
**COMMISSION of the
EUROPEAN COMMUNITIES**

**FORAGE PROTEIN
CONSERVATION and UTILISATION**

**SEMINAR HELD IN DUBLIN, 15-18 SEPTEMBER
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COMMISSION OF THE EUROPEAN COMMUNITIES

FORAGE PROTEIN CONSERVATION AND UTILISATION

Proceedings of a seminar in the EEC Programme of coordination
of Research on Plant Proteins organised by the Agricultural Institute
and held in Dublin, Ireland.

13 to 15 September, 1982

Edited by

T.W. GRIFFITHS and M.F. MAGUIRE

Department of Animal Nutrition,
The Agricultural Institute,
Dunsinea Research Centre,
Castleknock, Co. Dublin,
Ireland.

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PREFACE

This publication contains the proceedings of a seminar on forage protein conservation and utilisation held in Dublin on 13 to 15 September, 1982 under the auspices of the Commission of the European Communities as part of the EEC Common Research Programme on improvement of the production of plant proteins.

Forage crops are a major source of protein for ruminant livestock production in many areas of the community. It is important to ensure the best possible use is made of this resource by effective conservation, by developing more effective uses and by studying its utilisation by livestock. The objectives of the seminar were:-

- to up-date knowledge on changes in forage protein during ensilage
- to review progress on protein extraction and utilisation by crop fractionation procedures
- to examine the utilisation of forage protein by livestock and assess its adequacy in meeting requirements

A total of 17 papers were presented on these topics. An opportunity to discuss current research in these areas was afforded by the mounting of a poster session. Participants visited the Agricultural Institute's Animal Production Research Centre at Grange, Co. Meath to see work in progress on intensive beef production systems from grassland.

The Commission wishes to thank those representatives of the member states who took responsibility for the organisation and conduct of the seminar, notably Dr. M.F. Maguire (local organiser), the Chairman of sessions, those who prepared papers, posters and participated in the seminar discussions.

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OPENING STATEMENT

Prof. E. P. Cunningham

Deputy Director, The Agricultural Institute, Dublin

One of the consequences of the stressful times in which we live has been the questioning of the benefits of some aspects of the European Economic Community. This is probably less evident in Ireland than elsewhere, because the benefits of institutional cooperation with neighbouring countries are perhaps more easily seen in a small economy like ours. However, one of the activities which has unquestionably been of value far beyond its cost is the series of seminars on various aspects of applied science in the agricultural field. We are very pleased to have had the opportunity of hosting a number of these seminars in Ireland, and none more so than that which begins its work today.

The evolution of European agricultural production over the last twenty years has been remarkable. Total production of milk, beef, pigmeat, eggs and poultry have gone up respectively by 35%, 82%, 50%, 82% and 237%. The end result of these decades of development has been that the countries of Western Europe are largely self-sufficient for food stuffs of animal origin. However, this production has become increasingly dependent on external sources of animal feeds, and particularly for the protein elements in these feeds. It is therefore one of the major long term strategic aims of the Community to increase the indigenous production of protein feedstuffs.

In a recently completed study of the European Association for Animal Production these sources of protein feed for animal production are investigated. In the EEC as a whole, more than 60% of protein feeds are "non-marketable" i.e. very largely contained in forages produced and consumed on the farm. This proportion varies from a maximum of 90% in Ireland to a minimum of 40% in Denmark. These figures demonstrate clearly, therefore, both the strategic importance of the subject of this seminar for the evolution of European agriculture, and for the economy of animal production on the individual farm, as well as its particular relevance here in Ireland.

On behalf of my colleagues, I would like to welcome you to this seminar and to wish you the very best of success in your work.

A. ASPECTS OF CROP FRACTIONATION

Chairman: M. F. Maguire

THE CHARACTERISATION OF FORAGE PROTEIN

J.L. Mangan

Biochemistry Department, Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

Until recently agriculturalists considered feed proteins for ruminants mainly in terms of crude protein, i.e. Kjeldahl N x 6.25, on the assumption that rumen micro-organisms would convert most nitrogenous compounds, including urea, ammonia, amino acids, etc. into useful microbial protein. With the advent of present systems for assessment of protein requirements of ruminants for production (e.g. The Agricultural Research Council, 1980) it has become necessary to define the composition of feedstuffs in terms of rumen degradable and undegradable proteins. The biological value of the microbial protein and also that of the feed protein which is not degraded in the rumen must be evaluated in the same way as dietary protein in the nutrition of monogastric animals.

Plant physiologists and biochemists are well aware of the complexity of leaf proteins and their plant metabolic functions. In contrast to the proteins of storage organs such as tubers and seeds, leaf proteins are present to perform dynamic physico-chemical functions concerned with photosynthesis, respiration, cell multiplication, translocation, etc. Thus, although leaf proteins are complex, one would expect a degree of similarity between different leaves. By maceration of most leaves into ice cold buffer pH 7.4 in the presence of antioxidants, copper-chelating agents and protease inhibitors followed by high speed centrifugation, an extract of soluble proteins is obtained which on ultracentrifugation gives a relatively simple pattern. One large protein peak with a sedimentation constant 18S, M_r 550,000, is predominant and was called Fraction 1 leaf protein by Wildman & Bonner (1947). This fast moving peak is followed by a polydisperse peak with a sedimentation constant from 7S to 4S and M_r from 100,000 downwards. This is called Fraction 2 and consists of all the soluble proteins except Fraction 1. By fractional precipitation of the clear leaf extract, Fraction 1 almost free from Fraction 2 is obtained between 10% and 24% w/v ammonium sulphate. Fraction 2 is then precipitated from the supernatant by 40% w/v ammonium sulphate.

Although early workers assumed that the soluble proteins were cytoplasmic in origin and the green pigmented particulate protein was chloro-

plastic (see Chibnall, 1939), Lyttleton and Ts'o (1958) showed that Fraction 1, the most abundant soluble protein of leaves was present only in the chloroplasts and was released when the chloroplast outer membrane was ruptured. Fraction 1 was subsequently shown by Trown (1965) to be identical with ribulose-1,5-bisphosphate carboxylase (EC4.1.1.39) the first enzyme in the Calvin photosynthetic cycle and responsible for the fixation of CO_2 .

Kawashima & Wildman (1970) in a review pointed out that Fraction 1 had been isolated from at least 62 species and with genera ranging from photosynthetic bacteria, blue green algae, green algae, club mosses, bryophyta, gymnosperms and a large number of angiosperms. And since then the additions to the list are legion.

Microscopic study of leaf mesophyll cells shows that there is a large aqueous vacuole which contains sugars, salts and small M.W. compounds. The streaming cytoplasm occupies a relatively small volume around the

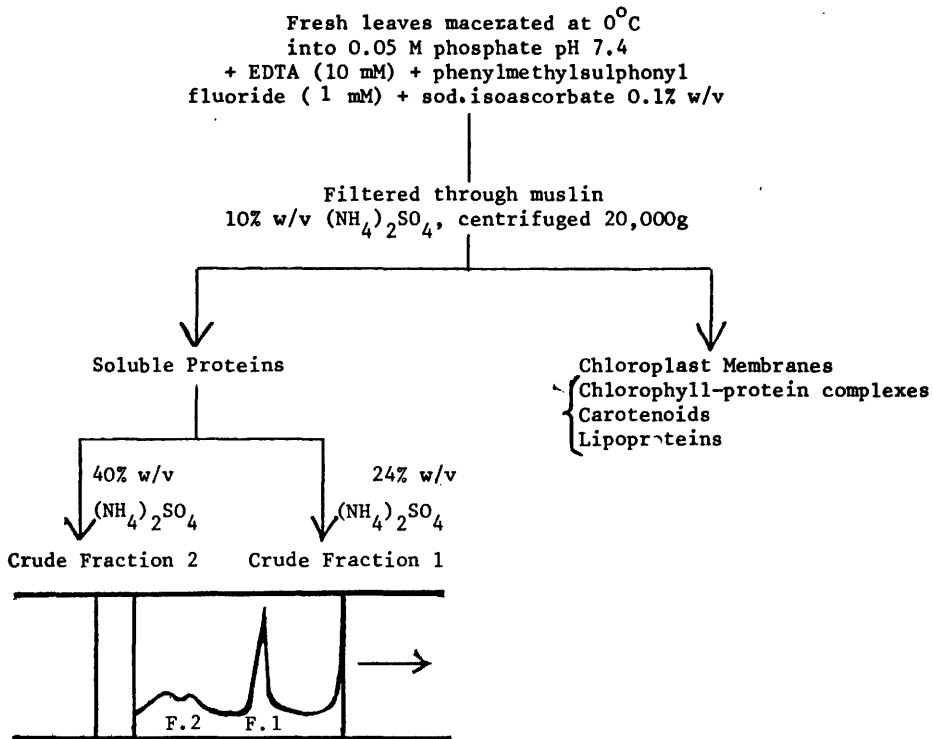


FIG. 1. Fractional precipitation of Leaf Proteins and ultracentrifugation pattern showing Fraction 1 (18S) and Fraction 2 (7S to 4S).

perimeter of the cell wall, but in fact most of the volume of the total cytoplasm consists of a large number of closely packed chloroplasts. The structure of chloroplasts has been studied extensively with the electron microscope. Within the limiting membrane is the characteristic thylakoid structure bunched into grana at intervals. The pigment-containing thylakoids are embedded in a colourless finely granulated stroma which has been shown by Willison and Davey (1976) using the electron microscope with a freeze-etch procedure to consist of Fraction 1 at very high concentration in a quasi crystalline state. Chloroplasts are known to contain about 75% of the total leaf protein and of this about half consists of Fraction 1. Although the composition of Fraction 1 may vary slightly it is essentially the same protein throughout the plant world and thus is considered to be the most abundant protein in existence. McArthur and Militmore (1966) directly determined the Fraction 1 content of lucerne leaves to be 32-39% of the total leaf protein. Fraction 1 is therefore an important dietary protein for ruminants and is degraded rapidly in the rumen (Nugent & Mangan, 1981). Fraction 1 protein, thylakoid membrane proteins and Fraction 2 proteins together comprise about 90% of the total leaf true protein with nuclear protein, cell wall protein and mitochondrial protein being well recognised but minor components. Free amino acids which may contribute 15-20% of the leaf total nitrogen, and hence the crude protein, are not part of the leaf protein as such and are rapidly fermented in the rumen. Their conversion to true protein of microbial cells will vary with the experimental conditions.

Fraction 1 leaf protein

Fraction 1 crystallises readily from extracts of tobacco and other nicotiana species and Baker, Eisenberg and Eiserling (1977) by X-ray diffraction and high resolution electron microscopy have shown that the molecule consists of 8 large and 8 small subunits arranged in a square shaped 2-layered molecule with a 20A cylindrical hole giving it a very characteristic appearance. Recently, by reacting with Polyethylene glycol M.W. 6000, Johal, Bourquet, Smith, Suh and Eisenberg (1980) have crystallised Fraction 1 from a number of other species, alfalfa, maize, cotton, potato, spinach, tobacco and tomato. The subunits are readily prepared by dissociating whole protein with sodium dodecyl sulphate and β -mercaptoethanol followed by chromatography on Sephadex G100 (Gray and

Kekwick, 1974) or by polyacrylamide gel electrophoresis. The large subunit has M_r 56,000 and the small subunit M_r 16,000. On the assumption that the molecule of Fraction 1 consists of 8 large and 8 small subunits, the M.W. would become 576,000 and the large subunits would comprise 77.8% by weight of the molecule. The amino acid composition of Fraction 1 from a large number of species has been shown (see Mangan, 1982) to be very uniform (Table 1).

Table 1. Amino Acid Composition of Fraction 1 Protein and the Large and Small Subunits (Molar Ratios: Phenylalanine = 1)

	FRACTION 1 Protein ¹ Mean \pm SEM	LARGE SUBUNIT ² Mean \pm SEM	SMALL SUBUNIT ³ Mean \pm SEM
Phenylalanine	1.00	1.00	1.00
Aspartic acid	2.22 \pm .121	2.13 \pm .063	2.21 \pm .226
Serine	1.05 \pm .066	0.84 \pm .039	0.97 \pm .128
Threonine	1.37 \pm .035	1.48 \pm .076	1.14 \pm .088
Glutamic acid	2.35 \pm .096	2.30 \pm .065	2.59 \pm .240
Proline	1.29 \pm .083	1.17 \pm .069	1.55 \pm .081
Glycine	2.14 \pm .074	2.27 \pm .062	1.47 \pm .142
Alanine	2.19 \pm .134	2.25 \pm .076	1.27 \pm .174
$\frac{1}{2}$ Cystine	0.45 \pm .056	-	-
Valine	1.56 \pm .040	1.47 \pm .046	1.38 \pm .097
Methionine	0.56 \pm .067	0.39 \pm .017	0.46 \pm .074
Isoleucine	0.94 \pm .043	0.87 \pm .033	0.73 \pm .086
Leucine	1.89 \pm .040	2.06 \pm .047	1.50 \pm .164
Tyrosine	0.91 \pm .059	0.86 \pm .029	1.37 \pm .128
Lysine	1.24 \pm .103	1.10 \pm .018	1.27 \pm .108
Histidine	0.61 \pm .041	0.66 \pm .024	0.39 \pm .048
Arginine	1.36 \pm .050	1.41 \pm .050	1.07 \pm .132
Tryptophane	0.40 \pm .054	-	-

¹ Analyses from Spinach beet, Spinach, Avena sativa, Nicotiana tabaccum, Medicago sativa, Euglena gracilis, Chlorella ellipsoidea, Chlamydomonas reinhardi, Rhodospirillum rubrum, Hydrogenomonas facilis, Hydrogenomonas eutropha.

² Analyses from Spinach beet, Spinach, Nicotiana tabaccum, Medicago sativa, Chlorella ellipsoidea, Phaseolus vulgaris, Halimeda cylindrica.

³ Analyses from Spinach, Nicotiana tabaccum, Spinach beet, Phaseolus vulgaris, Halimeda cylindrica, Chlorella ellipsoidea.

Application of the statistical analysis of Marchalonis & Weltman (1971) shows that the large subunit of all the species is a common molecular species and all the variation in Fraction 1 between species resides in the small subunits. Immunochemical studies also show that cross reaction between plant species resides in close similarity of the large subunits (Gray & Kekwick, 1974) and the small subunit acts as a species modifier.

The biochemical role of Fraction 1 is crucial to the plant's existence as it performs the CO₂ fixing step for all C₃ temperate plants. In addition to its primary function as ribulose-1,5-bisphosphate carboxylase in which 1 mole of CO₂ is combined with 1 mole of ribulose-1,5-bisphosphate to form 2 moles of 3-phosphoglycerate, Fraction 1 also functions as RUBP-oxygenase (Bowes, Ogren & Hageman, 1971) during photorespiration. The balance between these two functions is a major factor in the net CO₂ fixation by the plant.

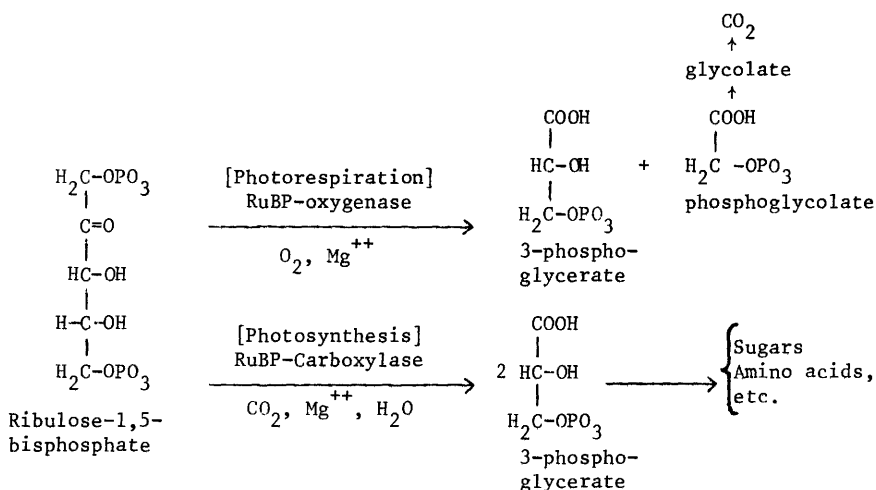


FIG. 2. Biochemical functions of Fraction 1 leaf protein

With tropical grasses such as sugar cane, maize and sorghum, it was shown by Slack & Hatch (1967) that the primary CO₂ fixation was carried out by phospho-enol-pyruvate carboxylase located in the leaf mesophyll cells to produce C₄ dicarboxylic acids, malate, oxaloacetate and aspartate. The temperature optimum for the C₄ system was 30-40°C compared to 15-25°C for the C₃ system. The rate of CO₂ fixation per unit of leaf surface area was

also doubled. The C₄ dicarboxylic acids thus produced are then translocated to the bundle sheath, where other chloroplasts occur which contain ribulose-1,5-bisphosphate carboxylase. The C₄ acids are then decarboxylated and the CO₂ refixed by the normal C₃ system. Fraction 1 can be isolated and even crystallized from maize, but a characteristic of the tropical grasses is the very low concentration of protein in the leaves, often less than maintenance requirements of stock, and a low protein: chlorophyll ratio.

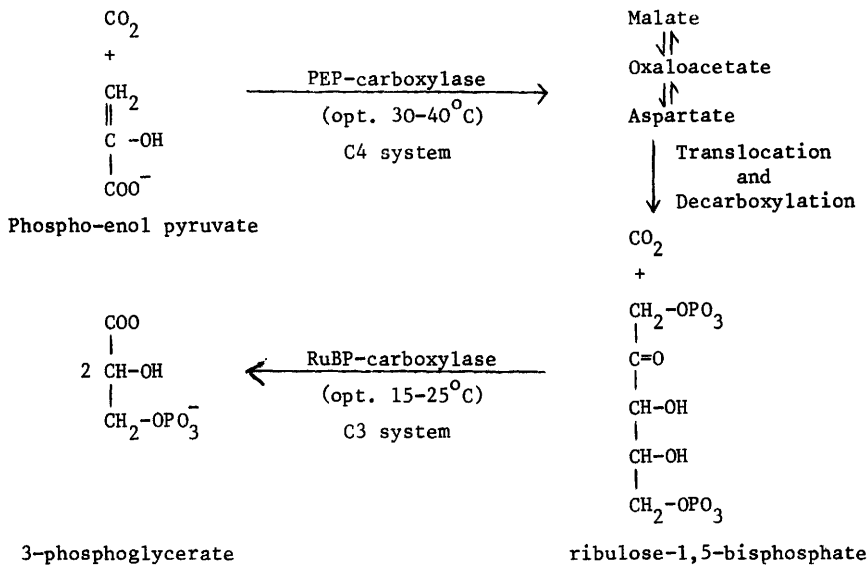


FIG. 3. PEP-Carboxylase system of CO₂ fixation in C₄ plants such as tropical grasses

Fraction 2 proteins

In contrast to Fraction 1 protein which is a single protein with only 2 enzyme functions, the Fraction 2 is derived both from chloroplasts and the cytoplasm and is a very complex mixture. Over 50 enzyme functions have been found in chloroplasts and over 1000 enzyme reactions in the whole leaf. In spite of this, however, De Jong (1977) by gel electrophoresis found only 7 major components in Fraction 2 of tobacco and Horsnell, Harrison and Mangan (1982) by crossed immuno-electrophoresis found only 9 major components in Fraction 2 of lucerne leaves.

Although most enzymes are present in leaves in small amounts and individually contribute little to the diet of a ruminant animal, collectively Fraction 2 constitutes about 25% of the total leaf protein. Some components such as plastocyanin, ferredoxin and the cytochromes are soluble proteins present in reasonable quantities in the chloroplast and major cytoplasmic constituents include actin, tubulin, protein elongation factors, ATP synthetase, carbonic anhydrase, etc.

The rate of proteolysis of these proteins in the rumen is not known, but it is now accepted generally that different soluble proteins degrade at different rates in the rumen (Nugent & Mangan, 1978; Mahadevan et al., 1980) and some components of Fraction 2 may contribute usefully to bypass protein if they have a slow rate of proteolysis.

Chloroplast membrane proteins

The chloroplast thylakoid (lamellar) membranes are complex structures which give the typical internal structure to chloroplasts and are insoluble in water. The protein constitutes about 40% of the chloroplast protein and is present as complex lipoprotein-chlorophyll complexes. By dissociation with sodium dodecyl sulphate and separation by polyacrylamide gel electrophoresis a relatively simple pattern of seven chlorophyll-protein complexes are found (Herrman, Börner & Hageman, 1980) and of these chlorophyll-protein complex 1 (CP1) and chlorophyll-protein complex 2 (CP2) comprise 28% and 49% respectively of the total thylakoid membrane protein. The five minor complexes, 20% of the total, are ill defined and little investigated.

Chlorophyll protein complex I (CP1) is present in all higher plants and algae. It has a M_r 110,000, contains 36% lipid and pigment and after solvent extraction gives a single protein M_r 70,000. CP1 contains only chlorophyll a and the molar ratio of chlorophyll: protein is 14:1. It contains or is identical with the Photosystem 1 (P-700 chlorophyll-protein complex). The degradation of CP1 in the rumen has not been investigated, but in general insoluble proteins are slowly broken down. If, however, sufficient entodiniomorphid protozoa are present chloroplasts are ingested and degraded rapidly in the digestive tract of the protozoa (Mangan & West, 1977). Hall, West & Coleman (1974) showed by electron microscopic studies that during this digestion the characteristic thylakoid membrane structure was rapidly destroyed in about 15 minutes.

Chlorophyll-protein complex 2 (CP2) has a much smaller molecular weight, $M_r = 24,000$, than CP1 and contains both chlorophyll a and b in equal amounts and also all the carotenoids. The protein moiety apparently consists of two polypeptides of M_r 26,000 and 24,000. CP2 is more abundant than CP1 and its fate in the rumen is not clear, but would be similar to CP1 as it is a component of the same thylakoid membrane system.

Nutritional value

Most of the true proteins of leaves of temperate C3 plants, about 70%, can be accounted for by the three main components, F1, CP1 and CP2. The amino acid compositions are known and it is therefore important to compare their nutritional value with a standard such as the FAO provisional pattern of essential amino acids (1957). Table 2 shows that Fraction 1 is nutritionally a very good protein with adequate supplies of all the essential amino acids. CP1 however is deficient in lysine and in the sulphur amino acids methionine and cystine. CP2 is not deficient in lysine and the sulphur amino acids methionine and cystine are the only deficiency. Ershoff, Wildman and Kwanyuan (1978) showed in rats that the protein efficiency ratio (PER, 28 days) of crystalline Fraction I from tobacco plants was 3.01, compared with casein 2.83, confirming the high nutritional value indicated by the amino acid composition.

Minor Protein Sources in leaves

1. Nucleoprotein: The nucleus is an essential part of the cell and is high in protein, about 50% of the dry matter. Its size, however, is small and it contributes only 1-2% of the total leaf protein. The protein moiety is a strongly basic histone combined with DNA. Its behaviour in the rumen is unknown. The histone of Pisum contains large amounts of the basic amino acids lysine and arginine and is deficient in cystine and methionine.
2. Extensin: This is a glycoprotein characteristic of primary plant cell walls (Lampert and Northcote, 1960) and contains the unusual amino acid hydroxyproline similar to animal collagen. Extensin has not been fully characterized but it contains only 5% protein and about 90% carbohydrate. The MW is about 230,000 and is firmly bound to cellulose. It is probably degraded slowly in the rumen.

3. Mitochondrial protein: Although mitochondria are numerous in the cytoplasm they are small in size and probably contribute less than 5% of the total protein of leaves (Lyttleton, 1973). Their behaviour in the rumen is not known but 40% of the protein is structural and insoluble and they contain the soluble enzymes of the tricarboxylic acid cycle.

Table 2. Amino Acid Composition (m-mole/g protein N) of leaf protein fractions compared with the FAO provisional pattern (1957)

	FAO (1957) Provisional Pattern	Fraction 1*	Chorophyll- [†] Protein Complex 1	Chlorophyll- [†] Protein Complex 2
Aspartate	-	5.79	4.73	5.60
Threonine	1.51	3.57	2.94	3.19
Serine	-	2.74	3.13	2.63
Glutamate	-	6.13	4.42	5.57
Proline	-	3.37	2.71	4.45
Glycine	-	5.51	6.29	7.84
Alanine	-	5.70	5.53	6.41
Valine	2.31	4.07	3.51	3.99
Methionine	0.97	1.46	0.72 ^{LIM}	0.95 ^{LIM}
Isoleucine	2.06	2.45	3.70	2.73
Leucine	2.33	4.93	6.52	5.88
Tyrosine	0.99	2.38	1.49	1.54
Phenylalanine	1.09	2.61	3.81	3.50
Lysine	1.85	3.24	1.68 ^{LIM}	3.19
Histidine	-	1.60	2.78	0.74
Arginine	-	3.52	2.02	1.79
Cystine/2	1.05	1.17	0.19 ^{LIM}	0.28 ^{LIM}
Tryptophane	0.44	1.04	0.57	0.60

* Mangan, 1982.

† Thornber, Steward, Hatton & Leggett-Bailey, 1967.

Immunology of Leaf Protein

Fraction 1 protein and the subunits are immunogenic and immunological cross reactions have been demonstrated between preparations from different

plant species. Murphy (1978) has used antisera against Fraction 1 to measure the relatedness of different plant species by their immunological distance. Gray and Kekwick (1974) showed by immunological methods that the large subunits of different species of Fraction 1 are closely related, but the small subunits do not cross-react. The active enzyme site of Fraction 1 was shown by the fact that ribulose-1,5-bisphosphate carboxylase activity was inhibited by antisera to whole Fraction 1 and the large subunit, but not by the antiserum to the small subunit.

Horsnell, Harrison & Mangan (1982) have raised antisera against Fraction 1, Fraction 2, and thylakoid membrane protein of lucerne and used high resolving crossed immunoelectrophoresis for highly specific analysis of these proteins in ruminant digestion. Fraction 1 gives a single immuno peak which changes to two peaks when the protein is treated with SDS to separate the subunits. Fraction 2 gives 9-11 distinct peaks and chloroplast membranes gives two somewhat diffuse peaks assumed to be CP1 and CP2. Areas under the immuno peaks give linear relations with the protein concentrations.

Gray and Wildman (1974) used anti-Fraction 1 IgG bound to Sepharose 4B in a column technique to absorb Fraction 1 from crude leaf extracts. Elution, however required 8M urea which dissociated Fraction 1 into subunits. An in situ immunofluorescent labelling of Fraction 1 in leaf sections (Hattersley, Watson & Osmond, 1977) has been used to locate precisely Fraction 1 in classical C3 and C4 plants and also in C3/C4 hybrids.

Total leaf protein

Following the pioneering work of Pirie and Byers at Rothamsted, many preparations of leaf protein concentrates have been made with various extraction procedures. This subject will be discussed in a following paper but Chibnall, Rees & Lugg (1963) showed that total leaf protein had a very uniform amino acid composition. Lyttleton (1973) correlated data from numerous sources and the amino acid composition was again found to be uniform over a range of species. Byers (1971) showed that the amino acid composition of total leaf protein was independent of the species and also that it was unaffected by leaf age.

In terms of nutrition of ruminants, however, this uniformity should not be allowed to disguise the fact that those preparations are mixtures of proteins with different properties.

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PAPER 1

AUTHOR: D.L. Mangan (United Kingdom)

DISCUSSION

A.S. Jones (UK)

Should N.R.C. or A.R.C. values rather than F.A.O. values have been used for the reference protein?

D.L. Mangan

For animals - yes.

F. van Sumere (Belgium)

Was air excluded during extraction?

D.L. Mangan

No, but it was ensured that antioxidants were present. All reagents contained 0.1% sodium isoascorbate and all processing was completed in one day.

A.G. Prendergast (EEC Commission)

Protein content of tropical grasses are generally low

D.L. Mangan

According to a review by Minson protein content may rise to around 14% under favourable conditions of cultivation.

R. Carlsson (Sweden)

Can chloroplasts store F1 protein? If so, it should be possible to manipulate plants genetically for higher production of F1 protein. Has maize F1 protein been recovered in crystalline form?

D.L. Mangan

Yes.

R. Carlsson

Elephant grass (*Pennisetum purpureum*) can contain up to 18% protein and young leaves up to 30% protein. The amino acid content of extracted protein varies possibly due to the conditions of extraction and the presence of phenolics.

D.L. Mangan

The amino acid composition of F1 protein is uniform but the composition of the F2 fraction is more variable.

PROTEOLYSIS AND CHANGES IN AMINO-ACID COMPOSITION DURING ENSILAGE
OF UNTREATED, FORMIC ACID AND/OR FORMALDEHYDE TREATED ALFALFA

Moreau I., Foulon M., Deswysen A., Vervack W., Vanbelle M.

Université Catholique de Louvain
Département de Biologie Animale Appliquée (BNUT)
Place Croix du Sud, 3, 1348, LOUVAIN-la-NEUVE, BELGIUM

ABSTRACT

Proteolysis and changes in amino-acids composition were studied in alfalfa ensiled with and without the addition of formic acid and/or formaldehyde. The ammonia-N and the true protein content were both lower in the formic acid- than in the formaldehyde silages, suggesting two distinct aspects in the nitrogen degradation: the proteolysis and the ammoniogenesis. A more precise measurement of the nitrogen degradation is given by the contents and recoveries of individual, essential and total amino-acids.

Recoveries were best in the silages treated with 0.2% formic acid + 0.2% formaldehyde followed by the silages with 0.4% formic acid.

INTRODUCTION

When conserving herbage as silages, extensive protein degradation is carried out by plant enzymes and micro-organisms reducing thereby the nutritional value of the silage compared with the original herbage. Some nitrogenous components are degraded to peptides and amino-acids which may be deaminated and decarboxylated. In lactate silages, ammonia content is generally less than 10% of total nitrogen and it mainly arises from deamination of arginine and serine. In silages in which clostridia had been active, amino-acids degradation is very extensive. The main metabolites are the alpha- and gamma-aminobutyric acid, histamine, tyramine, cadaverine, putrescine and delta-aminovaleric acid. The beta-alanine and beta-aminoisobutyric acid are present as traces (Ohshima, Mc Donald, 1978).

The main disadvantage of alfalfa ensiling without an additive is the high content of soluble nitrogen. This nitrogen is rapidly fermented in the rumen allowing only a poor N utilization (Valentine, Brown, 1973). Ensiling alfalfa with a formalin/formic acid additive reduces strongly the amino-acids loss and the soluble nitrogen content which is high in untreated silages (Ohshima et al., 1979). Barry et al. (1978) reported that increasing rates of formic acid addition, and the use of the precision-chop harvester reduced the loss of the amino-acids and minimized the increase in alanine, alpha- and gamma-aminobutyric acid. Formaldehyde treatment also reduced amino-acid degradation apart from apparently high losses of lysine, histi-

dine, tyrosine, tryptophane, cystine and methionine. Results of an experiment using crystalline amino-acids dissolved in water, indicated however that recoveries of lysine, histidine, tyrosine and tryptophane are reduced in the presence of formaldehyde (Ohshima et al., 1979). This means some analytical interference with the degradation per se for those four amino-acids, and thus needed caution in interpretation.

Protein degradation is thus restricted to some extent by the application of formic acid and/or formaldehyde at the time of ensiling. Addition of formic acid enhances the effects of formaldehyde in improving the nutritive value (Valentine et al., 1973), probably through a restriction of the protein degradation during ensiling.

The objective of the present investigation was to measure the proteolysis and the fate of individual amino-acids during the ensiling of alfalfa (*medicago sativa*) treated with and without formic acid, formaldehyde as well as combinations of both additives.

EXPERIMENTAL

1. Preparation of silages

Alfalfa was harvested on 11 August 1980 by sunny weather, chopped shortly with a laboratory forage chopper and ensiled directly in micro-silos (1.5 l WECK). The herbage was ensiled without additive and with the following applications on the fresh material: 0.0, 0.2, 0.4, 0.6, 1.0% formic acid, 0.0, 0.2, 0.4, 0.6, 1.0% formaldehyde and the combinations of 0.0, 0.2, 0.4% formic acid with 0.0, 0.2, 0.4% formaldehyde. Four silos were prepared for each treatment except for the treatment without additive where eight silos were made. This means a total number of 56 silos.

The ensiling period was 90 to 110 days. Representative samples were taken for analysis on fresh and freeze dried material of each silage. A representative sample of the fresh chopped alfalfa was taken, freeze dried, ground and analysed (6X) for amino-acid content.

2. Analysis

Dry matter content was determined by oven-drying at 105°C for 48 hours. Total nitrogen content was determined on freeze dried samples by the KJELDAHL method and true protein according the STUTZER method. Measurements of pH and ammonia content were carried out on the filtrate of a 100 g silage sample macerated for 24 hours in 1000 ml distilled water. Amino-acids were determined on 0,5 g freeze dried samples. The concentration of all non-sulphur-containing amino-acids was determined using an automatic amino-acid

analyser (Unichrom Beckman) after hydrolysis with 6N-HCL in sealed tubes at 110°C for 22 hours (Stein and Moore, 1958). Cystine/cysteine and methionine concentrations were determined separately using a performic oxydation (Schram et al., 1954) before 6N-HCL hydrolysis and separation on the automatic amino-acid analyser.

3. Statistical methods

Silage data were analysed by analysis of variance of a 3X3 factorial design with cross classification (formic acid and formaldehyde each at 0.0, 0.2, 0.4%) and a 2X5 factorial design with hierarchical classification (formic acid and formaldehyde each at 0.0, 0.2, 0.4, 0.6, 1.0%). Treatment means were compared two by two using the t-test.

RESULTS AND DISCUSSION

The composition of herbage and silages is given in tables 1 and 3. The recoveries of amino-acids in silage to corresponding amino-acids in original herbage are given in tables 2 and 4.

The fermentation pattern in the formic acid silages was different from the formaldehyde silages (table 1). The mean pH for the different levels of formic acid was lower than corresponding mean pH of formaldehyde silages ($P < 0.01$). The ammonia-N (% total N) and the true protein content were both lower in the formic acid silages. This would suggest that the nitrogen degradation during ensiling presents two different aspects: the proteolysis and the ammoniogenesis which may be partially independent. The amino-acid composition before and during ensiling gives even a better picture of the nitrogen conservation during ensiling (Tables 1 and 2). The contents of total analysed amino-acids and essential amino-acids were lower in the formic acid silages than in the formaldehyde silages (53.57 g against 58.24 g total amino-acids (N.S.) and 25.22 g against 26.94 g essential amino-acids (N.S.) per 100 g crude protein).

Differences between individual amino-acid contents were however only significant for four amino-acids: glycine, valine, isoleucine and leucine.

Both additives improved appreciably the fermentation during ensiling: lower pH, lower ammonia-N percentage, higher true protein content, higher content of total and essential amino-acids compared to the untreated silage.

The percentage recoveries of individual, essential and total amino-acids in silages to corresponding amino-acids in original herbage was lowest in the untreated silage (except for valine, alanine, and alpha-aminobutyric acid) and rather similar for the formic acid and formaldehyde silages

(table 2). In agreement with the results of OHSHIMA et al. (1979), the content of aspartic acid and glutamic acid was very high in the fresh alfalfa and much lower in the silages.

Table 1 - Composition of herbage and silages.

	Original herbage	Untreated silage	Formic acid treat- ed sila- ges	Formalde- hyde treat- ed sila- ges	S.E. treat- ment means B and signifi- cance of dif- ferences be- tween treat- ments C	
	A (n=6)	A (n=8)	(n=20)	(n=20)		
Dry matter (%)	21.32 [±] 0.36	21.34 [±] 1.23	22.87	22.31	0.45	N.S.
pH	n.d.	5.81 [±] 0.24	4.85	5.74	0.19	xx
Ammonia-N (% total N)	n.d.	33.70 [±] 2.19	17.19	20.50	2.31	N.S.
On freeze dried sample:						
Crude protein (%)	16.98 [±] 0.47	17.89 [±] 0.29	17.31	17.32	0.18	N.S.
True protein (%)	n.d.	5.13 [±] 0.58	7.43	10.16	0.28	N.S.
Amino-acid: (g amino-acid per 100 g cru- de protein)						
Lys D	6.60 [±] 0.20	1.91 [±] 0.20	3.74	2.45	0.25	N.S.
His	3.09 [±] 0.16	0.62 [±] 0.07	1.06	0.73	0.08	N.S.
Arg	2.22 [±] 0.15	0.95 [±] 0.13	1.96	2.71	0.18	N.S.
Met	1.42 [±] 0.06	1.16 [±] 0.11	1.31	1.14	0.07	N.S.
Cys	2.27 [±] 0.09	0.74 [±] 0.06	1.12	1.12	0.13	N.S.
Asp	15.44 [±] 0.91	2.67 [±] 0.40	7.79	7.49	0.49	N.S.
Thr	4.12 [±] 0.26	1.18 [±] 0.18	2.17	2.76	0.14	N.S.
Ser	3.86 [±] 0.24	0.88 [±] 0.09	1.92	2.65	0.13	N.S.
Glu	12.18 [±] 0.30	2.71 [±] 0.25	4.98	6.13	0.23	N.S.
Pro	4.84 [±] 0.13	2.36 [±] 0.30	3.34	3.37	0.16	N.S.
Gly	5.61 [±] 0.15	3.54 [±] 0.37	3.30	4.17	0.14	xx
Ala	6.30 [±] 0.21	5.54 [±] 0.73	4.79	4.98	0.29	N.S.
Val	6.48 [±] 0.24	4.72 [±] 0.41	3.96	4.55	0.21	x
Iso	4.94 [±] 0.25	2.80 [±] 0.30	2.72	3.44	0.16	xx
Leu	8.63 [±] 0.35	5.31 [±] 1.88	4.89	5.69	0.26	x
Tyr	2.84 [±] 0.09	0.58 [±] 0.06	1.29	1.13	0.09	N.S.
Phe	5.48 [±] 0.28	3.65 [±] 0.31	3.52	3.46	0.29	N.S.
α-AB	0.00 [±] 0.00	0.85 [±] 0.14	0.36	0.27	0.05	N.S.
Essential AA	42.52 [±] 1.19	22.02 [±] 1.52	25.22	26.94	1.19	N.S.
Total AA	96.79 [±] 3.26	42.46 [±] 3.29	53.57	58.24	2.29	N.S.

- A Mean value of 6 and 8 replicates \pm standard error
- B S.E. of treatment means in the 2X5 factorial design with hierarchical classification (formic acid and formaldehyde each at 0.0, 0.2, 0.4, 0.6, 1.0%)
- C N.S. non significant; x $P \leq 0.05$; xx $P \leq 0.01$.
- D Lys, lysine; His, histidine; Arg, arginine; Met, methionine; Cys, cystine; Asp, aspartic acid; Thr, threonine; Ser, serine; Glu, glutamic acid; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Iso, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; α -AB, α -aminobutyric acid; Essential AA, essential amino-acids: lysine, histidine, arginine, methionine, threonine, valine, isoleucine, leucine, phenylalanine; Total AA, total amino-acids.

Table 2 - Percentage recoveries of amino-acids in untreated, formic acid and formaldehyde treated silages to corresponding amino-acids in original herbage (%).

	Original herbage (n=6)	Untreated silage (n=8)	Formic acid treated silages (n=20)	Formaldehyde treated silages (n=20)
Lys	100	28.94	56.67	37.12
His	100	20.06	34.30	23.62
Arg	100	42.79	88.28	122.07
Met	100	81.69	92.25	80.28
Cys	100	32.60	49.34	49.34
Asp	100	17.29	50.45	48.51
Thr	100	28.64	52.67	66.99
Ser	100	22.80	49.74	68.65
Glu	100	22.25	40.88	50.32
Pro	100	48.76	69.00	69.63
Gly	100	63.10	58.82	74.33
Ala	100	87.94	76.03	79.04
Val	100	73.64	61.11	70.21
Iso	100	56.68	55.06	69.63
Leu	100	61.46	56.61	65.87
Tyr	100	20.42	45.42	39.78
Phe	100	66.60	64.23	63.13
α -AB		100.00	42.35	31.76
Essential AA	100	51.79	59.31	63.36
Total AA	100	43.87	55.35	60.17

Table 3 - Composition of formic acid and formaldehyde treated silages

Level (% fresh wt) formic acid	0.0 ^A	0.2	0.4	0.0	0.2	0.4	0.2	0.2	0.4	0.2	0.2	0.4	0.4	0.4	S.E. of treatment means	Significance of effects
B																
pH	5.81 ^{bc}	5.03 ^{abc}	4.16 ^a	5.81 ^{bc}	6.21 ^c	5.59 ^{bc}	5.94 ^{bc}	4.94 ^{ab}	5.10 ^{abc}	4.72 ^{ab}	0.42	N.S.	xx	xx		
Ammonia-N (% total N)	33.71 ^{cd}	19.20 ^{bc}	10.10 ^{ab}	33.71 ^{cd}	39.16 ^d	15.21 ^{ab}	16.92 ^{ab}	7.42 ^{ab}	3.40 ^a	3.70 ^a	5.27	xx	xx	xx		N.S.
On freeze dried sample: Crude Protein (%)	17.89 ^{bc}	16.75 ^a	17.41 ^{ab}	17.89 ^{bc}	16.63 ^a	16.75 ^a	18.37 ^{bc}	18.92 ^c	18.14 ^{bc}	18.26 ^{bc}	0.40	N.S.	xx	xx		
True Protein (%)	5.14 ^a	7.08 ^b	7.62 ^{bc}	5.14 ^a	9.19 ^c	11.25 ^d	11.85 ^d	11.18 ^d	12.79 ^d	12.85 ^e	0.55	xx	xx	xx		N.S.
Amino-Acid (g amino-acid per 100 g crude protein)	1.91 ^a	2.42 ^{ab}	6.39 ^c	1.91 ^a	2.60 ^a	2.87 ^a	2.10 ^a	1.67 ^a	3.37 ^b	1.81 ^a	0.43	xx	xx	xx		xx
Lys	0.62 ^a	0.70 ^a	1.54 ^c	0.62 ^a	1.08 ^b	0.77 ^{ab}	0.54 ^a	0.30 ^a	0.23 ^d	0.18 ^d	0.10	xx	xx	xx		xx
His																xx

Table 3 (suite) - Composition of formic acid and formaldehyde treated silages.

Arg	0.95 ^a	1.25 ^{ab}	2.23	bc	0.95 ^a	2.03 ^{bc}	2.81 ^{cd}	2.18 ^{bc}	3.09 ^{cd}	3.53 ^d	2.36 ^e	0.36	xx	N.S.	x
Met	1.16 ^b	0.80 ^a	1.52	d	1.16 ^{bc}	1.18 ^{bc}	1.40 ^{cd}	1.11 ^b	1.18 ^{bc}	1.25 ^{bc}	1.17 ^{bc}	0.09	N.S.	xx	xx
Cys	0.74 ^a	0.70 ^a	1.37	bc	0.74 ^a	0.98 ^{ab}	1.09 ^{abc}	1.53 ^c	1.11 ^{abc}	0.86 ^{ab}	0.99 ^{ab}	0.19	N.S.	N.S.	x
Asp	2.67 ^a	7.60 ^{bc}	12.30 ^e	bc	2.67 ^a	5.01 ^{ab}	8.59 ^{cd}	10.7 ^{de}	10.71 ^{de}	9.07 ^{cd}	8.53 ^{cd}	1.06	N.S.	xx	xx
Thr	1.18 ^a	2.21 ^b	2.92	bc	1.18 ^a	2.22 ^b	3.40 ^{de}	3.71 ^e	3.28 ^{cde}	3.16 ^{cde}	2.66 ^{bc}	0.24	xx	xx	x
Ser	0.88 ^a	1.88 ^b	2.58	bcd	0.88 ^a	2.22 ^b	3.40 ^e	3.40 ^e	3.17 ^{de}	2.86 ^{cde}	2.51 ^{cd}	0.28	xx	xx	xx
Glu	2.71 ^a	4.01 ^a	6.29	bc	2.71 ^a	5.48 ^b	6.74 ^{bc}	8.29 ^d	8.17 ^d	7.12 ^{cd}	6.65 ^{bc}	0.48	xx	xx	xx
Pro	2.36 ^a	3.64 ^{bc}	4.43	c	2.36 ^a	2.87 ^{ab}	3.52 ^{bc}	4.23 ^c	3.76 ^{bc}	3.63 ^{bc}	3.60 ^{bc}	0.33	N.S.	xx	xx
Gly	3.54 ^a	3.40 ^a	3.75	ab	3.54 ^a	3.69 ^{ab}	4.26 ^{ab}	4.76 ^b	4.13 ^{ab}	3.78 ^{ab}	3.31 ^a	0.31	N.S.	N.S.	N.S.
Ala	5.54 ^{bc}	5.89 ^c	4.88	abc	5.54 ^{bc}	4.17 ^{ab}	5.13 ^{abc}	5.45 ^{bc}	4.70 ^{abc}	4.23 ^{ab}	3.61 ^a	0.16	N.S.	N.S.	N.S.
Val	4.72 ^{ab}	3.74 ^a	4.21	a	4.72 ^{ab}	4.33 ^{ab}	4.38 ^{ab}	5.52 ^b	4.42 ^{ab}	4.00 ^a	3.70 ^a	0.10	x	N.S.	N.S.
Iso	2.80 ^{ab}	2.50 ^a	2.80	ab	2.80 ^{ab}	3.01 ^{abc}	3.83 ^{cd}	4.46 ^d	3.68 ^{bcd}	3.00 ^{abc}	2.85 ^{ab}	0.05	xx	N.S.	x
Leu	5.31 ^{ab}	4.54 ^a	5.16	ab	5.31 ^{ab}	5.22 ^{ab}	5.81 ^{abc}	7.14 ^c	6.24 ^{bc}	5.60 ^{ab}	5.13 ^{ab}	0.47	x	N.S.	N.S.
Tyr	0.58 ^a	0.86 ^{ab}	1.59	c	0.58 ^a	1.51 ^{bc}	1.77 ^{cd}	1.68 ^{cd}	2.18 ^d	1.25 ^{bc}	1.47 ^c	0.19	xx	xx	x
Phe	3.65 ^{ab}	3.08 ^a	3.54	ab	3.65 ^{ab}	3.84 ^{ab}	3.39 ^{ab}	4.56 ^b	3.30 ^{ab}	3.28 ^{ab}	2.99 ^a	0.48	N.S.	N.S.	N.S.
cAB	0.85 ^c	0.41 ^b	0.14	a	0.85 ^c	0.27 ^{ab}	0.15 ^a	0.01 ^a	0.05 ^a	0.00 ^a	0.00 ^a	0.05	xx	xx	x
Essential															
AA	22.18 ^a	21.34 ^a	30.30 ^c		22.18 ^a	25.5 ^{abc}	28.6 ^{bc}	31.33 ^c	28.85 ^c	25.59 ^{bc}	22.5 ^{ab}	2.14	N.S.	N.S.	xx
Total AA	41.98 ^a	47.22 ^{ab}	67.64 ^{ef}		41.98 ^a	51.5 ^{bc}	63.0 ^{def}	71.49 ^f	66.83 ^{def}	58.3 ^{bcde}	54.6 ^{abcd}	4.42	x	x	xx

A Mean value of 4 replicates (3X3 factorial design with cross classification)

B Values on the same line with different superscripts differ significantly ($P \leq 0.05$)

Table 4 - Percentage recoveries of amino-acids in untreated, formic acid and formaldehyde treated silages to corresponding amino-acids in original herbage (%)

Amino-Acids	Original herbage	Treatment level (% fresh wt)											
		formaldehyde	formic acid	0.0	0.2	0.4	0.2	0.4	0.2	0.4			
Lys	100	28.94	36.67	96.82	28.94	39.39	43.48	31.82	25.30	51.60	27.42	0.4	0.4
His	100	20.06	22.65	49.84	20.06	34.95	24.92	17.48	9.71	7.44	5.83		
Arg	100	42.79	56.31	100.45	42.79	91.44	126.58	98.20	139.19	159.01	106.31		
Met	100	81.69	56.34	107.04	81.69	83.10	98.59	78.17	83.10	88.03	82.39		
Cys	100	32.60	30.84	60.35	32.60	43.17	48.02	67.40	48.90	37.89	43.61		
Asp	100	17.29	49.22	79.66	17.29	32.45	55.63	69.75	69.36	58.74	55.25		
Thr	100	28.64	53.64	70.87	28.64	53.88	82.52	90.05	79.61	76.70	64.56		
Ser	100	22.80	48.70	66.84	22.80	57.51	88.08	88.08	82.12	74.09	65.03		
Glu	100	22.25	32.92	51.64	22.25	44.99	55.34	68.06	67.08	58.46	54.60		
Pro	100	48.76	75.21	91.53	48.76	59.30	72.73	87.40	77.69	75.00	74.38		
Gly	100	63.10	60.61	66.84	63.10	65.78	75.94	84.85	73.62	67.38	59.00		
Ala	100	87.94	93.49	77.46	87.94	66.19	81.43	86.51	74.60	67.14	57.30		
Val	100	72.84	57.72	64.97	72.84	66.82	67.59	85.19	68.21	61.73	57.10		
Iso	100	56.68	50.61	56.68	56.68	60.93	77.53	90.28	74.49	60.73	57.69		
Leu	100	61.53	52.61	59.79	61.53	60.49	67.32	82.73	72.31	64.89	59.44		
Tyr	100	20.42	30.28	55.99	20.42	53.17	62.32	59.15	76.76	44.01	51.76		
Phe	100	66.61	56.20	64.60	66.61	70.07	61.86	83.21	60.22	59.85	54.56		
α -AB	-	100.00	48.24	16.47	100.00	31.76	17.65	1.18	5.88	0.00	0.00		
Essential A.A.	100	52.16	50.19	71.26	52.16	60.00	67.33	73.68	67.85	60.18	52.94		
Total AA	100	43.37	48.79	69.88	43.37	53.26	65.18	73.86	69.05	60.25	56.47		

According to the level and nature of the additive, the fermentation pattern during ensiling was very different (table 3). The lowest pH was achieved in the 0.4% formic acid silage. The ammonia-N percentage was very variable and lowest in the silages with 0.4% formaldehyde + 0.2 or 0.4% formic acid (3.40 and 3.70%). These silages presented also the highest content of true protein (12.79 and 12.85%), but not the highest contents and recoveries of total and essential amino-acids. In the 0.4% formic acid silage, a higher percentage of ammonia-N (10.10%) and a significantly lower content of true protein (7.62%) were obtained. The recoveries of total and essential amino-acids were however the highest. This shows clearly distinct aspects in the nitrogen degradation during ensiling: proteolysis, amino-acid breakdown and ammoniogenesis, and thus the interest of an amino-acid characterizing.

The best contents and recoveries of essential and total amino-acids were obtained by two treatments: 0.2% formaldehyde + 0.2% formic acid and 0.4% formic acid (tables 3 and 4). The lowest recoveries occurred in the control silage where they were less than 50% for lysine, histidine, arginine, cystine, aspartic acid, threonine, serine, glutamic acid, proline, tyrosine. Recoveries of alanine and methionine were high (87.94% and 81.69%) and those of valine, glycine, phenylalanine, leucine and isoleucine intermediate. Percentage recoveries of individual amino-acids were all higher than 50% in the 0.4% formic acid silage as well as in the 0.2% formaldehyde + 0.2% formic acid silage, except for lysine and histidine in the later one (31.82% and 17.48%). Barry et al. (1978) and Ohshima et al. (1979) concluded that high losses of lysine, histidine and tyrosine arose through problems of estimation in formaldehyde-treated silages following HCl hydrolysis. This also, applies to our result and explain the lower recovery of lysine and histidine. The contents of lysine, histidine, methionine were significantly higher in the 0.4% formic acid silage compared to the 0.2% formaldehyde + 0.2% formic acid silage, those of arginine, cystine, aspartic acid, proline, glycine, alanine, tyrosine, phenylalanine, α -aminobutyric acid were similar and the recoveries of threonine, serine, glutamic acid, valine, isoleucine, leucine were significantly lower.

The net synthesis of alpha-aminobutyric acid during ensiling was highest in the control silage (0.85g/100g crude protein), intermediate in the 0.2% formic acid silage (0.41g/100g crude protein) and lowest in the other silages (0.00 to 0.27g/100g crude protein).

It appears that the contents of amino-acids in silage are giving a more precise measurement of the nitrogen degradation during ensiling than the con-

tent of ammonia which may even be sometimes misleading.

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PAPER 2

AUTHOR: A. Deswysen (Belgium)

DISCUSSION

B.C. Cottyn (Belgium)

Was digestibility measured?

A. Deswysen

No.

D. Beever (UK)

What are the results related to? Ammonia values are high for material treated with formaldehyde.

A. Deswysen

Results were related to the original crop.

R. Wilkins (UK)

Work at Hurley has shown low recoveries of amino acids in silages treated with formaldehyde?

A. Deswysen

Losses can also occur during analysis.

P.C. Thomas (UK)

The ammonia values are so high as to render the material atypical.

R. Wilkins

In sheep fed (ad libitum) a range of lucerne silages treated with formic acid and formaldehyde, nitrogen retention was negatively correlated with ammonia N (-0.86) amino butyric acid 0.89 and alanine (-0.90).



ASPECTS OF GREEN CROP FRACTIONATION IN RELATION TO
THE UTILIZATION OF GRASS

G.W. Wieringa

Centre for Agrobiological Research
Wageningen, The Netherlands

INTRODUCTION

Fractionation of herbage can be defined as a proces of rupturing and compressing of green plants yielding a protein-enriched juice fraction and a fibre-enriched pressed residue. In this way food grade protein could be obtained from crops which - as such - can be used by ruminants only. Due to the increasing demand for protein-rich food, a great deal of research on the fractionation of green crops has been carried out in many countries in the western as well as in the developing countries. The pioneer work of Pirie (1971) is worth mentioning here.

In the earlier investigations only little attention was paid to the utilization of the fibre-enriched pressed residue and almost all efforts were focused on the protein fraction to be used as human food. Later on other aspects were also taken into account: industrial fractionation as a means to save energy in green crop drying plants and the production of dried leaf protein concentrate (LPC) as a source of protein and xantophyl for poultry and broilers came to development in the USA, Hungary, France and England. On farm fractionation systems, the direct use of juice in pig rations and feeding of the fresh or dried residue to beef cattle were investigated mainly in Ireland, Scotland and England.

Most of the recent developments can be found in the proceedings of two symposia (Wilkins, 1977; Howarth, 1978).

The economic feasibility of an industrial scale fractionation was studied by Vosloh et al. (1976), Wilkins et al. (1977) and Connell et al. (1978). According to Monnier (1980) factories in Hungary, Denmark, France, Spain and England are producing LPC on a commercial scale. Although some of these closed down, the others are still in production. The developments on the world food- and fuel market will greatly influence the future of industrial LPC production.

From agricultural point of view there are other factors of importance in relation to the feasibility of green crop fractionation. Modern developments in grassland farming and animal husbandry inevitably lead to the production of highly digestible grass, which we need, but with a protein content far in excess of animal requirements. The industrial fractionation proces cannot serve to improve herbage protein utilization by grazing ruminants. For this reason on-farm extraction systems are worth being studied. On-farm fractionation was investigated in Ireland and Scotland (Maguire and Jones in Wilkins, 1977). In this paper a brief survey is given of the research on grass fractionation and utilization in the Netherlands from 1975-1981.

GRASS PRODUCTION AND QUALITY

In the Netherlands the average application of nitrogen fertilizer on grassland increased to about 250 kg N per ha. On the intensive dairy farms amounts of 400 kg N and even higher are used. Data of Van Steenberg (1977) and Wieringa et al. (1980) show that under farm conditions at this fertilizer level the DM yield response is small as compared to the protein yield response (table 1).

Table 1. Effect of N application on herbage yield and quality.

Nitrogen kg/ha	Yield in t/ha		Quality	
	DM	CP	ME MJ/kg DM	CP g/kg DM
0	8.8	1.39	10.7	158
100	10.4	1.69	10.8	163
218	11.4	2.07	10.9	180
323	12.6	2.39	11.0	190
411	12.8	2.63	11.1	206
518	13.1	2.88	11.2	220

DM = dry matter, CP = crude protein, ME = metabolizable energy.

In feeding high yielding dairy cows a high intake and a high metabolizable energy (ME) content is profitable. A crude protein (CP) content of 160 g/kg DM, however, is sufficient to meet the requirements of these animals. For maximizing grass production, nitrogen applications of up to 400 kg/ha are recommended. From table 1 it may be concluded that even at lower N rates the protein content of grass is above this level of 160 g/kg. Kemp et al. (1979) calculated a luxury consumption of 1.0 - 1.5 kg CP per cow and per grazing day. The average annual grass production in the Netherlands can be estimated at appr. 11-12 t DM/ha containing 2100-2200 kg protein. Considering the energy requirements of the dairy cow, 600-700 kg protein of the total protein yield could be saved and used in a more efficient way.

FACTORS AFFECTING THE FRACTIONATION

Apart from the influence of the fractionation machine itself, plant species, structure of the plant material, moisture content, and chemical composition play important roles in the separation of herbage into fractions. In general, leafy plants like lucerne are easier to crush than grass. This is the main reason that extraction ratios of lucerne are always higher than those of grass. On the other hand cell rupturing is not the only factor and has to be followed by a separation of the juice from the cell wall material. Lack of structure and a high viscosity of the juice may lead to a poor juice yield, like sometimes found with young grass in early spring. Fractionation experiments, carried out with grass grown under a wide range of fertilization

and cutting treatments showed that the moisture content is the main factor determining the DM extraction ratio (Wieringa, 1978) (table 2).

Table 2. Influence of moisture content on the extraction ratio's.

Grass moisture g/kg	Extraction ratio (E)*		
	E_{moisture}	E_{DM}	E_{N}
900	0.67	0.27	0.45
850	0.58	0.22	0.35
800	0.49	0.17	0.25
750	0.40	0.12	0.15

$$E_{\text{moisture}} = \frac{\text{juice moisture weight}}{\text{crop moisture weight}} \quad E_{\text{DM}} = \frac{\text{juice DM weight}}{\text{crop DM weight}}$$

$$E_{\text{N}} = \frac{\text{juice nitrogen weight}}{\text{crop nitrogen weight}}$$

The extractability of crop components is wholly dependent on their solubility in water. Thus, the extraction ratio's of sugars, water-soluble ash, free amino acids etc. are almost equal to the E_{moisture} , whereas the crude-fibre is not extractable at all ($E_{\text{CF}} = 0$). From this it will be clear that the composition of the juice DM depends on grass DM content as well as on the grass DM composition: e.g. the CP of the juice DM can be calculated by multiplication the CP content of the grass with a factor $E_{\text{CP}}/E_{\text{DM}}$. In this way can be calculated that the protein content of grass juice will be 1.25-1.65 times higher than the protein content of the grass from which it was produced. Water soluble compounds (sugar, ash, nitrate) are being concentrated in the grass juice by a factor 2 or even more. In a similar way the chemical composition of the pressed residue can be calculated. Dependent on grass DM content the crude fibre content will increase by a factor 1.1-1.3, the water soluble compounds will be lowered by a factor 0.5-0.75, and the protein content by a factor 0.75-0.97 (average 0.875). It is worth paying some attention to the latter value. At an average DM content of grass of 175 g/kg appr. 30 % of the total grass-protein yield will appear in the juice fraction. This is in good accordance to the above mentioned protein surplus of 600-700 kg per ha. However, because also non protein DM is extracted the protein content of the pressed residue is only lowered slightly (not by a factor 0.7 but only by $0.7/0.8 = 0.875$). From this it will be clear that it

is practically impossible to bring down grass protein contents of 200 g/kg DM and above to the desired value of 160 in the pressed crop.

THE UTILIZATION OF THE PRESSED HERBAGE

Although the pressed residue has proven to be a valuable roughage for growing cattle its value as a feed for dairy cows long remained questionable (Connel and Houseman, 1977; Jones, 1981). For this reason we carried out a number of feeding experiments with the aim to assess the influence of fractionation on feed intake, digestibility, mastication behaviour, rumen function and milk production. In four change-over experiments with 2 groups of 3 animals the ad lib intake of pressed fresh grass appeared to be higher than that of original grass. During a six week period in May-June the average difference amounted to 2.4 kg DM per animal per day in favour of the animals receiving pressed grass. After the change over the pressed grass DM intake was 1.1 kg per animal per day higher than the intake of grass DM. Determination of the in vitro digestibility of grass and of the pressed grass produced from it, showed a difference of organic matter digestibility of only 2%.

The main reasons for the high DM intake of the cows eating pressed grass are the lower water content and the structure of the treated grass. It was found that the difference in DM intake increased with increasing moisture content of the fresh grass. Apparently a grass moisture content above appr. 860 g/kg is becoming the limiting factor. The mechanical treatment of the pressed grass enabled the animals to eat faster and with less labour (table 3).

Table 3. Eating time and mastication

	pressed grass	grass
eating time (min/kg DM)	18	24
rumination (min/kg DM)	33	33
jaw movements/min:		
eating	58	73
ruminating	49	49

The greatest differences in mastication behaviour occur during eating, when less movements are needed for a considerably greater DM intake of pressed grass. The difference in mastication during rumination seems to be negligible. It was observed however, that although the number of movements was about the same in both groups, the movements on the pressed grass were less vigorous. It is of interest to compare eating behaviour and mastication of animals receiving fresh grass with those eating silage. Eating time of ensiled pressed grass in min per kg DM was only slightly higher than that of fresh pressed grass. Ensiled wilted grass, however, appeared to be consumed slightly faster than fresh pressed grass.

The mechanical treatment of the pressed grass did not exert any negative influence as to rumen functioning. No significant differences in course of rumen pH, volatile fatty acids, and fermentation pattern were observed. Even winterrations containing 10 kg of concentrates with appr. 9 kg of ensiled pressed grass as the sole roughage were acceptable to milking cows. In the experiments described in which DM intake of pressed forage was higher, the milk production was also higher, according to the expectation.

Using the results of these feeding experiments, calculations were made on the possible efficiency of the utilization of energy and of protein by dairy cows at different production levels (Wieringa et al., 1980). Zero-grazing of pressed grass in comparison to grazing or zero grazing of whole grass will lead to a better balanced ration, which means a ration with a smaller protein surplus. To prevent all luxury consumption of protein a limited feeding of pressed grass with the supplementation of an extra source of energy will be necessary. It is worth noting that in the latter case, a comparable amount of energy appears in the juice fraction. The juice fraction however, also contains protein up to appr. 350 g/kg DM.

PROSPECTS OF GREEN CROP FRACTIONATION

From agricultural point of view the potential production of protein by grassland is unsurpassed under our climatic conditions. However, the protein/energy ratio of grass is much higher than necessary for growth and milk production. The protein consumed in excess of requirements is being used as an energy source, the nitrogen part of it being rejected and excreted as urine.

Technically, it is possible to fractionate the herbage, to use the protein fraction as a feed for non-ruminants and to feed the pressed residue to dairy cows. In this way the total grass yield can be used more efficiently in terms of protein utilization and of energy utilization.

The realization of the better efficiency can only be achieved when over-consumption of protein can be prevented. Apart from mixed feeding systems with additional source of energy, in pure grassland husbandry systems this can be achieved by an on-farm fractionation system. The pressed grass can be fed directly or after a short period of ensilation. The extra labour and external energy needed for such a system may be compensated for by the extra yield gain obtained by preventing losses due to poaching, urine-scorching, regrowth retardation and the above mentioned luxury consumption.

The on farm utilization of the pressed residue asks for the development of an efficient farm scale fractionation machinery like the mobile system developed in New Zealand (Mills, 1980). The rather low capacity of this machine is a drawback and may lead to demand for a stationary equipment.

The utilization of the juice is not without problems. In our laboratory only a few experiments were carried out. Coagulation by heat or acidification and separation of the curd from the deproteinized juice (DPJ) is technically

feasible. It forms an attractive method to get rid of the major part of the ash, but the DPJ contains also up to 10 % of the total crop organic matter. From economic point of view such a loss is inadmissible. As far as known now, the best method for the preservation and utilization of the perishable juice still has to be developed. The economic feasibility of the process greatly depends on a good market for the protein concentrate.

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PAPER 3

AUTHOR: G. Wieringa (The Netherlands)

DISCUSSION

A.G. Prendergast (EEC Commission)

Small numbers of animals were used - are the results reliable?

G. Wieringa

Changeover designs were used.

R.J. Wilkins (UK)

What extraction ratios were used?

G. Wieringa

These were variable between 10 - 30% on average less than 20% depending on DM of the crop.

D. Beever (UK)

Do you have any data at equalised feed intake?

G. Wieringa

None. Digestibility was lower in the pressed pulp.

D.L. Mangan (UK)

What was the nature of the protein in the fibrous residues?

G. Wieringa

There were no large differences in the amino acid fraction between the pressed pulp and the fresh crop.

R. Carlsson (Sweden)

There are no differences between the extraction protein and the original herbage protein.

THE UTILISATION OF PROTEIN EXTRACTS OBTAINED
BY FRACTIONATION OF GRASS CROPS

M.F. Maguire, P. Finn and O. Carton

The Agricultural Institute, Dunsinea,
Castleknock, Co. Dublin, Ireland

ABSTRACT

The potential contribution of crop fractionation towards increasing the efficiency of utilisation of forage crop protein is briefly reviewed. By modifying existing systems of forage utilisation animal output could be increased and a protein-rich juice extract made available for more effective use. In two experiments grass juice extracted from an Autumn crop of *Lolium perenne*, cultivar Barvestra, was, however, poorly utilised after storage with chemical preservatives, metabisulphite with hydrochloric acid or formaldehyde with formic acid. It had a determined metabolisable energy (ME) value for pigs of only 8 MJ kg⁻¹ DM. This may have been due to the formation of indigestible reaction products between juice nutrients and the preservatives.

A wet leaf protein concentrate prepared from the same sward in the following year had 48% crude protein in the dry matter and a determined ME of 12.5 MJ kg⁻¹ DM. After storage for 220 days frozen or treated with 3% propionic acid at ambient temperature the ME values were 11.6 and 11.5 MJ kg⁻¹ respectively.

The application of crop fractionation technology to improve forage utilisation and silage making will necessitate the development of low cost systems for storing the products without loss of nutritive value.

INTRODUCTION

Research on the extraction and utilisation of protein from leafy crops has been advocated at various times since the early part of this century (Ereky, 1927; Slade, 1937; Pirie, 1942). From the late 1950s work has been carried out intermittently in a growing number of countries with a wide variety of nutritional and economic objectives in mind. These have ranged from attempting to combat malnutrition by making more protein available for direct human consumption in some of the developing countries to improving the efficiency of grassland utilisation and animal production systems elsewhere (Raymond and Tilley, 1956; Singh, 1964; Bruhn and Koegel, 1977). Greenhalgh (1976) has drawn attention to the economic and moral dilemma facing those countries seeking to increase their production of animal protein foods when the available feed ingredients are also those which could be much more effectively utilised directly by man to combat existing malnutrition. With the continuing growth in World population and escalating energy costs it is

generally agreed that increases in animal production should be based on greater use of the foods not directly consumable by man or simple stomached species. Feed competition between man and these species could be reduced by the use of crop fractionation technology to obtain protein and energy from forage crops which cannot be used directly due to their high fibre levels. This is the basis of much of the current interest in leaf protein for both human and animal nutrition in Third World countries.

Increasing feed efficiency

The yield of edible protein in animal production systems, expressed as a percentage of that consumed in the feed, varies widely for different species and with the products obtained. It is generally less than 5% in beef production and over 20% in milk. From an assessment of the yields and production achieved when fractionation procedures were applied to grass crops Houseman and Jones (1978) calculated that the yield of meat per hectare could be more than doubled by extracting part of the nutrients in a grass beef production system for use as a liquid feed by pigs. Ostrowski (1979) has claimed a five fold increase in efficiency of protein utilisation from fractionation applied to a milk production system under Australian conditions. Donnelly et al., (1980) in New Zealand estimate that for a given area of lucerne the returns over and above increased costs for a combined protein extraction and beef production system would be four times greater than from beef alone, the leaf protein being exported to the Japanese market. Exploitation of these projections has not, however, been possible because of the absence of suitable farm-scale processing equipment. Development work on a mobile crop harvester and juice extraction unit has been carried out in New Zealand at Ruakura (Mills, 1980). This has a processing capacity of five tonnes of lucerne per hour. A group at the University of Wisconsin has developed an efficient crop pulping unit capable of processing 20 tonnes alfalfa per hour (Straub et al., 1978) and designed a matching juice extractor (Pitt et al., 1982).

Commercial applications of crop fractionation technology to-date have primarily been in association with the green crop drying industries in Hungary, the USA, Denmark and France. Prior juice extraction reduces the cost of the subsequent high temperature drying stage. The alfalfa juice extract is used to produce a highly pigmented but expensive dried leaf protein concentrate

(LPC) (Giroud and Vinconneau, 1978). In a Spanish process the extracted alfalfa crop is ensiled rather than dried and sold to farmers as a high dry matter silage (Rivadulla, 1978). This reduces the fuel requirements to less than 20% of that required for conventional dehydration systems. Four plants reported to have been established in recent years have a capacity of 200,000 tonnes alfalfa/annum. Elsewhere the crop drying industry is declining because of increasing fuel costs and lack of capital to adopt fuel saving strategies.

JUICE UTILISATION

The direct utilisation of juice extracts from grass and lucerne crops without prior separation of protein has obvious advantages in terms of capital and production costs as compared with dried LPC production. Juice could conceivably be used in existing liquid feeding systems for pigs or calves provided a regular supply or satisfactory storage can be devised. Small scale juice feeding trials have indicated the feasibility of replacing part of the soya and fishmeal in pig rations (Maguire, 1971; Barber et al., 1974). Simple juice storage procedures for example, acidification to pH 3-4 and addition of metabisulphite, inhibits microbiological deterioration for several weeks but does not prevent gradual loss of true protein unless juice is first heated to 85°C (Cheeseman, 1977). Unheated metabisulphite-treated juice has been found to become unpalatable during storage (Carton, 1981). This may be due to partial protein hydrolysis, some protease hydrolysates being known to contain bitter tasting peptides. Too high an intake of potassium by pigs fed preserved lucerne juice may have been responsible for poor performance in trials reported from the NIRD at Reading (Barber et al., 1981).

Experimental results

In evaluating the potential feeding value of whole juice it is important to know its energy contribution to the diet. Juice was prepared from a late Autumn crop of *Lolium perenne*, cultivar Barvestra using the pulping and belt press equipment described by Pirie (1971). The juice extract with 5.8% dry matter was concentrated on an APV falling film evaporator to a DM of 11.7% at below 50°C to facilitate its inclusion at a high rate (30%) in a barley soya basal ration. It was acidified by addition of HCl to pH 4.0 and preserved by treating with sodium metabisulphite 3 g l⁻¹ and stored at 0°C. The analysis of the juice is shown in table 1.

Table 1: Analysis of preserved grass juice

	%
Dry matter	11.7
Ash	2.6
Organic matter	9.1
C. Protein	4.2
Gross energy (MJ kg ⁻¹ DM)	15.9

Digestible and metabolisable energy (DE, ME) values were determined for five pigs in individual metabolism crates. Following ten day feed adaptation periods total collections of excreta were made over five days. The percentage digestibility of dry matter and organic matter and DE and ME values for the preserved grass juice are shown in Table 2.

Table 2: The percentage digestibility of dry matter and organic matter and DE and ME values for preserved grass juice

	Mean ± SD	
Dry matter digestibility %	69.6	4.2
Organic matter digestibility %	68.1	5.1
DE (MJ kg ⁻¹ DM)	9.8	0.9
ME "	8.0	0.8

The ME value for the grass juice, 8 MJ kg⁻¹ DM, was lower than had been expected. The results of earlier feeding trials had indicated an energy value comparable with that of barley-fishmeal-soya diets (Maguire, 1971; Barber *et al.*, 1974). This suggests that there may have been a significant fall in energy value under the storage conditions employed. There appear to be no readily available published values for the ME of grass juice. Patterson and Walker, (1979) cite a value of 15.3 MJ kg⁻¹ DM given in a personal communication by Houseman (1978), this is believed to refer to fresh juice. In a study of silage effluents fed to pigs to provide 15% of the dry matter intake Patterson and walker obtained values of 11.4 and 14.6 MJ kg⁻¹ DM for two different effluents. Storage of the latter effluent which had been treated with formic acid and formaldehyde for 300 days gave an ME value of 14.2 MJ kg⁻¹ DM. It is difficult to account for the apparent difference in the ME values of stored grass juice and silage effluent. Both have

similar ash contents. Although the acid and metabisulphite treatment of grass juice effectively inhibited bacteria and mould growth it would not prevent further possible proteolysis of proteins, Maillard-type reactions or reactions of autoxidized lipids with proteins. Such reaction products if present in a silage are unlikely to appear in significant quantities in the silage effluent, being generally insoluble. On the other hand the multiplicity of reaction products known to result from the interactions of juice constituents with sulphur dioxide may have reduced the overall digestibility of the juice organic matter (McWeeney *et al.*, 1974).

In a subsequent pig production trial juice extracted from grass harvested in September and acidified to pH 4.0 with formic acid was preserved with formalin 3.5 ml l⁻¹. It was used to replace 25% of the organic matter intake from a barley soya ration fed to a control group. The juice and control group pigs were individually fed to a scale providing the same total organic matter intake. Because of poor palatability of juice the trial was terminated after 9 weeks. The average daily live weight gains and feed conversion rates of the juice fed pigs was significantly lower than that of the control group. From a comparison of the weight gains in each group and assuming the same efficiencies for ME utilization and carcass composition gain Carton (1981) computed that the weight gain of the juice fed pigs was consistent with the energy value determined for grass juice in the previous digestibility trial. Both batches of juice were from grass harvested in the Autumn but in different years. The same ME values may not, therefore, necessarily apply to crops processed at other times of the year.

WET PROTEIN CONCENTRATE

The separation of the protein components of juice by natural fermentation (Stahmann, 1979), heat coagulation or acid precipitation is an attractive alternative to the storage of whole juice but entails the disposal of the residual juice dry matter. This amounts to 5-10% of the total crop dry matter processed, a similar figure to that reported for the dry matter loss in silage effluent (Watson and Nash, 1960). It can however be used as a substrate for biogas or since cell protein production or disposed of as a fertilizer. Its separation may be desirable as Hanczakowski (1979) has reported that it decreased the biological value and true digestibility of protein.

The preparation of dried leaf protein concentrates (LPC) has to be carefully controlled otherwise inferior products are obtained because of interaction of juice constituents or heat damage during processing. Many products tested in the past have been badly prepared (Morris, 1977). Heat coagulation has been shown to give lower yields of dried LPC as compared with membrane filtration, the latter products having a higher content of essential amino acids and a better protein efficiency ratio (Ostrowski-Meissner, 1980).

Little attention appears to have been given to the direct use of undried LPC. The savings in the cost of fuel and processing equipment appear attractive provided satisfactory long term storage of the wet LPC product proves to be possible. Foot (1975) combined a steam coagulated wet protein precipitate with dried milled barley and proprionic acid (0.7%) to make a pelleted feed which was stored for two months before feeding to pigs. Protein precipitation by acid addition eliminates the possibility of heat damage by steam coagulation and may improve amino acid availability in the product. Recovery of acid precipitated protein is facilitated by the use of flocculating agents and forms the basis of the Italian polyprotein process (Anelli *et al.*, 1977). Inhibition of microbial deterioration of wet LPC by acid treatments has been demonstrated by Subbarao *et al.*, (1967) and Arkcoll (1973).

Experimental results

A wet leaf protein concentrate was prepared to determine its metabolisable energy value for pigs and to investigate the effects of prolonged storage. Juice extracted from a perennial ryegrass sward harvested in September was acidified with formic acid to pH 4.0. The protein was recovered on a Sharples super centrifuge as a wet cake with a dry matter of 21-27%. Its composition is shown in table 3.

Table 3: Percentage composition of wet leaf protein concentrate

	% (dry matter basis)
Crude protein	48
Total lipid	29
N.D. fibre	8
Ash	7
W.S. carbohydrate	3

It was stored at -20°C for 70 days prior to evaluation in a digestibility trial with six castrated littermate pigs. A basal barley ration supplemented with minerals and vitamins was used to which thawed LPC or soyabean meal was added so as to provide 20% of the total dry matter intake. The results for DE, ME and nitrogen digestibility are shown in table 4.

Table 4: Energy values and nitrogen digestibility for soyabean and wet LPC from grass

	SOYA	WET LPC	
Treatment	-	Frozen	S.E. and significance
Age (days)	-	70	
DE (MJ kg^{-1} DM)	14.5	13.7	0.4 NS
ME "	13.4	12.5	0.4 **
N-digestibility %	84.5	79.7	0.5 ***
Net protein value % (NPV)	60.3	52.3	1.9 NS
Biological value % (BV)	64.0	63.0	1.8 NS

The DE and ME values for LPC were only slightly lower than those found for the soyabean and identical with the values reported for rapeseed meal (May and Bell, 1971). However nitrogen digestibility was significantly higher for soyabean as was the NPV, the retained nitrogen as a percentage of that ingested. The biological values (BV) indicate comparable efficiencies of utilisation of the absorbed nitrogen. Separate aliquots of the thawed LPC refrozen, or treated with 3% proprionic acid (W/W) and stored at ambient temperature, were re-evaluated after a further storage period of 150 days. The results were similar 11.6 and 11.5 MJ kg^{-1} DM for the frozen and proprionic acid treated LPC respectively. This suggests that storage of wet LPC at ambient temperature without major loss of nutritive value may be possible. Further work using the principles of intermediate moisture food technology provides scope for optimizing the storage conditions. Some published values for the metabolisable energy content of LPC preparations are given in table 5.

TABLE 5: Reported ME values for LPC preparations (MJ kg⁻¹DM)

LPC SOURCE	ME	SPECIES	REFERENCE
UK Lucerne dried	11.4	Layers	Morris (1977)
France " "	10.9	"	Gastineau (1975)
Hungary (Vepex) Lucerne dried	6.9	Broilers	Fras & Hanczakowski (1979)
Poland " " "	6.9	"	"
Ireland grass wet	12.5	Pigs	Current work
Ireland grass stored	11.5	"	" "

FARM FRACTIONATION

The introduction of a crop fractionation step into farm silage making operations could greatly reduce the wastage of nutrients due to poor preservation and eliminate pollution caused by the effluent loss from low dry matter silages. Although silage now exceeds hay as the source of winter feed in Ireland it is still a very variable commodity with an average dry matter digestibility of only 66%. Earlier and more frequent harvesting would increase digestibility but is likely to result in even greater effluent loss. Juice extraction prior to ensilage would guarantee a well preserved silage of high digestibility, eliminate silage effluent losses and make available a potential substitute for imported protein concentrates. In the 10-year period 1971-81 manufacture of compounded animal feedingstuffs in Ireland increased by 100% and imports of protein concentrates by 140% to a value in excess of £60 million/annum. A very large part of this could be met by making better use of existing grass crop protein. The removal of readily extractable protein prior to ensilage would reduce degradation losses in the silage pit and permit the processing of the extracted protein to any required degree of rumen degradability before feeding (Lu et al., 1981). There are also indications that fractionation improves the nutritive value of the crop residue (Houseman and Jones, 1978). Higher efficiencies of feed utilisation may result from particle size reduction as has been previously reported for dehydrated pelleted forages (Thomson and Cammell, 1979; Beever et al., 1981).

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PAPER 4

AUTHOR: M.F. Maguire (Ireland)

DISCUSSION

B.C. Cottyn (Belgium)

In the digestibility trials what was the method of substitution used?

M.F. Maguire

25% of the organic matter of the barley - soya diet was replaced by an equivalent amount of OM as grass juice.

A.G. Prendergast (EEC Commission)

What is the cost of leaf protein concentrate compared with soyabean?

M.F. Maguire

It is not possible at present to give a realistic price but projections from New Zealand would suggest a favourable comparison.

G. Wieringa (Netherlands)

Difference in price between the pressed pulp and the protein concentrate was very small. The demands for labour of the processing occurred at the wrong time.

A.S. Jones (UK)

The xanthophyll content of the extract should also be taken into account.

M.F. Maguire

It is proposed that a contractor rather than a farmer would undertake processing centrally.

THE EFFECT OF MECHANICAL PROCESSING OF GRASS
ON THE NUTRITIVE VALUE OF FORAGE FOR RUMINANTS
AND THE DEGRADABILITY IN THE RUMEN

A. S. Jones

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

INTRODUCTION

Pirie's work on the extraction of protein from green leafy materials (Pirie, 1971) is well known. In general, Pirie's objective was to extract the maximum amount of protein from green crops and he was not particularly concerned with the nutritive value of the pressed residue. However, in monetary terms the value of the pressed residue is far greater than the value of the extracted protein and in the agricultural context it is better to partition a green crop in such a way that only the protein in the crop which is surplus to the ruminant's requirement is removed. The amount of surplus protein depends on species and the physiological state of the ruminant to which the pressed residue is to be fed. The age at which a crop should be cut so to yield the maximum amount of extractable protein and pressed residue will vary depending on the requirement of the ruminant to which the residue is to be fed (Jones, 1981). Different degrees of mechanical processing produce pressed residues of different types and data is presented on the effect of mechanical processing on the nutritive value of the pressed residue.

EXPERIMENTAL

The mechanical process used was the single-stage screw press (Bentall & Co. Maldon, Essex). Before screw pressing, grass is cut in the field with a precision chop forage harvester so that chop length is between 50-75 mm. Immediately after cutting, the chopped material is passed through the screw press. The pressed fibrous residue leaves the screw press with a fibre length of 4-7 mm and the fibres are laterally macerated so that most of the plant cells have been disrupted. It is possible that this material may be different from that produced in the two-stage pulper and press (equipment of the type used by Pirie) which reduces fibre length by chopping and dewaterers by squeezing between a belt and roller.

RESULTS AND DISCUSSION

There is considerable variation in the way in which a crop is partitioned by the single-strange screw press and there are two main factors which affect the process. Firstly, the moisture content of the crop; there is a significant relationship between the dry matter of the crop and the rate of extraction of nutrients into the juice; this has been reported by several workers, and the drier the crop, the lower the rate of extraction of protein and dry matter. The second factor is the pressure which is applied to the crop during processing. This pressure is increased as the distance between the end-plates is reduced and the effect of pressure on the extraction of protein is given in Table 1.

TABLE 1 The effect of the width of the end plate gap on the extraction rate of crude portien from grass

Distance between end plates (cm)	Extraction of crude protein (%)
2.0	25
1.7	37
1.2	42

The data which follow in this paper refer to an extraction rate of 25-30% for protein and 30-35% for soluble carbohydrate. With these rates of extraction, the compositions of the two fractions, juice and pressed residue, are as shown in Table 2.

TABLE 2 The effect of mechanical processing on the partitioning of Italian ryegrass and the chemical composition of the fractions

	Pressed residue (%)	Juice (%)
<u>Partitioning</u>		
Fresh weight	50	50
Crop dry matter	83	17
Crude protein	69	31
<u>Chemical Composition</u>		
Dry matter (DM)	5-11	25-35
Crude protein in DM	21-45	18-25
Soluble carbohydrate in DM	40	30
Ash in DM	15-20	5

The degree of processing required to give these extraction rates appears to have little or no effect on the digestibility of the fibrous part of the grass. Table 3 shows the digestibility of dry matter of the fibrous residue by sheep relative to that of the grass from which it was produced for grass cut at different times throughout the year.

TABLE 3 Digestibility of the dry matter by sheep of fibrous residue and unprocessed dried grass made from grass cut at approximately 5 week intervals throughout the year

	Digestibility coefficients	
	Fibrous residue	Dried grass
Cut 1 (May)	0.76	0.76
Cut 2	0.77	0.73
Cut 3	0.69	0.73
Cut 4	0.76	0.74
Cut 5 (October)	0.73	0.75

The nutritive value of the pressed residue for cattle appeared to be greater than that of the grass from which it was produced. Table 4 shows data for cattle initially weighing 350 kg which were zero grazed on pressed residue or grass or paddock grazed on grass. Growth rate and the efficiency of utilization of dry matter were significantly higher for those cattle given pressed residue while intake was, if anything, reduced relative to those given grass.

TABLE 4 Feed intake and live-weight gain of fattening cattle

Treatment	Live-weight gain (kg/d)	Dry matter intake (kg/d)
Grass (zero grazed)	0.73	6.8
Fibrous residue (zero grazed)	0.84	6.3
Grass (paddock grazed)	0.72	-
S.E.D. between means	0.06	0.19

Significant improvements were also obtained in the growth of cattle given pressed residue from clover when compared with fresh clover (Houseman *et al.*, 1978) and significant improvements were found in the growth of cattle given pressed residue as silage rather than grass silage. Similar improvements in growth have been reported for red clover by Mackie and Copeman (1976). From these results it is concluded that the nutritive value of the pressed residue is higher than that for grass, at least for fattening cattle, despite the removal of soluble carbohydrate and protein. Mechanical processing in some way improves the nutritive value of the fibrous material.

An interesting question is whether the nutritive value of the pressed residue would be further improved by the addition of the juice to the residue. Greenhalgh and Reid (1975) measured the voluntary feed intake of mature sheep given either chopped grass, pressed residue or recombined pressed residue and juice (RCG). There were small differences in the chemical composition of grass and RCG due to the loss of some soluble carbohydrate, which increased, but not significantly the proportions of acid-detergent fibre and crude protein. There were substantial increases in the voluntary feed intake of sheep given RCG compared with the other two treatments (Table 5). One might speculate that this

increase in intake was due to the production of a two-phase feed, pressed residue and juice, and that if ruminants eat to a particular rumen load (Baumgardt, 1970) that the liquid phase passed rapidly through the rumen, thus reducing rumen load which stimulated further eating. On the same basis, however, there should have been differences between the intake of sheep on chopped grass and pressed residue. The increase in intake may also have been due a faster colonisation of the fibres by rumen bacteria, but if this were the case then there would have been a similar increase in intake by the sheep given the pressed residue, unless to colonise rapidly the rumen bacteria required the protein expressed from the plant cells.

TABLE 5 Voluntary food intake of sheep given fibrous residue, grass or RCG

Treatment	Feed intake		Digestibility coefficient
	g/d	g/kg W ^{0.75} /d	
Chopped grass	1140	54.2	0.73
Fibrous residue	1060	50.5	0.72
RCG	1660	78.5	0.74
S.E. of difference	89		0.027

Whatever the reason for the increase in voluntary feed intake, it is pertinent to ascertain whether these differences in intake are specific to sheep and whether the differences in intake are reflected by differences in growth. The effect may be related to the relative size of the particles of forage and the size of the reticulo rumen orifice.

Experiments were therefore undertaken with fattening cattle given RCG or pressed fibre and intake and growth rate measured. The data are given in Table 6 and show that cattle given RCG grew 9% faster and utilized dry matter more efficiently than those given the pressed residue. At the end of the experiment, cattle given RCG were 14% heavier. Compared with cattle given grass, the improvements in growth rate of cattle given RCG were lower than those for cattle given the fibrous residue although it is difficult to make comparisons between experiments.

TABLE 6 Growth, feed intake and feed utilization of cattle initially weighing 350 kg over a 100 day zero-grazing period

Treatment	Grass	RCG
Daily gain (kg/d)	1.00	1.09
Feed intake (g dry matter/kg W ^{0.75} /d)	93.2	90.3
Feed utilization (kg dry matter/kg gain)	7.21	6.61

To determine whether the differences in growth and feed utilization were due to differences in the degradability of the materials in the rumen, the different materials were incubated in nylon bags in the rumen of sheep. Mature sheep were given a basal diet of grass, fibrous residue or RCG and the relationship between the degradability (percentage loss of dry matter) and time for the different materials determined. These relationships are given in Figure 1.

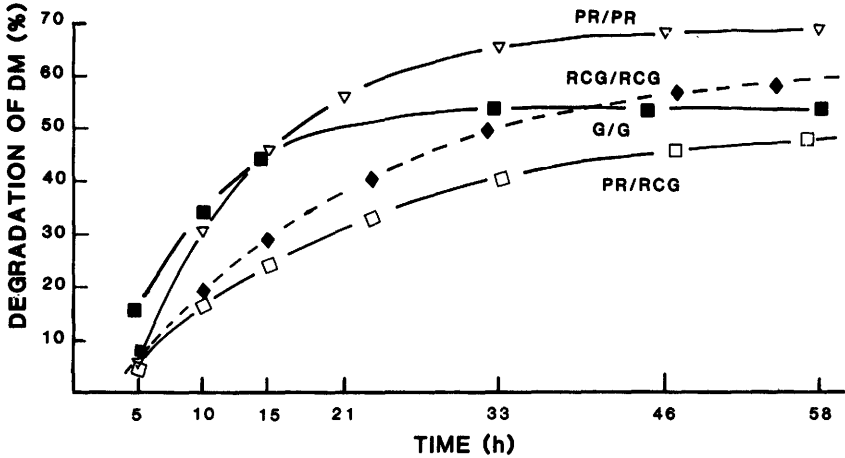
The extent of degradability of the dry matter of grass in sheep given a basal diet of grass was significantly lower than that of fibrous residue in sheep given a basal diet of fibrous residue which might explain why ruminants grew faster on the pressed residue. There were small differences in the initial rate of degradation of dry matter between grass and fibrous residue, the rate of loss being faster from grass and this would probably be due to the fact that the soluble material had already been removed from the fibrous residue by mechanical processing.

The extent of the degradability of fibrous residue in sheep given RCG was lower than that of pressed residue in sheep given pressed residue. RCG provides a readily available source of carbohydrate which may have reduced the activity of cellulolytic bacteria (see Mould and Ørskov, 1981). Certainly the pH of rumen contents were significantly lower in sheep given RCG (6.2) than in sheep given grass (6.5) or fibre (6.7).

There were difficulties in containing RCG in the nylon bags and the broken line in the figure is based on calculated data assuming that the dry matter of the juice (on average 21.7% of the dry matter of the grass) was totally fermented in the rumen. The shape of the curve is based on the assumption that the dry matter of the juice in RCG was degraded at the same rate as the fibre in RCG and obviously this is not the case, since the dry matter of the juice contains the

Fig. 1. Legend

- PR - Pressed residue
- RCG - Recombined pressed residue and juice
- G - Grass



**THE RELATIONSHIP BETWEEN TIME AND DEGRADABILITY
IN THE RUMEN**

highly soluble fraction of grass. Nevertheless while this calculation does not give a true picture of the rate of degradation, the estimate of the extent of the degradation should be correct, and the results suggest that RCG is more highly degradable than fresh grass - which is in line with the observations on growth.

These results suggest that the differences measured in the growth of ruminants on processed and unprocessed grass may be related to the extent of the degradability of these materials in the rumen. This being so any process which increases the extent of degradability in the rumen should also increase growth rate.

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PAPER 5

AUTHOR: A.S. Jones (United Kingdom)

DISCUSSION

M.F. Maguire (Ireland)

Could the reduction in particle size of the pressed pulp lead to improvements in utilization, as has been found with dried crops?

A.S. Jones

The outflow rate from the rumen may be improved.

G. Wieringa (The Netherlands)

What were the effects of alterations to the press on its capacity?

A.S. Jones

This depressed output from around 700 - 200 kg/hr.

R. Carlsson (Sweden)

What type of grass was used?

A.S. Jones

Italian ryegrass.

A Deswysen (Belgium)

With reference to the digestibility in nylon bags, how was the material processed?

A.S. Jones

Grass was cut into 4 mm lengths.

M.J. Drennan (Ireland)

Was slaughter data available?

A.S. Jones

Not in all experiments but sufficient time was allowed for stabilisation of gut fill.

G. Wieringa

What were the cutting intervals?

A.S. Jones

Grass was cut at a height of 20 cm every 4 - 6 weeks.

R. Jarrige (France)

The conflicting results can be explained by the presence of inhibitory factors in the juice or pulp.

TRENDS FOR FUTURE APPLICATIONS OF WET-FRACTIONATION OF GREEN CROPS

R. Carlsson

Department of Plant Physiology, University of Lund,
Box 7007, S-220 07 Lund, Sweden.

ABSTRACT

During the latest 15 years, research and development (R&D) activities of wet-fractionation of green crops for production of leaf nutrient/protein concentrate (LPC) have substantially expanded. More than 50 countries have R&D programmes in progress. Both selection of plant material and development of technical processes are equally important for a high yield of good quality LPC and a good quality of pressed crop, the second product of wet-fractionation.

New species and cultivars are going to be exploited. The importance of secondary plant substances and plant physiological stages at harvest for the quality of LPC is gaining recognition.

Low energy-consuming wet-fractionation systems, producing wet LPC and moist, pressed crop as end products will be developed parallel to more energy-consuming systems that supplement traditional, green crop driers, producing dried LPC and dried pressed crop. Biotechnological processes, using micro-organisms and enzymes, will be part of now, traditional wet-fractionation systems to increase the yield of LPC, and to de-toxify LPC from abundantly occurring plant material, otherwise less suitable for wet-fractionation. High technology processes for highly purified protein isolates will be further developed.

Processes for partially purified, whole LPC will allow a wider use of whole LPC as a full leaf nutrient concentrate for human consumption. LPC as non-ruminant animal feed and pressed crop as ruminant fodder will be consumed by more species of animals than today. The major product of wet-fractionation, the pressed crop, will be used for biogas or liquid fuel production, perhaps together with the third wet-fractionation product, the de-proteinized juice.

INTRODUCTION

During the International Biological Programme, Prof. H. Burström introduced leaf protein research in Sweden at the University of Lund in 1965. At that time about 10 countries had active research programmes going on (Stahmann, 1968; Singh, 1969; Pirie, 1971). According to a fresh global review (Carlsson, 1981A), more than 50 countries (not including separate states within India, USA, USSR) presently have research and development (R&D) programmes of wet-fractionation of green crops or on leaf nutrient/protein concentrate (LPC) in progress. The R&D trends during later years cover subjects as plant selection, utilization of by-product green matter, effects of

development stages of plants at harvest and presence of secondary plant substances and modifications thereof on yield and composition of LPC and other wet-fractionation products, development of plant processing, precipitation/coagulation and fractionation studies of extracted protein and other nutrients, conservation of leaf nutrients and proteins in the extracted/expressed juice or in the precipitate/coagulum, chemical analysis of LPC, in vitro and in vivo qualitative tests, studies of functional properties of LPC, utilization of LPC as a feed and as a food supplement in human diets, and production of crystalline or highly purified proteins for medical purposes (cf below).

Parallel to this line of production, extraction, processing and utilization of pure leaf nutrients, you can find utilization of microbial and enzymatic treatments of whole crop, extracted juice, wet LPC, pressed crop (fibre residue), and de-proteinized juice (brown juice/liquor, whey) (cf below). These biotechnological approaches aim at transforming carbohydrates, proteins, lipids, and secondary substances to alleviate processing to increase yield of protein-enriched products, to de-toxify materials, and to produce commercially useful substances, e.g., ethanol for fuel (cf below).

From these R&D activities one can discern trends for future development lines. Such R&D lines may aim at selection of or breeding of high-yielding and high-quality LPC crops, optimal harvesting stages for maximum LPC yield and quality, development of low energy consuming wet-fractionation systems, development of simple processing techniques, development of advanced harvesting/processing systems, maximum profit from wet-fractionation, production of cheap feeds, utilization of the potential of whole LPC as a full nutrient food supplement, production of LPC with good functional properties to fit into products of the food industry in developed countries, production of highly purified protein for medical treatments, utilization of de-proteinized juice for microbial protein production (including reduction of biological oxygen demand - BOD - of the final waste liquor) and as a base for production of chemicals, development of the pressed crop, not only as a storable ruminant fodder, but also as a biomass resource for production of biogas, liquid fuel and chemicals (cf below).

PLANT MATERIAL

Species

Plants for wet-fractionation will be chosen because they are readily available as traditional forage crops, e.g., *Medicago sativa* (lucerne, alfalfa), *Trifolium repens* (white clover), *T. pratense* (red clover), *T. alexandrinum* (berseem), *Lolium* sp. (rye-grasses), *Festuca* sp. (fescues), *Dactylis glomerata* (cocksfoot, orchard grass) and *Pennisetum* sp. (elephant/napier grasses) (Pirie, 1978; Carlsson, 1982).

By-product leaves will be the second choice of vegetation for wet-fractionation. Temperate species as *Solanum tuberosum* (potato haulm), *Beta vulgaris* (sugar/fodder beet), *Pisum sativum* (pea haulm), *Brassica* sp. (cabbage, cauliflower), *Lycopersicum esculentum* (tomato haulm), *Cucurbita* sp. (cucumber vines), *Apium graveolens* (celery), and energy forest species as *Populus* (poplar) and *Salix* (sallow, willow) and trees as *Pinus* (pine), *Picea* and *Abies* (spruces) will be utilized. Among tropical species one may use *Manihot esculenta* (cassava, manioc, tapioca, yuca), *Musa* sp. (banana, plantain), *Saccharum officinalis* (sugar cane tops), *Arachis hypogea* (groundnut, peanut), *Brassica* sp. (cabbage, cauliflower), *Ipomoea batata* (sweet potato vines), *Boehmeria nivea* (ramie), *Cichorium intybus* (chickory), and trees and shrubs as *Eucalyptus* sp., *Leucaena* sp. (ipil-ipil, koa haole), and *Euphorbia* sp. (shrubs for energy/rubber production) (Pirie, 1978; Carlsson, 1982; Telek and Martin, 1982).

All so far mentioned crops may not give the highest LPC yield and highest LPC quality, especially not woody species. The leaves from the latter species or the wet-fractionation products from them may be microbially treated with advantage for feed production (cf below).

Among 300,000 existing plant species, the traditional green fodder crops and the by-product leaf crops constitute an absolute minority. Therefore, progressive scientists started to look for plant species that would be especially adapted for wet-fractionation and production of high quality LPC. As far as known by the present author more than 300 new, promising species have been studied for LPC production, still only a promille of existing plant species. Our plant kingdom should have more to offer us for feed/food production.

Among investigated species, one can discern groups of species that gave a high yield of good quality LPC (Pirie, 1971, 1978; Carlsson, 1975, 1982; Telek and Martin, 1982). These species belong to certain plant families. The families of Leguminosae/Papilionaceae, Chenopodiaceae, Cruciferae, Solanaceae, Amaranthaceae and Cucurbitaceae are such families. The Leguminosae species are already known to be used for wet-fractionation, e.g., luserne, clovers, lupins (*Lupinus*), vetch (*Vicia*), cow pea (*Vigna*) and *Canavalia* sp. The yield of extracted leaf protein has varied from 1,000 to 3,000 kg per ha. The second group comprises South American and Indian pseudo-cereals (*Chenopodium*), leafy vegetables (*Chenopodium*, *Atriplex*, *Beta*, *Spinacia*, etc.), and salt bushes (*Atriplex*). The yield of extracted protein has varied from 1,000 to 1,500 kg per ha. Among Cruciferae species, those of *Brassica* (rape, kale, cabbage, mustard) and *Sinapia* (mustard) are widely investigated in Europe, India and USA. The extracted yield of protein could range from 600 kg per ha and upwards. Among Solanaceae species can be mentioned potato, tomato, egg plant, and tobacco. Tobacco plants in dense plantations have yielded up to 2.700 kg protein per ha (Staron, 1980), of which more than 50 percent may be extracted during homogenized leaf curing (HGL) processes for production of tobacco flakes. The yield of protein from potato tops could be up to 600 kg per ha. No yield figures are available for tomato and egg plants. *Amaranthus* and *Celosia*, pseudo-cereals and leafy vegetables, of the *Amaranthaceae* family, could yield up to 1,000 kg extracted protein per ha and harvest. Yield per ha is not known for Cucurbitaceae species. However, the protein has been shown to be extremely easy to extract from cucumber vines. From different other plant families only individual species can be recommended for wet-fractionation, i.e., if they can produce high amounts of protein in their plant shoots, e.g., *Helianthus annuus* (sun flower), *H. tuberosus* (Jerusalem artichoke) and its perennial hybrids with *H. annuus*, *Urtica dioica* (stinging nettle), *Herecleum Sosnowski* (Hanczakowski and Lutynska, 1976), green cereals (Byers and Sturrock, 1965), *Sesbania* sp. (Pirie, 1978), *Tithonia tagetiflora* (Pirie, 1978), and *Perescia aculeata* (ora pro nobis) (Dayrell and Vieira, 1977).

A fourth sources of plants for LPC production may be the aquatic plants and marsh plants (Firie, 1978; Carlsson, 1981B). At least the latter ones are looked upon as possible energy crops. For both types of plants a vigorous growth can result from water pollution, i.e., nutrient enrichment. Often the plants are cleaned off without any further use. By applying wet-fractionation both LPC and a fibre-enriched, pressed crop will be major products. The pressed crop has been used as cattle fodder as such. It could also be ensilaged, used for biogas production, or be used as a base for paper production in case of marsh weeds. Among aquatic species should be mentioned *Eichhornia crassipes*, *Ipomoea aquatica*, *Nasturtium aquatica*, *Nymphaea* species, *Pistia stratoides*, *Polygonum* sp., and small floating plants as *Lemna gibba*, *Wolffia colombiana* and *Azolla filiculoides*. *Eichhornia* (water hyacinth) can cover an area with 470 tonnes fresh weight per ha. Even if the dry matter content may be extremely low, the dry matter production still can be substantial (Wolverton and McDonald, 1979: 50 tonnes DM/ha/year). Among the marsh plants should be mentioned *Arundo donax* (canne de Provence) that can produce 20 tonnes of dry matter per ha (Arnoux et al., 1974), *Phragmites communis* and *Typha latifolia*.

Plant selection and breeding

Some preliminary studies to select especially suitable cultivars, provenances, breeding lines (strains), and even individual plants from established agricultural plants and some not-yet-agricultural plants have been performed since the beginning of 1970, in order to increase yield of LPC from plant shoots and to increase LPC quality (Carlsson, 1979). Cultivars of *Brassica* sp. (Hanczakowski, 1977; Lundborg, 1979), *Helianthus annuus* (Lundborg, 1979), *Medicago sativa* (Jelinowska, 1979; Kehr et al., 1979), and *Solanum tuberosum* (Carlsson, 1971; Hanczakowski and Makuch, 1980) have been investigated for that purpose. More than 150 provenances of *Amaranthus*, *Atriplex* and *Chenopodium* species have been investigated for the following factors: protein production per plant shoot, protein extraction ratio, nitrogen content of LPC, in vitro protein digestibility of LPC, saponin contents in shoot and LPC (Carlsson, 1979). Differences were found for both species and their provenances. Individual, field-grown

plants of *Medicago sativa* and *Trifolium pratense* have been investigated for similar factors (Carlsson, 1979). Bugge (1977, pers.com.) has investigated F₁ generations of individual plants of *Lolium multiflorum*. From the individual plant studies some examples are given in Table 1.

Table 1: Ranges of true protein content in LPC, and of L-methionine content in the protein of LPC.

<u>Species</u>	% protein nitrogen of LPC DM	g methionine/16 g N
Carlsson (1979):		
<i>Amaranthus</i> sp.	7.1 - 8.4	2.4
<i>Atriplex</i> sp.	9.0 - 10.0	2.7
<i>Chenopodium</i> sp.	9.0 - 10.0	2.6
<i>Medicago sativa</i>	5.8 - 9.8	1.0 - 2.0
<i>Trifolium pratense</i>	6.5 - 8.3	1.2 - 1.8
Bugge (1977):		
<i>Lolium multiflorum</i>	6.6 - 8.9	---

Irrespectively of what may have caused the differences, the ranges given in Table 1 can be of importance for practical application of wet-fractionation and production of LPC. The soluble leaf protein or fraction I protein that is the base for production of white fraction LPC has been subject for breeding, both for legumes and green cereals (Romman et al., 1971; Frey and Moss, 1976; Gutek et al., 1976).

Effects of physiological development stages of plants at harvest

The plant shoot accumulate protein until onset of senescence. However, the proportion of dry matter (as cell wall constituents) is increased, and then the content of protein of the dry matter is reduced. The possible amount of juice that can be expressed from the plant material thus will be diminished. As protein is expressed, dissolved or dispersed, together with the juice, the result is a reduce extraction ratio of protein (Heath, 1976). The increase of produced, accumulated plant shoot protein, and the decrease of the

extraction ratio, gave the optimal harvesting times, which also are species dependent, for maximum LPC yield (Arkcoll, 1971). A decrease in the extraction ratio has been demonstrated to be counterbalanced by addition of water during the extraction procedure (Carlsson, 1975).

Not only the yield of LPC has been shown to be affected by the growth stage, but also the LPC quality (Smith and Agiza, 1951; Henry and Ford, 1965; Carlsson et al., 1975). Qualitative changes could be due to plant physiological changes of the amount of secondary substances, e.g., phenolics and saponins (Carlsson et al., 1975), and due to a different composition of the protein that was extracted. In rat assays, optimal biological values, true digestibilities, and net protein utilizations have been found for plants harvested at different development stages (Henry and Ford, 1965; Carlsson et al., 1975; Table 2).

Table 2: Effect of plant physiological development stage on net protein utilization of LPC.

<u>Stage ...</u>	Vegetative	Bud forming	Flowering	Seed setting
<u>Species</u>				
Atriplex hortensis	55.3	63.3	56.5	51.1
Chenopodium quinoa	---	54.9	60.2	61.4

Note: Water and ethanol washed LPC; casein: 68.8 (Carlsson et al.1975)

Effects of species on LPC quality

As mentioned, the physiological development stage at plant harvest affected both the yield and quality of LPC. However, in cases where LPCs were produced from leafy plants at a vegetative stage, it could be justified to make a qualitative comparison of LPC from different species (cf Table 3 and 4; literature below).

Table 3: Amino acid composition of LPC from different species (g/16 g N).

<u>Amino acids ...</u>	Lys	Thr	Cys	Met
<u>Species</u>				
Amaranthus hypocondriacus	6.5	4.9	1.2	2.4
Amaranthus sp. Taiwan No 4	7.0	5.0	1.6	2.4
Atriplex hortensis	7.0	5.5	1.3	2.7
Brassica hirta	6.9	5.3	1.3	2.7
Brassica napus	6.7	5.2	1.3	2.4
Chenopodium quinoa	7.2	5.5	1.6	2.7
Helianthus annuus	6.6	5.4	1.3	2.6
Medicago sativa	6.2	5.4	1.1	2.3
Sorghum sudanense	6.6	5.1	1.2	2.7

Note: Sulphite added during processing (Carlsson et al., 1978)

Table 4: Protein efficiency ratio of LPC from different species.

<u>Species</u>	Non-treated LPC	Sulphite-treated LPC
Amaranthus hypocondriacus	1.78	1.88
Atriplex hortensis	1.51	1.95
Brassica hirta	2.10	2.14
Brassica napus	2.06	2.01
Chenopodium quinoa	1.88	1.96
Helianthus annuus	1.63	1.86
Medicago sativa	1.54	1.97
Casein	2.50	--

Note: Washed LPC (Carlsson et al., 1978)

LPCs from plants at vegetative stages have shown high nutritive values for species of Brassica, Chenopodium and Amaranthus, temperate, green cereals (wheat, rye, barley, oats), and for legumes as Medicago sativa and Lupinus species, compared with LPCs from legumes of the genera Melilotus, Trifolium and Vicia (Henry and Ford, 1965; Woodham, 1965; Subba Rau et al., 1972; Tao et al., 1972; Carlsson, 1975; Carlsson et al., 1975; Hanczakowski, 1975; Munshi et al., 1975; Horigome, 1977; Carlsson et al., 1978; Lundborg, 1979). Solanum tuberosum LPC also had fairly high nutritive values (Henry and Ford, 1965; Hanczakowski and Makuch, 1980). Species differences exist only for well processed plant materials, as bad processing conditions can damage any protein. Extraordinarily high nutritive values, the same as for casein, have been found for whole LPC from Chenopodium quinoa (Carlsson et al., 1975; Carlsson, 1980).

Effects of secondary substances on the nutritive value of LPC

Theoretically, e.g., due to the amino acid composition of a well processed leaf protein concentrate, the leaf protein should have a high nutritive value. However, secondary plant substances could reduce the nutritive value of the protein. Such secondary substances are, e.g., phenolics/tannins, saponins, trypsin inhibitors, plant oestrogens, cyano-glycosides, phytohaemagglutinins, and goitrogens (Liener, 1969). These substances could be metabolic endproducts, or could appear in connection with microbial and insect attacks of a plant as part of its defence mechanism.

The presence of several secondary substances in LPC have been demonstrated, e.g., phenolics (Igarashi et al., 1976, Monties and Rambourg, 1978), saponins (Carlsson, 1975; Livingston et al., 1979), trypsin inhibitors (Humphries, 1980; Jokl and Carlsson, 1981), plant oestrogens (Glencross et al., 1972; Knuckles et al., 1976; Igarashi and Yasui, 1978; Rambourg and Monties, 1980), cyano-glycosides (Balasundaram et al., 1976; Carlsson and Telek, 1978; Nandukumar et al., 1978), phytohaemagglutinins (Jokl and Carlsson, 1981). Generally, small amounts of secondary substances have been found in LPC compared with the amounts in original plant material. This is especially the case for non-green (white) fraction LPC.

Negative correlations have been found between the content of phenolics in LPC and its nutritive value (Carlsson et al., 1975: NPU; Carlsson et al., 1978: PER). A low saponin cultivar of *Medicago sativa* gave LPC with higher quality than a high saponin cultivar of *M. sativa* did (Hegsted and Linkswiler, 1980). Other secondary substances can give similar responses, e.g., reduced food intake, reduced weight gain, or reduced nutritive value. Secondary substances can, however, be washed off, or neutralized (Bickoff et al., 1975; Carlsson, 1975, Woodham et al., 1977; Carlsson et al., 1978), which gave whole or white fraction LPC with the same quality as casein (Bickoff et al., 1975; Carlsson, 1975; Carlsson et al., 1975).

Conclusion

One can assume that already established, often perennial, legume crops and grasses will be the major future sources for LPC production for feed. These major crops will be supplemented with by-product leaves, whenever available. The early and late parts of a wet-fractionation season, as well as "holes" in it will be covered by crops grown especially for LPC production, e.g., Brassica and Chenopodium species. Regarding water/marsh plants and forest trees, respectively, separate processing units will be built for local use at major harvest sites.

Much more care will be taken regarding the selection of plant material (e.g., cultivar breeding), and of harvesting dates (due to physiological development of plants), as presence of secondary substances can reduce the nutritive value of the produced LPC. Selection of species and cultivars, and harvesting dates will be very important, when LPC is paid for the availability of its nutrients, and not only for its proximate, chemical composition.

TECHNICAL PROCESSES

The trends of technical development are fairly divergent, depending on, for what purpose the wet-fractionation system is going to be used. Probably, low energy-consuming systems, or energy-saving systems, supplementing existing green crop driers, will be the close-future systems. High technology based systems may not come yet, although a much suitable LPC can be produced for modern food industry.

In the latter case probably more emphasis will be put on the functional properties of LPC than on its nutritive value.

Low energy-consuming or energy-saving systems

An individual, small farm can have its own stationary press for wet-fractionation of crops close to the farm animals to produce fresh, green juice daily for swine feeding, and to make silage of the pressed crop, if it is not consumed daily (Connell and Housemann, 1977; Maguire, 1977). The pressed crop can also be air-dried at ambient temperature (Housemann, 1975 pers.com.).

Instead of transporting the whole crop, which may be costly for longer distances, one can put a transportable wet-fractionation unit temporarily at the fields to collect the juice for transport to the farm, leaving the pressed crop for "grazing" cattle or sheep. This may work for farms with waste forage areas (Bruhn et al., 1978).

A more advanced harvesting system that seem to be coming is a mobile, combiner that cuts, macerates, and presses the crop (Mills, 1980). A combiner may be used cooperatively by several farms, where each farm takes care of its own pressed crop. The expressed juice can be transported by truck to a central LPC factory, which produces LPC for sale as hen and chicken feed. The de-proteinized juice from the factory can with advantage be brought back by truck to be used as a fertilizer for the field, where the crop was cut.

Today, however, most LPC is produced at wet-fractionation units that are built as supplements to commercial/cooperative green crop driers. Such units exist, e.g., in France, Denmark, Colorado (USA), Italy and Spain.

As wet-fractionation of a crop produces a pressed crop with broken cell membranes, which facilitate water evaporation, the green crop drier will save energy (oil, coal) used to produce hot air for drying the crop. This energy "gain" is used for carrying on the wet-fractionation and the LPC production. Waste heat from the green crop drier is used partly for recirculation in the drying drum, partly for preheating expressed juice (to +50 to 60 C), from which the wet LPC coagulum will be obtained, and partly for evaporating water from de-proteinized juice to concentrate it to molasses (50% DM) in a multiple step evaporator. Normally, the molasses are mixed into the

dried, pressed crop. Otherwise, it can be used separately as ruminant feed. An earlier, Hungarian LPC (VEPEX) factory utilized the de-proteinized juice for yeast growth before the waste liquor was concentrated to "molasses". The yeast was added to the LPC before drying it. Similar processes to increase the yield of LPC by fermentation of whole juice or de-proteinized juice are used in Italy with high LPC yields as the results (Galoppini et al., 1978).

In USSR, as well as in other not mentioned East European countries, several **small and large wet-fractionation** units are producing green juice, wet LPC and dried LPC (Novikov, 1981; Bekeris et al., 1982). The pressed crop is dried or made into silage.

Pilot plant and laboratory developments

Several solutions to reduce the energy used for wet-fractionation, and to increase the through-put of products in the system, and to increase the yield of LPC from crops are in progress since more than 5 years.

Plant shoot and cell maceration. The energy used for maceration of a crop can be reduced compared to traditional screw presses, roller presses and hammer mills. The extrusion model, with compression of the plant material followed by a sudden blowing up of it, to break up cell walls and cell membranes has both a low energy demand and a high through-put capacity, up to 20 tonnes crop per hour (Koegel et al., 1974). In other systems, a screw press, used for both shredding the plant material to open the plant cells, and to express the juice, has been shortened substantially at the fibre out-let end. That part of a normal screw press does not increase the maceration degree nor does it increase the juice yield. Instead, that part only causes frictional heat, i.e., wastes energy. Thus a shortened screw press is more efficient. The far end of a screw press can also have a cone regulating the through-put. In such case an optimal through-put speed can be selected. Screw presses have further been modified that they seem to be a mixture between a very slow "hammer mill" and a short screw press (Pirie, 1975; Joshi, 1981). Such a "macerator and press" utilize very little energy compared with a normal hammer mill in combination with any press. The "macerator

and press" units that now exist only handle hundreds of kilogrammes of crop per hour, which make them most useful for small operations.

A promising pre-treatment of plant material for increased cell membrane break up to increase the expression yield of juice and protein has been developed at The Academy of Science of Modavia, namely an electrolysis process (Licensintorg, 1982). This process is in progress in Latvia in USSR. It is a low energy-consuming process. Another pre-treatment has been to heat up the whole crop before pressing it (Pathak et al., 1978), which should leave part of the protein in the pressed crop, perhaps the less valuable part. Theoretically, an ethanol pre-treatment of a crop break up cell membranes, which may increase extraction yield of protein. Chibnall (1939) advocated ether for increased protein yield. Microbial/enzymatic treatments of crops for increased yields of LPC will be taken up together with treatments of the pressed crop (cf below).

Juice expression. Regarding pressing per se in wet-fractionation screw presses (single or twin-screw presses), band presses and roller presses are/have been used. Mostly screw presses are used as they are readily available, and have capacities for from 200 kg to 40,000 kg crop per hour. Presses that turn over the material during pressing are regarded as the most efficient ones (twin-screw presses, "macerator and press"). Band presses are more efficient with wet or slippery, pulped plant materials than screw presses are. On the other hand, Mills et al. (1980) used a short screw press that used much less energy than a band press. Roller presses are energy-consuming, and are used only when nothing else is available. A wide-angle cone press for pulped material that uses little energy (Koegel et al. 1978) may have a break-through for wet-fractionation.

Addition of extraction media for an increased expression yield of LPC is advantageous (Carlsson, 1975: water; Edwards et al., 1978: de-proteinized juice; de Mathan, 1981 pers.com.). From a qualitative point of view water is preferable to de-proteinized juice, as the latter contains secondary substances disturbing the LPC quality for non-ruminant feed.

Coagulation of leaf protein. The protein in the expressed juice can be coagulated/precipitated in many ways. Traditional ones are heat and acid coagulations. Less common ones are such that use calcium-rich minerals, bentonite, flocculants, limited amounts of organic solvents as ethanol, acid cheese-whey, vinasse from ethanol industry, tannin-rich waste, gelatinized starch, partial removal of membrane-bound chloroplastic proteins to get a less intensively greencoloured LPC (Carlsson, 1975, 1981C; Carlsson et al., 1981). The use of organic solvents has been extensively studied by Bray and Humphries (1978). Extensive studies of flocculants have been made in USA (Knuckles et al., 1980), and in Italy (Fiorentini, 1980). Stahmann (1976) is in favour of anaerobic fermentation and precipitation of LPC.

Juice concentration before protein precipitation can be obtained by ultrafiltration (Trägårdh, 1978), falling film evaporation (Uemaki et al., 1978), flash concentration and by auto-concentration (Anon., 1982). Ultrafiltration seems so far to be the only method used in practice. Various ways to preserve leaf protein and other leaf nutrients in the juice will not be dealt with in this paper.

Separation of coagulum/precipitate. The methods to separate the coagulum are different and adjusted to pilot plant lines locally. The coagulum can float, precipitate to the bottom, or even fill the the whole juice volume. It can be finely dispersed or be fairly coarse. Centrifuges and filters are mostly used to collect the coagulum, as they are efficient. Alternatively, skimming of a floating coagulum seems a viable process (Bruhn et al., 1978). This method is combined with low-temperature (below +105 C) drum drying, outside the drum (Straub et al., 1979).

The separated wet LPC can be used as such, mixed into cereal-based feed for short term storage, or treated with different chemical compounds to be long term stored, e.g., by addition of acids (formic acid, acetic acid, propionic acid, hydrochloric acid), sulphites and other reducing substances, sodium chloride and saccharose, and sorbate and benzoate, or treated by a Lactobacillus sp. addition, or made into a silage, or stored frozen (Hanczakowski, 1976;

Tutarova et al., 1977; Szadacs and Madas, 1979; Pirie, 1980). However, drying is the most common preservation technique for LPC. LPC has been spray-dried, fluidized-bed-dried and rotary drum-dried (inside the drum) by commercial enterprises in USA and Europe. Out-side drum-drying was mentioned above. Freeze-drying is mostly used for laboratory purposes. It would be feasible to store wet LPC under N_2 or CO_2 gas.

For smaller LPC production units (less than 1,000 kg crop processed/h) long term storage without drying may be the best alternative. However, large commercial units will dry the LPC as it is then easy to handle, store and transport. Animal feed mixturers may use either wet or dry LPC in the diet formula.

The residual de-proteinized juice from wet-fractionation and production of LPC will only get a few comments. Most easily it can be used as a fertilizer due to its contents of potassium, phosphorus and other elements. It has been used as fermentation medium with or without supplements. It can be concentrated into ruminant feed molasses, or be added back to the pressed crop before drying or conserving it, as the molasses contain much carbohydrates.

De-toxification and purification of leaf protein. The quality of LPC can be affected at different stages of the wet-fractionation process. To increase the quality, one may remove, neutralize or modify anti-nutritive factors. Sulphite addition during various stages of the process neutralize negative effects of phenolics and prevent oxidation reactions in general (Bickoff et al., 1975; Carlsson et al., 1975; Woodham et al., 1977; Carlsson et al., 1978). A high pH (8.5) during processing can remove saponins from LPC (Livingston et al., 1979). Before protein precipitation, secondary substances can be removed by ultrafiltration and diafiltration (de Fremery and Kohler, 1978). Organic solvent precipitation of leaf protein, and solvent washing of precipitated LPC can reduce the amounts of phenolics and saponins in LPC (Huang et al., 1971; Carlsson, 1975; Carlsson et al. 1975; Bray et al., 1978; Carlsson et al., 1978; Trägårdh, 1978). Normally, the quality in vivo is enhanced by such treatments. Jokl and Carlsson (1981) however found that LPC produced by ethanol precipitation and washing of leaf protein from plants grown in

Minas Gerais in Brazil (acid, iron-rich soils) gave lower nutritive values than heat precipitated LPC gave. By anaerobic fermentation of expressed juice the coagulum can be de-toxified (Stahmann, 1976). Staron (1975) used *Geotricum candidum* for de-composing hydrocarbons, phenolics, saponins and other secondary substances. Another Staron (1979) developed a process with oxygenated water. Wet LPC can be cleaned from saponin by *Lactobacillus* treatment (Szadacs and Madas, 1979). Carlsson and Nicoli (1981 unpubl.) used *Aspergillus* sp. for de-toxifying Eucalyptus LPC. Heat during processing destroys cyanoglycosides (Carlsson and Telek, 1978). Savangikar and Joshi (1979) used plastein reactions to transform LPC into a bland product. PEG and PVP have been used to neutralize phenolics in LPC during feeding experiments (Carlsson and Hanczakowski, 1982 unpubl.). From these examples one can see that plants with much secondary substances still could be used as a sources for LPC for animal feed, provided the coagulum is de-toxified or purified.

Pressed crop. The major part of the biomass from wet-fractionation of crops is recovered as pressed crop (about 80% of the plant biomass dry matter). In commercial wet-fractionation factories the pressed crop is dried as such or together with the concentrated de-proteinated juice (molasses). In USSR large wet-fractionation units also use ensiling for preserving pressed crop (Novikov, 1981; Bekeris et al., 1982). A definite advantage is to add formic acid to silages as the fermentation seems better, as well as the silage quality (Lu et al., 1980).

Many attempts are done to increase the yield of protein from the plant material. The interest in increased yield on non-fibrous plant constituents during wet-fractionation may be due to a wish to enhance LPC yield and profit, as LPC is sold for a high price. On the other hand, there is an interest to remove non-fibrous constituents from the pressed crop, as those can be a nuisance in the further processing of the pressed crop. Such a situation occurs, when the cellulose-rich fibre residue will be used for production of ethanol. This approach has been taken in Brazil for *Pennisetum* grass and in New Zealand for *Medicago sativa* and *Lolium perenne*/*Trifolium repens* pastures (Vaughan et al., 1982).

Miscellanea

The high technology processes used for production of highly purified leaf protein would take a paper of their own. Only a few comments will be given in this paper. There are good possibilities to produce highly biologically useful, white protein isolates (Bickoff et al., 1975). Prof. S. Wildman at UCLA initiated research on tobacco (*Nicotiana*) as a plant for production of crystalline fraction I protein. Some countries have continued to develop that aspect, e.g., USA (Kung et al., 1980), France (Staron, 1980) and Italy (Fantozzi and Sensidoni, 1981). Often white leaf protein isolates have good functional properties regarding: emulsifying activity and emulsion stability, water and fat absorption, solubility and foam stability (Franzén and Kinsella, 1976; Wang and Kinsella, 1976A, 1976B; Betschart, 1977; Savangikar and Joshi, 1979; de Fremery and Kohler, 1978; Humphries, 1978; Fiorentini and Galoppini, 1981).

It may be an advantage from a whole leaf nutrient point of view to partially purify whole LPC to get rid of disturbing secondary substances. By acid water washings, the LPC will contain less nitrate, oxalate, alkaloides, soluble toxic amino acids and water soluble substances in general. Ethanol can remove several anti-nutritive substances in a not too expensive process. The ethanol can be fairly cheaply recovered. A heat fractionation, or a similar fractionation with organic solvents, or a regulation of pH of the juice in combination with centrifugation can give a LPC with less membrane-bound constituents and heavy particles. In such a way a partly depigmented LPC can be obtained, still containing enough with beta-carotene for vitamin A and iron for haemoglobin, apart from well composed protein. Such a semi-depigmentation will increase the technical yield of LPC that will be used for direct human consumption. All of us are well aware that direct human consumption of LPC would be the best utilization of leaf protein and other nutrients.

Apart from the originally suggested subject for consumption of LPC, namely *Homo sapiens*, several other beings are assumed to be future consumers of LPC. From the existing pattern it can be distinguished that hens, chicken, swine and calves will be major consumers of LPC. However, ducks and other fowls, rabbits, fishes and perhaps

cray-fishes will compete with other animals for LPC as a feed.

Conclusion

This summarized description of some present wet-fractionation processes can give some trend for future R&D. A major advantage of wet-fractionation of green crops is that a weather independent harvest system in wet areas makes it possible to fully utilize the biomass production capacity of green crops.

Regarding technical aspects one can foresee low energy-consuming systems for local LPC and pressed crop production, e.g., for small farms or small villages (tropical countries). In this case the LPC will be consumed or stored as a wet product. The pressed crop will be consumed fresh or stored as silage (probably with formic acid added) for later consumption by ruminants (milk and beef cattle, sheep and goats). The pressed crop may also be used for biogas production. This option may give cooking gas for families, or gas for heating.

In market economy countries an increasing number of commercial or cooperative green crop driers may add wet-fractionation systems to their factories for production of dried LPC and dried, pressed crop. Plan economy countries probably will use both the low energy-consuming and high energy-consuming wet-fractionation systems mentioned above for medium and large scale fractionation units.

Although the support energy in-put for certain wet-fractionation systems will be high, it is still worth to use wet-fractionation as it maximizes protein out-put per unit energy as in-put (McDougall, 1980).

The technological development of systems for human LPC production will continue. Furthermore, microbiological/enzymatic biotechnological processes will have an increasing influence on wet-fractionation of green crops for animal feed production, and for a diversified utilization of the pressed crop.

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B. UTILISATION OF FORAGE PROTEIN BY RUMINANTS

Chairman: C. F. Van Sumere

PROTEIN METABOLISM IN RUMINANTS WITH SPECIAL REFERENCE
TO FORAGE PROTEIN UTILISATION

S. Tamminga

Institute for Livestock Feeding and Nutrition Research,
Lelystad, Netherlands

ABSTRACT

Forages can play an important role in the production of animal protein with ruminants in Western-Europe. There is also scope for improvement of forage protein utilisation. To achieve improvements one has to take into account the complicated digestive system of ruminants, particularly the possibilities, but also the limits of microbial fermentation in their forestomachs.

The best way to achieve an efficient utilisation of forage and forage protein clearly lies in increasing the quality of the forage. N-fertilisation and grazing or harvesting at an early stage of growth may be most helpful in this respect. A second way of improving the utilisation of forage protein is to balance the ratio of energy in protein to total energy in the forage to what is required for a specific form of ruminant production. In this respect supplementing the (conserved) forage with high energy low protein feedstuffs, wilting silages, chemical preservation of unwilted silages or crop fractionation may be beneficial.

INTRODUCTION

Forages give a major contribution to the feed supply of domesticated ruminant animals in most European countries. Forage protein gives an even larger contribution to the protein supply. According to EC statistics (1980, 1981) over 53% of the 37.6 million tons of crude protein fed to 93.5 million livestock units (1 livestock unit is the equivalent of a male bovine of two or more years of age), within the EC in 1976/77 was supplied by "animal feedstuffs normally not marketed", mainly forages. It may be assumed that these products were almost entirely fed to ruminants. Ruminants comprised some 70% of the livestock units, hence 70 to 80% of the crude protein fed to ruminants within the EC came from forages.

The importance of forages in ruminant feeding is not surprising. Because forages are often grown on (relatively) cheap land which is less suitable for some other form of primary production (i.e. arable farming) (Cunha, 1982) and growing forages does not require high investments, it forms a relatively cheap source of nutritive value. A substantial proportion of the nutritive value in forages is present in its cell wall constituents, structural carbohydrates like cellulose and hemicellulose

in particular. These polysaccharides are characterised by β -glycosidic bonds, for which animals do not possess hydrolytic enzymes. Their degradation and subsequent utilisation is only possible if at some stage in the digestion process microbial fermentation occurs.

In ruminants the normal enzymatic digestive process is preceded by microbial fermentation in the forestomachs, the rumen in particular. Because of their digestive system, ruminants are pre-eminently suited to digest and utilise forages, but their digestive system has important impacts on the supply of nutrients to the host animal. This is particularly true for protein and various aspects of protein metabolism in the forestomachs and its impacts on the utilisation of forage protein will be discussed in this paper.

PROTEIN DEGRADATION IN THE RUMEN

Under most conditions, microbial fermentation in the forestomachs is responsible for about two thirds of the total digestion. Particularly carbohydrates and proteins are degraded. The final result is the conversion of part of the carbohydrates and proteins into a mixture of volatile fatty acids (acetic acid, propionic acid, butyric acid), branched chain fatty acids, carbon dioxide (CO_2), methane (CH_4), ammonia (NH_3), microbial mass and fermentation heat. Occasionally significant quantities of lactic acid are also produced as end products. The (volatile) fatty acids (VFA) are absorbed in the blood and utilised by the host animal as a source of energy. Some of the end products of fermentation can be re-utilised by the microbes (CO_2 , NH_3) but if this is not the case they are excreted in urine (NH_3 after conversion to urea in the liver) or in expired air (CO_2 , CH_4).

Only feed protein escaping microbial degradation in the forestomachs contributes to the intestinal protein supply of the host-animal, together with newly formed microbial protein. Microbial protein synthesis will be discussed later.

Information on the proportions of feed N "bypassing" the rumen is urgently needed, but very difficult to obtain. Measuring techniques, both in vivo and in vitro were developed. In vivo techniques require surgically modified animals, but with such animals only an indirect estimate of the proportion of feed protein bypassing the rumen is obtained which is often less accurate than direct estimates. In vitro techniques usually give some estimate of the solubility of the feed protein. This direct solubility measurement does however not necessarily give a good estimate of the

proportion of the feed N bypassing the forestomachs. Advantages, disadvantages and limitations were summarised recently (Tamminga, 1980).

Crude protein in ruminant feeds can be regarded to consist of (at least) 3 fractions, a soluble degradable fraction, an insoluble degradable fraction and an insoluble undegradable fraction. A further fractionation is possible (Krishnamoorthy et al, 1982). The soluble fraction can be further separated in soluble true protein and soluble non protein N (NPN). Although differences were observed in the rate of degradation in vivo between various soluble proteins (Mahadevan et al, 1980) no indications are available yet that in vivo a significant part of soluble protein survives degradation in the rumen. At present it is generally accepted that the degradation of soluble feed protein is almost instantaneously and complete.

The proportion of insoluble but degradable protein bypassing the rumen depends on the rate of degradation on the one hand and the rate of passage on the other. Rate of degradation of this fraction differs between feedstuffs, but very little is known on what causes these differences.

Of the N in the insoluble but degradable fraction part is present in the cell wall constituents (CWC) and part in the cell contents. The ratio between these two fractions differs widely between different feedstuffs (Krishnamoorthy et al, 1982), but it is as yet uncertain if this can explain differences in rate of protein degradation in the rumen.

Rate of protein degradation also varies with the diet (Ganev et al, 1979; Siddons and Paradine, 1981). Roughage rich diets cause a higher rate of protein degradation than concentrate rich diets. The size of this effect seems however to depend on the type of feedstuff. Ganev et al. (1979) found such an effect with vegetable proteins, but not with animal proteins such as fishmeal. Siddons and Paradine (1981) observed also a reduction in protein degradation with concentrate rich diets, not only for vegetable proteins, but also for fishmeal. The results of Siddons and Paradine as well our own observations (Tamminga, unpublished results) suggest that with feedstuffs rich in starch such as maize and barley the effect of a roughage rich diet on protein degradation is small or absent.

The rate of passage of feed particles through the forestomachs also varies. It increases with an increasing level of feed intake and it is also influenced by the character a.o. the physical form of the diet (Evans, 1981). It is therefore not surprising that information on the actual degradation in the forestomachs of forage protein is not abundant.

Discussion here will be restricted to fresh forage, artificially dried

forage, ensiled forage and hay. Literature concerning estimates of the extent of protein degradation was reviewed recently (Anonymus, 1978, 1980). From these reviews it appears that all classes of forages show a rather variable extent of degradation in the forestomachs. According to the ARC table (Anonymus, 1980) 30 - 40% of protein in fresh forages, silage and hay is bypassing the forestomachs, but some 50% of protein in artificially dried forages escapes degradation. As yet no distinction is possible between different species of forages (legumes, grasses) because there are hardly any indications that they differ in susceptibility for microbial degradation. An exception may be forages which contain high amounts of tannins, which seems to protect the protein from degradation in the rumen. An example of such a legume is sainfoin.

It is somewhat surprising that no differences were found between fresh forage, silage and hay. In fresh forage 75 to 90% of the N is present in true protein (Ohshima and McDonald, 1978). Solubility figures range between 20 and 45% (MacRae, 1976; Tamminga, 1979b). With ensiling protein is rapidly degraded, both due to the action of plant proteases and microbial proteases. However, during wilting, a step preceding both hay making and making wilted silage, plant proteases are also thought to be active. Weather conditions seem to be very important in this respect (Carpintero et al., 1979). Wilting under humid conditions stimulated proteolysis. Under good dry weather conditions one may expect a high dry matter content in the ensiled product with only little proteolysis. It is therefore not surprising that a negative relationship was found between N-solubility (as some measure of proteolysis) and dry matter content in wilted silage (Tamminga, unpublished results).

As was stated earlier, the actual degradation of protein depends on its rate of degradation and on its rate of passage. Information on the rate of degradation can be obtained by incubating samples in dacron bags in the rumen for various lengths of time (Orskov et al, 1980). Fresh grass samples (DM 20-27%; CP/DM 0.22-0.24) were therefore incubated in grazing animals. Ingredients normally used in concentrates (barley, soyabeanmeal, maizeglutenmeal) were also incubated in the same grazing animals. The ranking order in rate of degradation of protein was soybeanmeal, barley, grass, maizegluten. After 17 hours of incubation 98, 94, 82 and 54% of the crude protein of these feedstuffs had disappeared from the bags respectively. Because the rate of passage of grass is probably much lower than the rate of passage of

the other ingredients, if included in concentrates, these experiments seem to confirm that a large proportion of crude protein in fresh grass is degraded in the rumen.

MICROBIAL PROTEIN SYNTHESIS

The formation of microbial mass, as part of the fermentation process includes the formation of microbial protein, usually the largest contributor to the protein supply of the ruminant animal. For this biomass production precursors and energy are required. Intermediates from the degradation of dietary carbohydrates and proteins can be used as precursors whereas the energy (ATP) can be extracted from this degradation. The extractable amounts are limited by the anaërobic conditions in the rumen. Estimates range from 4 to 6 moles of ATP per "hexose-equivalent" fermented (see Tamminga, 1979a). The first limiting factor for microbial growth and microbial protein production is usually the amount of energy (ATP) and occasionally the amount of rumen degradable protein. A generally accepted but rather rough estimate of the amount of energy extracted is the proportion of the apparently digestible organic matter (ADOM) which is apparently digested in the forestomachs. For most dietary situations a proportion of between 0.6 and 0.7 is found. Figures for the various types of forages are shown in table 1.

Table 1. Proportion of apparently digestible organic matter of different forages which is apparently digested in the forestomachs of ruminants

Forage	proportion digestion in forestomachs	No. of observations	References
fresh forage	0.65±0.02	8	1
silages	0.68±0.02	11	1,2,3,4
hay	0.68±0.02	13	1
art.dried forage	0.52±0.03	13	1

1: CAB, 1980; 2: Beever et al, 1977; 3: Unsworth and Stevenson, 1978;
4: Thomas et al, 1980.

The results show that only artificially dried forage behaves differently from other diets. As a consequence relatively less energy can be extracted in the forestomachs from the fermentation of artificially dried forages and hence relatively less microbial protein can be synthesized. The advantage of more feed protein "bypassing" the forestomachs with artificially dried forages is therefore likely to be counteracted to some extent by less microbial protein being produced. Due to the heating involved protein from artificially dried forages may not only become more resistant against degradation in the rumen, but the postruminal digestion may also become decreased (Harrison et al., 1973; Beever et al., 1976). Still feeding of artificially dried forage is likely to result in an increased absorption of protein from the small intestine.

With silages another aspect is of importance. Ensiling includes a microbial fermentation under anaerobic conditions. So a significant part of the energy extractable from fermentation is extracted already during the ensiling process. Up to 15% of the dry matter in unwilted silage may be present as acetic and/or lactic acid (Mc Donald, 1973, 1981). This may amount up to 25 to 30% of the organic matter which would normally be digested in the forestomachs. Hence feeding silage may deprive the microorganisms in the forestomachs of up to one third of their energy. It is therefore not surprising that the efficiency of microbial protein synthesis, if related to the organic matter disappeared between mouth and small intestine, is often lower with feeding silage than with feeding other types of diets (Thomas et al., 1980; Armstrong, 1981; Chamberlain, 1981). More research is needed to show the size of the effect of wilting before ensiling on the efficiency of microbial protein synthesis, because with wilted silage a smaller proportion of the dry matter is converted to acids than with unwilted silage (McDonald, 1973).

After feeding forage microbial protein is the major component in the intestinal protein supply. This is particularly true with feeding fresh forage or hay. It was found that 66 to 85% of the N entering the small intestine of sheep fed either fresh forage or long hay was of microbial origin with sainfoin as the one exception (Harrison et al., 1973; Beever et al., 1978). After feeding unwilted silage the contribution of microbial N to the total N in the small intestine was much lower and varied between 22 and 46% (Thomas et al., 1980). In this latter experiment DAPA was used as a marker for microbial protein and in the experiments of Harrison et al (1973) and Beever et al (1978) ³⁵S was used as a microbial marker. This could

however not explain the difference because in a direct comparison of both markers in silage fed sheep (Siddons et al., 1979) microbial N flow in the small intestine was about twice as much when based on DAPA compared with the flow based on ³⁵S. With feeding unwilted silage fermentation may have limited microbial protein synthesis and this could at least partly explain the low contribution of microbial N to the total N in the small intestine in this case.

PROTEIN SUPPLY AND UTILISATION AS RELATED TO ENERGY SUPPLY

Like all other living animals ruminants have a requirement for energy and for protein. In addition they have a requirement for a variety of other nutrients like minerals, trace elements, vitamins, etc. but discussing these requirements is beyond the scope of this paper. The requirements for energy and protein are linked as will be discussed hereafter.

Protein as contributor to the energy supply of the host animal.

With respect to energy and protein it must be stressed first that protein contributes to the energy supply. On the other hand part of the requirement for energy is a requirement for energy in protein. So, energy and protein are preferably required in a specific ratio (here after called RPE) which varies with the nature of the process for which the energy is required. It was calculated (Tamminga, 1982) that maintenance requires a RPE in absorbed nutrients of approximately 0.17. Calculated figures for growth varied from 0.08 (mature animals) to 0.30 (young, fast growing animals), whereas for the production of milk a figure of 0.26 was calculated.

A producing animal always has a mixed requirement for maintenance (usually met with a high priority) and for production processes (e.g. growth, wool, milk). The actual RPE required for a given animal therefore depends on the nature of its production and on the level of its production, but usually narrows with an increasing level of production as is demonstrated in table 2.

Table 2. Estimated required minimum ratio of energy in protein to total energy (RPE) for various types of ruminant production

Production type	level of production (x maintenance)			
	0	1	2	3
Maintenance	0.17	-	-	-
Growth (young animal)	0.17	0.24	0.26	-
Growth (mature animal)	0.17	0.12	0.11	-
Milk	0.17	0.22	0.23	0.24
Microbial growth (in rumen)	0.16 ¹⁾	0.16	0.16	0.16

1) ratio of energy in rumen degradable protein to energy in ADOM

Apart from the RPE required for the various types of ruminant production as such, the microbial population in the forestomachs also has a minimally required RPE. In a similar way as was done for the various types of ruminant production an estimate can be made of the required RPE for microbial growth. Microbial protein production was estimated to be 135 g of microbial crude protein per kg ADOM (Anonymus, 1978). If the energy content of 135 g of microbial CP is assumed to be 3.2 MJ (23.8 MJ/kg CP) and the energy content of 1 kg ADOM to be 20.0 MJ (20% protein with 23.8 MJ/kg; 5% lipids with 39.7 MJ/kg; 75% carbohydrates with 17.6 MJ/kg) this results in a required ratio of energy in rumen degradable protein (RDP) to energy in ADOM of 0.16. The major part of energy in RDP will originate from degraded dietary crude protein, but some may come from endogenous sources such as protein and NPN in saliva or urea transferred from the blood to the rumen. The minimal ratio energy in total dietary CP to energy in ADOM required to maintain an optimal microbial activity and protein production is likely to be higher than 0.16, because part of the dietary CP will not be available for the microbes and under most conditions this part will be more than can be compensated for from an endogenous supply of RDP.

As can be seen from table 2 under certain conditions the required RPE for microbial activity is likely to exceed the PRE required for ruminant production as such (e.g. growth in mature animals). Under such conditions the requirements of the microbial population becomes predominant. A similar

situation may occur with other types of ruminant production if a large proportion of the dietary protein is undegradable for the micro-organisms.

The ratio in which energy in protein and total energy are ultimately supplied to the ruminant animal as absorbed nutrients varies and may differ widely from the ratio in which both are present in the ingested feed. If the supply of protein in the small intestine of ruminants was related to the intake of metabolisable energy (ME), Oldham and Tamminga (1980), by reviewing data from the literature, found a maximum of between 2.5 and 3.0 g NAN (Non-ammonia-N) per MJ ME ingested. This could be calculated further to a maximum value for the ratio energy in protein to total energy in absorbed products. For this a maximum value of 0.25 was estimated (Tamminga, 1982). Because of the interference of microbial fermentation in the rumen this ratio cannot directly be assessed from the ratio energy in protein to gross energy in the diet or dietary components, such as forages.

In forages such as fresh grass or early cut silage or hay a ratio energy in protein to gross energy of 0.30 is no exception. Under such conditions protein will be lost in the rumen because microbial protein degradation exceeds microbial protein synthesis. The energy in this lost protein (mainly in volatile fatty acids) is however still available to the animal as an energy source.

Forages with a low N content also occur and a ratio energy in protein to gross energy of 0.15 is quite possible i.e. for mature hay, or in maize silage. Microbial protein synthesis may then exceed microbial protein degradation, provided an adequate N-supply for the microbes can be maintained by urea in saliva or from the blood after passing through the rumen wall. Under such conditions there is a net increase of protein in the forestomachs.

Energy as a determinant of microbial protein synthesis.

Microbial protein gives a large contribution to the protein supply of ruminants. It is therefore of interest to extent somewhat further on factors which may limit microbial protein synthesis. Various extensive reviews on this subject were published recently (Hespell and Bryant, 1979; Harrison and McAllen, 1980). From these it becomes clear that a number of factors may be important, but that the energy supply from the fermentation of ingested feed is usually the first limiting factor, and only in rather extreme cases will a shortage of rumen degradable protein limit microbial protein production (Satter and Slyter, 1974; McMeniman and Armstrong, 1977).

In animal feeding energy is often expressed in apparently digestible or metabolisable joules, irrespective of the origin. In "feeding" the microbes in the rumen with energy the situation is somewhat more complicated. Not all energy sources yield the same amount of energy in ATP if degraded by the microbes under anaerobic conditions which prevail in the forestomachs. The most efficient energy sources in this respect are carbohydrates, preferably carbohydrates which are degraded at a moderate rate. Easily degradable carbohydrates (sugars, starch) will yield ATP at such a fast rate that per unit of time ATP production will be maximum, but no longer per unit of carbohydrates (moles), because under these conditions degradation of carbohydrates follows faster but less efficient biochemical pathways. Carbohydrates which are degraded very slowly will probably yield their maximum amount of ATP per mole of carbohydrates fermented, but rate of ATP-production is so slow that an increasing proportion will be needed for maintenance of the microbial population and net microbial growth and protein production will remain low.

Compared with carbohydrates, proteins are an inferior source of energy in ATP for the microbes (Tamminga, 1979; Demeijer and van Nevel, 1979). Lipids are an even more inferior source of ATP for the microbes, because they cannot be degraded further than into long chain fatty acids and some volatile fatty acids due to a further degradation of glycerol.

Both proteins and lipids may however have a specific effect on microbial growth. Although microbes in the rumen can survive and grow quite adequately on NPN only, yields of microbial mass and of microbial protein are better when at least part of the N for microbial growth comes from true protein or at least from amino acids (Hume, 1970; Maeng et al., 1976). It has been suggested that lipids specifically inhibit protozoa (Demeijer, 1981). As a result the predation of bacteria by protozoa may become reduced, which in turn may decrease turnover of protein in the rumen and enable an (energetically) more efficient microbial protein synthesis. Indeed very efficient microbial protein productions were observed when fat rich diets were fed to sheep (Knight et al. 1979).

In considering forages with respect to the conditions mentioned above, it is beyond doubt that forages in a rather young stage of growth have advantages. Their cell wall carbohydrates are not encrusted yet with lignin and/or silica and their digestibility is therefore high. Under such conditions a maximum proportion of the ingested organic matter will be

digested, of which a rather fixed proportion of some 0.65 will be digested in the forestomachs. So a maximum amount of energy in ATP can be extracted from the ingested organic matter by the micro-organisms, resulting in a maximum amount of microbial protein to be produced. Moreover the rate of degradation of such forages is probably high enough to enable a high growthrate, resulting in an efficient growth and net protein production. Indeed indications were found that forage rich diets result in a more efficient microbial production than concentrate rich diets (Mc Meniman et al., 1976). In exceptional cases however, particularly with fresh grass, the level of free sugars may be so high that it affects rumen fermentation, with the possibility that microbial protein production becomes less efficient.

A young stage of growth of forages coincides with a high protein content, particularly when fertilised with high amounts of N (Deinum and Sibma, 1980). Consequently a significant amount of the organic matter digested in the forestomachs may be protein hence limiting the amount of energy in ATP extractable by the microbes from the organic matter.

Fertilisation with high levels of N also may result in a high nitrate content which may occur in (fresh as well as conserved) forages (Geurink et al., 1979; Deinum and Sibma, 1980). No harmful effects on the microflora in the rumen after the ingestion of high amounts of nitrate are known; it can be used to eliminate part of the surplus of "reduction equivalents" normally resulting from the fermentation process in the rumen. It may however cause detrimental and often lethal effects to the host animal due to a microbial conversion into nitrite so that high levels of nitrate in forages better be avoided. In this respect conserved forages (hay, silage) seem more dangerous than fresh forages (Geurink et al., 1979), as nitrate from the former is released more easily.

PRODUCTION OF ANIMAL PRODUCTION FROM FORAGE PROTEIN

The most important aspect of forage utilisation by ruminants is undoubtedly the "upgrading" of its protein, as such not suitable for human consumption, to high quality animal protein. The importance of microbial fermentation in this matter has been emphasized already. A crucial factor in protein utilisation by ruminants is a protein to energy ratio in which ruminal protein losses are avoided or at least kept to a minimum. The utilisation of forages is no exception in that respect.

The proportion of the protein supplied to the animal which is needed for maintenance is a second important factor by which the utilisation of protein is affected. For the farmer this maintenance protein in fact is a loss. The proportion required for maintenance becomes lower with an increasing level of production. Hence, if the animals genetic potential permits it, the conversion of feed protein into animal protein will be more efficient with higher levels of production.

Ruminant production from forages is often determined by a restricted feed intake rather than by the animals maximum genetic potential. Ways to overcome this problem are increasing the rate of digestion as well as increasing the density of digestible nutrients in forages. Both can be achieved by supplying the forage to the ruminant animal in an early stage of maturity. A drawback for an efficient forage protein utilisation is then very often the high ratio of protein to energy in the forage, causing substantial protein losses in the rumen. Ways to improve this situation are to protect the excess protein against ruminal degradation (1), to remove the excess protein before feeding the forage to a ruminant (2) or to supplement the forage with an additional source of energy (3).

In a grazing situation with an intensive grassland production only the last method seems feasible, but even this is often too expensive. It seems therefore more appropriate to accept protein losses in the rumen under these intensive grazing conditions.

In feeding conserved forages the situation is somewhat different. Protecting protein from degradation in the rumen seems possible either by artificial drying or by adding chemicals during conservation, such as formic acid or formaldehyde. Because of the increasing fuel prices artificial drying is losing its attraction very rapidly. Treatment of forages with chemical preservatives in order to decrease protein degradation will only result in an improved protein utilisation if the protein supply relative to the energy supply in the unpreserved forage was limiting. With our present knowledge this seems only the case under rather few conditions, such as fast growth in young animals or in high producing dairy cows in negative energy balance (see table 2).

The use of preservatives seems largely restricted to unwilted silages. It should be realised that an inadequate protein to energy ratio in the absorbed nutrients is easier achieved with feeding silage than with feeding other forages of similar quality i.e. crude protein content because of a

reduced microbial protein production with silages. The effect of additives in silage making is often twofold. It not only protects forage protein from degradation in the rumen, but microbial fermentation in the silage becomes also restricted (McDonald, 1981). As a result more substrate for rumen microbes remains available and hence more microbial protein can be synthesized. A disadvantage of protein protection is that under- as well as overprotection seems easily achieved, both with heat treatment and with the application of chemicals such as formaldehyde. Then the protein is either not protected enough or not only protected against degradation in the rumen, but also against enzymatic degradation in abomasum and small intestine. Moreover excessive protection may lead to a deficiency in rumen degradable protein which is required by the microbes in the rumen for their efficient growth and protein production. Too high levels of formaldehyde may also inhibit microbial activity in the rumen. We may however conclude that the use of chemicals in forage conservation does have some perspective, but that they should be applied with care.

Wilting the grass before ensiling seems a rather attractive alternative, provided this can be done under good weather conditions. Excessive protein breakdown in the silage is avoided and only relatively small amounts of substrate are converted into VFA, leaving enough substrate for the rumen microbes to maintain a sufficient growth and microbial protein production.

Another approach for eliminating the surplus protein in protein rich forages is to remove the cell contents, which contains relatively more protein than energy, by screwpressing or some other method. From the cell contents a protein concentrate can be produced and for instance be fed to monogastric animals. The residue remaining after extraction of the cell contents contains a large proportion of cell wall constituents but forms an adequate feedstuff for ruminants. Several aspects of the utilisation of forage protein by this procedure are dealt with in other contributions to this workshop.

Finally a surplus of protein in conserved forages can be balanced by supplementing the forage with low protein feedstuffs. This aspect of utilisation of forage protein is also dealt with in other papers of this workshop.

In forages not only a surplus of protein is possible, sometimes forages are deficient in protein, for instance maize silage or matured hay or straw.

In such cases supplementing the forage with some source of crude protein may be beneficial. Provided sufficient peptides and amino acids are available in the rumen degradable part of the protein to maintain optimal microbial growth in the rumen, a simple NPN source is very often quite adequate to eliminate the deficiency. A good alternative seems also to feed a mixture of maize silage with high quality grass hay or grass silage.

In mature hay or straw a low N content and a high degree of encrustation of plant cell walls with lignin and/or silica often coincide, (e.g. in the tropics). Under such conditions adding some crude protein still may not be effective as the microbes also lack energy.

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PAPER 7

AUTHOR: S. Tamminga (The Netherlands)

DISCUSSION

D. Beever (UK)

Could you comment on the maximum values given for the protein/energy ratio in intestinal contents?

S. Tamminga

The value of 0.25 represents a maximum value under optimum conditions.

R.J. Wilkins (UK)

What is the importance of energy source (i.e. fibre v sugar) with regard to the efficiency of microbial protein synthesis?

S. Tamminga

Optimum conditions for microbial protein synthesis occur where diets contains more than 25% of fibre and less than 25% sugar.

B.C. Cottyn (Belgium)

What methods are accepted for measuring protein solubility?

S. Tamminga

These are available in the literature. Measuring soluble protein (Fraction A) does not give data on degradability.

D.L. Mangan (UK)

All soluble protein is not necessarily degradable (e.g. bovine serum albumin).

D. Chamberlain (UK)

Under some conditions some 20-30% of the soluble fraction of soyabean escapes degradation in the rumen.

P.C. Thomas (UK)

Data given on the solubility of soyabean was higher than normal.

S. Tamminga

This was obtained with grazing animals, which is a different situation.

PROTEIN UTILISATION FROM PASTURE

D.E. Beever

Grassland Research Institute
Hurley, Maidenhead, Berks.

The conversion of forage crude protein by ruminants into carcass (including foetal), milk and wool protein comprises several important identifiable processes, which, when considered together, have been shown to dramatically influence the ultimate efficiency of utilisation of ingested protein.

The level, form and availability of the crude protein fraction of the forage will clearly influence the processes of digestion and synthesis which occur in the reticulo-rumen, and these in turn will reflect the relative contribution of the microbial and undegraded dietary protein flowing into the small intestine. Additionally, the amino acid composition of the two major protein sources, and their respective availabilities within the small intestine will influence the composition of the absorbed amino acids, whilst metabolic processes occurring within the small intestinal mucosa may modify the composition of the amino acids which finally enter the circulatory system.

The quantity, form and availability of the ingested energy will influence the processes of digestion and synthesis occurring within the reticulo-rumen, whilst the quantity and nature of the absorbed end products of energy digestion may influence the animal's ability to deposit or secrete the absorbed amino acids as protein. Overlying all these processes, the age, breed and physiological state of the animal will be important determinants of the efficiency with which ingested/absorbed protein is utilised, and in this context, the intake potential of the animal, the endocrinological control of tissue and milk protein synthesis, and tissue protein turnover per se are particularly relevant.

It is within this framework of events that the utilisation of pasture forage protein by ruminants will be considered in this paper, with specific emphasis being placed on aspects of nutrient digestion and supply.

COMPOSITION OF PASTURE FORAGE

The amount and the chemical and physical characteristics of the forage offered to grazing ruminants will clearly influence the quantity and the nature of the forage actually consumed by the animal, bearing in mind the grazing animal's ability to select the forage it consumes. Chemical composition of the forage will be influenced by the nature of the forage grown, the age of the forage at the time of grazing, the rate and pattern of fertiliser application, seasonal effects including the availability of water and locational effects including soil type and fertility. These factors, in turn, will have varying influences on the apparent digestibility of the forage being offered, this being a broad index of nutritive value. Thus, in studies with dairy cattle, Le Du et al. (1981) found the Nitrogen (N) content of perennial ryegrass grazed on a four-week rotation to vary between 13 and 30 g N/kg dry matter (DM), whilst organic matter (OM) digestibility was found to vary between 0.70 and 0.82. Legumes are generally found to have higher N contents (up to 50 g N/g DM) than grasses at similar stages of growth, whilst OM digestibility tends to be lower (0.54 - 0.68) with the exception of white clover which is characterized by persistent OM digestibilities in the range 0.75 to 0.82, whilst the more recently introduced large petiole varieties of white clover (e.g. Blanca) tend to have slightly lower OM digestibilities than the earlier, more prostrate growing varieties such as S100 (Thomson and Beever, unpubl. obs.). In grazing studies with sheep in Australia, Corbett et al. (1982) found the N/OM ratio of Phalaris to vary between 27 and 52 g/kg, whilst Lucerne gave values between 34 and 48 g/kg. On the other hand, these workers found native unimproved pasture to have an N/OM ratio between 23 and 28 g/kg, whilst with poor hill grazing in the United Kingdom, N contents ranging from 6 to 25 g/kg DM and OM digestibilities between 0.50 and 0.78 have been reported (HFRO, Annual Report, 1978). In a recent paper, Mayes and Lamb (1982) reported the N/OM ratio of a heather/agrostis pasture to be 13.5 g/kg with an OM digestibility of 0.49.

Whilst the N content of fresh grazed herbage can vary quite markedly, it is generally recognised that the proportion of non-protein nitrogen (NPN) in the total N is quite high, and that a large proportion of the total N is readily soluble within rumen, although precise estimates of total N solubility are not available owing to the number of different in vitro techniques which have been employed. Some exceptions to these

generalisations do exist, however. Heather and other hill species are generally considered to contain lower proportions of soluble N, and the same applies to the tannin-containing legumes such as sainfoin and lotus.

From this large variation in the form of the forage which may be presented to the grazing ruminant, the animal's positive selection of leaves rather than stems, their negative selection of faecal and urine contaminated forage, and their apparent preference for legumes rather than grasses will all have a major effect on the composition of the diet finally consumed. Furthermore, the pattern of grazing, with a few large meals per day, interspersed with several small meals, will have a major effect on the synchronisation of energy and protein utilisation both in the rumen and at the tissue level.

Clearly, many of these aspects require further elucidation, but if the true dynamics of pasture utilisation are to be fully understood, then effort should be put into these particular areas, an effort which clearly requires the combined talents of a multi-disciplined team including botanists, physiologists, agronomists and nutritionists.

NUTRIENT SUPPLY FROM FRESH FORAGE - indoor studies

Techniques to partition nutrient digestion and absorption in ruminants have been available for some 15 years or so, but it is only in recent years that these techniques have been successfully applied to grazing animals. Prior to this development, attempts to evaluate nutrient supply from fresh forage were restricted to stall feeding, initially with frozen herbage and subsequently with zero-grazed herbage harvested daily.

Early studies at Newcastle University (Beever et al., 1969; 1971; Proud, 1972) showed that when a frozen S24 perennial ryegrass regrowth was fed to sheep, the quantity of total N flowing to the small intestine was only 90% of the amount ingested, whilst N absorbed from the small intestine was equivalent to 62% of N intake. Further calculations by Thomson and Beever (1980), however, revealed that for the frozen grass fed by Beever et al (1971) and Proud (1972), duodenal amino acid N flow amounted to 63% of feed N intake, whilst absorbed amino acid N (i.e. from small intestine) was only 41% of N intake, and this led Beever (1979) to conclude that absorbed or metabolisable protein (MP) supply on this forage in relation to metabolisable energy (ME) supply was equivalent to 7.3 g /MJ.

In a subsequent study designed to consider the effect of reducing the solubility of the forage protein prior to feeding on N digestion and absorption, Beever et al. (1976), using a frozen primary growth S24 perennial ryegrass in which 72% of the total N was found to be soluble in dilute acid-pepsin, reported that duodenal N flow and small intestinal N absorption were 85 and 53% of N intake respectively. In this study, some quite substantial improvements in total duodenal N flow (up to 10 g N/d) and small intestinal uptake of N (up to 7 g N/day) were reported as a consequence of reducing dietary N solubility by heat or formaldehyde treatment, and the following relationship was developed : $TNR = 165 - 1.13S$ ($r = -0.98$) where $TNR = \text{g total N entering small intestine/g N intake}$ and $S = \%$ solubility of dietary N, indicating that the high solubility of fresh forage N leads to a considerable inefficient use of that nitrogen within the alimentary tract of ruminants. Subsequently, Beever (1979) reported that one of the consequences of forage dehydration in this study was to increase metabolizable protein supply from 8.2 g/MJ ME (frozen) to 12 g/MJ ME (oven dried).

In a subsequent study, Beever et al. (1974a) examined these effects in greater detail. A primary growth sward of S23 perennial ryegrass was harvested daily with a flail harvester, and part of the crop was fed directly (to represent fresh herbage) to surgically-modified sheep. In addition, part of each day's crop was blast frozen, whilst a further portion was oven dried, and these were fed later to the same sheep in the order of harvesting date to provide a direct comparison of fresh, frozen and dried herbage. N losses across the rumen amounted to 10 and 7% of the N intake on the fresh and frozen herbage respectively, whilst small intestinal absorption of N was found to be 61 and 67% of N intake respectively. On the other hand, duodenal flow and small intestinal absorption of N on the dried diet amounted to 136% and 93% of N intake, both marked improvements compared with the two non-dried diets. Using sulphur-35 to distinguish microbial protein flow to the small intestine (Beever et al., 1974b) revealed two interesting aspects. Firstly, microbial protein flow on the frozen diet was 25% greater than the value determined on the fresh diet, and this value was further enhanced on the dried diet (+57% compared with fresh forage). Furthermore, the efficiency of microbial nitrogen synthesis on the fresh forage was only 16 g/kg ruminally digested OM, whilst a value of 20 g/kg was recorded on the frozen

diet. Both of these compared rather adversely with the value of 32 g/kg noted on the dried diet.

At about the same time, MacRae and Ulyatt (1974) and Ulyatt and MacRae (1974) in New Zealand completed a study using sheep designed to examine the quantitative digestion of three fresh forages, viz. perennial ryegrass (R), short rotational ryegrass (M) and white clover (C) at DM intakes ranging from 0.45 to 1.0 kg/day. Expressing their results at fixed OM intakes of 0.5 and 0.8 kg/day by using a regression approach, MacRae and Ulyatt (1974) observed that in all situations examined (i.e. 3 forages at 2 levels of intake) non-ammonia nitrogen (NAN) flow to the small intestine was considerably less than N intake with greater between forage (R, 0.66, M, 0.88, C, 0.74) than between level of feeding (low, 0.78, high, 0.73) effects. Overall N digestibility was high on all diets (R, 0.84, M, 0.80, C, 0.82) whilst NAN absorption from the small intestine expressed per unit of N intake varied from a mean value across the two levels of feeding of 0.37 g/g (diet R) to 0.47 g/g (C) and 0.57 g/g (diet M). Consequently urine N was almost 60% of N intake on diet M and this rose to 70 and 74% for diets R and C respectively.

These results not only confirm the other studies reported earlier in this paper, but indicate that the magnitude of some of these effects may be considerably greater. That one third of the total N consumed on diet R failed to arrive at the duodenum as NAN whilst only 37% of N intake was absorbed as NAN from the small intestine on this diet were two important findings which MacRae and Ulyatt (1974) did not possibly emphasize sufficiently. On the other hand, however, their work showed quite clearly that large between-forage species effects with respect to N digestion did exist, with the obvious superiority, in this situation, of short rotational ryegrass (M). Attempts to reconcile these differences, especially at a ruminal level with respect to levels of degradable carbohydrate and nitrogen type substrates were inconclusive, although MacRae and Ulyatt (1974) drew attention to the higher N solubilities (as % of total N) generally recorded on fresh clovers (50%) compared with grasses (35%) and the possible contribution of the high pectin content of clover to the readily-fermentable carbohydrate fraction. Previously Ulyatt (1971) had reported that the readily-fermentable carbohydrate to structural carbohydrate rates on R and M averaged 0.57 and 0.79 respectively, compared with a value of 1.22 on diet C.

When the quantitative digestion data provided by MacRae and Ulyatt (1974) and Ulyatt and MacRae (1974) were compared with two lamb growth experiments conducted by Ulyatt (1971) where lambs grazed the three forages R, M and C, the effect of these changes in sites of digestion and absorbed nutrient profiles can be seen much clearer. These are presented in table 1, with slight modification to the data as presented earlier by MacRae and Ulyatt (1974), but show that the marked changes in live-weight gain observed by Ulyatt (1971) were closely related to the estimated quantity of protein absorbed from the small intestine ($r = 0.79$) poorly related to estimated VFA supply ($r = 0.02$) and showed a remarkably close relationship with absorbed protein to absorbed energy ratio.

In a more recent publication, Ulyatt and Egan (1979) examined the digestion of two contrasting ryegrasses, white clover and sainfoin, which is a tannin-containing legume (Osborn et al., 1971). All forages were fed fresh to mature sheep at two levels of intake and the results clearly established that the rumen was the principal site of water-soluble carbohydrate, pectin, hemicellulose and cellulose digestion. On the two ryegrasses and sainfoin the flow of N to the small intestine was more or less equivalent to N intake; only in the case of white clover was duodenal N flow found to be consistently less than N intake (up to 12% loss of dietary N across the rumen), but overall no statistically significant differences in duodenal N flow and small intestinal N absorption in relation to N intake were found between the four diets.

These results were clearly in sharp disagreement with those referred to earlier. In the study of Ulyatt and Egan (1979) the ratio of N/OM (g/kg) ranged from 38 (sainfoin) to 46 (Tama ryegrass), values which did not contrast widely with those reported earlier by Ulyatt and MacRae (1974) where the range of values for the two ryegrasses and the white clover was 39 to 53 g/kg. Consequently, differing N contents of the crops cannot be put forward as a possible cause of these different effects on N digestion, but Ulyatt and Egan (1979) did suggest that the higher OM digestibilities reported for the forages used in their study compared with the values obtained earlier by Ulyatt and MacRae (1974) and the use of hourly versus twice-daily feeding, as used by Ulyatt and MacRae (1974) may have resulted in a more efficient use of the soluble protein of forages, with an associated reduction in the quantity of N being lost from the rumen.

Table 1. Relationship between observed live-weight gains of lambs grazing perennial ryegrass (R), short-rotation ryegrass (M) and white clover (C) and the quantitative estimates of the end products of carbohydrate and protein digestion (after MacRae and Ulyatt,1974)

	EXPERIMENT 1			EXPERIMENT 2			Correlation with liveweight gain
	R	M	C	R	M	C	
Liveweight gain per day (g/d)	227	270	331	156	241	249	
Estimated VFA absorbed* from rumen and large intestine (MJ/d)	10.17	8.61	9.87	9.36	10.30	9.10	0.02
Protein absorbed (NAN x 6.25) from small intestine (g/d)	119	175	188	113	188	163	0.79
<u>Absorbed protein</u> **	9.18	13.8	13.2	9.41	12.8	12.6	
Absorbed energy (g/MJ)							

* Calculated as 75% of apparent digestion of gross energy from stomach and large intestine

** Includes absorbed VFA and protein energy

Table 2. Dietary characteristics and in vitro and in-vivo nitrogen digestion of fresh sainfoin, fresh red clover and two mixtures

Diet	S	SR	RS	R
Forage content (% DM)				
Sainfoin	100	40	20	
Red clover		60	80	100
<u>Dietary characteristics</u>				
Total nitrogen (g/100 g DM)	3.4	3.7	3.8	3.8
Rumen liquor TCA soluble N (%)	35	48	55	59
Buffer soluble N (%)	24	29	36	38
<u>In vitro observations (for units see footnote)</u>				
Proteolytic activity*	7.8	10.0	9.5	10.4
Deaminating activity**	4.1	5.3	5.0	4.7
<u>In vivo observations</u>				
Duodenal amino acid (AA) flow (% AA intake)	83	65	75	67
Microbial synthesis:				
a) g AA/100 g AA intake	41	33	35	32
b) g AA/100 g degraded AA	61	44	51	44
c) g N/kg ruminally digested OM	27	22	26	21

* mg casein N solubilized/ml rumen liquor

** mg NH₃-N produced/ml rumen liquor

Equally, the nil response with respect to N digestion noted on the sainfoin diet was somewhat surprising, especially in the light of studies by Jones et al., 1973; Reid et al., 1974 and Thomson et al., 1971. In the latter study, Thomson et al. (1971) reported a substantial improvement in N and total amino acid flow to the small intestine for sainfoin compared with lucerne both of which had similar total N contents, and the binding effect of the tannins of sainfoin to the protein, so rendering it less soluble in the rumen with an associated reduction in ruminal loss of N by deamination, was put forward as the most likely mechanism. However, it must be pointed out that the two forages used by Thomson et al. (1971) had been low temperature dried prior to feeding in a chopped state.

Recent experimentation at this Institute (Siddons, Beever and Thomson, unpubl. obs.), however, examined the digestion of fresh sainfoin and fresh red clover which were harvested daily without chopping and fed to sheep either separately or as two mixtures comprising 20:80 or 40:60 sainfoin:red clover. Such a study was undertaken to examine further the role of the tannins in sainfoin on the digestion of N, and whether or not the small addition of fresh sainfoin to a low tannin-containing legume such as red clover may give rise to an enhanced partition of N digestion in the clover. Clearly, such a finding could have beneficial effects with respect to reducing the bloat-inducing potential of legumes such as red and white clover and lucerne.

Some of the results obtained are presented in table 2. Total N content of the sainfoin was approximately 10% lower than that of the red clover, whilst the proportions of total dietary N found to be soluble in either rumen liquor or buffer were markedly lower on the sainfoin diet, with the trend across all four diets being in the expected direction with no suggestion of any interaction between the two contrasting legumes. This reduction in dietary N solubility was associated with quite substantially lower estimates of proteolytic (-25%) and deaminating (-13%) activity on the all sainfoin diet compared with the all red clover diet, and in turn these effects led to quite marked differences being observed in-vivo. Thus, on the all sainfoin diet the duodenal amino acid flow expressed per unit of amino acid intake was 20% greater compared with the three clover-containing diets, whilst microbial synthesis expressed in relation to total amino acid intake, estimated degraded amino acid intake and the amount of OM apparently digested in the rumen were 23%, 33 and 17% higher respectively.

In an attempt to collate all available data on the digestion of forages by sheep, Ulyatt and Egan (1979) produced a series of empirical relationships which examined the ruminal digestion of specific nutrients in relation to nutrient intake or apparent digestibility. From a total of 74 observations, covering both fresh and dried (both suncured and artificial) grasses and legumes, they observed that the ruminal digestion of OM (expressed per unit of OM intake) increased linearly with increasing OM apparent digestibility ($r^2 = 0.71$) and no substantial between-forage differences were noted. This is consistent with the findings of Thomson and Beever (1980) who examined the ruminal digestion of OM for grasses and legumes by sheep specifically, and concluded that for a four-fold range in digestible OM intake, the proportional digestion of OM within the rumen was similar for both types of forage. Ulyatt and Egan (1979) produced similar linear relationships for the ruminal digestion of hemicellulose and cellulose, whilst for N digestion, two distinct relationships for fresh and for dried forages were established with respect to N intake. Thus, at all levels of N intake, predicted total N flow to the duodenum was greatest on the dried forages, and this difference was exaggerated at higher N intakes. For fresh forages, at levels of N intake above 18 g/d, Ulyatt and Egan (1979) found that N flow to the duodenum was less than N intake and concluded that in this situation, for every extra gram of N supplied in the diet, ruminal loss of N amounted to 0.45 g. On the dried diets, the corresponding values were 25 g N intake/d, and 0.13 g N loss across the rumen per extra gram of N supplied, re-emphasizing the inefficient utilisation of N which occurs during the ruminal digestion of fresh forage.

The recent study by Mayes and Lamb (1982) represents one of the first attempts to consider the effect of supplementation on the digestion of poor quality fresh herbage. Thus, when a heather/agrostis diet was fed to sheep indoors, Mayes and Lamb (1982) reported that NAN absorption (3.6) amounted to 56% of N intake, whilst urea (3.5 g N/d) and starch supplementation increased NAN flow and absorption by 3.4 and 1.4 g/d respectively. Urea supplementation enhanced microbial N flow by 2.1 g N/day (equivalent to 60% of added urea N) and on both diets microbial N comprised 59% of total NAN flow, whilst gross efficiencies of microbial N synthesis were 34 and 31 g N/kg ruminally digested OM respectively. Calculation of feed N degradability, assuming complete degradation of all

supplement N showed the value to decline from 38% to 14%, whilst NAN absorption per MJ ME (assuming 15.1 MJ/kg DOM - see Corbett et al., 1982) amounted to 1.03 and 0.94 g/MJ for the control and supplemented diets respectively. These data clearly illustrate that NAN flow on the low quality control diet was poor and whilst urea and starch supplementation increased estimated ME intake by 52%, no significant improvement in NAN supply per MJ ME was detected.

- Grazing studies

Indoor studies on the digestion and utilisation of fresh (or frozen) forage can only represent first approximations of processes which occur within the grazing ruminant, a situation where the animal is constantly interacting with its environment, and in particular the forage which is on offer. Consequently, the nature and the amount of the diet selected, and the pattern of feed consumption may enormously influence the ultimate nutrient supply and utilisation which occurs in grazing ruminants.

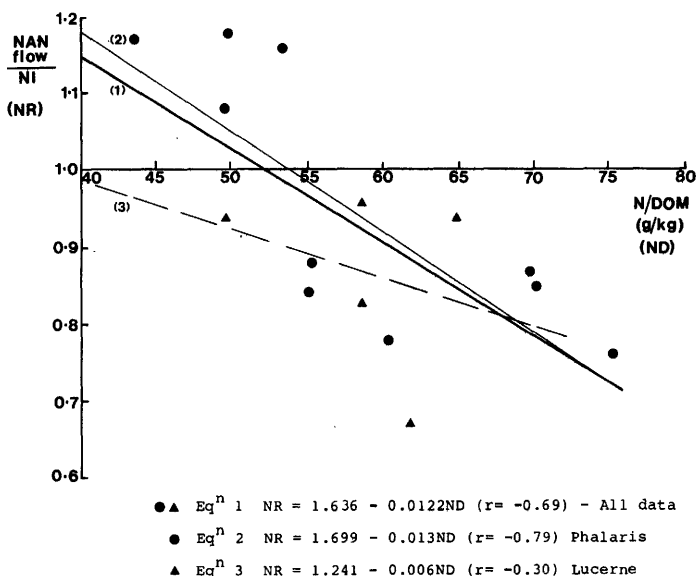
The development of portable infusion pumps for sheep (Corbett et al., 1976) and for cattle (Evans et al., 1981a) and portable duodenal sampling apparatus for cattle (Evans et al., 1981b) has greatly enhanced our ability to measure nutrient supply of animals at pasture. This progress has been quite recent, and consequently, experimental data which have been obtained using such techniques are limited. Furthermore, the experimentation which has been carried out has tended to concentrate on examining the nutrient supply of contrasting forages, and to date there has been no progress on examining in detail how the interaction of the animal with its environment may influence nutrient supply. Clearly, as the reliability of such techniques develops, and more research groups become involved, attention will need to be directed towards these aspects if the dynamics of the grazing system are to be better understood and more reliable assessments of the nutritive value of grazed forage are to be achieved.

Over the past four or five years, Corbett and his colleagues in Australia have carried out a considerable number of experiments designed to measure nutrient supply in sheep grazing a variety of pastures. Thus, Corbett et al. (1982) were able to present data relating to 23 experiments in which phalaris, lucerne and native pasture were examined. In presenting so much data, however, the authors tended to look for generalisations in their results and a full discussion of individual responses was not given. Thus, they reported that the mean fractional

outflow rates of rumen water was high on all three forages (range 0.196 to 0.240 h⁻¹) but failed to comment that the range in values for individual diets was from 0.10 to 0.31 h⁻¹. Equally, they reported that mean NAN flow to the small intestine was 0.93 of N intake, but did not discuss the range in values which was from 0.64 to 1.30 with mean values for the three diets being : phalaris, 0.90; lucerne, 0.84; and native pasture, 1.06. They reported that mean NAN digestibility in the small intestinal was 0.66. and added that the decline in individual values for small intestinal availability from 0.78 to 0.59 appeared to be directly related to decreasing quantities of NAN entering the small intestine.

Fig.1. Relationship between diet composition and non-ammonia nitrogen flow per unit of N intake for 16-22 kg sheep consuming Phalaris (●) or Lucerne (▲)

(Data from Corbett et al 1982. All data relating to heavier sheep has been omitted).



A closer examination of these data, however, is illustrated in fig. 1. where, adopting the approach suggested earlier by Hogan and Weston (1970), the flow of NAN per unit of N intake has been related to the chemical composition of the diet. Thus, for phalaris, a strong relationship between NAN flow and N/DOM composition was established, indicating that N losses between mouth and duodenum occurred in forages containing in excess of 54 g N/kg DOM, or at 34 g N/kg OM, assuming the average OM digestibility of phalaris reported by Corbett et al. (1982). Comparable values for lucerne were 37 g N/kg DOM and 26.5 g N/kg OM, but with the limited number of observations for lucerne (n = 5) it is difficult from these data to establish significant between-forage differences. Consequently, until more data become available, use of the overall relationship, indicating NAN flow equality with N intake at 52 g N/kg DOM, is recommended. Of the original 23 observations reported by Corbett et al. (1982), 8 were removed from the regression analysis due to their obvious non-conformity with the main body of the data. What is interesting about these observations, however, is that they were all obtained with heavier sheep (30 kg +), and suggests body weight in relation to level of feeding may be an important determinant of rumen function which hitherto has been ignored.

Table 3. Flow of organic matter and non ammonia nitrogen to the small intestine (g/kg live weight) of cattle grazing ryegrass or white clover

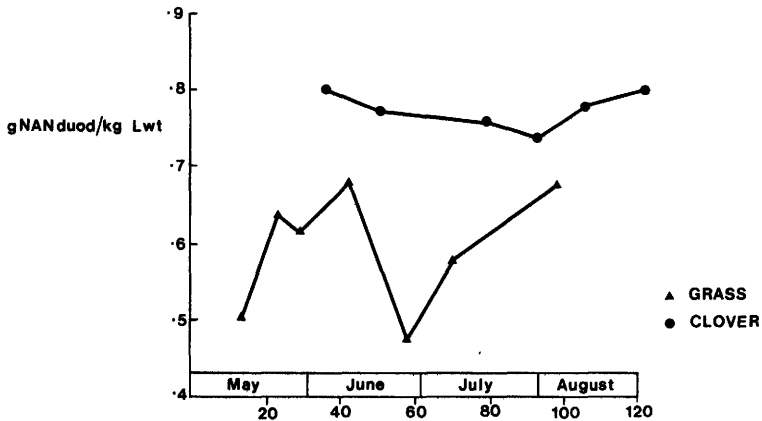
Diet	Month	OM flow (g/kg lwt)	NAN flow (g/kg lwt)	NAN flow/DOMI [†] (g/kg)
Ryegrass				
Primary growth	- May	13.0	0.93	34
Early regrowth	- Early June	10.2	0.69	33
Late regrowth	- Late June	9.9	0.65	38
White clover				
Primary growth	- July	13.3	0.75	48
Regrowth	- August	13.1	0.80	49

[†] DOMI calculated assuming partition of OM digestion reported by Beever et al. (1980) when identical diets were fed to housed cattle.

At the Grassland Research Institute studies on the nutrient supply to grazing ruminants commenced in 1979 when pure contrasting swards of perennial ryegrass and white clover were examined using growing cattle (Ulyatt *et al.* 1980). A total of five forages were examined and the results are presented in Table 3. OM flow to the duodenum ranged from 9.9 to 13.3 g/kg lwt. with both clovers and the primary growth grass giving values of 13 g/kg whilst NAN flow was highest on the primary grass (0.93 g/kg lwt.), intermediate on the 2 clovers (mean 0.78 g/kg) whilst the value on the late regrowth grass was only 70% of the value observed for early May grass. Assuming the OM digestion data obtained by Beever *et al.* (1980) with the same diets fed to housed cattle, Ulyatt *et al.* (1980) concluded that NAN flow per unit DOMI was remarkably similar for the three grasses (35 g/kg) and almost 40% less than the value recorded for the two clover diets (48.5 g/kg).

In the following year, Losada *et al.* (1982) extended this study to cover more of the grazing season, and in total, using 20 surgically modified cattle, examined the digestion of 7 ryegrasses and 6 white clovers. The results relating to NAN flow to the small intestine/kg liveweight when the animals were offered equal allowances of forage DM are shown in Fig. 2, where it can be seen that for the grass fed animals, the value

Fig.2. Changes in the flow of non-ammonia nitrogen to the small intestine (g/kg lwt) of cattle grazing either ryegrass or white clover between May and August 1980.



ranged from 0.47 to 0.68 g/kg. Large fluctuations were seen, especially in early May and late June, whilst at no time did the values observed for grass achieve the values for white clover which ranged from 0.74 to 0.80, with no apparent seasonal effects. When the results were expressed in relation to estimates of DOMI however, a large part of this between forage difference was removed, although the clover diets were generally superior to the grass diets and some seasonal effects were still discernable.

The indoor study mentioned earlier (Beever et al. 1980) also measured the ME content of the five diets, with methane production being measured by open circuit calorimetry (Cammell et al. 1980).

At a level of feeding equivalent to 22 g/kg body weight, Beever et al (1980) reported that ME proportion of digestible energy varied from only 0.78 to 0.81, and CH₄ production was constant for all diets (2.23 moles/kg DOMI) with the exception of the primary growth ryegrass, which had a value of only 1.87 moles/kg. This led Beever et al. (1980) to conclude that the five forages had ME contents ranging from 10.3 to 12.1 MJ/kg DM, whilst NAN absorption from the small intestine per unit of ME intake (g/MJ) assuming 70% availability of NAN in the small intestine ranged from 1.36 to 1.57 on the three grasses compared with a mean value of 2.00 on the two clovers. These compare with later estimates by Corbett et al. of 1.91 to 2.09 g/MJ for all three forage species examined.

In the data obtained by Losada et al. (1982) it was found that in 8 of the diets examined NAN flow was less than N intake and this led Losada et al. (1981) from a regression of N content in the forage OM (NO) on NAN flow in relation to N intake (NR) ($NR = 1.54 - 0.019 NO$ ($r = -0.89$)) to conclude that the grass and the white clover diets appeared to adhere to one single relationship, that with diets containing more than 28 gN/kg OM net losses of N between mouth and duodenum did occur and that with some diets this loss could be equivalent to up to 45% of N intake.

Additionally, Losada et al. (1982) reported that rumen ammonia concentrations fluctuated widely during the grazing season. Thus, the mean daily values for the grass diet varied from 4 to 10 mg NH₃-N/100 ml rumen liquor during May and June, and only in late August did values approaching 20 mg/100 ml occur. On the other hand, for the clover diets, all values exceeded 20 mg/100 ml, and in mid August, peak mean values approaching 40 mg/100 ml were observed.

To date there are surprisingly few estimates of feed N degradability for fresh forages and most of these are with stall fed sheep. Furthermore, the results are inconclusive with values ranging from 50% (Beever et al. 1974) for S24 perennial ryegrass to 70% (Ulyatt et al. 1975) for short rotational ryegrass and 74% for subterranean clover (Hume and Purser, 1975).

Data presented recently by Corbett et al. (1982) appears to be more consistent, where for Phalaris and lucerne (both early and late season) and Native pasture (early season) apparent feed protein degradability estimates ranged from 82 - 98% of protein intake and only with later grazed Native pasture was a significant reduction in degradability (69%) observed. These results are clearly in agreement with the generally high rumen ammonia concentrations noted by Corbett et al. (1972), and by Losada et al. (1982), and the considerable loss of N which may occur during ruminal digestion. Against these suggestions of a rapid rate of proteolysis and deamination occurring in the rumen of fresh forage animals, the microbial yields reported by Corbett et al. (1982) which ranged from 27 to 49 g microbial N/kg OM apparently digested in the rumen are surprisingly high and worthy of further investigation. In a recent experiment, Losada (unpubl. obs.) used ¹⁵N to distinguish microbial N in duodenal digesta of cattle consuming zero grazed ryegrass or white clover as early, mid or late season growths. Currently, the only results available suggest that microbial N comprised over 80% of duodenal N in all six situations examined, but data on apparent efficiencies of microbial synthesis are not yet available.

PROTEIN UTILISATION

It is still the currently held view that the majority of forages supply an excess of total N or digestible crude protein, and consequently performance of animals fed fresh forage is unlikely to be limited by the supply of amino acids. The data on the digestion of fresh forages presented in the previous section provides a rather different situation however and it would appear safe to conclude that in most situations, absorbed N supply in relation to N intake or ME supply is low, whilst legumes appear to be superior to grasses in these respects.

Several experiments have considered the effect of protein supplements to cattle and sheep consuming fresh forages, and generally the results have been quite encouraging. Gordon (1974) fed two concentrates contain-

ing either 90 or 210 g crude protein/kg DM to dairy cows consuming fresh grass and reported milk yield increases of 0.6 and 0.8 kg/kg concentrate fed. These were larger than the average milk yield responses to energy supplementation of 0.4 kg milk/kg concentrate reported by Thomson (1982) from a review of 63 experiments, Rogers et al. (1980a) on the other hand considered the form of the protein supplement, and when either un-protected or formaldehyde treated casein were fed at a standard rate of 1 kg/day to cows grazing a grass of high digestibility and total N content the milk yield responses were 0.5 and 2.0 kg/d respectively. Further support of a differential milk yield response to varying protein sources in the supplement is provided by Penning and Treacher (1982). Lactating ewes were fed fresh grass alone or with either a high energy supplement (barley and maize starch) or one of three barley:protein supplements in which the protein was provided either as soyabean meal, fishmeal or a mixture of the two protein sources. On average, all supplements reduced forage OM intake by 15% with no between supplement effects being detectable. Energy supplementation alone increased total OM intake by 23% but there was only a 4% increase in milk yield. On the other hand the protein supplements increased milk yield by 12, 25 and 24% respectively, and provided further evidence of the superiority of fish meal as a protein supplement to fresh forage. Equally, in a study with lactating ewes grazing ryegrass, Orr et al. (1982) used lamb growth rates over the first six weeks of lactation as an assay of milk output and showed fish meal supplementation to increase lamb growth by 12 and 19% respectively at low and high herbage allowances for the ewes. On the other hand, a response to energy supplementation (+11%) was only recorded at the high herbage allowance.

Barry et al. (1982) considered the effect of abomasal infusions of casein and methionine on protein utilisation in 4-month old lambs receiving a zero grazed ryegrass/white clover diet. Protein supplementation was calculated to increase small intestinal absorption of protein from 60 (control) to 99 g/day, and this was found to be associated with a 25% increase in liveweight gain (control 79 g/d; infused 99 g/d). Total protein deposition on the other hand was increased by almost 70% (12.6 and 21.0 g/d respectively), and a 10% reduction in fat deposition was noted. Overall estimates of Kf were unaffected by protein supplementation, but Barry et al. (1982) found the proportion of total body energy

retention present as protein to be increased from 0.27 to 0.41.

In attempting to explain these differences, Barry et al. observed no differences in whole body protein synthesis and only a small increase in glucose irreversible loss. On the other hand, a marked increase in thyroxine concentration was observed, along with small increases in insulin and glucagon levels, and a significant reduction in growth hormone levels. These results led Barry et al. (1982) to conclude that whilst the response in protein deposition may in part have been due to an increased supply of essential or total amino acids, changes in the endocrine system due to an elevated protein supply could be equally or more important.

In a similar experiment, Black et al. (1979) showed an abomasal infusion of casein to young lambs at pasture to enhance liveweight gain and wool growth by 34 and 1.9 g/day respectively, whilst an abomasal infusion of glucose gave no improvements in either parameters.

Thus it would appear from such data that animals consuming fresh grass will respond to protein supplementation, thus tending to confirm that in many situations, protein supply to animals consuming fresh forage alone may be inadequate. Two possible alternatives to using expensive protein concentrates are worthy of consideration. By a more careful choice of the forage species, it would appear that protein supply can be successfully manipulated. In their comparison of three forages, MacRae and Ulyatt (1974) reported a strong positive correlation between liveweight gain in grazing sheep and predicted amino acid absorption from the small intestine. In experiment 1, reported in Table 1 of this paper, it can be seen that a 69 g/day increase in protein absorption on the white clover diets, led to over 100 g/day improvement in liveweight gain. In a more recent forage species comparison, Rogers et al. (1979) considered ryegrass and white clover offered at ad libitum or restricted levels to lactating cows. At ad libitum levels, a 30% improvement in milk yield was observed on the white clover, whilst at equalised digestible DM intakes, the response was a 10% improvement on the clover diets. In a subsequent experiment, Rogers et al. (1980b) reported that at ad libitum levels of feeding, cows offered white clover, or a 50:50 white clover:ryegrass mixture consumed more DM than cows receiving ryegrass only, and milk yields were increased by 3.6 and 2.2 l/day respectively. These results are in agreement with the results of

Beever et al. (1980) which showed absorbed protein/MJ ME to be increased from 9 to 13 g/MJ as a consequence of changing from a ryegrass to a white clover diet.

The other alternative is to manipulate rumen fermentation, with the specific aim being, through a more controlled rate of proteolysis and deamination, to enhance the efficiency of transfer of ingested N to duodenal N. This would have two obvious benefits. The uptake of amino acids from the small intestine should be increased if duodenal supply is enhanced, unless the latter is brought about by a marked increase in the quantity of undegradable protein flowing to the duodenum, which in turn may have a reduced availability in the small intestine. Secondly, it should lead to a reduction in net ammonia absorption from the rumen. On the basis of ARC (1980) proposals, it can be calculated that when forages containing 30-35 g N/kg DM are fed to dairy cows, ruminally degradable N (RDN) supply may exceed RDN requirement by up to 300 g/day, assuming 90% degradability of dietary N, a microbial uptake of N equivalent to 30 g/kg ruminally digested OM and a DM intake of 18 kg/d. With smaller animals, fed at lower levels of intake, Beever et al. (1980) reported values of up to 50 g/day as the difference between N intake and NAN flow to the small intestine. Taking the calculated value for the dairy cow, if this excess RDN is lost as ammonia absorbed across the rumen wall, then this amounts to almost 13 g $\text{NH}_3\text{-N/hr}$ if constant absorption is assumed, whereas actual rates of absorption could vary considerably around this value if the effect of feeding pattern is taken into account.

In a recent paper, Symonds et al. (1981) reported that the liver of dairy cows could extract up to 15 mmol NH_3/min , and above this level arterial NH_3 concentrations were seen to rise, with quite dramatic clinical changes occurring in the cows until at a mean NH_3 infusion rate of 28 mmol/min, the animals became recumbant. These values equate with 12.6 and 23 g $\text{NH}_3\text{-N/hr}$, which are alarmingly close to the values calculated earlier. To what extent high NH_3 absorption on fresh forages may affect the animals, with the strong likelihood of a reduction in voluntary intake and hence production is impossible to determine at present, but clearly this is an important aspect which may be reducing overall animal performance from fresh forage.

Currently work is in progress at this Institute to consider the effects of either formaldehyde or monensin on N digestion in the rumen of cattle fed fresh ryegrass or white clover. The only results to date indicate a quite substantial decline in ruminal NH_3 levels on treatments, but final assessment of the overall effects in terms of NH_3 absorption and NAN flow to the small intestine is not yet available.

CONCLUSION

That amino acid N supply from fresh forage is low in comparison with N intake and ME supply appears indisputable. The mechanisms responsible for such inefficiencies are now being elucidated more fully, and the animal performance responses to supplemental N appear to justify more research effort into improving N utilisation in fresh forage. How these improvements are brought about is open to considerable debate but manipulation of rumen fermentation and a more careful choice of forage type appear to offer hope, whilst this may also be a long term problem which the plant breeders ought to address themselves to.

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PAPER 8

AUTHOR: D. Beever (United Kingdom)

DISCUSSION

S. Tamminga (The Netherlands)

Your data showed a reduced synthesis of microbial protein on herbage diets. Is this associated with energy supply? We have measured rumen pH value of 5.5 in grazing cows indicating rapid breakdown of soluble carbohydrates.

D. Beever

This is not thought to be due to carbohydrates. Rumen pH was not measured but ammonia levels were high. There was a shortage of amino acids which are required for microbial protein synthesis.

W. Sheehan (Ireland)

We have obtained improvements in animal production from premixing diets. Could this be due to the synchronising energy and protein supply in the rumen?

D. Beever

Yes, but this is not always the case.

THE UTILIZATION OF SILAGE NITROGEN

P. C. Thomas and D. G. Chamberlain

The Hannah Research Institute,
Ayr, Scotland

ABSTRACT

The ensilage of forage crops leads to hydrolysis of plant proteins with possible destruction of some of the amino acids released and to the formation of microbial fermentation products from plant soluble carbohydrates. This paper briefly considers the influence of the ensilage process on forage composition and then reviews information on the utilization of silage N in sheep and cattle, dealing mainly with aspects of nitrogen digestion in the rumen and intestines and with amino acid supply and metabolism in the tissues. It is concluded that silage nitrogen may be used with a low efficiency. There are limitations on the supply in the small intestine of some indispensable amino acids, methionine in particular and possibly lysine, valine and threonine, depending on the animals physiological state. With high protein silages the efficiency of nitrogen utilization is also reduced because of the losses of nitrogen arising from the absorption of ammonia in the rumen. The relationship between the composition of silages and the utilization of silage nitrogen is discussed and there is consideration of the effects of supplementary protein and energy foods.

INTRODUCTION

The nutritive value of conserved forage is heavily dependent on the species and variety of the crop conserved, the weather conditions during the crop's growth, and the crop-husbandry practices, for example the rates and timings of fertilizer applications and the stage of maturity and frequency at which the crop is harvested. Additionally, however, the method of conservation is important and particularly for crops conserved as silage the details and effectiveness of the silage-making procedure have a crucial impact on the characteristics of the conserved food. This paper is concerned with aspects of the utilization of silage nitrogen (N) and will concentrate primarily on the results of nutritional experiments with sheep and cattle given grass or grass-clover silages. First, however, it is appropriate to briefly consider the main effects of silage-making on herbage composition.

EFFECTS OF SILAGE MAKING ON HERBAGE COMPOSITION

Herbage cut at the immature state conventionally used for silage making has a high water-soluble carbohydrate content and a high N content. Approximately 75-90 % of the N is present in protein form, some as water-

soluble cytoplasmic proteins in the cells of the leaves. The remainder of the N is present in free amino acids, peptides, amines, ureides, nucleotides, chlorophyll, nitrates and amides, particularly glutamine and asparagine. Following the cutting of grass at harvest plant water-soluble carbohydrates are consumed by the plant's continuing respiration. Similarly, the action of the plant proteases leads to hydrolysis of proteins with the formation of peptides and free amino acids, though there is very little amino acid deamination. For example, over a 26 hour period of wilting Brady (1960) found that the proportion of non-protein nitrogen (NPN) in ryegrass increased from 89 to 173 g/kg total N; this was associated with an increase in α -amino N from 26 to 92 g/kg total N but with an increase in volatile N from 1 to 6 g/kg total N.

Metabolism of plant water-soluble carbohydrate continues after the grass has been placed in the silo. However, as conditions become anaerobic carbohydrate breakdown is increasingly accounted for by the action of homofermentative and heterofermentative lactic acid producing bacteria or, under adverse or poorly controlled ensilage conditions, by clostridia. The lactate producing organisms form lactate and acetate as main products of fermentation but small amounts of propionate, ethanol, mannitol and 2,3 butanediol can also be found in 'lactate' silages. The clostridia produce butyrate as a main fermentation product and clostridial fermentations typically show high levels of butyrate, often in association with acetate (see McDonald, 1981). Protein breakdown in the silo is always extensive with the exception of where silage additives containing high levels of formaldehyde have been used to provide a substantial degree of protein protection (see Barry, 1976). In well-preserved silages, including those made without additives but with a satisfactory lactate fermentation and those made with additives containing formic acid or mineral acids, protein breakdown reflects hydrolysis to amino acids, and possibly small peptides, with little amino acid degradation. The hydrolysis has been attributed to the activity of plant and microbial proteases, and the stability of the free amino acids can be explained by the restricted capacity of the lactic acid bacteria for amino acid fermentation (see McDonald, 1981). However, present understanding of the hydrolysis is far from satisfactory since there is clear evidence that the process continues after microbial fermentation of carbohydrate has ceased and at pH values as low as 3.5, where plant enzymes seem unlikely to be very active (Carpintero et al, 1979). The importance of chemical

hydrolysis of herbage proteins under these acid conditions does not appear to have been established. In inadequately-preserved silages, and particularly clostridial silages, protein hydrolysis is accompanied by amino acid degradation with deamination, decarboxylation and Strickland-type exchange reactions yielding ammonia and a range of amines including putrescine, cadaverine, histamine, β -phenylethylamine, ethanolamine, tryptamine and tyramine (McDonald, 1981).

Thus the main effects of silage-making on herbage composition can be regarded to be the loss in herbage water-soluble carbohydrate and the associated formation of fermentation end-products, and the breakdown of plant proteins. Moreover with respect to the latter, silages can be classified on the basis of the extent to which protein hydrolysis is accompanied by further degradation of the amino acids formed. The ammonia ($\text{NH}_3\text{-N}$) content of silages is a good index of amino acid deamination and a satisfactory classification can be made using the content of $\text{NH}_3\text{-N}$ in the total N. Typically in well-preserved lactate silages made without additives, or in silages made with acidic silage-additives applied at conventional U.K. rates, non-protein nitrogen (NPN) accounts for 400-650 g/kg total N but $\text{NH}_3\text{-N}$ is only 50-100 g/kg total N. In silages prepared with formaldehyde additives, or with acidic additives used at high application rates, NPN may be as low as 200-400 g/kg total N with $\text{NH}_3\text{-N}$ being 30-80 g/kg. With poorly preserved silages, NPN is generally 55-75 g/kg total N and $\text{NH}_3\text{-N}$ may be as high as 30 g/kg, depending on the degree of amino acid breakdown.

NITROGEN UTILIZATION IN THE RUMINANT

The utilization of N in ruminants has been investigated using a range of experimental techniques, each with its own particular advantages and limitations. As yet it has not been technically feasible to study more than a few aspects of N digestion or metabolism simultaneously in a single animal, and to gain an overall picture of N use requires an amalgamation and rationalization of data derived from the various types of experiments. A simple descriptive model of N utilization in the ruminant has been proposed as a basis for protein rationing (Agricultural Research Council, 1980) and more sophisticated computer simulation models dealing in detail with aspects of the digestion and/or metabolism of N are being developed (see Beever, 1978; Black, 1982). However, to date, almost all the data used as a basis for both the rationing and computer models have been

derived from studies with fresh or dried forages. Silage diets have only recently begun to receive the attention which their agricultural importance warrants and information on the digestion and metabolism of silage N is still scanty. The situation is also made difficult by the fact that the data available are derived from experiments with a range of silage types and comparisons between experiments which could give an insight into the effects on N utilization of, for example, silage energy and N content, are to a degree confounded by experimental differences in the method of ensilage or the quality of forage preservation achieved. Here therefore particular features of the digestion and metabolism of silage N will be illustrated using selected results from experiments employing a variety of investigational techniques, and following that some important aspects of silage N utilization will be highlighted for discussion.

NITROGEN RETENTION

There have been a large number of experiments in which the efficiency of utilization of N as measured by N retention has been used as a basis for comparisons between silages or between silage and corresponding fresh or dried forage (for references see Wilkins, 1974, 1978; Barry *et al*, 1978). However, in many of these studies feeding has been ad libitum and under those conditions the low efficiency of utilization of N from silages may be exaggerated because of limitations on silage consumption, and hence energy intake. Experiments in which forages have been given in rationed amounts indicate that N retention is affected by silage fermentation and whilst in some cases silages give a much poorer N retention than fresh forage or hay in others N retention varies little with the type of forage (Waldo *et al*, 1965; Durand *et al*, 1968). It is difficult to identify with complete certainty the features of silage that lead to reduced N retention because where fermentation is extensive and fermentation quality poor there are a large number of associated compositional changes. Nonetheless, there seems a good case a priori for scrutinizing the relationship between N retention and the composition of the silage nitrogen fractions. The evidence summarised in Table 1 suggests (1) that where silage $\text{NH}_3\text{-N}$ content is low and fairly constant (Expt 1) N retention declines as the proportion of NPN in the total N increases, and (2) that where NPN content is fairly constant (Expts 2 and 4) N retention declines with increasing $\text{NH}_3\text{-N}$ content. Where both NPN content and $\text{NH}_3\text{-N}$ content change together, as is often the case (Expt 3), there will be a dual effect on N retention.

Table 1 Nitrogen retention in sheep given silages containing varying proportions of non-protein nitrogen (NPN) and ammonia-nitrogen (NH₃-N)

Expt	Silage composition		Nitrogen retention (g/d)	Nitrogen retention (g/kg apparently digested N)
	NPN (g/kg total N)	NH ₃ -N (g/kg total N)		
1a	453	20	4.1	242
	551	41	2.9	169
	674	55	0.4	31
2b	694	137	2.5	119
	745	207	-1.6	-94
3b	560	108	0.8	49
	422	39	2.0	97
4b	641	118	2.2	94
	645	165	0.2	9

^a Results from Fujita (1976). The 3 silages were prepared by ensiling a sample of grass/clover for periods of 27, 66 and 132 days.

^b Results from Durand et al (1968). The silages were prepared from lucerne and were wilted and metabisulphite treated (Expt 2), wilted and AIV treated (Expt 3) and wilted and unwilted (Expt 4).

Studies on the influence of supplementary feeds on silage utilization have consistently shown responses in N retention to additional allowances of cereals (see Wilkins, 1978) but care must be taken in such circumstances to distinguish between responses arising simply as a result of increased dietary energy supply and those related to some true improvement in the efficiency of N use. In recent years at the Hannah Research Institute a number of calorimetric experiments have been made in which N retention and metabolizable energy (ME) intake have been determined simultaneously in animals receiving a silage diet at a maintenance level of feeding or the same diet supplemented with further silage or with barley. Selected observations from these experiments in which supplemented treatments have had equal N intakes are shown in Table 2. There is a clear demonstration that the response in N retention to increased silage intake is low and that, judged per unit of additional digestible N or ME, the efficiency of dietary N utilization is greatly improved by a barley supplement. Syrjala (1972) has shown that sucrose supplements have an even greater effect than starch supplements on N retention.

Responses in N retention to carbohydrate supplements could be interpreted to indicate that the retention of silage N is limited primarily by the amount of energy constituents in the diet, but retention has also been increased through the intraabomasal infusion of casein (Hutchison *et al.*, 1971) and that indicates that there are limitations in the amount or composition of the amino acid supply to the tissues. Consistent with this, increases in N retention can be achieved through supplementation of silage diets with high protein foods (Table 2) though the efficiency of utilization of the supplementary protein N is not very high.

Table 2 Nitrogen and metabolizable energy (ME) intake and nitrogen retention in sheep given diets of silage or silage with barley or groundnut meal

Expt	Diet	N intake (g/d)	ME intake (MJ/d)	N retention		
				(g/d)	(g/kg digested N)	(g/MJ increase in ME intake) ^b
1 ^a	Silage	14.3	7.43	2.1	213	
	Silage	19.6	10.21	2.9	213	0.15
	Silage + barley	19.9	11.96	5.0	377	0.65
	SED		0.22	0.3	21	
2 ^a	Silage	15.7	8.25	2.1	168	
	Silage	20.6	10.76	2.7	170	0.22
	Silage + barley	21.3	12.01	4.9	304	0.75
	Silage + groundnut	34.8	11.51	5.9	203	1.15
	SED		0.57	1.2	4.4	

^a Results from Kelly and Thomas (1978) and unpublished experiments. In each case the low level of silage intake supplied close to a maintenance level of ME intake. This low level was then supplemented with further silage, with barley or with groundnut meal.

^b Calculated as a response with respect to the low level of silage intake.

RUMEN AMMONIA CONCENTRATION

Ammonia absorption from the rumen, and the associated excretion of urea-N in the urine, increases with the rumen NH₃-N concentration, which in turn reflects the balance between the production of NH₃-N from the degradation of dietary N constituents and its fixation by the rumen microbes. Early evidence of especially high concentrations of NH₃-N in

the rumen in silage-fed animals was obtained by Fatianoff et al (1966) and subsequently the observation has been confirmed and extended in numerous experiments (Durand et al, 1968; Syrjala, 1972; Thomas et al, 1976; Beever et al, 1977; Farhan and Thomas, 1978; Siddons et al, 1979; Morgan et al, 1980a; Thomas et al, 1980; Wernli and Wilkins, 1980; Chamberlain et al, 1982b). The information now available indicates that rumen $\text{NH}_3\text{-N}$ concentration varies both with the N content of the silage and with the method of silage making. Ensilage techniques that reduce the proportion of NPN in the silage, in particular the application of formaldehyde (Beever et al, 1977) or of mineral acids (Durand et al, 1968) tend to reduce rumen $\text{NH}_3\text{-N}$ levels, though a consistent relationship between silage NPN proportion and rumen $\text{NH}_3\text{-N}$ concentration is not always observed. For example, in four silages made from the same grass but with formic acid added at rates of 0, 2.3, 4.6 and 5.9 l/t the proportion of NPN was progressively reduced from 560 to 400 g/kg total N but when the silages were given to sheep there were no significant differences in rumen $\text{NH}_3\text{-N}$ concentrations between treatments (Chamberlain et al, 1982b).

Rumen $\text{NH}_3\text{-N}$ concentrations are reduced when silages are supplemented with readily fermented carbohydrate foods, presumably because of an enhanced microbial fixation of ammonia (see below). The reduction in $\text{NH}_3\text{-N}$ concentration varies with the amount and type of carbohydrate supplement used and notably is greater with sucrose than with starch (Syrjala, 1972). This difference might be related to the relative rates of fermentation of sucrose and starch in the rumen since with silage diets the peak in ammonia production is reached rapidly (approximately 1 hour after feeding), and for optimum microbial fixation there is a need to synchronise NH_3 and energy supplies (Blackburn, 1965). Chamberlain et al (1980) investigated this topic in sheep by varying the time at which a meal of barley supplement was given relative to the time at which the animals received a meal of silage. However, it was found that irrespective of whether the barley was given 2 hours before or together with the silage the effect on rumen $\text{NH}_3\text{-N}$ concentration was the same. An alternative explanation for the differences between carbohydrate supplements is that their influence is exerted via effects on the composition of the rumen microbial population. This explanation has yet to be fully explored but there is circumstantial evidence in its support. As compared with sucrose supplements, starch supplements lead to a much increased protozoal population in the rumen (Table 3), and rumen $\text{NH}_3\text{-N}$ concentration varies with the number of protozoa (Table 3).

Table 3 Rumen ammonia concentrations and protozoal counts in animals given diets of silage with starch or sucrose supplements and in faunated and defaunated animals

Expt	Diet and treatment	Rumen NH ₃ -N (mg / l)	Protozoa (n x 10 ³ /ml)
1a	Silage	231	7.7
	Silage + starch	205	15.1
	Silage + sucrose	160	6.3
	SED	30	2.05
2b	Silage + barley (Faunated)	222	16.9
	Silage + barley (Defaunated)	176	0
	SED	20	

^a Chamberlain et al (1982a)

^b Chamberlain et al (1980)

THE DEGRADABILITY OF SILAGE NITROGEN

Since the introduction of the new protein rationing system (Agricultural Research Council, 1980) considerable attention has been given to the measurement of the rumen-degradability of dietary proteins. Various techniques based on laboratory procedures or on the incubation of feeds in sacco, in polyester bags suspended in the rumen, have been devised, but the in sacco method has been most widely accepted and used (see Thomas and Chamberlain, 1982a). The technique has been used to measure the degradability of silage N and high degradability coefficients of 0.75-0.85 have been obtained (Agricultural Research Council, 1980; Brett et al, 1981; Crawshaw et al, 1981). At first sight these values appear to be consistent with the high rumen NH₃-N concentrations observed with silage diets but a disturbing feature of the results is that the degradability values vary little with the type of silage; the values are uniformly high even for silages made with the addition of formaldehyde (c.f. in vivo values, see below). Recently Siddons et al (1982) have undertaken a comparison between in sacco incubations and various laboratory tests based on solubility in buffer, in vitro incubation with rumen fluid and incubation with solutions of acid or neutral proteases. Without exception laboratory methods gave lower degradability values for silage N than those measured

in sacco. In particular, solubility in buffer and in vitro incubation with rumen fluid gave degradability values of approximately 0.20 for silages made with a high level of addition of formaldehyde, whilst for the same silages in sacco tests gave values of approximately 0.75.

ABSORPTION OF NITROGEN IN THE RUMEN

Over the past few years a considerable amount of information about the absorption of silage N in the rumen has been obtained using animals fitted with cannulae in the abomasum or proximal duodenum. The digestion of more than 35 silage diets has now been studied in experiments with sheep or young cattle and a reasonably consistent picture has begun to emerge. It should be stressed, however, that the range of silage types investigated so far is narrow and is confined almost entirely to well-preserved silages of low $\text{NH}_3\text{-N}$ content, in many cases prepared with the application of additives containing formic acid and/or formaldehyde. The literature has been reviewed recently (Thomas, 1982; Thomas and Chamberlain, 1982b) and here attention will be confined to a summary of the main findings.

It is clear from the data available that with individual silages the flow of N from the rumen to the duodenum can be substantially less than the amount ingested in the diet, i.e. there is a pronounced net loss of N due to $\text{NH}_3\text{-N}$ absorption in the rumen. For example, in sheep given a silage of high N content (313 gN/kg DM) made without an additive Beever et al. (1977) reported that the N intake was 32.6 g/d and the duodenal flow of N was 27.8 g/d. This pattern of loss of N across the rumen is not inevitably observed, however, and in many experiments the amount of total N flowing to the duodenum is similar to the amount ingested in the diet. Data for duodenal N flow and dietary N intake for sheep given a wide range of silage diets are summarised in Figure 1. A clear relationship is demonstrated but the high correlation coefficient reflects in part differences in food intake between the various experiments from which the results are drawn. A more detailed analysis of results for selected silages, so as to avoid the confounding effects of protein protection by silage additives containing formaldehyde, indicates that net losses of N between the diet and the duodenum will begin to occur consistently as the crude protein content of the diet is raised above 160 g/kg DM (Table 4). There is insufficient information to undertake a similar analysis for formaldehyde silages but there are clear demonstrations in the literature that N absorption in the rumen can be reduced by the application of

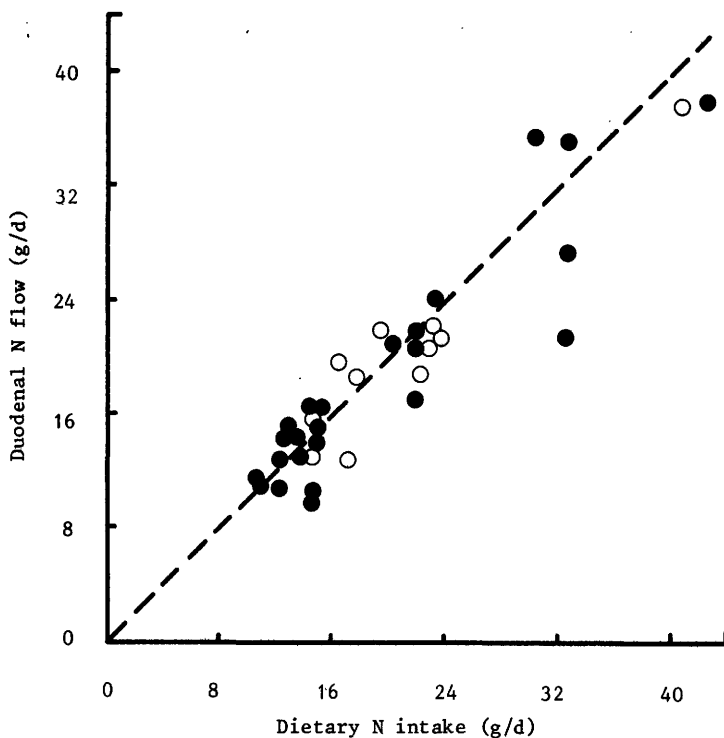


Figure 1 The relationship between the duodenal flow of total nitrogen and the dietary nitrogen intake in sheep given diets consisting of silage given either (●) alone or (○) together with a small quantity of cereal or protein supplement. (The broken line represents the line of equality. The relationship is described by the equation $Y = 2.91 + 0.859 X$ ($r = 0.93$, $n = 34$). Results are from Thomas et al, 1976; Beever et al, 1977; Gill and Ulyatt, 1977; Unsworth and Stevenson, 1978; Gill et al, 1979; Thomas et al, 1980; Morgan et al, 1980b; Chamberlain and Thomas, 1982a; Chamberlain et al, 1982b).

formaldehyde additives at appropriate levels (see Thomas, 1982).

Table 4 The ratio of duodenal N flow: dietary N intake for silage diets of different crude protein content. (Values are means \pm SE for diets of silage made without additive or with formic acid, and fed alone or with carbohydrate supplements. For references see Figure 1)

Dietary crude protein (g/kg DM)	Ratio of duodenal N flow : dietary N intake
130-140 [†]	1.08 \pm 0.02 (n = 7)
140-150	1.02 \pm 0.03 (n = 4)
150-160	1.11 \pm 0.01 (n = 2)
160-170	0.96 \pm 0.03 (n = 4)
190-200	0.75 \pm 0.04 (n = 3)

[†] Range e.g. 130 to < 140

MICROBIAL UTILIZATION OF NITROGEN IN THE RUMEN

In some experiments duodenal N has been fractionated to estimate the proportions of undigested dietary N and rumen-synthesised microbial N. Such fractionations have well recognised technical limitations related to the determination of microbial protein and to assumptions about the amounts and constancy of the endogenous secretion of N in the abomasum (Thomas and Chamberlain, 1982a), but they provide the only satisfactory basis for the estimation of the microbial degradation of dietary N in vivo. Using results from experiments in which microbial N had been estimated using ³⁵S or α - ϵ -diaminopimelic acid and assuming that endogenous secretions were approximately 1.5 gN/d, Thomas (1982) concluded that the degradability of silage N varied over a wide range and particularly with the nature of the additive applied at ensilage. For silages made without additive a mean degradability coefficient of 0.82 (0.78 - 0.86, n = 3) was calculated, whilst corresponding values for silages made with formic acid, and with additives containing formaldehyde were 0.63 (0.52 - 0.81, n = 8) and 0.33 (0.31 - 0.36, n = 3), respectively.

Measurement of the flow of microbial N to the duodenum allows the estimation of the rate of microbial protein synthesis in the rumen. For dried forage diets the rate of synthesis is typically 30-36g N/kg organic matter (OM) digested in the rumen (see Thomas, 1973) but it is evident that with silage diets much lower rates of synthesis are usual. The

results summarised in Table 5 indicate a mean value of 20.8 g N/kg OM digested for diets consisting solely of silage though there is an indication from the limited information available that the mean rate may be higher for diets containing silage in combination with a substantial proportion of concentrate foods. The cause(s) of the low rates of microbial synthesis with silage diets have yet to be fully established. Rates lower than those with dried forages are to be expected since the yield of ATP in the rumen from the digestion of silage fermentation products will be low (Thomas, 1982). Acetate and butyrate, for example, will make no contribution to ATP production though the main silage-fermentation product, lactate, is fermented in the rumen to propionate and butyrate (Chamberlain et al, 1981) and thus could furnish a net gain in ATP supply. However, the rates

Table 5 The rates of microbial protein synthesis in the rumen in sheep and cattle receiving diets of silage either alone or in combination with concentrate foods. (Results are from Thomas et al, 1976; Beever et al, 1977; Brett et al, 1979; Siddons et al, 1979; Armstrong, 1980; Thomas et al, 1980; Thomson et al, 1981; Chamberlain et al, 1982b; Chamberlain and Thomas, 1982a)

Diet	Rumen microbial protein synthesis (gN/kg OM apparently digested in the rumen)
Silage alone	20.8 ± 1.5 (n = 21)
Silage + concentrates [†]	30.8 ± 1.2 (n = 5)

[†] Diets containing concentrates at rates of 320 - 540 g/kg total diet

of microbial synthesis with some silages (Beever et al, 1977; Thomas et al, 1980) are much lower than can be explained simply in terms of the effects of the silage fermentation products, and also there is no clear evidence of a systematic variation in synthesis rate between silages made from the same grass but prepared with differing contents of lactic and acetic acids (see Chamberlain et al, 1982b). Supplementation of silage diets with urea has been without effect on the efficiency of microbial synthesis (Siddons et al, 1979) and whilst supplements of soyabean meal have induced positive responses in some experiments the effect has not been observed consistently (Siddons et al, 1979; Armstrong, 1980; Thomson et al, 1981). Supplements of sulphur and methionine have also failed to increase the

rate of protein synthesis though they have elicited a marked effect on the ruminal synthesis of microbial lipid (Chamberlain and Thomas, 1982a).

AMINO ACID SUPPLY AND UTILIZATION

Although the high content of free amino acids in silage leads to an elevation in the concentration of free amino acids in the rumen for a short period after feeding (see Durand *et al*, 1968) it seems unlikely that the absorption of amino acids across the rumen wall is of quantitative significance. Thus, as with other types of diet, the technique of duodenal and ileal cannulation can be used to obtain information on the animal's amino acid supply.

Table 6 The content (g/kg amino acids) of indispensable amino acids, histidine and arginine in duodenal digesta, grass silage, rumen bacteria, rumen, protozoa, pepsin and simulated duodenal digesta

	Duodenal digesta ^a	Silage ^a	Rumen bacteria ^b	Rumen protozoa ^b	Pepsin ^c	Simulated duodenal digesta ^d
Threonine	58	56	59	54	81	59
Valine	66	68	63	54	60	64
Methionine	18	16	25	22	15	20
Isoleucine	55	53	67	66	91	64
Leucine	88	84	83	92	90	85
Phenylalanine	54	55	57	66	56	57
Histidine	18	22	20	23	6	20
Lysine	68	38	84	112	7	62
Arginine	46	47	44	51	9	43

^a Mean results for silage diets from Thomas *et al* (1980) and Chamberlain *et al* (1982b)

^b Mean results from samples of bacteria and samples of protozoa isolated from the rumen in sheep receiving silage (Chamberlain and Thomas, 1982a)

^c Values from Taylor (1968)

^d See text

As pointed out by Chamberlain and Thomas (1981) the protein passing to the duodenum in animals receiving silage diets has relatively low concentrations of methionine and lysine. The reason for this can be understood by reference to Table 6 which shows the concentrations of the indispensable amino acids, histidine and arginine in the duodenal digesta from silage-fed sheep, together with corresponding values for the main

proteins contributing to the digesta. It can be seen that the rumen microbes contain the proteins rich in methionine and lysine, and the concentrations of these acids in the digesta simply reflects the presence of a relatively low proportion of microbial protein. To illustrate that this is the situation an amino acid composition for duodenal digesta was simulated by calculation. The proportion of bacterial amino acids was calculated from the mean bacterial content of the duodenal digesta (Thomas *et al*, 1980; Chamberlain *et al*, 1982b) by assuming that 0.8 of the bacterial N was protein N; the endogenous N and protozoal N contributions were each assumed to be 1.5 g/d (Thomas, 1982), and the remainder of the duodenal non-ammonia N was attributed to undigested silage protein. Endogenous secretions were assumed to have the same amino acid composition as pepsin. As can be seen (Table 6), the agreement between the simulated composition and that determined on samples of duodenal digesta was close, the small differences occurring with some amino acids possibly reflecting errors in the amino acid composition assumed for the endogenous secretions.

The coefficient of digestibility of total amino acids in the small intestine of animals receiving silage diets appears to be similar to that observed in animals given diets of hay and will normally lie in the range 0.65 - 0.75 (Harrison *et al*, 1973; Beever *et al*, 1977; Thomas *et al*, 1980). Values at the lower end of this range are observed with silages made with high rates of application of formaldehyde additives (Beever *et al*, 1977).

Table 7 The composition (g/kg total amino acids) of the mixture of indispensable amino acids absorbed from the small intestine in animals given silage diets, deposited in body tissues and secreted in milk and the ratios of absorbed amino acids to tissue amino acids and milk amino acids

	Amino acids absorbed from intestine (A)	Amino acids in body tissue (T) ^a	Ratio of A:T	Amino acids in milk (M) ^b	Ratio of A:T
Threonine	114	133	0.86	109	1.05
Valine	142	129	1.10	155	0.92
Methionine	49	65	0.75	60	0.81
Isoleucine	138	107	1.29	139	0.99
Leucine	235	230	1.02	229	1.03
Phenylalanine	139	113	1.22	116	1.20
Lysine	184	223	0.82	192	0.96

^a From Williams (1978)

^b From Kaufmann (1980)

The digestibilities for individual amino acids vary with the acid concerned but they are generally in the range 0.60 -0.80. In Table 7, mean digestibility values from Thomas et al (1980) have been used with the values for the amino acid composition of duodenal digesta given in Table 6 to calculate the average composition of the mixture of indispensable amino acids absorbed (A); values for the amino acid composition of carcass tissue (T) and milk (M) are also given for comparison. The effect of the low methionine and lysine contents in the duodenal digesta on the mixture of acids absorbed is clearly evident. The A:T and A:M ratios indicate that methionine is probably the 1st limiting amino acid for both tissue and milk synthesis, though it should be stressed that such ratios must be interpreted with caution since they do not allow for differences between amino acids in their efficiency of utilization in the body tissues. The supplies of acids other than methionine - lysine and threonine in the growing animal and lysine and valine in the lactating animal - might therefore also impose limitations on protein synthesis.

No measurements of the efficiency of amino acid utilization in animals receiving silages appear to have been made except in the work of Gill and Ulyatt (1979). Those workers found that in sheep given untreated grass silage, methionine was utilized with an efficiency of approximately 85%. A similar efficiency was also measured when animals were given a formaldehyde treated silage, which would have increased the total quantity of amino acids absorbed from the small intestine. The efficiency was slightly reduced, to 81%, when a specific intraperitoneal supplement of methionine was administered. There have been a number of experiments with sheep to determine the changes in N retention or blood plasma methionine concentration in response to intraperitoneal or intravenous supplements of methionine (Barry et al, 1973; Kelly and Thomas, 1975; Gill and Ulyatt, 1977; Barry et al, 1978). Most of these experiments have supported the concept that methionine is limiting for tissue protein synthesis, though increases in N retention have not been obtained in all circumstances, and with some silages responses have in fact been negative (Table 8). Some studies with methionine supplements given post-ruminally or intravenously have also been undertaken with lactating dairy cows. Rogers et al (1979) found that in Jersey cows given a diet solely of silage there was a significant increase in the yield and content of protein in milk in response to intraabomasal infusion of methionine (12 g/d). However, in contrast, in larger Friesian and Ayrshire cows given a silage-barley diet an

intravenous supplement of methionine (8 g/d) increased milk fat yield and content by approximately 10% but was without effect on milk protein (Chamberlain and Thomas, 1982b). Recent investigations at the Hannah Research Institute of blood plasma responses to methionine infusion have confirmed that the amino acid is limiting for protein synthesis in cows receiving silage-barley diets, but at the same time there has also been confirmation that the lactation response to methionine supplements is predominantly in milk fat (Wong *et al.*, 1982). In neither the experiments at the Hannah Institute nor in those conducted by Bryant *et al.* (1979) has there been any marked effect of methionine supplements on tissue N retention in the lactating cow.

Table 8 The effect of intraperitoneal supplementation with DL-methionine on nitrogen retention in sheep given silages prepared using flail or precision chop harvesting machines and different types of additives (From Barry *et al.*, 1978)

Silage	Nitrogen intake (g/d)		Nitrogen retention (g/d)	
	Control	+ Methionine	Control	+ Methionine
<u>Flail harvester</u>				
Untreated	26.2	27.8	2.9	4.6
Formaldehyde	33.4	34.4	6.2	6.1
Formic acid				
Low rate	20.8	19.3	3.7	1.8
Medium rate	20.7	20.6	3.5	2.9
High rate	27.0	27.0	6.0	4.6
<u>Precision chop harvester</u>				
Untreated	31.1	30.7	3.9	4.7
Formaldehyde	44.8	43.7	6.8	8.2
Formic acid				
Low rate	37.1	36.9	7.9	8.6
Medium rate	38.3	37.5	6.1	5.8
High rate	42.0	41.6	8.3	11.0
SE [†]	± 1.44		± 0.64	

[†] The SE applies to comparisons of methionine treatments within the same line.

SUMMARY AND DISCUSSION

Traditionally the view has been that silage N is utilized with a low efficiency by ruminant animals because the high content of NPN constituents in the food leads to excessive concentrations of $\text{NH}_3\text{-N}$ in the rumen with an associated N absorption and loss as urea in the urine (see Wilkins, 1974). The information given in this paper reaffirms that silage N is often poorly utilized but it also suggests that the basis of the poor utilization may be more complex than previously supposed. The studies with duodenally cannulated sheep now indicate that, at least for well-preserved silages of low $\text{NH}_3\text{-N}$ content, net absorption of N between the diet and the duodenum begins to be observed consistently only when the crude protein content in the diet is in excess of 160 g/kg DM. This implies that with silages of lower crude protein content $\text{NH}_3\text{-N}$ losses from the rumen are counterbalanced by the entry of N in endogenous secretions. The estimated values for endogenous secretions in the abomasum of the sheep are of the order of 1.5 gN/d (Harrop, 1974) so that in sheep given moderate or low protein silages the amount of dietary N absorbed from the rumen would be about 10% of the dietary intake. On the other hand, some ^{15}N studies have suggested that endogenous N secretions, including protein secretions in both the abomasum and the rumen, may be as high as 6.0 g/d (MacRae, 1980), and if this is the case there must be substantial absorption of silage N from the rumen even with low protein silages. The dilemma about the endogenous secretion of N cannot yet be resolved - there is conflict between the high rumen ammonia concentrations observed in sheep receiving silage and the low net absorption of N from the rumen but there is also difficulty in reconciling a large endogenous entry of protein into the gut with the amino acid composition of the duodenal digesta (Table 6).

At present the best interpretation of the information available is that in sheep given moderate or low protein silages the efficiency of N utilization is limited by deficiencies in the supply in the small intestine of some of the indispensable amino acids, methionine in particular, and possibly lysine, threonine and valine, depending on the animals physiological state. With high protein silages, in addition to the effects related to amino acid supply, there will be the effects on efficiency of utilization incurred as a result of the increased net absorption of N from the rumen. This interpretation must be treated with caution, however, since it is the incremental responses in N retention to increased silage N intake that are especially low (Table 1) and the effect of $\text{NH}_3\text{-N}$ loss from

the rumen on N utilization in the animal may increase as the level of feeding is raised. There is also some recent evidence from an experiment with dairy cows (Chamberlain and Thomas, 1982a) that in those animals net loss of N between the diet and the duodenum may occur at a lower dietary protein content than is indicated by the results for sheep summarised in Table 4. At a given silage protein content the rumen $\text{NH}_3\text{-N}$ concentration in cows appears to be higher than in sheep and that is probably the factor determining the quantitative importance of $\text{NH}_3\text{-N}$ absorption.

The amino acid supply in the small intestine reflects the intake of rumen-undegradable protein in the diet and the rumen synthesis of microbial protein. Both must be impaired with over-fermented, and particularly clostridial, silages where there has been destruction of amino acids and excessive formation of short-chain fatty acids in the silo. However, for silages of high preservation quality there are less readily answered questions about the relationships between silage type and composition and intestinal amino acid supply. The uncertainties about the estimation of rumen-degradability of silage-N have already been mentioned, at present they represent an important restriction on the systematic application of ensilage technology in animal production. Whilst grass protein can be readily protected from rumen degradation by the application of formaldehyde at ensilage it is clear that the degree of protection cannot be satisfactorily assessed for ration formulation by use of the in sacco incubation method. Presumably the formaldehyde treated silage contains soluble proteins which pass out of the incubation bag but which are of low degradability. With silages made without additives or with low rates of formic acid addition, the in sacco method may be a more reliable guide since a high proportion of the degradable N is NPN. However, even with formic acid silages there is a significant discrepancy between the values obtained in sacco and those estimated in vivo. Thomas *et al* (1980) suggested that for silages made with formic acid application at the conventional U.K. rate of 2.3 l/t, it might be possible to accept the true protein content of the silage as a measure of resistant, low-degradability protein, which had survived exposure to microbial attack in the silo. More recent experiments (Chamberlain *et al*, 1982) have shown that where fermentation is restricted by high levels of formic acid application the true protein proportion in the silage is increased but then represents a mixture of both 'resistant' and rumen-degradable proteins.

The relationship between microbial protein synthesis in the rumen and

silage composition is also not well understood. As is the case for dried forage diets, there is a relationship between microbial synthesis and energy supply in the diet but there are unexplained differences between silages in the efficiency of the synthesis process and there are uncertainties about the energy value that should be ascribed to the silage fermentation products.

There are also difficulties in reconciling some of the reported effects of silage composition on N retention in the animal with what is known about silage N utilization in the rumen. For example, the work of Fujita (1976) showed that N retention was reduced as the proportion of silage NPN was increased but changes in the proportion of silage NPN were found to have no significant effect on the passage of dietary or microbial amino acids from the rumen to the small intestine (Chamberlain *et al.*, 1982)

As far as supplements for silage are concerned the evidence shows that responses in nitrogen retention may be obtained from protein foods, which enhance amino acid supply in the small intestine directly, or from carbohydrate foods, which promote microbial protein synthesis in the rumen. The effect of the latter on the efficiency of N utilization should theoretically be more pronounced in silages of high protein content (see above). Major questions related to supplementary foods remain to be investigated nonetheless. In particular, there is a need to define more precisely the dose response relationships for the individual amino acids that are apparently limiting for production in growing animals and to understand more fully the responses of the dairy cow to amino acid supply. Such information is necessary to provide a scientific basis for the formulation of concentrate supplements and for the practical application of specific amino acid supplements (Chalupa, 1975). It would also allow a better definition of the limits for increasing animal production through the 'rumen-protection' of forage protein by formaldehyde treatment during ensilage.

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PAPER 9

AUTHOR: P.C. Thomas (United Kingdom)

DISCUSSION

A.S. Jones

Could 'in sacco' degradability data give misleading results due to such factors as the leakage of small particles from the bags'.

P.C. Thomas

Yes. There is no evidence that all soluble protein is degradable.

D. Beever (UK)

Part of the low efficiency of microbial protein synthesis has been explained by the presence of fermentation products in silage. Does the type of protein have any effect?

P.C. Thomas

Non-protein nitrogen compounds in the soluble N fraction are not important with reference to low microbial protein synthesis on silage.

D.L. Mangan (UK)

With regard to the biological value of microbial protein, what is known of the effects of D or L forms of amino acids?

P.C. Thomas

Data is available only for methionine in rats where little difference was found.

J. O'Shea (IRL)

What methods are available for protein protection other than formaldehyde?

P.C. Thomas

Tannins, gluteraldehyde and more recently dimethylolurea.

ASSESSMENT OF THE PROTEIN VALUE OF FORAGES AND ITS EXPRESSION
IN THE NEW PROTEIN FEEDING SYSTEMS

C. Demarquilly and R. Jarrige

Institut National de la Recherche Agronomique
Centre de Recherches Zootechniques et Vétérinaires
Theix, 63110 Beaumont, France.

ABSTRACT

In most countries the protein value of forages has long been expressed as apparent digestible crude protein (DCP). The DCP content depends mainly, if not exclusively, on the crude protein content (CP) from which it can be predicted very accurately. But, although generally suitable for comparing green forages or hays differing greatly in their CP content, the DCP content is not sufficient in many situations, especially for NPN rich feeds such as silages.

The protein value should be measured by the amount of absorbable amino acids supplied directly (undegraded protein) or indirectly (microbial protein) by the forage in the small intestine. New protein feeding systems based on this concept have been proposed in different European countries and in the U.S.A.

Four general approaches have been proposed to the laboratory assessment of protein degradability in the rumen :

- (1) N disappearance from forage suspended in the rumen in fibre bags.
- (2) ammonia release in *in vitro* incubation with rumen fluid.
- (3) N solubility in various solvents (buffers, etc...).
- (4) N digestion with proteolytic enzymes.

These techniques have advantages and limitations.

At present there is no reliable laboratory method to predict the *in vivo* microbial protein synthesis in the rumen. The latter has been obtained from the apparently digestible organic matter (DOM) content of the forage. Mean values of 120 - 135 g of microbial protein per kg of DOM have been assumed in most of the new protein feeding systems, in spite of considerable variation observed between forages.

Each forage contributes to microbial protein synthesis both by the degradable nitrogen and the available energy it supplies to the rumen microorganisms, the extent of this contribution depending on the other feed it is associated with in the diet. Therefore the French PDI system (truly digestible protein in the small intestine) ascribes two PDI values to each forage : one (PDIN) that depends only on the nitrogen content and its degradability, and the other (PDIE) that takes account of its rumen degradable energy content which is estimated from its DOM content. These values can be predicted from the crude protein content, the nitrogen solubility and the crude fibre content (as a predictor for DOM). The PDIN values of feeds are additive as are the PDIE values.

The PDI system, like the other new protein feeding systems, overcomes most of the shortcomings of the DCP system. It allows the present knowledge to be applied into practice. The values used for the different factors will be improved.

INTRODUCTION

The extent to which forages can meet ruminant amino acid requirements depends, firstly, on their crude protein content (CP % DM). It has long been measured and expressed as the apparently digestible crude protein content (DCP % DM). This DCP can be predicted very accurately from the forage CP (table 1). It is a crude description of the protein value. Nevertheless it is generally satisfactory to compare green forages or hays widely differing in their CP and to formulate diets based on these forages, supplemented with concentrates made of cereals and oil cakes, and fed to moderately producing animals.

Table 1 : Relationships between digestible crude protein content (DCP in g/kg organic matter), and crude protein content (CP in g/kg OM) of forages (Demarquilly et al., 1981)

Green forages

$$\text{DCP} = 0.939 \text{ CP} - 40.2 + \Delta \pm 4.8 \quad r = 0.996 \quad n = 1432$$

values of Δ

	1 st growth	2 nd growth	> 3 rd growth
grasses (year of sowing)	+ 3.9	+ 1.4	+ 0.3
grasses (other years)	+ 2.0	+ 1.0	- 2.9
natural pastures	+ 0.5	- 1.6	- 4.9
lucerne	+ 3.8	+ 3.8	+ 3.8
red clover	+ 1.8	- 5.2	- 5.2

Hays

$$\text{DCP} = 0.879 \text{ CP} - 37.3 + \Delta \pm 6.6 \quad r = 0.986 \quad n = 558$$

values of Δ

	1st growth	2 nd growth	> 3 rd growth
natural pasture : upland	- 0.3	← + 0.5 →	
lowland	- 3.1	← - 8.2 →	
grasses : upland	+ 1.1	← + 0.9 →	
lowland	- 1.5	← - 3.2 →	
lucerne	+ 5.1	+ 9.2	+ 3.3
red clover	- 10.1		

Silages : grasses or legumes

$$\text{DCP} = 0.824 \text{ CP} - 29.2 + \Delta \pm 5.7 \quad r = 0.986 \quad n = 118$$

values of Δ

- + 4.1 without additive
- + 1.7 with additives but without formaldehyde
- 5.8 with acid and formaldehyde

Maize silage

$$\text{DCP} = 0.936 \text{ CP} - 35.5 + \Delta \pm 3.2 \quad r = 0.995 \quad n = 113$$

values of Δ

- 0.9 without urea
 - + 0.9 with urea
-

However DCP is not suitable to accurately compare the protein value of forages having the same CP, to describe the influence of the conservation processes, in particular the silage processes, on the protein value of forages, and to formulate diets containing grass silages, roots and other NPN sources. These shortcomings were shown, in feeding experiments, several decades ago. In addition to CP, the protein value of feeds also depends on 1) the proportions of the different N substances, and the extent of their degradation in the rumen ; 2) the amount and origin of the energy available to the rumen microbes ; 3) all the other factors (pH, etc) influencing microbial metabolism ; 4) the metabolizable energy available to the animal, etc. Figure 1 shows the correlation coefficients between these factors and the retained N by growing sheep fed ad libitum fresh herbages, hays or silages (Grenet and Demarquilly, 1982).

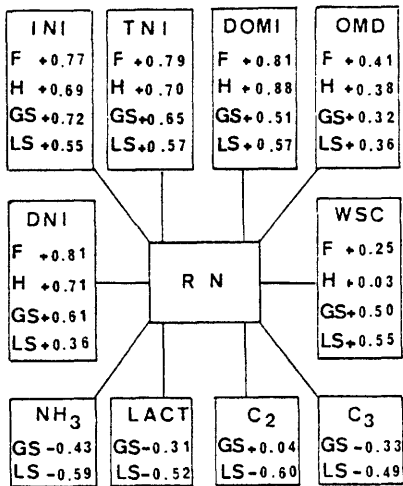


Fig. 1 : Factors affecting retained nitrogen (RN) g/d/kg $W^{0.75}$. Correlation coefficients between RN and various factors are on this figure. Intake in g/d/kg $W^{0.75}$: TNI (total N), INI (insoluble N), DNI (digestible N), DOMI (digestible organic matter) ; OMD : organic matter digestibility % ; WSC : water-soluble carbohydrates % DM ; NH_3 : NH_3 -N % totalN ; in g/kg DM : LACT (lactic acid), C_2 (acetic acid), C_3 (propionic acid). F : fresh forages (n = 29) ; H : hays (n = 25) ; GS : grass silages (n = 87) ; LS : lucerne silages (n = 21) (Grenet et Demarquilly, 1982).

The protein value of feeds is determined by the amount of amino acids available for absorption they supplied to the small intestine directly, as undegraded feed protein, and indirectly, as rumen microbial protein. New protein feeding systems based on that framework have been proposed in different countries to replace the DCP system.

This paper will examine the laboratory methods for predicting the amount of protein absorbed from the small intestine and its expression and use in the new feed evaluation systems.

ASSESSMENT OF THE PROTEIN VALUE OF FORAGES

The protein leaving the abomasum, or entering the small intestine, (PESI = non-ammonia N x 6.25) has been related to its two major determinants : the crude protein intake (CPI) and the digestible organic matter intake (DOMI) for estimating the microbial protein synthesis in the rumen. From their data on 23 dried forages fed to sheep in Australia, Weston and Hogan (1973) calculated a relationship which has been recently modified (Hogan and Weston, 1981) by the inclusion of data from additional herbage diets.

$$\text{PESI} = 0.36 \text{ CPI} + 0.16 \text{ DOMI} + 6 \quad (\text{equation 1})$$

A similar approach has been adopted by INRA (Journet and Vérité, 1977 ; Jarrige et al., 1978 ; Vérité et al., 1979) for forage and concentrate diets as well as for forage alone, but closer relationships were obtained by replacing the crude protein intake by the insoluble protein intake (ICPI).

$$\text{For sheep (158 data) PESI} = 0.57 \text{ ICPI} + 0.140 \text{ DOMI} \quad (\text{R} = 0.87) \quad (\text{equation 2})$$

$$\text{For cattle (72 data) PESI} = 0.72 \text{ ICPI} + 0.129 \text{ DOMI} \quad (\text{R} = 0.77) \quad (\text{equation 3})$$

The general equation adopted :

$$\text{PESI} = 0.65 \text{ ICPI} + 0.135 \text{ DOMI} \quad (\text{equation 4})$$

has been used to build the INRA's PDI system used in France.

These Australian and French equations have the advantage of giving simultaneous, and not independent, estimates of the two determinants of the feed protein value. However, these estimates are not biological constants but approximate mean values of widely varying results (see compilations by INRA, 1978 and ARC, 1980) as discussed later. The values predicted of PESI should be satisfactory for conditions similar to those of the experiments from which they have been established especially for forage-based rations. They should be more accurate than the values predicted by equations relating PESI from only one determinant, CPI (Ulyatt and Egan, 1979) or DOMI (Tamminga and Van Hellemond, 1977).

In the modern protein feeding systems, the rumen degradation of feed protein and the microbial protein synthesis in the rumen have been generally predicted separately (see reviews by Tamminga, 1979 ; Vérité, 1980).

Forage N degradability

Several methods have been proposed for the laboratory assessment of feed protein degradability, but almost exclusively for protein supplements. Their advantages and limitations have been discussed (Vérité, 1980 ; Hogan and Weston, 1981 ; Jarrige, 1980 ; Osbourn and Siddons, 1980 ; Broderick,

1980 ; Stern and Satter, 1980 ; Orskov, 1982). They can be divided into two categories roughly depending on whether the feed is expressed or not to rumen microbes.

In the first group of methods, the feed is incubated with rumen microbes either in vitro or in situ. In vitro incubation is a relatively simple technique which has been widely used (Hendricks and Martin, 1969). The net ammonia production is generally used as the index of ruminal N degradability. The limitations of this method are well-known : variations in the inoculum activity according to diet, viability of rumen microbes, dependance of the ammonia microbial uptake on the available energy, use of a single time interval, etc. Some of these limitations are overcome in the technique based on the nitrogen disappearance from polyester bags suspended in the rumen (Bailey and Hironaka, 1970 ; Orskov and Mehrez, 1977 ; Crawford et al., 1978 ; Broderick, 1980). The disappearance rate can be described (Fig. 2), interpreted and corrected for outflow rate from the rumen (Orskov and McDonald, 1979). The limitations of these fibre bag techniques are the control of the variables, the choice of the retention time of large forage particles in the rumen and the possible overestimation of degradability (see paper by Thomas). However they appear to be the choicest method of producing reference value (see Wilson and Strachan, 1980).

Most laboratories involved in routine feed evaluation cannot use rumen-cannulated sheep. They need simple procedures based on N solubility or on N digestion with proteolytic enzymes. A variety of solvents has been used to predict N degradability : water, sodium chloride, diluted alkali, artificial saliva, phosphate buffer, phosphate bicarbonate buffer, autoclaved rumen fluid ... (Waldo, 1978 ; Vérité, 1980). Only limited comparisons of some of these laboratories procedures have been made (Crawford et al., 1978 ; Crooker et al., 1978 ; Waldo and Goehring, 1979). Classification of feeds were roughly the same but significant interactions of feeds by methods were observed.

Mineral buffers similar to artificial saliva can be considered to dissolve all the NPN components (free amino-acids, peptides, amides, etc) and a small fraction of the forage proteins which is closely related to that soluble in the rumen fluid (Fig. 2). Therefore, they are valuable in estimating the fraction of forage N which is rapidly degraded in the rumen. However, an important proportion of the insoluble proteins may also be degraded. Buffer solubility values are thus lower than degradability values obtained with the rumen in situ procedures (Fig. 3). They are likely to be more reliable predictors of rumen

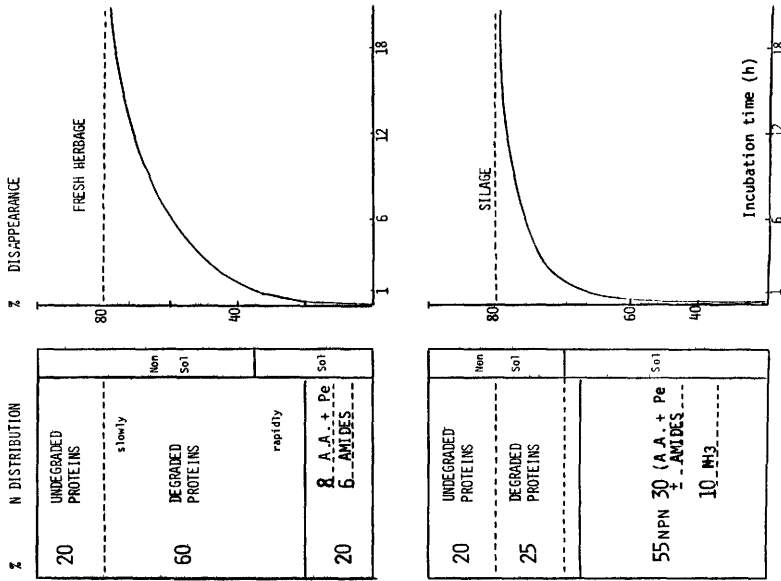


Fig. 2. Typical distribution, buffer solubility and disappearance curve in the rumen of the N components in a fresh herbage and a silage (direct-cut, untreated) made from it (A.A.: free amino-acids, Pe: peptides)

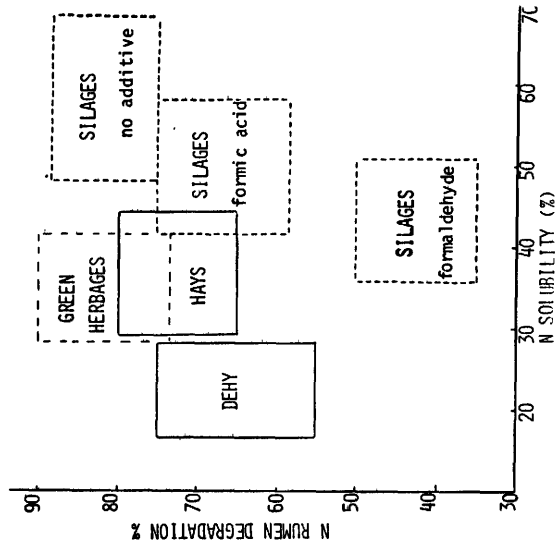


Fig. 3. Diagrammatic relationship between N rumen degradation (from literature - table 3) and N buffer-solubility (from Demarquilly et al., 1978)

degradability when the soluble N is closer to the degradable N content such as in untreated silages.

Solubility of forage N in Wise Burroughs mineral buffer has been found closely related to N disappearance from dacron bags suspended in the rumen (Crawford et al., 1978). The correlation was lower for hay than for silage and depended on the length of the rumen incubation, being highest at 2 h. for silages (0.94) and 4 h. for hays (0.88). For the 34 total mixed diets examined by Stern and Satter (1979) it was high at 1 h. (0.79) and 4 h. (0.74) and then progressively decreased. Solubility of forage N in a mineral buffer solution similar to saliva was also closely related to ammonia production during a 6 h. in vitro incubation in whole rumen contents (Vérité and Demarquilly, 1978). Nitrogen buffer solubility was not a reliable predictor of the in vivo rumen protein degradation for 11 feeds at Hurley (Siddons and Beever, 1977 ; $r = 0.55$) and for 34 mixed diets studied by Stern and Satter (1979) ($r = 0.26$). The latter found that ruminal protein degradation of alfalfa hay and low moisture silage was similar, ranging from 75 to 80 %, despite differences in N solubility, 40 % and 63 % respectively.

The use of proteolytic enzymes should be expected to provide a means of digesting a fraction of the insoluble protein thus better predicting rumen degradability. Pepsin, pancreatin, and proteases isolated from various bacterial and fungal sources have been used, even to partition the feed insoluble proteins according to their rate of degradation (Pichard and Van Soest, 1977). Although giving higher values, pepsin solubility and protease solubility were no more useful than buffer solubility for assessing the effect of additives on silage protein degradability (Siddons et al., 1982). Pepsin N solubility had a lower correlation with in vivo degradability than buffer N solubility in one comparison at Hurley (Siddons et al., 1976) but a higher in another one (Siddons and Beever, 1977) : $r = 0.75$ instead of 0.55 and of 0.42 for pancreatin and 0.32 for pronase. Correlation with undegraded feed protein was greater for solubility in a neutral fungal protease (0.92 to 0.94) than buffer solubility ($r = 0.57$) (and than dacron bag disappearance) in a comparison of 9 classes of protein supplements made by Poos et al., (1980) in Nebraska, but only slightly greater for protease in a comparison on 15 diets at Theix (0.80 instead of 0.75) (C. Genest, 1982).

From these conflicting data, it would appear that the proteolytic enzymes studied so far do not satisfactorily mimic the action of the rumen microbes on insoluble proteins. Buffer solubility, which is a simple laboratory technique, is useful to partition the forages into broad categories

according to their estimated in vivo degradability (Demarquilly et al., 1978, Table 2) and to assess the influence of treatments, such as the level of formaldehyde application, on silage protein degradability (Siddons et al., 1982). Its shortcomings are more important to predict the in vivo degradability of cereals and protein supplements. Especially for those feeds, there is a need for enzymatic methods which would match the adaptation to routine of solubility methods with the better relationships to in vivo degradation supplied by microbial methods.

Microbial protein synthesis

Several in vitro incubation procedures have been developed to estimate the amount of microbial protein synthesised in the rumen. Even conducted under precisely controlled conditions their results may be difficult to extrapolate to the in vivo conditions. These problems should be overcome by the use of continuous artificial rumen fermenters.

At present, the most reliable estimate of microbial protein synthesis can be obtained from the energy available to the rumen microbes once the supply in degradable N (as well as minerals and growth factors) is adequate to meet microbial requirements. Average values of 27 - 35 g of microbial N synthesized per kg of organic matter apparently digested in the rumen have been calculated from the in vivo data. Average values of 120 - 135 g of microbial protein per kg of apparently digestible organic matter (DOM) have been used to build the new protein systems (see Vérité et al., 1979). Therefore the yield of microbial protein allowed by the degradable energy of each forage can be calculated from its digestibility predictors (see inter alia Osbourn and Siddons, 1980 and Jarrige, 1980). In vitro incubation methods, such as the two-stage procedure described by Tilley and Terry (1963) generally estimate forage digestibility with the lowest error. Techniques based upon fungal cellulases appear to be as reliable and have the great advantage of sparing the maintenance of rumen-cannulated animals. But chemical determinations of fibre fractions (Weende crude fibre, Van Soest acid detergent fibre) still remain the basis for digestibility predictions in most routine laboratories.

Protein digestibility in the small intestine

Owing to their surgical and analytical difficulties, precise measurements of protein disappearance in the small intestine are still few and far between (see compilations by INRA, 1978 and ARC, 1980).

Microbial proteins entering the duodenum can be considered as relatively constant in composition and digestibility. There are only a few precise estimates of the digestibility of the rumen undegraded feed protein, because this fraction is determined by difference and is smaller than the microbial fraction. However there is little doubt that this digestibility is variable according to feed sources and treatments. These variations may be the main cause of those reported in the apparent digestibility in the small intestine of total non-ammonia N or total amino N, even if they fall in a narrow range, generally between 65 and 85 % (INRA, 1978 ; ARC, 1980 ; Zinn and Owens, 1980). They may also be the main cause of the variations in faecal N excretion, at least on forage diets. The forage content in crude protein which is apparently non-digestible in the whole digestive tract (NDCP = CP - DCP % DM) can vary from 3.2 to 7.0. It is increased by heating during forage processing and storage. The NDCP content measured on sheep has been used by INRA (1978) to calculate the true digestibility in the small intestine of forage undegraded protein.

There is a need for laboratory techniques to predict this digestibility. Measurement of the proportion of forage N which is insoluble in pepsin HCl or in the acid detergent fiber treatment (ADIN) may be of value to assess the proportion of feed N which escapes both rumen and small intestine digestion. For example, the increase in ADIN is an index of the decrease in N digestibility, caused by heating during forage processing or storage (Goering et al., 1972 ; Thomas et al., 1980).

They are presently no simple laboratory methods based on solvents or enzyme preparations for predicting the three determinants of forage protein value both accurately and cheaply. Better techniques, especially with enzymes, will be developed. However, protein value cannot be expected to be accurately predicted from simple laboratory measurements for all the feed-stuffs on account of the complexity of protein metabolism in the rumen and small intestine. The methods based on the in vitro exposure to rumen micro-organisms are more reliable but cannot be used presently by most of the routine laboratories.

These difficulties do not prevent the concepts of protein digestion from being used in farm practice through the new protein systems. Average values for the protein degradability can be assigned to the main forage categories (Table 2). They are reliable for fresh herbage, normal hays and

moist silages. They can be checked with a laboratory determination of N buffer solubility for treated silages and all heated forages (ADIN content can also be measured). The O.M. digestibility, which is needed to estimate microbial protein synthesis, can be predicted from fibre (Weende crude fibre or acid detergent fiber) and nitrogen content.

EXPRESSION AND USE OF THE PROTEIN VALUE OF FORAGES IN THE NEW PROTEIN FEEDING SYSTEM

Over the past decade, several new protein feeding systems have been proposed to incorporate the broad physiological concepts of ruminant digestion. The protein value of feeds and the protein requirements of ruminants are generally expressed as true protein digested (amino-acid x 6.25) absorbed or absorbable in the small intestine (so-called metabolizable protein). Most of these systems involve summing up the undegraded feed protein and the microbial true protein (generally assumed to account for 80 % of the total N). Those proposed by Burroughs et al. (1975) in the U.S.A., by ARC in U.K. (Roy et al., 1977 ; ARC, 1980) and by INRA in France (Journet, 1975, unpublished ; Jarrige et al., 1978 ; Vérité et al., 1979) are comprehensive. More limited proposals, especially for dairy cows, have been made in Western Germany (Kauffman, 1977) and in Switzerland (Landis, 1979 ; Schurch, 1980). Computerized systems without constant factors are being developed at Cornell (Fox et al., 1979-1980) and in Australia (Faichney et al., 1980). A different approach has been used in the Wisconsin system (Satter and Roffler, 1975) in which the protein value is keyed to ruminal ammonia concentration, or in the Nebraska growth system (Klopfenstein et al., 1980) which is limited to the evaluation of the undegraded protein ("by pass proteins"). The factors involved in several of these systems have been compared for lactating dairy cows (Vérité et al., 1979 ; Waldo and Glenn, 1980-1982) and for growing cattle (Geay, 1980). Most of these new systems need to be tested against conventional systems.

The British (ARC) and French (INRA) systems are structurally similar in the evaluation of the protein value of feeds. They are both factorial and additive and they use almost similar values for microbial protein synthesis. However, they differ in many respects : expression of protein value, digestibility in the small intestine (apparent in the ARC's and true in the INRA) protein requirement especially for maintenance, concept of diet formulation. The INRA system will be taken here as an example of the potential of the new protein systems for forage protein evaluation and use.

Principle and calculation of the PDI content of forage

The PDI content (protéines vraies réellement digestibles dans l'intestin grêle) estimates the quantity of amino nitrogen x 6.25 which is truly absorbed in the small intestine from two sources, 1) the truly digestible amino-N of the feed crude protein undegraded in the rumen (UFCP) and 2) the truly digestible amino-N of the microbial protein (MCP).

The main originality of the system is to ascribe two MCP values and thus two PDI values to each feed (Fig. 4).

- MCPN which corresponds to the maximum amount of microbial protein that could be synthesised in the rumen from the feed degradable N, when the latter is entirely incorporated into microbial protein and energy is not limiting.

- MCPE which corresponds to the amount of microbial protein that could be synthesised from the energy supplied by the fermentation of organic matter when degradable N is not limiting.

In the Feeding Tables (Table 2) the protein value of each feed is given by two values : PDIN = truly digestible UFCP + truly digestible MCPN

$$PDIE = \text{truly digestible UFCP} + \text{truly digestible MCPE}$$

Mean values of the degradability of crude protein in the rumen have been calculated (Table 2) for the main forage categories, from their buffer solubility according to equation(4). The true digestibility of undegraded dietary protein in the small intestine has been estimated from the faecal N, with the assumption that the dietary N which is not digested in the small intestine is entirely recovered in the faeces. It ranges from 0.60 to 0.90. The DMCP contents have been calculated from estimates of the microbial protein yield in the rumen (135 g MCP/kg DOM according to equation 4), the percentage of true protein in the microbial protein (0.80) and the true digestibility in the small intestine of this microbial true protein (0.70).

Table 2 shows the values adopted for the different factors and the PDI values obtained for some typical forages. Demarquilly et al. (1981) have recently published equations for predicting the PDIN content from the CP content ($PDIN = 0.58 \text{ to } 0.66 \text{ CP}$ according to forage category) and the PDIE content from the CP and the crude fibre contents.

When a forage is fed alone its actual protein value is the lower of its two PDI values. The higher one is a potential value which is reached when an adequate complementary feed is associated to the forage (Fig. 5). When calculating the PDI value of a mixed diet, the PDIN and PDIE values of the

Table 2 : Calculation of the PDI values of some typical forages (from INRA, 1978)

Feedstuffs	N buffer solubility	In vivo N degradation	True digestibility of UFCP (1)	DOM	CP	DCP	DUFCP (1)	PDIN	PDIE	
										(g/kg DM)
Green herbage										
Pasture grass, leafy	0.30	0.55	0.75	684	168	121	57	109	109	
Pasture grass, early heading	0.30	0.55	0.75	661	131	86	45	85	95	
Perennial rye-grass, early heading	0.30	0.55	0.75	673	112	68	38	72	89	
Italian rye-grass, leafy	0.30	0.55	0.75	710	169	122	58	109	111	
Italian rye-grass, early heading	0.30	0.55	0.75	651	97	59	33	63	82	
Lucerne, early flowering	0.30	0.55	0.85	567	190	146	73	131	116	
Hays, straws and dried forages										
Meadow, 1st cut, early heading	0.35	0.58	0.70	609	124	74	37	77	83	
Meadow, 1st cut, flowering	0.35	0.58	0.70	518	87	41	26	54	65	
Italian rye-grass, 1st cut, heading	0.35	0.58	0.70	590	81	36	24	50	69	
Lucerne, 1st cut, bud stage	0.35	0.58	0.80	542	178	129	60	118	101	
Lucerne, 1st cut, flowering	0.35	0.58	0.80	493	134	87	45	89	83	
Wheat straw	0.20	0.48	0.70	388	35	0	13	22	43	
Dried pelleted lucerne	0.20	0.48	0.80	546	176	114	73	121	114	
Silages										
Meadow, early heading, L.C.	0.65	0.77	0.60	661	131	84	18	75	68	
Meadow, early heading, F.C.	0.60	0.74	0.65	661	131	84	22	76	72	
Meadow, early heading, F.C., F.A.	0.50	0.68	0.70	661	131	84	30	79	80	
Italian rye-grass, 1st cut, heading, L.C.	0.65	0.77	0.65	651	97	57	13	55	62	
Italian rye-grass, 1st cut, heading, F.C.	0.60	0.74	0.65	651	97	57	16	57	66	
Italian rye-grass, 1st cut, heading, F.C., F.A.	0.50	0.68	0.70	651	97	57	22	59	71	
Lucerne, 1st cut, bud stage, F.C., F.A.	0.50	0.68	0.75	593	195	148	48	121	92	
Lucerne, 2nd cut, 5 weeks, F.C.	0.65	0.77	0.60	603	220	174	30	125	76	
Lucerne, 2nd cut, 5 weeks, F.C., wilted	0.50	0.68	0.70	585	220	174	50	133	94	
Maize > 28 % DM	0.50	0.68	0.75	672	82	42	20	51	71	
Roots										
Fodder beets (19 % DM)	0.85	0.90	0.75	832	90	51	7	52	69	
Sugar beet pulps (dried)	0.20	0.48	0.75	739	100	52	39	66	98	

F.C. : fine cut ; L.C. : long cut ; F.A. : formic acid
 (1) DUFCP truly digestible fraction of the rumen undegraded feed crude protein (UFCP)

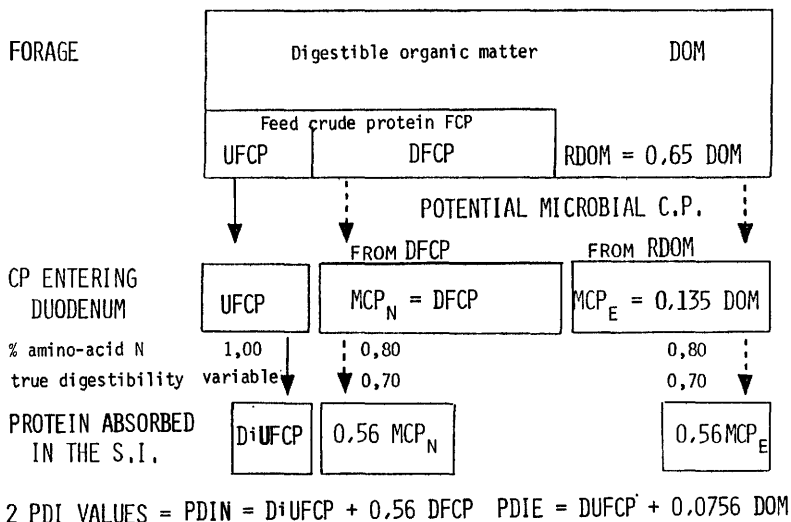


Fig. 4. Calculation of the PDI contents of feeds (INRA, 1978)
 UFCP : rumen undegraded feed crude protein ; DFCP : rumen degraded feed crude protein ; MCP : microbial crude protein.

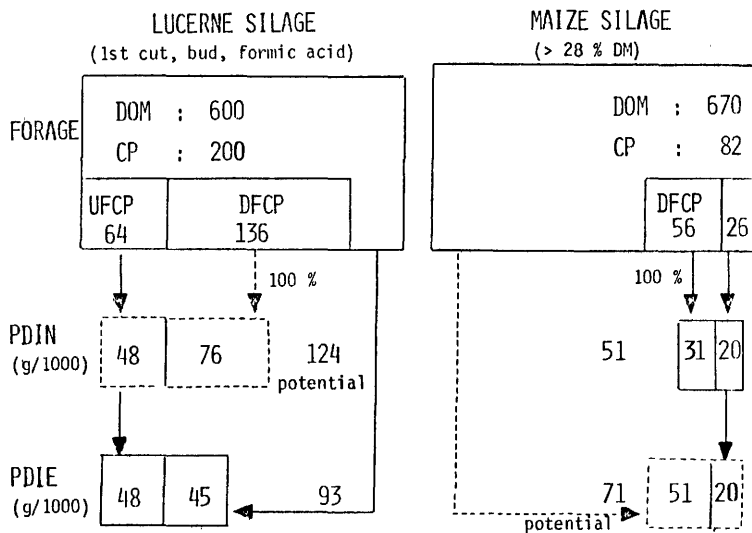


Fig. 5. Compared PDI content (g/kg DM) and complementary between lucerne silage and maize silage.

different diet ingredients are each summed separately. The actual PDI value of the diet corresponds to the lowest sum.

Some advantages of the PDI system over the DCP system

Description of the protein value of individual forages. The PDI system overcomes most of the shortcomings of the DCP system in the evaluation and utilisation of forage protein. It takes into account : 1) the differences in protein value between the different forms of feed nitrogen, and 2) the interactions between available energy and degradable N and other nutrients.

The DCP content overestimates the protein value of leafy grass and legume silages and cannot express its great variations resulting from ensiling methods and fermentation quality. The PDI content overcomes these shortcomings and gives a better description of the actual protein value of an individual silage fed alone and of its potential value in a mixed diet (Fig. 5). The extent of protein breakdown by silage fermentation is expressed by the content dietary PDI and therefore the PDIN and especially the PDIE contents. These values decrease as a result of clostridial fermentation and are improved by efficient additives (Fig. 6). They can also take into account the increase in undigestible dietary protein content (Maillard products) in heat-damaged silages, hays or dehydrated forages.

The comparisons between the two values, PDIN and PDIE, of each forage shows the factor limiting microbial growth in the rumen and the optimum association of the forage with other forages or concentrates. The protein value of high protein forages is limited by their digestible energy content. The difference between PDIN - PDIE expresses the surplus of rumen-degradable protein over rumen fermentable energy supplied by the forage to the microbes. This surplus is lost (as urea in the urine) when the forage is fed alone but it can be used by the rumen microbes when an other forage or concentrate supplies energy that exceeds the amount of rumen-degradable N. For example rumen microbes can utilize the excess available energy (PDIE - PDIN) from maize silage to synthesize proteins from the excess degradable protein (PDIN - PDIE) of lucerne silage (Fig. 5) or hay. The PDIN and PDIE contents are similar when the ratio CP : DOM is around 0.25. The PDIN content is limiting for lower values and the PDIE content for higher values.

Supplementation of forages by concentrates. A maximum intake of forage is generally sought, together with the minimum allowance of concentrates required to meet animal requirements. The PDI system makes it easier by fitting the

composition of the concentrate to the protein value of the forage, which is better assessed, and by optimizing the growth of rumen microbes.

The choice between protein sources according to their degradability is made from a comparison between the amounts of PDIE and PDIN supplied by the forage diet. Excess PDIN (grass silages) shows that a less degradable source should be used. Excess PDIE (maize silages) allows urea or other NPN sources to be used. The quantity of NPN that can be used is readily computed by dividing the difference between PDIE - PDIN by the PDIN value of the NPN source, e.g. 1.61 for urea, all values being in g. The experimental evidence supporting this approach is shown in the paper by V \acute{e} rit \acute{e} et al. The optimal supply per kg FCM in grass silage-based diets is close to be recommended allowance of PDI (50 g/kg FCM) but ranges from 55 to 75 g DCP. The use of protected proteins treated with formaldehyde can reduce the amount of meal required to supplement grass silage diets. The adequate supplementation of maize silage with urea increases rumen microbial activity, resulting in an increase both in the organic matter digestibility and voluntary intake of maize silage. Further knowledge might lead to a formulation of the optimum amino acid composition of the undegraded dietary protein.

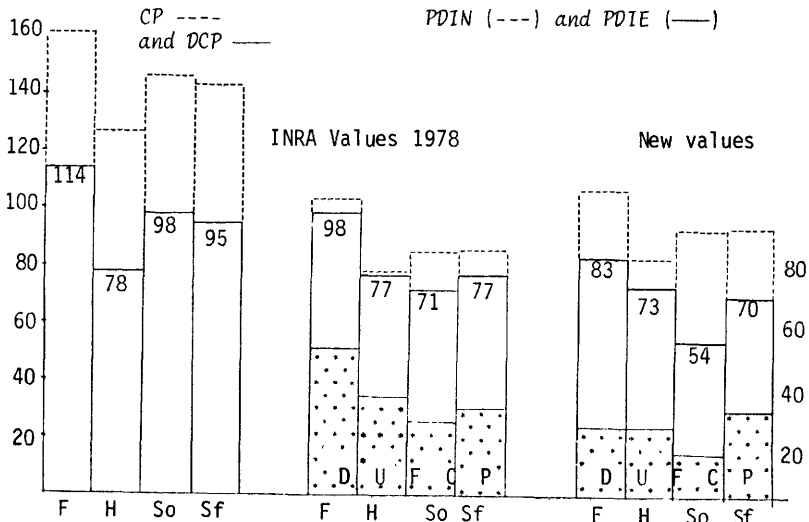


Fig. 6. Compared protein value (g/kg DM) of standing forage (F), hay and silages without (So), or with formic acid (Sf). Mean of 11 comparisons in which the standing herbage and the 3 conserved forages made from it were fed to sheep to measure OM and N digestibility and N balance (Grenet and Demarquilly, Fig 1). The PDI contents were calculated from the initial INRA values for degradability, etc (Table 2) or from the modified values suggested by more recent data (see text and Table 3).

On the whole, the PDI system, such as the ARC system, is based on a physiological framework arising from modern knowledge of nitrogen digestion and metabolism in ruminants. It allows : 1) the protein of forages, and other sources, to be more accurately assessed, supplemented and valorized, and 2) the protein feeding of the ruminant to be considered in terms of amino nitrogen, like that of the monogastric animal, and probably in the future in terms of limiting essential amino acids. It focuses on the necessity of providing the rumen microbes with adequate and balanced amounts of degradable N and energy substrates.

The PDI allowances for the animals, and the efficiency of PDI utilization, have been estimated from an analysis of the calculated quantities, available in different experiments which had previously been carried out to measure N balance or DCP requirements. This experimental approach has made the PDI system reliable for practical purposes.

Shortcomings and future improvements

As stated by Roy et al. (1977), for the ARC scheme : "It is fully recognized that the calculations involved in the proposed system necessitate the use of average values for factors, for which the supporting evidence is sometimes meagre and often variable". The use of these average values as constants make these systems appear static and inflexible.

Future improvement. The values used in the PDI system for degradability, microbial synthesis, etc., correspond to the knowledge available in 1976-1977. They can be modified, from now on, to take the new experimental data, such as those included in Table 3, into account. The following are a few examples :

. The values chosen for the protein degradability (Table 2) appear to be too low, particularly for fresh forages and hays (Table 3). This bias would explain, at least in part, why equation 4 has been found to overestimate the protein flow to intestines for high protein intakes (Whitlow and Satter, 1979 ; Corbett et al., 1981).

. The constant value of 135 g microbial protein synthesised per kg DOM for all the feeds, which is similar to that chosen in the other systems, was provisional. Large variations had been already found between forages, which have been confirmed (Table 3). The differences between authors could result from differences in the methods used and their accuracy. But there are undoubtedly actual differences between forages according to : 1) the composition of the DOM fermented (relative proportion of water-soluble carbohydrates,

Table 3 : Extent of degradation of forage N and microbial protein synthesis in the reticulo rumen (g/kg RDOM, OM apparently digested in the rumen) (1)

	N degradation				Microbial protein synthesis (g CP/kg RDOM)			
	Number	Range	Mean	References	Number	Range	Mean	References
Green forages								
• grasses	6	0.69 - 0.93	0.80	19,84,	8	171 - 328	240	19,84,93
• legumes	8	0.54 - 0.98	0.74	19,33,39	8	178 - 306	224	19,39
	5(b)	0.77 - 0.98	0.84					
• grasses + legumes	16		0.73		16		232	
	11(b)		0.82					
Hays and straws	8	0.59 - 0.87	0.73	49,52,53,54, 61,93	10	129 - 301	216	4,34,37,49,52,53, 63,93
Artificially dried forages (c)	24	0.24 - 1.00	0.62	2,3,9,10,17+81, 18+8,36,45,48, 49,50,51,93	19	72 - 491	303	3,17+81, 18+8, 36,45,48,49,51,93
	12(c)	0.46 - 0.94	0.65		9(c)	124 - 349	244	
Grass and legume silages								
• unwilted, no additive	2	0.80 - 0.88	0.84	11,16	2	166 - 209	187	16
• wilted, no additive	3	0.69 - 0.83	0.78	13,53,71	3	135 - 230	174	4,53,71
• unwilted or wilted with formic acid	7	0.55 - 0.83	0.68	16,80,82	9	68 - 230	150	16,80,82
• unwilted or wilted with formic acid and (or) formaldehyde	3	0.33 - 0.52	0.42	11,71,82	4	83 - 178	148	4,71,82
Maize silage	1		0.70	55	5	166 - 261	192	55

(1) given by the authors or calculated from duodenal passage of feed non-ammonia nitrogen (NAN), assuming protozoal nitrogen is 1.5 g/d if bacterial nitrogen is estimated with α - ϵ -diaminopimelic acid (DAPA) and endogenous nitrogen is 1.5 g/d in sheep.

(b) without wilted, post-wilted and mature samples of subtterranean clover of ref. 39.

(c) without data from ref. 48 and 9 (sainfoin) and data with microbial nitrogen estimated with the nucleic-acid method (10; 17+81; 18+8)

fermented cell-wall carbohydrates, etc.) ; 2) the content in degradable N and other nutrients required by the rumen microbes ; 3) the degree of coupling between ATP and ammonia release ; 4) the residence time in the rumen and the dilution rate, etc. All these factors can affect the efficiency of microbial synthesis (see reviews or discussion by Stern and Hoover, 1979 ; Hespell and Bryant, 1979 ; Orskov, 1982). Recent work suggest that the microbial protein yield per kg DOM sustained by grass silages is quite variable with fermentation quality and clearly lower than that with fresh forages or hays (see Beever, 1980 ; Thomas et al., 1980) (Table 3). Volatile and lactic acid produced from water-soluble carbohydrates by silage fermentations can be used only to a small extent as a source of energy by the microbes in the anaerobic conditions of the rumen. Therefore every increase in the silage fermentations would reduce the protein flow leaving the stomach and the PDI content by reducing both the rumen undegraded protein and the microbial protein yield. This could explain why the N retention in sheep fed silages was found to be related negatively to the content in the main fermentation products (ammonia, lactic acid, propionic acid) and positively to water-soluble carbohydrates and buffer-insoluble N contents (Grenet and Demarquilly, 1982, Fig. 1). By contrast, in addition to an increased proportion of undegraded protein, artificial dehydration appears to enhance microbial synthesis (see Beever, 1980).

Recent results show that the true digestibility of the microbial amino-acid N would lie in the upper range of the previous data, between 80 and 85 % (Salter and Smith, 1977 ; Elliot and Little, 1977 ; Tas et al., 1981 ; Storm and Orskov, 1982). Therefore the value of 0.70 chosen in the PDI system should be increased up to 0.80, which would increase the microbial PDI content of all the forages and compensate, in part, for the decrease in the dietary PDI content resulting from enhanced degradability.

However, the latter would lead to an appreciable reduction in the dietary PDI and the PDIE contents for the N rich forages, especially for young pasture grass (Fig. 6). This change is in agreement with the results of the feeding trials reported elsewhere (cf paper by Vérité et al.) suggesting that the present PDI value of grass silages is overestimated by around 10 % for milk production. Nevertheless it appears too large for good silages made without additives.

The PDI system is a whole. All the constant values chosen for the different factors were more or less interdependent : degradability

and microbial synthesis in the rumen (equation 4), degradability and the intestinal digestibility of dietary protein, PDI value of the feeds and PDI utilization and requirements. Therefore all the constants should be revised simultaneously.

Flexibility. "The ARC and INRA proposals are relatively inflexible because they have retained the classical factorial approach - they are static models in which the outcome of each of several successive processes is described by a factor. Such systems cannot take into account the feed-back effects due to interactions between the environment, the animal, its diets and its rumen" stated the Australian group (Faichney et al., 1980) who proposed a system based on the computer model of rumen function developed by Black et al., 1981 to overcome the shortcomings of the European systems.

Undoubtedly, the great number of factors determining ruminal protein metabolism and their dynamic interactions (cf. paper by Tamminga) must be considered simultaneously to predict accurately, in a wide range of conditions, the amount of protein leaving the stomach. Computer programs that simulate rumen function are required, particularly to take the relative rates of degradation and outflow of each substrate into account. They are efficient research tools that can be used to understand observed differences, to indicate the relative importance of the limiting factors, and to assess the consequences of changes in the variables (Beever et al., 1981 ; Black and Faichney, 1981). However, as the other systems, they are limited by the lack of accurate information about some factors (potential protein degradability, etc.) and of methods for measuring their influence.

The PDI system is quite flexible because it is analytical, involves a great number of factors, considers the different feeds separately and ascribes two values to each feed. As shown above, the constant values chosen for some factors, particularly for the microbial protein yield, are provisional. They will be diversified and adapted to each category of feeds as knowledge accrues. The actual PDI value of each feed in a ration is already not constant and can vary in the range delimited by PDIN and PDIE, according to associated feeds. Most of the interactions between forages and concentrates in mixed diets, as well as the influence of the level of feeding (dilution rate...) could be accounted for by correction factors, as it is done in the new energy systems. New understanding of protein or amino-acid intestinal digestion will be easily incorporated owing to the distinction between dietary and microbial proteins and the choice of true digestibility which has led, in addition, to a realistic evaluation of the PDI maintenance requirements.

CONCLUSION

The tremendous amount of research that has been carried out over the past 15 years has led to a satisfactory understanding of the major aspects of nitrogen digestion and metabolism in ruminants. It allows better assessment and use of forages as protein sources. Two contrasting positions regarding the application of present knowledge and the replacement of the DCP system may be taken :

The insufficiencies of present knowledge and the lack of laboratory methods to predict the feeds protein value could be emphasized, and surmounted, before adopting or building new systems ;

The importance and potential of present knowledge could be emphasized for immediate application, and incorporated into new systems, even if many values are only temporary.

The latter position has been adopted, at least by research groups in some countries (Australia, France, Western Germany, Switzerland, United Kingdom, U.S.A.). In France, the PDI system proposed (Journet, 1975) and comprehensively formulated (1978) by INRA is taught, advised and used in spite of a regulation difficulty concerning the PDI content of compound feeds. It is an efficient tool to advise improved protein nutrition of the ruminant and its rumen microbes.

However, it is fully recognized that in many practical circumstances little error may be incurred by using the DCP system with proper corrections for NPN rich feedstuffs and diets.

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PAPER 10

AUTHOR: R. Jarrige (France)

DISCUSSION

C. Cottyn (Belgium)

Could you comment on the adoption of the PDI system in France?

R. Jarrige

The system has been adopted by the agricultural advisors organisation and is taught in schools. Tables for practical use have sold 100,000 copies.

P.C. Thomas (UK)

The ARC system in the United Kingdom has had the effect of increasing the use of materials of low degradability, but poor quality. What is the situation in France?

R. Jarrige

PDI can not be declared because there is no simple reliable test but it can be calculated.

D. Beever (UK)

The PDI system requires the percentage of organic matter digested in the rumen (OMR_d). Can this be predicted from 'in vitro' tests?

R. Jarrige

Continuous fermenters may give values.

C. SUPPLEMENTATION OF FORAGE PROTEIN
AND ANIMAL PERFORMANCE

Chairman: S. Curran

PROTEIN SUPPLEMENTATION OF SILAGE DIETS FOR DAIRY COWS

R. VERITE⁺, J.P. DULPHY⁺⁺, M. JOURNET⁺

Institut National de la Recherche Agronomique
+ Station de Recherches sur la Vache laitière
Saint-Gilles, 35590 L'Hermitage, France

++ Laboratoire des Aliments
Centre de Recherches Zootechniques et Vétérinaires
Theix, 63110 Beaumont, France

An efficient protein supplementation of diet for ruminants, requires an accurate determination of the true protein value of the various feeds, primarily forages. This problem becomes the more important as cows produce more, the diversity in quality of protein sources is greater and the economic pressure is higher. For a long time these considerations were not very important, so the digestible crude protein (DCP) system was used. Although correct in average situations, DCP does have many shortcomings.

However, the recent trends in dairy husbandry have called for more accuracy in protein supplementation to avoid protein wastage and to prevent milk yield decrease. In practice this can be achieved owing to recent progress in protein digestion and metabolism, which have been grouped together in the new protein systems, particularly in France with the PDI system proposed by INRA (Jarrige et al., 1978 ; Vérité et al., 1979) and in Great Britain with the ARC system (Roy et al., 1977 ; ARC, 1980)

This paper will deal with : the protein supplementation of grass and maize silage diets, taking into account the effects of protein supply level, the nature of the supplementary protein source and, finally, the critical period of the beginning of lactation. All these aspects will be treated mainly from experiments carried out over the past ten years at the "Department of Elevage des Ruminants" in Theix.

GRASS SILAGE

The true protein value of silage seems easier to appreciate for maize than for grass since the level and the degradability of grass silage proteins are generally higher and more variable than those of maize silage.

The recent studies on grass silage (Castle et al., 1969, 1974, 1976 ; Laird, 1979 ; Adamson, 1979 ; Gordon et al., 1979, 1980) have

indicated that milk production increases when the supply of DCP largely exceeds the theoretical allowances of 60 g DCP/kg FCM. The reason is that the actual value of grass silages depends not only on their CP content, but also on their conservation quality as shown by measurements of the non-ammonia nitrogen arriving or absorbed in the small intestine.

Five trials were carried out between 1977 and 1980 to compare 2 or 3 levels of oilmeals, either soybean meal (trials I, II, III) or a formaldehyde treated mixture of soybean and rapeseed meals (trials IV, V). Various direct-cut grass silages from rye-grass treated with formic acid were used and given either in slightly limited amount for the first three trials or *ad libitum* for the two others (Dulphy, Andrieu and Demarquilly, 1979 and 1980).

The main results are given in table 1 and the FCM responses are related to protein supply in figure 1. Large differences have been noted between experiments. The optimum DCP supply ranges between 65 and 75 g/kg FCM in the first three trials and is as low as 50-55 g in the two others. Similarly the optimal CP content of the total CP is between 16 and 18 % for the first experiments, and close to 15 % only for the others. On the other hand, the different figures are much more consistent when the protein supply is expressed as PDI, as clearly shown in figure 1 : the optimal levels are included in the 50-55 g range PDI/kg FCM for any experiment. This value is slightly higher than the standard (50 g), possibly due to a slight over-estimation of the silage value (see paper by Demarquilly and Jarrige, 1982).

FIGURE 1 - MILK RESPONSE TO INCREASING PROTEIN SUPPLEMENTATION WITH GRASS SILAGE DIETS

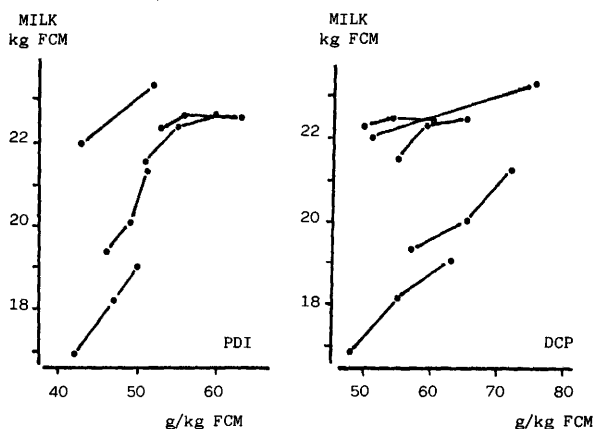


TABLE 1 - EFFECT OF PROTEIN SUPPLEMENTATION WITH GRASS SILAGE
DIETS ON MILK YIELD DURING MID-LACTATION

Experiment n°	I		II		III		IV		V			
	Low	High	Low	Medium	High	Low	Medium	High	Low	High		
SILAGE CHARACTERISTICS (1)												
- % DM	23,0	11,3	20,9	9,8	9,9	8,2	8,7	8,8	11,1	11,7	12,4	12,9
- CP % DM	13,1	3,9	15,4	4,4	4,8	4,9	5,0	5,1	4,3	4,2	4,5	3,6
- FU per kg DM	0,94	(0,64)	0,91	(0,18)	(0,84)	(1,1)	(0,13)	(1,48)	(1,31)	(1,63)	(2,01)	(1,12)
- ICP per kg DM	86		107									
- PDIN per kg DM	78		92									
- PDIE per kg DM	78		84									
Level of protein supplementation												
	Low	High	Low	Medium	High	Low	Medium	High	Low	Medium	High	High
INTAKE (kg DM/d)												
- silage	11,1	11,3	9,8	9,9	9,9	8,2	8,7	8,8	11,1	11,4	11,7	12,4
- total concentrate	3,9	3,9	4,4	4,8	4,9	4,7	5,0	5,1	4,3	4,2	4,5	3,6
* oilcake portion	(0,64)	(1,99)	(0,18)	(0,84)	(1,1)	(0,13)	(0,82)	(1,48)	(1,31)	(1,63)	(2,01)	(1,12)
Milk (kg FCM/d)	22,0	23,3	19,4	20,1	21,3	16,8	18,2	19,0	21,6	22,4	22,6	22,5
Body weight change (g/d) (2)	15	199	254	176	146	8	51	132	236	158	209	176
CP % DM	14,4	18,5	14,9	16,0	17,7	13,0	14,4	16,2	14,7	15,5	16,2	14,8
DCP supply (g/kg FCM) (3)	48	77	55	64	75	44	53	65	52	57	65	49
PDI supply (g/kg FCM) (3)	40	54	45	49	54	37	46	52	49	53	60	52

(1) always 1st cut of perennial rye-grass, fine cutting and (except for trial IV) with formic acid added

(2) taking gut content change into account

(3) computed as (protein-supply - maintenance requirements)/milk yield of the higher group within each experiment

MAIZE SILAGE

When CP content and protein degradability of forages are moderate, or high as in most grass silages, the microbial requirements for degradable N are generally met. On the other hand, with maize silage the degradable N supply is insufficient to sustain a normal microbial activity in the rumen. Meeting microbial N needs is then of primary importance. The deficit can be made up by using complementary feeds having a high degradability of their protein and also by industrial non-protein nitrogen, such as urea.

NPN supplementation

Practical rules for the use of urea have been derived from many feeding trials particularly in the U.S.A. (see Huber and Kung, 1981 ; Chalupa, 1972). To improve their accuracy, new approaches were needed. For example Satter and Roffler (1975), from studies of rumen ammonia levels, concluded that a maximum threshold of 12 -14 % CP in diet DM was needed to use urea efficiently. The new protein systems give another way, as shown by trials 6 and 7 (Table 2 and figure 2) in which different levels of urea (0 to 12 g/kg DM) lead to degradable N supplies ranging from 65 to 105 % of microbial requirements as estimated with the PDI system (Vérité, 1978,1980).

In trial 6, FCM increased progressively with urea level and with a concomittant increase in bodyweight gain. In trial 7, the low level of urea improved milk production of 7 %. However, with higher levels of urea, improvements occurred in bodyweight gain (300 g/d) but not in milk, probably because the yielding ability of the cows was reached. As a result, the energy valorization of the diet, calculated as the ratio of total energy requirements (maintenance plus the actual production) on DM intake, was increased by 6 to 7 % corresponding approximately to the energy equivalent of 1 kg concentrate.

These improvements can be related to the increases in the flow of protein entering the duodenum (10-15 %) and in the DM digestibility of the diet (2 and 5 units) resulting in a 4-7 % increase in the net energy content. They are consistent with the indications from the PDI system, since the production and digestibility parameters were increased each time the urea supply did not completely compensate for the deficit of degradable N expressed as the difference PDIE-PDIN (figure 2).

.../...

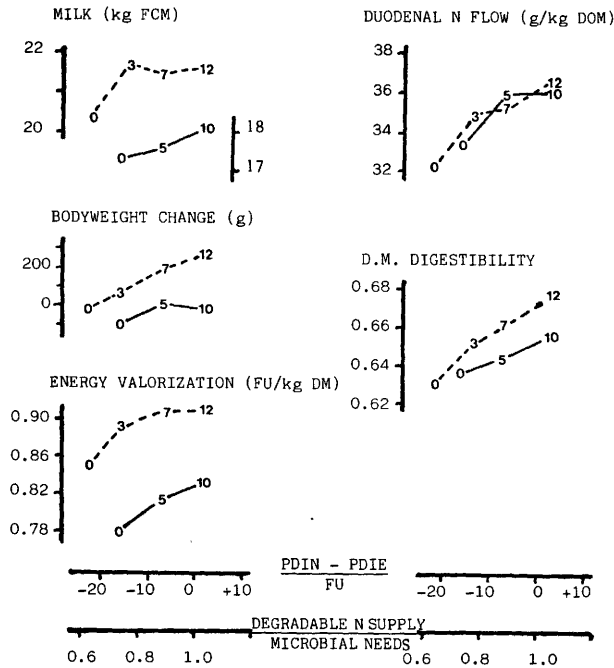


FIGURE 2 - EFFECT OF NPN SUPPLY ON PRODUCTION AND DIGESTION

——— trial 6 } the figures represent the amount of
 - - - - trial 7 } urea fed (g/kg total DM)

The frequently noted improvement in digestibility (Holter and Kabuga; 1974 ; Huber and Thomas, 1971 ; Wohlt et al., 1978) seems to occur up to a dietary crude protein threshold of 13-15 % which probably increases with the non-degradability of dietary proteins and with the production level of animals due to the differences in N recycling possibility.

Urea (or more generally NPN sources) is of interest when the PDIN value of the diet is lower than the PDIE value. The amount that can be fed corresponds to the ratio of the deficit in dietary PDIN to the PDIN value of urea (i.e. 1.61 g PDIN per g urea) :

$$\text{Amount of urea} = \frac{\text{PDIE supply} - \text{PDIN supply}}{1.61}$$

Thus the use of NPN is linked, not only to forage characteristics, but also to characteristics of supplementary protein sources (Vérité, 1978).

Practically, a low PDIN deficit need not to be compensated with dietary urea owing to endogenous urea recycling, but a very high deficit (>12 g/FU) must be avoided to prevent highly negative effects on intake, digestibility and production, even if animal requirements are met with a high non-degradable protein intake (Journet, 1981).

Optimal protein supply to the cow

As with grass, an optimal protein supply cannot be defined without considering the characteristics of supplementary protein sources. Complementary feeds can have a low N degradability either naturally (fish meal, brewers grains, ...) or because of processing (heating, formaldehyde treatments, etc.). There is practical evidence that there are differences in the true protein value of various sources according to their degradability. For example, compared to normal cakes, formaldehyde-treated cakes are good supplements to forages with high N degradability such as grass silages as shown by farm trials (Baraton and Pfmilin, 1980), and even to maize silage when associated with urea (Vérité and Journet, 1977 ; Journet, 1979).

Apart from those trials, the effect of the level of protein supply with maize silage was tested in six experiments between 1972 and 1977. In four of them, the different levels of protein were obtained by substituting low-N concentrate with different levels of oilcakes while in the two last experiments, previously mentioned, only the amount of urea fed was increased (table 2). Cows were always fed limited amounts of high DM maize silage (>28 % DM) to avoid an indirect effect on voluntary feed intake. Though these experiments were not carried out to the same end, in those conditions (cows producing 15-20 kg FCM and fed limited amount of maize silage), the optimal dietary CP content was 13-14 % DM, but with some variations due to the differences in protein sources and to the various responses such as bodyweight change. Milk responses seemed to be reasonably consistent with a standard of 60 g DCP/kg FCM since increasing DCP supply induced a response each time the supply was lower than 55 g but had no response when it exceeded 60 g. Experiment 7 was an exception but there were large deficits in degradable N for some groups and part of the response occurred as bodyweight change. The agreement seems a little better than with grass silage probably because the greater portion of the proteins was not from the silage but from the protein supplement. Similarly, responses fit a standard of 50 g PDI/kg FCM quite well.

TABLE 2 - EFFECT OF PROTEIN SUPPLEMENTATION WITH MAIZE
SILAGE DIETS ON MILK YIELD DURING MID-LACTATION

Experiment n°	2a			2b			3			5			6			7					
	Low (0,14)	Medium (0,68)	High (0,93)	Low (0,01)	Medium (0,25)	High (0,93)	Low (0,10)	Medium (0,44)	High (0,93)	Low (0,36)	Medium (0,77)	High (0,93)	0	70	150	0	150	0	70	120	200
Level of cakes (kg) (1).....	9,7	9,8	10,8	10,8	11,3	10,2	10,1	9,5	9,9	9,8	10,3	10,4	10,4	10,4	10,2	10,5	10,7	11,2	11,6	11,0	11,0
- total concentrate.....	3,3	3,5	4,2	4,0	4,0	2,2	2,2	3,3	3,4	3,5	4,8	4,7	4,7	4,8	4,6	4,6	4,9	5,0	4,9	5,0	5,0
Milk (kg FCM/d).....	15,4	16,6	17,1	17,3	17,3	14,1	15,1	15,3	16,9	16,7	17,3	17,5	18,1	18,2	18,1	20,3	21,7	21,4	21,4	21,6	21,6
Body weight change (g/d) (2)	- 55	96	100	55	55	121	86	131	261	156	- 96	12	- 19	- 10	121	- 19	67	196	261	261	261
CP % DM.....	12,6	14,0	12,1	12,7	12,7	12,2	13,3	13,3	14,0	15,0	11,3	12,6	13,7	12,2	14,9	11,3	12,1	13,2	14,7	14,7	14,7
DCP supply g/kg FCM (3)	46	59	50	56	44	44	53	53	62	70	42	53	62	49	72	37	45	54	63	63	63
FDI supply g/kg FCM (3)	38	48	44	48	48	36	40	42	49	55	39	45	50	45	56	37	43	49	53	53	53
Deficit of degradable N as g PDIN - PDIE/											- 16	- 7	2	- 15	3	- 22	- 14	- 7	2	2	2

(1) when unspecified, urea was included in the maize silage at the rate of 10-14 g/kg DM according to the trial

(2) taking gut content change (assuming 4.5 g/kg DM intake) into account

(3) computed as (protein supply - maintenance requirements)/milk yield of the higher group within each experiment

(4) soybean meals in trials 2a, 2b, 3 and a formaldehyde-treated mixture of soybean and rape-seed meals in trials 5, 6, 7

COMPARISON BETWEEN THE DCP AND PDI SYSTEMS FOR ASSESSING THE PROTEIN VALUE OF SILAGE DIETS.

To appreciate the marginal response of milk to protein supplementation, the results of the previous trials with grass and maize silages were pooled together. In order to take into account variations between trials, milk responses to increasing protein supplies were calculated as the difference with a "reference" yield, computed for each experiment. The "reference" yield was assumed to be achieved at the recommended allowances (60 g DCP or 50 g PDI per kg FCM) and was estimated by intrapolation or interpolation.

A total of 30 cow groups was taken into account (Tables 1 and 2 except for trial 3 where extrapolation would have been hazardous). Milk response (y - kg FCM/d) was related to the protein supply (g/kg FCM) using either the DCP or PDI system (figure 3). The relevant equations are :

$$\text{DCP} \quad y = 0.053 \text{ DCP} - 3.22 \quad R^2 = 0.62$$

$$\quad \quad \quad \left(\begin{smallmatrix} \pm 0.008 \\ \pm 0.42 \end{smallmatrix} \right)$$

$$\text{PDI} \quad y = 0.383 \text{ PDI} - 0.00304 \text{ PDI}^2 - 11.55 \quad R^2 = 0.75$$

$$\quad \quad \quad \left(\begin{smallmatrix} \pm 0.129 \\ \pm 0.00312 \\ \pm 0.36 \end{smallmatrix} \right)$$

Using the DCP system, surprisingly, the curvilinear component (DCP²) did not improve the coefficient of determination over that of the linear relation (r² = 0.620 vs 0.619). On the other hand, with the PDI system, the relation was curvilinear as expected from the law of diminishing returns, and the correlation was higher than with DCP.

Furthermore, the accuracy could be further improved with the PDI system, by taking better account of N recycling. Basically, the PDI system indirectly, takes into account a low and constant N recycling level by assuming an efficiency of 1 for the conversion of degradable N into microbial N. Occassionally, N recycling can be greater when large deficits of degradable N exist such those which occurred in trials 6 and 7 in the groups without urea. To manage with such an effect, new calculations including a maximum 10 % N recycling for those situations, improved the relationship which became as follows (figure 3) :

$$y = 0.546 \text{ PDI} - 0.00439 \text{ PDI}^2 - 16.40 \quad R^2 = 0.84$$

$$\quad \quad \quad \left(\begin{smallmatrix} \pm 0.122 \\ \pm 0.00122 \\ \pm 0.30 \end{smallmatrix} \right)$$

Thus, when comparing to recommended allowances, a variation in the supply of 10 g PDI/kg FCM (200 g PDI for 20 kg FCM) would result in a milk response of - 1.5 kg when below standard and + 0.6 kg when above standard. In other words, a 20 % variation in the PDI supply for milk leads to a milk response of - 8 % and + 3.3 % respectively. Such a response is 40 % and 15 % of the expected full response (i.e. 1 kg FCM for 50 g PDI). These marginal responses are in the range of those generally observed when the energy input is modified.

So, milk responses clearly appear to be much more consistent with the PDI evaluation than with DCP, even if some improvements remain to be made in the assessment of the PDI value of silages.

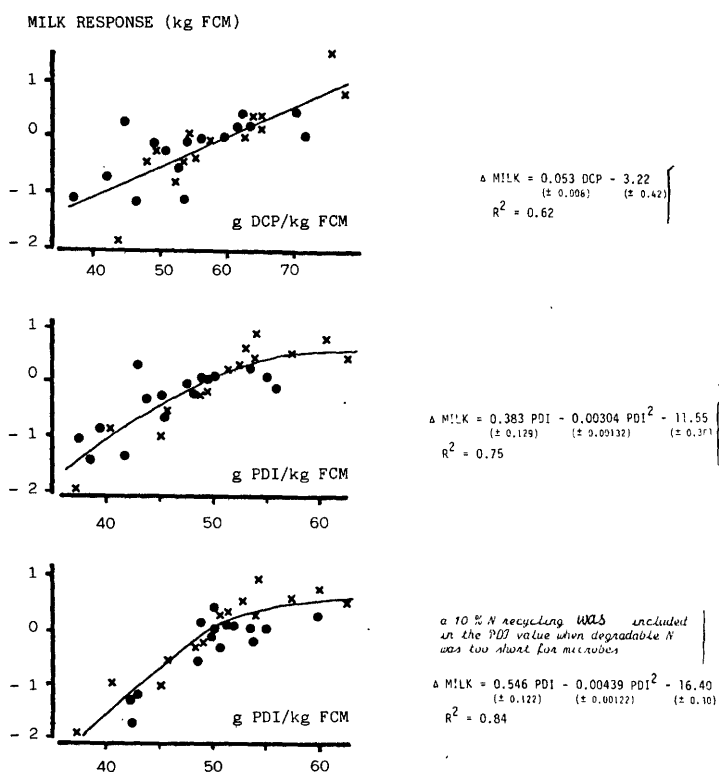


FIG. 3 - MILK RESPONSE TO INCREASING PROTEIN SUPPLY PER KG FCM

- Experiments with grass (x) or maize (●) silage.

The level of protein supply was computed thus :

$$\frac{\text{protein supply} - \text{maintenance requirements}}{\text{reference milk yield}} \quad (\text{see the text})$$

PROTEIN FEEDING DURING THE BEGINNING OF LACTATION

Protein requirements are high very soon after calving and the risk of protein underfeeding is then great because of the low intake capacity of the cow and of adaptation of microbes to dietary changes. Microbial proteins meet requirements for maintenance plus only 5 kg of milk during the first week compared to 12-15 kg milk after peak yield (Vérité, 1978). Undegraded protein from supplementary feed must provide more than 50 % PDI requirements for the first 3 weeks. Compared to the subsequent period, a greater amount of undegraded protein must be provided by a similar or even lower amount of concentrate. If no special attention is then paid to the CP content of the concentrate, a deficit in protein supply will occur, even with high concentrate levels.

Four experiments, starting at calving, with multiparous cows, were carried out to assess the effect of protein supplementation levels with maize silage diets. The main objective was to determine the extent to which a cow can tolerate protein underfeeding during the beginning of lactation without decreasing its production or affecting health and reproduction and bodyweight change (Rémond and Journet, 1981). In each experiment, cows were fed urea-maize silage as the sole forage and concentrates according their expected yield. From calving onwards, the low protein groups received a concentrate with a constant proportion of oilmeal (15-20 %) as usually done. In the well-fed groups, the ratio of oilmeal to total concentrate steadily decreased from 100 % during the first week to 15-20 % as the end of the second month in order to meet the protein requirements soon after calving. Consequently, the CP content of total diet decreased from 18-20 % the first week to 13.5 % eight weeks later whereas it remained constant from calving (near 13.5 %) in low protein groups.

Improving protein supplementation resulted in a sharp increase in the milk production : according the trial from 2 to more than 5 kg FCM per day as a mean for the two first months (Table 3). In the first two trials (7b, 8) using a slightly limited amount of silage in order to have similar energy input, the increase was obtained at the expense of body reserves as attested by bodyweight changes : losses increased 11 and 6 kg respectively (figure 4). In ad libitum feeding experiments (9 and 10), increasing the protein level brought on an increased silage intake (2 and 2.4 kg DM respectively). Therefore, the observed increase in milk yield no longer corresponded to a higher mobilization but rather to decrease in bodyweight loss (8 and 16 kg) (figure 4).

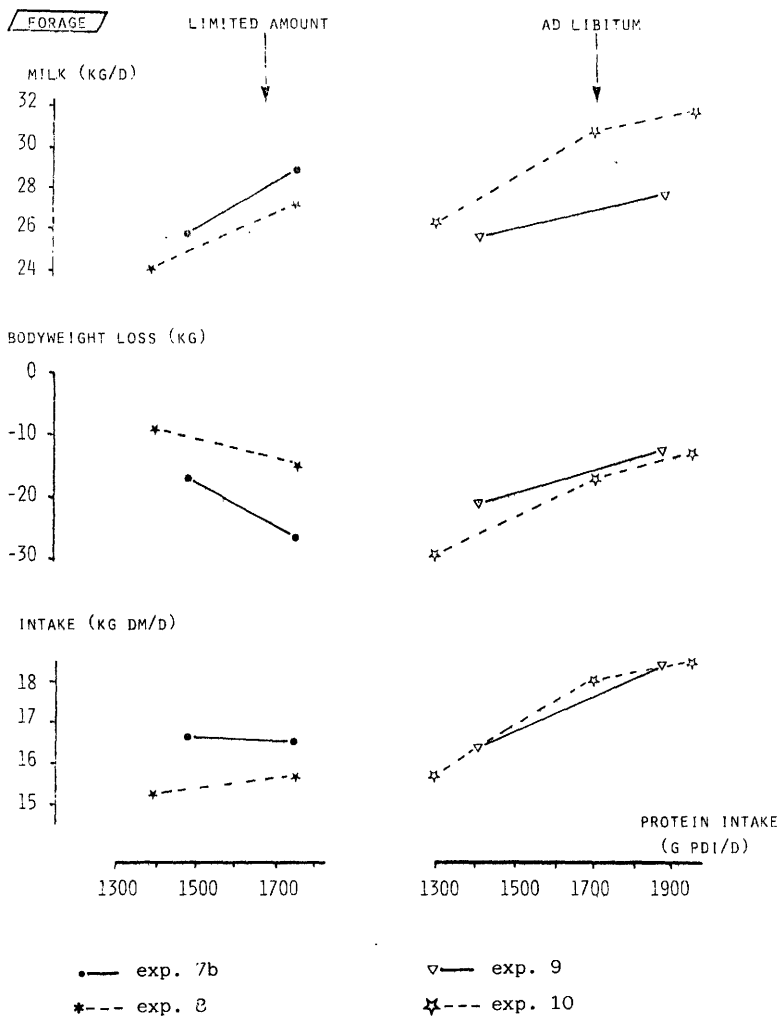


FIGURE 4 - EFFECT OF PROTEIN INTAKE ON MILK PRODUCTION, BODYWEIGHT LOSS AND DM INTAKE AFTER CALVING

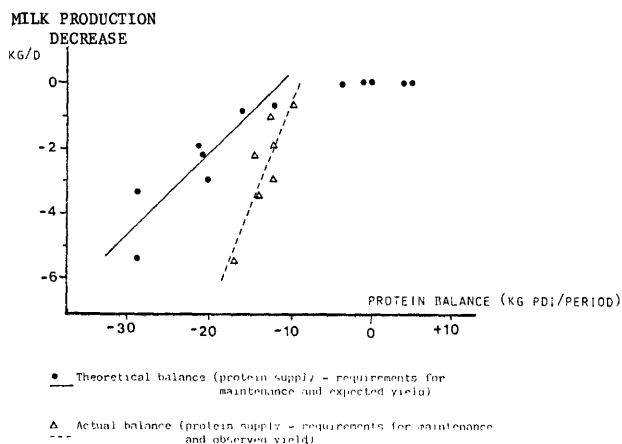
TABLE 3 - EFFECT OF PROTEIN SUPPLEMENTATION WITH MAIZE SILAGE DIETS
ON MILK YIELD DURING EARLY LACTATION

Experiment n°	7b		8		9		10 (1)		
	Low	High	Low	High	Low	High	Low (3)	Medium	High
Protein supplementation.....									
<u>INTAKE</u>									
- maize silage (2) (kg DM).....	11,87	11,47	9,18	9,51	10,68	12,73	11,70	13,81	14,14
- low-protein concentrate (kg DM).....	3,86	3,44	5,45	4,24	5,09	3,76	2,77	2,84	2,01
- high-protein concentrate (kg DM).....	0,98	1,68	0,60	1,84	0,39	1,73	1,20	1,20	2,06
<u>MILK PRODUCTION</u>									
- milk (kg FCM).....	25,6	28,9	24,1	27,0	25,7	27,6	26,1	30,8	31,7
- protein (g per kg).....	39,7	38,2	40,5	40,5	40,4	40,2	42,4	44,4	45,0
- fat (g per kg).....	31,9	31,6	33,1	33,0	31,1	32,2	33,3	33,7	33,2
- Liveweight change (1) (kg).....	- 17	- 26	- 9	- 15	- 21	- 13	- 29	- 17	- 13
Net Energy (FU).....	16,5	16,4	14,4	14,7	14,5	16,2	14,1	15,8	16,2
PDI Supply (g).....	1,478	1,758	1,384	1,744	1,414	1,882	1,286	1,698	1,950
Crude Protein (% DM).....	13,8	15,3	13,5	16,0	13,6	15,3	12,1	15,0	16,7

(1) weeks 1 to 8 except for trial 10 (weeks 1 to 6)
(2) with 150 to 200 g urea/day (the same amount with each trial) except low group in trial 10
(3) no urea

Further information about the possibility of protein saving by protein mobilization can be derived here and elsewhere (Rémond and Journet, 1981). The theoretical balance of protein supply with regard to the potential yield (PDI supply - PDI requirements for the expected yield) ranges from - 30 kg PDI to + 10 kg PDI for the first two months. The milk loss with low protein supply, compared to the well-fed group, remains low (< than 1 kg) when the theoretical protein balance is slightly negative (up to - 15 kg PDI), but increases sharply with more negative balance : approximately - 6 kg FCM/day at - 30 kg PDI/period (figure 5). This would suggest that cows cannot suffer such high deficits. Actually, since they decrease their milk production, the true deficit is more moderate : from 10 to 17 kg PDI according the actual balance (PDI supply - PDI requirements for the observed yield).

FIGURE 5 - RELATIONSHIP BETWEEN MILK PRODUCTION AND PROTEIN INTAKE AFTER CALVING (2 months)



Therefore, it seems that during the first weeks of lactation, a high producing cow can suffer a shortage in PDI supply approximating 10 kg PDI without decreasing its milk yield to a great extent.

This value can be compared with estimations of protein mobilization while bearing in mind possible differences in the efficiency of milk synthesis utilization, between the PDI supplied by the diet and the

body proteins mobilized. It correlates quite well with the lower part of the range of mobilizable protein (5 to more than 15 kg) reported by various authors (Bath et al., 1965 ; Beleya et al., 1978 ; Coppock et al., 1968 ; Paquay et al., 1972).

CONCLUSION

The milk response was more readily predicted with the new PDI system than with the DCP system because of a better assessment of the protein value of the silages and the protein supplements used.

With silages diets given to high milking cows at the beginning of lactation, the deficit of protein supply is relatively high due to the high N degradability and low grass silage intake and to the low crude protein content of maize silages. The great response of milk production observed with high quality protein supplements (and NPN for maize silages) supplies arises from the increase in both silage intake, diet digestibility and amino-acid supply and from the low capacity of cows to mobilize body protein.

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PAPER 11

AUTHOR: M. Journet (France)

DISCUSSION

P.C. Thomas (UK)

Could you comment on the negative protein balance in your experiments and if possible convert it to gN/day?

M. Journet

It was calculated from the group of animals on the low protein diet and it corresponds to the cumulative daily protein balance (requirement - supply during the duration of the experiment (6-8 weeks)). The reduction in milk production (kg/cow/day during the experimental period) corresponds to the difference between the high and low level of protein feeding.

THE EFFECT OF SOURCE OF SUPPLEMENTARY PROTEIN UPON
THE PERFORMANCE OF LACTATING COWS

D.J. Morgan

The Agricultural Institute, Moorepark Research Centre,
Fermoy, Co. Cork, Ireland

ABSTRACT

In experiment 1 72 cows in early lactation were fed grass silage ad libitum plus barley based supplements containing the following protein sources : none, soya, soya plus fermented, ammoniated, condensed whey (FACW), FACW, fish/animal protein blend or distillers grains. All protein supplements increased silage intake but only the soya treatment resulted in a significantly increased milk yield compared to the control. In experiment 2 52 cows were fed wilted silage ad libitum plus four supplements at 9 kg per day. The supplements were based on barley and wheat feed and varied in source and level of additional protein :

(a) Soya to 133 g CP per kg, (b) Formalin treated soya to 130 g CP per kg, (c) Soya to 187 g CP per kg and (d) Soya + formalin treated soya to 168 g CP per kg. The results demonstrated responses in milk yield to both increasing level of protein and to formalin treatment. Digestibility data are presented for both trials.

INTRODUCTION

A number of trials have been carried out at this Institute examining the response of lactating cows to supplementary protein when fed silage diets. While responses have been found to feeding up to 200 g CP per kg in the supplement (Judge and Gleeson, 1977) the optimum level of CP in the supplement has generally been found to be 150 to 160 g per kg (Butler and Gleeson, 1973 and 1974, Morgan, 1980).

In all these trials barley based supplements have been used with soyabean meal as the source of protein. Soyabean meal represents a protein source of moderately high degradability (Orskov et al, 1981) and as such may not necessarily be the ideal protein supplement for diets based on silage which can contain a high proportion of degraded protein (McDonald and Edwards, 1976). In this paper results are presented from two experiments in which comparisons were made of various sources of crude protein for milk production.

EXPERIMENT 1

Seventy two recently calved Friesian cows (30 in their first lactation) were used in a randomised block design trial to evaluate 6 supplement treatments fed from weeks 4 to 11 of lactation inclusive:

Treatment	Protein Source	Protein level (g CP/kg)
1	None	108
2	Soya	167
3	Soya plus FACW*	165
4	FACW*	155
5	Fish/animal protein blend**	164
6	Dried distillers grains	155

*Fermented, ammoniated whey, 'Lacto whey', Calor Gas Co.

** Fish Blend, 650 g CP per kg, UFAC Ltd; fishmeal and meat and bone meal present in approximately equal proportions

All supplements were formulated from the appropriate protein source, barley, minerals and vitamins. Supplements were fed in meal form at 7.5 kg per day while grass silage was offered ad libitum. The silage had the following analysis :

Dry matter 260 g per kg, Crude protein 210 g per kg DM
 In vitro DMD 0.79, pH 4.0

The main results of the production trial are shown in Table 1. Compared to the low protein control, only the soya treatment (T2) resulted in a significantly increased milk yield. Milk protein

Table 1. Performance of lactating cows fed various protein supplements

Treatment No. Protein Source	1		2		3		4		5		6		S.E. of diff.
	None	Soya	Soya	FACW	Soya	FACW	FACW	FACW	Fish blend	Fish blend	Dist. Grains	Dist. Grains	
Milk yield (kg/d)	19.95	21.31	20.36	19.81	19.79	20.56	0.69						
Milk fat (g/kg)	38.2	38.2	40.1	41.0	38.6	39.6	1.68						
Milk protein (g/kg)	31.4	32.2	32.0	31.4	32.3	32.5	0.83						
Milk fat yield (kg/d)	0.762	0.815	0.817	0.813	0.764	0.813	0.038						
Milk protein yield (kg/d)	0.627	0.687	0.652	0.622	0.640	0.669	0.028						
Silage DMI (kg/d)	7.79	8.77	8.57	8.71	8.44	8.94	0.40						
Liveweight change (kg/d)	-0.33	-0.17	-0.06	-0.07	-0.27	0.00	0.16						

yield was also significantly increased but not milk fat yield. Silage dry matter intake was lower on the control than the supplemented diets and this was reflected in a greater liveweight loss on this treatment.

Total diet digestibility was measured using five cows per treatment (Table 2). The digestibilities of DM, OM and energy were significantly greater in the diets containing FACW than in the control, while other treatments did not differ from the control. DE intake was lower for the control than for the other treatments due mainly to the difference in silage intake.

EXPERIMENT 2

A total of 52 recently calved cows were used in a randomised block design trial to evaluate four supplement treatments :

Treatment	Protein Source	Protein Level (g CP/kg)
A	Soya	133
B	Formalin-treated soya*	130
C	Soya	187
D	Soya plus formalin-treated soya*	168

*'Sopralin', BP Nutrition Ltd.

The supplements also contained barley plus constant amounts of wheat feed, molasses, minerals and vitamins. They were fed in pelleted form at 9 kg per head per day for an eight week period together with grass silage (fed ad libitum) which had the following analysis :

Dry matter 462 g per kg, Crude protein 140 g per kg DM

In vitro DMD 0.7, pH 4.5

A further 8 cows were used in a latin square design trial to measure digestibilities of the total diets. Cows were fed fixed amounts of silage plus supplements (mean daily intakes were 7.6 kg supplement plus 6.6 kg silage DM).

Results of the production trial are shown in Table 3. The lowest milk yield was observed on treatment A and both treatments C and D gave significantly greater yields. Milk yield on treatment

Table 2. Digestibility of dietary components by cows fed various protein supplements

Treatment No. Protein Source	1	2	3	4	5	6	S.E. of diff.
	None	Soya	Soya FACW	FACW	Fish blend	Dist. grains	
% digestibility of :							
DM	69.8	71.1	72.2	74.2	70.9	69.5	0.93
OM	71.7	72.9	74.2	75.8	72.3	71.5	0.95
GE	67.7	69.3	70.7	72.4	68.7	68.5	0.96
N	66.5	70.9	71.8	73.5	68.2	67.2	1.64
DE intake (MJ/day)	183.6	215	210.7	201.5	206.9	211.7	

Table 3. The performance of lactating cows fed concentrates containing soya or formalin-treated soya

	Treatment Code				S.E. of diff.
	A	B	C	D	
Milk yield (kg/d)	18.82a	19.63ab	20.3b	20.74b	0.709
Milk fat (g/kg)	36.9	38.0	37.5	37.3	1.17
Milk protein (g/kg)	33.2	32.3	32.6	31.6	0.83
Milk lactose (g/kg)	43.8	44.0	43.9	44.7	0.69
Fat yield (kg/d)	0.695a	0.745ab	0.762ab	0.774b	0.036
Protein yield (kg/d)	0.624a	0.633ab	0.661b	0.655ab	0.018
Lactose yield (kg/d)	0.823a	0.863ab	0.892ab	0.927b	0.037
Silage DMI (kg/d)	9.14	9.12	8.92	9.14	0.45
Livewt. change (kg/d)	+0.262	+0.173	+0.310	+0.306	0.191
Body score change	+0.04	0	+0.25	+0.17	

Table 4. The digestibility of diets containing soya or formalin-treated soya

	Treatment Code				S.E. of diff.
	A	B	C	D	
% digestibility of :					
Dry matter	72.9ab	72.5a	74.3b	74.3b	0.79
Organic matter	74.7ab	74.4a	76.2b	76.2b	0.77
Nitrogen	62.1ac	60.5a	69.8d	64.9c	1.80
Energy	70.7a	70.9a	73.1b	72.7b	0.84
Nitrogen retention (%)	29.7	30.6	31.7	30.5	2.59
Nitrogen retention (g/day)	95.4a	98.9a	126b	107.5ab	9.1
Digestible energy (MJ/kg DM)	12.52a	12.65a	13.18b	12.94c	0.105

B was intermediate and not significantly different to any other treatment. There were no differences between treatments for any parameter of milk composition. Yields of milk fat and milk lactose were significantly higher on treatment D than A while protein yield was significantly higher on treatment C than A. Silage DMI and liveweight changes were unaffected by treatment, liveweight changes being positive for all treatments over the eight week period.

The digestibilities of DM, OM and energy showed similar trends across treatments (Table 4). Treatment B had a significantly lower DM and OM than treatments C and D while in the case of energy digestibility, treatments A and B were significantly lower than C and D. There were large differences in nitrogen digestibility between treatments, C having the highest and B and A the lowest, but nitrogen retention (as a proportion of nitrogen intake) was not affected by treatment.

DISCUSSION

The protein sources used in Experiment 1 did not provide as wide a range of supplement CP degradabilities as had been anticipated. While inclusion of FACW led to an increased CP degradability as measured by incubation in situ, degradabilities of the supplements containing soya, fish blend or distillers grains were all similar (degradability after 16 hours: 0.69 T1, 0.64 T2, 0.82 T4, 0.6 T5, 0.64 T6). All protein supplements led to increased silage DMI and ME intakes compared to the control but only soya addition led to a significant increase in milk output. However when efficiencies of utilization of ME for milk production were calculated (taking into account liveweight changes and milk composition) the values were very similar for treatments 1, 2, 3 and 6 at approximately 0.61. The values were lower for treatment 4 at 0.57 and particularly treatment 5 at 0.51.

Since the silage fed in this experiment had a very high CP content the lack of response to additional NPN is not surprising. By contrast Huber et al (1976) found a response by cows fed a supplement of FACW when added to a maize, maize silage diet containing less than 10% CP in the ration DM. The lack of response to

the fish blend treatment is difficult to explain since on the basis of their relative degradabilities similar performances could have been anticipated for the soya and fish blend treatments.

In experiment 2 a response was obtained both to increasing the level of protein in the supplement and to formalin treatment. Regression of milk yield against concentrate protein level (assuming a linear response) gave the following equations :

For soya, milk (kg) = $15.054 + 0.028 \text{ CP (g/kg)}$

For formalin-treated soya, milk yield (kg) = $15.994 + 0.028 \text{ CP (g/kg)}$

At a mean concentrate CP level of 154.5 g/kg estimated milk yield was 19.4 for soya and 20.34 for formalin-treated soya, the difference being significant ($P < 0.05$) (SE diff. 0.52). The response to protein level was also significant ($P < 0.01$).

High levels of silage intake were achieved in this experiment and silage DMI was unaffected by treatment in contrast to the previous experiment. Digestibility of the total diets was increased by protein level thus calculated ME intakes were slightly higher on C and D than A and B. Milk yields were modest in relation to feed intake, and calculated efficiencies of ME utilisation for lactation plus gain were low, averaging 0.53 and ranging from 0.52 on treatment A to 0.56 on treatment D.

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PAPER 12

AUTHOR: D. Morgan (Ireland)

DISCUSSION

R. Carlsson (Sweden)

The efficiency of utilization of additional protein was different from that obtained by Journet. Could you comment on this?

D. Morgan

I have not done the calculations.

D.L. Mangan (UK)

What was the rate of formaldehyde treatment of your protein source?

D. Morgan

2.8 kg per 1000 kg soyabean meal.

T.W. Griffiths (Ireland)

Would not the experimental design used of flat rate concentrate feeding plus ad libitum access to silage tend to minimise the animal response?

D. Morgan

I do not think so.

P.C. Thomas (UK)

Could you explain the residual effects? Castle found no effects in changeover trials.

D. Morgan

No. We also had no effects in short-term trials.

A. Deswysen (Belgium)

Is it possible that the level of concentrate feeding was too high?

D. Morgan

Yes, but high level of feeding should give maximum effect.

Some studies on protein supplementation with diets of grass
silage for dairy cows

F. J. Gordon, A. C. Peoples and C. S. Mayne

Agricultural Research Institute of Northern Ireland,
Hillsborough, Co. Down.

In the lactating cow changes in feed input, either in terms of crude protein or energy, will generally result in changes in milk output. Broster (1972) reviewed the responses to additional protein and concluded that the response declined markedly at intakes of digestible crude protein (DCP) above 56 g per kg milk. This intake is closely in line with the generally accepted protein requirements for milk production (ARC, 1965). However the major proportion of the experiments reviewed by Broster (1972) were carried out using diets based mainly on hay, while within the United Kingdom and Ireland most dairy cows are offered diets based on grass silage. In view of the marked effects that type of forage may have on protein utilization within the animal (Beever, 1980) it is considered inadvisable to extrapolate the results from hay to silage based diets. A series of experiments have therefore been carried out to examine the response to supplementary protein when grass silage forms the basal part of the diet.

PRODUCTION RESPONSES TO PROTEIN

In an initial trial reported by Gordon (1979) pre-wilted grass silage containing 14.2% crude protein in the dry matter and with a D-value of 67 was offered ad-libitum to dairy cows during the first 75 days of lactation. In addition the animals received equal quantities of one of four supplementary concentrates of approximately similar energy content but with crude protein contents of 10, 14, 17 and 21% on a fresh

weight basis. In each case the concentrate consisted of ground barley, ground maize, extracted soya bean meal and minerals with the soya bean meal being used to progressively replace the barley when increasing the protein content. The effects of the differing supplements on milk output are given in Table 1.

TABLE 1

<u>Effect of increasing the crude protein content of a supplement given with grass silage</u>				
Protein content of concentrate (%)	9.5	13.7	17.4	20.9
Milk yield (kg d ⁻¹)	19.7	21.2	22.2	23.9

While in theory more than adequate DCP was supplied when the supplement contained 14% crude protein, milk yield continued to increase linearly as the crude protein content of the supplement was increased up to 21%. In addition the response to protein at 0.36 kg milk per day per percentage unit increase in the protein content of the supplement was extremely high and is similar to that reported when protein has been offered to animals on protein deficient, hay based diets (Broster, Balch, Bartlett and Campling, 1960). However within the literature there have been several other similar reports of large responses to protein when using silage based diets (Butler, 1973; Cuthbert, Thickett and Wilson, 1973; and Butler and Gleeson, 1973).

A further experiment (Gordon and McMurray, 1979) was carried out in order to provide information on the shape of the response curve at higher levels of supplementary protein. In this experiment concentrates containing six levels of crude protein ranging from 10 to 30% on a fresh weight basis were compared during the first 75 days of lactation. All animals had ad-libitum access to pre-wilted grass silage although its dry matter content at 19.4% and D-value at 61 were lower than that of the silage used in the previous study.

The results from this trial again showed a very marked response to supplementary protein although the response was curvilinear and was described by the following equation:

$$Y = 8.95 + 1.0 X - 0.0205 X^2$$

where Y = milk yield (kg day⁻¹) and X = crude protein content of the supplement.

From this relationship it was calculated that maximum milk output would be obtained when using a supplement containing 24.4% crude protein. However in a practical situation additional protein will incur increased costs and the economic return will be maximised at the point where the cost of an additional unit of protein in the concentrate is just offset by the return in terms of the additional milk produced. This point will obviously depend upon the prevailing economic situation and can be calculated from the data of this study for any given set of feed costs and milk prices. In the present economic conditions within the U.K. a supplement containing 21% crude protein, on a fresh weight basis, would be the economic optimum.

While both the studies reported above have shown large responses to supplementary protein in the concentrate two further studies have shown much lower responses. Gordon (1980a) compared supplements containing 15 and 21% crude protein when given to cows in early lactation in addition to a high quality pre-wilted grass silage (18% crude protein, D-value 77) and obtained a much smaller response of 0.15 kg milk per day per percentage unit increase in the crude protein content of the supplement. A further experiment (Gordon and Unsworth, unpublished) has shown no response to supplementary protein.

In view of the variability of the milk yield responses reported here and also available in the literature it would appear that while responses are generally obtained to high levels of protein with silage based diets, this response can be markedly influenced by a number of

other factors. These factors could either be within the animal, within the type of protein used as a supplement or alternatively within the types of silages used as the basal ration. The present series of studies were therefore extended to examine the effects of some of the factors associated with silage production on the response to supplementary protein.

Effect of wilting herbage

It has been suggested by Beever (1980) that wilting of grass prior to ensiling can result in a reduction in the quantity of amino acids entering the small intestine and this would therefore suggest that pre-wilting could influence the response to supplementary protein. Three production studies have therefore been carried out to test this hypothesis. In each of these studies animals were used in change-over design experiments to compare the response to supplementary protein when grass was either ensiled with or without pre-wilting but with formic acid being used as an ensiling additive in each case. A summary of the results from the three trials are given in Table 2.

TABLE 2

The response in milk yield per unit change in crude protein content of the supplement as influenced by pre-wilting of grass for silage

Source	Silage type	Milk yield response (kg d ⁻¹)
Gordon 1980b	Unwilted	0.08
	Wilted	0.29
Peoples and Gordon (Unpublished)	Unwilted	0.14
	wilted	0.08
Peoples and Gordon (Unpublished)	Unwilted	0.06
	wilted	<u>0.14</u>
Mean	Unwilted	0.09
	Wilted	<u>0.17</u>

While the trial reported by Gordon (1980b) showed a considerable increase in the response to protein when using wilted silage and hence supports the data of Beever (1980) this trend was not apparent in the subsequent two studies. It therefore seems likely that wilting per se does not influence the magnitude of the response to supplementary protein.

Effect of silage quality and season of harvest

The milk production value of forages is influenced greatly by the interval between harvests (Gordon 1980c) with reduced intervals between harvests resulting in an improvement in silage quality and a subsequent increase in milk production. Little is known about how such changes in ^{silage} quality may influence the response to supplementary protein. This aspect has been examined in the present series of studies with silages harvested after either 5 or 9 week growth periods. The full results are given by Gordon (1980b) and are summarised in Table 3.

TABLE 3

Effect of silage quality and season of harvest on the response to supplementary protein

Source	Silage type	Milk yield response (kg d ⁻¹)
Gordon 1980b	High quality	0.08
	Low quality	0.26
Peoples & Gordon (Unpublished)	Spring harvest	0.19
	Autumn harvest	0.06

The response to protein was greatest when a low quality silage was fed. However as an improvement in silage quality generally implies increases in both digestibility and crude protein content of

the silage it is not possible to determine which of these components may influence the response to protein. For example in the study reported above the silages not only differed in D-values (71 vs 62) but also in crude protein contents of the dry matter (18 vs 12%).

Season of the year during which silage is harvested can also influence silage composition with autumn harvested material generally being much higher in crude protein content and lower in fibre levels than spring harvested material of similar D-value. The effects of such changes on the response to protein has been examined with spring and autumn harvested materials being compared. The results of this study are shown in Table 3. Both silages were unwilted prior to ensiling, had formic acid applied as an additive, and were of similar digestibility. While the responses to supplementary protein were relatively small with both silages, there was a trend towards a greater response with the spring harvested material.

Level of supplementation and forage:concentrate ratio

In the series of experiments reported above level of concentrate supplementation was not constant between experiments and ad-libitum access was always allowed to the silage portion of the diet. Therefore trials have differed in both supplementation level and forage:concentrate ratio in the overall diet. The evidence available from hay based diets would suggest that the response to protein will increase with increasing levels of concentrate supplementation (Gordon, 1977). An experiment has therefore been carried out to examine if the level of supplementation given with silage diets influences the response to increasing crude protein content of the supplement (Mayne and Gordon, 1982). In this study concentrate supplementation levels of 7.0 and 10 kg day⁻¹ were offered to cows in early lactation in addition to a grass silage containing 13%

crude protein in the dry matter. A summary of the responses obtained are given in Table 4.

TABLE 4

The response in milk yield per unit change in crude protein content of the supplement as influenced by level of concentrate supplementation

Source	Level of Supplement (kg d ⁻¹)	Milk yield response (kg d ⁻¹)
Mayne and Gordon (1982)	7.0	+ 0.15
	10.0	+ 0.23

These results indicate that the response was greatest with the higher level of supplementation, although if the response is expressed per g of additional crude protein intake the differences between the responses are largely removed.

In a more recent study the influence of forage to concentrate ratio on the response to increasing levels of supplementary protein has been examined. Four levels of supplementary protein ranging from 10 - 21% were offered to cows receiving diets in which silage supplied either 40, 50 or 60% of the total dry matter. Within each forage:concentrate ratio increasing the level of protein resulted in a linear increase in milk yield with the mean response being 0.12 kg milk per percentage unit change in crude protein content of the concentrate. However there was no indication from the results that the magnitude of this response was influenced by forage:concentrate ratio.

ORIGIN OF RESPONSES TO PROTEIN

There are a number of possible factors which may influence milk yield the response to increasing levels of supplementary protein. These are outlined below:

Changes in milk composition
Effects on silage intake
Effects on ration digestibility
Direct effects of protein

Milk Composition

It is widely accepted that increases in crude protein intake will result in small increases in the crude protein content of milk when assessed as N x 6.38. Similar trends have been obtained in the present studies although it is likely that such changes mainly reflect an increase in the non-protein nitrogen rather than the protein content of the milk (Gordon and Forbes, 1970). In the present studies there were fairly consistent trends in the butterfat content of the milk with increased protein intake generally resulting in small reductions in milk butterfat content. The net effect of these changes has been such as to reduce the energy value of the milk per kg, with the result that the increases in milk energy output have not been as large as the increases in milk yield. This is borne out by the responses in terms of fat corrected milk yield (FCM) being only 65% of the responses in milk yield. For example Table 5 provides a summary of the responses determined within the present series of experiments both in terms of milk yield and FCM yield.

TABLE 5

Summary of responses in milk yield and fat corrected milk yield per unit change in crude protein content of the supplement

Source	Yield response (kg d ⁻¹)	
	Milk yield	FCM
Gordon (1979)	+ 0.36	+ 0.26
Gordon & McMurray (1979)	+ 0.30	+ 0.23
Gordon (1980a)	+ 0.15	+ 0.08
Gordon (1980b)	+ 0.21	+ 0.17
Peoples and Gordon (Unpublished)	+ 0.09	+ 0.01
Peoples and Gordon (Unpublished)	+ 0.12	+ 0.05
Mayne and Gordon (1982)	+ 0.15	+ 0.02
Mayne and Gordon (1982)	+ 0.23	+ 0.19
	Mean	+ 0.13

Effects on silage dry matter intake

In the present series of experiments ad-libitum access was allowed to the silage and it has therefore been possible to determine effects on intake. It has been found that increasing the level of supplementary protein results in marginal but consistent increases in silage intake. A summary of the data available is given in Table 6.

TABLE 6

Increase in silage dry matter intake (DMI) per unit
increase in the crude protein content of the
supplement

Source	Increase in silage DMI (kg d ⁻¹)
Gordon (1979)	+ 0.041
Gordon (1980)	+ 0.039
Peoples and Gordon (Unpublished)	+ 0.021
Hayne and Gordon (1982)	+ 0.025
Mean	+ 0.032

Effect on digestibility

In a number of the studies total ration digestibilities have been carried out with the lactating cows. These have shown increased supplementary protein to result in increases in the digestibility of the total diet. A summary of the recorded responses in digestibility are given in Table 7 and it would seem that the major proportion of increase arises from increased digestion of **fibre**.

TABLE 7

Increase in digestibility of the total diet per unit increase
in the crude protein content of the supplement

Source	Increase in digestibility (% units)
Gordon (1979)	+ 0.35
Gordon (1980)	+ 0.22
Peoples and Gordon (Unpublished)	+ 0.29
Mayne and Gordon (1982)	+ 0.13
Mean	+ 0.25

It is of interest to note that while considerable responses have been obtained with dairy cows the responses observed with sheep offered the same diets have only been 50% of that obtained with the cows (Unsworth, 1980).

Calculation of protein effects

The mean increase in dry matter intake due to protein in the present studies was 0.032 kg and this was associated with an increase in digestibility of 0.25 percentage units. This would suggest that an increase in crude protein content of the supplement by 1% would increase ME intake by approximately 1 MJ day⁻¹. In theory this should support an increased milk yield of 0.2 kg milk per day but it is generally accepted from production studies that this increased ME intake would only result in a milk yield response of approximately 0.09kg (Broster and Tuck, 1967; Gordon unpublished). In the present experiments the mean response was 0.2 kg milk (0.13 kg FCM) per unit increase in crude protein content of the supplement. This would therefore

suggest that approximately 50% of the response obtained in the present studies could be accounted for in terms of increased silage intake and ration digestibility and implies that a further portion of the response must be derived from other areas. It seems likely that this would be due to an increased flow of amino acids into the small intestine of the cow.

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PAPER 13

AUTHOR: F. Gordon (United Kingdom)

DISCUSSION

D. Morgan (Ireland)

Were any residual effects found?

F. Gordon

These were sometimes positive and sometimes negative. No conclusions could be drawn.

R. Jarrige (France)

Could you have interpreted your results in terms of the ARC protein system or the PDI system?

F. Gordon

The ARC protein system contains many variable factors that it is possible to obtain a range of possible answers.

EFFECTS OF SUPPLEMENTATION OF GRASS SILAGES
ON NITROGEN RETENTION IN GROWING HEIFERS

T.W. Griffiths

The Agricultural Institute, Dunsinea,
Castleknock, Co. Dublin, Ireland

ABSTRACT

Results are reported from two series of metabolism experiments which suggest that nitrogen (N) retention in growing heifers fed silage diets was limited in the first instance by metabolisable energy intake which restricted microbial protein (MP) production in the rumen. For silages containing a high N level supplementary protein could increase N retention. It is suggested that MP production was limited by factors other than $\text{NH}_3\text{ N}$ in the rumen in this case. Low protein silages when supplemented with barley gave significant responses in N retention to the addition of urea whilst high protein silages did not, indicating that rumen degradable protein could be limiting on some silage based diets, particularly if an additive containing formaldehyde had been used.

INTRODUCTION

Nutrient intake on basal silage diets is often low due to low voluntary feed intake (Murdoch 1962). Supplementation of these diets with cereals or cereal protein concentrates is, therefore, generally necessary to obtain acceptable levels of animal production. Supplementation of silage diets with protein has given responses in milk yield in dairy cows in some recent experiments (Castle and Watson 1976; Gordon and Mc Murray 1979) but the effect has not always been found (Laird et al. 1981).

It is the object of this paper to discuss the results of two series of metabolism experiments on supplementation of grass silages (Griffiths et al. 1973; Griffiths and Smith 1974) with reference to more recent concepts of protein metabolism and nutrition that are relevant to this conference (Agricultural Research Council 1980; Verité et al. 1979).

EXPERIMENTAL

Forty eight Friesian-type heifers with a live weight of 350-450 kg were used in six experiments. Barley was used as a supplementary energy source and groundnut meal as a protein source. Urea was used as a source of non-protein nitrogen. The major parameters measured were metabolisable energy (ME) intake, nitrogen (N) retention and volatile fatty acid (VFA) and ammonia N levels in

the rumen in some animals fitted with rumen cannulae.

RESULTS

Supplementation with energy

In all experiments barley was used as the supplementary energy source. Although barley addition depressed silage intake, without exception ME intake and N retention were significantly increased. The increase in N retention was the result of an almost equal reduction in urinary N excretion. In both series of experiments N retention (NR) was linearly related to ME intake:

Series 1	NR = 4.36 (\pm 0.18) ME - 472
Series 2	NR = 4.20 (\pm 0.42) ME - 493
	(g/kg W ^{0.75}) (Kcal/kg W ^{0.75})

It was also found that supplementation with barley significantly increased VFA but reduced rumen ammonia N (Griffiths and Bath 1973) frequently to levels approaching those considered limiting for maximum microbial protein synthesis in the rumen (Hume et al. 1970).

It was clear, therefore, that whilst energy was the first limiting factor for microbial protein production and N retention in growing cattle fed silage diets the second limiting factor was probably N.

Supplementation with protein

Table 1 summarises the results of one experiment on supplementation of silage diets with protein (as groundnut meal) and energy, which is of interest since the treatments were similar to those used by Castle and Watson (1976).

Table 1: Effects of protein and energy supplementation on N balance and associated data

	Treatments				S.E. of treatment
	Silage only	Supplements			
		Groundnut	Barley	Groundnut + Barley	
ME intake (M cal/kg w ^{.75})	129	153	194	214	7.0
N intake (g/d)	206	272	195	263	5.6
Urinary N excretion (g/d)	136	179	106	155	4.1
N retention (g/d)	5	18	22	30	3.6
Rumen NH ₃ N (mg %)	22	32	13	21	3.0
Rumen VFA (mE/l)	90	99	98	121	3.3

The interpretation of these results is not clear. The significant response in N retention to supplementation with groundnut is unlikely to be due to additional rumen degradable protein (RDP) since rumen NH₃ values were high. It is also unlikely to be due to additional undegraded dietary protein (UDP) since requirements of growing animals for UDP are low.

It is possible, however, that the groundnut might contribute some factor which increased the efficiency of microbial protein synthesis in the rumen since rumen VFA levels were also increased.

Supplementation of barley silage diets with urea

Supplementation of silages with barley increased N retention but reduced rumen NH₃ levels in some cases to values < 5 mg % (Griffiths and Bath 1973) suggesting that RDP might limit microbial protein synthesis. This possibility was examined using silages of high and low N content (Griffiths and Smith 1974). These silages were treated with a formaldehyde-sulphuric acid additive. Results are summarised in Table 2.

Table 2: Effect of urea supplementation of silage - barley diets on N retention and rumen NH₃

Supplements		Barley	Barley + Urea	S.E. of treatment mean
<u>Low protein silage</u> (11.8% of DM)				
ME intake	(Kcal/W ^{0.75})	218	220	3.3
N intake	(g/d)	124	143	1.3
N retention	(g/d)	45	55	2.1*
Rumen NH ₃ pool	(g)	3.9	6.3	
<u>High protein silage</u> (16.3% of DM)				
ME intake	(Kcal/W ^{0.75})	225	219	4.9
N intake	(g/d)	165	182	4.9
N retention	(g/d)	33	38	2.1 NS
Rumen NH ₃ pool	(g)	6.2	9.5	

Addition of urea to mixed barley silage diets significantly increased N retention for low protein silages but not for high protein silages. It is suggested that RDP might have been limiting on the low protein silage diets since the rumen ammonia pool was less than 4 g.

CONCLUSIONS

For growing heifers fed silage diets the first limiting factor in growth and N retention is undoubtedly energy. However, there are circumstances where responses have been obtained from protein, and from urea for low protein silages supplemented with barley. The evidence suggests that low protein silages supplemented with barley might be marginally deficient in RDP. However, microbial protein synthesis might be limited on some silages by factors other than RDP which are supplied by preformed protein.

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PAPER 14

AUTHOR: T.W. Griffiths (Ireland)

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DISCUSSION

S. Tamminga (The Netherlands)

How often was rumen ammonia measured?

T.W. Griffiths

Every two hours over 12-14 hours.

SUPPLEMENTATION OF LUCERN-BASED DIETS FOR INTENSIVE BULL FATTENING

Ch.V. Boucqué, B.G. Cottyn, L.O. Fiems and F.X. Buysse

National Institute for Animal Nutrition, Melle-Gontrode - Belgium

ABSTRACT

During 4 consecutive years, beef production experiments were carried out, to study different energy or protein sources as supplement in complete dry rations containing 50 % lucern pellets. Two important energy sources usually available on arable farms, namely dried sugar beet pulp and barley, were used as only energy source (50 % of the rations I and III) besides lucern pellets (50 %) and a premix (3 %) of minerals, trace elements and vitamins.

The composition of the two other rations (II and IV) was less simple ; a mixture of 50 % lucern pellets with only 25 kg dried beet pulp or rolled barley and further soybean oil meal, tapioca, molasses solubles, minerals, trace elements and vitamins.

In ration V, high quality feedstuffs were incorporated as well for the energy as for the protein supplementation : rolled barley (21 %), tapioca (13 %), tallow (2.5 %), linseed oil (1 %), herring meal (3 %), soybean oil meal (2 %) besides molasses solubles (5 %) and the deficient minerals, trace elements and vitamins.

All rations were ad lib. fed to Belgian white-blue store cattle (108 altogether) kept in straw bedded loose houses. Straw and drinking water were always available.

The relative feeding value of used lucern pellets, calculated from digestibility trials with wethers reached only 49 % of this of barley.

With each of the 5 rations we obtained a high feed - and dry matter intake. We established a significant relation between net energy intake per kg metabolic weight and growth rate. The cheapest feed cost price per kg gain was obtained with a simple less energetic ration (I) in spite of a lower daily gain (1.17 kg) ; such is due on the one hand to the cheapest energy source (dried beet pulp) and on the other hand to the simplicity of the ration (no manufacture costs).

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The best performances were obtained with ration V with the highest energy density. From the economical point of view however, the most simple ration based on 50 % lucern and 50 % dried beet pulp, supplemented with a vitamin mineral mixture should be preferred.

1. Introduction

During recent years, a large number of feeding trials were conducted at our Institute, to study complete dry rations for intensive fattening bulls. Most profitable beef production, results from best combination of ration and labour. Feeding dry rations requires minimal feeding labour. Following dry feedstuffs were already successfully used : dried sugar beet pulp and citrus pulp pellets (Boucqué et al., 1969, 1976) ; dehydrated whole maize plant pellets (Boucqué et al., 1973) ; dehydrated pelleted pea haulms with pods (Cottyn et al., 1973a) haypellets, lucern pellets, lucern-cocksfoot pellets (Cottyn et al., 1973b,c) ; barley in combination with beet pulp (Cottyn et al., 1976).

In spite of a low lucern production in Belgium (2,330 ha in 1981, I.E.A., 1981) a large quantity namely 82,700 t. is still yearly imported from France (Truchetto, 1980). While in 1969 1,228,000 ha lucern was cultivated in France, this area was decreased to 725,000 ha in 1979. From this production dehydrated lucern represented still more than 950,000 ton in 1978 versus 811,000 ton in 1977 from which 30 % is exported (Peyraud, 1979). Besides an ingredient for poultry and pig feeds, dehydrated lucern can be utilized by ruminants and horses.

Lucern pellets can be a potential stable constituent of complete dry rations. Other interesting aspects of introducing this feedstuff are a minimum feeding labour, the facilities and attractiveness of handling and storing this dry feedstuff on the farm.

High animal performances can however only be obtained with high energy rations (Boucqué et al., 1980a). Following Béranger and Marchadier (1970) dehydrated lucern has an average feeding value of 62 % compared with a compound feed. This is understandably insufficient to be used as sole basic component in dry rations for intensive beef production systems.

The objectives of this research were to investigate the relative feeding value of lucern pellets in comparison with beet pulp and barley and the effect of different energy supplements in complete dry rations for finishing bulls based on 50 % lucern pellets.

2. Materials and Methods

During 4 consecutive years, beef production experiments were carried out with in total 108 Belgian white-blue young store bulls, to study different energy or protein sources in complete dry rations containing 50 % lucern pellets. Two important energy sources usually available on large arable farms, namely dried sugar beet pulp and barley were used as energy source (50 % of the rations I and III) besides lucern pellets (50 %) and a premix (3 %) of minerals and trace elements. The composition of two other rations (II and IV) was less simple ; a mixture of 50 % lucern pellets with only 25 kg dried beet pulp or rolled barley and further soybean oil meal, tapioca, molasses solubles, minerals, trace elements and vitamins.

In ration V, high quality feedstuffs were incorporated as well for the energy as for the protein supplementation : rolled barley (21 %), tapioca (13 %), tallow (2.5 %), linseed oil (1 %), herring meal (3 %) and soybean oil meal (2 %) (table 1).

Table 1. Composition of the dry rations (%)

Ration	I	II	III	IV	V
Lucern pellets	50	50	50	50	50
Sugar beet pulp pellets	50	25	-	-	-
Rolled barley	-	-	50	25	21
Supplementary protein source	-	soybean oil meal		soybean oil meal	herring meal
Soybean oil meal 44 %	-	7	-	6	2
Herring meal 73 %	-	-	-	-	3
Tapioca	-	9.95	-	10.95	13.2
Molasses solubles	-	5	-	5	5
Linseed oil	-	-	-	-	1
Tallow	-	-	-	-	2.5
Mono-Na-phosphate	1.5	1.25	-	-	0.5
Feed phosphate	-	-	1	1	-
Salt	0.25	0.25	0.50	0.50	0.25
Trace elements	1	1	1	1	1
Vitamins A, D ₃ , E	0.55	0.55	0.55	0.55	0.55

All rations were fed ad lib. to the animals within the average liveweight interval 296 to 578 kg. The animals were kept in straw bedded loose houses. Straw in the rack and drinking water were always available. They were weighed at four-week intervals.

The feeds were chemically analysed following the Weende scheme. The digestibility of the 5 complete rations was determined with five wethers fed at maintenance level. After an adaptation period of 10 days, faeces were quantitatively collected and sampled once a day during an experimental period of 10 days. The digestibility of the main feedstuffs namely : lucern pellets, dried sugar beet pulp and rolled barley was also determined with wethers.

3. Results and discussion

3.1. Relative feeding value of lucern pellets

The chemical composition, digestibility and calculated net energy content (starch units) of the main used feedstuffs is shown in table 2.

Table 2. Chemical composition (g/kg), digestibility (%) and feeding value (g/kg) feedstuffs (\pm s_x)

	Dry matt.	Dry matter composition						Dig. crude prot. d.m.	Starch units d.m.
		Organ. matt.	Crude prot.	Crude fibre	Ether ext.	N-free ext.	Ash		
<u>Lucern pellets</u>	893	897	167	277	32	421	103	<u>104</u>	<u>397</u>
	+13	5	7	9	1	7	5	4	8
Dig. coeff.	55.0	57.4	62.3	35.1	51.4	69.6	-		
	+ 1.8	1.8	1.3	5.9	0.8	2.5			
<u>Sugar beet pulp</u>	873	935	101	195	9	630	65	<u>58</u>	<u>739</u>
	+11	17	6	15	4	16	17	1	9
Dig. coeff.	78.6	83.3	56.6	82.4	-	89.5	-		
	+ 1.9	1.9	6.7	2.3		1.4			
<u>Barley</u>	853	971	115	56	19	781	29	<u>80</u>	<u>804</u>
	+ 3	1	7	1	2	8	1	5	5
Dig. coeff.	82.2	84.3	69.4	-	86.3	90.3	-		
	+ 1.9	1.7	4.3		7.9	1.1			
<u>Soybean meal</u>	866	934	500	69	17	348	66		
	+ 2	3	12	9	6	10	3		
<u>Herring meal</u>	893	887	751	-	92	44	113		
	+15	2	40		24	25	2		

The average net energy content in the dry matter of the used lucern pellets amounted to only 397 starch units compared with 739 for sugar beet pulp and 804 for barley. The relative feeding value of lucern pellets reaches only 49 % of this of barley and 54 % of this of sugar beet pulp. From beef production experiments with young bulls, Béranger and Marchadier (1970) calculated for dehydrated lucern (with 18 % C.P. and 28-30 % C.F. in the d.m.) a relative feeding value of 62 % compared with a compound feed containing 70 % barley. From feeding experiments conducted with steers (Horton, 1978) we calculated a relative feeding value for dehydrated lucern of 61.6 % compared with barley.

In table 3, data about chemical composition and net energy content of dehydrated lucern derived from existing European feed value tables are summarized. The calculated feeding value of dehydrated lucern compared with barley obtained by dividing net energy content of lucern by net energy content of barley varied from 48 to 68 %.

Table 3. Chemical composition and net energy content of dehydrated lucern derived from feed value tables

	Dry matt. (g/kg)	Dry matter composition (g/kg DM)					Net energy (per kg DM)	Value v.s. barley (%)
		Crude prot.	Crude fibre	Ether ext.	N-free ext.	Ash		
M.A.F.F. (1975)	900	178	269	27	414	112	ME = 8.7 MJ	63.5
I.N.R.A. (1978)	910	176	330	-	-	90	UFV = 0.59	51
C.V.B. Nederland (1977)	902	173	303	33	377	114	VEVI = 695	64
Kellner u. Becker (1966)	900	167	322	27	392	92	SU = 369	48
Schiemann et al. (1971)	900	190	305	28	377	100	EFr = 471	68
DIG-Futterwert-tabellen (1982)	907	178	283	26	383	130	SU = 425	53

The low net energy content of lucern pellets is due to the low organic matter digestibility (57 %) principally owing to the high crude fibre content (277 g/kg which is very poor digestible (35 %). The high content of crude fibre (195 g/kg) in sugar beet pulp with an average digestibility of 82.4 % contrasts favourably with this of lucern pellets. The N-free

extract fraction of lucern pellets remains low 420 g/kg and its digestibility is moderate, comparing with the very high digestibility of the carbohydrates of sugar beet pulp and barley (89.5 and 90.3 %). The crude protein content of used lucern pellets (standard quality for ruminants) averaged 167 g/kg with a digestibility of 62 % resulting in a digestible crude protein in the dry matter of 104 g/kg. Protein quality of lucern seems further excellent (Ferrando and Spais, 1966 ; Tagari, 1969 ; Pacquay et al., 1973 ; Klopfenstein, 1981). Results of balance experiments conducted by Tagari (1969) showed clearly that sheep utilized the protein of lucern hay more efficiently than that of soybean oil meal when both feeds served as the sole source of protein in the diets. Hennaux (1966) however established very negative N-balances due to excessive urine N-losses when lucern silage was fed as the sole diet to lactating cows. A possible explanation can be the higher solubility of the protein in silage compared with the dehydrated product. Klopfenstein (1978) indeed established that when lucern was dehydrated, the heat in the dehydrating process reduced the protein solubility. The new PDI system used in France is following Peyraud (1979) also favourable for dehydrated lucern in comparison with soybean oil meal. The difference between the DCP content of SBM 50 % and dehydrated lucern 20 % amounted to 340 g per kg DM while expressed in PDIN the difference amounted to only 230 g per kg DM. Lucern outshines all other forages in Ca content ; we dosed an average content of 23 g/kg in de d.m. ; P content however remains rather low (3 g/kg in de d.m.).

3.2. Beef production results

The chemical composition (average of 4 experimental years) of the complete dry rations based on 50 % lucern pellets is given in table 4.

The crude protein content of ration II, IV and V is considerably higher than for ration I and III due to the protein content of SBM and herring meal which was higher than normally estimated (table 2).

Table 4. Chemical composition of the complete dry rations (g/kg)

Ration		I 50 % pulp	II 25 % pulp 7 % SBM	III 50 % barley	IV 25 % barley 6 % SBM	V 21 % barley 3 % her- ring meal
Dry matter		870	861	870	866	868
Dry matter composition	Organ. matter	906	898	924	907	909
	Crude protein	133	164	138	163	163
	Crude fibre	235	204	168	169	152
	Ether extract	21	20	25	23	67
	N-free extract	517	510	593	552	527
	Ash	94	102	76	93	91
	Ca	15	15	14	16	14
	P	5	4	5	5	4

The digestibility coefficients (wethers) and the calculated feeding value is shown in table 5.

Table 5. Digestibility coefficients and feeding value
of the dry rations (g/kg d.m.) $\pm s_x$

Ration	I	II	III	IV	V
Dry matter	68.9	69.8	70.4	69.5	73.9
	± 1.7	0.6	2.3	1.3	1.5
Organ. matter	71.8	72.1	73.0	72.3	76.0
	± 1.6	0.8	2.3	1.5	1.6
Crude protein	62.4	70.9	70.7	70.2	76.0
	± 3.5	1.2	3.7	2.5	2.1
Crude fibre	60.4	51.1	40.3	42.8	51.1
	± 2.2	1.9	5.0	2.6	4.6
Ether extract	29.4	43.8	55.8	58.4	84.0
	± 4.4	1.5	2.0	3.0	1.1
N-free extract	80.8	81.3	82.6	82.2	82.4
	± 1.2	0.6	1.5	1.0	0.9
<u>Feeding value</u>					
Dig. cr. prot.	83	116	98	114	124
Starch units	574	568	609	594	715

The digestibility of the dry matter, the organic matter and the N-free extract of the four first rations is very similar. All the nutrients of the high quality and energy rich ration V show a higher digestibility except the N-free extract fraction. The ether extract digestibility of ration V was considerably higher than of the other rations due to the incorporation of tallow and linseed oil.

Beef production results are presented in table 6.

With complete dry rations based on 50 % lucern pellets, we obtained an average daily liveweight gain of 1.23 kg varying from 1.17 kg to 1.29 kg. A high mean daily growth rate could be obtained for the four first dry rations (1.22 kg/d) with rather moderate energy content (average S.U. in the d.m. 586 g - table 5.).

The daily growth rate was higher than this obtained earlier with dry rations based on 60 % lucern pellets (Cottyn et al., 1973b) where we obtained for 20 bulls a mean daily gain of 1.14 kg between a weight interval of 248 - 559 kg. In that experiment we noted a lower daily dry matter intake per kg $W^{0.75}$ namely 92 g versus 98 g. Horton (1978) observed daily live weight gains with steers (290 - 445 kg) of 1.46 kg per day when 46.5 or 66.5 % dehydrated lucern respectively was included in the diet completed with rolled barley. Haurez et al. (1977) reported a daily gain of 1.15 kg with Normand bulls fed a diet containing 48 % dehydrated lucern completed with 48 % maize grain. With Salers bulls a daily gain of 1.07 kg and 1.15 kg was noted with rations consisting of 49 % dehydrated lucern and 49 % dried sugar beet pulp.

With each of the 5 rations we obtained a high feed intake and a high dry matter intake per kg metabolic weight. For the 5 experimental groups an average daily feed intake of 10.83 kg dry feed and a dry matter intake of 98.3 g per kg $W^{0.75}$ was noted ; this high intake data prove that complete dry rations based on 50 % lucern pellets are very palatable.

We established a significant relation between net energy intake per kg metabolic weight (x) and growth rate (y) (fig. 1).

Table 6. Beef production results ($\pm s_x$)

Ration (%)	I	II	III	IV	V
Lucern pellets	50	50	50	50	50
Beet pulp pellets	50	25	-	-	-
Rolled barley	-	-	50	25	21
Supplementary protein	-	SBM		SBM	herring meal
Number of bulls	22	21	22	21	22
Initial weight (kg)	292.2	295.8	297.5	296.7	296.2
Final weight (kg)	564.7	586.1	586.1	569.1	582.9
Experimental days	232.3	235.0	228.9	225.3	223.0
<u>Daily liveweight gain (kg)</u>	<u>1.17^a</u>	<u>1.24^a</u>	<u>1.26^a</u>	<u>1.21^a</u>	<u>1.29^a</u>
	± 0.03	± 0.05	± 0.04	± 0.04	± 0.04
Daily feed intake (kg)	10.58	11.37	10.51	11.14	10.55
<u>Intake/kg W^{0.75} (g/d)</u>					
- dry matter	97.9 ^a	101.8 ^a	94.9 ^a	101.6 ^a	95.5 ^a
- dig. crude prot.	8.1	11.8	9.2	11.9	11.9
- starch units	56.1 ^a	57.8 ^a	57.8 ^a	59.9 ^a	68.3 ^b
<u>Feed conversion (kg)</u>	<u>9.01^a</u>	<u>9.20^a</u>	<u>8.35^b</u>	<u>9.21^a</u>	<u>8.21^b</u>
	± 0.11	± 0.28	± 0.06	± 0.11	± 0.14
- dry matter	7.86 ^a	7.93 ^a	7.26 ^b	7.97 ^a	7.13 ^b
- dig. crude prot.	0.65	0.92	0.70	0.93	0.89
- starch units	4.50 ^{a,b}	4.50 ^{a,b}	4.42 ^a	4.70 ^b	5.10 ^c
<u>Carcass data (%)</u>					
Weight loss after 20 h. fasting (%)	3.72 ^{a,b}	4.08 ^a	3.26 ^{b,c}	3.75 ^{a,b}	2.94 ^c
	± 0.28	± 0.29	± 0.14	± 0.23	± 0.19
<u>Dressing percentage</u>	<u>61.9^a</u>	<u>61.8^a</u>	<u>62.2^a</u>	<u>61.7^a</u>	<u>62.4^a</u>
	± 0.3	± 0.3	± 0.3	± 0.3	± 0.3
<u>Feed cost price (BF)^x</u>					
- per kg feed	6.93	7.54	8.04	8.02	8.90
- per kg gain	62.4	69.4	67.1	73.9	73.1

a,b,c : means on the same line bearing different superscripts differ significantly (P < 0.05)

^x Unit prices : lucern pellets 16 % C.P. : 6.4 F ; sugar beet pulp : 6 F ; rolled barley : 8 F ; soybean oil meal 44 % C.P. : 10 F ; herring meal 73 % C.P. : 23 F.

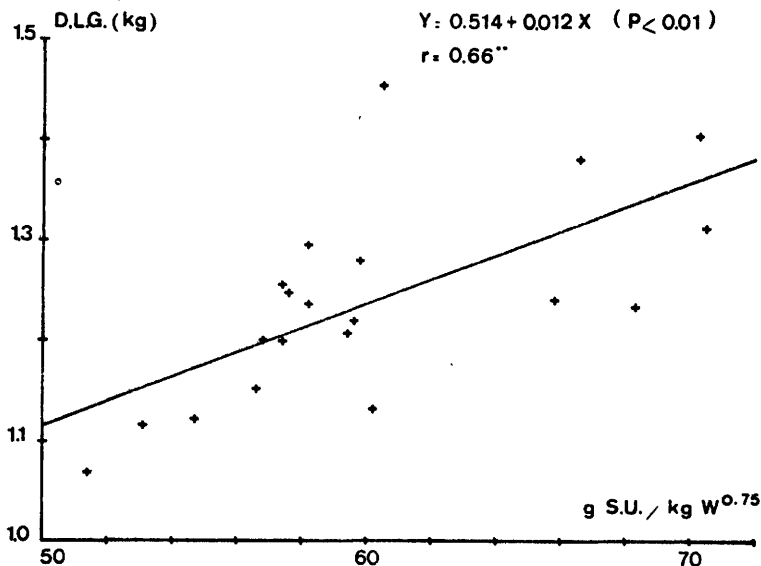


FIG. 1 RELATION BETWEEN S.U. INT. AND D.L.G.

The cheapest feed cost price per kg gain was obtained with the less energetic ration (I), in spite of the lowest daily gain (1.17 kg/d); such is due on the one hand to the cheapest energy source (dried beet pulp - 50 % of the ration) and on the other hand to the simplicity of the ration (no manufacturing costs). With the simple mixture of 50 % lucern and 50 % rolled barley (III) we obtained a high daily gain of 1.26 kg. Ration I and III further prove that lucern pellets can be used as a valuable sole protein source for intensive beef production.

Excessive amounts of protein were fed by supplementing groups II, IV and V with SBM or herring meal. From 11 feeding experiments with 340 fattening bulls de Boer and Hamm (1977) concluded that 11 g CP per $W^{0.75}$ is sufficient for a DLG of around 1100 g in fattening bulls of 275 kg and less. For fattening bulls in the live weight range of 275-375 kg about 9 g CP per $W^{0.75}$ seems to be appropriate, while 8 g CP per $W^{0.75}$ will suffice for fattening bulls of a heavier live weight. This is in agreement with our findings (Bouqué et al., 1980b) where a crude protein intake of 9 to 9.9 g per kg $W^{0.75}$ was sufficient for a daily gain of 1.11 to 1.27 kg between a weight interval of 240-630 kg.

The best performances were obtained with ration V characterized by the highest energy density. From the economical point of view however, the most simple ration based on 50 % lucern pellets and 50 % dried beet pulp, supplemented with a vitamin mineral mixture should be preferred. Before slaughter, the bulls were fasted for 20 hours. Fasting losses varied from 2.94 for the animals of the last ration (V) to 4.08 for ration II. The significant lower weight loss after fasting of ration V seems to be due to the energy density which resulted in a lower dry matter intake. The dressing percentage was high (average of 62 %) and differed not significantly.

3.3. Conclusions

- 1) The digestibility and net energy content of lucern pellets is rather low. We calculated an average S.U. in the d.m. of 397 g/kg ; such means a relative value of 49 % of this of barley (804 g/S.U. per kg d.m.).
- 2) With complete dry rations based on 50 % lucern pellets high animal performances could be obtained. An average high daily gain of 1.23 kg due to a high feed and dry matter intake was noted.
- 3) Lucern pellets can be used as a valuable sole protein source for intensive beef production. A mixture of 50 % lucern pellets and 50 % beet pulp or 50 % lucern pellets and 50 % barley were the most interesting formules.
- 4) Due to the increasing dehydration costs of last years however, the economical benefit of such dry rations based on lucern pellets will be limited.

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PAPER 15

AUTHOR: B.C. Cottyn (Belgium)

DISCUSSION

M.F. Maguire (Ireland)

Was the low feeding value of the lucerne reflected in its price?

B.C. Cottyn

Relative to barley lucerne is also a protein source.

S. Tamminga (The Netherlands)

What was the source of the lucerne?

B.C. Cottyn

The lucerne was a commercial product from France and had not been used for crop fractionation.

Keane (Ireland)

Was the sugarbeet pulp molassed and what was its energy value relative to barley?

B.C. Cottyn

The sugarbeet pulp was not molassed and its energy value was taken as 90% of barley.

W. Sheehan (Ireland)

Do you have any carcass composition data?

B.C. Cottyn

No.

Forage Protein Conservation and Utilization : Concluding Remarks

R. J. Wilkins
Grassland Research Institute,
Permanent Grassland Division,
North Wyke, Okehampton, Devon. EX20 2SB

The objectives of the Seminar were to discuss ways in which the utilization of protein in forages could be improved. Improvements in forage protein utilization would bring benefits through increasing the efficiency of animal production and through facilitating reduction in the need for supplementary feed of high protein content (a major import into the Community).

It has long been assumed that young forage (grasses and legumes) contains protein in excess of the requirements of ruminant animals. However, recent evidence, including that presented to the Seminar by Beever, indicates that amino-acid supply from such diets may limit production with lactating animals, young cattle and sheep. Considerable data from experiments with silages supports this view, and there are indications that such limitations may take place also with fresh forage, either grazed or fed indoors. This low amino-acid supply reflects inefficiency in the use of nitrogenous constituents of the feed and underlines the need to develop methods for increasing the efficiency of use of forage protein.

The Seminar considered the possibilities of improving efficiency through green crop fractionation and through the better use of unfractionated forage. This paper considers briefly these two approaches, and then some priorities for further research in the Community are discussed.

Green crop fractionation

Green crop fractionation may still be a valid approach to forage utilization even if the production of some ruminants fed unfractionated forage is limited by amino-acid supply. The high efficiency of protein use by the pig means that animal production per hectare may be increased by fractionation and feeding the pressed forage to ruminants and the juice to pigs. Also, fractionation may facilitate the exploitation of high-value components in forage, such as xanthophyll and the improved conservation of the crop as silage.

However, papers presented at the Seminar have shown that there are still problems in developing economically-efficient systems involving fractionation. Energy costs for the process are high, and there has been little progress in developing machinery for the initial fractionation into pressed forage and juice. In particular, machinery suitable for use on farm rather than factory scale has not advanced. There are difficulties with the storage of the juice and in organization of the complete operation involving the daily production of forage and its fractionation into feeds for different classes of stock. In some instances the performance of pigs fed forage juice has been disappointing; on the other hand, as indicated by Jones, rates of production by animals fed pressed forage have been higher than those found with unfractionated forage.

I suggest that fractionation is only likely to be an attractive method of forage use when most of the following conditions are satisfied:

1. There is a large-scale farming enterprise - in order to justify the high management skill required.
2. Cattle and pigs are both kept on the same farm (or in close proximity) - in order to facilitate the utilization of the products of fractionation in the fresh state or with minimum requirement for preservation.

3. Cattle are housed during the forage growing season. Thus fractionation can be adopted without the need to change from herbage utilization by grazing, which can be both a cheap and an efficient method of harvesting.
4. The Country, or Region, does not have a ready supply of high-protein feed.
5. There is a strong demand for xanthophyll - processes involving the production of dried leaf-protein concentrate cannot be economically justified on protein and energy value alone.

There are relatively few situations within the Community where these criteria are adequately satisfied, and it is likely that the adoption of green crop fractionation here will be limited. There are, in contrast, many more situations in Eastern Europe where the structure of agriculture favours fractionation and adoption of the process may be more likely in these countries. It is also possible, as suggested by Carlsson, that green crop fractionation will be important along with the processing of biomass for energy and as a chemical feedstock - pressed crops may be fermented to methane, deproteinised juice to ethanol, and the leaf-protein concentrate used for xanthophyll and protein.

Utilization of forage protein

The efficiency of utilization of the crude protein (CP) in forage will be influenced by the intake of CP and the metabolism of the consumed CP.

Intake. Intake of CP is particularly important as a high intake is needed to provide a margin over the maintenance requirement of the animal. Also, increases in forage intake result in increases in the intake of both CP and energy. The intake of CP in a particular forage will be affected by forage species (and possibly variety), the growth stage at harvest and the fertilizer regime used - particularly in relation to nitrogen. For a particular forage cut, intake will

also be influenced by the method of presentation and by changes during conservation. With grazed forage, the morphological structure of the sward and the quantity offered will be important; in silages there are effects from the extent of clostridial fermentation that has taken place; with hays the magnitude of loss during haymaking is important.

Metabolism. Discussion on CP metabolism centred on factors affecting the quantity of plant protein which may escape digestion in the rumen and on the yield of microbial protein. The rate of degradation in the rumen of the CP in fresh and ensiled forages is normally high. It is clear, however, that the protein in tannin-rich species such as Onobrychis sativa and Lotus spp. has low degradability in the rumen, but the extent of variation between other forage species is not known.

Method of conservation can have large effects. The CP in silages made without additives appears to be degraded somewhat more rapidly in the rumen than that in fresh forages. Marked reductions in protein degradation may result from high-temperature dehydration and from the addition of formaldehyde at harvesting, particularly for silage. Dehydration is, however, an extremely costly method of forage conservation and is not likely to increase in importance. Formaldehyde treatment, on the other hand, as well as reducing CP degradability in the rumen may reduce total rumen bacterial activity and render some of the plant protein and amino-acids unavailable in the intestines. Alternative processes for controlling forage protein degradability are needed.

The synthesis of microbial protein received considerable attention at the Seminar. The French P.D.I. system of evaluation discussed by Jarrige highlights the fact that shortage of energy in forages will commonly limit the quantity of microbial protein that is synthesised. Thomas indicated that fermentation in the silo will reduce the energy supply for microbial activity in the rumen, and thus the yield of microbial protein. There is also evidence for variation in the quantity of microbial protein synthesised per unit of organic matter digested in the rumen.

Microbial protein production is important not only in relation to its effects on total amino-acids available to the animal but also in terms of the supply of individual amino-acids, because the biological value of microbial protein is normally higher than that of forage protein.

Supplementation. The need for supplements will be determined by the requirements of the animal and the amino-acids supplied from forage. The responses to additional protein feeding with lactating animals and young ruminants with a high growth potential have already been noted, particularly with silages.

With forages of high protein content, it appears that deficiency in amino-acid supply can be rectified by increasing microbial protein production in the rumen, normally by providing a carbohydrate-rich supplement, or by providing a protein supplement with a low degradability in the rumen. The feeding of maize silage with grass silage (or fresh grass) as widely practised in The Netherlands will often provide a better ratio of energy to rumen-degradable CP, and probably improve overall yield of microbial protein. It seems likely that deficiency in the quantity of feed protein reaching the intestines can be recti-

fied more successfully by provision of a supplement of low degradability (possibly treated industrially by controlled heating, or with formaldehyde) than by attempting to reduce the degradability of forage protein (with possible adverse effects on microbial activity and on in vivo digestibility).

It is notable, however, that in many experiments - for instance those reported by Gordon - in which responses have been obtained to protein supplements, it has not been clearly established that the responses arise from increasing feed protein absorption from the intestines. Also, the effects of feed supplements, particularly energy supplements, on the voluntary intake of forage is important as high rates of substitution may limit the net benefit from the use of the supplement.

Research areas to improve forage protein utilization.

My personal interpretation of the evidence presented to the Seminar is that:

1. The highest priority is for research to further quantify the amino-acid supply to the ruminant from different forage diets, and to further develop techniques for predicting the degradability of CP in the rumen and for predicting microbial protein production. Progress in these areas, which has been substantial in recent years, would enable rational decisions to be made on the provision of supplements in forage-based diets, and probably result in the saving of imported protein concentrate feeds. This is an area of importance throughout Europe. Consideration should be given to ways in which the Commission can further encourage and assist this research.
2. High priority should be given to research to increase the voluntary intake of forage protein (and energy).

Lower levels of voluntary intake from forages in comparison with concentrate feeds can limit the efficiency of CP utilization and

result in low overall feed conversion efficiency. Plant breeding should have an important role to play here, and studies of inter-relationships between nutrition and endocrine factors may also have particular importance for forage diets.

3. High priority should be given to research to increase amino-acid supply to the ruminant from forage diets.

Increased attention should be given to maximising the yield of microbial protein rather than to reducing forage protein degradability.

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The scope in the Community for the use of techniques of green crop fractionation to improve protein utilization is rather low. The relationship between forage protein concentration and ruminant utilization of protein is not close, being influenced by many other factors.

PRODUCTION AND CONSERVATION OF FORAGE LEGUMES
FOR WINTER FEEDING OF CATTLE

D.P. Collins, An Foras Taluntais, Grange,
Dunsany, Co. Meath., Ireland.

SUMMARY

To ascertain the herbage productivity of predominantly legume swards and their feeding value as silage versus all-grass silage for cattle feeding, four swards were established. They were sown with (a) Lolium perenne (var. Sceempter) 22.5 kg/ha; (b) Medicago sativa (var. Europa) 16.8 kg/ha + L. perenne (var. Sceempter) 4.5 kg/ha; (c) Trifolium pratense (var. Hungeropoly) 15.7 kg/ha + L. perenne (var. Sceempter) 6.7 kg/ha; (d) Trifolium repens (var. Blanca) 4.5 kg/ha + L. perenne (var. Cropper) 11.2 kg/ha. All the swards established well with the exception of M. sativa. All were cut three times annually for silage. Fertiliser N was applied to the all-grass in the following amounts each year; 90, 80 and 70 kg/ha for cuts 1, 2 and 3 respectively.

In the first year the silages were fed to finishing steers of 440-450 kg liveweight for 94 days and in the second year to weanlings of 233 kg liveweight for 117 days.

In the first year the all-grass sward out-yielded the M. sativa sward by 55%, T. pratense sward by 26% and the T. repens sward by 78%. In the second year the T. pratense outyielded the all grass sward by 2%, the M. sativa by 10% and the T. repens by 29%.

All the swards preserved well, except M. sativa (all in excess of 4.4 on most occasions), while crude protein was high in all cuts except the initial one, when the mean level was 11.1%, and all D.M.D's were high except for M. sativa (64-65%) and higher in the first than second year.

With the finishing cattle, daily liveweight gain was highest with the all-grass silage (0.63 kg/head) but with the weanling animals T. pratense - silage was best (1.04 kg/head daily).

IN VITRO DETERMINATION OF PROTEIN DIGESTIBILITY
IN GRASS PROTEIN PREPARATIONS

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A study of the possible application of laboratory in vitro assays for measuring protein digestibility was, therefore, undertaken. Two assays were investigated. A pepsin pancreatin assay based on a 3 hr pepsin and 24 hr pancreatin digestion was modified to measure indigestible protein. A multienzyme assay based on measuring pH drop over 10 minutes on incubation with trypsin, chymotrypsin and peptidase was also carried out. Grass protein preparations made at various times after harvesting and treated with different preservatives were assayed and the effects of possible inhibitors studied with protein standards.

Wet green crop fractionation as part of an
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Galoppini C., Fiorentini R., Massantini F.

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The agronomic tests, performed on some 100 ecotypes and varieties of leguminosae and graminaceae, measured their production, the best harvesting time and their suitability for wet fractionation. It has thus been possible to find out the best combinations of crops in order to achieve a continuous and longer production of forage to be used in industrial plants.

Biochemical investigations have defined correlations between LPC yield and proteolytic activity, and have also led to the identification of a proteinase inhibitor in alfalfa.

On the basis of technological tests, a pilot plant has been set up for the fractionated recovery at room temperature of the chloroplastic and cytoplasmatic proteins present in the expressed juices. Chloroplastic proteins, to be used for animal feeding, are coagulated by a synthetic organic polyelectrolyte, while cytoplasmatic proteins, suitable for human consumption, are obtained by acidification at isoelectric pH.

Some experiments have also been performed on tobacco leaves, with the double aim of producing a deproteinized smoking residue and protein products with a high biological value.

RESPONSE IN SILAGE FED WEANLINGS TO LOW LEVELS OF SUPPLEMENTARY PROTEIN

M.G. Keane and M.J. Drennan
An Foras Taluntais, Grange, Co. Meath, Ireland.

In each of two experiments 54 weanling steers (initial L.W. 243 and 251 kg) in 6 treatment groups were fed grass silage ad libitum in a slatted house together with one of the following supplements per head daily for a 112 day treatment period

EXPERIMENT 1			EXPERIMENT 2		
SUPPLEMENT	ME (MJ/d)	CP (g/d)	SUPPLEMENT	ME (MJ/d)	CP (g/d)
1. BARLEY	5.5	60	1. NONE	-	-
2. SOYA	5.5	200	2. BARLEY	6.5	65
3. HCHO SOYA ^a	5.5	200	3. SOYA	6.5	250
4. FISH MEAL	5.5	340	4. HCHO SOYA ^b	6.5	250
5. FISH MEAL	3.2	200	5. BARLEY+FISH ^c	6.5	250
6. MEAT&BONE	5.5	340	6. BARLEY+UREA ^d	6.5	250

^aSoya home treated with 8g/kg formaldehyde; ^bSopralin, BP Nutr. Ire. Ltd.

^c250g Barley + 300g Fish Meal DM; ^d500g Barley + 70g urea DM

After the experimental period ended the animals were fed silage for 21 days and then grazed together for 91 days (Experiment 1) or they were fed silage + 1.5 kg barley for 42 days and grazed together for 126 days (Experiment 2). The animals fed the meat and bone supplement did not consume their full allowance. Average daily gains during the treatment period for groups 1 through 6 were 385, 649, 648, 743, 685 and 408 ± 40g (P<0.001) in Experiment 1 and 70, 211, 292, 490, 383 and 109 ± 33g (P<0.001) in Experiment 2. Corresponding daily gains from the end of the treatment period to the end of grazing were 987, 806, 905, 837, 793 and 870 ± 69g (N.S.) in Experiment 1 and 771, 811, 732, 694, 727 and 790 ± 44g (N.S.) in Experiment 2. In Experiment 1 all protein supplements except meat and bone were superior to barley and HCHO soya and fish meal were superior to barley in Experiment 2. Although post-treatment daily gains did not differ significantly the groups that performed poorest during treatment tended to exhibit compensatory gain subsequently. As a result there were no significant differences in liveweight at the end of grazing in Experiment 1 and the only differences in Experiment 2 were between the controls and the HCHO Soya and Barley + Fish Meal treatments. It is concluded that while weanling steers fed grass silage may respond to low levels of protein concentrates (particularly if low in degradability) the gain advantage is not always retained subsequently.

AMINO ACID ANALYSES OF FRESH AND ENSILED CROP AND JUICE
FROM GREEN CROP FRACTIONATION

Jørgen Mortensen

Statens Planteavlslaboratorium
Lottenborgvej 24
DK-2800 Lyngby, Denmark

Amino acid analyses of fresh crop and silage from ryegrass, pea and lucerne showed that the extent of degradation of amino acids during ensiling was different for the different crops, with the largest one found for lucerne, but the pattern of the degradation was roughly the same.

The largest degradation was found for the amino acids with polar groups in their side chains (arginine, serine, threonine, lysine, aspartic acid, tyrosine, glutamic acid, histidine and cystine, usually with a decreasing degradation in the named order), while the other amino acids normally were recovered with 90-110 per cent of the content in the fresh crop, except alanine, which was recovered with 135-224 per cent.

In the silage were found some amines proposed to be decarboxylation products of some of the amino acids and corresponding roughly in amount to the degradation of these.

In an experiment with green crop fractionation with ensiling of pressed and unpressed crops there was as a rule found a larger recovery of the amino acids in the silage from the pressed crop.

Analyses of stored juice from green crop fractionation showed that generally the same amino acids were liable to degradation as in silage.

Heating of the juice and lowering of the pH helped to preserve the amino acids. Addition of formaldehyde prevented the degradation of most of the amino acids, but about twenty per cent of lysine and histidine was not recovered.

References

- Nørgaard Pedersen, E. J.; Witt, N.; Mortensen, J. & Sørensen, C. (1980) - Tidsskrift f. Planteavl 84, 265-293.
—— ——— ——— (1981) - Tidsskrift f. Planteavl 85, 13-30.



ENSILING OF PRESSED CROPS

E. J. Nørgaard Pedersen

Statens Forsøgsstation, Ødum

DK 8370 Hadsten. Danmark

The ensiling of pressed crops was compared to the ensiling of unpressed crops in 9 experiments. The experimental crops were per. ryegrass (5 exp.), Ital. ryegrass (1 exp.), lucerne (1 exp.) and peas (2 exp.). A screw-press, Bentall Professor, was used to extract the juice. The crops were ensiled in 3 m³ airtight silos.

The amount of extracted juice varied from 21.6 to 46.5%. The extraction ratios for DM, OM and CP respectively were 10.0-26.6, 8.9-20.6 and 14.2-34.5. The extraction of juice reduced seepage but the reduction of the amounts of effluent was only ca. 40 % of the amounts of extracted juice.

In the 6 experiments with grass crops the OMD was determined by sheep in crops and silages. As the OMD of the untreated crops were high (average 80.3 %) the decrease of OMD which could be expected was low, assuming 95 % OMD in extracted juice about 1.5 unit. The average OMD of pressed crops, silages of unpressed crops and silages of pressed crops respectively were 79.3, 79.2 and 80.0. These values are neither significant different from the OMD of untreated crop, 80.3, nor from the expected value, 78.8.

The extraction of juice showed a slight positive effect on silage quality, especially seems the protein degradation reduced a little. In all experiments the content of true protein as % of crude protein was higher in silage of pressed crop than in silage of unpressed crop. Also the content of amines (determined in 4 experiments) seems somewhat lowered by extraction of juice. The amino acid composition seems almost unaffected.

The capacity of the screw-press varied from 950 to 1450 kgs of green material per hour. This is far too small a capacity, and - in spite of the great potential possibility of better utilization of home grown protein - our conclusion is that juice extraction under farm conditions will not be practicable until much more efficient machinery has been developed.

A report with all experimental details has been published:

Nørgaard Pedersen, E. J., Witt, Norman, Mortensen, J. & Sørensen, Chr.,
1980: Fraktionering af grønafgrøder ved udpresning af saft og konservering af pressede afgrøder og saft. I. Ensilering af pressede afgrøder. Tidsskr. Planteavl 84, 265-293.

3. Cattle are housed during the forage growing season. Thus fractionation can be adopted without the need to change from herbage utilization by grazing, which can be both a cheap and an efficient method of harvesting.
4. The Country, or Region, does not have a ready supply of high-protein feed.
5. There is a strong demand for xanthophyll - processes involving the production of dried leaf-protein concentrate cannot be economically justified on protein and energy value alone.

There are relatively few situations within the Community where these criteria are adequately satisfied, and it is likely that the adoption of green crop fractionation here will be limited. There are, in contrast, many more situations in Eastern Europe where the structure of agriculture favours fractionation and adoption of the process may be more likely in these countries. It is also possible, as suggested by Carlsson, that green crop fractionation will be important along with the processing of biomass for energy and as a chemical feedstock - pressed crops may be fermented to methane, deproteinised juice to ethanol, and the leaf-protein concentrate used for xanthophyll and protein.

Utilization of forage protein

The efficiency of utilization of the crude protein (CP) in forage will be influenced by the intake of CP and the metabolism of the consumed CP.

Intake. Intake of CP is particularly important as a high intake is needed to provide a margin over the maintenance requirement of the animal. Also, increases in forage intake result in increases in the intake of both CP and energy. The intake of CP in a particular forage will be affected by forage species (and possibly variety), the growth stage at harvest and the fertilizer regime used - particularly in relation to nitrogen. For a particular forage cut, intake will

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In an experiment with green crop fractionation with ensiling of pressed and unpressed crops there was as a rule found a larger recovery of the amino acids in the silage from the pressed crop.

Analyses of stored juice from green crop fractionation showed that generally the same amino acids were liable to degradation as in silage.

Heating of the juice and lowering of the pH helped to preserve the amino acids. Addition of formaldehyde prevented the degradation of most of the amino acids, but about twenty per cent of lysine and histidine was not recovered.

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- Nørgaard Pedersen, E. J.; Witt, N.; Mortensen, J. & Sørensen, C. (1980) - Tidsskrift f. Planteavl 84, 265-293.
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ENSILING OF PRESSED CROPS

E. J. Nørgaard Pedersen
Statens Forsøgsstation, Ødum
DK 8370 Hadsten. Danmark

The ensiling of pressed crops was compared to the ensiling of unpressed crops in 9 experiments. The experimental crops were per. ryegrass (5 exp.), Ital. ryegrass (1 exp.), lucerne (1 exp.) and peas (2 exp.). A screw-press, Bentall Protessor, was used to extract the juice. The crops were ensiled in 3 m³ airtight silos.

The amount of extracted juice varied from 21.6 to 46.5%. The extraction ratios for DM, OM and CP respectively were 10.0-26.6, 8.9-20.6 and 14.2-34.5. The extraction of juice reduced seepage but the reduction of the amounts of effluent was only ca. 40 % of the amounts of extracted juice.

In the 6 experiments with grass crops the OMD was determined by sheep in crops and silages. As the OMD of the untreated crops were high (average 80.3 %) the decrease of OMD which could be expected was low, assuming 95 % OMD in extracted juice about 1.5 unit. The average OMD of pressed crops, silages of unpressed crops and silages of pressed crops respectively were 79.3, 79.2 and 80.0. These values are neither significant different from the OMD of untreated crop, 80.3, nor from the expected value, 78.8.

The extraction of juice showed a slight positive effect on silage quality, especially seems the protein degradation reduced a little. In all experiments the content of true protein as % of crude protein was higher in silage of pressed crop than in silage of unpressed crop. Also the content of amines (determined in 4 experiments) seems somewhat lowered by extraction of juice. The amino acid composition seems almost unaffected.

The capacity of the screw-press varied from 950 to 1450 kgs of green material per hour. This is far too small a capacity, and - in spite of the great potential possibility of better utilization of home grown protein - our conclusion is that juice extraction under farm conditions will not be practicable until much more efficient machinery has been developed.

A report with all experimental details has been published:

Nørgaard Pedersen, E. J., Witt, Norman, Mortensen, J. & Sørensen, Chr.,
1980: Fraktionering af grønafrøder ved udpresning af saft og konservering af pressede afgrøder og saft. I. Ensilering af pressede afgrøder. Tidsskr. Planteavl 84, 265-293.

THE USE OF A FORMIC ACID/FORMALDEHYDE MIXTURE AS A SILAGE PRESERVATIVE

P. O'Kiely and V. Flynn
An Foras Taluntais, Grange, Co. Meath, Ireland.

Formaldehyde has been included in some silage preservatives on the assumption that its inclusion in silage will protect the forage protein from microbial degradation in the rumen. In the experiment reported here, one such preservative (Silaform: 55% formic acid + 35% formalin) was evaluated as a silage preservative and its "protein sparing" effect determined in terms of carcass production by intensively reared cattle.

Five cuts of silage were taken between 1980 and 1981 and the grass was treated at ensiling either with 85% formic acid applied at 2.3 l/tonne or with Silaform at 2.8 l/tonne. These silages were offered ad libitum to groups of Friesian bulls for 411 days along with a concentrate input of 2-5 kg per day (990 kg total input). Two groups of 8 bulls were allotted to each silage type and on each silage one group received a 16% crude protein concentrate (high protein) and the other a 10% crude protein concentrate (low protein).

All silages were similarly preserved as indicated by pH and $\text{NH}_3\text{-N}$, % N and all had high protein contents (over 15%). Dry matter digestibility for all silages was over 67% and within cuts was similar for both silage treatments. Table 1 summarises the animal performance and silage intake data for each treatment.

Table 1. Summary of Animal Performance and Intake Data for each Treatment

	Formic Acid		Silaform	
	Low Protein	High Protein	Low Protein	High Protein
Starting wt(kg) (116 days)	125.9	125.4	124.3	123.7
Final wt(kg) (527 days)	495.6	492.1	493.4	504.6
Daily gain (kg/day)	0.90	0.89	0.90	0.93
Carcass wt(kg)	274.4	279.1	270.8	281.9
KO%	55.4	56.7	54.9	55.9
Kidney fat (kg)	7.2	7.4	6.1	8.6
Total silage DM intake/ head(kg)	1475.1	1505.5	1478.7	1473.9

There was little response to inclusion of protein in the concentrate ration. Inclusion of formaldehyde in the silage preservative treatment had no effect on either silage intake or animal performance. There were no interactions between preservatives and concentrate protein levels.

AMOUNT AND QUALITY OF MICROBIAL PROTEIN IN DIFFERENT SILAGES

B. Ullrich

Institute of Grassland and Forage Research
Federal Research Center of Agriculture (FAL) Braunschweig-Völkenrode
Bundesallee 50, D-3300 Braunschweig, Federal Republic of Germany

Introduction

The microbial protein content in silage (perennial ryegrass in three treatments and maize) was determined with ^{15}N -tracer ammonium sulfate, using a mass spectrometer.

The separation of the microbes from silage was carried out by coarse homogenisation followed by differential centrifugation, resulting in microbial and plant protein fractions. Total nitrogen, ammonia and amino acids were analysed in fresh grass, silage and microbial fraction.

Results

1. The quantitative composition of nitrogen-content in different silages:

variant	% plant-N	% microbial-N	% NH_3 -N
	original material = 100 % N		
1. direct cut grass	65.6	28.4	6.0
2. direct cut grass with formic acid	71.7	23.4	4.9
3. prewilted grass	73.4	22.1	4.5
4. maize	71.1	22.6	6.3

2. The microbial activities were compared with the decomposition of the relative amounts of carbon. A differentiation of microbial protein content and activities in silage between the treatments seems evident, the ranking was: direct cut grass > direct cut grass with formic acid > prewilted grass > maize.
3. An estimation of the changes of silage protein quality was attempted by linear regression analysis with the amino acid data. During the fermentation the protein quality decreased in the grass silages but not in the microbial fraction and maize silage when compared to the original material.

N-FERULOYLGLY-L-PHEOH A SEQUENCE OF PLANT PROTEINS
AND N-FERULOYLGLYCINE AMIDOHYDROLASE (3.5.1.X.)
AN ENZYME HYDROLYSING N-FERULOYLGLYOH

C.F. Van Sumere, M. Martens, R. Hanselaer, K. Vande Castele,
D. Bral and M. Cottenie-Ruysschaert

Laboratorium voor Plantenbiochemie, Rijksuniversiteit Gent,
K.L. Ledeganckstraat, 43, 9000-Gent, Belgium.

ABSTRACT

Phenolic acids are widespread in nature in free and bound form (esters, β -glucosides and amides). They occur also in proteins (Van Sumere et al, 1973, 1980; Newby et al, 1980), e.g. N-feruloylGly-L-PheOH has been identified by METC* in barley globulins (Van Sumere et al, 1973) and Lucerne bulk leaf proteins (Van Sumere et al, 1980). Via the synthesis of N-feruloylpeptides (model substances), optimization of their hydrolysis and subsequent HPLC-TLC analysis (Van Sumere et al, 1982) of the hydrolysates, the development of a suitable and relative fast method for the detection in plant proteins of N-acylamino acids (of the cinnamoyl- or benzoyltype), is in progress.

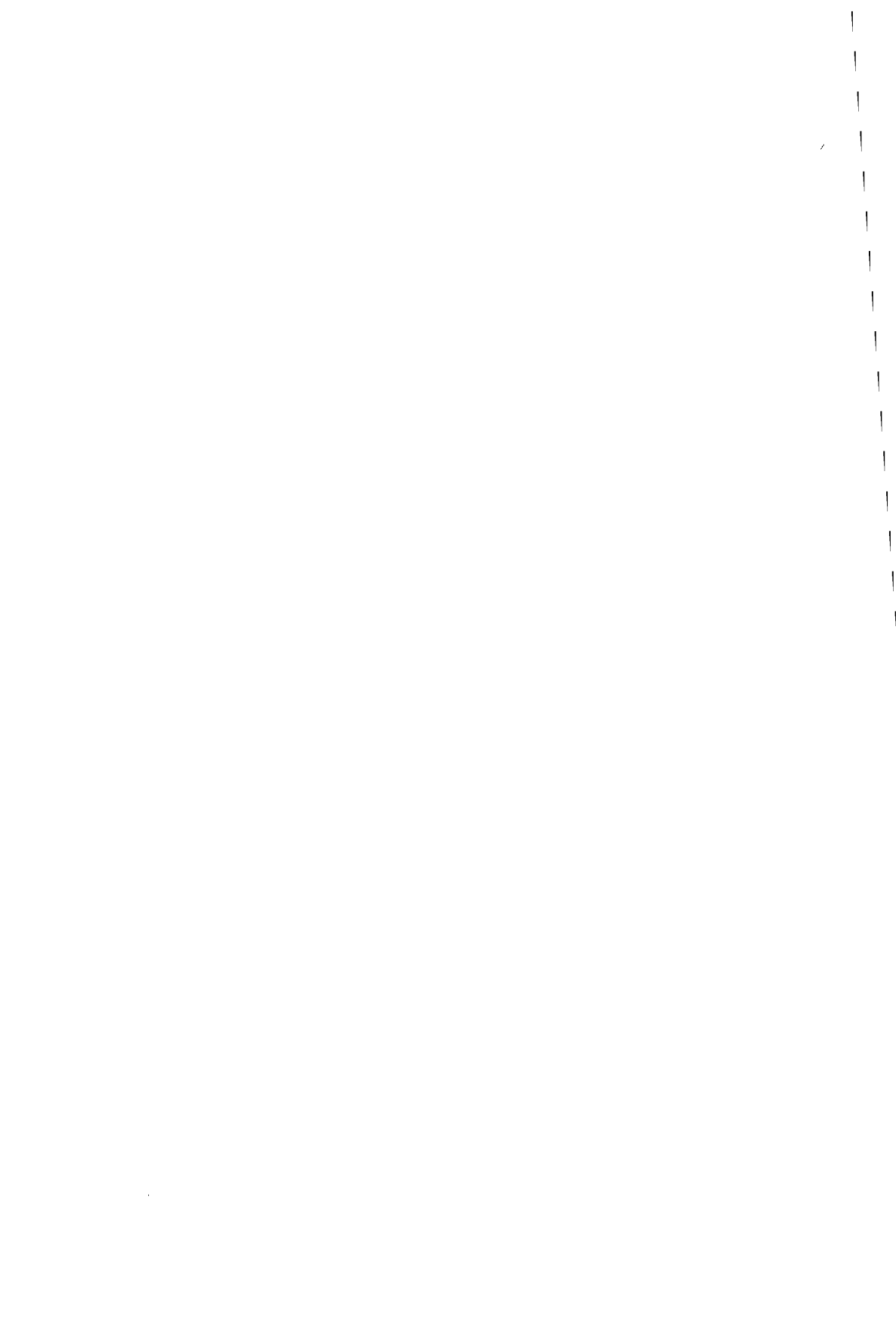
In addition N-feruloyl glycine amidohydrolase (3.5.1.X.) has been
(N-feruloyl glycine + H₂O $\xrightarrow[\text{amidohydrolase}]{\text{N-feruloyl glycine}}$ ferulic acid + glycine)

isolated from barley seeds. Enzyme activity was determined by either a radiobiochemical or a HPLC-technique. Some of the characteristics of the enzyme, which is difficult to purify (x110), are : pH optimum = 8; temp. opt. = 32°C; K_m (N-feruloyl glycine) = 8.62 x 10⁻⁵M; V_m = 0.04 μ moles/mg prot./min; Activation energy 43.6 kJ/mole; Stabilizer : Glycerol; Activator : (NH₄)₂SO₄ (0.1M). Minimal inhibitor conc. for 100% inhibition : HgCl₂ 5.10⁻⁵M; p.chloromercuribenzoate 10⁻³M; dithitritol 5.10⁻³M; o.phenantroline 10⁻²M. The importance of the N-feruloylamino acid containing proteins and the enzyme is discussed.

*Multiple Elimination Thin-Layer Chromatography.

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LIST OF PARTICIPANTS

- D. Beever
Grassland Research Institute,
Hurley,
Maidenhead,
Berks SL6 5LR,
UK.
- R. Carlsson
Department of Plant Physiology,
Lund University,
Box 7007,
S-22007 Lund,
Sweden.
- D.G. Chamberlain
The Hannah Research Institute,
Ayr KA6 5HL,
UK.
- D. Collins
The Agricultural Institute,
Grange,
Dunsany,
Co. Meath,
Ireland.
- B. Cottyn
National Institute of Animal Nutrition,
Scheldeweg 68,
9231 Melle-Gontrode,
Belgium.
- A.G. Deswysen
Université Catholique De Louvain,
Place Croix Du Sud, 3
B-1348,
Louvain-La-Neuve,
Belgium.
- M.J. Drennan
The Agricultural Institute,
Grange,
Dunsany,
Co. Meath,
Ireland.

R.A. Edwards Edinburgh School of Agriculture,
Kings Buildings,
Westmains Road,
Edinburgh EH9 3JG,
UK.

R. Fiorentini Istituto Di Industrie Agrarie Dell'Universita',
Via S. Michele No 4,
56100 Pisa,
Italy.

S. Fitzgerald The Agricultural Institute,
Belclare Research Centre,
Tuam,
CO. Galway,
Ireland.

C. Galoppini Istituto Di Industrie Agrarie Dell'Universita',
Via S. Michele No 4,
56100 Pisa,
Italy.

F. Gordon Agricultural Research Institute of N. Ireland,
Hillsborough,
CO. Down,
N. Ireland.

T.W. Griffiths The Agricultural Institute,
Dunsinea Research Centre,
Castleknock,
Co. Dublin,
Ireland.

R. Jarrige I.N.R.A.,
Centre De Recherches Zootechniques
et Veterinaries de Theix,
63110 Beaumont,
France.

A.S. Jones Rowett Research Institute,
Bucksburn,
Aberdeen AB2 9SB,
Scotland.

M. Journet I.N.R.A. Vache Laitiere,
Saint Giles,
35590 L'Hermitage,
France.

M.G. Keane	The Agricultural Institute, Grange, Dunsany, Co. Meath, Ireland.
M.F. Maguire	The Agricultural Institute, Dunsinea Research Centre, Castleknock, Co. Dublin, Ireland.
J.L. Mangan	Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, UK.
F. Massantini	Istituto Di Agronomia Dell'Universita', Via S. Michele No 2, 56100 Pisa, Italy.
D. Morgan	The Agricultural Institute, Moorepark Research Centre, Fermoy, Co. Cork, Ireland.
J. Mortensen	Statens Planteavlslaboratorium, Lottenborgvej 24, DK-2800 Lyngby, Denmark.
J. Murphy	The Agricultural Institute, Moorepark Research Centre, Fermoy, Co. Cork, Ireland.
W.E. Murphy	The Agricultural Institute, Johnstown Castle Research Centre, Wexford, Co. Wexford, Ireland.
P. O'Kiely	The Agricultural Institute, Grange, Dunsany, Co. Meath, Ireland.

J. O'Shea
The Agricultural Institute,
Dunsinea Research Centre,
Castleknock,
Co. Dublin,
Ireland.

M. O'Sullivan
The Agricultural Institute,
Johnstown Castle Research Centre,
Wexford,
Co. Wexford,
Ireland.

V. Pattakou
Cereal Institute,
Thessaloniki,
Greece.

E.J. Norgaard Pedersen
Ødum Forsøgsstation,
8370 Hadsten,
Denmark.

A.G. Prendergast
C.E.C.,
DG VI - F/4,
86 Rue de la Loi,
B-1049 Brussels,
Belgium.

W. Sheehan
The Agricultural Institute,
Creagh,
Ballinrobe,
Co. Mayo,
Ireland.

S. F. Spoelstra
Institute for Livestock Feeding
and Nutrition Research,
P.O. Box 160,
8200 AD Lelystad,
The Netherlands.

S. Tamminga
Institute for Livestock Feeding
and Nutrition Research,
Runderweg 2,
P.O. Box 160,
8200 AD Lelystad,
The Netherlands.

P.C. Thomas
The Hannah Research Institute,
Ayr,
KA6 5HL,
UK.

J.L. Tisserand E.N.S.S.A.A.,
 26 Bd Docteur Petitjean,
 F 21100 Dijon,
 France.

B. Toussaint Laboratoire d'ecologie des prairies,
 6654 Michamps,
 Belgium.

B. Ullrich Institute of Grassland and
 Forage Research,
 FAL Bundesallee 50,
 D - 3300 Braunschweig,
 Federal Republic of Germany.

C.F. van Sumere Laboratorium Voor Plantenbiochemie,
 Ri JKS Universiteit-Gent,
 KL Ledeganckstraat 35,
 B-9000 Gent,
 Belgium.

G. Wieringa CABO,
 P.O. Box 14,
 6700 AA,
 Wageningen,
 The Netherlands.

R.J. Wilkins Permanent Grassland Division,
 Grassland Research Institute,
 North Wyke,
 Okehampton,
 Devon EX20 2SB,
 UK.

R.K. Wilson The Agricultural Institute,
 Dunsinea Research Centre,
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