
Glu.	10.3	9.9	9.4	9.2	10.9	11.5
Gly.	4.9	4.9	5.0	4.6	5.2	5.8
Ala.	5.7	5.6	5.9	5.4	6.5	6.7
Val.	5.3	5.0	5.1	4.6	5.5	5.9
Met.	2.1	2.1	2.5	2.1	2.7	2.6
Ileu.	4.4	4.5	4.6	4.3	5.0	5.0
Leu.	7.7	7.5	8.0	7.4	8.3	8.9
Tyr.	4.4	4.5	4.0	4.1	4.7	5.0
Phe.	5.8	5.5	5.4	5.4	6.3	6.4
Pro.	4.4	4.4	4.4	3.9	4.3	4.2
Try.	2.5	2.5	2.5	2.5	2.5	2.5
Avail. lys.	4.7	4.7	5.2	4.6	5.7	5.9
Lys. avail.%	87	87	90	88	92	92

APPENDIX 12: The amino acid composition of LPC.

TABLE 2: White clover (spring '76)

Regrowth week	4		6		8	
Analytical duplicate	a	b	a	b	a	b
Amino acid (g/16g N)						
Lys.	6.0	5.6	6.0	6.1	6.2	6.1
His.	2.9	2.6	2.4	2.8	2.9	2.7
Arg.	6.5	6.4	6.4	6.5	6.6	6.4
Asp.	8.8	9.1	8.9	8.9	9.3	9.2
Thr.	5.8	5.3	5.1	5.4	5.3	5.4
Ser.	5.6	5.0	4.7	5.1	5.1	5.2
Glu.	10.3	11.0	9.7	10.1	10.7	10.5
Gly.	5.7	4.9	4.6	5.2	5.2	5.2
Ala.	5.6	5.7	5.2	5.3	5.8	5.6
Val.	6.2	5.8	5.7	5.7	5.6	5.7
Met.	2.1	2.2	2.1	2.0	2.1	2.1
Ileu.	4.9	4.2	4.4	4.9	4.8	5.0
Leu.	8.6	8.8	8.8	8.6	8.4	8.6
Tyr.	4.3	4.3	4.7	5.1	5.3	5.0
Phe.	5.7	5.5	5.4	5.8	6.0	5.9
Pro.	4.7	4.4	4.2	4.5	4.3	4.4
Try.	2.3	2.3	2.6	2.6	2.5	2.6
Avail. lys.	5.4	5.0	5.5	5.5	5.6	5.5
Lys. avail.%	90	89	92	90	90	90

APPENDIX 12: The amino acid composition of LPC

TABLE 3: Ryegrass/white clover (spring '76)

Regrowth week	4		6		8	
Analytical duplicate	a	b	a	b	a	b
Amino acid (g/16g N)						
Lys.	6.0	5.4	5.3	5.0	5.5	5.2
His.	1.9	1.9	1.8	1.8	1.9	2.0
Arg.	5.6	5.5	5.8	6.0	5.8	5.4
Asp.	8.2	8.0	8.7	8.4	8.7	8.6
Thr.	5.2	4.6	5.0	4.9	4.8	4.7
Ser.	4.8	5.0	5.5	4.8	5.4	5.1
Glu.	10.7	10.3	10.8	10.2	10.2	10.2
Gly.	5.1	4.7	4.9	5.0	4.7	4.6
Ala.	5.8	5.2	5.9	5.7	5.6	5.0
Val.	5.7	5.2	5.5	5.2	5.1	4.8
Met.	2.1	2.2	2.3	2.3	2.5	2.3
Ileu.	4.8	4.2	4.8	4.7	4.5	4.4
Leu.	8.3	7.6	8.1	7.9	7.5	7.4
Tyr.	4.3	4.1	4.2	4.3	4.1	3.8
Phe.	5.9	5.2	5.5	5.3	5.1	4.8
Pro.	4.7	4.3	4.3	4.3	4.2	4.1
Try.	2.6	2.6	2.5	2.5	2.3	2.3
Avail. lys.	5.3	4.7	4.5	4.2	4.6	4.4
Lys. avail.%	88	87	85	84	84	85

APPENDIX 12: The amino acid composition of LPC

TABLE 4: Lucerne (summer '77)

Regrowth week	4		6		8	
Analytical duplicate	a	b	a	b	a	b
Amino acid (g/16g N)						
Lys.	6.3	5.8	6.2	5.6	6.2	6.3
His.	2.7	2.3	2.8	2.4	2.4	2.7
Arg.	6.2	5.6	6.5	5.9	6.1	6.4
Asp.	8.8	9.0	9.6	9.3	9.1	9.1
Thr.	5.1	4.6	5.5	5.0	4.8	5.2
Ser.	4.8	4.2	5.2	4.7	5.1	5.2
Glu.	9.7	9.3	9.7	9.6	9.8	9.9
Gly.	4.9	4.4	5.3	4.6	4.7	5.1
Ala.	5.5	4.8	6.1	5.1	5.2	5.5
Val.	5.5	5.0	5.9	5.2	5.2	5.5
Met.	2.2	1.9	2.3	2.1	2.0	2.1
Ileu.	4.9	4.3	5.1	4.2	4.5	4.8
Leu.	8.3	8.2	8.5	8.4	8.3	8.4
Tyr.	4.7	4.6	5.0	4.6	4.6	4.9
Phe.	5.9	5.7	5.1	5.8	5.7	5.8
Pro.	3.8	3.9	4.1	3.7	3.5	3.9
Try.	2.4	2.4	2.5	2.5	2.4	2.4
Avail. lys.	5.9	5.3	5.7	5.1	5.6	5.7
Lys. avail.%	94	92	92	91	90	90

APPENDIX 12: Amino acid composition of LPC

TABLE 5: Means for all LPC averaged by regrowth weeks

Amino acid (g/16g N)	Regrowth week 4	6	8	SEM[a]
Lys.	5.7	5.7	6.0	0.1
His.	2.4	2.4	2.5	0.1
Arg.	6.1	6.3	6.3	0.2
Asp.	8.4	8.6	8.7	0.1
Thre.	5.0	5.0	5.1	0.1
Ser.	4.8	4.9	5.3	0.2
Glu.	10.2	9.8	10.5	0.3
Gly.	4.9	4.9	5.1	0.1
Ala.	5.5	5.6	5.7	0.2
Val.	5.5	5.4	5.4	0.2
Met.	2.1	2.2	2.3	0.1
Ileu.	4.5	4.6	4.8	0.1
Leu.	8.1	8.2	8.2	0.2
Tyr.	4.4	4.5	4.7	0.2
Phel.	5.7	5.6	5.8	0.2
Prol.	4.3	4.2	4.1	0.04
Try.	2.5	2.6	2.5	0.2
Avail. lys.	5.1	5.0	5.4	0.2
Lys. avail.%	89	89	89	1

a: SEM = standard error of mean

APPENDIX 13, TABLE 1: Performance data (± SD) from Rat Experiment One

Protein Source	Diet protein %	Food intakes[a] (g/3 wk)	Protein intake (g/3 wk)	Body water gain (g/3 wk)
Lactalbumin	3.67	137.3 ± 17.1	5.0 ± 0.6	4.3 ± 1.2
	5.62	178.2 ± 20.7	10.0 ± 1.2	20.0 ± 2.7
	9.14	214.5 ± 14.5	19.6 ± 1.3	42.7 ± 3.8
LPC[b] - RG	4.40	100.2 ± 11.6	4.4 ± 0.5	-0.4 ± 1.0
	6.64	110.9 ± 13.5	7.4 ± 0.9	2.8 ± 1.4
	8.82	143.4 ± 19.3	12.6 ± 1.7	10.1 ± 2.2
- WC	4.54	94.0 ± 8.4	4.3 ± 0.4	-1.3 ± 1.6
	7.04	115.6 ± 9.7	8.1 ± 0.7	2.9 ± 0.8
	9.15	147.4 ± 18.5	13.5 ± 1.7	9.2 ± 3.1
- L	4.79	117.1 ± 17.8	5.6 ± 0.8	2.7 ± 1.5
	7.04	125.5 ± 12.3	8.8 ± 0.9	8.0 ± 1.2
	9.30	142.4 ± 16.1	13.2 ± 1.5	13.6 ± 2.3
- RG/WC	4.51	94.4 ± 11.0	4.3 ± 0.5	-1.8 ± 1.3
	6.40	107.5 ± 16.5	6.9 ± 1.1	0.5 ± 1.3
	8.41	122.2 ± 9.4	10.3 ± 1.0	5.2 ± 1.7
Protein-free diet	0.42	79.3 ± 8.0	0.3 ± 0.0	-7.0 ± 1.0

Average initial liveweight of all rats 42.0 g (range = 34.6 to 50.0).

The regression of body water (g,Y) on liveweight (g,X) for the initial slaughter group was $Y = 0.62(\pm 0.02)X + 2.4(\pm 0.8)$; $r = 0.995$; $n = 12$; RCV = 1.7%.

a: On a dry matter basis

b: All LPC from herbage harvested in the summer '77 season in the fourth week of regrowth.

APPENDIX 13, TABLE 2: Performance data (± SD) from Rat Experiment Two

Protein Source	Diet protein %	Food intake (g/3 wk)	Protein intake (g/3 wk)	Body water gain (g/3 wk)
Lactalbumin	3.54	156.8 ± 18.6	5.4 ± 0.6	6.9 ± 1.6
	5.58	174.4 ± 25.6	9.5 ± 1.4	17.6 ± 5.4
	8.55	184.8 ± 31.2	15.5 ± 2.6	32.7 ± 9.4
LPC[a] - RG+M[b]	4.22	131.4 ± 22.0	5.6 ± 0.9	2.1 ± 2.7
	6.31	169.9 ± 24.3	10.7 ± 1.5	14.1 ± 5.0
	8.03	201.4 ± 12.6	16.2 ± 1.0	27.1 ± 3.2
- WC+M	4.22	147.7 ± 16.6	6.2 ± 0.7	6.4 ± 1.4
	6.28	188.0 ± 15.6	11.8 ± 1.0	20.0 ± 1.5
	8.13	212.6 ± 29.3	17.3 ± 2.4	32.6 ± 4.7
- L+M	4.38	143.7 ± 8.6	6.3 ± 0.4	6.0 ± 1.6
	6.50	179.7 ± 18.5	11.7 ± 1.2	20.2 ± 1.4
	8.47	176.1 ± 20.0	14.9 ± 1.7	26.9 ± 7.2
- RG/WC+M	4.22	134.5 ± 20.1	5.7 ± 0.8	3.2 ± 1.9
	6.00	166.3 ± 13.0	10.0 ± 0.8	14.8 ± 2.2
	8.13	211.6 ± 16.7	17.2 ± 1.4	29.6 ± 4.0
Protein-free diet	0.32	77.4 ± 8.8	0.2 ± 0.0	-6.6 ± 2.9

Average initial liveweight of all rats = 42.6 g (range = 34.2 to 50.8)

The regression of body water (g,Y) on liveweight (g,X) for the initial slaughter group was $Y = 0.58(\pm 0.01)X + 4.2(\pm 0.5)$; $r = 0.998$ $n = 11$; RCV = 1.2%.

a: LPC from herbage harvested in the summer '77 season in the fourth week of regrowth.

b: M indicates methionine supplementation.

APPENDIX 13, TABLE 3: Faecal dry matter and protein production (± SD) and estimates of true digestibility (F) of protein for Rat Experiment One.

	Diet protein %	Faecal dry matter (g/3 wk)	Faecal protein content (%)	Faecal protein (g/3 wk)	Protein true digestibility (%) (F)
Lactalbumin	3.67	6.3 ± 1.0	14.2 ± 1.5	0.9 ± 0.1	96.6 ± 3.0
	5.62	8.3 ± 0.9	14.0 ± 0.9	1.2 ± 0.1	97.8 ± 0.9
	9.14	10.1 ± 1.1	17.2 ± 0.4	1.7 ± 0.2	96.9 ± 0.9
LPC - RG	4.40	9.0 ± 1.0	17.5 ± 1.1	1.6 ± 0.2	76.4 ± 1.1
	6.64	12.2 ± 1.6	19.7 ± 0.6	2.4 ± 0.4	75.2 ± 1.2
	8.82	19.2 ± 2.6	21.1 ± 0.8	4.0 ± 0.6	74.1 ± 0.6
- WC	4.54	9.0 ± 0.7	18.3 ± 1.4	1.6 ± 0.2	73.2 ± 3.3
	7.04	14.0 ± 1.2	18.3 ± 1.2	2.6 ± 0.3	76.0 ± 2.5
	9.15	20.5 ± 2.6	19.0 ± 1.1	3.9 ± 0.6	76.8 ± 2.1
- L	4.79	8.3 ± 1.5	15.8 ± 1.6	1.3 ± 0.2	87.7 ± 2.7
	7.04	10.8 ± 1.3	18.5 ± 1.2	2.0 ± 0.4	84.9 ± 2.5
	9.30	14.7 ± 2.0	20.2 ± 1.6	3.2 ± 0.8	83.4 ± 2.8
- RG/WC	4.51	9.0 ± 1.1	18.5 ± 0.6	1.7 ± 0.2	72.5 ± 1.4
	6.40	13.0 ± 2.1	19.4 ± 1.5	2.5 ± 0.4	71.8 ± 3.5
	8.41	17.5 ± 1.5	20.3 ± 0.5	3.5 ± 0.3	71.8 ± 1.2
Protein-free diet	0	3.5 ± 0.5	11.8 ± 0.5	0.4 ± 0.1	-

Faecal protein excretion for the protein-free group was 0.53 ± 0.10 g/100g dry matter intake.

APPENDIX 13, TABLE 4: Faecal dry matter and protein production (± SD) and estimates of true digestibility (F) of protein for Rat Experiment Two.

	Diet protein %	Faecal dry matter (g/3 wk)	Faecal protein content %	Faecal protein (g/3 wk)	Protein true digestibility (%) (F)
Lactalbumin	3.54	7.0 ± 1.2	12.6 ± 1.4	0.9 ± 0.2	97.6 ± 2.6
	5.58	8.1 ± 1.0	15.2 ± 3.1	1.2 ± 0.3	95.4 ± 5.3
	8.55	9.3 ± 0.6	18.6 ± 3.9	1.7 ± 0.4	93.9 ± 4.9
LPC - RG+M	4.22	12.2 ± 1.6	18.3 ± 2.4	2.2 ± 0.4	71.0 ± 5.8
	6.31	20.1 ± 2.1	20.9 ± 1.7	4.2 ± 0.4	68.0 ± 5.9
	8.03	28.3 ± 1.8	19.9 ± 0.5	5.6 ± 0.4	71.1 ± 1.4
- WC+M	4.22	13.3 ± 1.5	15.6 ± 0.7	2.1 ± 0.3	77.9 ± 3.6
	6.28	21.8 ± 2.0	17.4 ± 1.0	3.8 ± 0.4	75.6 ± 1.4
	8.13	29.7 ± 3.8	17.6 ± 0.6	5.2 ± 0.7	75.5 ± 2.9
- L+M	4.38	10.4 ± 0.9	16.1 ± 2.0	1.7 ± 0.3	84.1 ± 4.5
	6.50	15.4 ± 1.7	16.4 ± 1.2	2.5 ± 0.3	85.7 ± 1.5
	8.47	17.4 ± 1.3	19.0 ± 1.8	3.3 ± 0.2	83.4 ± 3.0
- RG/WC+M	4.22	12.4 ± 2.2	17.9 ± 1.5	2.2 ± 0.5	72.3 ± 5.4
	6.00	19.7 ± 2.3	18.7 ± 0.6	3.7 ± 0.4	71.3 ± 1.6
	8.13	29.5 ± 2.7	18.6 ± 0.4	5.5 ± 0.6	74.0 ± 1.0
Protein-free diet	0.32	3.2 ± 0.6	11.6 ± 1.5	0.4 ± 0.1	-

Faecal protein excretion by the protein-free group was 0.49 ± 0.12 g/100g dry matter intake.

APPENDIX 13, TABLE 5: Performance data (± SD) from Rat Experiment Three.

Protein Source	Diet protein %	Food intake (g/3 wk)	Protein intake (g/3 wk)	Body water gain (g/3 wk)
Lactalbumin	2.25	99.4 ± 20.8	2.2 ± 0.5	-2.6 ± 0.7
	5.44	140.6 ± 17.6	7.6 ± 1.0	9.6 ± 2.2
	8.31	184.4 ± 36.6	15.3 ± 3.0	29.5 ± 5.7
LPC - RG/WC -4[a]	3.60	77.6 ± 13.8	2.8 ± 0.5	-4.5 ± 1.9
	6.44	98.0 ± 9.4	6.4 ± 0.6	0.9 ± 0.8
	9.50	104.7 ± 19.7	10.0 ± 1.9	2.6 ± 3.7
- RG/WC -6	4.31	86.3 ± 13.3	3.7 ± 0.6	-3.0 ± 1.7
	6.62	94.0 ± 9.5	6.2 ± 0.6	0.7 ± 1.6
	9.50	105.4 ± 17.0	10.0 ± 1.6	3.4 ± 3.0
- RG/WC -8	4.38	84.3 ± 6.8	3.7 ± 0.3	-2.8 ± 1.3
	6.94	92.7 ± 20.0	6.4 ± 1.4	-0.1 ± 2.5
	9.56	110.2 ± 16.7	10.5 ± 1.6	5.1 ± 3.8
- RG/WC + M[b] -4	3.50	98.1 ± 22.9	3.4 ± 0.8	-1.7 ± 2.1
	5.50	136.5 ± 8.1	7.5 ± 0.4	6.9 ± 1.9
	8.88	178.5 ± 19.8	15.8 ± 1.8	22.7 ± 4.6
- RG/WC + M -6	3.94	95.2 ± 10.8	3.8 ± 0.4	-0.8 ± 0.9
	5.81	141.4 ± 17.4	8.2 ± 1.0	7.9 ± 1.2
	9.06	152.2 ± 37.0	13.8 ± 3.4	17.4 ± 8.0
- RG/WC + M -8	3.88	92.2 ± 14.9	3.6 ± 0.6	-2.3 ± 2.2
	5.94	127.8 ± 15.6	7.6 ± 0.9	5.0 ± 1.2
	8.56	167.8 ± 24.5	14.4 ± 2.1	20.2 ± 4.1
Protein-free diet	0.37	71.2 ± 7.3	0.3 ± 0.0	-9.7 ± 0.9

Average initial liveweight of all rats = 42.7g (range = 34.1 to 49.5)

The regression of body water (g,Y) on liveweight (g,X) for the initial slaughter group was: $Y = 0.59(\pm 0.02)X + 3.3(\pm 0.8)$; $r = 0.995$; $n = 12$; RCV = 1.7%.

a: 4, 6 or 8 - indicates week of regrowth when harvested; all LPC from summer '77 season.

b: M - indicates methionine supplementation.

APPENDIX 13, TABLE 6: Performance data (± SD) from Rat Experiment Four.

Protein Source	Diet protein %	Food intake (g/3 wk)	Protein intake (g/3 wk)	Body water gain (g/3 wk)
Lactalbumin	2.44	115.4 ± 16.5	2.8 ± 0.4	-0.3 ± 1.4
	5.75	159.6 ± 17.2	9.2 ± 1.0	15.7 ± 3.7
	8.81	169.1 ± 28.2	14.9 ± 2.5	30.8 ± 6.5
LPC - L(4)[a]	4.69	97.8 ± 20.3	4.6 ± 1.0	-0.6 ± 2.6
	7.44	120.0 ± 23.0	8.9 ± 1.7	7.7 ± 3.1
	9.94	131.6 ± 14.4	13.1 ± 1.4	12.8 ± 3.4
- L(6)	4.63	103.2 ± 10.1	4.8 ± 0.5	1.6 ± 0.6
	7.38	129.9 ± 14.7	9.6 ± 1.1	9.0 ± 2.1
	10.19	156.9 ± 16.9	16.0 ± 1.7	18.0 ± 2.3
- L(8)	4.81	106.4 ± 10.0	5.1 ± 0.5	1.2 ± 2.1
	7.50	106.8 ± 19.0	8.0 ± 1.4	4.8 ± 4.6
	10.56	146.7 ± 22.0	15.5 ± 2.3	15.2 ± 6.0
LPC - L(4) + M[b]	3.50	167.0 ± 23.3	3.7 ± 0.8	-0.02± 2.1
	5.75	144.8 ± 33.6	8.3 ± 1.9	10.5 ± 5.1
	8.88	157.4 ± 27.4	14.0 ± 2.4	24.7 ± 5.8
- L(6) + M	3.56	115.2 ± 8.2	4.1 ± 0.3	1.0 ± 1.1
	5.75	143.8 ± 21.9	8.3 ± 1.3	10.3 ± 3.7
	9.44	179.1 ± 44.1	16.9 ± 4.2	27.2 ±10.8
- L(8) + M	3.56	121.7 ± 22.0	4.3 ± 0.8	1.5 ± 2.1
	5.94	166.1 ± 32.3	9.9 ± 1.9	14.2 ± 4.2
	9.38	171.6 ± 37.0	16.1 ± 3.5	30.8 ± 5.0
Protein-free diet	0.56	70.8 ± 5.5	0.4 ± 0.0	-6.7 ± 1.9

Average initial liveweight of all rats = 41.5g (range 32.1 to 53.5).

The regression of body water (g,Y) on liveweight (g,X) for the initial slaughter group was Y = 0.59(±0.02)X + 3.26(±0.76); r = 0.995; n = 12, RCV = 1.7%.

a: 4, 6 or 8 - indicates week of regrowth when harvested; all LPC from summer '77 season.

b: +M - indicates methionine supplementation.

APPENDIX 13, TABLE 7: Performance data (± SD) from Rat Experiment Five.

Protein source	Diet protein %	Food intake (g/3 wk)	Protein intake (g/3 wk)	Body water gain (g/3 wk)
Lactalbumin	3.38	103.8 ± 12.0	3.5 ± 0.4	1.1 ± 1.2
	6.38	153.7 ± 22.0	9.8 ± 1.4	17.0 ± 3.3
	10.31	173.5 ± 24.8	17.9 ± 2.6	32.1 ± 5.8
LPC - RG/WC(A)[a] + M	3.81	103.0 ± 14.6	3.9 ± 0.6	-0.1 ± 1.0
	6.00	135.3 ± 14.2	8.1 ± 0.9	7.2 ± 1.7
	8.75	167.3 ± 19.5	14.6 ± 1.7	22.3 ± 4.6
- RC/WC(S)[b] + M	3.69	108.8 ± 12.6	4.0 ± 0.5	0.4 ± 1.4
	5.88	133.8 ± 32.8	7.9 ± 1.9	8.7 ± 3.1
	9.38	183.1 ± 10.3	17.2 ± 1.0	26.8 ± 2.9
- L(A) + M	3.63	108.6 ± 10.2	3.9 ± 0.4	-0.7 ± 1.1
	5.63	153.0 ± 15.1	8.6 ± 0.8	9.5 ± 1.7
	8.81	177.2 ± 17.6	15.6 ± 1.6	23.4 ± 5.4
- L(S) + M	3.88	88.2 ± 11.5	3.4 ± 0.4	-3.0 ± 2.8
	6.00	104.7 ± 16.8	6.3 ± 1.0	3.6 ± 3.9
	9.94	131.6 ± 16.2	13.1 ± 1.6	16.4 ± 4.8
Protein-free diet	0.31	57.8 ± 8.2	0.2 ± 0.0	-6.1 ± 0.7

Average initial liveweight of all rats = 40.4g (range 35.0 to 47.7).

The regression of body water (g,Y) on liveweight (g,X) for the initial slaughter group was $Y = 0.58(\pm 0.01)X + 3.80(\pm 0.44)$
$r = 0.999$; $n = 8$; RCV = 0.53%.

a:A indicates LPC from herbage harvested in the autumn

b:S indicates LPC from herbage harvested in the spring

APPENDIX 13, TABLE 8: Performance data (± SD) from Rat Experiment Six.

Protein source	Diet protein %	Food intake (g/3 wk)	Protein intake (g/3 wk)	Body water gain (g/3 wk)
Lactalbumin	3.19	113.7 ± 19.3	3.6 ± 0.6	-0.9 ± 2.4
	6.44	147.2 ± 15.1	9.5 ± 1.0	13.3 ± 4.2
	9.00	175.7 ± 12.3	15.8 ± 1.1	31.3 ± 3.8
LPC - L	4.91	87.6 ± 15.9	4.3 ± 0.8	-3.6 ± 2.9
	7.69	96.7 ± 30.1	8.0 ± 1.8	3.3 ± 5.1
	10.63	103.5 ± 16.0	11.0 ± 1.7	7.0 ± 5.6
- L.MBS	3.81	92.8 ± 23.2	3.5 ± 0.9	-3.9 ± 3.6
	6.69	114.9 ± 7.9	7.7 ± 0.5	5.7 ± 3.3
	9.50	129.8 ± 27.8	12.3 ± 2.6	13.3 ± 6.2
- RG/WC	4.85	85.8 ± 10.9	4.2 ± 0.5	-2.2 ± 1.9
	7.53	101.6 ± 14.0	7.6 ± 1.1	2.3 ± 2.3
	10.16	117.5 ± 19.9	11.9 ± 2.0	8.5 ± 4.4
- RG/WC.MBS	3.56	96.2 ± 12.4	3.4 ± 0.4	-1.5 ± 1.0
	6.31	123.7 ± 28.1	7.8 ± 1.8	6.8 ± 5.0
	9.00	134.7 ± 35.8	12.1 ± 3.2	15.1 ± 6.4
Protein-free diet	0.36	71.1 ± 7.6	0.3 ± 0.0	-8.2 ± 1.6

Average initial liveweight of all rats = 40.5g (range 35.4 to 45.8)

The regression of body water (g,Y) on liveweight (g,X) for the initial slaughter groups was $Y = 8.62(\pm 0.01)X + 2.84(\pm 0.49)$; $r = 0.999$; $n = 10$; RCV = 0.33%

APPENDIX 13, TABLE 9: Faecal dry matter and crude protein production (± SD) from Rat Experiment Six.

Protein source	Diet protein %	Faecal dry matter g	Faecal protein content (%)	Faecal protein g
Lactalbumin	3.19	5.3 ± 0.6	17.43 ± 3.64	1.0 ± 0.2
	6.44	7.5 ± 0.8	19.39 ± 2.60	1.5 ± 0.3
	9.00	8.6 ± 1.2	21.07 ± 5.00	1.8 ± 0.6
LPC - L	4.91	5.8 ± 0.4	22.72 ± 2.56	1.3 ± 0.1
	7.69	8.4 ± 1.8	24.01 ± 2.02	2.0 ± 0.4
	10.63	10.9 ± 1.6	27.18 ± 2.59	3.0 ± 0.5
- L.MBS	3.81	5.9 ± 1.4	21.25 ± 3.05	1.2 ± 0.2
	6.69	8.7 ± 0.7	21.85 ± 3.05	1.9 ± 0.4
	9.50	11.5 ± 2.4	24.94 ± 2.01	2.8 ± 0.4
- RG/WC	4.85	6.2 ± 0.6	22.45 ± 1.74	1.4 ± 0.1
	7.53	9.2 ± 1.1	25.67 ± 1.23	2.4 ± 0.3
	10.16	13.2 ± 2.2	28.52 ± 1.80	3.8 ± 0.8
- RG/WC.MBS	3.56	6.0 ± 0.8	19.30 ± 1.64	1.2 ± 0.1
	6.31	9.6 ± 2.3	22.53 ± 1.38	2.1 ± 0.4
	9.00	14.0 ± 1.5	24.83 ± 2.09	3.5 ± 0.5
Protein-free diet	0.36	3.2 ± 0.3	14.21 ± 3.50	0.5 ± 0.1

APPENDIX 13, TABLE 10: Performance Data (± SD) from Rat Experiment Seven.

Protein Source	Diet protein %	Food intake (g/3 wk)	Protein intake (g/3 wk)	Body water gain (g/3 wk)
Lactalbumin	3.06	116.7 ± 6.3	3.6 ± 0.2	-0.6 ± 1.3
	6.31	145.0 ± 23.1	9.9 ± 1.4	14.9 ± 5.7
	9.00	185.2 ± 42.4	16.7 ± 3.8	36.6 ±11.6
LPC - WC	4.69	91.3 ± 8.1	4.3 ± 0.4	-1.1 ± 0.5
	7.50	101.2 ± 14.5	7.6 ± 1.1	2.4 ± 2.3
	10.31	128.0 ± 15.7	13.2 ± 1.6	11.4 ± 2.0
- WC.MBS	3.75	87.8 ± 12.7	3.3 ± 0.5	-1.9 ± 1.7
	6.50	116.0 ± 29.1	7.5 ± 1.9	4.6 ± 4.4
	9.94	131.2 ± 26.6	13.0 ± 2.6	12.2 ± 5.3
- RG	4.44	85.9 ± 12.9	3.8 ± 0.6	-2.4 ± 1.5
	7.13	104.4 ± 20.9	7.4 ± 1.5	2.4 ± 2.5
	9.38	148.1 ± 27.9	13.9 ± 2.6	13.8 ± 6.6
- RG.MBS	3.50	88.7 ± 15.6	3.1 ± 0.6	-2.9 ± 2.2
	6.06	116.1 ± 17.3	7.0 ± 1.1	4.4 ± 2.7
	8.63	163.1 ± 21.7	14.1 ± 1.9	18.5 ± 5.8
- RG/WC	4.56	87.9 ± 9.1	4.0 ± 0.4	-2.1 ± 0.6
	7.31	108.4 ± 13.2	7.9 ± 1.0	3.8 ± 1.6
	9.81	133.8 ± 19.9	13.1 ± 2.0	11.0 ± 3.4
- RG/WC.MBS	3.44	98.8 ± 15.4	3.4 ± 0.5	-2.4 ± 2.8
	6.00	137.5 ± 15.0	8.2 ± 0.9	8.8 ± 1.4
	8.81	164.9 ± 18.3	14.5 ± 1.6	20.5 ± 1.6
Protein-free diet	0.56	72.1 ± 11.9	0.4 ± 0.1	-8.8 ± 0.6

Average initial weight of all rats = 41.5 (range 35.8 to 49.9).

The regression of body water (g,Y) on liveweight (g,X) for the initial slaughter group was Y = 0.61(±0.02)X + 3.04(±0.69); r = 0.997; n = 10; RCV = 0.6%.

APPENDIX 13, TABLE 11: Faecal dry matter and crude protein production (± SD) in Rat Experiment Seven.

Protein Source	Diet protein %	Faecal dry matter g.	Faecal protein content %	Faecal protein g.
Lactalbumin	3.06	4.6 ± 0.7	14.69 ± 1.23	0.7 ± 0.2
	6.31	7.1 ± 0.8	18.22 ± 2.02	1.3 ± 0.2
	9.00	8.8 ± 1.5	18.13 ± 0.79	1.6 ± 0.3
LPC - WC	4.69	5.2 ± 0.6	21.71 ± 1.73	1.1 ± 0.1
	7.50	7.1 ± 1.0	26.50 ± 1.63	1.9 ± 0.2
	10.31	11.5 ± 1.6	26.26 ± 3.89	3.0 ± 0.4
- WC.MBS	3.75	5.3 ± 0.8	21.95 ± 2.04	1.2 ± 0.2
	6.50	8.4 ± 2.0	24.03 ± 1.59	2.0 ± 0.4
	9.94	11.7 ± 2.1	27.79 ± 1.34	3.2 ± 0.5
- RG	4.44	5.9 ± 0.8	22.28 ± 1.90	1.3 ± 0.1
	7.13	9.0 ± 1.5	24.70 ± 1.04	2.2 ± 0.4
	9.38	16.5 ± 2.7	27.13 ± 1.93	4.5 ± 0.7
- RG.MBS	3.50	6.2 ± 0.9	21.36 ± 1.62	1.3 ± 0.2
	6.06	9.8 ± 1.4	23.71 ± 1.29	2.3 ± 0.4
	8.63	16.4 ± 1.1	24.49 ± 1.19	4.0 ± 0.3
- RG/WC	4.56	6.3 ± 0.7	23.00 ± 1.08	1.4 ± 0.2
	7.31	8.9 ± 1.2	25.51 ± 1.38	2.3 ± 0.3
	9.81	14.5 ± 2.5	27.05 ± 0.47	3.9 ± 0.7
- RG/WC.MBS	3.44	6.5 ± 0.7	19.31 ± 1.91	1.2 ± 0.1
	6.00	10.2 ± 1.7	20.71 ± 1.24	2.1 ± 0.5
	8.81	15.4 ± 2.2	23.06 ± 1.12	3.6 ± 0.6
Protein-free diet	0.56	3.3 ± 0.2	15.45 ± 3.44	0.5 ± 0.1

APPENDIX 13, TABLE 12: Performance data (± SD) from Rat Experiment Eight.

Protein Source	Diet protein %	Food intake (g/3 wk)	Protein intake (g/3 wk)	Body water gain (g/3 wk)
Lactalbumin	3.13	98.6 ± 16.3	3.1 ± 0.5	-0.6 ± 1.0
	5.88	138.0 ± 23.2	8.1 ± 1.4	12.1 ± 2.5
	8.63	166.1 ± 15.1	14.3 ± 1.3	27.5 ± 3.0
LPC - RG + M	3.42	125.7 ± 19.0	4.3 ± 0.6	-0.7 ± 1.4
	5.76	156.3 ± 32.4	9.0 ± 1.9	10.5 ± 3.1
	8.44	186.7 ± 14.0	15.8 ± 1.2	24.4 ± 2.6
- RG.MBS	3.44	92.7 ± 14.0	3.2 ± 0.5	0.4 ± 0.2
	6.13	122.8 ± 17.4	7.5 ± 1.1	7.0 ± 2.4
	8.75	151.4 ± 17.5	13.2 ± 1.5	17.6 ± 2.1
- RG.MBS + M	3.50	89.7 ± 11.9	3.1 ± 0.4	-1.2 ± 1.0
	6.19	139.4 ± 19.3	8.6 ± 1.2	9.9 ± 2.1
	8.81	165.2 ± 26.3	14.6 ± 2.3	22.3 ± 5.6
- WC + M	3.69	128.4 ± 3.3	4.7 ± 0.1	0.4 ± 0.2
	6.06	168.6 ± 13.1	10.2 ± 0.8	13.7 ± 1.2
	8.50	193.1 ± 8.8	16.4 ± 0.7	26.1 ± 2.2
- WC.MBS	3.75	82.3 ± 3.7	3.1 ± 0.1	-1.4 ± 0.7
	6.69	108.9 ± 9.0	7.3 ± 0.6	4.2 ± 1.2
	9.38	126.9 ± 14.3	11.9 ± 1.3	11.7 ± 3.9
- WC.MBS + M	3.94	114.1 ± 7.7	4.5 ± 0.3	0.4 ± 0.8
	6.56	129.1 ± 17.5	8.5 ± 1.2	9.4 ± 3.3
	9.38	157.2 ± 21.1	14.7 ± 2.0	24.3 ± 2.9
Protein-free diet	0.38	62.2 ± 7.0	0.2 ± 0.0	-6.5 ± 0.8

Average initial liveweight of all rats = 44.3g (range 32.1 to 55.0)

The regression of body water (g,Y) on liveweight (g,X) for the initial slaughter group was $Y = 0.60(\pm 0.02)X + 3.41(\pm 0.69)$; $r = 0.99$; $n = 8$; RCV = 0.65%.

APPENDIX 13, TABLE 13: Performance data (± SD) from Rat Experiment Nine.

Protein Source	Diet protein %	Food intake (g/3 wk)	Protein intake (g/3 wk)	Body water gain (g/3 wk)
Lactalbumin	2.44	112.5 ± 22.4	2.7 ± 0.6	0.2 ± 1.2
	5.94	144.6 ± 18.2	8.6 ± 1.1	13.7 ± 2.6
	8.75	182.0 ± 15.1	15.9 ± 1.3	30.8 ± 1.8
LPC - RG/WC + M	3.63	103.5 ± 12.3	3.8 ± 0.4	0.4 ± 1.2
	6.69	143.9 ± 19.4	9.6 ± 1.3	11.3 ± 2.4
	9.50	166.3 ± 18.6	15.8 ± 1.8	23.7 ± 5.8
- RG/WC.MBS	3.56	100.3 ± 11.1	3.6 ± 0.4	-0.1 ± 1.1
	6.44	122.9 ± 14.4	7.9 ± 0.9	7.4 ± 2.7
	9.00	153.6 ± 12.3	13.8 ± 1.1	17.4 ± 3.3
- RG/WC.MBS + M	3.69	102.7 ± 10.9	3.8 ± 0.4	-0.3 ± 0.8
	6.56	155.1 ± 20.4	10.2 ± 1.3	12.7 ± 2.3
	9.25	174.1 ± 16.8	16.1 ± 1.6	24.0 ± 2.2
- L + M	3.81	112.2 ± 16.6	4.3 ± 0.6	-0.1 ± 0.7
	6.75	153.2 ± 18.7	10.3 ± 1.3	13.0 ± 2.0
	9.44	151.7 ± 21.2	14.3 ± 2.0	21.4 ± 5.9
- L.MBS	3.81	107.7 ± 19.5	4.1 ± 0.8	-0.7 ± 1.7
	6.81	133.0 ± 2.8	9.1 ± 0.2	6.3 ± 4.0
	9.56	150.7 ± 13.3	14.4 ± 1.3	15.5 ± 2.3
- L.MBS + M	3.94	108.8 ± 23.0	4.3 ± 0.9	-0.3 ± 3.4
	6.90	156.7 ± 7.9	12.2 ± 0.6	19.3 ± 3.0
	9.69	168.5 ± 16.7	16.3 ± 1.6	25.7 ± 4.8
Protein-free diet	0.38	69.0 ± 6.0	0.3 ± 0.02	-6.8 ± 0.9

Average initial liveweight of all rats 45.5g (range 34.2 to 55.9).

The regression of body water (g,Y) on liveweight (g,X) for the initial slaughter group was $Y = 0.57(\pm 0.02)X + 3.76(\pm 1.0)$; $r = 0.994$; $n = 8$; RCV = 0.9%.

APPENDIX 14, TABLE 1: Regression coefficients, RNV estimates, protein true digestibility (PTD) results and significance tests on selected treatment comparisons for Rat Experiment Six.

Protein Source	Growth data		Faecal data	
	$b^1 \pm$ Se	RNV $\pm$ Se	$b^2 \pm$ Se	PTD (1-b)%
Lactalbumin	2.52 $\pm$ 0.07	1.00	0.095 $\pm$ 0.011	90.5 $\pm$ 1.1
LPC - L	1.54 $\pm$ 0.08	0.61 $\pm$ 0.03	0.215 $\pm$ 0.030	78.5 $\pm$ 1.3
- L.MBS	1.88 $\pm$ 0.10	0.75 $\pm$ 0.04	0.194 $\pm$ 0.014	80.6 $\pm$ 1.4
- RG/WC	1.56 $\pm$ 0.08	0.62 $\pm$ 0.03	0.269 $\pm$ 0.012	73.1 $\pm$ 1.2
- RG/WC.MBS	2.06 $\pm$ 0.10	0.82 $\pm$ 0.04	0.223 $\pm$ 0.014	77.7 $\pm$ 1.4
Regression intercept =	-9.6		0.44	
Regression RSD =	2.5		0.35	

1: b = coefficient from the regression of body water gain on protein intake.

2: b = coefficient from the regression of faecal protein on protein intake.

Difference tests

(a) on regression coefficients from growth data:

	Diff. $\pm$ Se(d)	Sig.
L - L.MBS	= -0.34 $\pm$ 0.09	**
RG/WC-RG/WC.MBS	= -0.50 $\pm$ 0.09	***

L v RG/WC and L.MBS v RG/WC.MBS were not significantly different

(b) on protein true digestibility results:

L - RG/WC	=	5.4 $\pm$ 1.1	***
L - L.MBS	=	-2.2 $\pm$ 1.3	NS
RG/WC - RG/WC.MBS	=	4.5 $\pm$ 1.3	***
L.MBS - RG/WC.MBS	=	-3.0 $\pm$ 1.5	*

APPENDIX 14, TABLE 2: Regression coefficients, RNV estimates, protein true digestibility results and significance tests on selected treatment comparisons for Rat Experiment Seven.

Protein Source	Growth data		Faecal data	
	$b^1 \pm$ Se	RNV $\pm$ Se	$b^2 \pm$ Se	PTD (1-b)%
Lactalbumin	2.70 $\pm$ 0.05	1.00	0.075 $\pm$ 0.008	92.5 $\pm$ 0.8
LPC - RG	1.62 $\pm$ 0.06	0.60 $\pm$ 0.02	0.275 $\pm$ 0.009	72.5 $\pm$ 0.9
- RG.MBS	1.94 $\pm$ 0.06	0.71 $\pm$ 0.02	0.256 $\pm$ 0.009	74.4 $\pm$ 0.9
- WC	1.53 $\pm$ 0.06	0.57 $\pm$ 0.02	0.192 $\pm$ 0.010	80.8 $\pm$ 1.0
- WC.MBS	1.69 $\pm$ 0.06	0.63 $\pm$ 0.02	0.213 $\pm$ 0.010	78.7 $\pm$ 1.0
- RG/WC	1.53 $\pm$ 0.06	0.57 $\pm$ 0.02	0.258 $\pm$ 0.010	74.2 $\pm$ 1.0
- RG/WC.MBS	2.02 $\pm$ 0.06	0.75 $\pm$ 0.02	0.215 $\pm$ 0.009	78.5 $\pm$ 0.9
Regression intercept =	-8.8		0.43	
Regression RSD =	1.76		0.28	

1: b = coefficient from the regression of body water gain on protein intake.

2: b = coefficient from the regression of faecal protein on protein intake.

Difference tests

(a) on regression coefficients from growth data:

RG - WC	= 0.08 $\pm$ 0.06 NS
RG - RG/WC	= 0.09 $\pm$ 0.06 NS
WC - RG/WC	= 0 NS
RG - RG.MBS	= -0.32 $\pm$ 0.06 ***
WC - WC.MBS	= -0.15 $\pm$ 0.06 *
RG/WC - RG/WC.MBS	= -0.49 $\pm$ 0.06 ***
RG.MBS - WC.MBS	= -0.25 $\pm$ 0.06 **
RG.MBS - RG/WC.MBS	= -0.08 $\pm$ 0.06 NS
WC.MBS - RG/WC.MBS	= -0.34 $\pm$ 0.06 ***

(b) on protein true digestibility results:

RG - WC	= -8.3 $\pm$ 1.1 ***
RG - RG/WC	= -1.7 $\pm$ 1.1 NS
WC - RG/WC	= 6.6 $\pm$ 1.1 ***
RG - RG.MBS	= -1.9 $\pm$ 1.1 NS
WC - WC.MBS	= 2.1 $\pm$ 1.1 NS
RG/WC - RG/WC.MBS	= -4.3 $\pm$ 1.1 ***

APPENDIX 14, TABLE 3: Regression coefficients, RNV estimates and significance tests on selected treatment comparisons for Rat Experiment Eight.

Protein Source	$b^1 \pm$ Se	RNV $\pm$ Se
Lactalbumin	2.44 $\pm$ 0.05	1.00
LPC - RG + M[2]	2.01 $\pm$ 0.06	0.82 $\pm$ 0.02
- RG.MBS	1.93 $\pm$ 0.06	0.79 $\pm$ 0.02
- RG.MBS + M	2.06 $\pm$ 0.06	0.84 $\pm$ 0.02
- WC + M	2.05 $\pm$ 0.06	0.84 $\pm$ 0.02
- WC.MBS	1.66 $\pm$ 0.07	0.68 $\pm$ 0.03
- WC.MBS + M	2.11 $\pm$ 0.06	0.86 $\pm$ 0.01
Regression intercept	-7.7	
Regression RSD	1.68	

1: b = coefficient from the regression of body water gain on protein intake.

2: M indicates methionine supplementation.

Difference tests

on regression coefficients from growth data:

RG + M - WC + M	= -0.04 $\pm$ 0.06 NS
RG.MBS - WC.MBS	= 0.27 $\pm$ 0.06 **
RG.MBS + M - WC.MBS + M	= -0.05 $\pm$ 0.06 NS
RG + M - RG.MBS	= 0.07 $\pm$ 0.06 NS
RG + M - RG.MBS + M	= 0.05 $\pm$ 0.06 NS
RG.MBS - RG.MBS + M	= -0.13 $\pm$ 0.06 +
WC + M - WC.MBS	= 0.39 $\pm$ 0.06 ***
WC + M - WC.MBS + M	= -0.06 $\pm$ 0.06 NS
WC.MBS - WC.MBS + M	= 0.46 $\pm$ 0.06 ***

APPENDIX 14, TABLE 4: Regression coefficients, RNV estimates and significance tests on selected treatment comparisons for Rat Experiment Nine.

Protein Source	$b^1 \pm SE$	RNV $\pm$ Se
Lactalbumin	2.40 $\pm$ 0.06	1.00
LPC - L + M	1.97 $\pm$ 0.07	0.82 $\pm$ 0.03
- L.MBS	1.58 $\pm$ 0.07	0.66 $\pm$ 0.03
- L.MBS + M	2.06 $\pm$ 0.07	0.86 $\pm$ 0.03
- RG/WC + M	1.95 $\pm$ 0.07	0.81 $\pm$ 0.03
- RG/WC.MBS	1.81 $\pm$ 0.08	0.75 $\pm$ 0.03
- RG/WC.MBS + M	1.93 $\pm$ 0.07	0.80 $\pm$ 0.03
Regression intercept	-7.5	
Regression RSD	2.1	

1: b = coefficient from the regression of body water gain on protein intake.

Difference tests

on regression coefficients from growth data:

L + M - RG/WC + M	= 0.02 $\pm$ 0.07 NS
L.MBS - RG/WC.MBS	= -0.23 $\pm$ 0.07 **
L.MBS + M - RG/WC.MBS + M	= 0.13 $\pm$ 0.07 NS
L + M - L.MBS	= 0.39 $\pm$ 0.07 ***
L + M - L.MBS + M	= -0.09 $\pm$ 0.07 NS
L.MBS - L.MBS + M	= -0.48 $\pm$ 0.07 ***
RG/WC + M - RG/WC.MBS	= 0.14 $\pm$ 0.07 +
RG/WC + M - RG/WC.MBS + M	= 0.02 $\pm$ 0.07 NS
RG/WC.MBS - RG/WC.MBS + M	= -0.12 $\pm$ 0.07 NS

BIBLIOGRAPHY

ALLISON, R. M. (1971) *Leaf Protein: its agronomy, preparation, quality and use* I.B.P. Handbook No. 20, p. 78. Ed. N. W. Pirie. Blackwell Scientific.

ALLISON, R. M. & VARTHA, E. W. (1973) *New Zealand Jl exptl Agric. 1:* 35.

A.O.A.C. (1965) *Official Methods of Analysis of the Association of Official Agricultural Chemists.* Tenth Edition, p. 785.

ANONYMOUS (1975/6) *New Zealand Department of Statistics, Statistical Bulletin.* Agricultural Statistics. Table 55, p. 81.

ARKCOLL, D. B. (1973) *Jl Sci. Fd Agric. 24:* 437.

ARKCOLL, D. B. & FESTENSTEIN, G. N. (1971) *Jl Sci. Fd Agric. 22:* 49.

ARKCOLL, D. B. & HOLDEN, M. (1973) *Jl Sci. Fd Agric. 24:* 1217.

ARKCOLL, D. B. (1971) *Leaf Protein: its agronomy, preparation, quality and use* I.B.P. Handbook No. 20, p. 9. Ed. N. W. Pirie. Blackwell Scientific.

BAARS, J. A. (1976) *New Zealand Jl exptl Agric. 4*: 151.

BAILEY, R. W. (1973) *Chemistry and Biochemistry of Herbage.* Vol. 1. p. 157. Ed. G. W. Butler, R. W. Bailey. Academic Press.

BAKER, D. H. (1977) *Advances in Nutritional Research 1:* 299. Ed. H. H. Draper. Plenum Press.

BAGCHI, D. & MATAI, S. (1976) *Jl Sci. Fd Agric. 27:* 1.

BARBER, R. S., BRAUDE, R. & MITCHELL, K. G. (1958) *Proc. Nut. Soc. 18*: iii.

BECK, A. B. (1964) *Aust. Jl agric. Res. 15:* 223.

BETSCHART, A. A. & KINSELLA, J. E. (1975) *Jl Fd Sci. 40:* 271.

BICKOFF, E. M., BOOTH, A. N., de FREMERY, D., EDWARDS, R. H., KNUCKLES, B. E., MILLER, R. E., SAUNDERS, R. M. & KOHLER, G. O. (1975a) *Twelfth Tech. Alf. Conf. Proc.* p. 110. Overland Park Kansas, 1974. Pub. U.S.D.A. & A.D.A.

BICKOFF, E. M., BOOTH, A. N., de FREMERY, D., EDWARDS, R. H., KNUCKLES, B. E., MILLER, R. E., SAUNDERS, R. M. & KOHLER, G. O. (1975b) *Protein Nut'l Quality of Foods and Feeds* Part 2: 319. Ed. M. Friedman. Marcel Dekker N.Y.

BLOCK, R. J. & MITCHELL, H. H. (1946) *Nut. Abs. & Rev. 16:* 273.

BOOTH, V. H. (1971) *Jl Sci. Fd Agric. 22:* 658.

BOOTH, A. N., SAUNDERS, R. M., CONNER, M. A. & KOHLER, G. O. (1972) *Eleventh Tech. Alf. Conf. Proc.* p. 51. Albany California, 1971, Pub. U.S.D.A.

BRAY, W. J., HUMPHRIES, C. & INERITEI, M. S. (1978) *Jl Sci. Fd & Agric. 29:* 165.

BROUGHAM, R. W., BALL, P. R. & WILLIAMS, W. M. (1978) *Plant Relations in Pasture* Symp. Proc., Brisbane 1976. Ed. J. R. Wilson. Pub. C.S.I.R.O. Melbourne.

BRYANT, A. M. & PARKER, O. F. (1971) *Proc. Ruakura Farmers' Conf.* p. 110.

BUCHANAN, R. A. (1969a) *Jl Sci. Fd Agric. 20:* 359.

BUCHANAN, R. A. (1969b) *British Jl Nut. 23:* 533.

BYERS, M. (1961) *Jl Sci. Fd Agric. 12:* 20.

BYERS, M. (1970) *Rothamsted Exp. St. Report for 1969* Pt. *1.* p. 132.

BYERS, M. (1971a) *Jl Sci. Fd Agric. 22:* 242.

BYERS, M. (1971b) *Leaf Protein: its agronomy, preparation, quality and use* I.B.P. Handbook No. 20, p. 95. Ed. N. W. Pirie. Blackwell Scientific.

BYERS, M. & STURROCK, J. W. (1965) *Jl Sci. Fd Agric. 16:* 341.

CARR, J. R. & PEARSON, G. (1976) *New Zealand Jl exptl Agric. 4:* 45.

CHEEKE, P. R. (1974) *Nut. Rep. Intl 9:* 267.

CHIBNALL, A. C., REES, M. W. & LUGG, J. W. H. (1963) *Jl Sci. Fd Agric. 14:* 234.

CLIFFORD, A. J., WEIR, W. C., VASCONCELLOS, J. A., FORMAN, L. P. & LUMIJARV, D. (1975) Manuscript of paper presented to Annual Meeting of Amer. Soc. Agric. Eng. Univ. California, Davis. June 22-25, 1975.

CONNELL, J. & FOXELL, P. R. (1976) *Biennial Reviews Natl Inst. Res. Dairyg* p. 21.

COUCH, R. (1975) *Jl of the Assoc. of Off. Agric. Chem. 58:* 599.

COWLISHAW, S. J. & EYLES, D. E. (1956) *Empire Jl of Exptl Agric. 14:* 234 (cited by Morris, 1977).

COWLISHAW, S. J., EYLES, D. E., RAYMOND, W. F. & TILLEY, J. M. A. (1954) *Nature* (London), *174:* 227.

COWLISHAW, S. J., EYLES, D. E., RAYMOND, W. F. & TILLEY, J. M. A. (1956) *Jl Sci. Fd & Agric. 7:* 775.

CROOK, E. M. & HOLDEN, M. (1948) *Biochem. Jl 43:* 181.

DALY, G. T. (1973) *Pastures and Pasture Plants* p. 1. Ed. R. H. M. Langer. Pub. A. H. & A. W. Reed, Wellington.

DAVIES, M. & Evans, W. C. (1952) *Biochem. Jl 52:* xxiii.

DAVYS, M. N. G. & PIRIE, N. W. (1965) *Jl agric. Engng Res. 10:* 142.

DAVYS, M. N. G. & PIRIE, N. W. (1969) *Biotech. Bioengng 11:* 517.

DAVYS, M. N. G., PIRIE, N. W. & STREET, G. (1969) *Biotech. Bioengng 11:* 529.

de FREMERY, D., BICKOFF, E. M. & KOHLER, G. O. (1972) *Jl agric. Fd Chem. 20:* 1155.

de FREMERY, D., EDWARDS, R. H., MILLER, R. E., KNUCKLES, B. E., BICKOFF, E. M. & KOHLER, G. O. (1975) *Twelfth Tech. Alf. Conf. Proc.* p. 72. Overland Park Kansas, 1974. Pub. U.S.D.A. & A.D.A.

DELIC, I., VUCEREVIC, N. & STOJANOVIC, S. (1976) *Hrana i Ishrana 17:* 103, (cited in Nut. Abs. & Rev. (1977) 47: No. 5148).

DESHMUKH, M. G., GORE, S. B., MUNGIKAR, A. M. & JOSHI, R. N. (1974) *Jl Sci. Fd Agric. 25:* 717.

DEV, D. V., USHA RANI BATRA & JOSHI, R. N. (1974) *Jl Sci. Fd Agric. 25:* 725.

DEVERAJ, M. J., SUBBA RAU, B. H., RAMANA, K. V. R. & SINGH, N. (1970) *Proc. Third Intl Cong. Fd. Sci. & Tech.* (Washington), p. 1.

DUCKWORTH, J., HEPBURN, W. R. & WOODHAM, A. A. (1961) *Jl Sci. Fd Agric. 12:* 16.

DUCKWORTH, J. & WOODHAM, A. A. (1961) *Jl Sci. Fd Agric. 12:* 5.

EDWARDS, R. H., de FREMERY, D., MACKEY, B. E. & KOHLER, G. O. (1978) *Trans. Amer. Soc. Agric. Eng. 21:* 55.

EDWARDS, R. H., MILLER, R. E., de FREMERY, D., KNUCKLES, B. E., BICKOFF, E. M. & KOHLER, G. O. (1975a) *Twelfth Tech. Alf. Conf. Proc.* p. 99. Overland Park Kansas, 1974. Pub. U.S.D.A. & A.D.A.

EDWARDS, R. H., MILLER, R. E., de FREMERY, D., KNUCKLES, B. E., BICKOFF, E. M. & KOHLER, G. O. (1975b) *Jl agric. Fd Chem. 23:* 620.

EGGUM, B. O. (1970) *British Jl Nut. 24:* 761.

ELLINGER, G. M. & DUNCAN, A. (1976) *Chromat. Jl 155:* 615.

EPPENDORFOR, W. H. (1977) *Jl Sci. Fd & Agric. 28:* 607.

ERBERSDOBLER, H. (1976) *Protein Metab. & Nut.* p. 139, Ed. D. T. Cole *et al.* E.A.A.P. Publ. No. 16. Butterworths.

ESKIN, N. A. M., HOEHN, E. & FRENKEL, C. (1978) *Jl agric. Fd Chem. 26:* 973.

EVERITT, G. C. (1972) *Proc. New Zealand Soc. Anim. Prod. 32:* 185.

FAFUNSO, M. & BYERS, M. (1977) *Jl Sci. Fd Agric. 28:* 375.

FESTENSTEIN, G. N. (1976) *Jl Sci. Fd Agric. 27:* 849.

FOXELL, P. R. (1977) *Green Crop Fractionation* p. 97. Symp. Proc., Harrowgate, 1976. Ed. J. R. Wilkins. Brit. Grassld Soc. & Brit. Soc. Anim. Prod.

GARCHA, J. S., KAWATRA, B. L. & WAGLE, D. S. (1971) *Jl Fd Sci. Tech. 8:* 23.

GASTINEAU, C. (1975) *Twelfth Tech. Alf. Conf. Proc.* p. 123. Overland Park Kansas, 1974. Pub. U.S.D.A. & A.D.A.

GERLOFF, E. D., LIMA, I. H. & STAHMANN, M. A. (1965) *Jl agric. Fd Chem. 13:* 139.

GJOEN, A. U. & NJAA, L. R. (1977) *British Jl Nut. 37:* 93.

GLENCROSS, R. G., FESTENSTEIN, G. W. & KING, H. G. C. (1972) *Jl Sci. Fd Agric. 23:* 371.

GOEL, U., KAWATRA, B. L. & BAJAJ, S. (1977) *Jl Sci. Fd Agric. 28:* 786.

GORE, S. B., MUNGIKAR, A. M. & JOSHI, R. N. (1974) *Jl Sci. Fd Agric. 25:* 1149.

HARRIS, W. (1978) *Plant Relations in Pastures* p. 67. Symp. Proc., Brisbane 1976. Ed. J. R. Wilson. Pub. C.S.I.R.O. Melbourne.

HARTMAN, G. H., AKESON, W. R. & STAHMANN, M. A. (1967) *Jl agric. Fd Chem. 15:* 74.

HAWKE, J. C. (1973) *Chemistry & Biochemistry of Herbage 1:* 213. Ed. G. W. Butler, R. W. Bailey. Academic Press.

HEATH, S. B. & KING, M. W. (1977) *Green Crop Fractionation* p. 9. Symp. Proc., Harrowgate 1976. Ed. R. J. Wilkins. Brit. Grassld Soc. & Brit. Soc. Anim. Prod.

HEGSTED, D. M. & WORCESTER, J. (1967) *Proc. Seventh Intl Congress of Nut. 4:* 318. Ed. J. Kuchnau & H. D. Cremer. Vieweg & Son, Hamburg.

HEGSTED, D. M., NEFF, R. & WORCESTER, J. (1968) *Jl agric. Fd Chem. 16:* 190.

HENRY, K. M. (1963) *Proc. Sixth Intl Nut. Cong.* Edinburgh, (cited by Woodham, 1971).

HENRY, K. M. & FORD, J. E. (1965) *Jl Sci. Fd Agric. 16:* 425.

HOGLUND, J. H., CRUSH, J. R., BROCK, J. L. & BALL, R. (1979) *New Zealand Jl exptl Agric. 7:* 45.

HOLDEN, M. (1967) *Rothamsted Exp. Stn. Rep. for 1966* p. 310.

HOLLO, J. & KOCH, L. (1971) *Leaf Protein: its agronomy, preparation, quality & use* I.B.P. Handbook No. 20, p. 63. Ed. N. W. Pirie. Blackwell Scientific.

HORIGOME, T. & KANDATSU, M. (1966) *Jl Agric. Chem. Soc. Japan 40:* 246.

HORIGOME, T. & KANDATSU, M. (1968) *Agric. biol. Chem. 32:* 1093.

HOUSEMAN, R. A. & CONNELL, J. (1976) *Proc. Nut. Soc. 35:* 213.

HOVE, E. L., LOHREY, E., URS, M.K. & ALLISON, R. M. (1974) *British Jl Nut. 31:* 147.

HUDSON, B. J. F. & KARIS, I. G. (1973) *Jl Sci. Fd Agric. 24:* 1541.

HUDSON, B. J. F. & KARIS, I. G. (1976) *Jl Sci. Fd Agric. 27:* 443.

HUDSON, B. J. F. & WARWICK, M. J. (1977) *Jl Sci. Fd Agric. 28:* 259.

HUGHES, G. P. & EYLES, D. E. (1953a) *Jl Agric. Sci.* (Camb.) *43:* 144.

HUGHES, G. P. & EYLES, D. E. (1953b) *Jl Agric. Sci.* (Camb.) *43:* 136.

HUNT, O. J. & Peaden, R. N. (1975) *Twelfth Tech. Alf. Conf. Proc.* p. 30. Overland Park Kansas, 1974. Pub. U.S.D.A. & A.D.A.

HUTTON, J. B. (1961) *New Zealand Jl agric. Res. 4:* 583.

HUTTON, J. B. (1970) *Proc. Eleventh Intl Grassld Cong. p. A78.* Brisbane, Ed. J. T. Norman.

HUTTON, J. B. (1972) *Proc. New Zealand Soc. Anim. Prod. 32:* 160.

HUTTON, J. B., JURY, K. E. & DAVIES, E. B. (1967) *New Zealand Jl agric. Res. 10:* 367.

JAGUSCH, K. T., RATTRAY, P. V. & MacLEAN, K. S. (1978) *Proc. New Zealand Soc. Anim. Prod. 38:* 129.

JOHNS, A. T. (1955) *New Zealand Jl Sci. & Tech. A37:* 301.

JOHNS, D. C. (1978) *Proc. Nut. Soc. New Zealand 3:* 112.

JONES, A. S. & HOUSEMAN, R. A. (1975) *Rowett Res. Inst. Ann. Rep. of Studies on Nut. & Allied Sci. 31:* 136.

KABA, H. & PELLETT, P. L. (1975) *Ecology of Fd & Nut. 4:* 109.

KAMALANATHAN, G. & DEVADAS, R. P. (1971) *Leaf Protein: its agronomy, preparation, quality and use* I.B.P. Handbook No. 20, p. 145. Ed. N. W. Pirie. Blackwell Scientific.

KHANNA, S. K., VISWANATHAN, P. N., KRISHNAN, P. S. & SANWAL, G. G. (1968) *Phytochem. 7:* 1513.

KOHLER, G. O. (1975) *Twelfth Tech. Alf. Conf. Proc.* p. 65. Overland Park Kansas, 1974. Pub. U.S.D.A. & A.D.A.

KOHLER, G. O., EDWARDS, R. H. & de FREMERY, D. (1978) *Proc. Second Intl Green Crop Drying Congress.* Saskatoon Canada. (In press).

KUZMICKY, D. D. & KOHLER, G. O. (1977) *Poultry Sci. 56:* 1510.

KUZMICKY, D. D., KOHLER, G. O. & BICKOFF, E. M. (1972) *Eleventh Tech. Alf. Conf. Proc.* p. 58. Albany California, 1971. Pub. U.S.D.A.

KUZMICKY, D. D., LIVINGSTON, A. L., KNOWLES, R. E., KOHLER, G. O., GUENTHNER, E., OLSON, O. E. & CARLSON, C. W. (1978) *Poultry Sci. 56:* 1504.

LAHIRY, N. L. & SATTERLEE, L. D. (1975) *Jl Fd Sci. 40:* 1326.

LARSON, R. L. & HALVERSON, A. W. (1962) *Agric. & Fd Chem. 10:* 422.

LEA, C. H. & PARR, L. J. (1961) *Jl Sci. Fd Agric. 12:* 785.

LEACH, G. J. (1978) *Plant Relations in Pastures* p. 290. Symp. Proc. Brisbane. Ed. J. R. Wilson. C.S.I.R.O. Melbourne.

LEXANDER, K., CARLSSON, R., SCHALEN, V., SIMMONSSON, A. & LUNDBORD, T. (1970) *Ann. appl. Biol. 66:* 193.

LIMA, I. H., RICHARDSON, T. & STAHMANN, M. A. (1965) *Jl agric. Fd Chem. 13:* 143.

LIVINGSTON, A. L., KNUCKLES, B. E., EDWARDS, R. H., de FREMERY, D., MILLER, R. E. & KOHLER, G. O. (1979) *Jl agric. Fd Chem. 27:* 362.

LOHREY, E., TAPPER, B. & HOVE, E. L. (1974) *British Jl Nut. 31:* 159.

McARTHUR, J. M. & MILTIMORE, J. E. (1969) *Can. Jl Anim. Sci. 49:* 69.

McDONALD, R. M. & DONNELLY, P. E. (1978) *Proc. Nut. Soc. New Zealand 3:* 70.

McKENZIE, D. R. (1976) *Proc. First Australasian Poultry & Stockfeed Convention* p. 128. Melbourne.

McKENZIE, D. R. (1977) *Aust. Jl exptl Agric. 17:* 268.

McKENZIE, D. R. (1978) *Proc. Second Australasian Poultry & Stockfeed Convention* p. 232. Sydney.

McKENZIE, S. L. (1977) *Jl Chromat. 130:* 399.

McNAUGHT, K. J. & DOROFAEFF, F. D. (1968) *New Zealand Jl agric. Res. 11:* 551.

MAGUIRE, M. F. (1977) *Green Crop Fractionation* p. 149. Symp. Proc. Harrowgate, 1976. Ed. J. R. Wilkins. Brit. Grassld Soc. & Brit. Soc. Anim. Prod.

MAGUIRE, M. F. & BROOKES, I. M. (1973) *Proc. First Intl Green Crop Drying Cong.* Oxford, 1973. p. 346. Ed. C. L. Skidmore.

MATAI, S., BAGCHI, D. & CHANDA, S. (1976) *Jl Sci. Fd Agric. 27:* 736.

MATHESON, N. A. (1974) *British Jl Nut. 31:* 393.

METSON, A. J. & SAUNDERS, W. H. M. (1978) *New Zealand Jl agric. Res. 21:* 355.

MILLER, E. L. (1976) *From Plant to Animal Protein* Reviews in Rural Science II. Proc. of Symp. at Univ. of New England p. 47.

MILLS, R. A., McDONALD, R. M. & DONNELLY, P. E. (1980) *Trans. New Zealand Inst. Eng.* 7(1): 9.

MINSON, D. J., HARRIS, C. E., RAYMOND, W. F. & MILFORD, R. (1964) *Jl British Grassld Soc. 19:* 298.

MINSON, D. J., RAYMOND, W. F. & HARRIS, C. E. (1960) *Jl British Grassld Soc. 15:* 174.

MORRISON, J. E. & PIRIE, N. W. (1961) *Jl Sci. Fd Agric. 12:* 1.

MORRIS, T. R. (1977) *Green Crop Fractionation* p. 67. Symp. Proc. Harrowgate 1976. Ed. R. J. Wilkins. Brit. Grassld Soc. & Brit. Soc. of Anim. Prod.

MUNGIKAR, A. M., BATRA, U. R., TEKALE, N. S. & JOSHI, R. N. (1976) *Exptl Agr. 12:* 353.

MYER, R. O. & CHEEKE, P. R. (1975) *Jl Anim. Sci. 40:* 500.

MYER, R. O., CHEEKE, P. R. & KENNICK, W. H. (1975) *Jl Anim. Sci. 40:* 885.

NAGY, S., TELEK, L., HALL, N. T. & BERRY, R. E. (1978) Manuscript of paper presented to Amer. Chem. Soc. Symp.

NANDA, C. L., TERNOUTH, J. H. & KONDOS, A. C. (1977) *Jl Sc. Fd Agric. 28:* 1075.

NETKE, S. P. & SCOTT, H. M. (1974) *Poultry Sci. 53:* 592.

NGWIRA, T. N. & BEAMES, R. M. (1978) *British Jl Nut. 40:* 443.

NJAA, L. R. (1963) *Fiskeridirectoratets Skrifter IV* No. 5. A monograph entitled 'A study on the Mitchell method for determination of the biological value of protein'.

N.R.C. (In Press) Revised National Research Council Publication No. 1100. *Nutritional Evaluation of Protein Foods.*

N.R.C. (1971) *Nutrient Requirements of Domestic Animals* No. 3 Nutrient Requirements of Dairy Cattle. Fourth revised edition. Natl. Acad. Sciences, Washington, D. C.

N.R.C. (1972) *Nutrient Requirements of Domestic Animals* No. 10. Nutrient Requirements of Laboratory Animals. Second Revised Edition. Natl Academy Sciences, Washington, D.C.

Oelshlegel, F. J., SCHROEDER, J. R., STAHMANN, M. A. (1969) *Jl Agric. Fd Chem. 17:* 791.

OHSHIMA, M. & OOUCHI, K. (1976) *Nut. Rep. Intl 14:* 611.

OSTROWSKI, H. T. (1966) *Proc. New Zealand Soc. Anim. Prod. 36:* 30.

PIERPOINT, W. S. (1971) *Rothamsted Exp. St. Rep. for 1970* Part 2: p. 199.

PIERPOINT, W. S. (1979) Personal communication.

PIRIE, N. W. (1966) *Science* (New York) *152:* 1701.

PIRIE, N. W. (1971a) *Leaf Protein: its agronomy, preparation, quality and use* I.B.P. Handbook No. 20, p. 1. Ed. N. W. Pirie. Blackwell Scientific.

PIRIE, N. W. (1971b) *Leaf Protein: its agronomy, preparation, quality and use* I.B.P. Handbook No. 20, p. 29. Ed. N. W. Pirie. Blackwell Scientific.

PIRIE, N. W. (1971c) *Leaf Protein: its agronomy, preparation, quality and use* I.B.P. Handbook No. 20, p. 53. Ed. N. W. Pirie. Blackwell Scientific.

PIRIE, N. W. (1971d) *Leaf Protein: its agronomy, preparation, quality and use* I.B.P. Handbook No. 20, p. 86. Ed. N. W. Pirie. Blackwell Scientific.

PIRIE, N. W. (1975) *Protein Nutritional Quality of Foods & Feeds 2:* 341. Ed. M. Friedman. Marcel Dekker.

PIRIE, N. W. (1977) *Expl Agric. 13:* 113.

PLESHKOV, B. P. & FOWDEN, L. (1959) *Nature* (London) *183:* 1445.

RADCLIFFE, J. E. (1974) *New Zealand Jl exptl Agric. 2:* 341.

RADCLIFFE, J. E. (1975a) *New Zealand Jl exptl Agric. 3:* 239.

RADCLIFFE, J. E. (1975b) *New Zealand Jl exptl Agric. 3:* 259.

RADCLIFFE, J. E. (1976) *New Zealand Jl exptl Agric. 4:* 163.

RADCLIFFE, J. E. & SINCLAIR, D. P. (1975) *New Zealand Jl exptl Agric. 3:* 247.

RAMA RAO, P. B., NORTON, H. W. & JOHNSON, B. C. (1964) *Jl Nut. 82:* 88.

RANKIN, B. C. & POCOCK, K. S. (1970) *Aust. Wine Brewing & Spirit Review 88* (8): 40.

RICKARD, D. S. & RADCLIFFE, J. E. (1976) *New Zealand Jl exptl Agric. 4:* 329.

ROUND-TURNER, N. L., SCOTT, R. S. & RADCLIFFE, J. E. (1976) *New Zealand Jl exptl Agric. 4:* 321.

ROSS, A. J. & WALLACE, G. M. (1978) *Proc. Nut. Soc. New Zealand 3:* 122.

ROSS, D. J., MOLLOY, L. F., COLLIE, T. W. & BALL, R. (1978) *New Zealand Jl agric. Res. 21:* 231.

SASSE, C. E. & BAKER, D. H. (1973) *Jl Anim. Sci. 37:* 1351.

SENTHESHANMUGANATHAN, S. & DURAND, S. (1969) *Jl Sci. Fd Agric. 20:* 603.

SHAH, F. H. (1968) *Jl Sci. Fd Agric. 19:* 199.

SHURPALEKAR, K. S., SUNDARAVALLI, O. E. & SINGH, N. (1969) *Science & Culture 35:* 644.

SINGH, N. (1960) *Biochim. Biophys. Acta. 45:* 422.

SINGH, N. (1962) *Jl Sci. Fd Agric. 13:* 325.

SINGH, N. (1971) *Leaf Protein: its agronomy, preparation, quality and use.* I.B.P. Handbook No. 20, p. 131. Ed. N. W. Pirie. Blackwell Scientific.

SNOW, J. T., FINLAY, J. W. & KOHLER, G. O. (1976) *Jl Sci. Fd Agric. 27:* 649.

SOWERS, J. E., STOCKLAND, W. L. & MEAD, R. J. (1972) *Jl Anim. Sci. 35:* 782.

STAHMANN, M. A. (1975) *Twelfth Tech. Alf. Conf. Proc.* p. 92. Overland Park Kansas, 1974. Pub. U.S.D.A. & A.D.A.

STOCKLAND, W. L., MEADE, R. J., WASS, D. F. & SOWERS, J. E. (1973) *Jl Anim. Sci. 36:* 526.

SUBBA RAO, M. S., SINGH, N. & PRASANAPPA, G. (1967) *Jl Sci. Fd Agric. 18:* 295.

SUBBA RAU, B. H., MAHADEVIAH, S. & SINGH, N. (1969) *Jl Sci. Fd Agric. 20:* 355.

SUBBA RAU, B. H., RAMANA, K.V.R. & SINGH, N. (1972) *Jl Sci. Fd Agric. 23:* 233.

SUBBA RAU, B. H. & SINGH, N. (1970) *Ind. Jl exptl Biol. 8:* 34.

SUBBA RAU, B. H. & SINGH, N. (1971) *Jl Sci. Fd Agric. 22:* 569.

SYNGE, R.L.M. (1975). *Qual. Plant. Mater. Veg.* (Plant Fd human Nut.) *24:* 337.

SYNGE, R. L. M. (1977) *Proc. Nut. Soc. 36:* 107.

TAO, M., BOULET, M., BRISSON, G. J., HUANG, K. H., RIEL, R. R. & JULIEN, J. P. (1972) *Jl Inst. Can. Sci. Tech. Aliment. 5:* 50.

TILLEY, J. M. A. & RAYMOND, W. F. (1957) *Herb. Abs. 27:* 235.

TRUCHETTO, M. (1977) *Industries de L'Alimentation Animale* No.12. p. 23.

ULYATT, M. J. (1973) *Chemistry & Biochemistry of Herbage 3:* 31. Ed. G. W. Butler & R. W. Bailey. Academic Press.

VARTHA, E. W. & Allison, R. M. (1973) *New Zealand Jl exptl Agric. 1:* 239.

VARTHA, E. W. & BRUSSE, M. J. (1977) *New Zealand Jl exptl Agric. 5:* 151.

WIERINGA, G. W. (1978) *Proc. Seventh General Meeting Europ. Grassld Fed.* (Gent 1978), p. 6.63.

WILSON, R. F. & TILLEY, J. M. A. (1965) *Jl Sci. Fd Agric. 16:* 173.

WITT, S. C., SPENCER, R. R., BICKOFF, E. M. & KOHLER, G. O. (1972) *Eleventh Tech. Alfalfa Conf. Proc.* p. 95. Albany California, 1971. Pub. U.S.D.A.

WOODHAM, A. A. (1965) *Proc. Nut. Soc. 24:* xxiv.

WOODHAM, A. A. (1971) *Leaf Protein: its agronomy, preparation, quality and use.* p. 115. I.B.P. Handbook No. 20. Ed. N. W. Pirie. Blackwell Scientific.

WOODHAM, A. A. (1976) *Qual. Plant.* (Plant Fd human Nut.) *25:* 311.

WOODHAM, A. A., KARMALI, R. A., CLARKE, E. M. W. & ARKCOLL, D. B. (1974) *Proc. Fourth Intl Cong. Fd Sci. & Tech. 1:* 653.