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**STUDIES IN PROTEIN SUPPLEMENTATION OF
LOW-QUALITY ROUGHAGE FEEDS**

A thesis submitted to the University of Glasgow

for the degree of

DOCTOR OF PHILOSOPHY

In the Faculty of Veterinary Medicine

by

JOHN PETER ALAWA

Dept. Animal Husbandry,
University of Glasgow
Veterinary School.

December, 1985

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TABLE OF CONTENTS

	<u>PAGE</u>
Acknowledgements	
Summary	(i)
Abbreviations	(iv)
Introduction	1
 REVIEW OF THE LITERATURE	 3
Potential and deficiencies of poor-quality roughages	3
Methods to improve the nutritive value of poor-quality roughages	4
Supplementation of poor-quality roughages	5
Effect of supplementation with protein	5
Old methods for assessing protein requirements	7
New methods for assessing protein requirements	8
Rumen degradation of proteins	9
Formaldehyde treatment of proteins	11
Heat treatment of proteins	12
Reduction in particle size of proteins	12
An assessment of protein source with regard to rumen degradation	13
Non-protein nitrogen supplements	13
Vegetable protein supplements	14
Animal protein supplements	15
Effect of phosphorus supplementation	16
Conclusions	18
 EXPERIMENTAL SECTION	
 SECTION 1	 19
FORMALDEHYDE-TREATED SOYA BEAN MEAL : EFFECT ON THE NUTRITIVE VALUE OF STRAW DIETS	19
Introduction	19

Experiment 1. The influence of formaldehyde treatment of soya bean meal on the digestibility of straw diets and nitrogen metabolism of sheep	20
Introduction	20
Materials and methods	20
Results	22
Discussion	23
 Experiment 2. The voluntary intake and digestibility of straw diets and the performance of lambs as influenced by formaldehyde treatment of soya bean meal	 24
Introduction	24
Materials and methods	24
Results	25
Discussion	26
 Experiment 3. The effect of dietary protein degradability and supplementary energy source on the voluntary intake and digestibility of straw and the performance of pregnant beef cows	 31
Introduction	31
Materials and methods	32
Results	35
Discussion	37
 Experiment 4. The effect of supplementary protein and energy source and dietary protein degradability on the voluntary intake, digestibility and utilization of straw by lactating beef cows	 43
Introduction	43
Materials and methods	44
Results	46
Discussion	47

SECTION 2	52
EVALUATION OF ALTERNATIVE PROTEIN SUPPLEMENTS	52
Introduction	52
Experiment 5. The use of brewers grains as a protein supplement	53
Introduction	53
Experiment 5.1. The rumen degradation of wet and dry brewers grains	53
Materials and methods	53
Results	56
Discussion	57
Experiment 5.2. The use of brewers grains as a protein supplement to straw fed <u>ad libitum</u> to pregnant beef cows	58
Introduction	58
Materials and methods	58
Results	60
Discussion	62
Experiment 6.1. The proximate composition and ruminal degradation of some tropical and temperate peas	66
Introduction	66
Materials and methods	66
Results	67
Discussion	68
Experiment 6.2. Further comparisons of brewers grains and peas with other nitrogen sources as supplements to straw fed <u>ad libitum</u>	70
Introduction	70
Materials and methods	71
Results	72
Discussion	74

SECTION 3	79
PHOSPHORUS SUPPLEMENTATION	79
Experiment 7. The effects of supplementary phosphorus and source of nitrogen on the performance of growing wether sheep given a low phosphorus diet	79
Introduction	79
Materials and methods	80
Results	81
Discussion	81
 SECTION 4	
 GENERAL DISCUSSION AND CONCLUSION	84
 Suggestions for further work	88
 References	89
 Appendix 1	105
 Appendix 2	105
 Appendix 3	107
 Appendix 4	108

ACKNOWLEDGEMENTS

I am most grateful to my supervisor, Professor R.G. Hemingway, for his continued advice, guidance and help and also for his efforts towards a problem-free conduct of experiments throughout the course of this study and the preparation of this thesis. I also wish to thank him for the provision of facilities at the Glasgow University Veterinary Field Station and in the Department of Animal Husbandry at the Veterinary Faculty of Glasgow University. I am indebted to the University of Science and Technology, Port Harcourt, Nigeria, for an award covering the first two years of this study, and to the Federal Nigerian Government for continuing the award during the final year.

I also greatly appreciate the assistance of Dr. G. Fishwick in conducting the experiments and Dr. J.J. Parkins for his help throughout.

I would like to acknowledge the splendid help of Miss M. Young, Mr. C. Cameron, Mrs M. Cunningham and Mrs M. Bell for carrying out the many laboratory analyses and Mrs C. Turpie for her own contributions. My thanks also go to Mrs C. Marshall of the Department of Veterinary Biochemistry for plasma total-protein estimations and Dr. J.M. Kelly of the Royal (Dick) School of Veterinary Studies, University of Edinburgh, for plasma beta hydroxy butyrate estimations. For help with the collection of rumen liquor from sheep, I wish to thank Dr. J.S. Boyd of the Department of Veterinary Anatomy. The assistance of Mr. M. McColl, Mr. T. Collett and Miss S. Osbourne with the care and management of the experimental animals is greatly appreciated, as is their companionship throughout the course of the experiments. Drs E.R. Orskov and G.W. Reid of the Rowett Research Institute, Aberdeen, advised on chemical treatment of brewers grains for which I express my appreciation.

I was very much encouraged by the kindness of the Departmental secretary, Miss E. Fairgrieve and other teaching staff who, in working as a team with the staff with whom I had direct contact, were very helpful. Mrs M. Findlay and Miss M. McKerracher of the Veterinary Branch Library of Glasgow University were also of great assistance to me. To these people and to others who assisted in various ways I am most grateful.

Special thanks are due to Mrs J. Bounds and Mrs N. Verrico for efficiently typing this thesis. I am further grateful to Mrs Bounds for typing two manuscripts of experiments described in this thesis for publication.

Finally, I must acknowledge with deep appreciation the patience and care of my mother-in-law in looking after my family during my absence and the indispensable aid of my wife, Theresa, during this study.

SUMMARY

The work described in this thesis investigates the influence of the source of supplementary protein (N x 6.25) on the nutritive value of low protein roughage feeds. Protein supplements degrade to varying degrees in the rumen and a recognition of the protein needs of the ruminant animal separate from that of the rumen micro-organisms which reside in their digestive tract implies that various protein supplements may differ in their effects on feed utilisation and productivity of animals given roughage diets low in crude protein.

In Section 1 proteins differing in rate and extent of ruminal degradation were compared as supplements to straw fed to sheep and beef cows. In Experiment 1, formaldehyde-treated soya bean meal, with approximately 80% reduced ruminal degradation due to treatment, was compared with untreated soya bean meal in mixed diets of straw and concentrate (3:1 on fresh weight basis) and adult wether sheep were restricted-fed. Total diet digestibility was not affected but nitrogen digestibility was depressed while rumen ammonia-nitrogen production and blood urea levels were reduced as a result of formaldehyde treatment. In Experiment 2, involving diets similar to those fed in Experiment 1 but in a ratio (straw:concentrate) of 3:2, growing wether sheep were fed the straw portion approximately ad libitum. Total diet digestibility was also unaffected although voluntary straw intakes were marginally reduced due to formaldehyde treatment. Rumen liquor ammonia-nitrogen and blood urea were also reduced due to treatment. In both Experiments 1 and 2 there was a gradual adjustment to low dietary protein with time, indicating a nitrogen economy by the sheep.

In Experiment 3 with beef cows during months 5-8 of pregnancy, urea, untreated and formaldehyde-treated soya bean meal were compared with molassed and unmolassed sugar-beet pulp and rolled barley as the main energy supplements. The overall results showed that, for the three main protein supplements, straw DM intakes were highest for urea and lowest for formaldehyde-treated soya bean meal, with untreated soya bean meal being intermediate. This apparent increase in straw intake in response to ruminal degradability of the source of supplementary protein was confirmed in a significant linear relationship between straw DM consumption and RDP intake for the twelve dietary treatments.

Rolled barley promoted higher straw intakes as an energy source than sugar-beet pulp.

In Experiment 4, with lactating beef cows and their calves, untreated and formaldehyde-treated soya bean meal were also fed with either molassed sugar-beet pulp or barley and straw was given ad libitum. Formaldehyde treatment did not reduce straw consumption and its effect in increasing milk yield was marginal. There was also evidence that straw consumption responded to RDP intakes although a protein x energy supplement interaction reduced the sensitivity of this relationship. For the main energy supplements, barley promoted higher intakes than molassed sugar-beet pulp.

In Section 2, other less popularly used protein sources, such as brewers grains and peas, were evaluated as protein supplements, in view of the increasing costs of feed supplements. In Experiment 5, the rumen degradabilities of both wet and dry brewers grains and their effects on voluntary straw intakes were studied. In Experiment 5.1 it was found that over half of the CP contained in wet or dry brewers grains was degraded in the rumen and that drying did not affect the effective degradability of protein in brewers grains. In Experiment 5.2 beef cows in months 5-8 of pregnancy were offered straw ad libitum and either dry or wet brewers grains unsupplemented or supplemented with urea. Neither voluntary straw intake nor digestibility was affected by treatment but significant linear relationships were found between RDP intake on the one hand and rumen ammonia nitrogen production, plasma urea and straw DM consumption on the other. There was also a significant linear relationship between rumen ammonia-nitrogen production and plasma urea concentrations.

In Experiment 6 the chemical composition of tropical and temperate varieties of peas was studied. One particular variety of temperate peas was further evaluated as a protein supplement by comparison with brewers grains and a combination of rolled barley and soya bean meal, with straw fed ad libitum to pregnant cows. The result of Experiment 6.1 showed that over half the CP contained in peas was degraded in the rumen and that there was little, if any, difference in chemical composition or rumen degradability between tropical and temperate pea varieties. In Experiment 6.2 in which lactating beef cows were fed straw ad libitum supplemented with either peas, dry brewers grains or rolled barley plus soya bean meal, the highest straw intakes and digestibility were recorded for cows fed barley plus soya bean meal,

while the highest milk yield and calf growth rates were recorded for cows fed brewers grains. Although peas per se were well digested, they were not well utilised and resulted in the highest live weight losses, despite their lower milk yield compared to other treatments.

In Section 3 the simultaneous influence of phosphorus and protein source on voluntary food intake by growing lambs was studied. Although there were no significant treatment effects of phosphorus supplementation or the source of protein, there were indications that, in the presence of phosphorus, marginal improvements in food intakes and growth rates were made. Similarly, urea marginally improved growth rates and food intakes over blood meal. The lack of effect of reduced phosphorus intake was explained in terms of the low blood phosphorus levels recorded which were known to have depressed feed intakes by lambs in previous experiments.

It is concluded that the ruminal degradation of supplementary dietary protein affects the intakes of low-protein roughage feeds. The rumen microbes need to be satisfied in terms of rumen ammonia-nitrogen concentrations if maximum potential intakes are to be achieved. Ruminants aged three months and over are able to utilise stored bone phosphorus in the short term to overcome inadequate phosphorus intakes.

ABBREVIATIONS

Statistical conventions

The following statistical conventions are used to indicate the probability of differences between means occurring by chance:

<u>Statistical Convention</u>	<u>Abbreviation</u>
Not significant	NS
Significant at the 5% level of probability	$P < 0.05^*$
Significant at the 1% level of probability	$P < 0.01^{**}$
Significant at the 0.1% level of probability	$P < 0.001^{***}$

Statistical terms

Degrees of freedom	df
Standard error of a mean	SE (s.e.)
Standard error of difference between means	SED (s.e. of difference)
Significance	Sig.
Least significant difference	LSD

Technical term

Dry matter	DM
Fresh matter	FM
Apparent digestibility of dry matter	DMD
Intake of dry matter	DMI
Organic matter	OM
Apparent digestibility of organic matter	OMD
Apparent digestibility of organic matter in the dry matter	DOMD
Intake of organic matter	OMI
Apparent metabolisable energy	ME
Crude fibre	CF
Crude protein	CP
Digestible crude protein	DCP

Technical termAbbreviation

Rumen-degraded (degradable) protein	RDP
Rumen-undegraded (undegradable) protein	UDP
Nitrogen	N
Biological value	BV
Ammonia nitrogen	NH ₃ -N
Total nitrogen	Total N
Beta hydroxy butyrate	3-OHB
Free fatty acids	FFA
Live weight	LW
Metabolic live weight	W ^{0.75}
Live weight gain	LWG
Head	hd
Day	d
Kilogramme	kg
Gram	g
Gram per kilogram	g/kg
Litre	l
Millilitre	ml
Centimetre	cm
Millimetre	mm
Hour	h
Variety	var.

Chemical terms

Molar	M
Hydrochloric acid	HCl
Hydrogen ion concentration, negative exponent	pH
Calcium	Ca
Phosphorus	P

Other termsAbbreviation

Soya bean meal

SBM

Formaldehyde-treated soya bean meal

FT-SBM

Urea

U

Blood meal

BM

Unmolassed sugar-beet pulp

USEP

Molassed sugar-beet pulp

MSBP

Dried brewers grains

DBG

Wet brewers grains

WBG

Experiment

Expt.

Figure

Fig.

INTRODUCTION

An important factor controlling animal productivity from poor quality roughages in general, but especially in the developing countries, is the amount of these roughages that animals will voluntarily consume. These roughages have often been considered as suitable components only of low output rations, a traditional concept that has thrived on their deficiencies, low digestibility and voluntary intake and a consequent poor feeding value.

In the case of crop residues, the deficiencies, mainly nitrogen, phosphorus and the vitamins are generally known to become more severe with increasing maturity of the crop. In the tropics, high ambient temperatures during a long dry season further aggravate the shortage of these nutrients by hastening the maturity of roughages already low in them. Without adequate supplementation of such roughages, the growth and activities of rumen micro-organisms are seriously inhibited and intakes are affected. Low intakes, especially at a time when seasonal effects further reduce the amount of pasture available, carry serious penalties of loss of body weight and condition for the animal. For young female breeding stock, this is also accompanied by delayed maturity relative to parturition.

While urea has often been demonstrated to be a suitable nitrogen source for maximising the voluntary intake of poor quality roughages such as straw, there are indications that natural nitrogen sources may be advantageous. More recently, arising from differences in microbial yield of various feedstuffs, the concept of total protein requirement of the animal, previously expressed as a percentage of the dietary dry matter or as an absolute intake, appears to be gradually changing in recognition of the independent needs of the rumen micro-organisms and those of the host animal. Differences between nitrogen sources are implicated in this gradual modification of the concept of the animal's protein needs. The importance of this in the use of inherently low protein feeds is therefore obvious. This is the basis for the studies reported in this thesis.

The experiments concerned the influence of the source of protein supplement on the voluntary intake, digestibility and utilization of poor quality roughage feeds. Young and adult sheep and subsequently pregnant and lactating beef cows were used to study these effects. The

choice of young sheep and pregnant and lactating cows for most of the experiments reported in this thesis was in view of the elevated nutrient needs of these animals and the possible stress that reduced intakes, presumably from dietary effects, might further cause. This, it was hoped, would make the effect of supplementation of poor quality roughage feeds more easily apparent and allow differentiation between alternative protein sources.

REVIEW OF THE LITERATURE

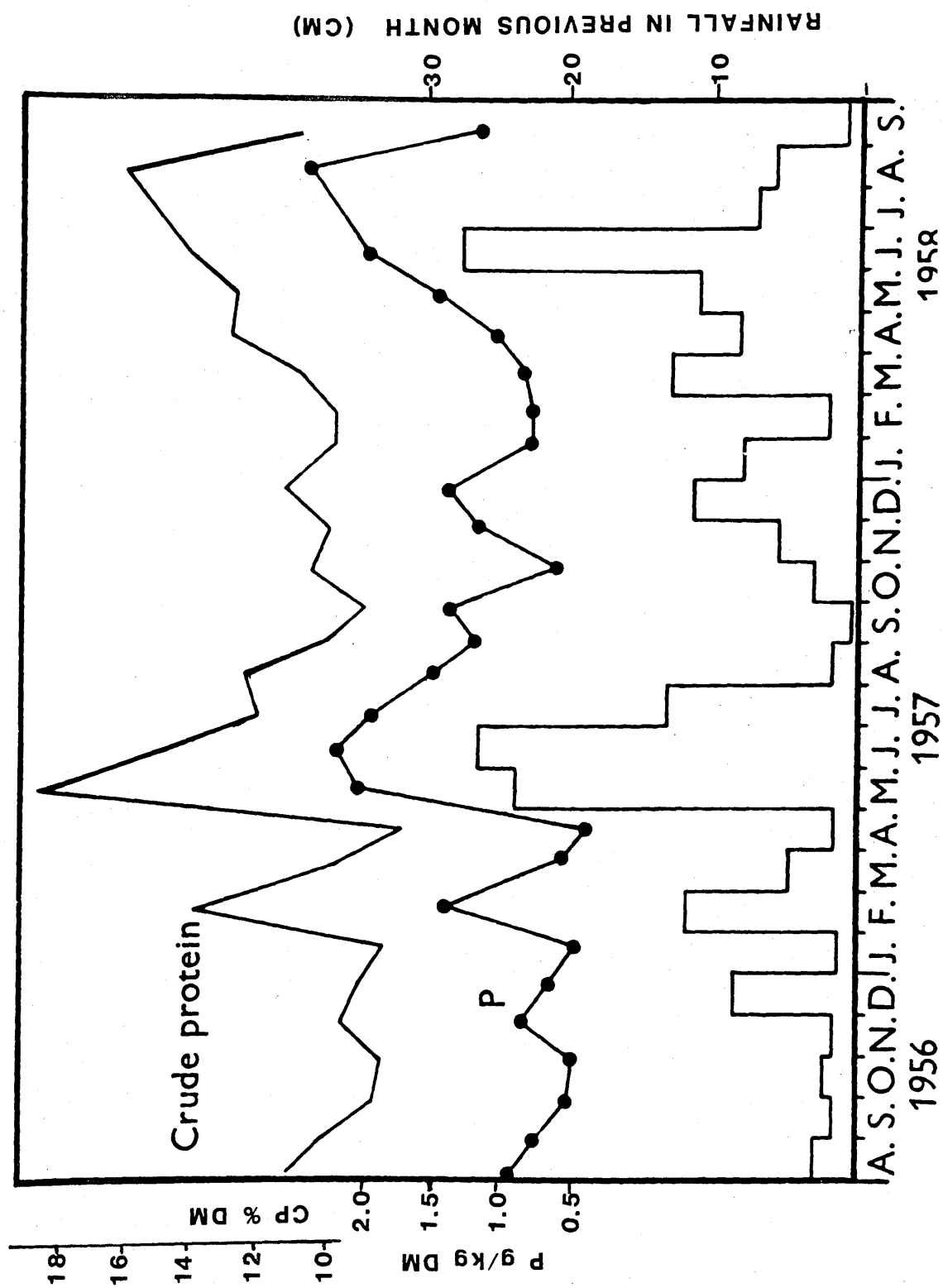
Potential and deficiencies of poor quality roughages.

The utilization of poor quality roughages in ruminant nutrition has taken on special importance because of the present efforts and the clear necessity to increase world food supplies. This class of roughages, which consists mainly of fibrous by-products of agricultural origin such as straw and other crop residues had hitherto not been extensively used but quite often burnt in the fields, dumped as wastes or disposed of in ways that constitute environmental problems. It is now known that when such 'wastes' are fed with the necessary supplements, they have a potential nutritional value capable of maintaining animals or drastically reducing live weight losses during periods of the year when natural grazing is not available or the quantity of feed is impaired. While this further emphasises the compatibility of crop and animal production, crop residues would cease to be wastes in this context.

Prominent among crop residues are remnants of grain production, mainly straw. The estimated quantity of straw DM produced in 1971, between 1300 and 1400 million tonnes, exceeded that of the total quantity of grain, 1113 million tonnes (FAO, 1971). The problem with straw lies in its well-defined nutritional limitations with proteins, the minerals especially phosphorus and the vitamins. It is also high in cell wall material, which is highly lignified. In the tropical regions of the world, grasses mature quickly and the hay and pastures available during dry periods of the year have such high fibre and low protein contents, they are at best nutritionally comparable to straw. For example, Ademosun (1973) noted that for Nigerian grasses, there is a rapid accumulation of highly lignified fibre at an early stage of growth with CP levels of between 10 and 30 g/kg which further decrease as the dry season advances. The same can be said of tropical grasses in general.

More recently, Bae, Gilman, Welch and Palmer (1983) also associated decreasing CP concentrations and digestibility of DM with increasing maturity, high cell wall and lignin. In an earlier report, Lampkin, Howard and Burdin (1961) also showed, as illustrated in Figure 1, that long dry periods, when there was no rainfall, were associated with a fall in the CP and P contents of East African pastures. It is also notable that they observed a significant correlation ($r = 0.72$)

Figure 1 Average phosphorus and crude protein content
of grass samples from three fields superimposed
on a histogram showing rainfall in the preceding
month (Lampkin et al., 1961)



between herbage P and CP concentration in those pastures. This further illustrates the effect of seasonal variation on the chemical composition of roughages mainly in tropical and subtropical environments. Of equal importance is the observation by Hemingway, MacPherson, Duthie and Brown (1968) that a high proportion of hays, about 98%, provide less than the recommended levels of P (ARC, 1965) for maintenance of cattle when fed alone, while almost 25% contain less than 50 g/kg crude protein. These deficiencies and the chemical nature of the roughages in question are frequently the main causes of poor digestibility, slow rate of passage and consequently a low voluntary intake by animals.

Methods to improve the nutritive value of poor-quality roughages.

Efforts have been made to overcome the problems of low digestibility and voluntary intake resulting from the deficiencies and chemical nature of straw and other poor-quality roughages. Such methods rely mostly on physical processing, chemical treatment and/or supplementation with the deficient nutrients.

Physical processing of poor-quality roughages which embraces particle size reduction, pressure cooking and irradiation has recently been reviewed by Walker (1984). The influence of milling, pelleting and wafering in improving the voluntary intake of roughages has also been the subject of other reviews by Minson (1963), Moore (1964), Beardsley (1964) and Greenhalgh and Wainman (1972), but there are doubts as to the extent to which the energy value of such processed roughages is increased (Greenhalgh, 1980). In addition the energy and time cost of modifying the physical characteristics of poor-quality roughages may make the use of such processed roughages uneconomic (Palmer, 1976).

Although several chemicals have been screened in the laboratory and in feeding experiments for potential to improve digestibility and voluntary intake, the most widely used appears to be sodium hydroxide (Klopfenstein, 1978). The use of sodium hydroxide (Rexen and Knudsen, 1984), ammonia (Sundstol and Coxworth, 1984) and other chemicals (Owen, Klopfenstein and Urio, 1984) to improve the digestibility and voluntary intake of straw and other fibrous roughages have recently been reviewed. The economics of chemical treatment is however questionable due to its support energy cost. In an evaluation of the 'value added' to straw by treatment with sodium hydroxide, Taylor, Lewis, Langley and

Yates (1977) estimated negative gross margins of £27 and £20/head for finishing and wintering store cattle respectively, fed concentrates and treated straw. Greenhalgh (1980) also concluded that the value of treated straw was too low to justify its inclusion in complete diets. More recently, losses (output less variable costs) of £14 and £19/head were also estimated for finishing beef cattle as a result of substituting 33% of the DM of silage based diets with farm and factory-treated straw respectively (Alawa, 1980).

In spite of the possible improvements in nutritive value from physical and chemical processing, poor quality roughages are still inherently deficient in nitrogen, the minerals and vitamins and will need supplementation with these nutrients. In any case, if straw of improved digestibility resulting from chemical treatment replaces cereals (with about 100 g CP/kg) there will be a need for extra supplementary protein which is itself expensive. Improvements in physical and chemical characteristics might therefore be regarded as 'optional extras' to supplementation (Greenhalgh, 1980). Consequently the remainder of this review and indeed the work to be described in this thesis will be restricted to supplementation of poor-quality roughages with the nutrients in which they are deficient.

Supplementation of poor-quality roughages

An important principle in the feeding of poor-quality roughages is that the potential intakes and digestibility can only be realised if other factors are not limiting. The most important factor in this context appears to be the quantity of nitrogen available to the rumen micro-organisms but there are also strong indications that rumen microbial activity is diminished by the lack of phosphorus in the diet (Komisarczuk, Merry and McAllan, 1985; Breeves, Hoeller and Lessman, 1985).

Effect of supplementation with protein

The complete absence or the presence of small amounts of nitrogenous compounds greatly limits the growth of the normal fauna in the rumen. As a consequence not enough of the consumed plant cell walls are ruptured and dissolved and their contents made accessible to the digestive processes in the abomasum and the intestine. When nitrogen is provided either as non-protein nitrogen or true protein, the rumen micro organisms are stimulated. They multiply and attack

fibre more vigorously. The primary effect is to increase digestibility of the roughage (Blaxter, Wainman and Wilson, 1961). An increase in digestibility may be accompanied by an increase in voluntary intake (Campling, Freer and Balch, 1962; Huber and Thomas, 1971; Oldham, Broster, Napper and Siviter, 1979; Wohlt, Clark and Blaisdell, 1978). In a study of the voluntary intake of low protein roughage feeds, Elliott and Topps (1963) obtained a close correlation ($r = 0.650$) between dietary nitrogen concentrations and voluntary intake. In the same experiment, although there was a small positive association between nitrogen content of the diet and digestibility, the authors concluded that there was no evidence of any relationship between digestibility and voluntary intake. They further suggested, based on their results, that with low protein foods, improved intake is not necessarily accompanied by increased digestibility. Coombe and Christian (1969) also found that increased digestibility was not important in determining food intake as shown by a significant negative relation between digestibility and intake of straw-based diets. However since dietary nitrogen concentration and digestibility are broadly correlated for roughage diets, this might be viewed as an expression of the general positive relationship between digestibility and voluntary consumption of roughage feeds. Egan (1965) considered that the magnitude and consistency of the intake response is greater when the nitrogen source is provided in the diet rather than when it is infused into the rumen. In contrast, nitrogen balance data (Chalmers, Cuthbertson and Synge, 1954; Reis, 1969) shows that postruminal administration of protein and amino acids gives better utilization of nitrogen than when the same source of nitrogen is offered in the diet. This may be more important in growth trials.

The problem of low protein content of roughage feeds is not always so easily solved. Given a satisfactory level of nitrogen in the diet, the amount of protein synthesized by the rumen microbes and indeed the rate at which they are able to ferment roughages is limited primarily by the amount of fermentable energy in the feed, (Burroughs, Nelson and Mertens, 1975; Egan, 1977).

Old methods for assessing protein requirements

There has been a continuous flow of publications on the magnitude, source and nature of nitrogen additions to various diets. In general, the methods for assessing the protein needs of animals, based on nitrogen balance trials, assumed that once the total protein needs were met, the nitrogen needs for all physiological and metabolic functions including digestibility and voluntary intake would be satisfied. At least until recently, only the total protein needs for maintenance and production were considered. Quantitative measurements were made in balance trials and the protein needs of the animal were subsequently expressed either as a percentage of the diet DM or as an absolute intake.

A wide range of concentrations of CP ($N \times 6.25$) in the diet DM have been used by various workers. However Burroughs, Gerlaugh, Edington and Bethke (1949) and Hungate (1966) suggested that for cattle, if less than 11 to 12% CP in feed DM were fed, rumen fermentation and total digestion were likely to decrease with a possible reduction in voluntary feed intake and utilization of roughage nutrients.

As an absolute intake, assessed values of the required levels of CP ($N \times 6.25$) in the diet were presented as DCP, and shown in standard tables of nutrient requirements (e.g. ADAS, 1976). Such values may have been more important for growth and productivity and less important in deciding dietary levels for voluntary intake. The DCP needs of animals were arrived at by using a factorial method which recognises (i) losses of protein from the body (ii) retention of protein during growth, pregnancy and lactation (iii) secretions of protein in milk and the BV of dietary protein (ARC, 1965). While a margin of excess may have been allowed in the tabulated values of DCP requirements, a study of the literature (Klosterman, Bolin, Buchanan, Bolin and Dinusson, 1953; Elliott and Topps, 1964; Robinson and Forbes, 1967) shows variations in the values that have been considered adequate. Elliott and Topps (1964) observed that nitrogen requirements for maintenance were dependent upon the composition of the diet, as increasing the proportions of ground roughage in the diet resulted in substantial increments in digestible nitrogen required for nitrogen equilibrium. It is as a result of this observation that Elliott and Topps (1964) and Broster, Tuck, Smith and Johnson (1969) suggested that any statement of nitrogen requirements for maintenance of ruminants or as a percentage

of protein in feed DM should be qualified by information on the type and amount of other dietary components and particularly of energy-yielding components of the diet.

New method for assessing nitrogen requirements.

The protein value of a feedstuff is determined by the amount and pattern of amino acids it provides for absorption in the small intestine (Miller, 1982). But for ruminants, microbial protein accounts for a significant amount, about 70% (BP Nutrition, personal communication), of the amino acids absorbed in the small intestine. Microbial protein synthesis is therefore an important consideration in ruminant feeding, complemented only by protein which passes through the rumen intact.

However differences in microbial protein yield with different feedstuffs have become apparent (McAllan and Smith, 1974) and although a high protein degradability is wasteful since much of the degraded protein, as nitrogen, may be lost to the animal, the reverse could possibly reduce microbial protein yield. On the other hand a relatively less degradable protein generates a higher non-ammonia nitrogen (NAN) flow to the duodenum (Mathers and Miller, 1982; Sriskandarajah, Kellaway and Leibholz, 1982) which can then be digested directly by the host animal and used to further improve productivity (Slen and Whiting 1954; Ross, Topps and Paterson, 1981).

These findings have rendered the previous procedure for evaluating feedstuffs as protein sources for ruminants, the DCP system, inadequate. Consequently new methods for determining nitrogen requirements (ARC, 1980) have focussed attention on microbial needs for nitrogen separately from the needs of the host animal, an earlier proposition by Orskov (1970). Additionally, this concept recognizes the need for specific energy input (Buttery and Lewis, 1982; Smith, 1982) if the nitrogen available in the rumen is to be efficiently utilized for synthesis of microbial protein. In this regard, the new concept has one factor, energy restrictions on protein utilization, in common with the DCP system, as discussed earlier.

Given a readily available source of energy, microbial protein yield is related to the amount of organic matter fermented and vice versa. Hagemeister, Luping and Kaufmann (1980) used data from 75 of their own trials involving the use of cows with duodenal re-entrant cannulae to show a close relationship ($r = 0.81$) between microbial

protein synthesis and the amount of organic matter (OM) fermented. Viewed from the broad positive relationship between digestibility of OM and voluntary food intake, a higher yield of microbial protein in the rumen could result in increased intake. A highly significant relationship ($P < 0.01$) between microbial protein synthesis and voluntary intake of a roughage diet observed by Singh, Verma, Varma and Ranjhan (1977) is of direct relevance.

Rumen degradation of proteins

In evaluating various protein sources in terms of the extent to which they meet the needs of the rumen micro-organisms and those of the host animal, the basic principle is that of ruminal degradation. Mehrez and Orskov (1977) have described the use of a nylon bag technique to study the degradation of protein supplements. In conjunction with the outflow rate of chromium-treated particles from the rumen (Garnev, Orskov and Smart, 1979) or in faeces (Eliman and Orskov, 1984), the technique may be used to obtain estimates of the effective comparative nitrogen degradation.

When protein-rich feed is consumed, the ingested feed is exposed to rumen micro-organisms. These micro-organisms break down the protein to ammonia which they incorporate into microbial protein using any available source of energy. The proportion of protein broken down in this way, rumen-degraded protein, varies with different protein sources. Although it is claimed that the ease of protein breakdown is a function of its solubility (Henderickx and Martin, 1963; Ring and Buttery, 1968; Craig and Broderick, 1981), MacDonald, Edwards and Greenhalgh (1973) suggest that such a claim does not survive critical examination since casein which is readily degraded in the rumen is not readily soluble, while albumin which is resistant to breakdown in the rumen is readily soluble. Also in a previous study on the rate of proteolysis of casein and ovalbumin, Mangan (1972) concluded that although ovalbumin is a soluble protein, its cyclic structure, in which there are no terminal amino- or terminal carboxyl- groups precluded degradation by proteolytic enzymes of the carboxyl peptidase or the leucine amino peptidase types which explained why ovalbumin was not readily degraded by rumen micro-organisms. Further evidence to counter claims of a relationship between protein solubility and degradation have been shown by Mertens (1977), Pichard and Van Soest (1977) and Mahadevan, Erfle and Sauer (1980) who found that considerable amounts

of the protein degraded by rumen microbes in vivo were insoluble in mineral solvents. In the study by Mahadevan et al. (1980), which tested the resistance of a variety of proteins to the protease from Bacteroides amylophilus, they concluded that the structural characteristics of a protein are important in influencing its rate of breakdown and that changes in these often increase its susceptibility to attack. The observation by Mehrez, Orskov and Opstredt (1980) that the breakdown of fishmeal in the rumen is markedly influenced by the processing method may be due to this, since processing would normally affect the physical characteristics of a protein. Chalupa (1975) in his review on rumen bypass of proteins also observed that normal processing procedures used in the manufacture of feed ingredients such as solvent extraction of grains may also influence the magnitude of degradation of proteins through alteration of the structural characteristics of these feedstuffs.

Reports indicate that the rate and extent of protein degradation in the rumen is affected by rumen retention time and the basal diet. For example, Ganey et al. (1979) found that rates of degradation are slightly lower when diets are fed ad libitum than when they are given as restricted feeding, because of the enhanced rate of passage from the rumen. They also showed that the rate of digestion was faster in the rumen of sheep receiving dried grass than in those fed concentrate-based diets because of the rapid digestion of cellulose. The latter effect, type of diet, is related to rumen pH (Orskov, 1982) since a rumen environment that can support a high rate of cellulose digestion also results in a higher rate of ruminal protein degradation. Other reports (Wallace, 1979) also suggest that degradation of gluten protein is lower at a low rumen ammonia concentration than with higher levels of ammonia.

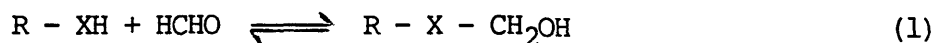
Despite the extensive microbial activities in the rumen, some proteins escape the action of the rumen microbes and pass on to the small intestine where the enzymes of the host animal digest them. The proportion of protein that resists degradation in the rumen, rumen-undegraded protein, also varies with different protein supplements.

It would be extremely useful if the rumen microbes degraded protein supplements only to the extent required to optimise their cell yields. Such a situation would also help to overcome problems of synchronization between the availability of energy and degraded

nitrogen. However, the rumen microbes quite often degrade protein supplements beyond their needs. In this way some proteins which are highly susceptible to rumen degradation are almost completely degraded, thus leaving little or no undegraded protein for post-ruminal digestion. Consequently, attention has been given to the protection of some proteins so that an increased proportion can escape microbial action. A summary of the methods employed to achieve this is given in Table 1. Of these methods, chemical treatment appears to be the most popular so far employed. It is claimed that formaldehyde is more efficient in protecting proteins than other aldehydes (BP Nutrition, personal communication).

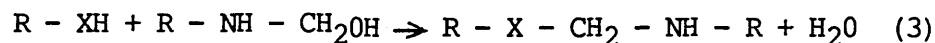
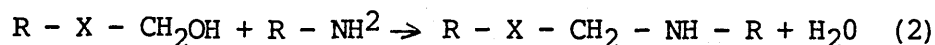
Formaldehyde treatment of proteins

A survey of the literature on reactions between formaldehyde and proteins has been made (Van Dooren, 1972) and the chemical reactions involved have been summarised (Barry, 1976). It is essentially a Maillard reaction between sugar aldehyde groups and free terminal amino groups of proteins. The initial step is thought to be a rapid formation of a methylol compound:-



where R is the acidic carboxyl unit and XH may be the terminal amino group.

This step is followed by two condensation reactions:-



with the formation of stable methylene cross-linkages.

These cross-linkages are thought to be acid-reversible rendering the protein insoluble at the pH of the rumen, 5.5 to 6.5 (MacRae, Ulyatt, Pearce and Hendtlass, 1972) but they are destroyed in the abomasum (pH 2 to 3) and the small intestine where the protein is digested (Ferguson, Hemsley and Reis, 1967).

According to BP Nutrition (personal communication), the minimum maturation time, that is the time needed to allow the methylene cross-linkages to stabilise, is from 5 to 8 days at ambient

Table 1

Examples of processing methods for changing rumen degradability of protein.

A Chemical Treatments.

<u>Formaldehyde</u>	Objective of experiment	Effect (positive(+) negative(-) no effect (o))	Author(s)
Soya bean meal	Growth	+	Peter <u>et al</u> (1971)
	"	--	Schmidt <u>et al</u> (1973a)
	"	o	" " (1973b)
	"	-	" " (1974)
	"	-	Wachira <u>et al</u> (1974)
	"	--	Thomas <u>et al</u> (1979a)
	"	-	" " (1979b)
	"	++	Spears <u>et al</u> (1980)
	Milk production	o	Clark <u>et al</u> (1974)
	"	o	Folman <u>et al</u> (1981)
	"	+	Lundquist <u>et al</u> (1982)
	"	-	Oldham <u>et al</u> (1982)
	"	+	Rees and Rowlinson (1983)
	"	o	Crooker <u>et al</u> (1983)
	"	o	Crawford & Hoover (1984)
	Nitrogen digestibility	--	Nishimuta <u>et al</u> (1973)
	"	-	Rooke <u>et al</u> (1981)
	"	-	" " (1983)
	Nitrogen balance	+	Amos <u>et al</u> (1974)
	Degradability study	+	Freer & Dove (1984)
Casein	Wool production	+	Reis & Tunks (1969)
	Total digestibility	o	Faichney & Weston (1971)
	Nitrogen digestibility	o	MacRae <u>et al</u> (1972)

Rapeseed meal	Nitrogen		
	digestibility	-	Rooke <u>et al</u> (1983)
Sunflower meal	Nitrogen balance	o	Amos <u>et al</u> (1974)
Whey protein	Milk production	++	Muller <u>et al</u> (1975)

Acetic acid

Soya bean meal	Nitrogen		
	digestibility	-	Tilman & Kruse (1962)

Tannic acid

Soya bean meal	Growth	+	Dreidger <u>et al</u> (1969)
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Glyoxal

Fishmeal	Growth	++	Nimrick <u>et al</u> (1972)
Soya bean meal	"	--	Schmidt <u>et al</u> (1973b)

Hexamethylenetetramine

Soya bean meal	Growth	-	Schmidt <u>et al</u> (1973b)
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B Heat Treatments

Soya bean meal	Growth	++	Sherrod & Tilman (1962)
	"	++	Glimp <u>et al</u> (1967)
	"	+	Hudson <u>et al</u> (1969)
	"	-	Thomas <u>et al</u> (1979b)
	Total		
	Digestibility	-	Tilman & Kruse (1962)
	"	-	Nishimuta <u>et al</u> (1973)

C Grinding

Soya bean meal	Total digestibility		
	and intake	--	Netemeyer (1980)
	Milk production	o	Crawford & Hoover (1984)

D Pelleting

Soya bean meal	Degradability		
	study	++	Freer & Dove (1984)

temperature. Although formaldehyde treatment of proteins has been found to increase the amount of protein escaping rumen microbial action (Peter, Hatfield, Owens and Garrigus, 1971), it may also reduce the lysine content of proteins such as soya bean meal and rapeseed meal (Rooke, Brookes and Armstrong, 1983).

Heat treatment of proteins

The use of heat to increase protein resistance to ruminal degradation is based on changing the physical configuration of the protein. It has been shown that heat treatment reduces solubility of nitrogen (Tagari, Ascarelli and Bondi, 1962; Danke, Sherrod, Nelson and Tilman, 1966), an effect that is claimed to improve nitrogen utilisation (Little, Burroughs and Woods, 1963) and feed efficiency (Hudson, Glimp, Little and Woolfolk, 1969). Treatment temperature is the critical factor as high temperatures may change the protein to the extent that it is much less digestible than originally intended. Harris and Mattill (1940) concluded that heat-treated proteins may also have a lower nutritive value than the untreated form as a result of the formation of new enzyme-resistant configurations involving amino acids. The amino acids may become either less digestible or are less utilisable after digestion.

Reduction in particle size of proteins

The concept of increasing the amount of protein that bypasses the rumen through a change in its particle size appears to be based on the effect of particle size on digestion and intake. Netemeyer, Bush and Owens (1980) reported a decrease in ruminal degradation of soya bean meal due to fine grinding, presumably due to an increased passage rate. Also Crawford and Hoover (1984) reported positive effects of grinding soya bean meal on intake, body weight and efficiency of feed-to-milk conversion. They suggested that the effect of particle size in general is mediated through changes in (i) rate and extent of digestion due to a change in surface area and (ii) rate of passage. While a reduction in particle size, as in grinding, would be expected to increase the rate of degradation because of the greater surface area that is exposed, it appeared that the increased rate of passage from the rumen as a result of grinding overcame any increase in the rate of degradation in the work of Netemeyer et al. (1980).

An assessment of protein sources with regard to rumen degradation

In a review of rumen bypass and protection of protein, Chalupa (1975) concluded that between 40 and 80% of dietary protein might be degraded in the rumen. As discussed earlier, the various protein supplements differ in the degree to which they are susceptible to rumen degradation and numerous reports abound in the literature regarding comparisons of the degradability of various protein supplements. An examination of the values presented in these reports suggests an enormous variability between laboratories. For example, Wilson and Strachan (1980) reported that for soya bean meal, various workers have obtained in vivo degradability values of 0.39, 0.43, 0.55 and 0.74. They also reported that for fish meal, values of 0.29, 0.31, 0.38 and 0.67 have been obtained, thus indicating the variation in reported protein degradability values. This is to be expected since aspects of the methodology for estimating protein degradability have not been standardised. As such, the assessment of protein sources in terms of ruminal degradability, in this review, will be approached separately for each class of protein.

Non-protein nitrogen (NPN) supplements

The most popularly used of these, urea, is rapidly and completely hydrolysed in the rumen (Gonzalez, Robinson, McHattie and Mehrez, 1979) leading to possibly excessive amounts of ammonia in the rumen (Armstrong and Trinder, 1966) which may cause ammonia toxicity and, in some cases, death (Hemingway, Parkins and Ritchie, 1972). Consequently, it is often advised that urea be given frequently in small amounts. Moreover, it is often claimed that giving urea frequently in small amounts improves nitrogen utilisation (Bloomfield, Welsch, Garner and Muhrer, 1961) since urea hydrolysis occurs at a rate 4 times faster than the corresponding uptake of ammonia by rumen micro-organisms (Bloomfield, Garner and Muhrer, 1960). In a subsequent trial involving straw diets, Kendall (1977) found no difference in nitrogen utilisation when cows were fed 60 g of urea daily either in one meal or in ten small meals. The voluntary intakes and digestibility of straw DM were 7.31 kg and 0.56 for cows fed urea once per day and 7.34 and 0.56 for those fed urea in ten small meals.

Some modified NPN compounds which have been shown to be more slowly hydrolysed include biuret (Broome, 1968), isobutylidene diurea (Parkins, Ritchie and Hemingway, 1971), urea phosphate (Ritchie,

Parkins and Hemingway, 1972; Hemingway and Law, 1975). Other alternatives to rapid hydrolysis of urea have been to give it in complexed forms such as 'Dehy-100' (consisting of pelleted urea with alfalfa meal, dicalcium phosphate, sodium sulphate and sodium propionate) (Conrad, Hibbs and Neuhardt, 1969), 'Starea' (consisting of expanded grains and urea) (Helmer, Bartley, De Yoe, Meyer and Pfost, 1970), 'Triple nuts' (consisting of cubed molassed sugar-beet pulp with added urea, dicalcium phosphate, trace elements and vitamins) (Parkins, Hemingway and Ritchie, 1974) and liquid supplement (consisting of urea, phosphoric acid, calcium and sodium chloride, molasses and water) (Fishwick, Parkins, Hemingway and Ritchie, 1978). A large number of other organic and inorganic compounds, about sixty, have been screened in the laboratory (Belasco, 1954) for the potential to supply dietary nitrogen, with urea as the standard.

The extent of hydrolysis of NPN compounds and the complete absence of amino acids means that it does not provide any bypass protein for the animal which therefore has to depend on the spectrum of amino acids provided by microbial protein. Although the amino acid content of microbial protein is fairly consistent and does not appear to be influenced by diet (Purser, 1970), provision of bypass proteins in straw diets supplemented with urea has been demonstrated to increase feed intakes and growth rates of cattle (Smith, Broster and Hill, 1980; Sriskandarajah, Kellaway and Leibholz, 1982; Smith, Siviter and Merry, 1985). Thomas, Katz, Auld and Peterson (1984) found that urea caused significantly higher intakes by lambs on diets containing 15% straw with a consequent higher growth rate than cotton seed meal, rapeseed meal or safflower meal. This finding suggests that for low quality roughages, the amount of nitrogen degraded in the rumen may be important.

Vegetable protein supplements

Vegetable protein supplements consisting mainly of oilseed cakes and meals, e.g. soya bean meal, cottonseed meal, groundnut meal, palm kernel meal and linseed meal, appear to be intermediate in degradability between non-protein nitrogen and animal protein supplements. However, they vary largely in rate and extent of degradation. The ruminal degradation of some vegetable protein supplements that have been studied include:- Groundnut meal and soya bean meal, 0.47 at 24 h (Gonzalez et al., 1979); rapeseed meal, 0.64

at 9 h (Setälä and Syrjälä-Qvist, 1984); sunflower meal, 0.66 at 24 h (Garnev et al., 1979). Orskov, Hughes-Jones and McDonald (1980) have given effective comparative degradability values for some other vegetable protein supplements estimated at various outflow rates from the rumen.

The most popularly used vegetable protein supplement, soya bean meal, is degraded to a considerable extent. Consequently, attempts have been made to protect soya bean meal by treatment with chemicals. Formaldehyde-treated soya bean meal, manufactured commercially, is marketed under the trade name 'Sopralin' by BP Nutrition (U.K.) Ltd. It is claimed by BP Nutrition that treatment reduces the 24 h ruminal degradability of soya bean meal from about 95% to around 28%.

A considerable number of studies have been carried out on the effect of reduced degradability, mostly of vegetable proteins, on milk yield. However, Twigge and Van Gils (1984) have surveyed experimental results and concluded that the effect of undegraded protein on milk yield is inconsistent. They also cited a situation (Baraton and Pflimlin, 1978) in which formaldehyde treatment of soya bean meal had a negative effect on milk yield of dairy cows fed a basal diet of maize silage, as a result of inadequate RDP supply (less than 500g) for rumen microbes. In this situation a supplement of RDP proved more beneficial. Despite this understanding of the importance of RDP on performance, presumably through its effect on the rumen environment, there is limited information on how the degradability of dietary protein supplied from vegetable sources affects the voluntary consumption of roughages.

Animal protein supplements

Fish meal, bone meal and meat and bone meal are among animal protein sources that have been shown to be relatively resistant to degradation (Gonzalez et al., 1979; Orskov, 1982). Data presented by Orskov et al., (1980) show that fishmeal and meat and bone meal were more resistant to degradation, possibly because of heat treatment, than vegetable protein supplements such as cotton seed meal, linseed meal, soya bean meal and groundnut meal. Although the effect of the relative resistance of animal protein supplements to ruminal degradation on rumen liquor characteristics and voluntary feed intake has not been studied in detail, the high level of bypass protein they provide has been claimed to improve milk production in sheep (Robinson, McHattie,

Calderon Cortes and Thompson, 1979; Gonzalez, Robinson, McHattie and Fraser, 1982). In many of the experiments involving the use of relatively resistant forms of protein, feeding was equalised. As such, the possible affects of protein degradability on voluntary food consumption could not be assessed.

Effect of phosphorus supplementation

The overall implications of phosphorus (P) inadequacy in ruminant diets especially when poor quality roughages are fed have been discussed by Hemingway (1967). They culminate in retarded growth, poor reproductive performance, reduced milk yield and wool growth and impaired skeletal and dental health. The effects appear to be more easily apparent during periods of stress to the animal or when nutrient needs are high. For example, Fishwick, Fraser, Hemingway, Parkins and Ritchie (1977a) fed two groups of cows on 1.35 kg molassed sugar-beet pulp for the last 16 weeks of pregnancy and 3.15 kg for the first 6 weeks of lactation, together with oat straw ad libium. In addition, one group received supplementary dicalcium phosphate increasing their total P intake from about 12 to 28 g/day, while the other group did not. In the absence of supplementary P, there was a marked and significant reduction in blood P during the 4th week of lactation. During pregnancy, a severely reduced P intake did not reduce digestibility and voluntary intake of straw or the birth weight of calves. Bass, Fishwick, Hemingway, Parkins and Ritchie (1981a) similarly fed two groups of cows during pregnancy and lactation with one group receiving 3.7 g supplementary P in addition to the basal diet. Although there was a reduction in blood P concentration of cows which did not receive supplementary P, digestibility and voluntary intake of straw were not affected during pregnancy. After calving, the voluntary intake and digestibility of straw and blood P concentrations of the cows which received no P supplement were severely reduced.

Wilson (1981) investigated the effects of a low phosphorus intake on the voluntary feed consumption of ewes in pregnancy and lactation. Blackface ewes 2-4 weeks pregnant were given either a basal diet of barley husk siftings, molassed sugar-beet pulp and urea or supplemented with dicalcium phosphate. The amounts of feed given were increased progressively from 0.9 to 1.5 kg/day as pregnancy proceeded and to 2.0 kg after lambing. The comparable phosphorus intakes in pregnancy were

from 0.5 to 0.85 g P/day (low P diet) and 3.3 to 5.5 g P/day (supplemented diet). Over the first ten weeks of pregnancy the blood phosphorus concentration fell steadily from about 5.9 to 1.2 mg/100 ml (low P group). In contrast, the concentration in the supplemented group fell to only about 5.0 mg/100 ml. From about 7 weeks prior to lambing, when the blood P concentration was below 1.7 mg/100 ml, the low P group had a reduced appetite and failed to consume more than 1.0 - 1.2 kg of the diet/day. The supplemented group consumed all the feed presented to them up to the limit of 1.5 kg/day in late pregnancy or 2.0 kg in late lactation. The lambs born to the low P ewes were smaller, death rates much higher and lamb live-weight gains were severely reduced.

These results are in apparent contrast to those recorded by Fishwick et al. (1977a) and Bass et al. (1981a) for beef cows and their calves, where reductions in voluntary feed intake did not appear in pregnancy but only in lactation. The reason is probably that pregnancy causes a greater stress on the ewe than on the cow. For example, a 500 kg cow may produce a calf weighing 35 kg (14:1 ratio) but a 50 kg ewe may produce a single lamb of say 4.5 kg (11:1 ratio) or two 4.0 kg twins (6:1 ratio). Wilson (1981) recorded that appetite was reduced more rapidly in ewes carrying twin lambs than in those carrying only a single lamb. What is noteworthy is that for both cows (Fishwick et al., 1977a and Bass et al., 1981a) and ewes (Wilson, 1981), the reduction in voluntary feed intake occurred when the blood phosphorus fell to about 3.0 mg/100 ml or less.

In other work, Henry, Gueguen and Rerat (1979) associated a decrease in voluntary food intake by rats offered a low phosphorus diet with severe phosphorus deficiency. Also Field, Suttle and Nisbet (1975) found that appetite in growing lambs was reduced one week after the introduction of a low phosphorus diet.

The reductions in voluntary intake in the experiments cited above were accompanied by marked reductions in the intake of metabolisable energy which carry serious consequences for lactating animals in terms of body weight and condition. The depressive effects of phosphorus inadequacy on voluntary food intake in particular may be a direct consequence of phosphorus depletion on microbial protein synthesis recently reported by Breeves et al (1985) in a phosphorus depletion-repletion study. The above authors demonstrated a highly significant reduction in microbial protein synthesis resulting from

feeding a phosphorus-deficient diet. A further observation by Komisarczuk et al (1985) indicates significant reductions in total volatile fatty acid concentrations at phosphorus concentrations below 1 mg/litre, while ATP concentrations were significantly reduced when phosphorus concentrations were less than 48 mg/litre. Since rumen bacterial cells contain 2-6% phosphorus on a dry weight basis (Hungate, 1966), their growth and activities would presumably be diminished by inadequate dietary phosphorus levels.

Conclusions

Rumen fermentation results in the conversion of some of the feed proteins to ammonia nitrogen, part of which is captured and incorporated into microbial protein. If microbial protein accounts for about 70% of the protein digested and absorbed distal to the rumen, then there must be equal interest in the degraded component of supplementary protein as there is in the undegraded portion since (1) qualitatively and quantitatively, microbial protein accounts for a significant amount of amino acids absorbed by the animal and (2) rumen digestion and voluntary intakes depend largely on rumen microbial growth. However, there seems to have been more concern over the bypass portion of supplementary protein, since it is often assumed that rumen microbes degrade protein supplements sufficiently to satisfy their needs. Less attention has consequently been given to the influence of protein degradability or the effect of reducing protein degradability on the voluntary intake of roughages. The following sections of this thesis are, therefore, intended to investigate the effects of supplementary protein degradability on voluntary food intake. In view of the critical importance of supplementary phosphorus in poor quality roughage diets, it is also intended to examine the effects of supplementary phosphorus on voluntary food intakes.

SECTION 1
FORMALDEHYDE-TREATED SOYA BEAN MEAL: EFFECT ON THE
NUTRITIVE VALUE OF STRAW DIETS

Introduction

The digestibility and voluntary intake of straw-based diets by ruminants is generally dependent upon the provision of supplementary nitrogen. Whilst urea has been demonstrated (e.g. Campling, Freer and Balch, 1962) to be an appropriate source, it is rapidly and completely hydrolysed in the rumen leading to potentially serious wastage of nitrogen. One method of reducing the rumen degradation of proteins, thereby increasing the quantity of protein that bypasses the rumen, is to treat the protein supplement to make it less degradable. However, extensive investigations indicate that, while formaldehyde treatment of proteins such as casein has resulted in consistently improved lamb weight gains and wool growth (Wright, 1971; MacRae *et al*, 1972; Hemsley, Reis and Downes, 1973), treatment of soya bean meal without adversely affecting microbial metabolism, microbial protein production, intestinal digestion and absorption may be difficult to achieve (Schmidt, Jorgensen, Benevenga and Brungardt, 1973a; Schmidt, Benevenga and Jorgensen, 1973b). However, none of the experimental work appears to have examined the possible effects of reduced rumen degradability of nitrogen on the digestibility and voluntary intake of low-quality roughage feeds for which supplementary nitrogen is perhaps vital. It is possible that reduced nitrogen degradability may, to some extent, sacrifice the needs of the rumen microbes for rumen-degraded nitrogen.

This section of the thesis, therefore, investigates the effects of changing the rumen degradation of the protein in soya bean meal by treatment with formaldehyde on the intake and nutritive value of straw generally, and the nitrogen metabolism of sheep when given over extended periods.

Experiment 1 - The influence of formaldehyde treatment of soya bean meal on the digestibility of straw diets and nitrogen metabolism of sheep

Introduction

The objective of Experiment 1 was to examine the digestibility of straw as affected by supplementation with formaldehyde-treated soya bean meal. At a low level of supplementary CP (about 4%) in the diet DM, it was intended to determine whether formaldehyde treatment might so limit the RDP available to rumen microbes that the plasma nitrogen pool might be depleted over a period of time and that diet digestibility might be reduced as a consequence.

Materials and Methods

Animals and treatments

Eight 16-month old Suffolk x Greyface wether sheep averaging 60 kg live weight (55 to 72 kg) were paired on a live weight basis and randomly allocated to either a control diet supplemented with untreated soya bean meal (SBM) or to a diet (FT-SBM) prepared by substituting untreated soya bean meal with the same quantity of formaldehyde-treated soya bean meal (0.25% of a 30% solution) (Sopralin, BP Nutrition (U.K.) Ltd.). The sheep were dozed with 10 ml/hd of Thiabendazole before the experiment commenced and they were housed in metabolism cages throughout the experimental period.

Diets and feeding

A mixed diet consisting of chopped (2-3 cm) barley straw and concentrate (3:1 on fresh weight basis) was used. The total daily intake of straw and concentrate mixture was held constant at 1.0 kg fresh weight. The amounts of dietary constituents and the estimated amounts of RDP and UDP (N x 6.25) and ME content of the diets, which were isonitrogenous and isocaloric, are shown in Table 2.

The degradabilities (g/kg) of the protein (N x 6.25) in the concentrate feeds were SBP ^{0.092} 92, SBM ^{0.515} 51.5, FT-SBM ^{0.092} 92. The RDP and UDP were estimated using the nylon bag technique as detailed in Appendix 1. The RDP from straw was considered negligible as the estimated degradability of CP in chopped samples of straw used in this experiment showed a net gain (g/kg incubated) of 0.0006 g (SE \pm 0.0011) of nitrogen in the nylon bags after incubation for 24 h.

Table 2 Experiment 1

The amounts of concentrate supplements and straw given to each sheep/day and the estimated amounts (g) of crude protein (CP), rumen-degraded protein (RDP) and undegraded protein (UDP) and metabolisable energy (MJ/kg DM) supplied by each diet.

	SBM	FT-SBM
Soya bean meal	50	-
Formaldehyde-treated soya bean meal	-	50
Unmolassed dried sugar-beet pulp (SBP)	200	200
Trace elements, vitamin and mineral supplement	19.5	19.5
Barley straw	750	750
CP	57.1	57.3
Estimated RDP	13.4	3.7
Estimated UDP	43.7	53.6
Estimated ME	7.7	7.7

+ CP in straw assumed to be completely undegradable.

All the concentrate was given in one feed/day and fully consumed at 07.30 h. The straw was fed in two approximately equal feeds at 08.00 and 16.00 h. The animals were fed for a preliminary period of 18 days in individual pens and then in cages for 35 days during which a series of five digestibility and nitrogen balance trials, each consisting of 17 days were conducted.

The proximate compositions of the various dietary constituents are given in Table 3.

Animal procedure

The sheep were harnessed for collection of faeces. Quantitative measurements were made of food consumed and urinary and faecal losses. The routine procedure used in this and subsequent digestibility trials is described in Appendix 2. Additionally, urine free of feed particles and faeces was collected daily in dilute (25%) HCl sufficient to maintain the liquid at a pH of 2.0, as determined by a pH test paper to minimise loss of ammonia.

At the end of every feeding period, the volumes of urine were measured and total faeces weighed and a representative fresh sample of each was obtained. One hundred grams of the fresh faeces were macerated with water and a small amount of toluene as described by the Grassland Research Institute (Commonwealth Bureau of Pastures and Field Crops, 1961) for nitrogen determination, while the remainder was dried to constant weight at 95°C. for chemical analysis. The results of the analysis were used to calculate the digestibility of diets.

Heparinised blood samples were obtained from the jugular vein of each animal two hours after the start of feeding on the last day of each collection period. The blood was immediately centrifuged and the plasma stored at -20°C. for plasma urea determination. In order to avoid stress on animals arising from sampling of rumen fluid during total collection periods, samples were taken, using a stomach tube under vacuum, on the day after the final collection period, as described in Appendix 3.

Immediately following withdrawal, rumen liquor from each animal was strained through four layers of muslin and 1 ml added to 9 ml of a solution containing 1.5 g sodium tungstate and 15 ml of 0.5M sulphuric acid made up to 200 ml with distilled water for determination of the concentration of ammonia nitrogen.

The sheep were weighed before and at the end of the experiment.

Table 3 Experiment 1

Proximate analysis of dietary constituents (g/kg DM)

	Straw	SBP	SBM	FT-SBM
Dry matter	861	832	871	877
Organic matter	938	929	928	929
Crude protein	27	100	528	530
Crude fibre	428	203	53	55
Ether Extract	11	2	12	10
⁺ N-free extract	471	624	335	334
Ash	62	71	72	71
Gross Energy (MJ/kg DM)	17.7	16.6	19.3	19.5

⁺ N-free extract was calculated by difference.

Sampling of feeds

Throughout the experiment samples of dietary constituents were obtained every other day. These were bulked at the end of the experiment and dried to constant weight.

Analyses

Dried feed and faecal samples were subsequently milled through a 0.8 mm screen in a laboratory mill (Christy and Norris 8-inch laboratory mill) for chemical analyses. The analytical methods used for the proximate constituents of feed and faeces and for plasma urea and $\text{NH}_3 - \text{N}$ in rumen liquor are outlined in Appendix 4.

Statistical analysis was done on the data for each collection period and on the pooled means for all periods by means of t-tests for paired comparisons.

Results

There was no change in live weight of the animals at the end of the experiment. The data for apparent digestibility coefficient of dry matter and organic matter are presented in Table 4 and for crude fibre and energy in Table 5. The digestibilities of dry matter, organic matter, crude fibre and energy were not affected by dietary treatment during any of the five assessment periods. The pooled means for these data consequently reflected the individual period means with no significant difference between treatments.

The apparent digestible organic matter in the dry matter and the calculated metabolisable energy of the diets (Table 6) were also similar for both treatments during the five periods and for the pooled means.

The apparent digestibility of nitrogen and nitrogen balance data are shown in Table 7. The digestibility of nitrogen (Nitrogen intake minus faecal) was consistently lower for FT-SBM than for SBM during the five periods of assessment, the difference being significant in Period IV ($P < 0.05$) and also when the means were pooled ($P < 0.05$). The data for nitrogen balance shows that higher faecal and lower urinary nitrogen losses were consistently recorded for the FT-SBM diet than for SBM but the differences between pooled means were not statistically significant. More nitrogen was retained from SBM when expressed both as an absolute value and as a percentage of intake but the large variations between replicates resulted in the differences not being

Table 4 Experiment 1

The apparent digestibility coefficient of dry matter (DMD) and organic matter (OMD).

Period	DMD			OMD		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	0.551	0.552	0.0102	0.566	0.563	0.0121
II	0.506	0.523	0.0138	0.519	0.540	0.0142
III	0.536	0.524	0.0148	0.553	0.543	0.0153
IV	0.525	0.535	0.0146	0.545	0.550	0.0149
V	0.524	0.524	0.0129	0.537	0.529	0.0172
Pooled						
Means	0.528	0.532	0.0093	0.544	0.545	0.0097

Table 5 Experiment 1

The apparent digestibility coefficient of crude fibre (CF) and energy.

Period	CF digestibility			Energy digestibility		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	0.555	0.567	0.0183	0.518	0.518	0.0097
II	0.487	0.513	0.0225	0.473	0.488	0.0111
III	0.544	0.535	0.0208	0.521	0.511	0.0140
IV	0.549	0.565	0.0192	0.520	0.531	0.0140
V	0.549	0.523	0.0215	0.513	0.493	0.0195
Pooled						
Means	0.537	0.541	0.0167	0.509	0.508	0.0121

Table 6 Experiment 1

The digestible organic matter in the dry matter (DOMD) and the calculated metabolisable energy (ME; MJ/kg DM) content of diets.

Period	DOMD			ME ⁺		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	0.519	0.517	0.0109	7.8	7.8	0.16
II	0.473	0.492	0.0126	7.1	7.3	0.19
III	0.506	0.496	0.0138	7.6	7.5	0.21
IV	0.498	0.502	0.0133	7.5	7.5	0.20
V	0.489	0.482	0.0154	7.3	7.6	0.41
Pooled						
Means	0.497	0.498	0.0097	7.5	7.5	0.13

⁺ ME calculated as DOMD (g/kg) x 15

Table 7 Experiment 1

The apparent digestibility coefficient of nitrogen and
nitrogen balance (g)

Period	<u>Digestibility</u>			<u>Intake</u>		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	0.507	0.479	0.0603	62.5	62.7	2.13
II	0.444	0.359	0.0463	61.8	61.3	0.70
III	0.454	0.383	0.0556	66.0	64.9	0.51
IV	0.515	0.355	0.0597*	63.2	64.1	0.59
V	0.376	0.338	0.0275	61.0	61.9	0.60
Pooled						
Means	0.459	0.383	0.0350*	63.1	63.0	0.70
Period	<u>Faecal</u>			<u>Urinary</u>		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	30.8	32.6	3.41	30.3	27.2	2.41
II	34.4	39.4	3.11	32.2	31.3	1.99
III	36.0	40.2	2.59	28.2	28.1	1.84
IV	30.7	41.4	3.96*	20.2	18.4	6.81
V	38.1	40.9	1.98	13.2	15.6	3.78
Pooled						
Means	34.0	38.9	2.24	25.3	23.7	4.67
Period	<u>Retained</u>			<u>Retained as % of intake</u>		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	1.4	2.9	5.73	2.3	4.5	9.32
II	-4.8	-9.5	2.90	-8.0	-15.4	5.23
III	-1.8	-3.3	2.34	-2.6	-5.0	4.52
IV	12.3	4.3	7.63	19.7	6.8	12.50
V	9.7	7.7	4.04	12.0	12.5	6.62
Pooled						
Means	3.6	0.4	3.43	5.7	0.7	4.13

significant. There was an apparent gradual decrease in urinary nitrogen loss and a gradual increase in the nitrogen retained from Period II to V for both diets.

Plasma urea concentrations are presented in Table 8. Plasma urea concentrations of sheep fed the FT-SBM diet were significantly lower in Period III ($P < 0.05$) and in Period IV ($P < 0.01$) than for those fed SBM. A similar trend was also observed for other periods, although the differences were smaller.

Feeding SBM increased rumen ammonia nitrogen concentrations by two hours after feeding (5.9 increasing to 9.6 mg/100 mls rumen liquor, S.E. of difference 3.32). For FT-SBM the increase was only from 6.0 to 6.5 mg/100 mls (S.E. of difference 1.85). None of these increases was, however, statistically significant.

Discussion

In view of the similarity of Experiments 1 and 2 (immediately following) and their results, both will be discussed together after the section on Experiment 2 results.

Table 8 Experiment 1

The concentrations of plasma urea (mg/100 ml)

Period	SBM	FT-SBM	SED
I	18.6	15.0	3.27
II	21.9	19.8	3.22
III	25.8	22.1	1.49*
IV	20.1	15.3	1.26**
V	16.5	16.1	1.66
Pooled Means	20.6	17.7	2.10

Experiment 2 - The voluntary intake and digestibility of straw diets and the performance of lambs as influenced by formaldehyde treatment of soya bean meal

Introduction

An earlier report (Peter et al, 1971) using 88% corn diets (12% CP) showed that lambs fed formaldehyde-treated soya bean meal-supplemented diets achieved significantly higher growth rates than those fed the untreated soya bean meal diets. In contrast, Schmidt et al. (1973a) using similar diets, together with ground corn cobs (approximately 10% CP), recorded significantly higher growth rates for steers fed untreated soya bean meal than for those fed the formaldehyde-treated soya bean meal diets. In view of these conflicting reports and the more sensitive nature of the protein needs of younger animals, Experiment 2 was conducted to determine the response of growing lambs to straw-based diets supplemented with formaldehyde-treated soya bean meal.

Materials and Methods

Animals

Eight 4-month old Suffolk x Greyface wether lambs with mean live weight 35 kg (33-37 kg) were selected from pasture based on uniform appearance. They were dozed with 10 ml/hd of Thiabendazole and allocated to treatments.

Treatment diets and feeding

The treatments were identical to those used in Experiment 1 but the proportion of straw:concentrate was adjusted to 3:2 (air dry basis) to enable the lambs to consume energy in excess of maintenance needs. The dietary energy allowance, approximately 8 MJ/day was estimated to permit a daily live weight gain of approximately 100 g. The amounts of the concentrates fed are shown in Table 9.

The lambs were initially penned together for 10 days during which they were fed soya bean meal and unmolassed sugar-beet pulp (1:3 air dry basis) plus hay ad libitum in a gradual change from their previous pasture diet to straw. They were subsequently transferred to metabolism cages and fed the treatment diets for a preliminary period of 14 days. During the preliminary period, diets were fed as in Experiment 1 but the straw portion was fed ad libitum, twice daily so

Table 9 Experiment 2

The composition of the concentrate mixture fed to each sheep/day and the estimated amounts of crude protein (CP), rumen degradable protein (RDP) and undegradable protein (UDP) (g) and metabolisable energy (ME; MJ) supplied by each diet.

	SBM	FT-SBM
Soya bean meal	50	-
Formaldehyde-treated soya bean meal	-	50
Unmolassed sugar-beet pulp	310	310
Vitamin and mineral supplements	25	25
CP	63.0	63.1
Estimated RDP	14.4	4.6
Estimated UDP	48.6	58.5
Estimated ME	8.0	8.0

that residues were 10 to 15% in excess of intake. Due to the high levels of selection of straw components (leaf:stem) shown by the lambs, especially when offered straw in excess of about 10% of anticipated intake, the amount of straw fed was reduced so that residues were about 5 to 10%. It was hoped that this attempt to control straw selection by the lambs would minimise errors associated with nutrient intakes and digestibility. The proximate composition of dietary constituents are shown in Table 10.

Following the 14-day preliminary period, six digestibility and nitrogen balance trials were conducted for one period of 6 days and five periods of four days. These shorter than normal periods were due to the somewhat irregular eating habit of the lambs fed the FT-SBM diet. Straw intake was recorded for 34 days.

Animal Procedure

Urine and faeces were collected as in Experiment 1. Samples of feed, faeces, urine, blood and rumen liquor were also obtained as described in Experiment 1.

Analyses

Chemical and statistical analyses were done as described in Experiment 1.

Results

One of the lambs fed the FT-SBM diet began to lose appetite after the first collection period and was removed from the trial. It later died. The data for FT-SBM are therefore for four lambs for Period I and three lambs for Periods II to VI.

Data for daily live-weight gain and voluntary food intakes are shown in Table 11. Daily live-weight gain, measured as the difference between initial and final live-weights were higher for FT-SBM than for SBM but the difference was not significant. Voluntary intakes were marginally higher, but not significantly so, for lambs fed SBM than for those fed FT-SBM.

The apparent digestibilities of dry matter and organic matter are shown in Table 12, while those for crude fibre and energy are presented in Table 13. Apart from the significantly ($P < 0.05$) higher digestibility of dry matter for FT-SBM than for SBM during Period IV, there were no apparent effects of treatment on the digestibility of dry

Table 10 Experiment 2Proximate analysis of dietary constituents (g/kg DM)

	Straw	SBP	SBM	FT-SBM
Dry matter	864	847	851	856
Organic matter	942	914	928	929
Crude protein	27	104	543	543
Crude fibre	485	214	58	60
Ether Extract	14	6	9	9
N-free extract	429	590	318	316
Ash	58	86	72	71
Gross Energy (MJ/kg DM)	17.6	16.6	19.5	19.6

⁺ N-free extract was calculated by difference.

Table 11 Experiment 2

The voluntary intake of straw dry matter and growth rate of the lambs.

	SBM	FT-SBM	SED
DMI (g/d)	639	607	28.0
DMI (g/kg $W^{0.75}$ /d)	43.3	41.1	1.56
Daily LWG (g)	86	122	20.4

Table 12 Experiment 2

The apparent digestibility coefficient of dry matter (DMD) and organic matter (OMD).

Period	DMD			OMD		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	0.606	0.598	0.0159	0.638	0.633	0.0186
II	0.581	0.601	0.0183	0.607	0.631	0.0194
III	0.569	0.586	0.0152	0.593	0.616	0.0177
IV	0.563	0.598	0.0150*	0.595	0.625	0.0150
V	0.588	0.579	0.0154	0.618	0.606	0.0084
VI	0.560	0.577	0.0172	0.590	0.605	0.0182
Pooled Means	0.578	0.590	0.0083	0.607	0.619	0.0082

Table 13 Experiment 2

The apparent digestibility coefficient of crude fibre (CF) and energy.

Period	CF digestibility			Energy digestibility		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	0.620	0.604	0.0222	0.606	0.597	0.0179
II	0.607	0.639	0.0266	0.559	0.586	0.0259
III	0.593	0.613	0.0205	0.564	0.587	0.0205
IV	0.518	0.618	0.0191	0.551	0.582	0.0143
V	0.621	0.598	0.0277	0.582	0.569	0.0136
VI	0.611	0.613	0.0161	0.548	0.571	0.0210
Pooled Means	0.607	0.614	0.0080	0.569	0.582	0.0100

matter, organic matter, crude fibre or energy for the various periods or for the pooled means.

A similar trend was evident for the apparent digestibility of organic matter in the dry matter and the calculated metabolisable energy (Table 14). The apparent digestibility of nitrogen was similar for both treatments, although marginally higher for the SBM diet (Table 15). Pooled means for faecal nitrogen were also similar for both treatments but a slightly lower urinary nitrogen loss for FT-SBM was accompanied by a slightly improved nitrogen retention. However, none of the differences in nitrogen balance data was significant. There was a gradual decrease in the amount of nitrogen lost in urine by the lambs from Period I to V for both SBM and FT-SBM. This was accompanied by a corresponding increase in nitrogen retained for both diets.

Plasma urea concentrations (Table 16) were consistently lower for lambs fed FT-SBM during the six assessment periods but the difference reached significance ($P < 0.05$) only when the means were pooled.

Rumen ammonia-nitrogen concentrations measured before and approximately 2 hours after feeding increased from a pre-feeding level of 8.4 to 9.9 mg/100 ml (S.E. of difference 1.23) for SBM and from 8.8 to 9.2 (S.E. of difference 1.20) for FT-SBM. None of these increases was significant.

Discussion (Experiments 1 and 2)

Rumen-degraded protein supply and digestibility of diets

For both experiments, the FT-SBM diet provided only about one third of the total quantity of RDP as the SBM diet. Calculations indicate that in Experiment 2 the daily supply of RDP from the SBM and FT-SBM diets amounted to only about 22 and 7% respectively of the requirement (65 g) for lambs of similar weights growing at a rate of 100 g/day when given a diet with a metabolisability of $Q = 0.5$ (ARC, 1980), which would be similar to the diets used in these experiments. It is possible that the straw component may have provided some RDP, albeit small, in the diet although degradability estimates of the straw did not provide information on which to base this assumption. Any such additional RDP is probably negligible when considered against the background of grossly inadequate RDP supply for both diets, particularly for FT-SBM.

Table 14 Experiment 2

The digestible organic matter in the dry matter (DOMD) and the
calculated metabolisable energy (ME; MJ/kg DM) content of diets.

Period	DOMD			ME ⁺		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	0.577	0.571	0.0164	8.7	8.6	0.25
II	0.540	0.560	0.0172	8.1	8.4	0.26
III	0.532	0.552	0.0158	8.0	8.3	0.24
IV	0.539	0.566	0.0136	8.1	8.5	0.20
V	0.562	0.551	0.0137	8.4	8.3	0.21
VI	0.535	0.537	0.0198	8.0	8.1	0.30
Pooled						
Means	0.548	0.556	0.0085	8.2	8.4	0.13

⁺ ME calculated as DOMD (g/kg) x 15

Table 15 Experiment 2

The apparent digestibility coefficient of nitrogen and nitrogen balance (g).

Period	Digestibility			Intake		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	0.584	0.562	0.0182	67.6	66.8	0.74
II	0.664	0.674	0.0178	63.3	62.7	0.53
III	0.668	0.657	0.0183	61.6	61.3	0.54
IV	0.681	0.686	0.0177	62.3	61.8	0.58
V	0.688	0.683	0.0192	63.9	63.4	0.51
VI	0.661	0.648	0.0205	63.4	63.1	0.44
Pooled Means	0.658	0.652	0.0244	63.7	63.3	1.66

Period	Faecal			Urinary		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	28.1	29.3	1.38	19.7	22.0	3.26
II	21.3	20.4	0.92	17.7	12.6	2.95
III	20.5	21.0	1.20	16.9	13.6	1.31
IV	19.6	19.4	1.13	15.4	11.7	1.43
V	20.0	20.1	1.30	13.8	11.5	2.71
VI	21.5	22.3	1.42	15.5	12.0	1.94
Pooled Means	22.1	21.8	1.77	16.5	13.9	1.87

Period	Retained			Retained as % of intake		
	SBM	FT-SBM	SED	SBM	FT-SBM	SED
I	19.7	15.6	3.57	29.2	23.2	5.29
II	24.3	29.7	2.89	38.2	47.2	4.21
III	24.2	26.7	1.55	39.2	43.5	2.31
IV	26.9	30.7	1.95	43.2	49.7	3.11
V	30.6	32.2	2.43	47.9	50.2	3.75
VI	26.3	28.9	2.20	41.6	45.8	3.41
Pooled Means	25.4	27.3	2.87	39.9	43.3	4.87

Table 16 Experiment 2

The concentrations of plasma urea (mg/100 ml)

Period	SBM	FT-SBM	SED
I	23.3	21.0	4.36
II	28.2	24.9	4.09
III	29.8	25.2	3.33
IV	31.6	26.5	3.35
V	25.9	23.6	4.56
VI	30.1	24.9	3.09
Pooled			
Means	28.1	24.4	1.47*

It is most significant, however, that despite the larger RDP deficit for the FT-SBM diet, there was a consistent similarity in the apparent digestibility of both diets by sheep in these experiments. The results of several trials involving formaldehyde-treated soya bean meal have been inconsistent with regard to total digestibility. However, the lack of any difference between SBM and FT-SBM in the digestibility of diets observed in Experiments 1 and 2 is in agreement with some previous studies (Amos, Burdick and Huber, 1974; Clark, Davis and Hatfield, 1974; Rooke, Brookes and Armstrong, 1983) but not with others (Nishimuta, Ely and Boling, 1973) summarised in Table 17. It would appear that grossly inadequate levels of RDP given to ruminants such as those used in Experiments 1 and 2 may not seriously reduce diet digestibility.

One important concern in the chemical treatment of protein-rich feeds is the nitrogen available in the rumen for maximum microbial protein synthesis, digestion and utilisation of the diet being dependent on this. Maximum growth rate of rumen micro-organisms can be sustained at 5 mg NH_3 - N/100 ml rumen fluid (Roffler, Schwab and Satter, 1976) although a much higher level of NH_3 - N (23.5 mg/100 ml rumen fluid) was suggested by Mehrez, Orskov and McDonald (1977). However, Satter and Slyter (1974) demonstrated by means of infusion of urea into incubating mixtures in fermentors that bacteria were actually satisfied at 2.0 mg NH_3 - N/100 ml rumen fluid and thus a value of 5 mg/100 ml probably allows a margin of excess. Ammonia nitrogen production with both diets in these present experiments was in excess of 5 mg/100 ml rumen fluid, which would suggest that the rumen was not deficient in ammonia for any of the diets. This could explain the consistent similarity in digestibility for both diets in these experiments.

Table 17 Summary of some previous results of trials involving formaldehyde-treated soya bean meal.

Type of Animals Used	Level of formaldehyde (% of SBM)	N		DM		OM		Daily LWG (g)		Source
		SBM	FT-SBM	SBM	FT-SBM	SBM	FT-SBM	SBM	FT-SBM	
Rats	1.0		86	-	-	-	-	7.1	7.1	Schmidt et al (1973a)
	2.0		77	-	-	-	-	7.1	6.7	
	3.0	86	66	-	-	-	-		6.1	
	4.0		55	-	-	-	-		5.1	
Rats	0.2		84	-	-	-	-		5.0	Thomas et al (1979a)
	0.8	84	77	-	-	-	-	5.6	4.3	
	1.4		62	-	-	-	-		3.7	
Lambs	0.6	-	-	-	-	-	-	246	290	Peter et al (1971)
Lambs	1.0	75	47	71	65	-	-	-	-	Nishimuta et al (1973)
Lambs	1.5		60	-	-	-	-			Schmidt et al (1974)
	3.0	64	57	-	-	-	-	280	270	
Wether Sheep	1.1	57	38	66	60	-	-	-	-	Amos et al (1974)
Wether Sheep	0.25		54	-	-	-	-	-	-	Amos et al (1974)
	0.50	59	51	-	-	-	-	-	-	
	0.75		44	-	-	-	-	-	-	
Calves	0.2	-	-	-	-	-	-		730	Thomas et al (1979b)
	0.4	-	-	-	-	-	-	1010	820	
	0.8	-	-	-	-	-	-		990	

Apparent nitrogen digestibility and balance

Formaldehyde treatment of soya bean meal depressed apparent digestibility of nitrogen in these experiments. The significant depression in Experiment 1 is more relevant as a greater amount (40%) of the dietary crude protein was supplied from soya bean meal, in contrast to 35% in Experiment 2. This observation is in agreement with most of the reports cited in Table 17 in which the effect of formaldehyde treatment on the digestion of soya bean crude protein was examined. Although the extent of depression may differ between experiments, most have been consistent in reporting that formaldehyde treatment marginally reduces nitrogen digestibility. It is likely that the level of formaldehyde applied to soya bean meal may account for the extent of depression of nitrogen digestibility. It seems certain, however, that the bypass fraction of formaldehyde-treated soya bean meal is less well digested in the small intestine.

In the present experiments, the increase in faecal nitrogen loss due to formaldehyde treatment was accompanied by a reduction in urinary nitrogen loss. The lower urinary nitrogen excretion by sheep fed the FT-SBM diet in these experiments, especially in Experiment 2, suggests a more efficient utilisation of the nitrogen that was digested and absorbed on that diet. Consequently, lambs fed the FT-SBM diet in Experiment 2 retained more nitrogen than those fed the SBM diet.

For both experiments, the N balance data for SBM and FT-SBM showed a gradual decrease in the quantities of N lost in urine and a corresponding increase in the amount retained with time. This decrease in urinary N appeared to have started from Period II for Experiment 1 (25 days after the diets were introduced) and from Period I for Experiment 2 (24 days after the diets were initially fed) and it suggests a gradual adjustment by the sheep to the low protein diets fed in these experiments. This seems to emphasise the importance of conserving nitrogen to meet the needs of animals fed such low protein roughage feeds. Perhaps the return of urea to the rumen of animals fed such diets may also create a better rumen environment for the rumen microbes. It is probable that conserved nitrogen, evident from the gradual improvement in nitrogen balance with time in these two experiments, may also have contributed in maintaining normal digestibility for the FT-SBM diet in particular.

Ammonia nitrogen in rumen liquor and plasma urea

Ammonia nitrogen concentrations in rumen fluid increased slightly after the start of feeding, the increases and levels tending to be lower on the FT-SBM diet. These increases are consistent with the general reductions in blood urea concentrations brought about by formaldehyde treatment.

Dry matter intake and live weight gain (Experiment 2 only)

Although voluntary intake did not differ significantly between both treatments, the marginally higher intakes by lambs fed SBM may indicate that the lower rumen ammonia nitrogen values for FT-SBM which was reflected in reduced plasma urea resulted in an inadequate return of salivary nitrogen to the rumen. This could have led to a less favourable rumen environment for lambs fed FT-SBM, thus marginally reducing their intakes. In this regard, the importance of plasma urea in maintaining rumen ammonia nitrogen should not be overlooked and the levels of ammonia nitrogen observed soon after feeding should not be regarded as remaining unchanged with time.

Although formaldehyde treatment of soya bean meal has been found to improve lamb growth rates (Peter et al., 1971) and food conversion ratio by steers (Spears, Hatfield and Clark, 1980), it has depressed live weight gains of steers in other experiments (Schmidt et al., 1973a; Schmidt, Benevenga and Jorgensen, 1974; Thomas, Trenkle and Burroughs, 1979b). It is, however, difficult to interpret the difference in live weight gain in Experiment 2, reported in this thesis, up to 36 g/day in favour of the FT-SBM diet, as true growth since the variable gutfill of animals fed high straw diets introduces a possible source of error. Of course there is the possibility that bypass protein digested in the small intestine can improve growth rate. However, the extra apparent increase in live weight of lambs fed the FT-SBM diet in this experiment was achieved with dry matter intakes slightly lower than those of lambs fed the SBM diet, with little, if any change in intake of digestible nutrients. Additionally, the small number of replicates in the experiment casts further doubt on the significance of the observed live weight changes.

The results of Experiments 1 and 2, discussed above, indicate that formaldehyde treatment of soya bean meal did not depress total digestibility. Nitrogen digestibility, but not necessarily nitrogen utilisation, was lowered due to formaldehyde treatment. Blood urea

concentrations were also reduced due to lower degradability of treated protein but intakes were not seriously affected. It is possible that the level of formaldehyde applied to soya bean meal and the differing types of diets (e.g. the proportion of concentrate to roughage and the type of roughage or concentrate) as well as the levels of dietary crude protein ($N \times 6.25$), all of which may affect the efficiency of nitrogen utilisation, may be largely responsible for the inconsistencies in literature concerning the effect of formaldehyde treatment on the nutritive value of soya bean meal.

Experiment 3 - The effect of dietary protein degradability and supplementary energy source on the voluntary intake and digestibility of straw and the performance of pregnant beef cows

Introduction

It has been shown that reduced rumen degradability of dietary protein can improve milk yield (e.g. Gonzalez *et al.*, 1979, 1982; Forster, Grieve, Buchanan-Smith and MacLeod, 1982). In a study of the digestion of formaldehyde-treated soya bean meal, Rooke, Norton and Armstrong (1981) found that, although increased flow of feed non-ammonia nitrogen into the small intestine was associated with reduced degradability of dietary protein, the effect was balanced by a decrease in the flow of microbial non-ammonia nitrogen. This indicates a higher yield of microbial nitrogen for the diet containing more degradable dietary protein. However, there is little information on the effect of reducing dietary protein degradability on the voluntary intake of low-quality roughage feeds. In Experiments 1 and 2 (reported in this thesis), it was shown that, although reduced protein degradability due to formaldehyde treatment of soya bean meal did not depress the total digestibility of straw diets for sheep, it may have marginally reduced voluntary food intake. It was suggested that reduced protein degradability caused lower rumen ammonia nitrogen levels which resulted in reduced plasma urea levels and that this may have resulted in an inadequate return of salivary nitrogen to the rumen with a consequent marginal reduction in food intake.

The varying effects of energy source on nitrogen utilisation are also becoming more apparent. As an energy source, dried molassed sugar-beet pulp has been shown to be comparable to barley in some experiments (Ronning and Bath, 1962; Bhattacharya and Sleiman, 1971; Castle, 1972) but not in others (Ducker, Fraser and Hemingway, 1976). The report by Ducker *et al.* (1976) showed that the inferiority of molassed sugar-beet pulp with added urea relative to barley plus urea was presumably an energy effect. In view of the total contributions of energy and degraded and undegraded protein from these feeds, it is possible that differences in degradability and their effects may become more apparent when various protein supplements are given in association with these energy supplements, especially in diets containing

low-protein roughage feeds.

Experiment 3 was therefore undertaken to evaluate proteins differing in the rate and extent of ruminal degradability when included as supplements with sugar-beet pulp or barley in ad libitum straw diets. Pregnant beef cows were chosen for the investigation in view of their increased nutrient needs and their capacity to consume straw.

Materials and Methods

Animals and dietary treatments

Twelve pregnant beef cows (Hereford x British Friesian and Irish Blue-Grey) of mean live weight 481 kg (392-551 kg) and 16 weeks from calving were arranged in three balanced groups. Each of 4 cows was then allocated to one of three experiments (A, B and C), each designed as a 4 x 4 Latin square with 21-day feeding periods. Each feeding period consisted of an initial 14-day introductory period followed by a 7-day digestibility and intake assessment period.

In Experiment A, the cows were given a concentrate diet in which molassed sugar-beet pulp (MSBP) was the main energy source. In Experiments B and C the main energy sources were unmolassed sugar-beet pulp (USBP) and rolled barley (BARLEY) respectively. These various energy sources were either fed alone at 1.7 kg DM (CONTROL) or in combination with urea (U), soya bean meal (SBM) or formaldehyde-treated soya bean meal (FT-SBM) (Sopralin, BP Nutrition (U.K.) Ltd.). Each energy source provided approximately 200 g crude protein (CP)/day. Each diet was further supplemented with (fresh basis) 0.1 kg of a mineral supplement (Rainbow Dairy Hiphos, BP Nutrition (U.K.) Ltd.) and 0.2 kg of a barley chromic oxide mix. In addition, all the cows received barley straw ad libitum in the long form.

Composition of diets

Table 18 shows the composition of each dietary component and the estimated degradability and apparent digestibility of each concentrate component. The estimated degradabilities of MSBP, USBP, BARLEY, SBM and FT-SBM were obtained using a nylon bag technique as described in Appendix 1. Each dietary constituent was replicated in five rumen-fistulated cows. The urea contained (g/kg fresh matter) 2852 CP (N x 6.25) and was assumed to be completely degradable. The digestibilities of the concentrate components were obtained in a 17-day digestibility trial involving 7 days total collection using adult

Table 18 Experiment 3

Proximate analyses (g/kg DM) of dietary components, degradability (%) and apparent digestibility coefficient of the concentrate components.

	MSBP	USBP	BARLEY	SBM	FT-SBM	STRAW
Proximate analyses						
Dry matter	878	854	823	884	862	818
Crude protein	106	100	119	523	533	36
Crude fibre	137	204	57	31	41	455
Ether extract	3	4	9	10	13	10
N-free extract	650	606	793	396	340	444
Ash	105	86	22	68	73	55
Gross energy (MJ/kg DM)	16.2	16.6	18.3	19.4	19.5	17.8
Degradability (%)						
Dry matter	63.9	41.6	83.0	67.5	39.9	-
Crude protein	38.6	9.2	89.8	53.9	10.6	-
Apparent digestibility coefficient						
Dry matter	0.850	0.905	0.926	0.854	0.850	-
Organic matter	0.871	0.925	0.950	0.871	0.870	-

castrated male (Suffolk x Greyface) sheep (four sheep per treatment) in metabolism cages. The trial was conducted using the procedure described in Appendix 2. The digestibilities of MSBP, USBP and BARLEY were determined by feeding 700 g of each with 300 g of pelleted dried grass of known digestibility. The digestibility of the SBM and FT-SBM was determined by feeding 250 g of each with 250 g of barley husk siftings and 500 g MSBP, the digestibility of barley husk siftings and MSBP having been determined previously.

Table 19 shows the composition of the concentrate mixtures given to the cows in each Latin square and the estimated RDP supplied by the concentrate portion of each diet. The RDP content of straw was considered negligible in view of the negative degradability values obtained with the straw used in these experiments.

Management and feeding

The cows were tied in individual standings in a conventional byre with facilities for individual feeding. The concentrate part of the diet was offered and fully consumed at 07.30 h while the straw was fed ad libitum three times a day at 08.00, 12.00 and 16.00 h in amounts at least 10-15% in excess of individual consumption. The entire straw for each cow during each 7-day assessment period was pre-weighed two days earlier to ensure uniformity, uneaten straw being reweighed at the end of each period to determine the amounts of straw fed to each cow. Straw residues were also weighed daily, sampled and stored in polythene bags numbered for each cow. At the end of each feeding period, the bulked straw residues for each cow were thoroughly mixed, subsampled and DM determined on the subsample. The DM obtained was used to calculate refused straw and by difference to obtain the actual quantities of straw consumed.

Determination of digestibility was by means of chromic oxide included in cubed barley (10 g chromic oxide in 200 g cubed barley) which was added to all concentrate diets daily, as described earlier. The apparent digestibility of straw dry matter and organic matter was obtained by difference after allowing for the digestibility of the concentrate components (shown in Table 18). During the seven days of intake recording and digestibility assessment, rectal grab samples of faeces were obtained from each cow three times daily at 10.00 h, 13.00 h and 16.00 h. These 21 samples were amalgamated at the end of each period, thoroughly mixed and dried at 95°C. for 48 h.

Table 19 Experiment 3

Composition of the concentrate mixture, the amounts of molassed or unmolassed sugar beet pulp or barley (kg, air dry basis) fed, and the estimated daily intakes of rumen degradable protein (RDP) and crude protein (CP) in the three experiments.

Dietary treatments	CONTROL	U	SBM	FT-SBM	Comp. Mixture
<u>Basal ingredients</u>					
(EXPERIMENT A) MSBP	2.02	2.02	1.62	1.62	0.540
(EXPERIMENT B) USBP	2.05	2.05	1.64	1.64	0.546
(EXPERIMENT C) BARLEY	2.14	2.14	1.71	1.71	0.570
<u>Protein supplements</u>					
Urea	-	0.07	-	-	0.023
Soya bean meal	-	-	0.52	-	0.173
Formaldehyde-treated soya bean meal	-	-	-	0.53	0.177
Chromic oxide/barley cubes	0.2	0.2	0.2	0.2	0.2
Mineral supplement ⁺	0.1	0.1	0.1	0.1	0.1
<u>Intake of RDP (g)</u>					
(EXPERIMENT A) MSBP	73	272	188	84	198
(EXPERIMENT B) USBP	16	215	143	39	198
(EXPERIMENT C) BARLEY	189	388	281	177	198
<u>Intake of CP (g)</u>					
(EXPERIMENT A) MSBP	188	387	393	396	400
(EXPERIMENT B) USBP	174	373	382	385	400
(EXPERIMENT C) BARLEY	210	409	410	413	400

+

Containing (by manufacturer's declaration) (g/kg) 150 Ca; 120 P; 30 Mg; 290 NaCl and (mg/kg) 4000 Fe; 120 Co; 6000 Mn; 1200 Cu; 1600 Zn; 500 I; 8 Se and 450,000 IU/kg Vitamin A; 80,000 IU/kg Vitamin D₃ and 500 IU/kg Vitamin E.

On the last day of each 21-day period, the cows were weighed and heparinised blood samples were obtained from the jugular vein of each cow at 09.30 h. These were centrifuged and the plasma stored for determination of urea, beta hydroxy butyrate (3-OHB), total protein and free fatty acids (FFA).

At the end of the experimental period, all the cows were fed a concentrate mixture (COMP MIXTURE) containing equal proportions (DM basis) of MSBP, USBP and BARLEY and equal amounts of CP from each of the protein sources, providing approximately 400 g CP/d, for a further three weeks. This was done to confirm that any differences in voluntary straw intake between energy sources (or between the groups of cows used in the three experiments) could be properly accounted for. Straw intakes were recorded and digestibility assessment by means of rectal grab samples were undertaken during the last seven days. Blood samples were also taken as described earlier.

Feed sampling and chemical analyses

Samples of the concentrate constituents of diets were obtained daily during the last 7 days of each period. Straw samples were also obtained during pre-weighing of straw for each period and from the straw left over at the end of each period. Samples of concentrate constituents and straw were separately bulked and dried. All dried feed samples and faeces were subsequently milled through a 0.8 mm screen in a laboratory mill. These were analysed for crude protein, crude fibre, ether extract, ash and chromium, as outlined in Appendix 4.

Analyses for plasma urea, 3-OHB, FFA and total protein were also done as outlined in Appendix 4.

Statistical analyses

Statistical analyses were performed on the data by microcomputer using an interactive analyses of variance programme (W.A. Greig, personal communication). The three experiments were firstly analysed as three separate 4 x 4 Latin squares, and then as a 4 x 4 x 3 analysis of variance to determine the direct effects of protein supplement and energy source and first order interactions.

Results

Live-weight changes and the intake and digestibility of barley straw

Table 20 shows the daily changes in the mean live weight of the cows. Generally, the cows gained some weight during the 84-day experimental period, although when the control diet was given in Experiments A (MSBP) and C (BARLEY), the cows lost weight marginally. There were no significant differences in daily live weight gains between treatments in Experiments A (MSBP) and B (USBP) but the daily live weight loss by cows fed the CONTROL diet in Experiment C (BARLEY) was significantly ($P < 0.05$) different from the daily live weight gains of cows fed FT-SBM.

During the final three weeks, when all the cows were fed a common concentrate mixture, there were no differences between the three groups of cows used in the three experiments in the intake or digestibility of straw DM or OM (Table 21). This suggests that the cows used in Experiments A, B and C were uniform and a CV of 4.4% in intake of straw DM by the cows when fed the common concentrate mixture seems reasonably low. It therefore seems reasonable to draw relevant conclusions from these experiments on the assumption that the cows used were similar.

The data for voluntary straw intakes and apparent digestibility of straw are given in Table 21. One cow occasionally stole straw from its neighbour during the first period before a faulty feeding rack was rectified. Consequently, two missing values had to be estimated using Yate's missing plot technique (Paterson, 1939) before subjecting the data to statistical analysis.

In Experiment A (MSBP) all the protein supplements improved the intake of straw DM and OM, although this improvement was significant ($P < 0.05$) only for U which was also significantly ($P < 0.05$) better than FT-SBM. Although there were no significant differences between treatments in the digestibility of straw DM or OM, absolute values were higher for U and SBM and, surprisingly, lower for FT-SBM than for the CONTROL. The calculated DOMD and intake of ME from straw followed the same pattern as the straw intake.

In Experiment B (USBP) all the protein supplements also increased intakes of straw DM, the increases being significant ($P < 0.001$) for U and SBM and ($P < 0.05$) for FT-SBM. Both U and SBM also resulted in significantly ($P < 0.05$) higher intakes than FT-SBM. The intakes of OM were also significantly ($P < 0.01$) improved by supplementation with U and SBM but the difference between FT-SBM and the CONTROL failed to be significant. Improvements in the apparent digestibility of straw DM

Table 20 Experiment 3

Daily changes in mean live weight of the cows (kg) over the 84-day experimental period.

	Initial Live weight (kg)	CONTROL	U	SBM	FT-SBM	SEM	Level of sig
Expt A (MSBP)	482	-0.04	0.18	0.06	0.12	0.064	NS
Expt B (USBP)	480	0.09	0.10	0.10	0.14	0.637	NS
Expt C (BARLEY)	481	-0.04 ^b	0.18 ^{ab}	0.17 ^{ab}	0.25 ^a	0.072	*

NS, not significant; * $P < 0.05$

a,b Means with the same superscript were not significantly different.

Table 21 Experiment 3

The mean daily voluntary intakes (kg) and apparent digestibility coefficient of straw and the calculated metabolisable energy from straw (ME, MJ) and the apparent digestible OM in the dry matter.

	CONTROL	U	SBM	FT-SBM	SEM	SIG	Comp	SE _± and SIG mixture
<u>Straw DM intake</u>								
Expt A	4.77 ^b	6.04 ^a	5.58 ^{ab}	5.03 ^b	0.236	**	5.80	
Expt B	4.83 ^c	5.84 ^a	5.93 ^a	5.32 ^b	0.120	***	6.01	0.258 ^{NS}
Expt C	5.53	5.94	6.07	6.77	0.501	NS	5.97	
<u>Straw OM intake</u>								
Expt A	4.51 ^b	5.71 ^a	5.27 ^{ab}	4.75 ^b	0.223	**	5.48	
Expt B	4.56 ^b	5.53 ^a	5.60 ^a	5.03 ^{ab}	0.117	**	5.68	0.250 ^{NS}
Expt C	5.23	5.61	5.77	6.39	0.462	NS	5.64	
<u>Straw DM digestibility</u>								
Expt A	0.481	0.540	0.499	0.448	0.0214	NS	0.567	
Expt B	0.289 ^b	0.427 ^a	0.411 ^a	0.397 ^a	0.0142	***	0.522	0.029 ^{NS}
Expt C	0.381	0.367	0.422	0.460	0.0278	NS	0.514	
<u>Straw OM digestibility</u>								
Expt A	0.471	0.531	0.493	0.443	0.0216	NS	0.581	
Expt B	0.284 ^b	0.433 ^a	0.414 ^a	0.397 ^a	0.0150	***	0.541	0.034 ^{NS}
Expt C	0.373 ^{ab}	0.357 ^b	0.407 ^{ab}	0.448 ^a	0.0256	*	0.541	
<u>Straw DOMD</u>								
Expt A	0.445 ^{ab}	0.502 ^a	0.466 ^{ab}	0.418 ^b	0.0204	*	0.523	-
Expt B	0.269 ^b	0.409 ^a	0.391 ^a	0.359 ^a	0.0155	***	0.486	0.031 ^{NS}
Expt C	0.351 ^{ab}	0.337 ^b	0.387 ^{ab}	0.423 ^a	0.0236	*	0.486	-
<u>ME from Straw</u>								
Expt A	31.7 ^b	46.0 ^a	39.0 ^{ab}	31.5 ^b	3.21	*	45.5	-
Expt B	21.4 ^b	35.6 ^a	35.1 ^a	29.0 ^{ab}	2.36	**	43.8	2.70 ^{NS}
Expt C	29.8	30.2	35.5	42.9	4.36	NS	43.5	

and OM followed the same pattern as intakes of DM, being significantly ($P < 0.001$) higher for U and SBM and ($P < 0.01$) for FT-SBM than for the CONTROL but there were no significant differences between the three protein-supplemented diets in apparent digestibility. The calculated DOMD was significantly improved ($P < 0.001$) for U and ($P < 0.01$) for SBM and FT-SBM. The calculated intake of ME from straw was significantly higher for U and SBM than for the CONTROL ($P < 0.01$).

Although all the protein supplements increased the intakes and digestibilities of DM and OM in Experiment C (BARLEY), the only significant difference was in the digestibility of organic matter, between FT-SBM and U ($P < 0.05$), in favour of FT-SBM. In contrast to Experiment A (MSBP) and B (USBP) where absolute values for straw intakes and digestibility were higher for U and SBM than for FT-SBM, cows fed the FT-SBM diet in Experiment C (BARLEY) had the highest mean values for straw intakes and digestibility. Cows fed FT-SBM digested straw OM significantly ($P < 0.05$) better than those fed the U diet. Supplementation with FT-SBM appeared to increase the ME obtained from straw but by amounts which were not significant.

The concentrations of blood metabolites

Table 22 shows the concentration of blood metabolites. Supplementation with U significantly ($P < 0.001$) increased plasma urea concentrations in the three experiments. The effect of SBM in increasing plasma urea concentrations was also significant in Experiment A (MSBP) ($P < 0.001$) and in Experiments B (USBP) and C (BARLEY) ($P < 0.01$). In the three experiments, cows given the U diet had significantly higher ($P < 0.01$) plasma urea levels than those fed FT-SBM. The difference between SBM and FT-SBM in plasma urea levels was also significant ($P < 0.01$) in Experiment A (MSBP) and ($P < 0.05$) in Experiment C (BARLEY). The FT-SBM and CONTROL diets resulted in similar plasma urea levels in Experiments B (USBP) and C (BARLEY) but FT-SBM caused significantly higher plasma urea levels than the CONTROL ($P < 0.01$) in Experiment A (MSBP).

Free fatty acid (FFA) concentrations were highly variable even between animals fed the same diet and the lack of any significant differences between treatments may have been due in part to this variability. The levels of 3-OHB were similar between treatments in Experiments A (MSBP) and C (BARLEY) but significantly higher for U than for FT-SBM ($P < 0.01$) and SBM ($P < 0.05$) in Experiment B (USBP).

Table 22 Experiment 3
Concentrations of blood metabolites

	CONTROL	U	SBM	FT-SBM	SEM	Level of sig
<u>Plasma urea (mg/100 ml)</u>						
Expt A (MSBP)	7.65 ^c	19.35 ^a	17.63 ^a	12.75 ^b	0.889	***
Expt B (USBP)	8.15 ^c	18.90 ^a	16.28 ^{ab}	12.23 ^{bc}	1.19	***
Expt C (BARLEY)	10.05 ^c	20.40 ^a	16.65 ^b	12.00 ^c	1.048	***
<u>FFA (u-equiv/l)</u>						
Expt A (MSBP)	250	307	435	113	116.8	NS
Expt B (USBP)	327	262	156	136	71.7	NS
Expt C (BARLEY)	131	83	41	364	116.3	NS
<u>3-OHB (mg/100ml)</u>						
Expt A (MSBP)	1.83	1.50	2.08	2.08	0.18	NS
Expt B (USBP)	1.68 ^{ab}	2.05 ^a	1.48 ^b	1.33 ^b	0.11	**
Expt C (BARLEY)	1.73	1.43	2.00	1.18	0.379	NS
<u>Total protein (g/100 ml)</u>						
Expt A (MSBP)	7.43	7.30	7.13	7.38	0.177	NS
Expt B (USBP)	6.68	6.88	6.98	6.88	0.293	NS
Expt C (BARLEY)	6.73 ^{ab}	6.50 ^a	6.30 ^b	7.15 ^a	0.174	*

NS, not significant. * P<0.05, ** P<0.01, *** P<0.001

a,b,c Means with the same superscripts were not significantly different.

Total protein levels in plasma were also similar for all treatments in Experiment A (MBSP) and B (USBP) but significantly higher for FT-SBM than for SBM and U ($P < 0.01$) in Experiment C (BARLEY).

Discussion

Effect of protein source on straw intake and digestibility

The overall means (Table 23) show an appreciable improvement in the voluntary intake, digestibility of straw and ME derived from straw due to supplementation with the various sources of protein. The improvements were more marked for urea than for untreated or formaldehyde-treated soya bean meal. Although formaldehyde treatment lowered the degradability of soya bean meal, the effect of which was reflected in reduced plasma urea concentrations, the consequent reduction in straw intake and digestibility was only marginal, thus confirming the results of Experiment 2.

There are numerous previous comparisons of the effect of urea and natural protein sources on animal productivity, when included in low protein fibrous feeds. Pope, Gallup and Read (1952) using cottonseed meal and urea in 10% CP diets and Parkins, Fraser, Ritchie and Hemingway (1974) using groundnut meal and urea found that for pregnant ewes fed hay-based diets, urea was comparable to cottonseed meal and groundnut meal but voluntary food intakes were not measured in their experiments. Giardini, Lambertini, Gaspari and Lo Bruno (1976) summarised the results of an unspecified number of trials with a total of 120 heads of growing cattle and showed from the computed data on DM intakes, growth rates and efficiency of food conversion that soya bean meal had no advantage over urea in predominantly silage diets containing 11% CP. One other report in which urea was compared with a less degradable protein supplement such as fish meal failed to show any difference in terms of DM intakes or estimated ME between the two nitrogen supplements (Smith, Broser and Hill, 1980) in 70% straw diets, although fish meal promoted higher growth rates. More recently, Tudor, McGuigan and Norton (1985), using diets consisting mainly of cassava tubers and tops, in a growth study with cattle concluded that groundnut meal was not better than urea in terms of nitrogen retention or efficiency of utilisation. In one trial (Thomas, Katz, Auld and Peterson, 1984) involving a 15% straw diet containing 14% CP, urea was found to promote significantly higher growth rates and voluntary food intakes by lambs than cottonseed meal, extracted rapeseed meal or

Table 23 Expt. 3 Main effects of supplemental protein and energy sources

	Straw DM intake (kg/d)	Straw DMD	Straw OMD	Straw ME intake (MJ/d)	Daily live weight gain (kg)	Urea (mg/100 ml)	Total Protein (g/100 ml)	3-OHB (mg/100 ml)	FFA (u-equiv/l)
<u>Supplemental Protein source</u>									
CONTROL									
U	5.04 ^b	0.383	0.375 ^b	27.6 ^b	0.01 ^b	8.6 ^d	6.9	1.7	236
SBM	5.94 ^a	0.444	0.440 ^a	37.3 ^a	0.15 ^a	19.6 ^a	6.9	1.7	217
FT-SBM	5.86 ^{ab}	0.444	0.438 ^a	36.5 ^a	0.11 ^a	16.9 ^b	6.8	1.9	210
	5.70	0.435	0.429 ^a	34.5 ^a	0.17 ^a	12.3 ^c	7.1	1.5	204
SEM and level of significance	0.236 ^{**}	0.013 ^{***}	0.0127 ^{***}	2.16 ^{**}	0.036 ^{**}	0.56 ^{***}	0.151 ^{NS}	0.205 ^{NS}	62.4
<u>Energy source</u>									
MSBP									
USBP	5.35 ^b	0.492	0.485 ^a	37.1 ^a	0.08	14.3	7.3 ^a	1.9	276
BARLEY	5.48 ^b	0.381	0.382 ^b	30.3 ^{ab}	0.11	13.9	6.9 ^b	1.6	220
	6.08 ^a	0.407	0.396 ^b	34.6 ^{ab}	0.14	14.8	6.7 ^b	1.6	155
SEM and level of significance	0.204 [*]	0.011 ^{***}	0.0110 ^{**}	1.87 [*]	0.131 ^{NS}	0.49	0.130 ^{**}	0.178 ^{NS}	54 ^{NS}
Level of sig. of interactions (Protein source x energy supplement)	NS	***	**	*	NS	NS	NS	NS	NS

a, b, c, d: means with the same superscript letters were not significantly different

safflower meal in isonitrogenous diets.

It is often assumed that, because of its rapid hydrolysis in the rumen which may lead to wastage of feed nitrogen, urea is inferior to natural proteins, especially if the required amount is fed in one meal. For example, in three trials, Umunna, Klopfenstein, Hasimoglu and Woods (1982) showed that urea was inferior to SBM in promoting growth rates of lambs and calves when included in 60-70% corn cob diets containing 12% CP but could demonstrate a difference in DM intakes between the two nitrogen sources in only one of the trials.

In these present experiments a daily inclusion of 70 g urea in one meal, providing approximately half the CP supply in the concentrate portion (400 g CP/day) when straw was given ad libitum resulted in intakes of straw DM comparable to soya bean meal and higher than formaldehyde-treated soya bean meal.

The general relationship between protein degradability and straw intake

There was clear evidence of a general relationship between the degradability of protein in the concentrate portion of the diets and voluntary straw consumption, a relationship that did not exist when straw intakes were related to the concentrate CP concentrations. The trend, which was evident in the individual experiments, became more apparent when the overall means (Table 23) were considered. Although the relationship was not linear, it appeared to indicate that intake of RDP influenced straw consumption.

To further examine the general relationship which appeared to exist between voluntary intake of straw and RDP concentrations of the diets, linear regressions were fitted over the range of estimated RDP intakes from the various diets using the mean of the data for straw DM intakes of all animals fed each dietary combination. The relationship obtained, shown in Fig. 2, indicates that straw DM intakes increased as RDP supply in the diets increased. The regression equation was:-

$$\text{straw dry matter intake (kg)} = 5.03 + 0.0035 \times \text{RDP intake (g)}$$

The regression line was significant ($P < 0.05$) ($r = 0.661^*$; $\text{rsd } 0.459$; $\text{df } 10$). Although straw DM intakes also apparently increased with CP concentration, as shown in Fig. 3, this effect was entirely due to the highly significant interaction ($P < 0.01$) between supplementary energy and protein source on digestibility. This interaction, which may also be responsible for the anomalous value (the highest point) in Fig. 2,

(Square symbols indicate USBP; circles, MSBP; and triangles, BARLEY in both Figure 2 and Figure 3)

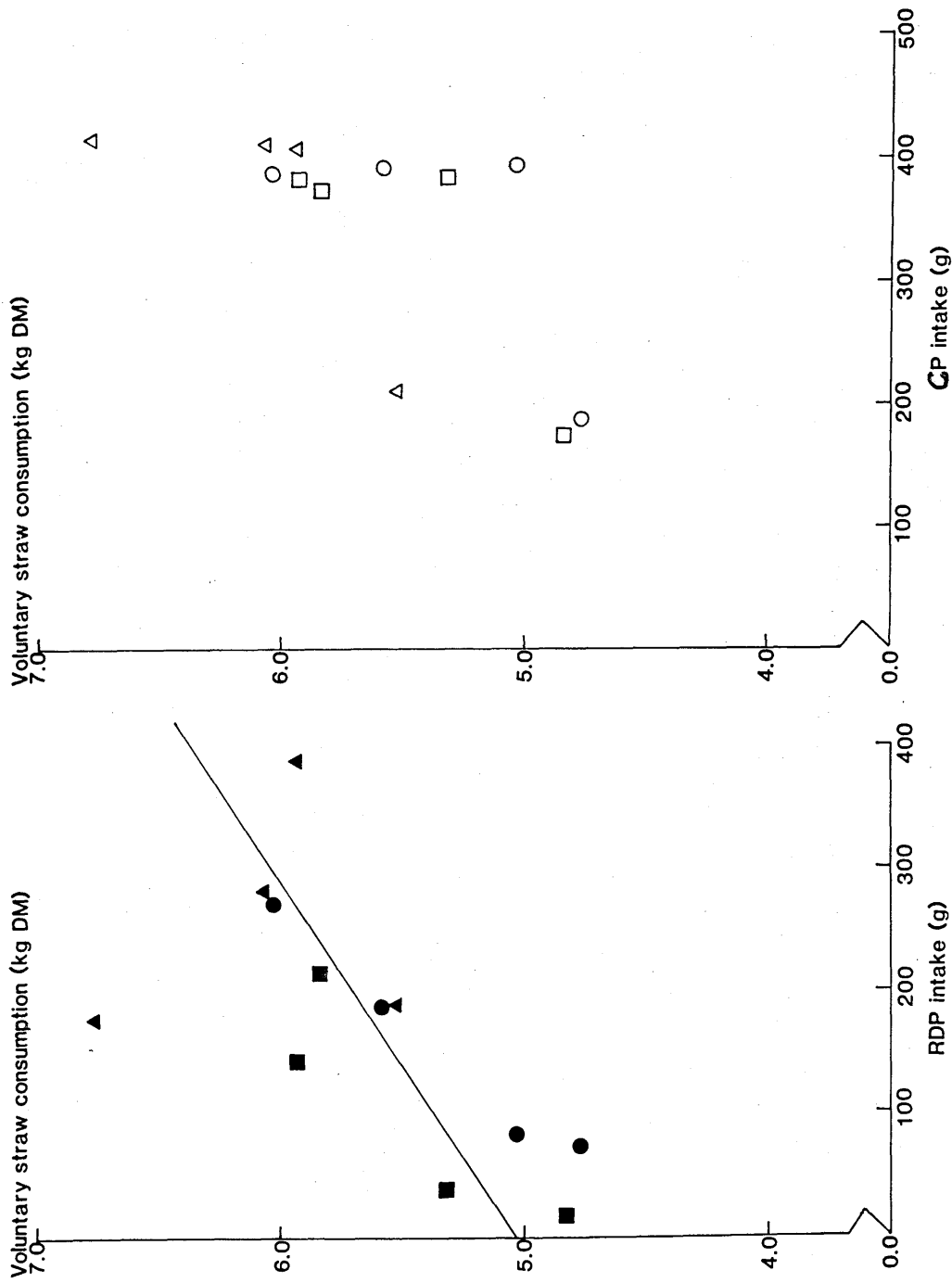


Fig. 2 The relationship between RDP intake and voluntary straw consumption

Fig. 3 The relationship between CP intake and voluntary straw consumption

apparently reduced the sensitivity of the relationship between RDP and straw DM intakes.

Data from 10 previous experiments (listed in Table 24) involving 18 different dietary treatments with urea and either about 2 kg barley or molassed sugar-beet pulp with 4-17 pregnant cows per treatment were also tested for this relationship. The RDP concentrations of the supplements used in these earlier experiments were calculated by using the same degradability values as obtained in these present experiments. The results are shown in Fig. 4. The regression equation was:-

$$\text{dry matter intake (kg)} = 3.67 + 0.0062 \times \text{RDP intake (g)}$$

The regression line was significant ($r = 0.641^{**}$; $\text{rsd } 0.918$; $\text{df } 16$). When straw DM intakes were plotted against CP intakes, the regression line only just approached significance ($r = 0.507^{*}$).

There was no significant difference between the slope of the line for the regression of straw DM intake on RDP for the present experiments and that of the previous experiments cited above, although there was a significant difference in the intercept. This relationship suggests that dietary protein degradability is an important factor in straw intake and emphasises the importance of satisfying the microbial need for nitrogen if optimum straw consumption and digestibility are to be achieved.

On the assumption that 0.65 of the straw OM apparently digested was digested in the rumen (A.R.C. 1980), the calculated values for RDP/kg straw OM apparently digested in the rumen ranged from 5 to 106 g. This is equivalent to 3.25 to 68.9 g RDP (0.52 to 11.0 g RDN)/kg OM apparently digested in the whole tract. Although it is possible that the straw portion of the diets used in these experiments provided additional RDP, the above values are low compared to a value of 19.6 g RDN/kg OM digested in the whole tract (Roy, Balch, Miller, Orskov and Smith, 1977) which would be required to optimise intakes through increases in microbial protein synthesis. It is also possible that these requirements vary depending on the nature of the diet fed.

Although the assessment of the degradability values for feeds may vary between laboratories, the values for DM loss and CP degradability for soya bean meal and formaldehyde-treated soya bean meal obtained in this study (Table 18) are in close agreement with those obtained in another study using food samples from the same batch as those used in these present experiments (respectively 66 and 56% for soya bean meal and 34.5 and 10% for formaldehyde-treated soya bean meal) (F.G. Perry,

Figure 4 The relationship between RDP intake and the
voluntary consumption of straw in previous
comparable experiments

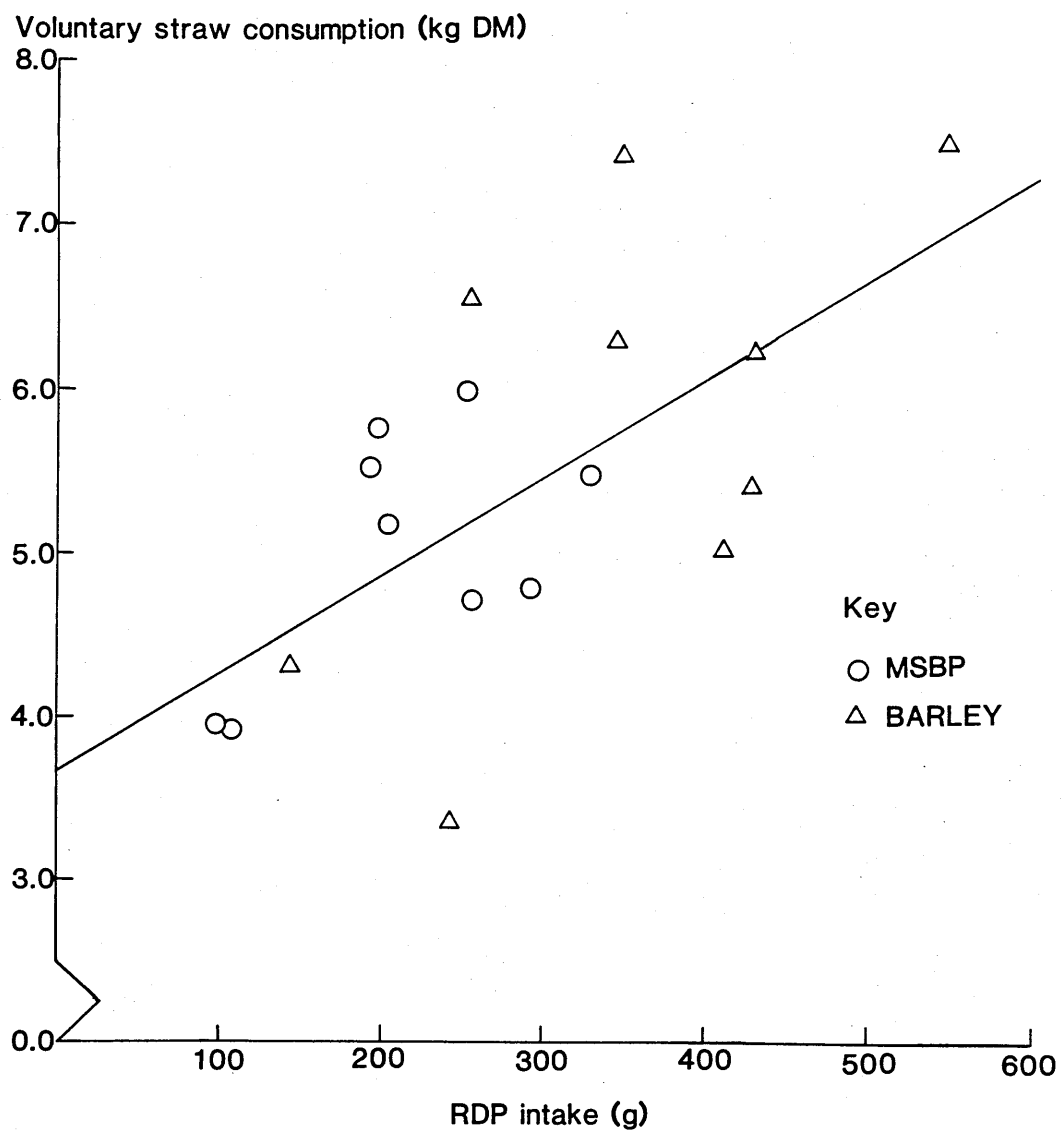


Table 24 (Previous comparable experiments)¹

The calculated intakes of RDP and the corresponding voluntary intakes of straw dry matter (DM) by pregnant cows in ten previous experiments.

RDP intake (g)	Straw DM intake (kg)	Type of supplement	Author (s)
97	3.97	MSBP+U	Fishwick <u>et al</u> (1974)
107	3.91	MSBP+U	" " (1974)
143	4.33	B+U	Bass <u>et al</u> (1981a)
192	5.47	MSBP+U	Fishwick <u>et al</u> (1977a)
196	5.75	MSBP+U	" " (1977b)
203	5.18	MSBP+U	" " (1973)
242	3.28	B+U	Bass <u>et al</u> (1981b)
251	5.99	MSBP+U	Fishwick (1985, unpublished)
254	6.55	B+U	Fishwick <u>et al</u> (1977b)
255	4.72	MSBP+U	" " (1974)
291	4.79	MSBP+U	" " (1974)
327	5.47	MSBP+U	" " (1977b)
342	6.30	B+U	" " (1978)
346	7.43	B+U	² Kay <u>et al</u> (1968)
410	5.02	B+U	Bass <u>et al</u> (1981b)
425	6.27	B+U	Fishwick <u>et al</u> (1977a)
426	5.41	B+U	Bass <u>et al</u> (1981a)
544	7.30	B+U	³ Early and Anderson (1978)

¹ With the exception of two, (2,3), all the experiments were conducted at Glasgow University Veterinary Field Station with the same set up and facilities as the present experiments, using oat straw.

² Used barley straw, ³ Used bluegrass straw

personal communication).

The differing levels of plasma urea observed for the various diets, 2 hours after feeding, are consistent with the degradability of the dietary protein. A significant relationship between the estimated intake of RDP and plasma urea concentrations ($r = 0.792^{**}$; $\text{rsd} \pm 2.830$; $\text{df } 10$) (Fig. 5) was obtained. The regression equation was:-

$$\text{plasma urea concentration (mg/l)} = 8.84 + 0.0321 \times \text{RDP intake (g)}$$

Although there was a fortuitously significant ($r = 0.733^{**}$) relationship between CP and plasma urea, this relationship, shown in Fig. 6, was not as good as that between RDP and plasma urea. Indeed, the third lowest plasma urea level was recorded for cows fed the diet containing the highest CP.

The relationship between the estimated RDP intake and plasma urea may have been important in maintaining an adequate return of urea as salivary nitrogen to the rumen and perhaps accounts in part for the highest overall straw intake by cows fed the urea-supplemented diet. Indeed, the relationship between daily intake of straw DM and plasma urea concentrations was virtually significant ($r = 0.574$; $\text{rsd } 0.499$; $\text{df } 10$ with a value of $r = 0.576$ required for significance at $P < 0.05$). The lowest straw intakes were associated with the lowest plasma urea values (about 10 mg/100 ml plasma) (Figs. 2 and 5). The importance of nitrogen cycling in ruminants fed low-quality roughage diets has been noted. In a study of fermentation and nitrogen dynamics in sheep fed low-quality roughage diets, Nolan and Stachiw (1979) observed that urea is transferred to the rumen directly through the rumen wall and indirectly through saliva and hydrolysed making available $\text{NH}_3\text{-N}$ which is taken up by microorganisms or reabsorbed and utilised in the body. They also found that 60-70% of blood urea is returned to the rumen through saliva. Of equal importance is a similar observation by Cheng and Wallace (1979) that the recycling of urea to the rumen is an important means of supplying ammonia for microbial protein synthesis in ruminants fed low protein diets. The diets used in these present experiments contained approximately 9% crude protein in the dry matter. The regression lines shown in Figs. 2 and 5 suggest that a daily intake of about 450 g RDP was required to maintain plasma urea concentrations of about 20-25 mg/100 ml necessary to maximise straw intakes by the cows in these present experiments. The Agricultural Research Council (1980) suggested that between 12 and 4 weeks before calving, which was the period covered by these experiments, pregnant cows maintaining

(Square symbols indicate USBP; circles, MSBP; and triangles,
BARLEY in both Figure 5 and Figure 6)

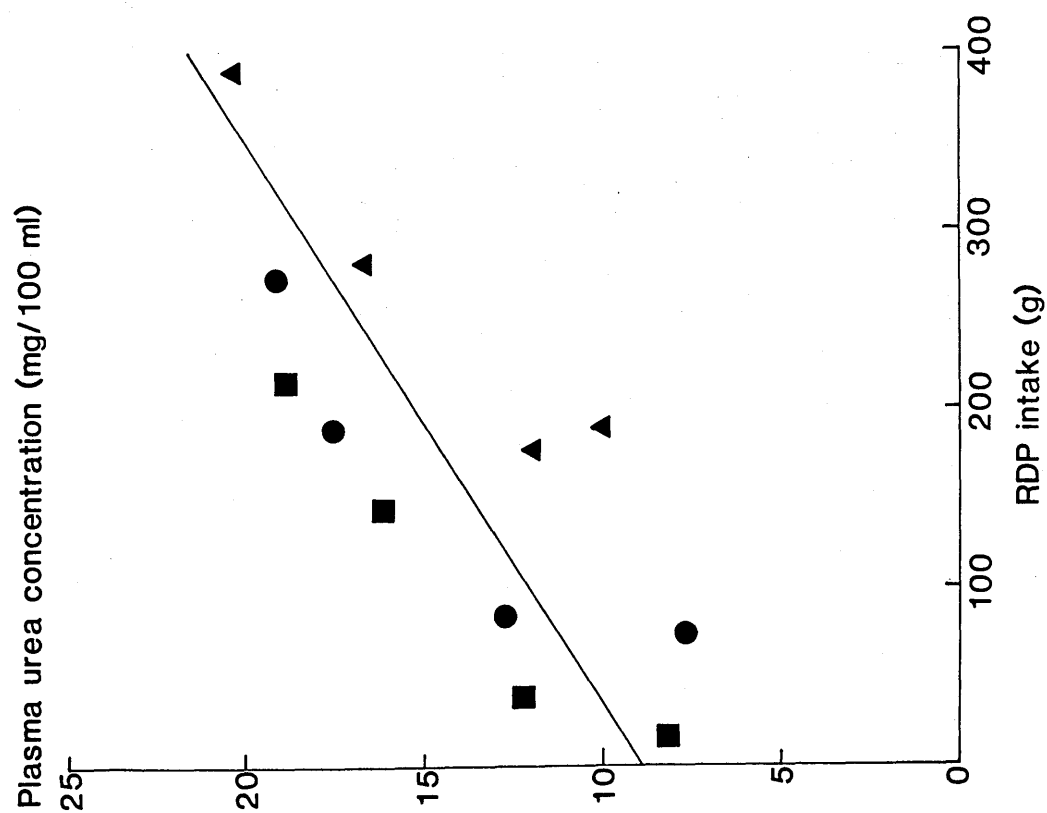


Fig. 5 The relationship between RDP intake and plasma urea concentration

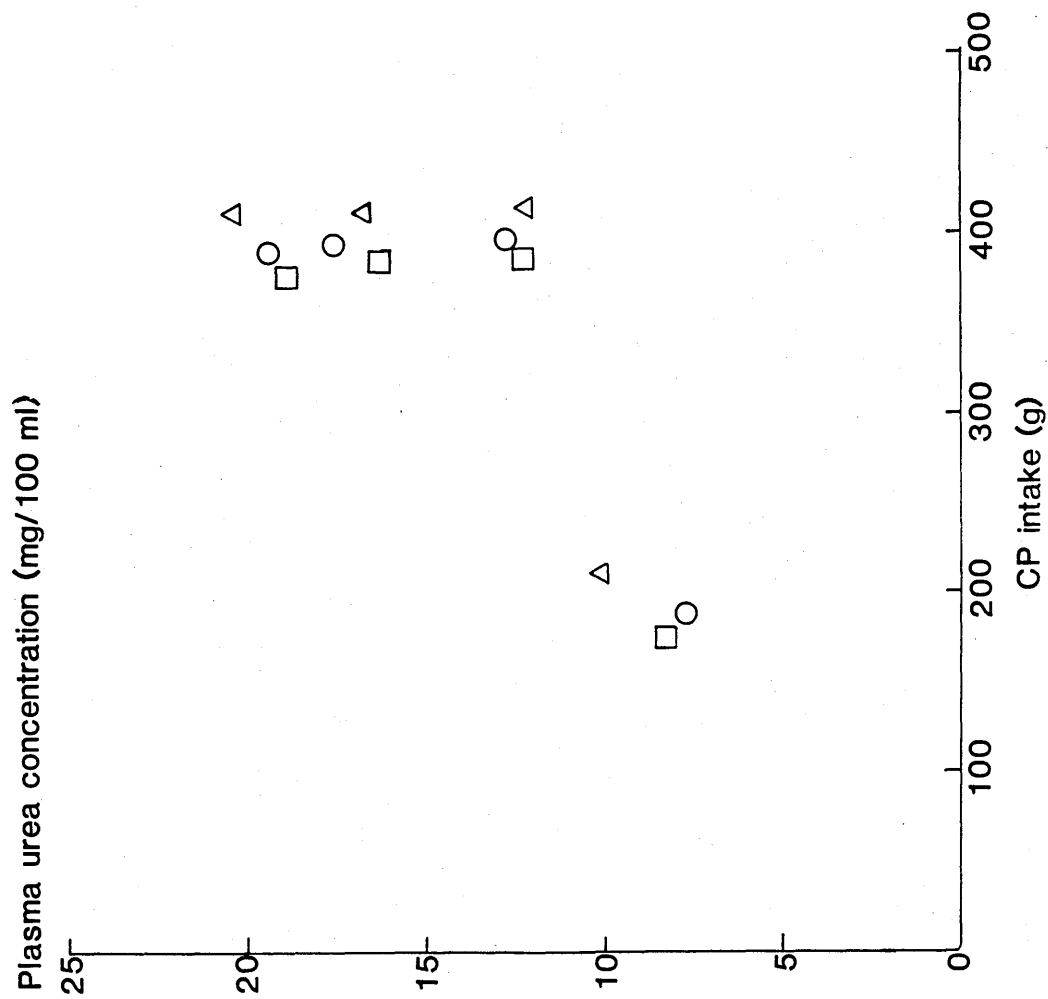


Fig. 6 The relationship between CP intake and plasma urea concentration

constant live weights of 500 kg and fed diets with a metabolisability $Q = 0.5$ (which is similar to diets used in the present experiments) require 445 to 550 g RDP (increasing to 665 at term).

The failure of urea supplementation in Experiment C (BARLEY) to improve straw digestibility is difficult to explain. There is also no obvious explanation for the lower digestibility of straw observed when the cows were given FT-SBM compared with the CONTROL, especially since RDP supply was slightly higher for the FT-SBM diet.

The effect of energy source and its interaction with protein supplement

The lack of a significant difference in straw intakes and digestibility between the three groups of cows when given a common concentrate mix at the end of the experiments suggests a superiority of BARLEY over MSBP and USBP (Table 23) in Experiments A, B and C. Although this finding is in general agreement with the observations of Ducker *et al.* (1976), the details are in contrast. In the report by Ducker *et al.* (1976), BARLEY plus urea was superior to MSBP with added urea for ewes, while in these present experiments voluntary straw intake and, in particular, digestibility was lower for BARLEY plus urea compared to MSBP and urea. The lower overall digestibility of straw by cows fed BARLEY than those fed MSBP may be due to the relatively detrimental effects of grain feeding on rumen microbial activity and consequently the digestibility of straw (Terry, Tilley and Outen, 1969). In other experiments, Edwards and Poole (1983) observed that rolled barley is subject to rapid fermentation and rapidly fermentable carbohydrates have been known to depress the digestibility of straw (Williams, MacDermid, Innes and Brewer, 1983).

The highly significant interaction between the energy and protein supplement on straw digestibility (Table 23) was probably responsible for the contrasting trends in straw intake response to protein source in Experiments A (MSBP) and B (USBP) on the one hand and in Experiment C (BARLEY) on the other. This interaction also helps to explain the apparently anomalous value (the highest point) in Fig. 2 for the BARLEY plus FT-SBM diet. The interaction between supplementary energy and protein source on voluntary intake was not significant.

Free fatty acids, 3-hydroxy butyrate and total protein in blood

A marginally higher FFA concentration for the CONTROL than for

other diets (Table 23) was associated with the lowest intakes of straw, in absolute terms, and may indicate a slightly increased body fat mobilisation to meet the needs of the cow on that diet. The levels of 3-OHB and total protein observed for the various treatments in these present experiments are similar. It has been indicated that there is no simple relationship between serum beta hydroxy butyrate levels and feed energy level that could be used to predict the energy status of cows (MAFF, 1984). Furthermore, Russel and Wright (1983) observed that, except under conditions of severe malnutrition, changes in blood metabolites are normally not appreciable and therefore may not serve as good indices of nutritional status in situations where differences in food intake are not sufficiently large, as in these present experiments.

The results obtained in this study indicate that dietary protein degradability may be one of the many factors which influence the voluntary intake of low quality roughage diets, especially when the rumen degradable protein content is the critical factor. Although rumen $\text{NH}_3\text{-N}$ was not measured in this experiment, this influence would appear to be exerted through the provision of adequate rumen $\text{NH}_3\text{-N}$ both as a result of the extent of protein degradability and a return of the resultant elevated plasma urea to the rumen through salivary nitrogen. However, where various energy and protein sources are fed in the same evaluation, interactions may complicate the results and raise problems of interpretation.

Experiment 4 - The effect of supplementary protein and energy source and dietary protein degradability on the voluntary intake, digestibility and utilisation of straw by lactating beef cows

Introduction

It was shown in Experiment 3 that for pregnant beef cows fed straw-based diets, crude protein supplied from sources that have low rumen-degradability do not enable the animal to achieve its full potential voluntary intake of straw dry matter. This was presumed to be due to failure of such diets to provide adequate levels of rumen ammonia nitrogen and to maintain an adequate return of salivary nitrogen to the rumen. Both energy and protein requirements are higher in lactation than in pregnancy and therefore reduced energy intakes due to low protein degradability restricts the ability of the beef cow to give more milk in response to the increasing appetite of the calf (Baker, Le Du and Barker, 1982; Somerville, Lowman, Edwards and Jolly, 1983). On the other hand, previous reports have shown that the provision of protein with a reduced degradability in the rumen results in increased milk yield during early lactation (e.g. Robinson, et al., 1979; Orskov, Reid and McDonald, 1981; Gonzalez et al., 1982) through additional mobilisation of body fat. Orskov et al. (1981) suggested that this response in milk yield to rumen-undegraded protein does not occur when energy intake is close to requirement, although Webster, Simmons and Kitcherside (1982) analysed several experiments and concluded that the differences in milk yield occur only when production is not limited by the lack of energy.

In view of these separate influences of protein degradability on voluntary energy intake and milk yield and the effect of energy intake on milk yield, Experiment 4 was designed to study the response of lactating beef cows and their calves to formaldehyde-treated or untreated soya bean meal when fed with barley or dried molassed sugar-beet pulp as supplements to ad libitum straw diets. Since these particular treatments were among those studied in Experiment 3, it was also intended to confirm the interaction between the supplementary source of energy and protein and how this affects the response in voluntary straw intake to increases in dietary RDP supply.

Materials and Methods

Animals and dietary treatments

Twelve cows and four heifers (Hereford x British Friesian and Irish Blue-Grey) with a 2-week spread in calving and their calves were weighed immediately after calving. They were blocked on the basis of calving date, live weight and body condition score (Lowman, Scott and Somerville, 1973) and randomly allocated to one of four treatment groups in a 2 x 2 factorial design, the heifers being separately allocated. The treatments consisted of 2.5 kg fresh matter (1.8 kg dry matter) of either rolled barley (B) or shredded molassed sugar-beet pulp (MSBP) each supplemented with 0.5 kg (fresh basis) of untreated soya bean meal (SBM) or formaldehyde-treated soya bean meal (FT-SBM) (Sopralin). Additionally, all the cows received 0.1 kg of a mineral supplement and 0.2 kg of a barley cube containing 10 g chromic oxide. Barley straw was fed ad libitum in long form.

Composition of feeds

Table 25 shows the composition of dietary constituents, the calculated metabolisable energy (ME) and digestible crude protein (DCP) and estimated degradability of the concentrate components. The ME and DCP of the concentrate components used in this experiment were determined in a 17-day digestibility trial involving 7 days total collection using adult castrated (Suffolk x Greyface) wether sheep averaging 57 kg live weight (four sheep per treatment). The digestibility trial was conducted according to the procedure described in Appendix 3. The quantities of the various concentrate components and the ratio in which they were given with other feeds of known digestible energy (DE) and crude protein (DCP) were the same as those specified for each supplement in a similar digestibility trial described in Experiment 3. The ME was calculated as $DE \times 0.832$ (Wainman, Dewey and Boyne, 1981) having first obtained the DE of the concentrate components by difference. The degradability values used were the same as those obtained in Experiment 3 as the feeds used were from the same lots purchased. Table 26 shows the apparent digestibility of energy in concentrate dry matter, the calculated ME and DCP and the estimated RDP and UDP of the concentrate parts of the various diets. The four concentrate supplements each supplied about 490-540 g crude protein containing approximately 400-460 g DCP/day. The proportions of concentrate CP supplied as RDP for the various diets

Table 25 Experiment 4

Chemical composition of dietary constituents (g/kg), calculated metabolisable energy and digestible crude protein content of the concentrate components and the estimated degradability of the concentrates.

	Straw ⁺	B	MSBP	SBM	FT-SBM
Dry matter	845	872	882	888	864
Crude protein	28	137	114	545	556
Crude fibre	447	56	131	40	38
Ether extract	11	17	4	15	13
N-free extract	459	768	675	332	320
Gross energy (MJ/kg DM)	18.7	18.4	16.3	19.3	19.2
Ash	55	22	76	68	73
Metabolisable energy (MJ/kg DM)	-	12.6	12.5	11.8	12.4
Digestible crude protein (g/kg) <i>coefficient</i>	-	0.796	0.767	0.947	0.891
Rumen degradable protein coefficient	-	0.90	0.39	0.54	0.11

⁺ Straw crude protein assumed to be completely undegradable.

Table 26 Experiment 4

The digestibility of energy in concentrates and the calculated intake of metabolisable energy (ME) (MJ) and nutrients (g) from the combined concentrate supplements.

	B		MSBP	
	SBM	FT-SBM	SBM	FT-SBM
Digestibility of energy (%)	76.7	76.5	75.9	75.6
ME	27.7	27.9	25.9	26.1
Digestible crude protein	465	449	422	407
Rumen degradable protein	396	290	227	122
Undegraded protein	142	246	266	370

were 0.74, B/SBM; 0.54 B/FT-SBM; 0.46, MSBP/SBM; 0.25 MSBP/FT-SBM. Fortuitously perhaps, about the same amounts of UDP were supplied by B/FT-SBM as MSBP/SBM.

Experimental Procedure

The cows were kept in individual stalls. The calves were tied up behind the cows, being released to suckle the cows three times a day, a procedure previously shown to lead to a reduction in the variation associated with the calf-suckling technique and to provide a satisfactory estimation of milk yield (Le Du, Baker and Barker, 1978). Milk yield during the third week after calving was measured as the difference between the weight of the calf before and after suckling.

The concentrate supplement together with the barley/chromic oxide cubes and the mineral supplement were given in one feed at 07.30h. The straw portion of feeds was weighed, sampled and fed as described in Experiment 3. Straw intakes were recorded during the third and fifth weeks after calving. Digestibility was estimated by analysis of faecal grab samples taken three times a day for 7 days, as described in Experiment 3. Straw digestibility was calculated by difference after allowing for the digestibility of the various concentrates. The cows were weighed and blood samples were taken on the last day of the third and fifth weeks after calving.

Chemical Analysis

Blood samples were centrifuged (3000 rpm) and the plasma analysed for urea, total protein, beta hydroxy butyrate (3-OHB) and free fatty acids (FFA) as described in Appendix 4. Dried and milled faeces were analysed for crude protein, crude fibre, ether extract, ash, energy and chromium (faeces only) as described in Appendix 4.

Statistical Analysis

Results were analysed as a 2 x 2 factorial analysis of variance to determine main effects and interactions. Individual treatment means for voluntary straw intake were also compared to determine the effect of rumen degraded protein intake on voluntary straw consumption and milk production.

Results

Voluntary intakes and digestibility of straw

The mean values for the voluntary intakes of straw DM are given in Table 27. Intakes increased rapidly following calving, with the cows consuming, in addition to 2.9 kg concentrate DM, an overall daily average of 5.8 and 6.2 kg of barley straw DM during the third and fifth weeks after calving respectively. Although there was a greater increase in the intake of straw DM when B rather than the MSBP was the energy supplement, the effect was significant ($P < 0.01$) only during the fifth week after calving. The source of protein supplement had no effect on straw intake but a non-significant protein x energy supplement interaction on intake of straw dry matter was evident during the fifth week after calving.

When the individual treatment means were compared, straw intakes tended to increase in response to the level of RDP in the diet (shown in Table 26), the amount being significantly higher for B/FT-SBM than for MSBP/SBM ($P < 0.05$) and MSBP/FT-SBM ($P < 0.01$) during the fifth week after calving. Although absolute values for straw DM intakes were slightly higher for B/FT-SBM than for B/SBM due to the non-significant interaction between protein and energy supplement, the difference was not statistically significant.

The calculated digestibilities of the straw DM and OM are also shown in Table 27. Straw digestibilities, estimated by faecal chromium concentrations in this experiment, generally appeared lower than expected. This could possibly have been due to a small error in sampling of barley chromic oxide cubes used in this present experiment. However, supplementation with MSBP significantly ($P < 0.05$) improved the apparent digestibility of straw DM during the fifth week after calving and of straw OM during both periods of assessment. The source of protein supplement had no effect on straw digestibility. The interaction between protein and energy supplement on straw digestibility did not approach significance but was significant ($P < 0.05$) and ($P < 0.01$) on the intake of straw ME during the third and fifth weeks respectively after calving.

Changes in live weights and milk yield of the cows and the growth rates of their calves

The live weight changes of the cows and the growth rates of their calves and the daily milk yield are presented in Table 28. By the

Table 27 Experiment 4

Mean cow live weight after calving (kg), voluntary intake of straw dry matter (DM) and digestible organic matter (OM) (g/kg $W^{0.75}$) and the apparent digestibility of straw DM and OM and metabolisable energy (ME) intake from straw.

	B		MSBP		SE of	ES	PS	ExP
	SBM	FT-SBM	SBM	FT-SBM				
Cow live weight								
after calving	449	446	427	429	-	-	-	-
<u>Straw DM intake</u>								
3 weeks after calving	56.2	68.8	56.4	54.6	3.09	NS	NS	NS
5 weeks after calving	69.8 ^{ab}	79.2 ^a	63.2 ^b	57.8 ^b	2.28	**	NS	NS
<u>Straw DM digestibility</u>								
3 weeks after calving	0.25 ^b	0.30 ^b	0.39 ^a	0.30 ^b	0.025	NS	NS	NS
5 weeks after calving	0.31 ^b	0.35 ^{ab}	0.40 ^a	0.37 ^{ab}	0.013	*	NS	NS
<u>Straw OM digestibility</u>								
3 weeks after calving	0.24 ^b	0.30 ^{ab}	0.40 ^a	0.31 ^{ab}	0.025	*	NS	NS
5 Weeks after calving	0.31 ^b	0.35 ^{ab}	0.41 ^a	0.38 ^a	0.013	*	NS	NS
<u>Straw digestible OM intake</u>								
3 weeks after calving	12.0	18.8	20.7	17.0	1.68	NS	NS	NS
5 weeks after calving	20.9 ^b	26.5 ^a	25.3 ^a	21.1 ^b	0.84	NS	NS	**
<u>ME intake from straw¹</u>								
3 weeks after calving	17.8 ^b	27.0 ^a	29.1 ^a	23.5 ^{ab}	2.14	NS	NS	*
5 weeks after calving	30.7 ^b	40.3 ^a	35.2 ^{ab}	31.3 ^b	1.10	NS	NS	**

⁺ Calculated as DOMD x 0.15 MAFF (1975)

a,b Means on the same row with different superscripts were significantly different (P<0.05).

ES = Energy Source; PS = Protein Source; E x P = Energy x Protein.

third week after calving, the cows fed the B diet had lost some weight in contrast to those fed MSBP that gained considerable live weight. The difference was significant ($P < 0.05$). Although there was progressive loss in weight for all the cows from the third week after calving, the cows fed the MSBP diet apparently maintained their weights until the end of the fifth week after calving. Live weight losses also tended to be lower when the cows were fed FT-SBM than when given SBM but the difference was not significant during any of the periods of assessment.

Average daily milk yield of the cows, measured during the third week after calving, was about 6.4 kg. Although milk yield did not differ significantly between treatments, cows fed the B diet produced 0.3 kg more milk/day than those fed MSBP. Formaldehyde treatment of soya bean meal also increased milk yield by an average of only 0.35 kg/day.

The average daily growth rate of the calves was about 0.7 kg with a conversion ratio (milk:gain) of approximately 9:1. Calf growth rates did not differ significantly between treatments but the trend was in agreement with milk yield on the various diets.

Blood metabolites

The concentrations of blood metabolites of the cows are presented in Table 28. The plasma concentrations of the various metabolites did not differ significantly between treatments. There was no relationship between the degradability of the concentrate protein and plasma urea, neither was there any relationship between the amount of undegraded protein supplied by the concentrate fed and plasma total protein. Plasma concentrations of beta hydroxy butyrate (3-OHB) were slightly higher for cows fed B than for those fed MSBP. The cows fed SBM also had slightly higher plasma 3-OHB concentrations than those fed FT-SBM. Absolute values of plasma free fatty acid (FFA) concentrations were higher for cows fed B than for those fed MSBP.

Discussion

The effect of energy supplement

The results show that at 3 to 5 weeks after calving, supplementation with rolled barley promoted higher straw intakes than with molassed sugar-beet pulp and confirms the results of Experiment 3 and an earlier report when comparable diets were given (Fishwick,

Table 28 Expt. 4 Changes in live weight of the cows, daily milk yield and calf growth rates (kg) and the mean composition of blood plasma

	B		MSBP		S.E. of mean	Significance of main effects		
	SBM	FT-SBM	SBM	FT-SBM		Energy supplement	Protein supplement	Energy x Protein
Cow live weight change to:								
3 weeks	-12.8 ^b	-7.0 ^{ab}	+11.8 ^a	+9.5 ^{ab}	5.65	*	NS	NS
5 weeks	-20.3 ^b	-9.5 ^{ab}	+ 3.0 ^a	+1.8 ^{ab}	6.65	NS	NS	NS
Daily calf growth rate to 5 weeks	0.69	0.74	0.65	0.71	0.047	NS	NS	NS
Daily milk yield at 3 weeks	6.30	6.76	6.13	6.36	0.356	NS	NS	NS
+ Composition of blood plasma (mg/100 ml):								
Urea	7.01	7.76	7.67	7.51	0.367	NS	NS	NS
Total protein	7.7 ^a	7.2 ^{ab}	7.4 ^{ab}	7.1 ^b	0.17	NS	NS	NS
3-OHB	2.95	2.84	2.74	2.54	0.277	NS	NS	NS
FFA (u-equiv/l.)	317	254	197	256	42.4	NS	NS	NS

⁺ Mean of 3 and 5 weeks after calving

Fraser, Hemingway, Parkins and Ritchie, 1977b) which showed that for pregnant beef cows, barley improved the voluntary intake of straw compared to sugar-beet pulp. However, for dairy cows barley and sugar-beet pulp have been shown to be comparable energy sources as measured ^{for} with milk production when part of the barley was substituted with sugar-beet pulp on an equal DM basis (Bhattacharya and Sleiman, 1971; Castle, 1972). The difference in straw intake in this present experiment was, however, not reflected in ME intake due to the lower digestibility of the barley-supplemented diet (Table 27). Although the cows fed the B diet lost more weight than those fed MSBP, this was consistent with the marginally higher milk yield and calf growth rates for the B diet.

The effect of protein source

There was no difference between formaldehyde-treated and untreated soya bean meal-supplemented diets in the voluntary intake of straw or milk production in this experiment. Castle and Watson (1984) also failed to find any difference in the milk yield of cows fed ad libitum high digestibility silage diets supplemented with a concentrate mix containing either 167 g/kg formaldehyde-treated or untreated soya bean meal (mean milk yields were respectively 23.8 and 23.9 kg/day for formaldehyde-treated and untreated soya bean meal-supplemented diets). In an earlier report, Clark, Davis and Hatfield (1974) failed to find any response in milk yield to formaldehyde treatment of soya bean meal when dairy cows were fed a roughage/concentrate diet (47:53 air dry basis) containing formaldehyde-treated or untreated soya bean meal. On the other hand, Hunter, Rowlinson, Brett, Harrison and Armstrong (1981) reported a significant increase in milk yield when untreated soya bean meal replaced formaldehyde-treated soya bean meal in a grass silage/barley diet containing 90 g/kg soya bean meal. Perhaps the failure of formaldehyde treatment of soya bean meal to significantly affect milk yield in this present experiment is because the cows were closer to energy equilibrium (Orskov et al., 1981) as calculated from estimates of energy intakes. In addition to the reports cited above, the results of this present experiment and most of those cited in Table 1 on the effect of formaldehyde treatment of soya bean on milk yield combine to suggest that the response in milk yield to formaldehyde treatment of soya bean meal has not been consistent.

The effect of protein degradability

These experiments in which there are interactions are not quite suitable for measuring responses to graded levels of any factor. However, despite the apparent interaction between protein and energy supplement on the digestibility and voluntary intake of straw observed in the present experiment, there was clear evidence of a response in straw consumption to increases in RDP content of the concentrate supplements. This is consistent with the results of Experiment 3 where a similar interaction tended to reduce the sensitivity in the positive relationship obtained between RDP and straw intake. The response in straw DM intake was not exactly reflected in milk yield but the LW changes tended to follow the same pattern as straw intake, except that the highest total milk yield appeared to have caused more live weight loss than would be expected.

The comparative effects of barley and molassed sugar-beet pulp on straw digestibility

The lower digestibility of straw observed for barley than for MSBP also confirms the results of Experiment 3. In a recent study of the effects of different supplements on straw digestion, Fahmy, Lee and Orskov (1984) concluded that more molassed sugar-beet pulp than rolled barley could be incorporated into diets before the degradation of straw is reduced and that the depression in digestion of straw is more pronounced with rolled barley than with molassed sugar-beet pulp. The relatively unfavourable rumen conditions caused by feeding rapidly fermentable carbohydrates (Williams *et al.*, 1983) of which rolled barley is an example, may be due to a depression in rumen ammonia production which has been observed when starch, another rapidly fermentable carbohydrate, was fed with proteins differing in solubility (El-Shazly, 1958). Although it was not possible to measure rumen ammonia production in this present experiment or in Experiment 3, these rumen conditions, particularly that reported by El-Shazly (1958) involving starch and proteins differing in solubility, may indeed explain the interactions in the present experiment and in Experiment 3. In both experiments, the interaction was due mainly to the feeding of barley with either SBM (present experiment) or SBM or urea (Experiment 3), which, in either case, adversely affected straw digestibility and intake, tending to alter the response in straw consumption to concentrate RDP supply. It is difficult to attribute the change in

straw intake response (higher absolute straw intake for B/FT-SBM compared to B/SBM or B/U (Experiment 3)) to the effect of bypass protein on the intake of low protein roughages, considering these interactions. Egan and Moir (1965) suggested that bypassing the rumen with protein increases the voluntary intake of low protein roughages.

Blood metabolites

The similarity in blood urea concentrations despite the wide differences in the concentrations of RDP for the various diets is in contrast to results obtained in Experiment 3. But considering that Blaxter (1962) attributed an increase in the concentration of urea in the peripheral plasma of the dairy cow during early lactation to gluconeogenesis from amino acids to make up the energy deficit of the cow, the contrast is not surprising. On this assumption, differences in plasma urea concentrations of lactating cows in relatively small negative energy balance would perhaps be minimal, although much higher dietary CP concentrations containing highly degradable proteins may give different results. Differences in blood urea levels between high and low-producing cows may also be found during lactation (Freeman, Kelley, Ledet, Evans, Appell, Wass and Pearson, 1978) but this may be more closely related to the genetic potential for milk production.

It has been suggested that blood metabolites are better indicators of nutritional status if used as indices of the severity of undernutrition (Russel and Wright, 1983). As such, the close range of plasma beta hydroxy butyrate of cows fed the various diets in this experiment is not surprising since the calculated energy intakes could be considered close to requirements. The marginally higher plasma beta hydroxy butyrate concentrations of cows fed the barley diet agrees with the marginally higher milk yield and greater loss in weight since an increase in circulating beta hydroxy butyrate is thought to result from a shift in the balance of acetyl Co-enzyme A metabolism towards ketogenesis consequent upon increasing gluconeogenesis (Russel and Wright, 1983). Plasma free fatty acid concentrations tended to show a similar trend as beta hydroxy butyrate, the marginally higher FFA values for cows fed the barley diet indicating a greater extent of tissue mobilisation which is in agreement with the marginally higher milk yield and greater loss in live weight (Hart, Bines, Morant and Ridley, 1978).

The results obtained in this experiment indicate that barley

improved the consumption of straw over molassed sugar-beet pulp, although this improvement was not reflected in the intake of energy and consequently milk yield due to lower digestibility. Formaldehyde treatment of soya bean meal did not reduce voluntary straw consumption and its effect on milk yield was less clear cut. Voluntary straw intake tended to increase in response to concentrate rumen degraded protein supply, although a combination of highly degraded protein supplement and rapidly fermentable grains tended to depress digestibility and consequently intake through a protein x energy supplement interaction.

Suggestions for further work

Despite the significant effects of energy source on straw intake and digestibility in the present experiment, any conclusions drawn from the results would have been more valid, and perhaps more definite information on the effects of the protein treatments would also have been obtained, had the number of animals involved been increased. With hindsight, it would have been preferable to feed the treatments beyond the fifth week after calving since the apparently increasing appetite of the cow following calving appeared to have amplified the differences in straw intake with time. The above considerations may be necessary in a repeat of this type of experiment.

SECTION 2

EVALUATION OF ALTERNATIVE PROTEIN SUPPLEMENTS

Introduction

The sustained competitive demands for feedstuffs between the livestock feeds industry and human nutrition, especially in the developing countries, has necessitated the search for cheaper and unconventional sources of protein and animal feeds. Also, the relative costs of feed protein supplements from animal and plant sources, and even the varying costs among the plant protein sources, further emphasise, at the least for the poorer countries, the importance of investigating other presently less popular sources of protein. Since the aim is to restrict animal production costs, the availability of by-product sources of protein would equally be advantageous. By-products of the brewery and distillery pass through processes that concentrate their crude protein contents to about 200 g/kg and may thus provide adequate protein to meet the overall needs of adult animals. Although peas are not by-products, they possess a CP content of about 22-29 g/kg. As such, they may also be quite useful as protein supplements but they are presently less often used in animal feeding. As leguminous plants, they are cheaply grown without the need for fertilizer and many varieties also grow uncultivated in certain tropical regions, including Nigeria. In comparison with popularly used protein supplements, they may provide a cheap source of protein in ruminant diets.

This section of the thesis therefore investigates the use of brewers grains and peas as protein and energy supplements to straw. Experiment 5 is designed to study the degradability and use of brewers grains as supplements to straw fed ad libitum to pregnant cows. Experiment 6 examines the degradability of peas and further compares peas and brewers grains each as a protein and energy source with a conventional plant protein and energy source.

Experiment 5 The use of brewers grains as a protein supplement

Introduction

Brewers grains, a by-product of the brewing industry, have long been used as a component of animal feeds. Since the brewer's aim is to maximise the removal of soluble sugars from the grains, the residues consist largely of protein, some lipids and the fibrous parts of the original grains, which places them in a special category as potential protein supplements for use in ruminant diets. A survey of the nutritive value of brewery and distillery by-products (MacLean, 1969) indicates the paucity of scientific information available concerning the nutritive value of these by-products. There is, however, evidence to suggest that wet distillers grains, when fed both as an energy and a protein supplement with ad libitum straw to pregnant cattle, is better utilised than barley plus urea in terms of calf birth weights and live weights at transfer to grass in the spring (Ball, Broadbent and Dodsworth, 1971) but not in terms of straw consumption. In a recently updated report on distillery by-products as feeds for livestock, Black, Crabtree, McKelvie and Topps (1984) compared the nutritive value of brewers grains with other by-products of the distillery but there is little further information on the use of these by-products as the sole protein supplements, especially considering their crude protein contents. Moreover, it is not clear to what extent the crude protein in brewers grains is degraded in the rumen and whether the heat which may be applied in drying the 'spent' grains adversely affects its degradability. These considerations are important in view of the effect of protein degradability in increasing the rate of rumen digestion (Mehrez and Orskov, 1978) and consequently the voluntary intake of straw as observed in Experiments 3 and 4.

Experiment 5.1, therefore, investigates the rate and extent of ruminal degradability of wet and dry brewers grains.

Experiment 5.1 The rumen degradation of wet and dry brewers grains

Materials and methods

Both the wet (WBG) and dry brewers grains (DBG) used for this present experiment and the subsequent one were obtained from Brewers Grains Marketing (Misson Feeds and Storage, Wetmore Road, Burton-on-Trent, Staffordshire). On delivery, the wet brewers grains

was ensiled under a black polythene sheet, being well compacted to exclude air. Bales of straw were placed on the polythene sheet with adequate care to ensure, as far as possible, the exclusion of air pockets from within the heap. The initial DM of the WBG on delivery was 191 g/kg. During storage, effluent, mainly water, drained away resulting in a DM content of 247 g/kg by the third week after ensiling. From the fourth to the seventh week after delivery, when the current experiment was commenced, the DM content had settled to approximately 250 g/kg DM.

The dry form of the grains from the same source was delivered at the same time. This had been commercially dried and bagged before delivery and was stored in an open building protected from rain and bad weather. Since the same batches of brewers grains were used in this present experiment as in the next, their proximate compositions have been presented together with their digestibilities in Table 33.

Incubation procedure

The procedure for estimating the rate and extent of degradation of dry matter (DM) and crude protein (CP) in WBG and DBG is essentially the same as that described in Appendix 1, except that a higher quantity of substrate (10 g per replicate) was used in this experiment. Four rumen fistulated cows (mean live weight 467 kg) given hay plus 1 kg molassed sugar-beet pulp/hd daily were used. The nylon bags (4 replicates/substrate) were withdrawn after either 3, 6, 9, 15 or 24 h of incubation.

Measurement of retention time in the rumen

In order to estimate the rate of passage from the rumen, both forms of brewers grains were first treated with sodium dichromate using a modification of the procedure for the preparation of Chrome Mordanted straw (E.R. Orskov, personal communication).

Sodium dichromate was dissolved in warm water and the solution was mixed with each form of brewers grain (72.1 g sodium dichromate/kg DM) to achieve a porridge consistency. Approximately 2.4 l of solution/kg (air dry brewers grains) was required for DBG and 2.14 l/kg for WBG to achieve the appropriate porridge consistency. The mixture was put into drying trays, covered with aluminium foil to prevent drying out and placed in the oven at 100°C. for 24 hours. The heated brownish material was subsequently washed in a 1 mm sieve with a jet of cold

water until the effluent ran clear. Warm water was then added and the material stirred. Ascorbic acid was added to reduce the pH from approximately 8.0 to 4.0. The mixture was stirred again and left overnight. It was subsequently washed as before and dried at 100°C. for 24 hours.

During each of the drying processes, water was occasionally added to the wet brewers grains to maintain a consistency as near as possible to its original form.

Eight rumen fistulated cows with mean live weight 508 kg (445-594 kg), arranged in two balanced groups (four per treatment) were used to estimate the fractional outflow rates of brewers grains from the rumen, using faecal chromium concentrations. Eliman and Orskov (1984) had indicated that marker concentrations in faeces provide an accurate estimate ($r = 0.99$) of the rate of outflow of chromium-treated protein supplements from the rumen, thus eliminating the need for surgical preparations. The cows were fed 1 kg molassed sugar-beet pulp plus 0.3 kg soya bean meal at 07.30 h and 7 kg hay (fresh basis) in two approximately equal feeds at 08.00 and 16.00 h daily.

Two hundred and fifty grams of each form of chromium-treated brewers grains were suspended in warm water and poured into the rumen as a single dose through the rumen cannulae at 09.00 h, after the morning feed. Thereafter, rectal grab samples of faeces were obtained every 3 h for 36 h and every 12 h from then for 5 days. All 160 faeces samples were weighed, dried and analysed for chromium as described in Appendix 4.

Statistical analysis

The disappearance of substrates from nylon bags after each time interval was compared by standard analysis of variance. The curves of the disappearance of DM and N were described by a single exponential equation of the form $p = a + b(1 - \exp^{-ct})$. The constants a (the rapidly degradable fraction), b (the amount which in time will degrade) and c (the fractional rate of disappearance per unit time (h)) were fitted by iterative least squares procedure with the constraint, if necessary, that $a + b$ should not exceed 100 (Orskov and McDonald, 1979). The exponential model did not provide a good fit for DBG because of an apparent lag phase and linear regressions of the form $p = a + ct$ were fitted to both forms of brewers grains for purposes of calculating degradation.

The fractional outflow rate (k) of undegraded protein from the rumen was calculated from the linear regression of the logarithmically transformed faecal chromium concentrations on time, using the points after the peak in the descending line for the regression analysis. Freer and Dove (1984) have suggested that for foods in which the rate of nitrogen disappearance was linear, the equation for calculating effective degradability (P) could be derived thus:-

$$\begin{aligned} \text{Cumulative percentage protein} & \quad t \\ \text{degraded up to time } t & = \int_0^t \exp(-kt) \, c \, dt \\ & = a + (c/k) (1 - \exp(-kt)) \\ \text{therefore effective degradability} & \\ \text{(coefficient) } P & = 0.01 (a + c/k) \end{aligned}$$

Results

The disappearance of DM and N from nylon bags incubated in the rumen of the cows are given in Table 29. The uncorrected nylon bag values show that the disappearance of DM from the nylon bags was the same for WBG and DBG for the various incubation periods except the value for 15 h which was significantly ($P < 0.05$) higher in favour of WBG. The disappearance of nitrogen was also similar for both forms of brewers grains for the various incubation periods except that at 9 h, nitrogen disappearance was significantly ($P < 0.05$) higher for WBG.

The fitted and the calculated values have also been presented together in Table 29 for purposes of comparison. Both the fitted and calculated values for the disappearance of DM were initially marginally lower for DBG but the 24 h values were not different. The calculated disappearance of nitrogen at 24 h was slightly lower for DBG. The differences between the fitted and the calculated values for each time period, especially for the 24 h period, were marginal.

The fitted constants are shown in Table 30. Although the exponential model did not provide a good fit for the disappearance of DM or nitrogen, the fitted constants have been given only in connection with the fitted values shown in Table 29. The linear model showed the 'a' (rapidly degradable) and 'c' (fractional rate of disappearance) values for the DM of both forms of brewers grains to be essentially similar. The value for 'a' is negligible in both cases. Although the value for 'c' for nitrogen is also the same for both treatments, that

Table 29 Expt. 5.1 The disappearance of dry matter and nitrogen
(g/kg) from nylon bags with time following incubation

Time(h)	Measured		SED	Fitted ¹		Calculated ²	
	WBG	DBG		WBG	DBG	WGB	DGB
<u>Dry matter:</u>							
3	73	64	7.6	39	27	62	42
6	92	78	17.4	125	107	123	105
9	152	135	15.0	201	181	183	168
15	397	355	16.1 [*]	328	310	305	294
24	449	463	9.5	469	467	486	483
<u>Nitrogen:</u>							
3	197	151	19.8	144	120	167	114
6	207	176	17.5	241	185	238	185
9	248	184	17.4 [*]	328	252	308	256
15	537	452	43.5	473	393	450	399
24	633	609	30.4	633	621	662	613

¹Values calculated from the fitted equation:

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

²Calculated using a linear model: $p = a + ct$

(All fitted constants a, b and c for both the exponential and linear models are shown in Table 30)

Table 30 Expt. 5.1 Fitted constants (g/kg) for dry matter and nitrogen
loss from nylon bags

	Dry Matter			RSD	Nitrogen			RSD
	a	b	c		a	b	c	
<u>Exponential Model:</u>								
WBG	-57.5	852.5	0.401	69.7	33.2	966.8	0.404	69.2
DBG	-60.6	1060.6	0.287	45.7	57.1	-1944.3	-0.106	68.0
<u>Linear Model:</u>								
WBG	1.60	-	20.2	54.2	95.9	-	23.6	69.4
DBG	-21.0	-	21.0	42.0	42.2	-	23.8	56.3

for 'a' is much lower for DBG, being about half the value for WBG.

The chromium concentration in sodium dichromate-treated brewers grains was 19.3 g/kg DM for DBG and 15.4 g/kg DM for WBG compared with an estimated, from calculation, 24.7 g/kg DM. However, treatment seemed effective in protecting both forms of brewers grains from degradation in the rumen. The recovery of DM and nitrogen from nylon bags after incubation for 24 h were (g/g) respectively 1.005 (\pm 0.0324) and 1.0028 (\pm 0.0259) for DBG and 1.034 (\pm 0.0341) and 1.010 (\pm 0.0523) for WBG. The outflow rate 'k', calculated to be 0.0438 for DBG and 0.0418 for WBG were derived from Figs. 7a and 7b respectively. There was, however, a small number of anomalous values for faecal chromium concentrations, mostly during the first 24 h for both DBG and WBG. It is possible that the slightly higher chromium concentration for DBG compared to WBG after treatment is responsible for the slightly higher estimated outflow rate for DBG.

The calculated effective degradabilities 'P' are shown in Table 31. The degradabilities of both DM and nitrogen are slightly higher for WBG than for DBG but this may be due to the higher analysed chromium concentration of treated dry brewers grains relative to the treated wet brewers grains referred to earlier which may have given a slightly higher 'k' value for DBG.

Discussion

Since both dry and wet brewers grains were also compared in Experiment 5.2, it is intended to discuss the results of this present experiment with those of Experiment 5.2.

Changes in log chromium concentration with time in faeces

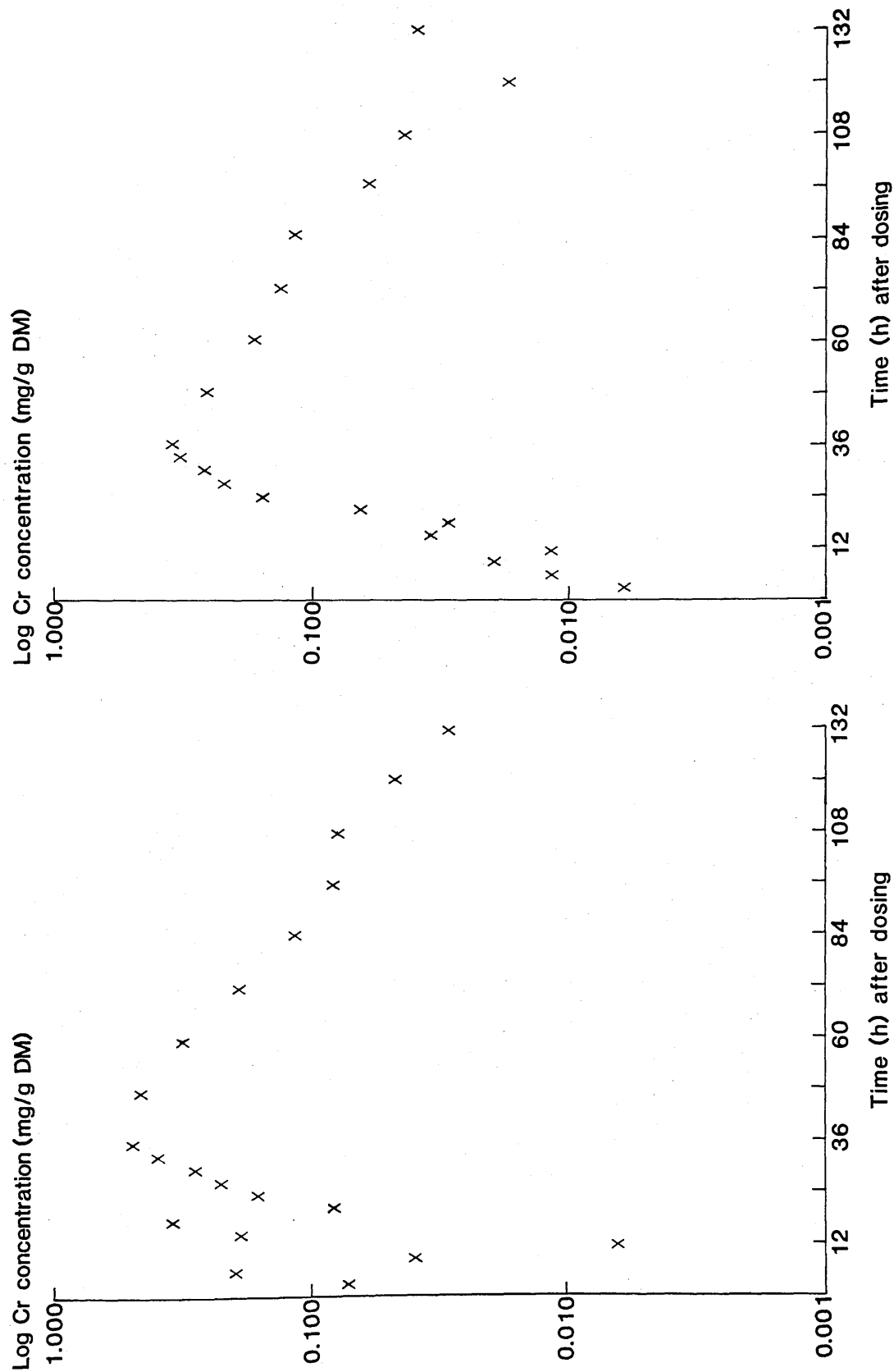


Fig. 7a (DBG)

Fig. 7b (WBG)

Table 31 Expt.5.1 Calculated effective degradability (P, g/kg)
using a linear model: $P = a + \frac{c}{k}$

	WBG	DBG
Dry Matter	485	454
Nitrogen	660	584

Experiment 5.2 The use of brewers grains as a protein supplement to straw fed ad libitum to pregnant beef cows

Introduction

In Experiment 5.1 it was shown that at least half the crude protein in brewers grains is degraded in the rumen. It was also shown that, although there was an initial lag phase in the degradation of the protein in dry brewers grains, wet and dry brewers grains were almost similarly degraded in the rumen with time.

Experiment 5.2 investigates the use of wet and dry brewers grains as protein supplements in straw diets fed to pregnant beef cows. It was also intended in this experiment that brewers grains should provide the supplementary energy required in the diet, although without comparison with any other energy supplement.

Materials and methods

Animals and treatments

Sixteen pregnant beef cows (Hereford x Friesian and Irish Blue-Grey) with mean live weight 464 kg (423-533 kg) and eight weeks from calving were paired on the basis of live weight, body condition score and anticipated calving date and randomly allocated to four diets in a 2 x 2 factorial design. Four non-pregnant fistulated cows of similar breed were separately allocated (one per treatment) to the four diets, mainly to provide information concerning rumen metabolites. The dietary treatments consisted of 2.2 kg dry matter of either wet (WBG) or dry brewers grains (DBG), either unsupplemented (NIL) or supplemented with 0.1 kg urea (U) (Table 32). Each diet was further supplemented with 0.1 kg of a mineral supplement and 0.2 kg of a barley/chromic oxide mix. In addition, all the cows were fed barley straw ad libitum in the long form. The experiment consisted of two consecutive 21-day periods, each divided into a 14-day adjustment period and a 7-day intake recording and digestibility assessment period. At the end of the first period, the cows were reallocated to the same four diets such that cows fed the unsupplemented WBG or DBG diet in the first period were respectively given the urea-supplemented WBG or DBG diet in the second period and vice versa, thus giving twenty observations per treatment in the factorial arrangement (ten per treatment for the four diets).

Table 32 Expt. 5.2 Composition of the concentrate mixture,
the amounts of brewers grains (kg, air dry basis)
and the estimated contents of CP and RDP (g)

	WBG		DBG	
	NIL	U	NIL	U
Dry brewers grains	-	-	2.5	2.5
Wet brewers grains	8.8	8.8	-	-
Urea	-	0.1	-	0.1
Chromic oxide/barley cubes	0.2	0.2	0.2	0.2
Mineral supplement	0.1	0.1	0.1	0.1
Total CP	486	771	433	718
Estimated RDP ¹	310	595	298	583

¹Unadjusted nylon bag values at 24h have been used for purposes of uniformity and comparison of the results with those of earlier experiments in which rumen bag values were used to estimate the RDP intake

Composition of feeds

Table 33 shows the proximate analyses of the dietary constituents, the total digestibility and the digestibility of energy and the ME content of the brewers grain portion of the concentrates. The digestibility of brewers grains was determined in a 17-day digestibility trial involving 7 days total collection using castrated male (Suffolk x Greyface) wether sheep averaging 49 kg live weight (four sheep per treatment). The procedure was as described in Appendix 2. The quantity of brewers grains (fresh basis) given to the sheep was either 1.28 kg wet or 0.380 kg of the dry material which was equivalent to 0.334 kg DM, and was the maximum the sheep could consume together with the quantity of dried grass (0.375 kg FM, equivalent to 0.333 kg DM) fed daily. The DCP and DE of the dried grass had been determined previously. The ME was calculated as in Experiment 4.

Management and feeding

Housing of the cows and the feeding of concentrates were as described in Experiment 4. The urea portion of the concentrates was 'mixed in' at the time of feeding, for the urea-supplemented WBG diet to avoid loss of nitrogen. Occasionally, about 200 ml of a very dilute molasses solution was added to the wet brewers grains to ensure complete consumption since about three cows did not readily consume their daily allocation due, presumably, to the bulky nature of the feed. Straw feeding and estimation of straw digestibility were also as described in Experiments 3 and 4. The cows were weighed at the end of each 21-day period.

Sampling of rumen contents and blood

Samples of digesta were obtained from the rumen of each fistulated cow just before the morning feed at 07.30 h and subsequently at 1, 2, 3 and 7 h after the start of feeding on the fourth day of each 7-day intake recording and digestibility assessment period. At each sampling, digesta was obtained from a depth of about 18 inches at five different intraruminal sites. Exposure to air was minimised by immediately squeezing the digesta, and the liquor strained through two and then four layers of muslin. One ml of the strained rumen liquor was preserved as described in Experiment 1 for determination of ammonia nitrogen ($\text{NH}_3\text{-N}$) and total nitrogen. The pH of the remaining sample was immediately obtained using a Pye Model pH meter.

Table 33 Expt. 5.2 Chemical composition of dietary constituents (g/kg DM) and the apparent digestibility coefficient of brewers grains

	Straw	Brewers grains	
		Wet	Dry
Dry Matter	827	250	880
Organic Matter	938	973	964
Crude Protein	30	221	197
Crude Fibre	437	212	215
Ether Extract	12	69	63
N-free extract	459	471	489
Ash	62	27	36
Gross energy (MJ/kg DM)	17.5	21.1	20.7
DM digestibility	-	0.579	0.580
OM digestibility	-	0.601	0.597
Energy digestibility	-	0.615	0.612
Estimated ME (MJ/kg DM)	-	10.6	10.4

Heparinised blood samples were obtained from the tail of each cow at 1.5 h after feeding on the third day of each 7-day measurement period and 2 ml of whole blood added to 4 ml of a resin suspension. This was decanted and washed four times for blood ammonia determination while the remaining blood was centrifuged for plasma urea determination.

Chemical analyses

Feeds, faeces, plasma and rumen liquor were analysed for the various constituents as described in Appendix 4.

Statistical analysis

The data were analysed by microcomputer using an interactive analysis of variance programme to determine the main effects of form of brewers grains and urea supplementation and also to detect any interactions.

Results

Voluntary intake and digestibility of straw

The data for voluntary intakes and digestibility of straw are shown in Table 34. The cows consumed a daily average of 6.2 kg barley straw DM in addition to 2.2 kg brewers grain DM between 8 and 2 weeks before calving. Urea supplementation or the form of brewers grains (wet or dry) did not significantly affect voluntary intakes or the digestibility of straw DM or OM although intakes were marginally higher for the urea-supplemented than for the unsupplemented diet. The digestibility of energy, the DOMD and the daily intakes of digestible OM and ME from straw (Table 34) were not significantly affected by treatment. There were small daily live weight gains by the cows and, although these tended to be higher for the U diet, the differences between treatments were not significant. Generally, there were no interactions between the form of brewers grains and urea supplementation on straw intake or digestibility or on the live weight change of the cows.

The concentrations of plasma urea, blood ammonia and rumen liquor total nitrogen and ammonia nitrogen

The concentrations of plasma urea, blood ammonia, rumen liquor total-N and $\text{NH}_3\text{-N}$ and the pH of rumen liquor are shown in Table 35.

Table 34 Expt. 5.2 Mean daily voluntary intakes (kg) and apparent digestibility coefficient of straw, DOMD (g/kg) and calculated ME intakes (MJ/day) from straw and daily live weight (LW) change of the cows

	WBG		DBG		SE ⁺
	NIL	U	NIL	U	
DM intake	6.05	6.28	5.97	6.32	0.172
OM intake	5.54	5.76	5.48	5.79	0.158
DM digestibility	0.392	0.422	0.439	0.416	0.0133
OM digestibility	0.410	0.431	0.460	0.426	0.0133
Energy digesti- bility	0.360	0.391	0.407	0.385	0.0141
DOMD	377	394	423	392	12.3
DOMD intake	2.30	2.52	2.51	2.44	0.111
ME intake	34.5	37.7	37.4	36.6	1.67
LW change (kg)	+ 0.67	+ 0.73	+ 0.57	+ 0.83	0.1170

None of the differences due to the form of the brewers grains or urea supplementation or interactions between them was significant

The values for rumen liquor total-N and pH are the averages of two samples obtained for each cow during the two digestibility and intake assessment periods. The values for rumen $\text{NH}_3\text{-N}$ for the five different periods from feeding at 07.30 h to 14.30 h have been presented graphically in Fig. 8. Supplementation with urea significantly ($P < 0.001$) increased blood urea concentrations by 1.5 h after feeding. Although the blood samples taken 3 h after feeding were from only one cow per treatment (the fistulates only), plasma urea levels of cows fed the urea-supplemented diet appeared to have fallen considerably at about 3 h from feeding, though it was still higher than that of cows fed the unsupplemented diet. The levels of plasma urea also tended to be higher for WBG than for DBG at 1.5 h after feeding but the difference was not significant. An increase in blood ammonia concentrations was also evident as a result of supplementation with urea but the difference was not statistically significant.

The concentrations of rumen $\text{NH}_3\text{-N}$ after feeding (Fig. 8) showed a gradual increase following the morning feed. The increases appeared to have peaked by 2 to 3 hours after feeding, the highest levels being noted for the urea-supplemented diet. The average values (Table 35) were also higher for the diet containing urea than for the one without it. The amounts of total-N in rumen liquor and the pH of the rumen contents also tended to be higher for the urea-supplemented diet.

Effect of dietary protein degradability on straw intake, plasma urea and rumen $\text{NH}_3\text{-N}$

For estimating the RDP content of the diets fed in this experiment, uncorrected rumen bag values determined in Experiment 5.1, rather than effective degradability, have been used for purposes of uniformity and comparison of the results with those previously reported in Experiments 3 and 4. As a result of the small difference in protein degradability between wet and dry brewers grains (Table 29), the addition of urea, assumed to be completely degradable, resulted in four different levels of RDP in the diets (Table 32). When the relevant data corresponding to these four levels of RDP were examined, some linear effects (Table 36) were evident. Significant increases in straw DM intake ($P < 0.05$), rumen liquor $\text{NH}_3\text{-N}$ ($P < 0.05$) and plasma urea ($P < 0.01$) were associated with increases in RDP intake. There was also a significant relationship ($P < 0.01$) between rumen $\text{NH}_3\text{-N}$ and plasma urea. The relationship between straw DM intake and plasma

Table 35 Expt. 5.2 Mean concentrations (mg/100 ml) of
plasma urea and ammonia (NH₃) and average values
for rumen ammonia nitrogen (NH₃-N) and total
nitrogen (Total N)

	WBG		DBG		SE ⁺
	NIL	U	NIL	U	
<u>Plasma Urea</u> ¹					
1.5 h after feeding	19.7 ^b	38.5 ^a	18.4 ^b	34.5 ^a	1.31
3 h after feeding	18.2	29.9	17.4	31.4	-
<u>Blood NH₃</u> ¹					
1.5 h after feeding	84.6	114.6	84.7	144.5	13.65
3 h after feeding	77.5	155.5	100.5	112.5	-
<u>Rumen NH₃-N</u> ²					
3 h after feeding	26.6	58.6	25.9	48.2	-
<u>Total N</u>					
0730 h	60	70	65	60	-
0830 h	68	108	63	75	-
0930 h	68	93	78	70	-
1030 h	68	90	83	78	-
1430 h	45	90	50	63	-
Mean	62	90	68	69	-
<u>Rumen pH</u>					
0730 h	7.2	7.1	7.2	7.1	-
0830 h	6.7	7.4	6.8	7.0	-
0930 h	6.7	7.0	6.8	6.9	-
1030 h	6.6	7.1	6.7	6.8	-
1430 h	6.8	7.1	6.7	6.8	-
Mean	6.8	7.1	6.8	6.9	-

¹ Samples taken 3 h after feeding were for fistulates only

² Individual values obtained for various sampling times after feeding are shown graphically in Figure 8

Apart from plasma urea which was significantly ($P < 0.001$) increased by urea supplementation, none of the differences in this table was statistically significant

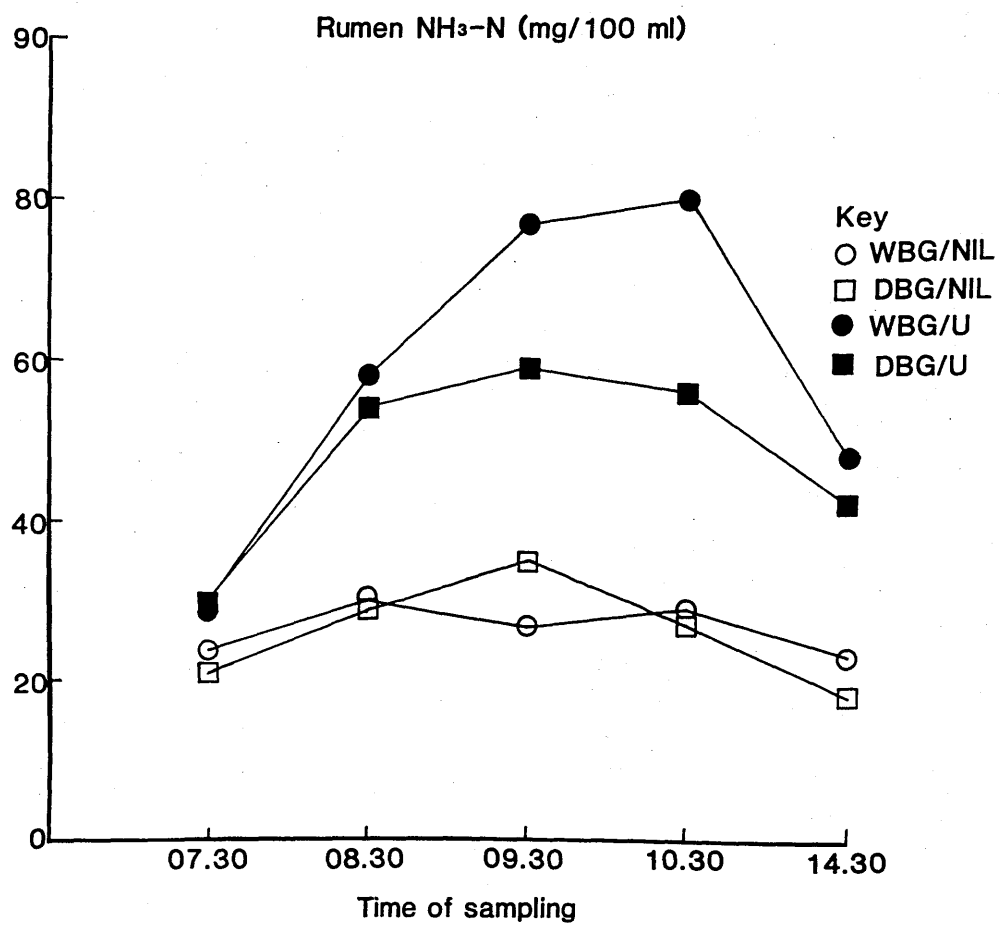


Fig. 8 Expt 5.2 Increase in rumen $\text{NH}_3\text{-N}$ with time after feeding

Table 36 Expt. 5.2 The relationships between RDP intake (g)
and straw DM intake (DMI, kg/d), rumen NH₃-N (mg/100 ml
rumen liquor) and plasma urea (mg/100 ml) and between
rumen NH₃-N and plasma urea

Parameters	Equation	r	rsd	df	Sig.
Straw DMI/RDP	$Y = 5.70 + 0.0010x$	0.97	0.0428	2	*
Rumen NH ₃ -N/RDP	$Y = 2.99 + 0.0959x$	0.96	4.639	2	*
Plasma urea/RDP	$Y = 2.85 + 16.0x$	0.99	26.68	2	**
Rumen NH ₃ -N/Plasma urea	$Y = 2.87 + 0.626x$	0.99	1.378	2	**

urea concentrations failed marginally to be significant ($r = 0.940$ as against 0.950 for significance at $P < 0.05$).

Discussion

The degradation of wet and dry brewers grains

The negative values ascribed to 'a' (the rapidly degradable fraction) for the DM of dry brewers grains by both models used to fit values to the rumen bag measurements in Experiment 5.1 indicates a lag phase in degradability due to drying, although this particular portion appears to be negligible for both forms of brewers grains. The lag phase which was probably due to the time dry brewers grains require to soak sufficiently in rumen liquor for microbial enzymes to penetrate them, is a reflection of the time that lapses before degradation starts. This seems to be apparent from the physical behaviour of dried brewers grain particles shortly after feeding when, on opening the rumen fistula, dry brewers grains could be seen floating on the rumen liquor surface and could be collected almost completely from the liquid phase of the rumen contents. Despite the effect of drying in causing a lag phase in the degradability of brewers grains, the 24 h measured or fitted value or even the calculated degradability of both DM and N were not largely affected.

It may be unusual for the effective degradability (P) to exceed the uncorrected rumen bag value, as observed for degradability of N in WBG in this present experiment. It should, however, be noted that the choice of 24 h for the upper time limit in this experiment was made arbitrarily and may not be justified considering further possible degradability beyond 24 h. Moreover, brewers grains is highly fibrous and it was not milled before incubation since it seemed more appropriate to incubate it 'as fed'. As such, it would perhaps have required more time for its effective degradability, in the limit, when compared to the course of degradation for supplements such as soya bean meal or even other milled feeding stuffs. However, the effective degradability (P) of 660 g/kg N corresponds to the asymptotic value obtained from the fitted equation (662 g/kg N).

Merchen, Hanson and Klopfenstein (1979) calculated a protein bypass value of 609 g/kg for dry brewers grains. This is equivalent to a degradability of 391 g/kg, a value much lower than that obtained in the present experiment. Their estimates for undegraded protein were, however, based on the flow of liquid and particulate abomasal fractions

and of the nitrogenous components of these rather than on rumen bag measurements adjusted for outflow rate. The effective degradability value of 660 and 584 g/kg for wet and dry brewers grains respectively obtained at an outflow rate of approximately 0.04 in the present experiment are within the range of 708 and 541 g/kg corresponding to outflow rates of 0.02 and 0.05 respectively obtained with a similar material (R.T. Pass, personal communication).

The voluntary intake and digestibility of straw, rumen $\text{NH}_3\text{-N}$ production and blood urea

The addition of urea to brewers grains to provide additional nitrogen did not improve the intake or digestibility of straw in Experiment 5.2. The amount of brewers grains fed (2.2 kg DM) provided approximately 460 g CP/day containing an estimated 304 g RDP. With straw, Campling *et al.* (1962) demonstrated a 40% increase in straw intake resulting from a daily infusion of 75 g urea into the rumen of cows consuming straw containing 30 g CP/kg. Although the highly fibrous nature and low digestibility of brewers grains (Table 33) may affect the straw intake response to additional nitrogen provided as urea, the conspicuous lack of effect of urea supplementation on voluntary intakes or the apparent digestibility of straw in this experiment suggests that no beneficial effect can be achieved by adding urea to brewers grains fed to supply about 460 g CP. There is little information available on the use of brewers grains or distillery by-products as protein sources in productive rations. However, Waller, Klopfenstein and Poos (1980) reported that distillers dry grains with solubles replaced urea and soya bean meal as sources of supplementary protein for growing lambs without significantly affecting digestibility or voluntary intakes of diets based on ground corn cobs. Intakes and digestibility were, however, slightly higher for soya bean meal than for dry distillers grains with solubles.

The comparable voluntary intakes and digestibility of straw for both forms of brewers grains in the present experiment suggests that either form of brewers grains can be used to replace the other without adverse effects on intakes and the performance of pregnant beef cows. The slightly lower rumen degradability of dry brewers grains, due to drying, probably did not affect the utilisation of its CP.

The lack of a treatment effect on intakes and digestibility in this experiment may be explained in terms of the concentrations of

rumen $\text{NH}_3\text{-N}$ generated in the rumen liquor of cows fed the various diets, although the rumen liquor samples were obtained from only one cow per treatment during each period which would limit the value of the data on rumen liquor measurements. The rumen $\text{NH}_3\text{-N}$ concentrations necessary for maximum microbial synthesis is still unresolved. Satter and Slyter (1974) suggest 5 mg/100 ml rumen liquor but other workers (Mehrez *et al.*, 1977) suggest 23.5 mg/100 ml, although with a further observation that the concentrations need not be 23.5 mg/100 ml rumen fluid all the time since this level is only necessary for the maximum rate of fermentation. However, Hunter and Siebert (1985) found no further increase in the rate of digestion of low-quality roughage feeds beyond 6-8 mg NH_3 /100 ml rumen fluid which would suggest that values of about 5-8 mg NH_3 /100 ml rumen fluid are probably adequate. On the basis of the studies cited above, it seems reasonable to suggest that rumen $\text{NH}_3\text{-N}$ concentrations for the various diets given in the present experiment were satisfactory, perhaps even in excess of required levels, for maximum rumen microbial protein synthesis (respectively 19.1 and 36.5 mg/100 ml for the unsupplemented and urea-supplemented diets and 29.1 and 26.5 mg/100 ml for the wet and dry brewers grains diets). Any differences in voluntary intakes would probably therefore be marginal and, considering the slightly lower degradability of the CP in dry brewers grains, the responses would perhaps be exhibited as linear relationships instead of significant treatment differences.

The increases in voluntary straw intake and plasma urea (Table 36) in response to increases in RDP confirm the results of Experiment 3. In connection with the increases in rumen $\text{NH}_3\text{-N}$ in response to increasing RDP, it seems that the increases in voluntary straw intake are maintained through the provision of adequate levels of rumen $\text{NH}_3\text{-N}$ resulting from the degradability of concentrate protein. The highly significant relationship between rumen $\text{NH}_3\text{-N}$ and plasma urea seems also to indicate that the relationship between straw DM intake and RDP is maintained firstly through the provision of adequate $\text{NH}_3\text{-N}$ concentrations in the rumen liquor. This, in turn, causes the elevation of blood urea, a considerable amount of which is returned to the rumen via salivary nitrogen. These interrelationships seem to stress (a) the need to satisfy the rumen microbes with N if optimum straw intakes are to be achieved and (b) the importance of recycled nitrogen as a buffer to the biological efficiency of the system.

It was suggested from the results of Experiment 3 that straw

consumption may be influenced by the degradability of the concentrate supplement but since rumen liquor samples were not obtained in that experiment, it was difficult to support the suggestion with evidence from the contents of rumen liquor. The interrelationships obtained in this present experiment seem to confirm that assumption and also to establish the mechanism by which this control of intake appears to be brought about.

Experiment 6.1 - The proximate composition and ruminal degradability of some tropical and temperate peas

Introduction

Although a large proportion of most cultivated peas is used for human feeding in certain tropical regions, including many African countries, some shrivelled and damaged seeds are left after harvest and these are consumed by livestock. Also since most livestock in such countries are normally traditionally herded through uncultivated pastures, they quite often consume peas growing wild in the sparse vegetation where they graze. Although the chemical composition of some varieties of such tropical peas have been published (Imperial Bureau of Animal Nutrition, 1936), their rumen degradabilities have not been studied. There is also little published information on the rumen degradation of temperate peas in general. Experiment 6.1 was therefore conducted to comparatively study the proximate composition and rumen degradation of some some tropical and temperate type peas. Quite often feed values and broader research findings obtained in temperate regions are applied in some tropical regions without reference to genetic or inherent qualities of the feedstuff or its geographical location and conditions. It was therefore intended to determine if temperate type peas differ in degradability from those that grow in tropical conditions and if work done on temperate peas could be related to tropical peas and vice versa. The tropical peas used in this experiment were collected at the mature stage, at about the middle of the dry season in Nigeria and they have not yet been specifically identified on a taxonomic basis.

Materials and methods

Five varieties of peas were collected locally in the southern part of Nigeria. They were also locally identified (U.I. Oji, personal communication) as brown mottled type (PBP); Ukpa (PUP); Brown Akidi type (PBAP); Odudu (POP) and Pigeon Pea (PPP). Seven temperate peas, randomly selected from five varieties of combining peas, were also obtained from the Processors and Growers Research Organisation (The Research Station, Thornhaugh, Peterborough). These were Vedette; Finale; Birte; Consort; Progrete; Maro and Imposant. The peas were all crushed through a barley bruiser (E.H. Bentall & Co., Malden, Essex).

Three replicates of 10 g each for the various varieties were then incubated as described in Appendix 1, except that the nylon bags used had a mean pore size 43 μ m. Because the number of nylon bags available for the incubations were insufficient, the estimation of degradabilities of the temperate varieties were done separately from the tropical peas and these were done for 24 h only. The tropical varieties were incubated for various time periods as described in Experiment 5.1.

Due to the brittle nature of the pea seeds, bruising them resulted mostly in very fine powdery, almost dusty, particles. As such, separate estimates were made on each type of pea of the loss of DM and N from bags that were suspended with agitation in warm water for 6 and 12 h. Since no further loss of DM or N appeared to occur beyond 6 h, the loss of DM and N at 6 h was taken as the loss through the pores of the nylon bags and this was used to adjust the DM and N degraded by rumen micro-organisms.

Analyses

Dried samples were milled in a coffee grinder and analysed for nitrogen, as outlined in Appendix 4. Statistical analysis was done on the DM and N loss from the nylon bags for the various time periods by standard analysis of variance. Since the incubations were made separately and at different times for the tropical and temperate varieties, statistical analyses were also done separately for the tropical and temperate varieties.

Results

The proximate compositions of the various varieties of peas are shown in Table 37. Apart from PBAP, one of the tropical varieties, which had a considerably higher CP concentration than all the other varieties, the CP contents were similar for both the tropical and temperate varieties. The DM was within a close range for all the varieties of both the tropical and temperate types, the slightly higher DM content of the tropical varieties being due probably to the stage at which they were harvested relative to the temperate types, or perhaps to the direct effect of a higher environmental temperature on the mature tropical peas. The crude fibre was generally higher (almost twice) for the temperate varieties but within the tropical and temperate types the values were generally similar. The ether extract

Table 37 Expt. 6.1 Proximate composition of peas (g/kg)

	Dry matter	Crude protein	Crude fibre	Ether extract	N-free extract	Gross energy (MJ/kg)	Ash
<u>(a) Tropical Variety:</u>							
PBP	903	234	94	12	621	18.3	38.6
PUP	894	235	54	61	614	19.1	35.8
PBAP	897	288	56	9	617	18.5	30.0
POP	887	207	49	16	700	19.0	28.4
PPP	903	214	84	11	646	18.1	44.7
<u>(b) Temperate Variety:</u>							
VEDETTE	858	216	116	12	629	17.1	27.3
FINALE	862	221	109	10	634	16.9	26.3
BIRTE	869	230	87	9	648	17.0	26.5
CONSORT	861	223	114	11	624	17.0	28.1
PROGRETA	876	210	98	9	659	17.0	24.4
MARO	876	231	130	10	601	17.1	28.0
IMPOSANT	867	212	140	11	609	17.1	27.9

content of PUP was considerably higher than the rest of the peas, otherwise all the tropical and temperate varieties contained similar levels of ether extract. The tropical varieties had marginally higher gross energy and ash than the temperate varieties.

The degradability of DM and N of the peas are shown in Table 38. For the tropical varieties, although there were significant differences between varieties in the degradability of DM during the initial periods (3h and 6h) of incubation, the longer periods (9 - 24 h) showed no significant differences between varieties. Nevertheless, the highest degradability of DM was noted for the POP variety.

The degradability of N of the tropical varieties showed significant differences throughout the various periods of incubation. However, calculations indicated that, although the differences continued to be significant throughout the incubation periods, the absolute differences between the varieties and the range of these differences were reduced with time.

The degradability of the temperate varieties measured for 24 h only was more variable, ranging from 512 to 721 g/kg for DM and 446 to 777 g/kg for N and this was reflected in the unusually large standard errors associated with them.

Discussion

Although the tropical varieties of peas used in this experiment may not be completely representative, their CP contents (207 - 288 g/kg) indicate their potential value as protein supplements. In particular, a CP content of 288 g/kg DM noted for PBAP is high in relation to CP values recorded for some tropical peas by the Imperial Bureau of Animal Nutrition (1936) (about 230 - 250 g/kg) although it is still within the range for tropical legumes in general (between 180 and 310 g/kg). Considering the CP content, PBAP is perhaps the best choice for a breeding and improvement programme that seeks to improve peas for their protein content.

Although it is likely that the temperate varieties of combining peas used in this experiment are the result of years of breeding and crossbreeding for such qualities as higher yield, early maturity, 'standing ability', straw length and resistance to lodging, among others, the close range of values for some of the components of the DM within the temperate varieties and among all the peas used in this experiment is indicative of the similarity of peas in general. The

TABLE 38

Expt. 6.1 The degradation of DM and N of peas (g/kg)

	Hours after incubation				
	3	6	9	15	24
DM degradability:					
<u>Tropical var.</u>					
PBP	192 ^b	317 ^b	421	515	609
PUP	244 ^a	327 ^{ab}	468	533	599
PBAP	196 ^b	337 ^{ab}	467	552	637
POP	280 ^a	367 ^a	476	559	642
PPP	182 ^b	243 ^c	435	520	606
SE of difference	17.2	18.9	26.3	20.2	28.4
<u>Temperate var.</u>					
VEDETTE		-			534
FINALE		-			546
BIRTE		-			585
CONSORT		-			721
PROGRETA		-			534
MARO		-			586
IMPOSANT		-			512
SE of difference					92.2
N degradability:					
<u>Tropical var.</u>					
PBP	255 ^b	376 ^{bc}	477 ^c	533 ^b	590 ^{bc}
PUP	417 ^a	491 ^{ab}	572 ^a	590 ^a	622 ^{ab}
PBAP	238 ^b	361 ^c	503 ^{bc}	568 ^{ab}	630 ^a
POP	422 ^a	498 ^a	550 ^{ab}	581 ^{ab}	625 ^{ab}
PPP	240 ^b	337 ^c	478 ^c	535 ^{ab}	581 ^c
SE of difference	23.3	51.0	22.2	24.6	15.6
<u>Temperate var.</u>					
VEDETTE		-			640
FINALE		-			600
BIRTE		-			660
CONSORT		-			777
PROGRETA		-			446
MARO		-			621
IMPOSANT		-			514
SE of difference					139.1

a, b, c - Means with different superscripts differ significantly

considerably higher crude fibre contents of the temperate peas compared to tropical varieties may be due to the ever increasing emphasis generally on the dietary crude fibre level in this country, mainly for human nutrition, which would tend to encourage breeding for fibre content. It should be noted that all the temperate varieties of peas used in the present experiment are combining peas, and not only for use in animal feeding. The CF values are similar to those given by Fairweather-Tait and Wright (1985) for the leafless pea (Pisum sativum) (CF 137.6 g/kg). The above authors also indicated that one novel use of peas in the U.K. is in the production of high-fibre white bread which tends to support the suggestion that breeding for fibre content may partly account for the difference in CF content of the temperate pea varieties and the unimproved tropical types.

While the degradability of DM, on average, appears to be slightly higher for the tropical varieties, this may not be considered as a difference since the incubations were done separately for the tropical and the temperate varieties and any of a number of possible factors could be responsible for the marginally higher values noted for the tropical varieties. The same reason could possibly apply to values obtained for N degradability. Of course, it is possible that the considerably higher CF contents of the temperate varieties may cause small resistance to rumen degradation of temperate peas compared to the tropical types. Nevertheless, these uncorrected rumen bag values show that at least half of the CP of peas, in general, may be degraded in the rumen. Apart from Progreta, the N content of most of the temperate and tropical peas used in this present experiment appeared to be degraded to similar extents. The marginal differences in degradability at 24 h are presumably too small to cause any important differences in the total quantities of RDP that they would individually provide if included in animal diets.

Although, on the basis of the results obtained in this experiment, it would seem justifiable to relate work regarding the degradability of temperate peas to tropical varieties, caution is advisable on the basis that the tropical peas fortuitously used in this experiment may not be representative. Their compositions do not, however, seem to differ and their CP content indicates that peas in general may serve as valuable protein supplements.

Experiment 6.2 - Further comparisons of brewers grains and peas with other nitrogen sources as supplements to straw fed ad libitum

Introduction

It was shown in Experiment 5.2 that there was no benefit in terms of improvement in the intake or digestibility of straw or the performance of pregnant beef cows from adding urea to 2.2 kg DM supplied from brewers grains when used as a protein supplement. The daily supply of CP from brewers grains alone was estimated to be 460 g and it contained an estimated 289 g rumen degraded protein (RDP). It was suggested that the lack of effect of urea was due to adequate concentrations of $\text{NH}_3\text{-N}$ (Satter and Slyter, 1974; Mehrez et al., 1977) generated by RDP supplied from brewers grains alone, in the rumen liquor. There is need to further compare brewers grains with other protein sources to assess its value in supplying both RDP and undegraded protein (UDP) requirements of adult animals fed low quality roughages. This is important in view of the increasing need to utilise industrial by-products in livestock feeding, especially in the developing countries. This is even more relevant to the Nigerian situation where there has been considerable increase in the number of breweries over the past ten years and there is inadequate information about the potential and utilisation of the unknown, but tremendous, quantities of the resulting brewers grains.

There is also an increasing interest in the use of field peas and beans as protein supplements. Although some workers (Heitman and Howart, 1960; Kakade and Evans, 1965; Bell and Youngs, 1970) have shown them to be inferior to fish protein concentrate and casein, others (Cunha, Warwick, Ensminger and Hart, 1948; Clark, 1970; Bell and Wilson, 1970) concluded that cull peas and field beans were comparable to soya bean meal and fish meal with greater economic advantages. The toxic effects of dietary legume protein on the morphology and secretory responses of the rat small intestine (Pusztai and Greer, 1984) may well apply to peas, thus lowering the nutritive value of pea protein when compared to other protein supplements. Moreover, little published information is available on the extent to which peas are degraded in the rumen and, consequently, to what extent it satisfies the rumen microbial needs for nitrogen. Although it was shown in Experiment 6.1 that about half the crude protein contained in several varieties of tropical and temperate peas were degraded in the rumen, there may be

other problems in the utilisation of RDP provided by peas.

Experiment 6.2 was therefore designed to compare brewers grains and peas, each as a protein and energy supplement, with a combination of soya bean meal and rolled barley when fed as supplements, to cows offered straw ad libitum.

Materials and methods

Fifteen lactating cows (Hereford x British Friesian and Irish Blue-Grey) with mean liveweight 466 kg (375 - 544 kg) and their calves were weighed immediately after calving. The cows, which calved within 3 weeks of each other, were paired on the basis of calving date, liveweight and body condition score and randomly allocated to three treatments to form five blocks. Three non-lactating cows each fitted with a rumen fistula were separately allocated, one to each of the three treatments, thus giving a total of six cows per treatment. The treatments which were calculated to provide similar amounts of CP in the concentrate portion of the diets consisted of approximately 2.6 kg DM based on either flaked peas (var. Birte) (Dalgety Agriculture Ltd., Turiff, Aberdeenshire) (PEAS); dry brewers grains (DBG) or rolled barley plus soya bean meal (RB/SBM). The amount of brewers grain was adjusted to allow the addition of 0.26 kg rolled barley (DM) in order to provide, as near as possible, similar estimated levels of CP, DM and ME on all three treatments. The composition of the concentrate mixtures are shown in Table 39. In addition, all the cows received mineral supplements, barley/chromic oxide cubes and long straw ad libitum as in Experiment 5.2.

The ME of peas, rolled barley and soya bean meal were determined in a 17-day digestibility trial by feeding 667 g of each with 333 g dried grass to wether sheep (4 per treatment) in metabolism cages following the procedure described in Appendix 2. ME was calculated as in Experiment 4. Rumen degradability and digestibility of dry brewers grain protein was calculated by using estimates of degradability and digestibility obtained in Experiment 5.1 and 5.2 respectively, since the same batch of brewers grains was used in these experiments. The degradabilities of peas, rolled barley and soya bean meal (Table 40) were estimated as described in Appendix 1. As in Experiment 5.2, unadjusted rumen bag measurements, rather than effective degradabilities calculated with an outflow rate component, have been used for purposes of standardising the values with others given earlier

Table 39

Expt. 6.2 Composition of the concentrate mixture (kg, air dry basis), the amounts of CP (g) and ME (MJ) and the estimated quantities of RDP and UDP (g) supplied by the concentrates

	PEAS	DBG	RB/SBM
Peas	3.0	-	-
Dry brewers grains	-	2.8	-
Rolled barley	-	0.3	2.4
Soya bean meal	-	-	0.6
Barley/chromic oxide cubes	0.2	0.2	0.2
Vitamin and mineral supplemt.	0.1	0.1	0.1
Total concentrate DM	2.893	2.995	2.862
CP	588	573	565
RDP	369	362	451
UDP	219	211	114
ME	38	31	32

Table 40

Expt. 6.2 The chemical composition of dietary components (g/kg DM), and the apparent digestibility coefficient and estimated degradability of the concentrates

	Peas	Brewers grains	Rolled barley	Soya bean meal	Barley/chromic oxide cubes	Straw
Dry matter	873	880	858	879	870	844
Crude protein	216	210	133	510	126	27
Crude fibre	66	193	40	69	39	462
Ether extract	11	56	14	9	15	14
N-free extract	681	510	792	346	756	452
Ash	26	31	21	66	64	45
Gross Energy (MJ/kg DM)	17.9	20.7	18.1	19.3	17.3	18.0
DM digestibility	0.919	0.580	0.844	0.778	-	-
OM digestibility	0.927	0.597	0.885	0.788	-	-
ME (MJ/kg DM)	13.9	10.4	11.4	11.8	-	-
CP digestibility	0.837	0.731	0.796	0.928	-	-
Estimated degradability	0.620	0.609	0.839	0.752	-	-

on in this thesis.

Experimental procedure

The management of the cows and their calves and the estimation of milk yield during the fourth week after calving, by means of the calf suckling technique, were as described in Experiment 4. The concentrates and straw were also fed as in Experiment 4, straw intakes being recorded during the fourth and sixth weeks after calving. The determination of digestibility by means of analyses of 21 faecal grab samples bulked over 7 days was as described in Experiment 3. The digestibility of straw was determined by difference after allowing for the digestibility of the concentrate portions (Table 40) of the diet. Heparinised blood samples were obtained immediately after weighing the cows on the last day of the fourth and sixth weeks following calving. Samples of rumen liquor were obtained as described in Experiment 5.2

Feed sampling and chemical analyses

Samples of dietary constituents were obtained as described in Experiment 3, while chemical analyses of feed, faeces, rumen liquor and blood were undertaken as described in Appendix 4.

Statistical analysis

Results were analysed as 3-treatment randomised block trial analysis of variance. Individual treatment means were compared by means of the least significant difference.

Results

The data for the various measurements have been presented separately for each of the two periods of assessment (4th and 6th weeks after calving) and also as a combined average for both periods where appropriate.

The mean voluntary intakes and digestibility of DM and OM are shown in Table 41. Although differences between treatments in the voluntary intake of straw DM and OM were not significant, substantially higher quantities of straw were consumed by cows fed RB/SBM than by those fed either PEAS or DBG.

Although the digestibility of total diet has also been presented in Table 41, any differences between treatments in respect of total

Table 41

Expt. 6.2 Mean daily voluntary intakes (kg) and apparent digestibility of dry matter and organic matter by the cows

Weeks after calving	Dry Matter			Organic Matter		
	+4	+6	Combined	+4	+6	Combined
<u>Intake</u>						
<u>Whole diet</u>						
PEAS	9.82	9.86	9.84	9.29	9.34	9.32
DBG	9.74	9.97	9.85	9.24	9.43	9.34
RB/SBM	10.59	11.27	10.93	10.06	10.68	10.37
SE of difference	0.600	0.930	0.726	0.571	0.888	0.694
LSD ($P < 0.05$)	1.384	2.145	1.674	1.317	2.048	1.600
<u>Straw</u>						
PEAS	6.93	6.96	6.95	6.62	6.65	6.64
DBG	6.74	7.00	6.87	6.43	6.65	6.54
RB/SBM	7.71	8.41	8.06	7.38	8.03	7.70
SE of difference	0.599	0.935	0.730	0.572	0.888	0.695
LSD ($P < 0.05$)	1.381	2.156	1.683	1.319	2.048	1.603
<u>Digestibility</u>						
<u>Whole diet</u>						
PEAS	0.531	0.566 ^{ab}	0.551 ^{ab}	0.537	0.574 ^{ab}	0.556 ^{ab}
DBG	0.477	0.532 ^b	0.504 ^b	0.492	0.539 ^b	0.515 ^b
RB/SBM	0.537	0.614 ^a	0.576 ^a	0.621	0.621 ^a	0.578 ^a
SE of difference	0.0290	0.0261*	0.0217*	0.0374	0.0269*	0.0266*
LSD ($P < 0.05$)	0.0669	0.0602	0.0500	0.0862	0.0620	0.0613
<u>Straw</u>						
PEAS	0.388	0.435 ^b	0.414	0.374	0.431 ^b	0.403
DBG	0.423	0.500 ^{ab}	0.461	0.426	0.491 ^{ab}	0.460
RB/SBM	0.437	0.548 ^a	0.493	0.410	0.537 ^a	0.474
SE of difference	0.0449	0.0448*	0.0360	0.0585	0.0472*	0.0438
LSD ($P < 0.05$)	0.1035	0.1033	0.0830	0.1349	0.1088	0.1010

diet are not the only criterion in view of the differences in digestibility of the supplements shown in Table 40. The digestibilities of straw DM and OM were significantly ($P < 0.05$) lower for PEAS than for RB/SBM during the 4th week after calving but this difference was not reflected in the combined values. The digestibilities of straw DM and OM were similar for RB/SBM and DBG during both periods of assessment, being only marginally higher for RB/SBM than for DBG. Although the difference between DBG and PEAS in straw digestibility was not statistically significant, absolute values were higher for DBG.

The apparent digestibility of energy and the DOMD are shown in Table 42. The digestibility of straw energy and the straw DOMD followed a similar pattern as the digestibility of DM and OM, being significantly higher for RB/SBM than for PEAS. The difference between RB/SBM and DBG were not significant. The digestibility of straw energy and the straw DOMD were also higher, but not significantly so, for DBG than for PEAS.

Data for the intake of ME and digestible organic matter are also shown in Table 42. The differences between treatments in the calculated ME intakes from straw were not significant but cows fed the RB/SBM diet achieved the highest straw ME intakes, while those fed PEAS achieved the lowest. The intakes of digestible OM from straw followed a similar pattern as the intake of ME, with cows fed PEAS consuming the least amount of digestible OM.

The daily changes in live weight of the cows over six weeks and their milk yield and the growth rates of their calves during the first 4 weeks are shown in Table 43. While the differences between treatments in the daily changes in live weights were not significant, these changes appear to have been influenced by the ME intake from the straw portion of diets rather than the total ME intakes shown in Table 42. The loss in live weight was least for cows fed RB/SBM and highest for those fed PEAS. Milk yield, estimated by the calf suckling technique during the 4th week after calving, was significantly ($P < 0.05$) higher for DBG than for PEAS. Cows fed DBG also produced more milk than those fed RB/SBM and although the difference failed to be significant, it was 0.93 kg per day higher for DBG than RB/SBM.

Calf growth rates were apparently related to milk yield, with calves suckling cows fed DBG achieving the highest growth rates and those suckling cows fed PEAS the lowest. The differences were however

Table 42

Expt. 6.2 The apparent digestibility coefficient of energy, the digestible organic matter in the DM (DOMD, g/kg) and the calculated daily intakes of metabolisable energy (ME, MJ) and digestible organic matter (DOMI, kg)

	Energy Digestibility			DOMD		
Weeks after calving	+4	+6	Combined	+4	+6	Combined
<u>Whole diet</u>						
PEAS	0.515	0.536 ^{ab}	0.526 ^{ab}	511	544 ^{ab}	528 ^{ab}
DBG	0.476	0.513 ^b	0.495 ^b	467	510 ^b	488 ^a
RB/SBM	0.526	0.591 ^a	0.558 ^a	507	589 ^a	548 ^a
SE of difference	0.0270	0.0270*	0.0200*	35.6	25.4*	25.4*
LSD (P<0.05)	0.0623	0.0623	0.0461	82.1	58.6	58.6
<u>Straw</u>						
PEAS	0.354	0.384 ^b	0.369 ^b	357	411 ^b	384
DBG	0.396	0.452 ^{ab}	0.424 ^{ab}	407	469 ^{ab}	438
RB/SBM	0.436	0.531 ^a	0.484 ^a	392	513 ^a	452
SE of difference	0.0444	0.0463*	0.0355*	55.8	42.4*	41.7
LSD (P<0.05)	0.1024	0.1068	0.0819	128.7	97.5	96.2

ME intake (MJ/d)			DOMI (kg)			
<u>Whole diet</u>						
PEAS	75.4	81.2	78.3	5.03	5.37	5.20
DBG	74.0	81.1	77.6	4.55	5.11	4.83
RB/SBM	81.9	97.5	89.7	5.40	6.67	6.03
SE of difference	9.90	10.88	9.93	0.580	0.725	0.582
LSD (P<0.05)	22.83	25.09	22.90	1.337	1.672	1.342
<u>Straw</u>						
PEAS	37.4	43.2	40.3	2.52	2.92	2.72
DBG	43.0	50.1	46.6	2.75	3.34	3.04
RB/SBM	50.1	65.5	57.8	3.08	4.37	3.72
SE of difference	9.88	10.87	9.91	0.580	0.735	0.585
LSD (P<0.05)	22.78	25.07	22.85	1.337	1.695	1.349

Table 43

Expt. 6.2 Total live weight (LW) loss at 4 and 6 weeks after calving and milk yield and the daily growth rates (kg) of the calves at 4 weeks after calving

Period after calving (weeks)	LW change		Milk Yield	Calf Growth rates
	0-4	0-6	At 4 weeks	0-4
PEAS	-8.6	-23.6	5.62 ^b	0.722
DBG	-3.2	-10.6	7.44 ^a	0.909
RB/SBM	-0.6	- 8.4	6.51 ^{ab}	0.792
SE of difference	5.66	10.88	0.785*	0.1156
LSD ($P < 0.05$)	13.05	25.08	1.810	0.2666

not significant.

The concentrations of plasma urea and of total N and $\text{NH}_3\text{-N}$ are shown in Table 44. The total N and $\text{NH}_3\text{-N}$ concentrations for the single fistulated cow per treatment have been averaged for the five different collections during the day, as the increases were in a regular pattern that was consistent with the overall average value for each treatment. Cows fed RB/SBM had the lowest plasma urea levels at 4 weeks after calving, while those fed PEAS had the highest but differences were not significant. The overall values were also similar. The highest total N was associated with the lowest $\text{NH}_3\text{-N}$ concentration which was generated in the rumen liquor by the DBG diet. Although the total N produced in the rumen for the PEAS diet was the same as that produced for RB/SBM, rumen $\text{NH}_3\text{-N}$ seemed to be higher for PEAS.

Discussion

Effect of type of concentrate supplement on the voluntary intake and digestibility of straw

The combined means for the fourth and sixth weeks show that, although the differences in voluntary intakes were not significant, the combination of rolled barley and soya bean meal (RB/SBM) resulted in substantially improved straw DM intakes, approximately 16 and 17% higher than PEAS and DBG respectively. DBG was similar to PEAS in terms of promoting straw consumption. Experiments in which brewery or distillery by-products have been compared with popularly used protein supplements, as they affect straw consumption, are scarce. In one of such few experiments, wet distillers grains, when fed as the only protein supplement to pregnant cows, was found to be inferior to either barley plus urea or barley plus earthnut cake (Ball *et al.*, 1971) in promoting straw intake but not in terms of the performance of the cows or the birth weight of their calves. Daily intakes of barley straw (fresh matter) were 5.4 kg for the cows fed wet distillers grains compared to 7.7 kg for those fed either barley plus urea or barley plus earthnut cake. The above authors attempted to explain this difference in terms of the fibre content of the supplements but they were unable to test statistically an assumed relationship between straw consumption and the CF content of the concentrates.

The situation is similar to what obtained in the present

Table 44 Expt. 6.2 The concentrations (mg/100 ml) of plasma urea and of total nitrogen (Total-N) and ammonia nitrogen (NH₃-N) in the rumen liquor

Weeks after calving	Plasma urea			Total-N ⁺	NH ₃ -N ⁺
	+4	+6	Combined		
PEAS	17.7	15.4	16.5	19.8	17.3
DBG	15.4	15.2	15.3	59.2	7.8
RB/SBM	12.4	15.5	14.0	18.5	11.8
SE of difference	2.86	0.81	1.38	-	-
LSD (P<0.05)	6.60	1.87	3.18	-	-

+ Total-N and NH₃-N concentrations are for one cow (fistulate) per treatment and these are mean values for samples obtained at 0730, 0830, 0930, 1030 and 1430 h

experiment. However, it is not possible to ascertain whether the substantial but non-significant improvement in straw intake by cows fed RB/SBM over those fed DBG was due to the fibre content of the concentrate supplements (42 g/kg DM for RB/SBM and 165 g/kg for DBG) which would, presumably, cause a greater 'rumen fill' and greater physical restriction on intake for DBG, or whether it was due to a specific energy or protein effect. It is possible that the higher amount of readily fermentable carbohydrates yielded by rolled barley may have been partly responsible for the difference.

It is even more difficult to explain the 16% difference in straw intake between RB/SBM and PEAS, in favour of RB/SBM, in terms of their CF contents, although PEAS was marginally higher in fibre, or in terms of a direct energy effect. Unprocessed legume proteins are known to be toxic to rats (Pusztai, Clark, King and Stewart, 1979) and cattle (Williams, Pusztai, Macdearmid and Innes, 1984) and are poorly utilised by rats (Pusztai and Greer, 1984). Even when no evidence of toxicity was noted, increasing proportions of black-eyed peas in a predominantly ground barley diet reduced feed consumption and utilisation by pigs (Heitman and Howart, 1960). Bell and Youngs (1970) attributed the poor nutritive value of pea protein concentrate to an inferior biological value compared to other proteins and they recommended supplementation with methionine when pea protein is used as a protein supplement for mice. It seems, however, that the problem of feed utilisation by animals fed unprocessed peas or other legumes as protein supplements goes beyond their deficiencies and may, indeed, be connected with inherent, perhaps toxic, factors as well as utilisation of the contained nitrogen.

Experiments 1 - 5 reported earlier in this thesis have shown that straw intake may be influenced by the concentrate RDP content. Although these present results would tend to confirm that finding, the other possible causes of the differences in intake discussed above would tend to preclude any such specific explanation.

The combination of rolled barley and soya bean meal also resulted in higher straw digestibility than PEAS, the difference between RB/SBM and DBG being much smaller and not significant. There is no reason to suggest that rumen $\text{NH}_3\text{-N}$ production (Table 44) resulted in a better rumen environment for cows fed RB/SBM, although the data on rumen liquor characteristics are of little value due to the limited number of observations. The reasons that were suggested earlier for the

differences in straw intake may also be responsible for the differences in straw digestibility. In this regard, the DBG diet would be more slowly digested leading to a longer retention time that would physically affect straw intake, in contrast to RB/SBM. When the digestibilities of the total diets are compared to those of straw, it appears that peas caused a 'substitution effect' to a certain degree, being preferentially digested in relation to straw (peas itself being very highly digested (Table 40)). However, this reason alone may not explain the low digestibility of straw by cows fed PEAS and the effect of unprocessed peas on its utilisation, mentioned earlier, may also have contributed.

As a result of the differences in intake and digestibility of straw between the treatments, the overall intakes of straw digestible OM by cows fed RB/SBM was respectively 36 and 22% higher than by those fed PEAS and DBG. The equivalent differences for straw ME intakes were 43 and 24%. The slightly improved straw digestibility by cows fed DBG over those fed PEAS resulted in an improvement in straw DOMD and ME intakes of 12 and 16% respectively.

Live weight change and milk yield of the cows and growth rates of their calves

Although the extent of live weight loss cannot be discussed in isolation from milk yield, the calculated dietary ME intakes which are virtually the same for DBG and PEAS does not relate in any way to the data on live weight loss. Straw ME intakes appeared to relate better to the live weight losses. It seems more likely that total ME intakes were less efficiently utilised by cows fed PEAS, assuming that the ME derived from the 'PEAS' concentrate (Tables 39 and 40) did not change when it was fed with straw. Generally, live weight losses for the various treatments appeared to have been influenced by the proportions of energy intake derived from straw.

The data on milk yield showed that DBG was superior to PEAS as supplements to straw fed to lactating cows. Clearly, milk yield did not respond to energy intake ^{nor} ~~but rather~~ to concentrate UDP supply. Although the difference in milk yield between DBG and RB/SBM was not statistically significant, it was appreciable (almost 1 kg/day). These results agree with previous reports. For example, increases in milk yield by sheep, resulting from UDP supplied in concentrate supplements, have been reported for diets containing 500 g hay/kg DM

(Robinson *et al.*, 1979) or 570 g hay/kg DM (Gonzalez, Robinson and McHattie, 1985). The response in milk yield to UDP in this present experiment is, however, in contrast to that in Experiment 4. Although results of Experiment 4 cannot be compared directly with those of the present experiment, cows appeared to have consumed more energy both from straw and from the total diet in the present experiment. The effect was a less severe live weight loss by cows in the present experiment (0.338 kg/hd/day on average) compared to the live weight loss by cows in Experiment 4 (about 0.714 kg/hd/day). On the assumption that milk yield response to undegraded protein occurs mostly when production is not limited by the lack of energy (Webster *et al.*, 1982), the energy balance of the cows in the present experiment was more likely to enable the cows to give more milk in response to UDP than in Experiment 4. The growth rates of the calves seemed to agree with the milk yield for the three treatments. Calves suckling cows fed DBG gained 187 and 117 g daily more than calves suckling cows fed PEAS and RB/SBM respectively. On the average the efficiency of conversion of milk to gain was approximately 8:1.

Plasma urea, rumen total- and ammonia-nitrogen

Plasma urea concentrations were not influenced by the degradability of the concentrate dietary protein. This lack of effect of protein degradability on plasma urea concentration of lactating cows was also observed in Experiment 4. As discussed in that experiment, the plasma urea concentrations of lactating cows, which are presumably in some degree of negative energy balance, are unlikely to be related to dietary protein degradability because of the confounding influence of gluconeogenesis from amino acids (Blaxter, 1962) on peripheral plasma urea concentrations.

The highest level of total N and the lowest level of $\text{NH}_3\text{-N}$ for the DBG diet tend to support the estimated low degradability of the concentrate portion of that diet and, consequently, the conclusion that milk yield was related to undegraded protein in the present experiment. The low level of $\text{NH}_3\text{-N}$ for the DBG diet (7.8 mg/100 ml rumen liquor), however, meets the requirement (5 mg/100 ml rumen liquor) suggested by Satter and Slyter (1974) and Roffler, Schwab and Satter (1976) to be satisfactory for maximum microbial synthesis.

The results of this experiment appear to indicate that brewers grains may be comparable to, or even better than, soya bean meal in

terms of milk yield and consumption of low quality roughage feeds. However, since brewers grains is highly fibrous, its relatively low digestibility may, when fed with straw, further slow down the rate of passage and thus prevent the potential intake of straw from being achieved.

SECTION 3PHOSPHORUS SUPPLEMENTATIONExperiment 7 - The effects of supplementary phosphorus and source of nitrogen on the performance of growing wether sheep given a low phosphorus dietIntroduction

Although prolonged reduced phosphorus intakes may not affect the voluntary intake or digestibility of straw by cows during pregnancy (Fishwick et al., 1977a; Bass et al., 1981b) it has been found to reduce appetite in growing heifers (Kleiber, Goss and Guilbert, 1936) and young growing sheep (Fishwick and Hemingway, 1973a, 1973b) and also in lactating cows (Fishwick et al., 1977a). In the experiment reported by Fishwick et al. (1977a) voluntary intake by cows was affected when blood P dropped to less than 2 mg/100 ml. Wilson (1981) also observed depressed appetite in pregnant ewes after ten weeks on a low phosphorus diet, when mean plasma phosphorus dropped below 1.7 mg/100 ml. The ewes could consume only 50-60% of their daily ration. This is in contrast to reports cited above where reduced P intakes did not affect the appetite of pregnant cows. Although this contrast may be due to the greater stress on the pregnant ewe relative to the pregnant cow, especially considering the relative sizes of the dam and the foetus and the frequent possibility of twin foetuses for the ewe, the end effect of depressed appetite in young and pregnant sheep and also in lactating cows is a reduction in energy intake. This can impair growth rate or milk production or cause a loss of condition but the relative lack of effect of dietary phosphorus inadequacy on voluntary intake of the non-lactating cow may be due to the large daily turnover of P in the ruminant saliva (Cohen, 1980) which probably maintains normal blood P levels and growth and development of rumen micro-organisms. For the young growing ruminant, the turnover of P in saliva may fail to meet the requirements of rumen microorganisms after a period of time, since it has a relatively smaller amount of bone P to draw upon to meet its requirements. This may cause a reduction in microbial activity with consequences on food intake.

It was shown in Experiments 2, 3, 4 and 5.2 that reduced protein degradability may restrict energy intake. Experiment 5.2 indicated

that this may be due to less than adequate concentrations of rumen $\text{NH}_3\text{-N}$ required most by rumen microbes. It is apparent therefore that a response in food intake, and consequently growth rate, to dietary RDP supply can be anticipated only up to the level where other nutrients, especially P, restrict energy intake. Experiment 7 was therefore designed to test the effect of supplementary P and two protein sources of differing rumen-degradabilities (blood meal and urea) on the performance of growing wether sheep. Blood meal has been shown to be as very low as only 0.01 degradable (Gonzalez *et al.*, 1979), while urea is known to be almost completely hydrolysed in the rumen and their effects may well be influenced by P supplementation.

Materials and methods

Sixteen 6-month-old wether lambs (mean live weight 38 kg) were ranked into four weight groups and randomly allocated, one lamb from each group, to one of four treatments in a 2 x 2 factorial design. The treatments consisted of (fresh basis) either 45.3 g blood meal (BM) or 15.0 g urea (U) as nitrogen sources. Each of these was either unsupplemented (NIL) or supplemented (P) with 11.0 g of dicalcium phosphate to provide an additional 2.0 g P/d. These were fed with unmolassed sugar-beet pulp (USBP) and barley husk siftings (BHS) (2.3:1 on fresh weight basis). Each diet was further supplemented with 5.0 g salt and 2.5 g trace elements and vitamins. The various amounts of the dietary constituents and the amounts of CP, phosphorus (P) and calcium (Ca) supplied are shown in Table 45. The proximate compositions of USBP and BHS are shown in Table 46.

Experimental procedure

The sheep were individually penned and fed their various treatment diets ad libitum for three weeks. Thereafter the total feed offered to each sheep was restricted to 1.5 kg (air dry)/day. This was the approximate amount the sheep could consume completely and this restriction was to remove appetite differences and thus ensure that possible effects of P addition or the source of nitrogen or both on food intake might be detected from food residues. The sheep were fed for ten weeks.

Small quantities of feeds refused daily were removed and weighed to record intakes. Heparinised blood samples were obtained weekly from each sheep to determine changes in plasma P and Ca and differences in

Table 45 Expt. 7 The amounts (g/kg air dry basis)
of the various constituents of each diet

		Blood meal		Urea	
		NIL	P	NIL	P
<u>Dietary constituents:</u>					
Blood meal		45.3	45.3	-	-
Urea		-	-	15.0	15.0
Unmolassed sugar-beet pulp		700	700	700	700
Barley husk siftings		300	300	300	300
Dicalcium phosphate		-	11.0	-	11.0
Salt		5.0	5.0	5.0	5.0
Trace elements and minerals		2.5	2.5	2.5	2.5
Total	DM(g)	889	900	862	873
"	CP	103	103	109	109
"	P	0.9	2.9	0.9	2.9
"	Ca	6.1	9.3	6.0	9.2

plasma urea.

Dietary constituents were sampled twice weekly and the samples bulked and dried at the end of the experiment for chemical analysis.

Chemical analyses

Plasma samples were analysed for P, Ca and urea as described in Appendix 4. The proximate composition of the feed samples (Table 46) were also analysed by methods described in Appendix 4.

Results

The main results are presented in Tables 47 and 48. There were small food residues from all treatments from the 4th week but these did not occur in any pattern that could be attributed to the effect of P supplementation or the source of nitrogen. Dry matter intakes were not affected by treatment. Daily growth rates were slightly higher for lambs fed the U diet than for those fed BM but the difference was not significant. Phosphorus supplementation improved the efficiency of food conversion but not significantly. The difference between U and BM in feed efficiency was not significant but it was better for U.

The blood P concentration of the low P group decreased gradually from the end of the first week of the experiment. The decrease was significant ($P < 0.01$) from the third week of the experiment, when compared with the P supplemented group. The mean concentrations of blood P at the end of the Experiment (Table 48) were significantly lower ($P < 0.05$) for the low P group.

Blood urea concentration (Table 47) was significantly ($P < 0.01$) increased by supplementation with urea. The mean concentrations of blood urea tended to be lower in the presence of adequate P supplementation.

Discussion

Over the entire period covered by this experiment (ten weeks), the appetite of the lambs fed the low P diet was not depressed. Phosphorus intakes were approximately 50% less than the recommended level (1.8 g/d for sheep growing at approximately 100 g/d) (ARC, 1980) for the low P group.

There are few experiments involving a study of the combined effects of phosphorus and the source of supplementary nitrogen. However, in experiments conducted to study the effects of reduced P

Table 47 Expt. 7 Mean live weights, daily intakes of
dry matter, plasma urea concentrations and
performance of the sheep

	Blood meal		Urea		SE \pm
	NIL	P	NIL	P	
DM intake (kg)	1.10	1.16	1.18	1.16	0.066
DM intake (g/kgW ^{0.75})	68.0	70.8	73.4	70.7	3.81
Initial live weight (kg)	38.3	38.2	37.8	37.8	0.38
Final live weight (kg)	43.5	44.8	43.5	45.5	1.09
Daily growth rate (kg)	0.11	0.14	0.12	0.16	0.016
Food conversion ratio	12.2	9.0	10.6	7.6	1.66
Plasma urea (mg/100ml)	23.9 ^b	19.3 ^b	41.4 ^a	38.4 ^a	2.97**

Table 48 The estimated daily intakes (g) of P and Ca
and the corresponding changes in blood plasma
concentrations (mg/100 ml)

	Blood meal		Urea		SE ⁺
	NIL	P	NIL	P	
P intake	1.36	4.17	1.34	4.14	-
Ca intake	8.97	22.4	8.97	22.4	-
Initial plasma P	5.2	6.5	5.8	6.0	-
Plasma P at end of experiment ⁺	4.0 ^b	6.2 ^a	3.3 ^b	6.0 ^a	0.30
Decrease (%)	30.0	4.8	75.8	0.0	-
Initial plasma Ca	9.5	9.4	9.2	9.2	-
Plasma Ca at end of experiment	11.2	10.1	11.1	10.1	
Increase (%)	17.9	7.4	20.7	9.8	-

+ P < NIL ***

intakes per se on voluntary food consumption and growth of 3-4 month old lambs (initial live weight 27.5 to 33 kg), Fishwick (1978) and Fishwick and Hemingway (1973a,1973b) noted that, in the absence of P, the lambs left some feed in less than three weeks from the start of the experiment, recovering full appetite 2 to 3 days after changing to full supplementation. In a similar trial but with 3-week old rats, Henry et al. (1979) also reported a decrease in food intake caused by severe P deficiency (one quarter of the normal level). This was accompanied by retarded growth and reduced food and energy utilisation.

The failure to observe reduced appetite on low P diets in the present experiment may be due to the relative age of the lambs used. At six months and with a mean live weight of 38 kg, the lambs used were relatively mature compared to those used in the experiments cited above, having presumably stored more bone P. It is, however, more important to note that, whilst blood P fell, the lowest level recorded at the end of the experiment (3.65 mg/100 ml for the low P diet) was still higher than those recorded by Fishwick and Hemingway (1973a,1973b) (less than 3 mg/100 ml) which caused a reduction in appetite of growing sheep. For lactating cows fed straw (Fishwick et al., 1977a), appetite was also affected only when the blood P concentrations fell to below 2 mg/100 ml. It is possible, therefore, that unless the P status of the lamb is so low that blood phosphorus is reduced to below 3 mg/100 ml, the voluntary food intake of lambs may not be affected.

The small non-significant improvement in live-weight gain due to P supplementation may suggest a more efficient nutrient utilisation, confirming earlier observations by Hegsted (1974). As a result, 27% less food was used per kg live weight gain by lambs fed P supplemented diets.

The source of nitrogen (blood meal or urea) had no effect on food intakes. However, urea supported marginally higher live weight gains and food conversion ratios than blood meal. There appears to have been an interaction, albeit small, between P and nitrogen source on live weight gain when the size of the difference between the P supplemented U and BM is compared with that of the unsupplemented equivalents. The certainty and extent of possible interactions between P and the source of nitrogen is, however, not clear from the present results, especially since no clear differences were recorded.

Normal blood P was maintained in lambs fed the P supplemented

diets. For lambs fed the low P diet, plasma P dropped by 51% at the end of the experiment. This was accompanied by a corresponding rise in plasma Ca levels of 19%. These changes suggest some degree of bone mineralisation. The tendency for a lesser decrease in plasma P and lower increase in plasma Ca on the unsupplemented BM diet may have been due to small but negligible P content of ~~bone~~^{blood} meal.

The differences between U and BM in plasma urea concentrations are only expected in view of the high solubility of urea. The slightly lower plasma urea concentration in the presence of adequate P supplementation may indicate a greater efficiency of nitrogen utilisation, probably accounting in part for the improvement in live weight gain on the P supplemented diets.

Although less than adequate intake of P did not affect appetite in 6-month old lambs for the period of ten weeks covered by this experiment, feed efficiency appeared to have been lowered with corresponding reductions in growth rate. Urea seemed to have resulted in marginal improvements in food intake and growth rate over blood meal. There is the possibility of an interaction between the source of nitrogen supplement and level of dietary P in low-quality diets.

The value of these results is somewhat limited and longer treatment periods may be necessary to evaluate the effects suggested in this experiment. Quite different results might have been obtained if the experiment had been conducted with younger lambs of, say, 20 kg initial live weight. That should form the basis of a further study.

SECTION 4GENERAL DISCUSSION AND CONCLUSION

The results obtained in the experiments reported in this thesis generally show that the degradability of dietary protein in the rumen affects the voluntary intake of low-protein roughage feeds. Although the new (ARC, 1980) system of estimating protein needs of ruminants, which requires the description of dietary CP in terms of rumen-degraded and undegraded protein, implies an RDP component, it seems that this portion of the protein intake is too often assumed to satisfy voluntary intake requirements for all diets. The risk has been that the importance of rumen-degraded protein with regard to microbial needs and voluntary intakes, especially of low-quality roughage feeds, as a result of which improved productivity is possible, is less often considered. An example is the work of Baraton and Pflimlin (1978) where formaldehyde treatment of soya bean to improve milk yield caused the opposite effect because reduced degradability of the dietary protein resulted in a relatively RDP deficient diet (less than 500 g RDP/cow/day).

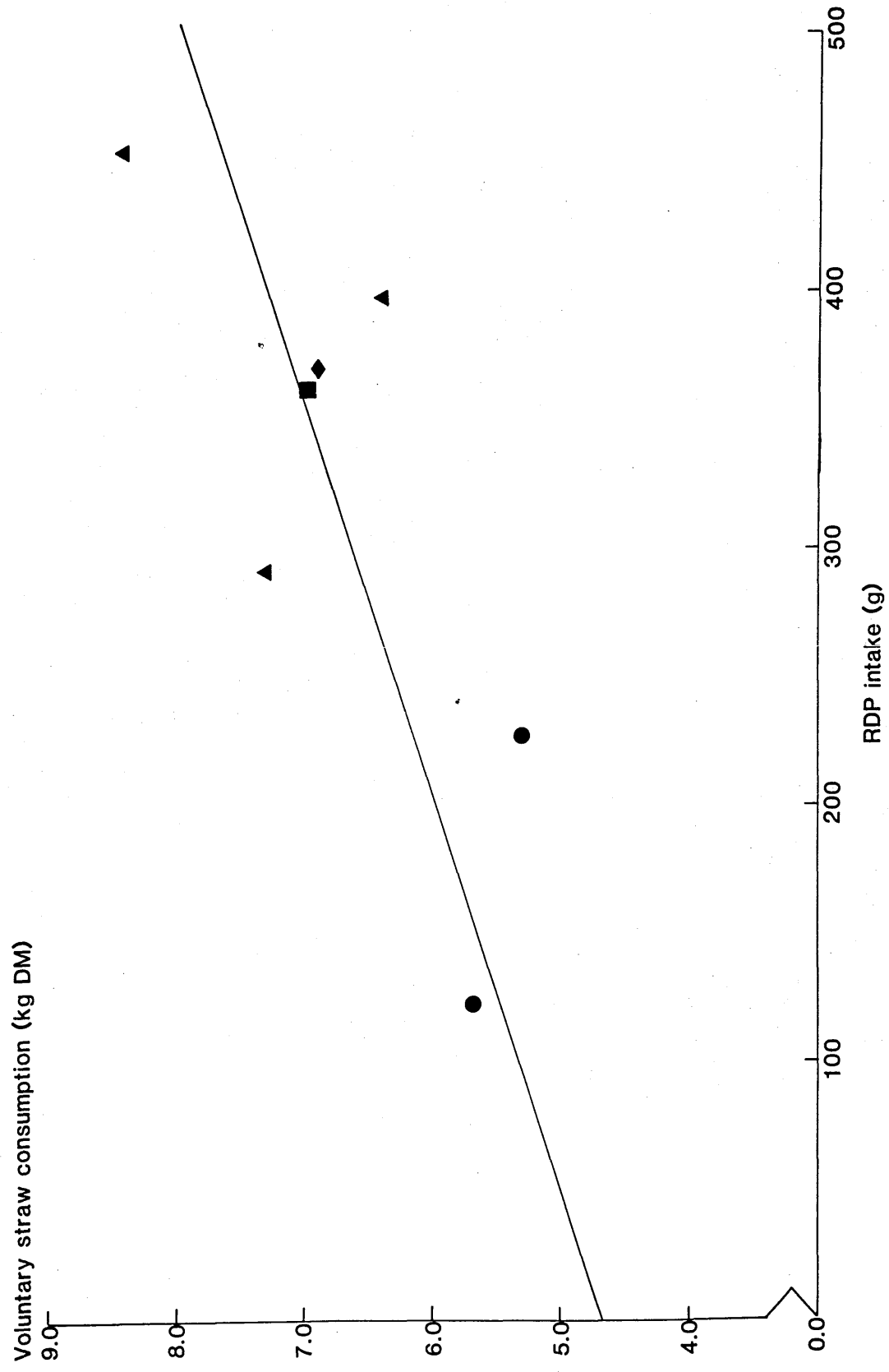
It is such problems that the evidence gathered from the results of experiments reported in this thesis highlights. This evidence concerning the influence of RDP intake on voluntary consumption of low-protein roughage feeds was noted mainly with sheep in Expt. 2, the pregnant beef cows in Expts. 3 and 5.2 and, to a lesser extent, with the lactating cows in Exp. 4. A protein x energy source interaction was probably responsible for the failure of the relationship between RDP intake and straw consumption to be significant in Experiment 4, but the trend was evident. While the results of Exp. 6.2, with lactating cows, would also confirm this relationship, it is recognised, as discussed in that experiment, that other factors may have been involved.

In view of the interactions on straw intake encountered with the lactating cows in Expt. 4 and the possible influence of other factors on intake also with lactating cows in Expt. 6.2, a further test of the relationship between straw consumption and RDP intake was made by plotting voluntary straw intakes against RDP in the two experiments (Table 26, 27, 39 and 41). It was thought that pooling these data obtained with lactating cows and treating them as coming from one

Figure 9 The relationship between RDP intake and voluntary straw consumption (Data taken from Expts. 4 and 6.2)

Key

- MSBP
- DBG
- ▲ ROLLED BARLEY
- ◆ PEAS



experiment could either help to validate the relationship or cause a divergence. The regression equation obtained was DM intake (kg) = $4.67 + 0.0066 \times \text{RDP intake (g)}$ and the regression line (Fig. 9) was significant ($P < 0.05$) ($r = 0.802$, $\text{rsd } 0.7385$, $\text{df } 5$). There was no such linear relationship when voluntary straw intakes were plotted against CP, as evidenced in Fig. 10. As a result of the linear relationship obtained with the data on intake by the lactating cows taken from the two experiments above, the opportunity was taken to test for this relationship with data taken from three previously reported and one unpublished trial involving 6 different diets conducted with lactating cows fed oat straw ad libitum and supplemented with either barley or sugar-beet pulp. The protein supplements used in these other experiments, listed in Table 49, were soya bean meal and urea. The degradability of the concentrates were obtained by using values given in Table 18 since these values were estimates made on the same type of feedstuffs. The cows used in the experiments listed in Table 49 were mostly the same as those used in these present experiments and were similarly housed in the same general conditions with the same facilities at the Glasgow University Veterinary School Field Station. The allocation of concentrate to the cows (about 3.0 kg FM) was also essentially the same as in these present experiments. A linear relationship (Fig. 11) was also obtained between straw DM intake and intakes of RDP for these particular results of previous experiments. The regression equation was straw DM intake (kg) = $5.52 + 0.0038 \times \text{RDP intake (g)}$. The regression line was significant ($P < 0.05$) ($r = 0.821$; $\text{rsd } 0.3657$, $\text{df } 4$).

The regression line did not differ significantly from that obtained with the data taken from Expts. 4 and 6.2 (shown in Fig. 9) (the variance ratio for the difference between both lines being 0.2025 F 1, 9 df). There was, however, a significant difference in the intercept of the two lines. It is probable that this difference is due to the type of straw used. Barley straw, used in all the experiments reported in this thesis, contained approximately 30 g CP/kg DM compared with approximately 20 g CP/kg DM for the batches of oat straw used in the various previous experiments cited in this thesis.

The regression line obtained with lactating cows in the present experiments (Fig. 9) was also compared with that obtained while the cows were pregnant in Experiment 3 (Fig. 2). Although both lines, shown in Fig. 12, were similar (variance ratio 1.35:F 1, 15 df), their

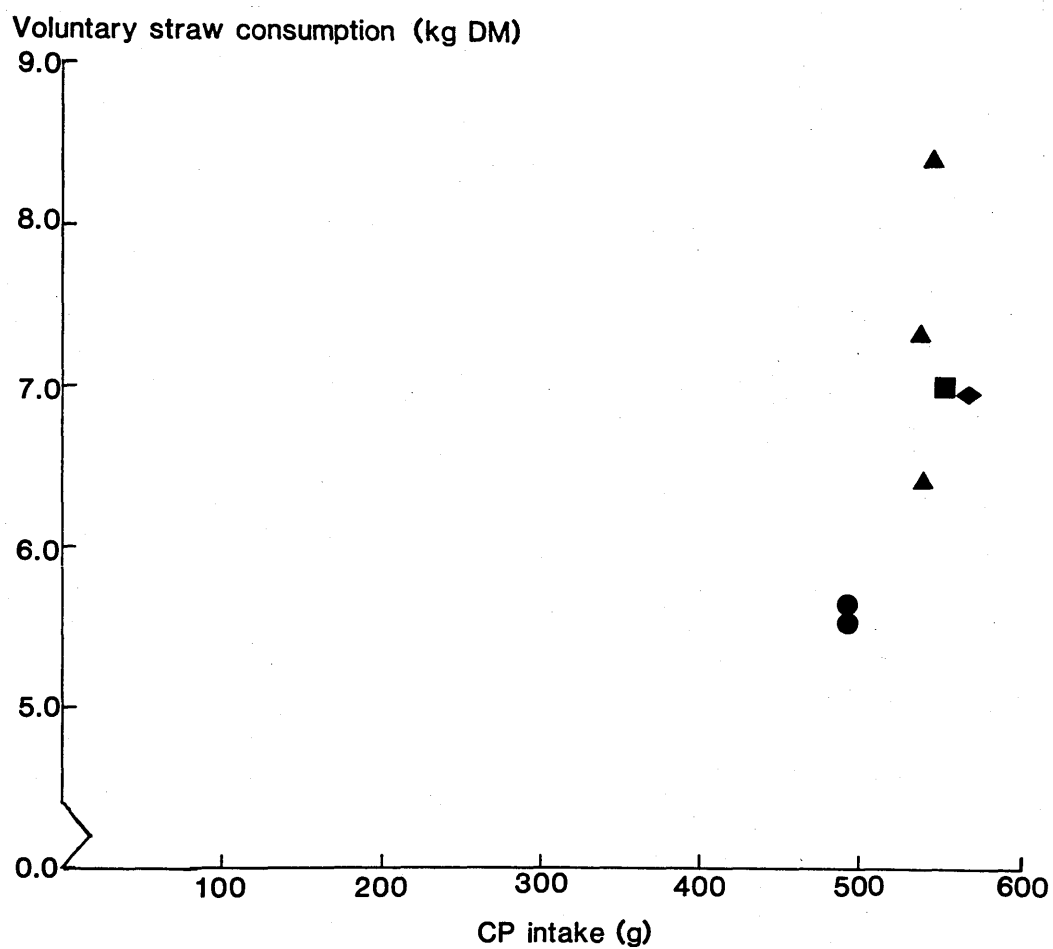


Fig. 10 The relationship between CP intake and voluntary straw consumption
(Data taken from Expts 4 and 6.2)

(● MSBP, ■ DBG, ▲ ROLLED BARLEY, ◆ PEAS)

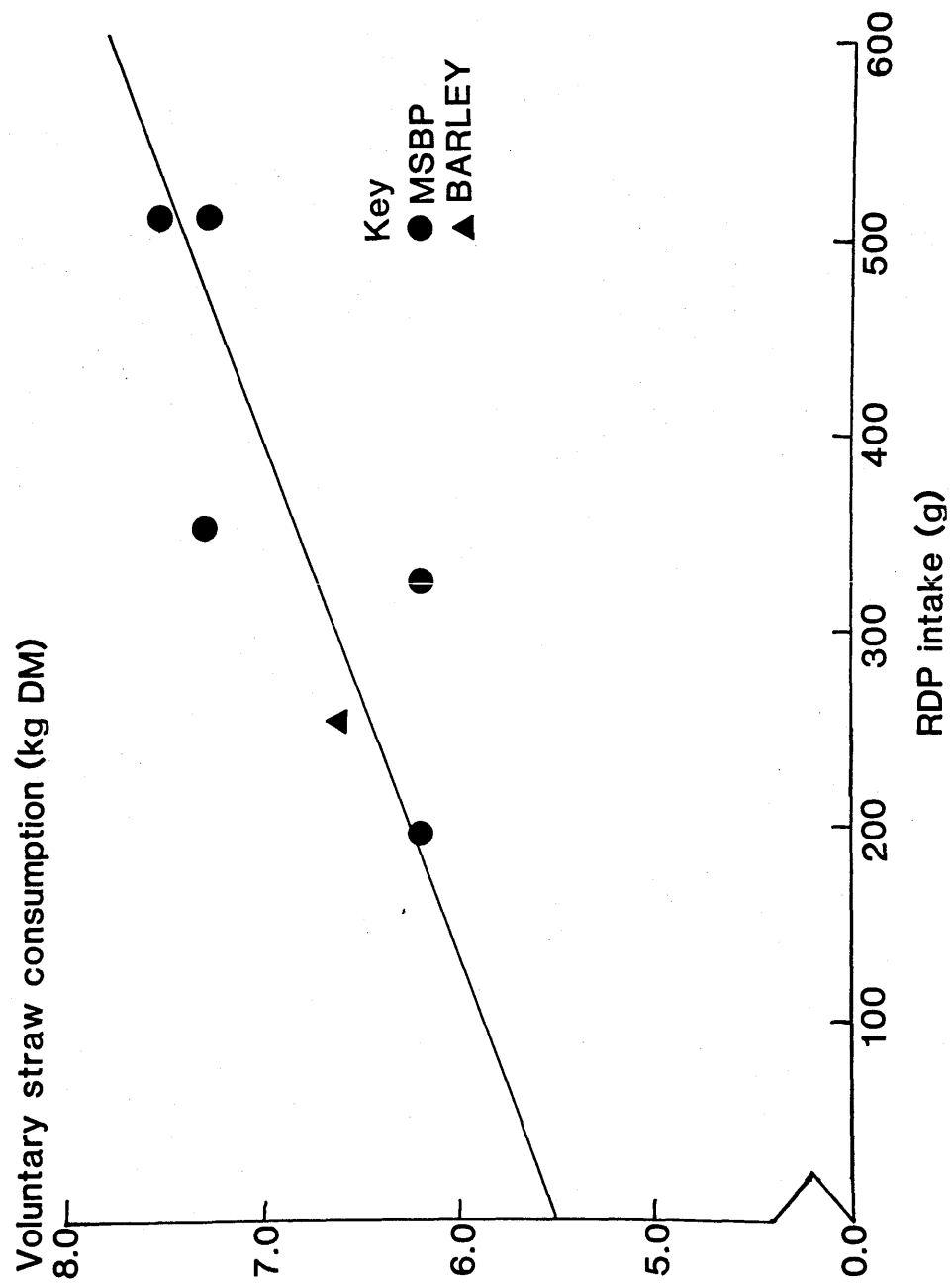


Fig. 11 The relationship between RDP intake and voluntary straw consumption
(Previous comparable expts with lactating cows)

Table 49 Previous comparable experiments with lactating
beef cows

RDP intake (g)	Straw DM intake (kg)	Supplements used	Reference
196	6.23	MSBP + U	Fishwick <u>et al.</u> (1977b)
254	6.65	B + U	Fishwick <u>et al.</u> (1977b)
327	6.23	MSBP + U	Fishwick <u>et al.</u> (1977a)
352	7.33	MSBP + SBM	Fishwick (G. Fishwick (1985, unpublished results)
508	7.31	MSBP + U	Bass <u>et al.</u> (1981)
508	7.55	MSBP + U	Bass <u>et al.</u> (1981)

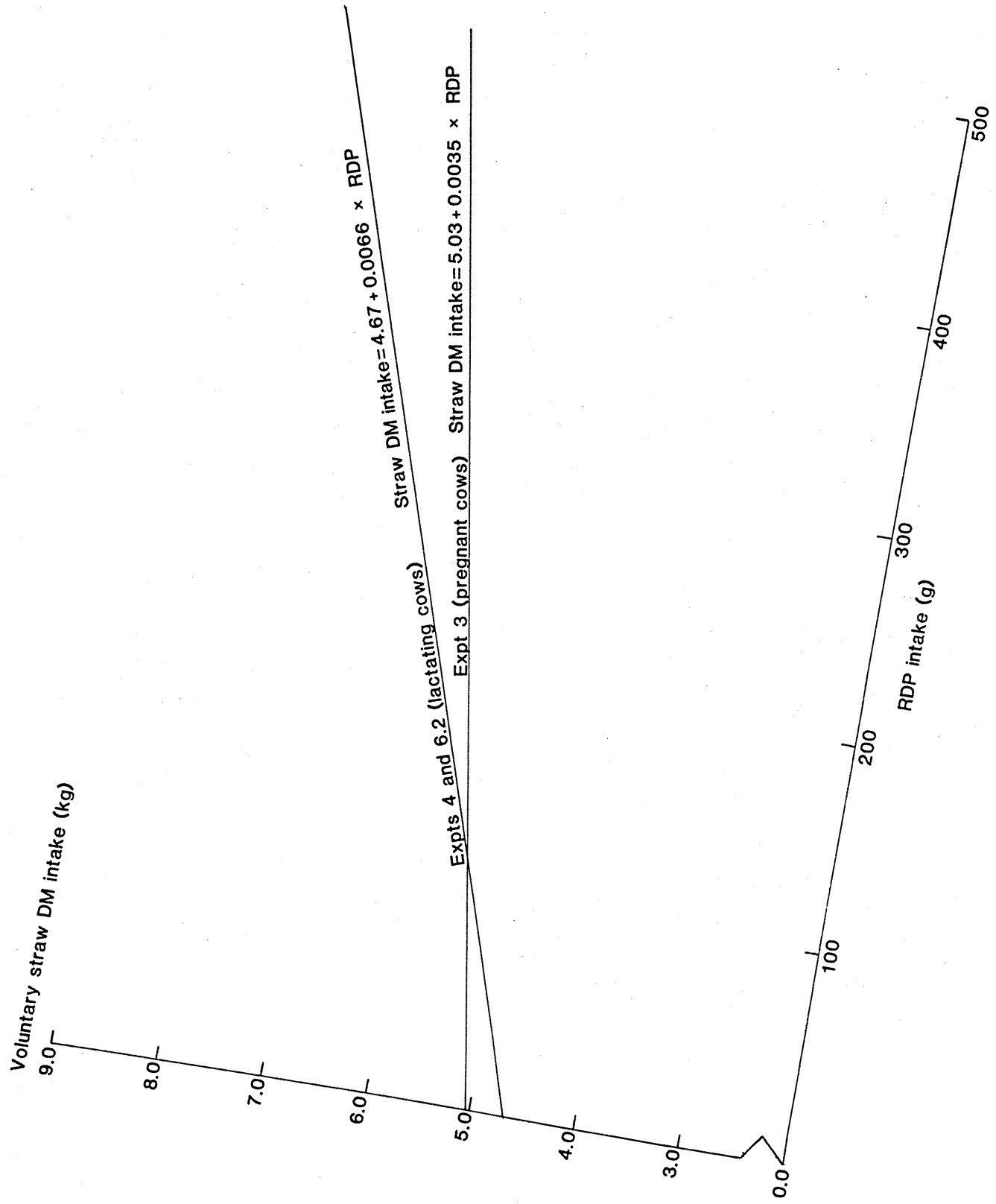
constant terms were significantly different. It is interesting, however, that the lines indicate higher straw intakes for the cows during lactation than during pregnancy at higher RDP contents in the diet. The difference between the two lines approximated to 1.0 kg straw DM. Considering that the cows were at an advanced stage of pregnancy during Expt. 4, this difference between the lines may be attributed to a relatively restricted rumen capacity for the pregnant cow, an effect which was not existent during lactation. This may account for the difference in the intercept of the two lines.

These results and the main ones reported in Expts. 1 - 6 in this thesis show clearly that for low-protein roughage feeds, such as straw, the RDP available to the animal affects voluntary food intake. It should be noted that, as a result of interactions between nutrients or the broad relationship between CP and digestibility of low-protein roughage feeds, or perhaps even between CP and RDP, there may be an apparent but spurious relationship between CP and voluntary straw intake. However, it would seem that a better and more predictable relationship exists between voluntary straw intake and RDP consumed by the animal.

Linearity of the relationships between voluntary straw DM intake and RDP has been assumed within the range of RDP content of concentrates encountered in these present experiments, especially considering the evidence in Fig 4, where the highest intake was associated with 595 g RDP. Curvilinearity would be more reasonable but the departure from linearity could not be proved with the evidence presented in this thesis.

With protein deficiency, the quantity and quality of protein absorbed from the intestines limits intake (Egan, 1965). With low-protein straw used in these experiments it is reasonable to assume that this will be largely supplied from rumen microbial protein synthesis, especially since about 70% of amino acids absorbed from the intestines is microbial protein. Pure culture studies have shown that ammonia is the major source of nutrients for microbial growth and, although data relating rumen ammonia concentrations to microbial synthesis on straw diets is scarce, the significant relationship obtained between protein degradability and rumen $\text{NH}_3\text{-N}$ and other relationships shown in Table 36 and also the effect of chemical treatment of SBM on rumen $\text{NH}_3\text{-N}$ in Experiments 1 and 2 show the course of the effect of RDP on intake. It is probable, therefore, that in terms of (1) ruminal ammonia levels

Figure 12 Regression lines for straw DM intakes on RDP



necessary to encourage maximum fermentation and consequently intake, and (2) the amino acids absorbed from the intestines which may further increase intake, the RDP available is the first limitation to intake and response to UDP is more likely to occur only when RDP needs have been satisfied. The response in milk yield to UDP observed in Expt. 6.2 occurred probably because the rumen $\text{NH}_3\text{-N}$ concentrations, averaged for most of the day (from 07.30 - 14.30 h) was in excess of minimum requirements for microbial needs. Also, the adjustment to, and economic use of, the limited amounts of feed nitrogen by sheep in Expts. 1 and 2 is further evidence to indicate that recycled nitrogen, plasma urea and protein degradability are probably more important to the biological efficiency of the animal fed low-quality roughage feeds over a period of time.

The most probable hypothesis for the positive relationship between concentrate protein degradability in the rumen and straw DM intakes observed in these present experiments is likely to have a basis in the importance of the relative quantities of nitrogen needed to sustain maximum microbial activity, especially of micro-organisms digesting the cell wall contents of plant feedstuffs. For poor-quality roughage feeds the addition of nitrogen exercises its influence through a specific effect in increasing rumen digestion (Campling, 1970), the rate of which depends on the degradability of dietary protein (Mehrez and Orskov, 1978). A more degradable protein ($\text{N} \times 6.25$) source is more likely to increase the rate of microbial digestion through a general increase in microbial protein production, although beyond 23.5 mg $\text{NH}_3\text{-N}/100$ ml generated in the rumen fluid, there is little if any increase in microbial fermentation (Mehrez, Orskov and McDonald, 1977). The point at which a plateau in straw intake is likely to occur is, however, not within the scope of the experiments reported here. But the effect of a more rapid fermentation would be to increase both total and digestible food intakes observed by Balch and Campling (1962), and indeed, a highly significant ($P < 0.01$) linear relationship between rumen bacterial production and DM intake has been reported (Singh, Verma, Varma and Ranjah, 1977).

It is therefore reasonable to conclude, based on results of experiments reported in this thesis, that for low-quality roughage feeds, the degradability of dietary CP is an important, perhaps the first limiting, factor in voluntary food intake. Protein that is not degraded in the rumen is likely to be better utilised to enhance

production when microbial needs for ammonia which depend largely on the degradable protein supply are satisfied.

The results also seem to indicate that for the voluntary intake of low-quality roughage feeds, urea or soya bean meal may account for a significant proportion of, or perhaps even all, the nitrogen needs of mature ruminants for maintenance.

After most of the experiments reported in this thesis had been conducted and the trends observed, an interesting observation was made in a recent brief communication by Newbold (1985). Working with steers fed all-concentrate barley-based diets, he reported a highly significant ($P < 0.001$) linear relationship between food intake and dietary RDP concentration.

Suggestions for further work

At present there are insufficient results from which the possible levels of RDP necessary for maximum intakes of various feeds, but especially of low-quality roughage feeds, can be estimated. Further work in this respect would probably be of advantage if done simultaneously with investigations on the rumen $\text{NH}_3\text{-N}$ concentrations likely to encourage the highest intakes. At the point that RDP satisfies microbial needs as judged by, among other things voluntary food intake, investigations should be made into the possible effects and the efficiency of UDP in further increasing voluntary food intake and production. Experiments similar to the ones reported in this thesis could also be conducted using younger animals to determine how intakes, and possibly growth rates, are affected by a deficiency of RDP for rumen micro-organisms. It is also important to standardise the methodology for RDP determination if meaningful interpretations of results involving RDP and UDP are to be made. Perhaps results of measured nitrogen flows should, when possible, be related to estimates of protein degradability derived by calculations based on rumen bag measurements or vice versa. These would ensure that the best combinations of feeds, especially of the amounts of highly degradable and undegradable nitrogen components, are provided for a specific level of production so that feeds are given to advantage. More importantly, livestock that graze on poor pastures most of the year can be better maintained to reduce the stress from inadequate nutrition.

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APPENDIX 1

METHODS USED IN DETERMINING THE DEGRADABILITY OF FEED SAMPLES

Five grams air dry substrate per replicate of each sample were placed in nylon bags made of polyester filament (24 μ m) (Henry Simon Ltd., Stockport, Cheshire) each bag (16 cm x 11.5 cm) having been washed and dried to constant weight at 60°C. Each replicate was then suspended on a 70 cm nylon cord in the rumen of each fistulated cow for 24 hours. After removal, they were placed in water for 6 hours after which they were washed, gently squeezed and dried at 60°C. for 48 hours.

APPENDIX 2

PROCEDURE USED IN DIGESTIBILITY TRIALS WITH WETHER SHEEP HOUSED IN METABOLISM CAGES

Allocation of feeds and feeding

The feedstuff under investigation was allocated to the wether sheep either in restricted amounts (1.0 kg fresh matter/hd/day) for feedstuffs that would later be fed with straw to cows or in amounts determined by the specific needs of the trial.

The feedstuffs were allocated either with dried grass of known digestibility, usually in the ratio (compound feed FM to dried grass/FM) of 7.0:3.0 or with a combination of molassed sugar-beet pulp and barley husk siftings (containing 500 g MSBP and 250 g barley husk siftings) in a ratio of 2.5:7.5, the digestibility of MSBP and barley husk siftings having been determined previously. The digestibility coefficient of the particular feed under investigation was determined by difference.

The feed allocation for the duration of the trial was pre-weighed into paper bags at the beginning of the experiment, a subsample being taken simultaneously for proximate analysis and gross energy determination.

The feed was offered twice daily in approximately equal halves at 07.30 and 16.00 h. Water was provided in containers, being replenished twice daily.

Collection of faeces and urine

The wether sheep, clipped free of wool around the hindquarters prior to housing in metabolism cages, were each fitted with a standard type of leather harness which included a chest strap. A faecal collection bag was attached to the harness by four quick-release spring-loaded cast scissor-grip hooks. After a preliminary period of at least 7 days, complete faecal collections, and urine where appropriate, were taken from each wether sheep during the subsequent 7-day period. The faecal collection bags were removed daily and faeces were emptied into numbered plastic buckets, each fitted with an air-tight lid and permanently kept at the rear of each animal. The bags were then refitted to the animals. Urine, where appropriate, was similarly collected in dilute (25%) HCL and stored in numbered plastic containers.

The faeces and urine collected over the 7 days were weighed separately, thoroughly mixed and an appropriate subsample taken. The faeces were dried and ground for chemical analysis. Samples of fresh faeces, macerated with a small amount of toluene (Commonwealth Agricultural Bureau, 1961) and urine were preserved for nitrogen determination.

APPENDIX 3PROCEDURE USED IN COLLECTING RUMEN LIQUOR FROM SHEEP

The procedure for the withdrawal of rumen fluid involved placing a Probang gag in the mouth of the sheep held in a standing position with its rear end against the gate of the pen and head slightly raised. A polythene stomach tube (210 cms in length and 9.5 cm in diameter) (Portex Ltd., Hythe, Kent) reinforced with a centre of rigid polythene tubing (3 mm in diameter) to prevent it from collapsing was passed through the gag into the mouth of the animal. The reinforcing polythene tubing was heat-sealed at the open end to prevent entry of rumen contents or saliva. Following a swallowing action by the animal, the tube was passed down the oesophagus until a pre-marked length of 100 cm had passed through the gag. Air was subsequently briefly pumped down the tube with an Enema pump (Aerosol Products Ltd., 680 Garratt Lane, London, SW17 ONP) to clear the rumen end of any solids present in the rumen which might block the tube. The external end of the tube was subsequently connected to a vacuum evacuation pump through a glass jar and rumen fluid withdrawn by gentle suction into the jar. About 15 to 20 mls of rumen fluid was withdrawn into the jar and the vacuum control turned off to prevent entry of saliva into the tube on withdrawal.

Between withdrawals of rumen fluid, the stomach tube was thoroughly washed with water and dried with an air jet. The collecting glass jar was similarly washed and dried with a hand towel.

APPENDIX 4ANALYTICAL PROCEDURE USED FOR CHEMICAL ANALYSISDry matter

The dry matter of feed and faecal samples was determined by heating weighed quantities (usually 0.5 to approximately 1.0 kg) in a hot air oven at 90-95°C. for 24 - 48 h until a constant weight was obtained.

Ash

The ash content of samples was determined by ignition at 550°C. in a muffle for at least four hours.

Gross energy

The gross energy of feed and faecal samples was measured by combustion in a Gallenkamp Adiabatic Bomb Calorimeter.

Ether extract and crude fibre

The ether extract and crude fibre contents of feed and faecal samples were determined by normal standard methods (The Fertilizer and Feeding Stuffs Regulations, 1976).

Chromium

The chromium content of feed and faecal samples was determined by atomic absorption spectrophotometry according to the method of Williams, David and Iismaa (1962) after dry ashing the samples.

Rumen liquor ammonia nitrogen

Ammonia nitrogen in strained rumen liquor samples was determined, following deproteinisation with acidified sodium tungstate, using a modification of the method of Waite and Wilson (1968).

Total nitrogen in rumen liquor, feed and faeces

Total nitrogen in strained rumen liquor samples and CP (N x 6.25) in feeds were determined using an automated Kjeldahl technique (Kjel-Foss Automatic 16210). Nitrogen in fresh faeces was determined following maceration of faecal samples with distilled water and a little toluene (C.A.B., 1961).

Rumen liquor pH

The pH of rumen liquor samples was determined using a Pye Model pH meter (Pye Unicam Ltd.) with a combined glass and reference electrode. The electrode was immersed directly into the rumen liquor.

Phosphorus

Phosphorus in feed samples was determined by a modification of the colorimetric method of Cavell (1955). Phosphorus in the blood was determined by the colorimetric method of Fiske and Subbarow (1925).

Calcium

The calcium content of blood samples was determined by atomic absorption spectroscopy (Perkin-Elmer, 1976).

Blood ammonia

$\text{NH}_3\text{-N}$ in whole blood was determined using the cation exchange method of Hutchinson and Labby (1962) in which the ammonium is exchanged for sodium and potassium ions. The exchanged ammonia was subsequently determined directly by colorimetry after reaction with Nessler's reagent (Parkins, 1972).

Blood urea

The urea content of blood samples was determined by a urease-Nesslerization method.

Beta hydroxy-butyrate

Plasma beta hydroxy butyrate was determined using the automatic colorimetric method of Zivin and Snarr (1973).

Free fatty acids

Free fatty acids in plasma was determined using the colorimetric micro-determination method of Duncombe (1964).

Plasma total protein

Plasma was analysed for total protein in the Technicon AA2 automatic analyser using the Biuret method of Reinhold (1953).