NITROGEN AND AMINO ACID METABOLISM IN DAIRY COWS

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Oan Heit en Mem
Voor Jitske en
Karsjen Ytzen

DANKWOORD

Aan de totstandkoming van dit proefschrift hebben zeer velen hun steentje bijgedragen. Door in een dankwoord daarvan slechts enkelen met name te noemen zou ik de anderen onrecht aandoen. Ik geef er daarom de voorkeur aan om iedereen die op wat voor manier dan ook aan de totstandkoming van dit proefschrift heeft meegewerkt vanaf deze plaats voor zijn of haar aandeel dank te zeggen.

STELLINGEN

1. De beste waarborg voor een zo gunstig mogelijke eiwitvoorziening van melkkoeien is een maximale energieopname.

   Dit proefschrift.

2. Het verbeteren van de eiwitvoorziening van herkauwers door middel van chemische stoffen die selectief de proteolyse in de pens remmen biedt weinig perspectief.

3. De opvatting dat de in de voormagen van herkauwers werkelijk verteerde organische stof een betere maat is voor de voor microbiële groei beschikbaar komende ATP dan de in deze voormagen schijnbaar verteerde organische stof, berust op onjuiste veronderstellingen.

   Nutrition requirements for farm livestock (1980)
   Commonwealth Agricultural Bureau, Slough, p. 125.

4. De gunstige invloed van tegen afbraak in de voormagen beschermd eiwit op de vruchtbaarheid van melkvee is onvoldoende bewezen.

   Hagemeister, H., Lüpping, W. en Kaufmann, W. (1980)
   In: Recent advances in animal nutrition, Butterworth, London, pp. 67-97.

5. De verblijfsduur van krachtvoer in de voormagen van melkkoeien wordt vaak onderschat omdat ten onrechte wordt aangenomen dat de passagesnelheid van krachtvoerdeeltjes door de voormagen die van vloeistof benadert.

   Bull, L.S., Rumpler, W.V., Sweeney, T.F. en
   Zinn, R.A. (1979) Fed. Proc. 38: 2713-2719.

6. De opvatting dat meer dan 8% vet in rantsoenen voor melkvee de ruwe celstof vertering vermindert heeft geen algemene geldigheid.

   Rohr, K., Daenicke, R. en Oslage, H.J. (1978)
   Landbauforsch. Völkenrode, 28: 139-150.
   Honing, Y. v.d., Wieman, B.J., Steg, A. en
   Donselaar, B. v. (1981)
   Neth. J. Agric. Sci., 29: 79-92.

7. Er wordt doorgaans te weinig aandacht geschonken aan minder gewenste neveneffecten die optreden bij het gebruik van farmaceutische stoffen om het fermentatieproces in de pens te beïnvloeden.

Chalupa, W. (1980)
In: Digestive Physiology and metabolism in ruminants. MTP Press Ltd., Lancaster, pp. 325-348.

8. Recombinant DNA technieken zullen in industriële fermentatieprocessen wel, maar in de pensfermentatie niet leiden tot belangrijke efficiëntieverbeteringen.

9. Verhoging van het linolzuurgehalte van melk- en zuivelprodukten door menging met meervoudig onverzadigde vetzuren, als zodanig of in de vorm van vetten, verdient de voorkeur boven het voeren van melkvee met tegen verzadiging in de voormagen beschermde meervoudig onverzadigde vetzuren.

10. De vertering in de dikke darm is bij het Nederlandse vleesvarken meer van wetenschappelijk dan van praktisch belang.

11. Een juiste voeding van hoogproduktief melkvee wordt bemoeilijkt door, als selectiecriterium voor fokstieren, gebruik te maken van 100 dagen lijsten.

12. Embryo-transplantatie kan een bijdrage leveren om bij onderzoekingen met herkauwers met een niet genetisch doel, waarbij een vergelijking binnen dieren niet mogelijk is, de tussen-dier-variatie te verminderen.

13. Voorspellingen over de snelheid waarmee informatiemedia door computersystemen zullen worden vervangen zijn vaak te optimistisch omdat ze onvoldoende rekening houden met de weerstand die bij vele gebruikers tegen dergelijke vernieuwingen bestaat.

Evans, C. (1981)
Het micromillenium, Kluwer, Deventer;
Soc. Ec. Magazine, Utrecht.

14. De vaak ongenuanceerde en niet zelden suggestieve berichtgeving over de aanwezigheid van schadelijke stoffen in ons voedselpakket is schadelijker voor de geestelijke gezondheid van veel mensen dan de aanwezigheid van deze stoffen zelf voor hun lichamelijke gezondheid.

S. Tamminga
Nitrogen and amino acid metabolism in dairy cows.
Wageningen, 25 november 1981.

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INTRODUCTION

Domesticated animals have served mankind for thousands of years. Productivity of these animals requires an adequate feeding. The feeding strategy of domesticated animals is gradually changing towards diets composed of ingredients which are less suitable as human food, making animal production less competitive with human nutrition. In this respect ruminants such as sheep and dairy cows have a high potential, due to a symbiosis with micro-organisms in their forestomachs. Probably because of this symbiosis the digestive tract of ruminants has developed into the present very complicated shape.

To advantageously exploit the full potential of ruminants as non-competitive food producers for mankind, a detailed knowledge of their digestive system is required. This is particularly true in the case of dairy cows, because of their relatively high production intensity.

Information on the digestive process in ruminants seems abundant. The vast majority of this information unfortunately concerns sheep fed according to or slightly above their maintenance requirements. Information on dairy cows, lactating animals in particular, is very scarce. It is believed that in qualitative terms the digestive process in lactating dairy cows is very similar to that in sheep. However in quantitative terms the situation may differ substantially between an animal fed according to its maintenance requirement and an animal fed 3 or even 4 times its maintenance requirement, as is often the case in a high producing dairy cow.

The first limiting nutrient in animal feeding is usually energy. Energy metabolism in dairy cows has been studied extensively and due to this a large number of feeding problems associated with energy metabolism could be solved. This is also illustrated by the recent introduction of a new energy evaluating system for dairy cows in The Netherlands and other European countries.

With respect to protein, usually regarded as the most important nutrient next to energy, many problems still remain, or even new problems may arise. This is because on the one hand certain aspects of the digestion process in dairy cows are still poorly understood and on the other because of the introduction of new types of feeds and diets in the feeding of dairy cows.

The aim of the studies of this thesis was to collect more information on

the protein digestion process in ruminants, both by surveying the literature and from own experiments. Several aspects were studied and discussed.

In order to follow the protein digestion process, access to the digestive tract at other sites than before (feed) and after (faeces) this tract is necessary. Therefore surgically modified animals were needed for these studies. From the research point of view the best way to surgically modify the experimental animals would be the insertion of a rumen fistula and of two pairs of re-entrant cannulae, one pair at the beginning of the small intestine, immediately after the pyloris and a second pair at the end of the small intestine. It was however realized that maintaining such animals would be very difficult. Besides the insertion of cannulae immediately behind the pyloris requires a perforation through the skin between rather than posterior to the ribs which may cause excessive growth of cartillage and eventually block the cannulae. As a compromise experiments were done with animals fitted with a rumen fistula and one pair of re-entrant cannulae at the beginning of the small intestine although not immediately after the pyloris but just beyond the exit of the pancreatic and biliary duct.

Two important aspects of protein metabolism in ruminants, the microbial degradation of dietary protein in the forestomachs and microbial protein synthesis are discussed in chapter 1 and 2 respectively. The following chapters 3 to 8 report on various factors which may influence the intestinal protein supply of dairy cows. The possible influence of the dietary protein source is discussed in chapter 3. The chapters 4 and 5 deal with the possibilities of increasing the intestinal protein supply in dairy cows by processing the roughage part of the diet. Chapter 6 deals with the possible influence of varying the roughage/concentrate ratio. Two more factors which may influence the intestinal protein supply in dairy cows are discussed, the level of feed intake in chapter 7 and the frequency of feeding in chapter 8. The last three chapters are of a more concluding nature. In chapter 9 methods to manipulate the amino acid supply of dairy cows are reviewed. In chapter 10 the concept of amino acid requirements of dairy cows is discussed. Finally in chapter 11 a general discussion tries to integrate the various findings and an attempt is made to formulate some practical recommendations for the feeding of ruminants with special regard to the protein feeding of high yielding dairy cows.

The experimental results presented in this thesis cover a research period of about 10 years, during which certain scientific views changed, reason why the results in the various chapters, most of which were published in journals,

are not always presented in the same way.

First of all the attitude towards the use of indigestible markers in research as described in this thesis changed. In some of the earlier chapters digestion results were corrected if the recovery of the marker used was incomplete. Comparing the digestive results obtained in surgically modified animals with those obtained in normal animals showed that corrections for an incomplete recovery of markers was not necessary. The results obtained in later years were therefore presented without any correction.

The way in which microbial protein synthesis is expressed also shows a development. In the first years of the research, microbial protein synthesis was related to organic matter disappeared between intake and duodenal flow. In later years it was thought to be more appropriate to relate microbial protein synthesis to carbohydrates disappeared rather than organic matter disappeared. Reasons for this are given in chapter 2.

Amino acid compositions are presented in various chapters. Various ways are possible to do this. In our research it was important to know what proportion of the total N was present in amino acids. Amino acids were therefore expressed in amino acid N as proportion or percentage of total N and not in g amino acid per 16 g of N, a more often used way of expressing amino acid compositions.

With respect to the sequence of the chapters, a compromise had to be found between the sequence in time during which the different papers were published and a logically composed thesis. The last was considered more important, reason why the various chapters are not in the order in which they were published.

The nature of the research reported in this thesis requires cooperation in a team. Otherwise it would hardly be possible to perform such experiments. As a result some of the chapters in this thesis were published together with others. Chapter 4 and 7 were published together with one or two members of the team which carried through the research. My contribution in this chapter was to initiate and plan the experiments, to take part in the execution of the experiments, to collect and process the data, to draw the conclucions and to write the report. The chapters 9 and 10 were published together with Dr. J.D. Oldham from the National Institute for Research in Dairying (Reading, G.B.), as a result of an invitation by the chief editor of Livestock Production Science. Writing of chapter 9 was largely done by Dr. Oldham, but a substantial part of the data used in this chapter, particularly those used for table 1

and figure 1, result from our research at the Institute for Livestock Feeding and Nutrition Research. Chapter 10 was mainly written by myself.

In some chapters mean results rather than the results of individual animals are given. In order not to lose the possibility for others to use the results for certain calculations, the results on intake, apparent digestion and apparent digestion in the stomachs of organic matter and nitrogen of all individual experiments are given in an appendix. In this appendix also reference is made in which chapter(s) the individual results are used.

1. PROTEIN DEGRADATION IN THE FORESTOMACHS OF RUMINANTS[1]

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Summary

Dietary protein ingested by ruminant animals is extensively degraded by microorganisms inhabiting their forestomachs. Mechanism of microbial breakdown of dietary protein is very complicated and not yet entirely understood. Experimental results, both *in vitro* and *in vivo* show a varying degradation of dietary protein, with differences in degradation between individual amino acids. Part of this variation, particularly *in vivo*, must be attributed to inadequate measuring techniques. Among other factors influencing degradation are nature and solubility of dietary protein, rate of passage of digesta through the forestomachs and level of feed intake.

Decreasing the extent of degradation of dietary protein can be achieved in various ways. Two possibilities include formulation of diets from ingredients with low protein solubility and chemical treatment of the dietary protein, for instance, with formaldehyde. Under present feeding regimens this seems profitable only if level of animal production is high (early lactation, fast growing young animals). Protection may result in an inadequate supply of nitrogen or even amino acids for microbial growth in the forestomachs. Shortage of N can easily be overcome by addition of some nonprotein nitrogen such as urea to the diet, provided that the energy supply to the microbes is not a limiting factor as well.

(Key Words: Amino Acids, Microbial Activity, Nitrogen, Protein Degradation, Protein Protection, Rumen.)

Introduction

Protein metabolism in digestive tracts of ruminants has received much attention in past decades. Numerous research activities have been devoted to the subject and various aspects of it have been reviewed in recent years (Chalupa, 1975; Hogan, 1975; Kaufmann and Hagemeister, 1975; Satter and Roffler, 1975; Smith, 1975; Armstrong, 1976; Tamminga and Van Hellemond, 1977). Results of research activities have led to the conclusion that protein evaluating systems presently in use in most countries have some limitations in predicting the real protein value of a feed component in ruminant feeding. It must be emphasized, however, that these systems have served their purpose adequately for a long time and in many countries still do. Because present systems may cause overfeeding of protein, improvements in the efficient utilization of nutrients for animal protein production seem possible. But higher animal production levels, introduction of new feeds and application of more sophisticated research techniques have resulted in the development of new protein evaluating systems in various countries (Hagemeister and Kaufmann, 1974; Burroughs *et al.*, 1975a,b; Roy *et al.*, 1977; Jarrige *et al.*, 1978). These new systems take into account the physiological and biochemical principles of the complicated digestive system in ruminants, of which much has been learned in recent years. All of these systems attempt to quantify the protein absorbed from the small intestine as a measure of protein supply to the animal's tissues.

Although principles of most of these new systems are similar, some of the factors used in quantifying calculations differ considerably (Waldo, 1978). A main factor in all these systems is the quantity of microbial protein synthesized in the forestomachs of ruminants as a result of microbial fermentation. This process of fermentation involves a growing microbial population with a concomitant formation of biomass including microbial protein. This biomass proceeds to the lower parts of the

[1] Presented during the 1978 ASAS-ADSA meetings held at Michigan State University, East Lansing, as part of the Ruminant Nutrition Symposium on "Quantitative Aspects of Nitrogen Metabolism in the Rumen".

digestive tract and contributes to the protein supply of the ruminant animal. This aspect of nitrogen (N) metabolism in the digestive tract of ruminants will be dealt with in one of the other papers of this symposium (Smith, 1979).

Fermentation in the forestomachs also involves degradation of ingested feed, including protein. This results in a varying proportion of ingested protein escaping microbial breakdown and entering the small intestine, where it contributes to the protein supply of the animal. The proportion of feed protein escaping microbial breakdown is therefore also an important factor in these new protein evaluating systems.

The main objective of this paper will be to discuss protein degradation in the forestomachs of ruminants, with particular reference to dairy cows. Attention will be paid to the mechanism of microbial protein degradation, to methods for measuring microbial protein breakdown in the forestomachs, to factors influencing this breakdown and to implications of the preceding items for practical feeding.

Mechanism of Microbial Breakdown of Dietary Protein in the Forestomachs

Dietary protein entering the forestomachs is often extensively degraded by both bacteria and protozoa. This degradation involves two steps. Initially the protein chain is broken by hydrolysis of peptide bonds (proteolysis), resulting in peptides and amino acids. It is uncertain which of the two processes (proteolysis or amino acid degradation) is the rate limiting step. Based on the increased levels of free amino acids appearing in the rumen shortly after feeding (Demeijer, 1976) it has been proposed that proteolysis is not the rate limiting step; recently it was postulated however (Nugent and Mangan, 1978) that proteolysis is the rate limiting step.

Proteolysis and deamination were both found to be affected by pH, but experimental results are conflicting. The optimum pH for both proteolysis and deamination has been reported to be between 6 and 7 (Blackburn and Hobson, 1960; Henderickx, 1962; Lewis and Emery, 1962). *In vitro* experiments showed pH maxima for ammonia production at 4.5, 5.6, 6.7 and 7.7 (Henderickx and Demeijer, 1967). In other reports it was stated that deamination by rumen bacteria becomes negligible below pH 4.5 (Lewis and Emery, 1962), whereas at a pH higher than 7.2 deamination also stopped (Chalmers, 1969). It may be concluded that under most nutritional circumstances pH in the rumen will allow an extensive breakdown of dietary protein.

Mechanism of protein degradation differs somewhat between bacteria and protozoa. With bacteria the protein chain is broken into smaller parts by hydrolysis of some or all of its peptide bonds. This process takes place outside the bacterial cell. Resulting peptides and amino acids are transported inside the bacterial cells and peptides are hydrolysed further to amino acids. The amino acids in turn are either incorporated into bacterial protein or degraded to volatile fatty acids (VFA), ammonia (NH_3), carbon dioxide (CO_2), methane (CH_4) and some fermentation heat. End products of this degradation are excreted back into the surrounding medium.

The role of protozoa is not well documented. Protozoa are capable of engulfing small feed particles and bacteria (Coleman, 1975) and proteolysis of dietary protein takes place inside the protozoal cell. If the resulting amino acids are not incorporated into protozoal protein they are often excreted into the surrounding medium rather than being degraded further (Coleman, 1975).

The reason that microorganisms in the forestomachs hydrolyze dietary protein and further degrade its amino acids is not well understood. It seems plausible that degradation of protein is necessary to provide microbes with required precursors for their own protein synthesis, either ammonia and presumably α-keto acids or even intact amino acids. However, degradation is often in excess of these requirements. This observation is probably because degradation of amino acids yields energy (ATP) which can be utilized by microbes for their synthetic processes. At least one strain of rumen bacteria requires amino acids as a source of energy (Prins, 1977).

Under anaerobic conditions, such as found in the rumen, the energy extractable from degradation of protein is very limited. In mammals the synthesis of one peptide bond requires 4 to 6 moles of ATP (Campbell, 1977) and because of the more rapid turnover of RNA in bacteria their requirement may be even higher. Formation of 1 mole of terminal pyrophosphate bonds in ATP at body temperature requires at least 12 kcal (Armstrong, 1969), whereas hydrolysis of peptide bonds yields only 3 kcal of free energy per mole (Baldwin, 1968). Consequently, proteolysis

cannot yield ATP. Important biochemical reaction mechanisms in the further degradation of amino acids by microbes are deaminations, transaminations and decarboxylations (Demeijer, 1976; Prins, 1977). Under normal conditions decarboxylation of amino acids to yield amines does not seem to be an important way of amino acid degradation, but may become significant under acid conditions (Prins, 1977). The most important degradative pathway for amino acid degradation is thought to be deamination of the amino acid, followed by decarboxylation of the resulting α-keto acid (Demeijer, 1976; Prins, 1977). From the latter reaction a yield of 1 ATP per decarboxylation is firmly established; in the other reaction mechanisms yield of ATP is absent or uncertain. Reduced cofactors are formed, concomitant with some of the degradative pathways and their reoxidation with a simultaneous formation of propionate, methane and possibly butyrate will yield some additional ATP (Demeijer, 1976; Prins, 1977).

Although most end products of degradation of amino acids are known (Henderickx, 1973), not much is known about stoichiometric relationships of this degradation. Estimates of the ATP yield of fermentation of protein in the forestomachs are therefore far from accurate and have only limited value. Such an estimate was made for the degradation of casein, by assuming that all its amino acids were deaminated, followed by decarboxylation of the resulting α-keto acids, yielding 1 mole of ATP per mole of amino acids fermented and assuming an additional yield of 1 mole of ATP per mole of propionate or methane generated. *In vitro* experiments on fermentation of casein were performed by Demeijer (1976), and it was shown that .43 moles of amino acids in casein yielded .14 moles of propionate and .09 moles of methane. Casein contains some .85 moles of amino acids per 100 g and fermentation of 100 g of casein would therefore yield 1.3 moles of ATP (.85 due to decarboxylation of α-keto acids, .27 from formation of propionate and .18 from formation of methane). This is considerably less than the generally accepted minimal yield of 4 to 5 moles of ATP per mole of hexose or hexose equivalent (=162 g of polysaccharide) fermented in the rumen (Prins, 1977).

A further reason for degradation of excess amino acids by rumen bacteria may be the lack of mechanisms to transport amino acids from the cytoplasm across the cell wall into the surrounding medium. It has been found that some strains of rumen bacteria require NH_3, even if amino acids are present in their growth medium. One of these is the strain *Bacteriodes ruminicola*, which, however, can utilize peptides from the growth medium (Pittman *et al.*, 1967), suggesting a lack of transport systems for individual amino acids across the cell wall. In order to excrete excess amino acids, they would need to be degraded first.

Measuring the Degradation of Dietary Protein. Various methods, both *in vitro* and *in vivo*, have been developed for measuring the degradation of dietary protein in the forestomachs of ruminants.

In vitro methods are generally based on either the release of ammonia after incubation with rumen liquor, or on the estimate of the proportion of N which goes into solution after incubation at body temperature for a fixed time. With respect to the latter method, various solutions have been applied as an incubation medium, such as diluted NaOH (Lyman *et al.*, 1953), artificial saliva (Tagari *et al.*, 1962; Wohlt *et al.*, 1973), autoclaved rumen fluid (Wohlt *et al.*, 1973), diluted solution of pepsin in .1 N HCl (Beever *et al.*, 1977) and water at various temperatures (Mertens, 1977). The various methods have been discussed (Mertens, 1977; Waldo, 1978) and incubation with artificial saliva at body temperature was considered the most attractive. A complication of this method is the presence of N containing salts in the incubation mixture, causing high blank values.

Studying protein degradation by ammonia release after incubation with rumen liquor has the disadvantage of microbial growth occurring simultaneously with protein degradation. Because of this growth, part of the released ammonia may become incorporated into microbial protein. A general limitation of all *in vitro* methods is that, although they may yield a value for degradability, they do not yield data representing actual degradation *in vivo*. Attempts were made to overcome this problem by studying the kinetics of protein degradation resulting in a measure of degradation rate (Broderick, 1978). Combining this rate with the rumen liquor turnover rate may yield figures that give an estimate of degradation close to the actual degradation *in vivo*.

Measurements *in vivo* are usually performed with surgically prepared animals, equipped with

cannulae in the abomasum or small intestine. With such animals undegraded dietary protein can be estimated as the difference between total and microbial protein entering the abomasum or small intestine, after estimating microbial protein. The latter can be estimated by use of specific markers such as nucleic acids, diaminopimelic acid (DAPA), aminoethylenephosphonic acid (EAP) or one of the radioisotopes ^{35}S, ^{32}P or ^{15}N (Clarke, 1977). Estimating undegraded dietary protein by regression calculation techniques also seems possible (Jarrige *et al.*, 1978; Hvelplund *et al.*, 1976). Accuracy of measuring the flow of total protein and microbial protein entering the abomasum or small intestine is limited. The measuring techniques are laborious and, as a consequence, abomasal or duodenal flow measurements are often restricted to a period of 24 hours. To achieve meaningful results the flow of digesta usually needs correcting. For this purpose indigestible markers such as chromium oxide are applied with the feed or directly in the rumen and the abomasal or duodenal digesta flow corrected by dividing it by the proportion recovery of the marker. Reducing the variation due to nonsteady state conditions seems possible by frequent feeding, but makes the results less meaningful for practical conditions. Therefore, estimates of the proportion of dietary protein escaping microbial degradation in the forestomachs are subject to high error. Analytical techniques involved also have limited accuracy. Moreover, different methods often yield different results (Harmeyer *et al.*, 1976; Ling and Buttery, 1978; Tamminga, 1978). Finally the metabolic functions of the markers applied to estimate microbial protein makes interpretation of results difficult (Demeijer and Van Nevel, 1976; Hagemeister, 1975; Nikolic, 1977 ;Van Nevel and Demeijer, 1977).

Improving accuracy of experimental results seems possible by increasing the number of experimental animals. To achieve an accuracy of estimating the proportion of undegraded dietary protein under standard conditions with 5% would require 10 to 12 animals (Miller, 1978). Because the experimental techniques involved are very laborious, difficulties arise in handling such numbers of animals, particularly if large ruminants such as lactating cows are used. It must be stressed, however, that systematic errors in measuring or analytical techniques cannot be eliminated by increasing the number of animals.

Recently a new technique was proposed by Mehrez and Orskov (1977). In this method a direct measurement of protein degradation is achieved by incubating a sample of the feedstuff enclosed in a dacron bag directly in the rumen. An important advantage of such a method is that it yields a direct estimate of protein degradation, not biased by inaccuracies of the estimate of microbial protein. By suspending a number of bags with the same sample in the rumen and removing them after different times of incubation, an estimate of both the rate and extent of protein degradation can be obtained (Mathers *et al.*, 1977; Mehrez and Orskov, 1977). It is questionable, however, if rate of disappearance of protein from the dacron bag represents rate of degradation, because soluble protein may be washed out without actually being degraded (Mohamed and Smith, 1977). Moreover, protein in the dacron bag is not entirely subjected to the dynamic system characteristic of digestive metabolism in the ruminant animal. This problem may be overcome to a certain extent by estimating protein degradation at the moment when 90% of the truly digestible organic matter has disappeared from the dacron bag, thus simulating the *in vivo* situation in the normally fed animal (Orskov, 1977).

Factors Influencing Protein Degradation in the Forestomachs. The degradation of dietary protein in the forestomachs of ruminants is influenced by a number of factors, some of which are related to diet, others to the animal. An important dietary factor seems to be solubility of the protein, which is usually measured in artificial saliva at body temperature (37 or 38 C). Apart from solubility, structural differences to a certain extent caused by disulphide bridges and crosslinking of the protein, may be important determinants of degradability (Nugent and Mangan, 1978).

Solubility of feed protein is partly determined by the relative amount of soluble albumins and globulins on the one hand and the less soluble prolamins and glutelins on the other. Feeds whose major protein fractions are albumins and globulins have a higher protein solubility than feeds containing mainly prolamins and glutelins in their protein (Wohlt *et al.*, 1976). An influence of pH on protein solubility has also been reported (Isaacs and Owens, 1972). Solubility of feed protein is further affected by treatments during manufacturing, both of forages and of concentrates.

With forages the main treatments for conservation are haymaking, silage making and artificial drying. Forage conservation always starts with cutting. If the next step is drying in the field, which is required for making hay or wilted silage, plant proteases become active and N-solubility increases (Sullivan, 1973).

Depending on weather conditions, a larger or smaller part of the soluble N may be washed out, leaving a less soluble N-containing residue. If, on the other hand, the freshly cut grass is used directly for artificial drying, activity of plant proteases is limited and because of the heat treatment involved, part of the protein will become denatured, resulting in low N-solubility. In silage making part of the carbohydrates and proteins will be degraded due to fermentation (McDonald and Whittenbury, 1973). The N-containing end products of the fermented protein will then be found in the soluble fraction. Substantial increases in N-solubility may be the result, particularly if a Clostridium-type of fermentation takes place, with the production of substantial amounts of butyric acid. However, other important differences are possible because of the varying influence of weather conditions during the field period and the type of fermentation within the silage as is shown in table 1. With respect to this table it should be realized that part of the variation may be the result of differences in technical procedures among various laboratories. Although solubility is an important determinant of protein degradation in the rumen, both characteristics are not identical. Attempts have been made to relate degradation of dietary N in the forestomachs *in vivo* to N-solubility measured in artificial saliva *in vitro*. Mertens (1977) proposed that *in vivo* all of the soluble N is degraded, and 40 to 50% of the insoluble N is degraded in the rumen. Based on multiple regression calculations in which intestinal flow of protein was related to digestible organic matter and insoluble dietary protein, it was calculated that 65% of the insoluble dietary protein escapes microbial degradation (Jarrige *et al.*, 1978). However, degradation of dietary protein is not entirely determined by characteristics of the feedstuff. In addition, some factors related to the animal are important.

Under practical feeding conditions the extent of protein breakdown in the rumen may be considered as a function of rate of proteolysis and rumen retention time. The latter is influenced by particle size of dietary ingredients and level of feed intake (Balch and Campling, 1965; Church, 1970; Hungate, 1966). The effect of level of feed intake on protein breakdown in the forestomachs of dairy cows was studied in our laboratory with animals equipped with re-entrant cannulae in the small intestine (Tamminga *et al.*, 1979b). Three animals were fed mixed diets of long meadow hay and concentrates with three different levels of protein at two levels of feed intake, approximately 2 and 3.3 times their energetic maintenance requirement, respectively. The concentrates were composed of a number of ingredients and

TABLE 1. N-SOLUBILITIES (%) OF GRASS PRODUCTS CONSERVED IN VARIOUS WAYS AS FOUND IN VARIOUS LABORATORIES

Treatment	N-solubility, %				
Freshly cut grass	21–46	65–100	53–58	35–42	...
Unwilted silage	48–75	70– 80	...	64–69	54
Wilted silage	...	50– 70	48	58	67
Artificially dried and pelleted grass	19–28[a]	40[a]	23[b]	34	...
Hay	23–44	60[c]	17–36	20–50	...
References	Demarquilly *et al.*, 1978	Kempton *et al.*, 1977	Mertens, 1977	Tamminga and Van der Koelen, 1975 and *unpublished results*	Waldo[d], 1977

[a]Dehydrated alfalfa.

[b]Alfalfa meal.

[c]Alfalfa hay.

[d]Mean values of various laboratories published in a review article.

N content was varied by replacing corn, wheat, sugar beet pulp and tapioca with corn gluten feed, soybean meal, linseed meal and coconut meal. Undegraded dietary N entering the small intestine was estimated using DAPA as a marker for microbial protein, or by regression analyses developed by Hvelplund *et al.* (1976). In the latter technique proportions of undegraded dietary N and microbial N entering the small intestine are estimated from the relationship between the ratio of duodenal N to N ingested and the N content of the diet. Before applying both methods, duodenal N flow was corrected for NH_3-N and endogenous N. Although the two methods did not yield exactly the same results (Tamminga *et al.*, 1979b), both showed a decreased degradation of dietary protein at the higher level of feed intake. At the low level of intake (8.6 kg of dry matter/day), the proportion of undegraded dietary N entering the small intestine (mean result of both methods) was .26; at the higher level of intake (12.9 kg of dry matter/day) this figure was .42. Because of limitations in the experimental techniques applied, these figures must be treated with caution.

Based on the estimated solubility figures of Mertens (1977) for a variety of feedstuffs, the proportion of soluble protein in our mixed diets was calculated. This resulted in a figure of .30 for all six diets. Therefore, with the low level of intake .26 of the total dietary N or .37 of the insoluble dietary N escaped microbial degradation in the rumen. At the high level of intake the corresponding values were .42 and .60, respectively. These results confirm the proposal of Mertens (1977) that with increasing intake the proportion of insoluble N degraded in the forestomachs decreases, presumably due to a decreased rumen retention time.

Ruminal retention time of dietary ingredients is quite variable and varies not only from one diet to another, but also between animals (Balch and Campling, 1965) and apparently between species (Church, 1970). In cattle rumen retention time seems higher than in sheep; the reason for this is not understood. In reviewing the data, Hungate (1966) reported values for cattle ranging from 1.3 to 3.7 days and for sheep from .8 to 2.2 days. The turnover rate of rumen fluid is usually much higher, but it too probably affects the passage rate of food particles. In our research with dairy cows rumen fluid turnover rate, if expressed as porportion of the total volume disappearing per hour, was quite variable and ranged from .04 to .20. However, over two-thirds of the variation could be explained by two single factors, the intake of long roughage (kilograms dry matter/day) and the intake of ground and pelleted concentrates (kilograms dry matter/day). Between the two factors a significant difference was found. For each additional kilogram of dry matter ingested from long roughages, the rumen liquor turnover rate increased by .017 ± .0027; each additional kilogram of dry matter ingested from ground and pelleted concentrates caused an increase of only .007 ± .0011 (Tamminga *et al.*, 1979a). It was shown possible to increase rumen fluid turnover rate in sheep by infusing PEG or artificial saliva in the rumen (Harrison *et al.*, 1975, 1976). Infusion of artificial saliva did increase the total flow of amino acids into the small intestine, but this could be attributed entirely to an increase in flow of microbial protein. No significant effects were shown on degradation of dietary protein.

Degradation of Individual Amino Acids in the Forestomachs. Some new information of the subject of amino acid degradation by rumen bacteria has recently become available (Chalupa, 1976; Scheifinger *et al.*, 1976). In these experiments, disappearance of amino acids from an incubation medium was studied. The amino acids could be either incorporated into bacterial protein or degraded, but because of an excess of amino acids compared to energy in the batch culture, degradation must have been predominant. Not all amino acids were utilized by all five strains of rumen bacteria tested and different amino acids disappeared at different rates. Some of the strains tested showed a net synthesis of the amino acid methionine rather than a utilization (Scheifinger *et al.*, 1976). Incubating mixed rumen bacteria with physiological quantities of amino acids showed that specific amino acids were degraded at different rates, and interactions existed between certain amino acids. Of the essential amino acids only valine and methionine seemed rather resistant to microbial degradation. Of these amino acids, even after 7 hr of incubation, less than 60% were degraded. The metabolism of amino acids under *in vivo* conditions appeared approximately 1.5 times faster than under *in vitro* conditions, but a close fitting relationship was found between results of the *in vitro* and the *in vivo* experiments.

Information on *in vivo* degradation of amino

acids under normal feeding conditions when the microorganisms are offered amino acids incorporated in protein rather than as a mixture of free amino acids is very limited and difficult to obtain. In our laboratory experiments were carried out with re-entrant cannulated dairy cows in which the duodenal flow of total N and amino acid N was measured and the contribution of bacterial protein to the total protein entering the small intestine estimated using DAPA as a marker. Bacteria were isolated from the rumen and amino acid composition of their protein was determined. Thus, it became possible to estimate the bacterial contribution to individual amino acids flowing through the duodenum. The remainder of the amino acids were considered as being apparently undegraded dietary amino acids (AUDAA), neglecting the possible contribution of protozoal and endogenous amino acids. These apparently undegraded dietary amino acids were expressed as proportion of the amino acids ingested (DAA). Because proportions of bacterial protein to total duodenal protein flow varied between experiments, a better comparison was obtained by expressing the proportion of the individual apparently undegraded amino acids (AUDAA) as a percentage of the proportion of total dietary amino acids (TDAA) that remained apparently undegraded (TAUDAA). The mean result of 22 experiments with dairy cows, fed with long meadow hay and mixed concentrates in various ratios and at various levels of intake are shown in table 2. Results indicate that arginine, aspartic acid, glutamic acid, proline and alanine were degraded to a larger extent and methionine, serine, glycine, tyrosine and cystine to a lesser extent than the total amino acid N. The increase in glycine compared with the amount ingested must be mainly attributed to glycocholic acid, excreted with bile into the duodenum. Part of the high apparent resistance of cystine to degradation in the forestomachs may be due to the contribution of digestive enzymes such as trypsin and chymotrypsin. These enzymes contain three to four times more cystine than most feed proteins or bacterial protein and their contribution to duodenal cystine is measured as cystine apparently resistant against degradation in the forestomachs. The ranking order of apparent degradation of essential amino acids as found in these experiments differs somewhat from results of experiments where mixtures of free amino acids were incubated either *in vitro* or *in vivo* (Chalupa, 1976), particularly for valine and threonine.

Results in table 2 show that nonamino acid N is degraded to a lesser extent that amino acid N. Since the protein value is determined by its amino acids, the residue of dietary protein escaping microbial degradation may have a lower nutritive value than the original dietary crude protein. Evidence that this is the case was obtained recently by Smith and Mohamed (1977) with the dacron bag technique. From these experiments it also appeared that methionine content (g/16 g of N) remained constant, suggesting that degradation of methionine is less than the degradation of most other amino acids, which would confirm our results and those of Chalupa (1976).

Various factors may be responsible for the apparent differences in degradation of amino acids when supplied in protein form compared with offering them to microbes as a mixture of free amino acids, and for the differences in rates of degradation among individual amino acids. The distribution, amino acid composition and amino acid sequence of various classes of protein (albumins, globulins, prolamins, and glutelins) may be partly responsible. For barley, Folkes and Yemm (1956) found characteristic differences in the content of a number of amino acids between the more soluble proteins (albumins, globulins) and the less soluble fractions (prolamins, glutelins). A rather good agreement in amino acid composition of prolamin and glutelin exists between barley and various types of wheat (Folkes and Yemm, 1956; Eward, 1967), suggesting that amino acid composition of the various protein fractions is rather constant in grains. Soluble protein fractions contain far higher levels of lysine, arginine, aspartic acid and glycine and much lower levels of glutamic acid and proline than do less soluble fractions.

Differences in the rate of transport across the bacterial cell wall or differences in activities of various enzymes or enzyme systems involved in degradation of amino acids may also have an influence. If transport is the rate limiting step, results from experiments using free amino acids do not necessarily represent the situation under practical conditions in which the dietary amino acids are offered to the bacteria in the form of protein. Transport of amino acids across the bacterial cell wall may be either as individual amino acids or as peptides. There is evidence that the latter way of transporting amino acids across cell walls is at least as important as

TABLE 2. ABSOLUTE PROPORTIONS AND RELATIVE PERCENTAGES OF DIETARY AMINO ACIDS ENTERING THE SMALL INTESTINE OF DAIRY COWS APPARENTLY UNDEGRADED

Amino acid	AUDAA[a] / DAA[b]	SEM	AUDAA X 100 / TAUDAA[c]/TDAA[d]	SEM
Lysine	.48	.043	106	4.1
Histidine	.46	.029	106	2.1
Arginine	.28	.020	65	2.6
Threonine	.47	.031	109	3.1
Valine	.39	.030	89	2.6
Methionine	.54	.050	122	7.8
Isoleucine	.43	.032	98	4.1
Leucine	.41	.026	95	1.5
Phenylalanine	.41	.033	93	3.4
Aspartic acid	.31	.026	70	2.2
Serine	.49	.029	115	3.3
Glutamic acid	.31	.021	72	1.8
Proline	.26	.020	62	4.3
Glycine	1.25	.071	298	14.6
Alanine	.32	.028	72	3.5
Tyrosine	.66	.081	143	11.5
Cyst(e)ine	.88	.081	203	12.8
Nonammonia N	.46	.023	110	4.2
Total amino acid N	.44	.028	100	.0
Essential amino acid N	.39	.027	88	1.6
Nonessential amino acid N	.48	.030	112	1.6
Nonamino acid N	.54	.039	139	15.9

[a]AUDAA = Apparently undegraded dietary amino acids.

[b]DAA = Ingested dietary amino acids.

[c]TAUDAA = Total apparently undegraded amino acids.

[d]TDAA = Total ingested dietary amino acids.

transport of individual amino acids (Matthews and Payne, 1975; Payne, 1975), presumably because transport mechanisms required for transporting peptides are less specific than those required for transport of individual amino acids.

Practical Methods for Decreasing Protein Degradation in the Forestomachs. From the previous sections it becomes evident that it should be possible by taking appropriate measures to reduce protein degradation in the forestomachs to obtain greater amino acid absorption from the small intestine. It should be understood that these measures should not result in a decrease of microbial protein production in the forestomachs, or make feed protein so undegradable that it can no longer be hydrolyzed in the small intestine.

A simple method for decreasing protein degradation would be to formulate diets from ingredients containing protein with a natural resistance to ruminal breakdown. Other measures usually include some form of processing like grinding, heat treatment or treatment with chemical agents such as aldehydes, tannins or volatile fatty acids. The general idea behind treatment of proteins with chemicals is to create a reversible pH dependant chemical modification that will inhibit breakdown of the protein at the pH usually found in the reticulorumen (very oftern close to neutral), but still enable proteolysis at the much lower pH found in the abomasum and proximal duodenum. Based on the same principle, treatment of individual amino acids is possible, either by application of some protective agent, or by chemical modification which inhibits degradation by rumen microbes. Attempts have also been made to inhibit deaminative activity of microbial enzymes and the use of the esophageal groove reflex has been proposed as a means of bypassing the rumen.

All methods have been reviewed extensively in recent years (Broderick, 1975; Chalupa,

1975; Clark, 1975; Ferguson, 1975; Barry, 1976a; Kempton *et al.*, 1977; Tamminga and Van Hellemond, 1977; Waldo, 1977) and discussion here will therefore be restricted to some selected topics, mainly referring to developments in the last few years, with special reference to the situation in dairy cows.

The method to which most attention has been paid is treatment of feedstuffs with formaldehyde. The classical example is treatment of casein, resulting in dramatic reductions of degradability in the rumen, both *in vitro* and *in vivo* (Ferguson, 1975). In addition, a variety of other feedstuffs have been subjected to treatment with formaldehyde. Substantial increases in postruminal protein flow after treatment of dietary protein with formaldehyde, ranging from 6 to 34% seem possible as discussed recently by Kaufmann and Hagemeister (1976) and Hagemeister (1977).

The best production responses were usually reported for woolgrowth (Ferguson, 1975), particularly with casein as the protein source. Responses did increase with an increasing content of sulfur containing amino acids in the treated protein (Barry, 1976b). This is not surprising, considering the high content of such amino acids in wool proteins, implying a high requirement for S-containing amino acids. Responses in meat production, measured as N retention or rate of growth were often variable and usually much smaller than responses in wool growth as reviewed by Chalupa (1975) and Clark (1975). The small response in growth compared to wool growth is likely the result of a less important role of S-containing amino acids in meat production.

Little information is available on the effect of formaldehyde treatment of dietary protein on milk yield and milk protein production. Available data on the effect of formaldehyde treatment of dietary protein on milk protein production, compared with the untreated diet, are summarized in table 3. Results show that responses are usually small, even at high levels of milk production. Only the responses in the experiments of Verite and Journet (1977) were statistically significant. One of the reasons for an absence in significant responses in the other experiments may be the short experimental periods. There is also evidence, however, that no single amino acid is clearly limiting for milk protein production (Tamminga and Van Hellemond, 1977), such as S-containing amino acids are for woolgrowth (Barry, 1976b). Moreover, under most feeding conditions protein supply in the small intestine of dairy cows seems sufficient for milk production of at least 25 kg/day (Tamminga and Van Hellemond, 1977). Unless milk production exceeds this level, which may be the case in early lactation, protecting protein will be without any production response, except when the dietary protein content is lowered.

Results in table 3 give the impression that at formaldehyde levels of over 10 g/kg of protein a negative rather than a positive response can be expected. This may be the result of overprotection, causing not only a reduced degradation of protein in the forestomachs, but also a decreased susceptibility to proteolytic enzymes in the abomasum and small intestine. Suggested optimum levels for the application of formaldehyde are .8 to 1.2% formaldehyde per protein (w/w) for the protection of casein, 2% for oil seed meals and 3% for legume grass silage (Broderick, 1975). However from table 3 it seems that the application of 2 g formaldehyde/100 g of protein for feedstuffs to be used in concentrates is fairly high. This is in agreement with recommendations made by Barry (1976a) who also suggests application rates expressed in grams formaldehyde per kilogram degradable true protein to be more appropriate.

Not only can formaldehyde be applied to concentrates, but protein in forages, particularly silages, can also be protected by this method. Formaldehyde used as an additive in silage making, serves two purposes. Initially it prevents excessive degradation of protein and other ingredients during fermentation of the silage and when fed, solubility of the silage protein is reduced (table 4), probably resulting in protection against microbial degradation in the forestomachs.

In experiments of Beever *et al.*, (1977) the pepsin soluble N decreased from 82% in the untreated silage to 78% in the formaldehyde treated. A further reduction in solubility was achieved by drying the formaldehyde treated silage at high temperature. Ruminal protein degradation, as measured *in vivo* with re-entrant cannulated sheep, was reduced from 85% for the untreated to 22% for the formaldehyde treated silage and to 16% for the formaldehyde treated silage after heat treatment. *In vitro* solubility and *in vivo* ruminal degradation though showing a similar tendency differ widely. Pepsin soluble N may be regarded as a

conclusive (Annison, 1975), the deaminating enzyme systems (NAD^+- and $NADP^+$- linked glutamate dehydrogenase (GLDH) are thought to be the most important NH_3-fixing pathways. Inhibiting these key enzyme systems will very likely reduce microbial protein synthesis and possibly inhibit microbial degradation of other dietary ingredients, such as cellulose. This may cause an increase in rumen retention time of cellulose rich dietary components, followed by a reduction in feed intake, a phenomenon often observed if deamination suppressing agents are applied (Chalupa, 1975).

Nutritional Implications of Degradation and Protection of Dietary Protein in Ruminant Feeding. In ruminant feeding one has to consider two protein requirements, the requirement of the animal itself and the requirement of the microbial population in the forestomach. Meeting the requirement of the animal means supplying adequate blood levels of essential amino acids and N, carbon (C) and energy for synthesis of necessary nonessential amino acids. This requirement can be met if sufficient protein enters and is subsequently absorbed from the small intestine. The main sources of protein entering the small intestine are undegraded dietary protein and microbial protein synthesized in the forestomach. The microbial population in the forestomach of a ruminant has the capacity to synthesize all essential amino acids. Growth of some strains of rumen microbes is stimulated by amino acids (Hungate, 1966) and the addition of small amounts of protein stimulated microbial protein synthesis from NPN (Hume, 1970). So it seems advisable to provide the microbes with a small amount of protein-N, but the bulk of the required N may be supplied as NH_3 which can originate from NPN sources such as urea as well as from degraded dietary protein.

Not only do the two protein requirements differ in a qualitative sense, the quantities required may also differ. The requirement for protein absorbed from the small intestine is mainly governed by the level of production and will be relatively high for milk production, particularly in early lactation, and for fast growing young beef animals. Under those circumstances net protein supply from the small intestine, being the result of absorption of dietary protein escaping microbial breakdown and of microbial protein synthesized in the forestomachs, may be insufficient to meet the high requirement (Hogan, 1975; Tamminga and Van Hellemond, 1977). Under less intensive animal production systems the N requirement of the rumen microbes may become predominant. Under such conditions undegraded dietary protein plus microbial protein will supply more amino acids to the animal's tissue than is actually needed. Reducing the degradation of protein becomes useless or even harmful. Due to protection flow of dietary protein into the small intestine will increase, but flow of microbial protein may decrease because of an insufficient supply of NH_3 for microbial growth and fermentation in the rumen.

For maximum microbial growth and microbial protein production in the rumen a minimal NH_3 concentration of approximately 5 mg of NH_3-N/100 ml of rumen fluid seems required (Satter and Slyter, 1974). Under normal feeding practices a relationship between ruminal NH_3 concentration and dietary crude protein content can be established, and this indicates that the minimum level of 5 mg/ 100 ml rumen fluid is achieved with a dietary crude protein content of between 11 and 14% in the diet dry matter, varying with the density of dietary digestible nutrients (Satter and Roffler, 1975).

An inadequate N supply for the microbes may also have a negative effect on degradation of other dietary components, particularly the cellulose rich cell wall constituents. To achieve maximum microbial activity much higher NH_3 concentrations than required for maximum microbial protein production seem necessary (Mehrez *et al.*, 1977). Maximum rumen fermentation rates in the experiments of Mehrez *et al.* (1977) were achieved at a NH_3 concentration of 23.5 mg/100 ml fluid. Data in table 5 also show that a dietary crude protein content of 13.4% in the diet dry matter is clearly too low to sustain maximum microbial fermentation of dietary crude fiber, but no effect was seen on degradation of N-free extractives (NfE). The experiments were done in our laboratory with three dairy cows, equipped with re-entrant cannulae in the small intestine, fed mixed diets consisting of long meadow hay and mixed concentrates. Of the total dry matter intake (mean intake 12.9 kg per day), 33% was as long roughage. If the dietary N level is inadequate for the microbial population in the forestomach, additional N must be supplied. Application of NPN will be sufficient under these circumstances.

If protein requirement of the animal is not met, then the intestinal flow of undegraded

TABLE 5. EFFECT OF DIETARY N CONTENT ON DIGESTION OF CRUDE FIBER AND N FREE EXTRACTIVES (NfE) IN THE FORESTOMACH OF DAIRY COWS

Cow	Intake, kg DM/day	Dietary N content, g/kg of DM	Dietary CF content, g/kg of DM	Fermented in the forestomachs, proportion of intake	
				Crude fiber	NfE
A	14.3	23.2	142	.52	.73
B	12.8	20.3	141	.48	.69
C	10.8	21.0	170	.37	.77
Mean	12.6	21.5	151	.46	.73
A	12.8	33.3	135	.61	.70
B	13.0	30.1	150	.50	.73
C	12.9	29.6	149	.64	.76
Mean	12.9	31.0	145	.58	.73

dietary protein needs to be increased, either by increasing dietary protein content or by reducing degradation of the protein already present in the diet. The latter seems to be the most attractive because an additional protein supply in the diet will be largely degraded, resulting in poor utilization of the extra protein.

Because the proportion of dietary protein degraded in the forestomach differs among feedstuffs (Chalupa, 1975; Mertens, 1977), increasing the flow of dietary protein into the small intestine must be possible by formulating diets with feedstuffs having relatively resistant proteins. Alternatively, an increase in flow of dietary protein is possible by protecting the dietary protein with formaldehyde, for example. However, feeding more resistant protein will result in lower NH_3 levels in rumen fluid (Wohlt *et al.*, 1976; Bakker and Veen, 1977; Beever *et al.*, 1977; Verité *et al.*, 1977), and NH_3 may fall below the minimum level required for maximum microbial protein synthesis. Evidence for a reduced microbial protein synthesis following feeding of formaldehyde treated grass silage has been obtained (Beever *et al.*, 1977). However, this may have been a specific effect of formaldehyde, because the rumen NH_3 level did not fall below the level recommended for maximum microbial protein synthesis and the level of formaldehyde applied was very high.

An adequate N supply for the rumen microbes after feeding naturally resistant or protected protein can easily be achieved by including some NPN such as urea in the diet, and protein protection and use of NPN would seem a useful combination.

Protection of dietary protein will increase the relative contribution of undegraded dietary protein to the total protein entering the small intestine. This situation may also have an effect on the amino acid composition of the total protein that enters and is subsequently absorbed from the small intestine. This response is because of a potential difference in amino acid composition between the residue originating from dietary protein and from microbial protein. Experimental results obtained so far do not clearly indicate a single amino acid as being first limiting for milk production (Tamminga and Van Hellemond, 1977) because amino acid composition of the protein absorbed from the small intestine seems to resemble the pattern of required amino acids. Changing the ratio between undegraded dietary protein and microbial protein entering the small intestine may change this balance and may result in one single amino acid becoming limiting. This situation could increase the need for protection of individual amino acids against microbial degradation in the forestomach. So far production responses to protected amino acids have been quite variable and often absent (Barry, 1976a), mainly because there was no distinct requirement for extra amino acids.

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2. MEASUREMENT OF MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN

SUMMARY

Microbial protein production forms a major part of microbial growth in the rumen. The principles of microbial growth in the rumen are expected to be similar to those in a continuous culture system under anaërobic conditions. Because energy is often the first limiting factor for microbial growth under such conditions, energy supply and energetic efficiency of growth become important. In continuous culture systems in vitro the energy supply is mainly determined by the amount and nature of the substrate fermented and the nature of the endproducts of this fermentation. The energetic efficiency of biomass production in such a system depends on the nature of the precursors for microbial cellular compounds, the composition of microbial cells and the growth-rate.

Factors influencing microbial growth and microbial protein production in the rumen are much less understood. This apparent discrepancy is believed to result from the difficulty to perform experiments in vivo under strictly controlled experimental conditions. Studying microbial growth in the rumen is usually done with experimental animals equipped with cannulae by surgical preparation. Variation not related to experimental treatments is often high in such experiments. This may be caused by the short measuring period, the use of indigestible markers and the method of sampling ruminal and duodenal digesta. Errors in the estimate of microbial protein by using markers such as RNA, DAPA and radioisotopes, cause additional random variation. Variation coefficients of up to 20% were found for the RNA-N/total-N content in bacteria and protozoa and for the DAPA-N/total-N content of bacteria, isolated from the rumen of dairy cows. Apart from possible analytical errors, the variation seems to be of between animal, between experiment and diurnal origin. A substantial difference was found in the RNA-N/total-N ratio between bacteria and protozoa.

The use of RNA as microbial marker in the bovine is likely to overestimate the contribution of microbial-N to total-N entering the small intestine. Even if that was taken into account the agreement between the results based on RNA,

DAPA and on regression calculations was poor. Reproducibility was also poor for the methods based on RNA and DAPA, but may be better with ^{35}S as a marking substance for microbial-N.

The efficiency of microbial growth in microbial cultures is usually expressed in g microbial dry matter/mole of ATP. In ruminant nutrition it has become common practice to study microbial protein production rather than growth and its energetic efficiency is often expressed as g microbial N/100 g organic matter apparently digested in the forestomachs, the latter being considered as a measure of energy (ATP) available for microbial growth. Under anaërobic conditions the protein and lipid part of this organic matter are poor energy yielding substrates. Moreover endogenous proteins and fatty substances do interfere with the estimate of organic matter fermented in the forestomachs. Relating the microbial N entering the small intestine to the total of crude fibre and NfE, which disappear between intake and small intestine (a measure of carbohydrates fermented), seems therefore more appropriate. In experiments with dairy cows, fitted with cannulae in the small intestine, this relationship was calculated, using various ways of estimating microbial protein. As could be expected, agreement between the methods was poor. Within each method the reproducibility was also poor.

In continuous culture systems a relation exists between the energetic efficiency of microbial growth and the dilution rate. In dairy cows a close positive relation was found between the dilution rate of rumen liquor and feed intake, which would suggest a more efficient microbial protein production at a higher level of intake. Contradictory to this the efficiency of microbial protein synthesis tended to be lower at higher feed intakes in the experiments referred to. No explanation for this discrepancy could be given.

It is concluded that for a better understanding of the principles of microbial growth and of the efficiency of microbial protein production in the rumen, measuring techniques and analytical procedures applied in research in this field need to be improved. If no such improvements can be made, relating the total N or amino acids entering the small intestine, to dietary factors, thus avoiding the use of microbial markers, may also give a satisfactory estimate of the protein supply in the ruminant animal. The organic matter apparently digested seems to be a useful estimate of protein supply in this respect.

INTRODUCTION

In recent years knowledge on N metabolism in the digestive tract of ruminants has accumulated. It has become clear that protein supply in ruminants is governed by the extent of microbial degradation of dietary protein and by the efficiency of microbial protein synthesis. Both are subject to variation, but particularly for the efficiency of microbial protein synthesis, the nature of this variation is poorly understood.

There is little doubt however that under most feeding practices microbial protein forms a substantial part of the protein entering the small intestine. Consequently a way of improving protein supply in ruminants can be achieved by maximizing microbial protein synthesis in the rumen. To realize this a better understanding of the principles of microbial growth in the rumen is necessary. However, this requires a reliable and accurate estimate of microbial protein. This becomes even more urgent because in vivo, undegraded dietary protein is usually estimated as the difference between total protein, sometimes corrected for endogenous protein, and microbial protein entering the small intestine. So, any error in estimating microbial protein will affect the estimate of undegraded dietary protein.

In this paper attention will be paid to the principles of microbial growth in the rumen. Subsequently techniques used to estimate microbial protein and the efficiency of microbial protein synthesis in vivo will be discussed.

MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN

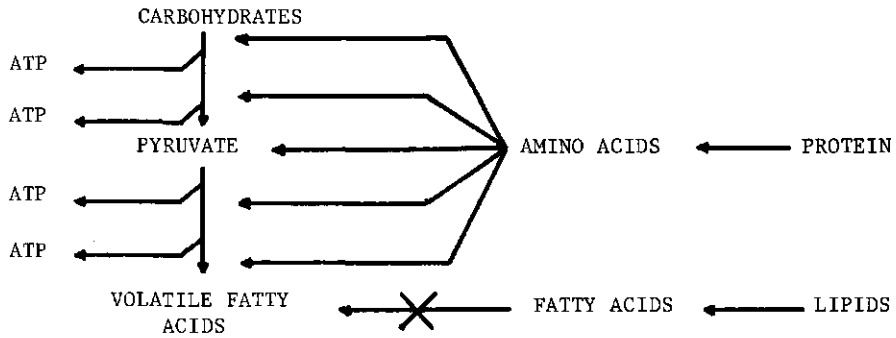
Microbial protein synthesis forms a major part of microbial growth in the rumen. The rumen resembles a continuous culture system, so the principles of such a system should be applicable. In anaërobic microbial systems such as the rumen, energy (ATP) is often the first limiting factor for microbial growth, reason why energy supply and the efficiency of its utilisation for both microbial growth and protein production are of great interest. The supply depends on the nature of the substrate and the end products in which the substrate is fermented.

The energetic efficiency in microbial growth studies is usually given as Y_{ATP}, which is the yield in g of dry matter per gmol of ATP. Important determinants of this energetic efficiency of microbial growth in continuous cultures are the nature of the precursors for microbial cellular compounds, their composi-

tion and the growthrate.

The energy extractable from the substrate in the rumen is limited by anaërobiosis. Under such conditions carbohydrates form the best substrate for microbial growth. During their degradation ATP is generated at several places (figure 1).

Figure 1. Simplified representation of the energy (ATP) yield of various dietary ingredients for anaërobic microbial growth in the rumen



Breakdown of protein results in intermediates whose conversion into VFA yields less ATP. There is very little conversion of lipids in the rumen. The amount of ATP produced varies even within carbohydrates, depending on their nature and on the composition of the end products formed. Part of the substrate, carbohydrates as well as proteins, are not broken down into end products, but in intermediates, which in turn are used as precursors for microbial cell synthesis. Such a degradation will yield considerably less ATP if any at all. Consequently it is virtually impossible to give an accurate estimate of the ATP generated in the rumen, reason why it has become common practice to relate microbial growth in the rumen to the organic matter fermented in it rather than to the ATP generated from this fermentation. Its value is limited, because neither its nature, nor the nature of the end products is taken into account.

Not only varies the ATP yield from the substrate fermented in the rumen, so does the amount of ATP required for microbial growth and microbial protein production. Both, intermediates and end products from rumen fermentation are

used as precursors for microbial cell synthesis (Allison, 1969), but their relative importance is not known precisely. Fermentation to end products yields more ATP than fermentation to intermediates, but the difference is not enough to compensate for the much higher ATP requirement if end products rather than intermediates of fermentation are used as precursors for microbial growth (Stouthamer, 1973). Variation in the ratio between net protein synthesis and net growth which is known to occur in rumen microbes (Smith, 1975), also affects the energetic efficiency of microbial protein production.

From studies with continuous cultures it has become clear that the energetic efficiency of microbial growth is dependent on the growthrate. The ATP requirement for microbial growth can be divided in a requirement for maintenance and a requirement for growth (Stouthamer, 1973). With a high growthrate the ATP required for maintenance becomes relatively less important, so the efficiency of the net growth increases. The relation between efficiency of microbial growth and growthrate is well documented in vitro, including for cultures of rumen microbes (Isaäcson et al., 1975). In vitro a positive relation was found between the dilution rate as a measure of growthrate and the efficiency of microbial growth. There is evidence that the efficiency of microbial growth in the rumen is also influenced by the dilution rate of rumen fluid (Harrison et al., 1976).

Factors influencing the rumen liquor dilution rate (RLD) have therefore become of interest, and this was studied in dairy cows, using Cr-EDTA as a soluble but indigestible marker. In these experiments, the animals were fed with varying amounts of long roughage and concentrates. Using multiple regression analysis a satisfactory relationship was found ($R^2 = 0.66$), between the RLD and the intake of long roughage and concentrates. Each additional kg of DM in long roughage ingested increased the RLD by $1.7 \pm 0.27\%$ units; for ground and pelleted concentrates this increase was only $0.66 \pm 0.11\%$ units. However, attempts to relate the efficiency of microbial protein production based on the DAPA method, to the dilution rate calculated with the regression formula remained as yet unsuccessful.

MEASURING MICROBIAL PROTEIN

Various techniques for measuring microbial protein have been developed, both in vitro and in vivo. They are usually based on the use of marking substances characteristic for bacteria, protozoa or both. For this purpose

nucleic acids (RNA, DNA), 2.6-diaminopimelic acid (DAPA), 2-aminoethylphosphonic acid (EAP) and various radioisotopes (^{15}N, ^{32}P, ^{35}S) have been applied. In recent papers shortcomings of the various methods were discussed (Demeyer & Van Nevel, 1977; Ling & Buttery, 1978).

An assumption in a rather large number of reports dealing with the measurement of microbial protein in the rumen is the constancy of the marker content in microorganisms. Recent reports show that the variation in RNA and DAPA content is often large (Smith, 1975; Ling & Buttery, 1978). This was also found in microorganisms isolated from the rumen of dairy cows fed with long hay and concentrates (Table 1). The RNA values were slightly higher than most of the values reported elsewhere (Smith et al., 1975; Ling & Buttery, 1978). The results show a considerable variation for both markers. This may be

Table 1. RNA-N/total-N (mg/g) in isolated rumen bacteria and protozoa and DAPA-N/total-N (mg/g) in isolated rumen bacteria.

	RNA-N/total-N		DAPA-N/total-N
	bacteria	protozoa	bacteria
no. of observations	17	16	17
mean value	134.1	64.9	7.13
range	79-189	35-94	3.7-10.5
coeff. of variation (%)	20	22	23

partly due to a difference in the level of feed intake. At a lower level of intake (8.2 $\pm$ 0.3 kg DM/day) the mean content of all markers was some 20% lower than at a higher level of intake (12.9 $\pm$ 0.4 kg DM/day). Moreover the variation was considerably higher at the higher intake. Differences in the technique of isolating bacteria and protozoa from the rumen may also have an effect. Particularly the time of sampling seems important, because of diurnal variation in RNA and DAPA content of bacteria in the rumen (Smith et al., 1975).

A marked difference in the RNA-N/total-N appears to exist between bacteria and protozoa, which confirms results found elsewhere (Ling & Buttery, 1978). This is also confirmed by the difference in amino acid content. It was found that in bacteria 67% of the N was present in amino acids, whereas in protozoa this figure was 78% (Tamminga, 1975).

Data on the agreement of the various methods for estimating microbial protein by comparing them in the same experiments are limited. Comparisons in vitro for ^{15}N, ^{32}P and ^{35}S (Harmeyer et al., 1976) did not show good agreement between the various methods. Similar discrepancies were reported in vivo

by comparing the application of RNA and DAPA in dairy cows (Smith, 1975) and by comparing RNA, DAPA and ^{35}S in sheep (Ling & Buttery, 1978).

From the RNA-N/total-N and DAPA-N/total-N ratios in duodenal content and isolated rumen bacteria, the contribution of microbial N to the N entering the small intestine could be estimated. The objective of the experiments referred to was to study the influence of 3 protein levels (2.15 ± 0.06; 3.06 ± 0.11 and 3.89 ± 0.05% N/DM) at the two levels of intake mentioned already, on the N entering the small intestine. The contribution of microbial N at the various dietary N levels at the two levels of intake, using either RNA or DAPA as a marker are shown in Table 2.

Table 2. Microbial N as proportion of total N entering the small intestine of dairy cows, using RNA and DAPA as markers (mean results of 3 observations with their standard error).

dietary N (%/DM)	low intake		high intake	
	RNA	DAPA	RNA	DAPA
2.15	1.16 ± 0.24	0.59 ± 0.03	0.67 ± 0.07	0.51 ± 0.01
3.06	0.75 ± 0.04	0.54 ± 0.05	0.63 ± 0.15	0.41 ± 0.02
3.89	0.94 ± 0.15	0.48 ± 0.03	0.64 ± 0.08	0.37 ± 0.03

The results based on RNA show a large standard error and are probably overestimations. In these experiments a significant part of the N passing through the small intestine must be of endogenous origin, because the cannulae were situated beyond the pancreatic and biliary duct, thus values near one are unreliable. It has been reported that the RNA method overestimates the microbial contribution of N entering the small intestine up to 15% (Smith et al., 1976). Results based on DAPA show much less variation and are very much in line with results found in dairy cows elsewhere (Hutton et al., 1972; Smith, 1975). The mean difference between the results based on RNA and DAPA are of the same order as reported for dairy cows by Smith (1975).

MEASURING THE EFFICIENCY OF MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN

The organic matter fermented in the rumen, often used as a measure of ATP available for microbial growth, is usually estimated as the difference between intake and organic matter entering the small intestine, in animals fitted with cannulae. The measuring methods, including the use of indigestible markers and sampling procedures from rumen and small intestine, applied to such animals, cause an important source of variation which makes the estimate

of the organic matter fermented in the rumen less accurate. Its value as a measure of ATP available for microbial growth becomes also limited because of variation in its composition and due to organic matter of endogenous origin. Particularly if the measuring point in the small intestine is beyond pancreatic and biliary duct, endogenous protein and fatty substances become a serious problem, because they decrease the amount of organic matter apparently fermented in the forestomachs. To eliminate some of the problems it seems preferable to relate growth or microbial protein production to some estimate of the carbohydrates fermented. The simpliest choice seems the total of crude fibre and N-free extractives (NfE). The latter is calculated by subtracting crude fibre, protein (N x 6.25) and lipids from organic matter. Part of the N in duodenal digesta is NH_3-N and when NfE is calculated for duodenal digesta ammonia free protein (NAN x 6.25), rather than crude protein (N x 6.25), should be used as measure of protein.

Microbial N, undegraded N and endogenous N contribute to the NAN entering the small intestine. The contribution of microbial N and from this the microbial protein production per 100 g substrate fermented can be estimated with RNA or DAPA. In experiments with a varying dietary N content it becomes also possible to estimate degradation and synthesis of N in the forestomachs by relating the ratio duodenal NAN/ingested N to the dietary N content, using regression techniques (Hvelplund et al., 1976). In our calculations the dietary N content (% N/DM) was replaced by the ratio N intake/(crude fibre + NfE) fermented in the rumen (g/100 g), also resulting in an estimate of the energetic efficiency of microbial protein synthesis in the forestomachs. The method was applied after correcting the NAN entering the small intestine for an endogenous contribution of 4 g N/kg DM ingested. The results of the various methods of estimating the efficiency of microbial protein synthesis (Table 3) do not show good agreement. Moreover the results based on RNA show a high variation, apparently at random. Although differences are not significant, all methods show a tendency of a slight more efficient microbial protein production at the lower level of intake, which is difficult to explain. Because all methods yield results within the range to be expected on theoretical grounds (Stouthamer, 1973), it is impossible to decide which method is the most reliable.

In surveying literature also a wide variation is found in estimated efficiencies of microbial protein production. Although some of the variation could be ascribed to factors associated with the substrate fermented, such as its

Table 3. The efficiency of microbial protein synthesis (microbial N/100 g 'carbohydrates' apparently fermented) in the rumen of dairy cows, calculated by various methods.

method	high intake	low intake
RNA	4.9 ± 0.59	5.6 ± 0.53
DAPA	3.1 ± 0.20	3.5 ± 0.14
regression	2.4 ± 0.20	2.7 ± 0.08
number of observations	9	8

nature, the nature of the end products, extent and rate of fermentation, rumen dilution rate, etc., a substantial part of the remaining variation must be due to errors in the measuring and analytical techniques involved. Particularly if RNA and DAPA are used as microbial markers, the reproducibility seems poor. The limited data available suggest the reproducibility of ^{35}S to be considerably better (Ling & Buttery, 1978).

The accuracy of estimations of the protein supply in ruminants, by using estimates of undegraded dietary protein and microbial protein, based on the use of microbial markers seems rather poor. Alternatively the protein supply can be estimated by relating the total N or amino acids entering the small intestine to dietary factors, thus avoiding the use of microbial markers. For dairy cows a close fitting regression equation (R^2 = 0.90) was calculated between amino acid N entering the small intestine and the intake of apparently digested organic matter (ADOM). For each kg of ADOM ingested 32.3 ± 1.60 g of amino acid N entered the small intestine (Tamminga & Van Hellemond, 1977).

CONCLUSIONS

Because of their influences on protein supply in ruminants more and accurate information is needed on factors governing the microbial breakdown of dietary protein and the synthesis of microbial protein.
Measuring techniques applied in cannulated animals and analytical procedures, using marking substances for microbial protein need to be improved. Without this, the results of research in this field will yield qualitative rather than quantitative differences, both for the degradability of dietary protein and for the efficiency of microbial protein production.

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3. The influence of the protein source on the protein digestion in the ruminant

By S. TAMMINGA

Receipt of Ms. 30. 7. 1973

1. Introduction

Protein in the ration offered to ruminants is assumed to be broken down to a wide extent into volatile fatty acids (VFA) and ammonia. Quantitative estimations about this breakdown, go as far as 90 % in some cases (McDONALD und HALL, 1957; WELLER, GRAY and PILGRIM, 1958; WELLER, GRAY and PILGRIM, 1962). In vitro experiments showed that the proportion of the breakdown depends on the solubility of the protein source in rumen fluid (HENDERICKX, 1962). Casein was found to be broken down nearly completely while from maize isolated protein, zein was digested only to a small extent. This difference in ruminal protein breakdown was confirmed in vivo with sheep (McDONALD, 1954; McDONALD and HALL, 1957). In vitro only a small difference was found between the ruminal breakdown of soya-protein and casein (Henderickx, 1962).

Beside the breakdown of protein, there is a synthesis of microbial protein in the rumen. This means that the amino acid composition of the protein leaving the stomachs and arriving at the beginning of the small intestine is mainly determined by the amino acid composition and quantity of unattacked feed protein and synthesized microbial protein.

To study the influence of a less attackable protein in the ration on the digestion in the ruminant an experiment was carried out with a fistulated cow. It was examined to see if there was a difference in the quantity and amino acid composition of protein reaching the duodenum after feeding maize-protein or soya-protein.

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2. Materials and methods

A non-lactating mature cow, fitted with a rumen fistula and a re-entrant cannula at the beginning of the small intestine, about 50 cm beyond the pilorus and just beyond the pancreatic and biliary duct, was fed with semi-synthetic rations containing maize-protein (ration I) or soya-protein (ration II) as a nitrogen source. The composition of the rations is given in table 1.

The concentrate mixture was fed as a porridge which was mixed with the straw. The animal was fed twice daily with equal portions at 7.30 a.m. and 7.30 p.m. Each experiment consisted of a transition and adaptation period of 18 days and a collection period of 10 days. In the collection period, faeces were collected, weighed and

Table 1

Composition of the ration

	Ration I	Ration II
Wheat straw	2.00 kg	2.00 kg
Maize gluten feed	1.28 kg	—
Maize gluten meal	1.22 kg	—
Soyabean meal	—	2.50 kg
Maize-starch	3.05 kg	3.05 kg
Soyabean oil	0.29 kg	0.29 kg
Mineral mixture	0.15 kg	0.15 kg
Vitamin A + D_3 premix	0.01 kg	0.01 kg

sampled daily. The daily samples were conserved with 0.25 % toluene and stored at 0° C. The daily samples were made up to a proportional period sample and this was analyzed for dry matter, inorganic matter, nitrogen and amino acids. During the last 5 days of the collection period the duodenal flow was measured continuously for 96 hours. The digesta flowed, due to the peristaltic movements of the gut, through a plastic tube (internal diameter 16 mm) which was connected with the proximal part of the cannule, in a flask. This flask was kept at 38° C in a waterbath. Each 15 minutes the flask was changed for an empty one and the contents of the filled flask were thoroughly mixed and sampled. Each day a proportional sample of 2 % of the duodenal content was collected in this way. The remainder was carefully poured back into the animal by means of a funnel which was connected to the distal part of the cannula by a plastic tube. The sample was conserved with 0.25 % toluene and stored at 0° C. A composite sample was made of the four day samples. This sample was analyzed for dry matter. Part of this sample was freeze-dried and analyzed for dry matter, inorganic matter, nitrogen and amino acids. Nitrogen was determined according to the method of Kjeldahl. The amino acid composition was determined by columnchromatography on a Carlo Erba amino acid analyzer.

On the 3rd day of the collection period a rumen sample was taken about 3 hours after feeding. In this sample the ammonia content was determined according to the method of KAPLAN (1965).

3. Results and discussion

3.1. Digestion of N-containing feed components

In table 2 a survey is given of the digestion of dry matter, organic matter, total-N and amino acid-N in the various parts of the digestive tract. These figures show that the quantity of total-N and amino acid-N passing through the duodenum is influenc-

Table 2

Digestion of DM, OM, total-N, total amino acid-N (TAA-N), essential amino acid-N (EAA) and non essential amino acid-N (NEAA-N)

	Ration	DM	OM	N	TAA-N	EAA-N	NEAA-N
Intake (g/day)	I	7192	6800	173	136	60	76
	II	7096	6640	201	145	76	69
Apparently digested (% intake)	I	70	73	65	79	77	81
	II	75	79	77	82	82	81
Apparently digested in the stomachs (% intake)	I	26	34	−55	−51	−56	−46
	II	30	40	− 7	− 4	0	− 9
Apparently digested in the intestines (% duodenal flow)	I	60	59	78	86	85	86
	II	64	65	78	83	82	84

ed by the protein source in the ration. After feeding the maize-protein there appeared to be more N and amino acid-N in the duodenum than after feeding the soya-protein. These results agree with the findings in sheep fed with maize-protein or soya-protein in the ration (CLARKE, ELLINGER and PHILLIPSON, 1966). The difference in quantity of nitrogen and amino acid-N is ascribed to differences in protein breakdown in the rumen after feeding maize-protein or soya-protein. When the ruminal breakdown is important, ammonia is formed and part of this passes through the rumen wall. After this passage, ammonia is transported to the liver, incorporated in urea and mainly excreted in the urine. This excreted nitrogen has been of no use to the animal. A relative measure for the protein breakdown in the rumen, is the ammonia content in rumen fluid. The measured levels were 3.0 mg per 100 ml after feeding maize-protein and 18.1 mg per 100 ml after feeding soya-protein. Both levels seem to be low, however the feed intake was low and the ration contained a high percentage of starch, which prevents the protein breakdown and stimulates the protein synthesis in the rumen (HENDERICKX, 1962). This means that breakdown does not exceed synthesis very much and the result is a low ammonia level in the rumen.

3.2. The origin of nitrogen in the duodenum

The quantity of protein in the digestive tract is the result of the quantity unattacked feed protein, synthesized microbial protein and endogenous protein. In most cases the limiting factor in ruminal protein synthesis is not the presence of nitrogen, but the supply of available energy especially as Adenosine triphosphate (ATP). The

energy for protein synthesis has to be generated by the anaerobic breakdown of carbohydrates in VFA. This anaerobic process produces considerably less ATP per mol carbohydrate than aerobic oxidation. The anaerobic circumstances therefore are a limiting factor for the ATP formation and thus for the microbial protein synthesis (HUNGATE, 1966). As a measure for the energetic efficiency of microbial protein synthesis the term YATP is often used. This means the quantity of microbial dry weight which is produced for each mol ATP formed. This energetic efficiency varies from about 10 to 20 for the different bacterial strains (BAUCHOP and ELSDEN, 1960; HOBSON and SUMMERS, 1967). Since the bacterial population in the rumen generally consists of several hundreds of different strains, one may expect that the variation in energetic efficiency for the rumen population as a whole is rather small.

In vitro values were found between 13 and 15 (WALKER and NADER, 1968). On account of the quantity of organic matter disappearing from the fore stomachs, and the rather constant levels of protein and diaminopimelic acid (DAPA) content in mixed rumen bacteria, the quantity of synthesized microbial protein in the rumen of sheep was estimated to be at least 3.7 g microbial N for each 100 g organic matter, that is apparently digested in the fore stomachs (HOGAN and WESTON, 1970). This value has to be considered as a minimum value, because DAPA is only a measure for the quantity of bacterial protein and the protein which is formed by the rumen protozoa from earlier synthesized bacterial protein is not taken into account.

Taking the view that 3.7 g microbial-N is formed for each 100 g organic matter disappeared between intake and beginning of the duodenum, in our experiment the quantity of microbial protein in the duodenum after feeding maize-protein was

Table 3

Amino acid compositions. Amino acid-N expressed as a percentage of total-N

	Intake		Duodenal content		Rumen
	Ration I	Ration II	Ration I	Ration II	Bacteria
Lys	2.5	6.2	5.0	7.1	8.4
His	3.4	3.9	2.9	2.8	2.1
Arg	6.5	12.1	6.3	6.9	7.3
Asp	4.2	6.6	5.1	5.8	6.6
Thr	2.4	2.4	2.7	3.0	3.0
Ser	4.3	3.8	4.0	3.5	2.9
Glu	12.7	10.0	9.8	6.4	6.4
Pro	7.9	4.3	4.4	2.5	2.0
Gly	3.6	4.5	6.9	8.8	5.2
Ala	8.8	4.0	7.0	5.0	5.6
Val	3.7	3.3	3.6	3.9	3.5
Met	1.7	0.9	1.7	1.6	1.2
Ileu	2.7	2.7	2.7	2.9	2.8
Leu	8.7	4.0	5.9	3.5	4.0
Tyr	2.2	1.5	2.0	1.7	1.8
Phe	3.1	2.3	2.4	1.8	1.9
TAA-N	78.4	72.5	72.4	67.2	64.7
EAA-N	34.7	37.8	33.2	33.5	34.2
NEAA-N	43.7	34.7	39.2	33.7	30.5

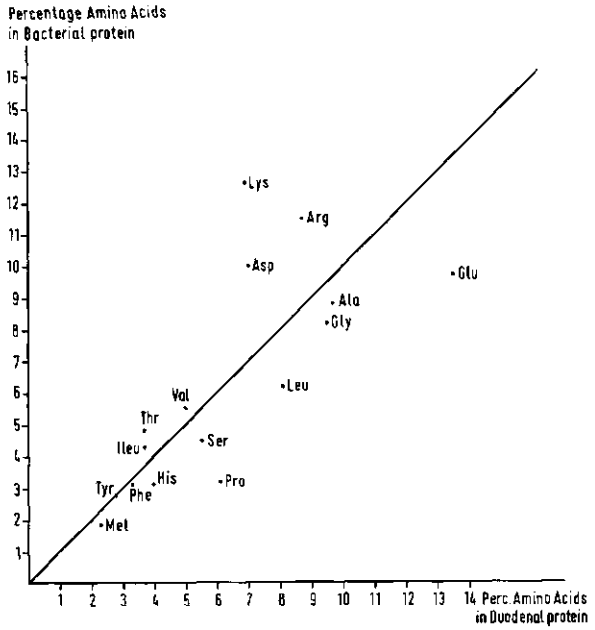

Fig. 1. Association between duodenal protein and bacterial protein in a cow after feeding maize-protein. Amino acid-N expressed as percentage of total amino acid-N

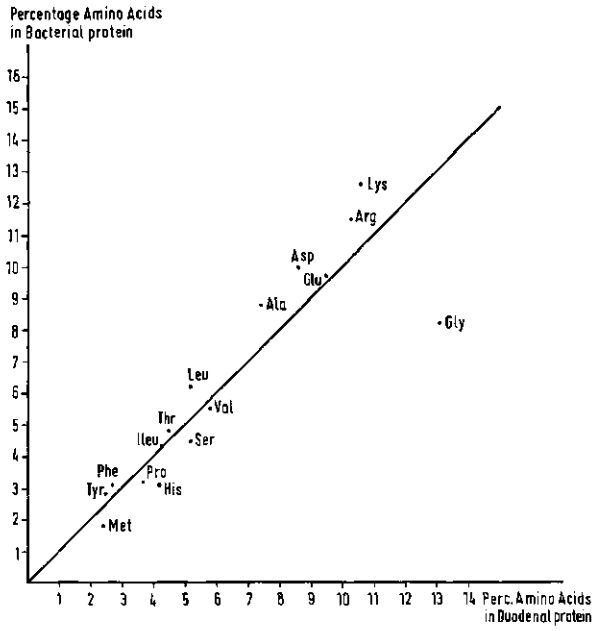

Fig. 2. Association between duodenal protein and bacterial protein in a cow after feeding soya-protein. Amino acid-N expressed as percentage of total amino acid-N

estimated at 86 g and after feeding soya-protein at 98 g. In that case the percentage of microbial N in the total N in the duodenum was calculated as 32 and 46 % respectively. If it is assumed that in both cases the quantity of endogenous N was the same and the protozoa had a rather small influence on the entire, the differences are caused by a difference in ruminal breakdown between the two rations.

After feeding maize-protein there is a net increase of amino acid-N in the daily duodenal flow, compared to the daily intake, of 69 g amino acid-N, of which at least 34 g (49 %) are essential amino acids (Tryptophane was not determined, so this amino acid has not been taken into account). After feeding soya-protein however, the much smaller increase of amino acid-N of 6 g consisted entirely of non-essential amino acids. Assuming that part of the protein in the duodenum is of endogenous origin, there must have been a loss of amino acid-N after feeding soya-protein, particularly essential amino acid-N.

In table 3 a survey is given of the amino acid composition of the protein in the rations, the protein in the duodenal content after feeding both rations and the protein in mixed rumen bacteria. The last figures were

Table 4

Quantitative changes in individual amino acids in the digestive tract

Ration	Intake (g/day)		Duodenal flow (% intake)		Absorbed from intestine (% intake)		(% flow)	
	I	II	I	II	I	II	I	II
Lys	22.3	64.4	323	121	32	76	79	81
His	21.8	29.0	124	89	84	88	87	86
Arg	35.3	75.2	153	77	72	89	82	86
Asp	68.9	125.3	196	99	68	83	83	83
Thr	35.0	40.7	180	134	69	74	83	81
Ser	56.0	56.9	145	87	82	83	88	80
Glu	231.4	208.9	125	64	84	87	87	80
Pro	112.2	69.6	96	73	89	87	88	83
Gly	33.7	48.1	320	223	68	76	90	89
Ala	97.8	50.7	129	147	81	65	85	76
Val	53.1	55.2	170	122	76	76	86	80
Met	31.4	20.0	172	162	72	65	83	78
Ileu	43.4	50.3	166	112	77	79	86	81
Leu	141.7	73.9	114	101	89	82	90	82
Tyr	49.7	38.6	145	128	84	82	89	86
Phe	64.1	54.2	126	104	83	82	87	83
AA-N	136.0	144.6	151	104	79	82	86	83

obtained from mixtures of rumen bacteria, isolated in our laboratory from lactating cows fed with different rations. Compared to the intake, the proportion of amino acid-N in total-N has decreased. This decrease was probably caused by the microbial-N present in the duodenal content, which contains a substantial amount of non amino acid-N (nucleic acids).

The results from table 3 were recalculated as amino acid-N expressed as a percentage of the total amino acid-N present in the sample and the amino acid composition of both duodenal contents were compared with the amino acid composition of rumen bacteria (Fig. 1 and Fig. 2). From these figures it appears that the best association exists between the amino acid composition of duodenal protein and bacterial protein after feeding soya-protein, probably due to the bigger contribution of unchanged feed protein, to the duodenal protein after feeding maize-protein.

3.3. The fate of the individual amino acids

The quantitative changes in individual amino acids during the digestive process in the animal are given in the table 4. After feeding maize-protein there is, except in the case of proline, always found a net increase in the duodenal flow, compared to the intake. After feeding soya-protein however, in a number of amino acids, a net increase is found, but in some cases a net decrease was measured. It is suggested that the greater breakdown in the rumen after feeding soya-protein causes the difference in amino acid content in the duodenal fluid after feeding both rations.

After feeding soya-protein a net increase was found for lysine, threonine, glycine, alanine, valine, methionine and tyrosine. Aspartic acid, isoleucine, leucine and phe-

nylalanine appeared in the duodenum in about equal amounts as in the ration. A net decrease was established for histidine, arginine, serine, glutamic acid and proline.

These results agree rather well with that of PFEFFER, KAUFMANN and DIRKSEN (1972), obtained with cows fed with 1—2 kg meadow hay and 6 kg soyabean meal containing concentrates.

The resorption coefficients of the individual amino acids, expressed as percentage of the duodenal flow, are rather high compared to what was found in sheep (COELHO DA SILVA, SEELEY, THOMSON, BEEVER and ARMSTRONG, 1972; COELHO DA SILVA, SEELEY, BEEVER, PRESCOTT and ARMSTRONG, 1972), which were fed with several forms of artificial dried roughage. However, the sheep were fitted with re-entrant cannulae at the beginning and the end of the small intestine and the difference may be the result of amino acid-N disappearing between the end of the small intestine and the rectum. In sheep the disappearance of total-N in caecum and colon varied from 5–20 % of the duodenal flow (COELHO DA SILVA et al., 1972 a, 1972 b). If it is assumed in our cow, that this disappearance was about 10 % of the duodenal flow, then the percentage apparently digested in the small intestine would be 68 %. This agrees rather well with the results in sheep. It is assumed that in the large intestine a microbial fermentation takes place. This is probably interpreted as the breakdown of ileal protein and the synthesis of bacterial protein. Therefore a good agreement in amino acid composition between the faeces after feeding both rations is to be expected. Compared to the situation in the duodenal content there was a closer resemblance in the faeces which is demonstrated in Fig. 3 and Fig. 4.

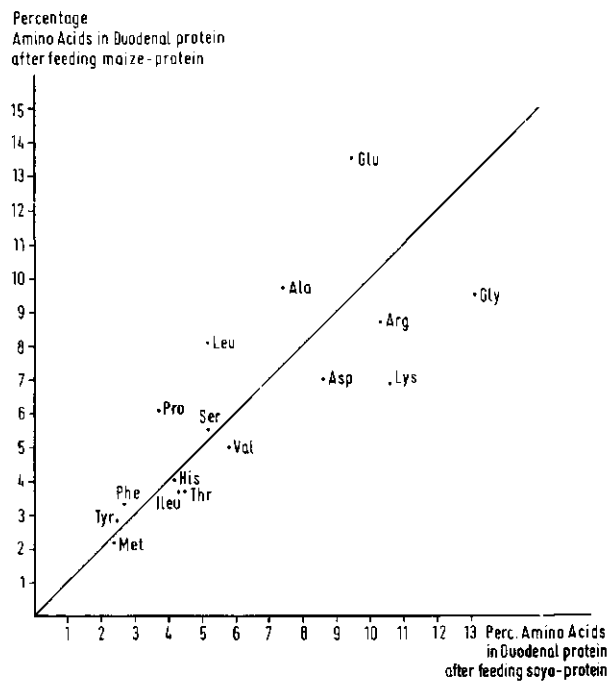

Fig. 3. Association between amino acid composition of duodenal content after feeding maize-protein or soya-protein. Amino acid-N expressed as percentage of total amino acid-N

4. Conclusions

It was demonstrated that the quantity and amino acid composition of the protein which is resorbed from the small intestine of a cow can be influenced by the ration.

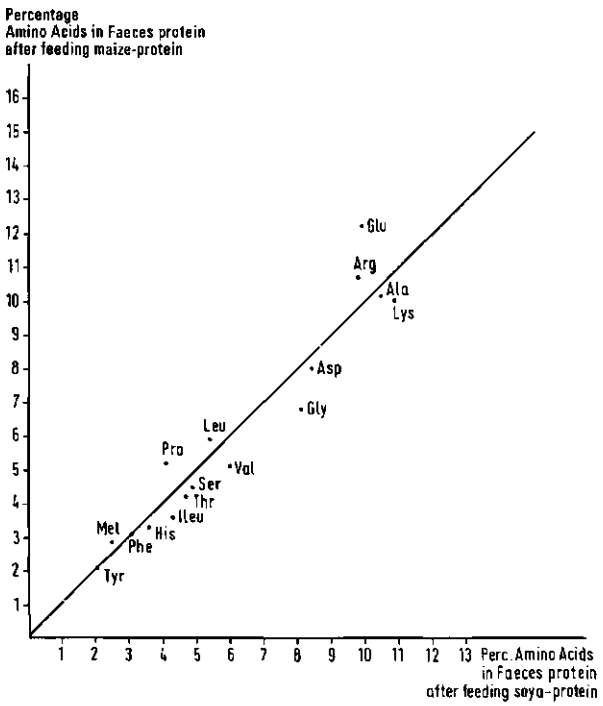

Fig. 4. Association between amino acid composition of faeces of a cow fed with maize-protein or soya-protein. Amino acid-N expressed as percentage of total amino acid-N

In the case of high yielding lactating cows or fast growing beef cattle or lambs this can be of value, since a high demand for protein exists in such animals. This need has to be fullfilled by the quality of protein reaching the intestines. Both are dependant on protein breakdown and protein synthesis in the rumen and attention has to be paid to this. Further research is necessary to clarify the differences existing between different protein sources for high producing and fast growing ruminants.

In spite of a lower apparent N-digestibility for the maize-protein containing ration, the quantity of amino acid-N disappearing from the intestines was higher and the potential protein value is considered to be higher. This means that the real value of different protein sources cannot be measured by digestibility trials, but fistulated animals in which the intestinal flow can be measured will be necessary.

Summary

In a non lactating cow, fitted with a rumen cannula and a re-entrant cannula in the duodenum the quantity and amino acid composition of the protein reaching the duodenum was measured after feeding maize-protein or soya-protein.

After feeding maize-protein the quantity of amino acid-N in the duodenal flow was 150 % of the intake, after feeding soya-protein 104 %. The difference was ascribed to a difference in protein breakdown in the rumen, which was confirmed by the ammonia levels in the rumen fluid. The proportion of microbial protein in the duodenal protein after feeding maize-protein was estimated at 36 %; after feeding soya-protein at 56 %.

A better agreement was found in regard to the amino acid composition between the protein of duodenal content and the protein of mixed rumen bacteria after feeding soya-protein, than after feeding maize-protein.

From the entire intestinal tract 86 % of the amino acid-N reaching the duodenum disappeared after feeding maize-protein. For 16 individual amino acids this varied from 79—90 %. After feeding soya-protein 83 % of the amino acid-N disappeared with a variation for the individual amino acids from 76—89 %.

Zusammenfassung

Der Einfluß der Proteinquelle auf die Proteinverdauung beim Wiederkäuer

Eine nicht laktierende Kuh, versehen mit einer Pansenfistel und einer Umleitungskanüle am Duodenum wurde mit Mais-Eiweiß oder Soja-Eiweiß gefüttert. Anschließend wurden die Menge und die Aminosäurenzusammensetzung des Proteins im Duodenalinhalt untersucht.

Nach Fütterung des Mais-Proteins betrug die Menge des Aminosäuren-N im durchgeflossenen Duodenalinhalt 150 % der Aufnahme, nach Fütterung mit Soja-Protein nur 104 %. Diese Differenz wird einem unterschiedlichen Eiweiß-Abbau im Pansen zugeordnet. Der Anteil an mikrobiellem Eiweiß am Duodenal-Eiweiß nach Fütterung von Soja-Eiweiß auf 56 % und nach Fütterung von Mais-Eiweiß auf 36 % wird geschätzt.

Es zeigte sich eine bessere Übereinstimmung zwischen der Aminosäurenzusammensetzung des Duodenal-Eiweißes und isolierter gemischter Pansenbakterien nach der Fütterung von Soja-Eiweiß als nach der Fütterung von Mais-Eiweiß.

Nach der Verfütterung von Mais-Eiweiß wurde 86 % des Aminosäuren-N im gesamten Intestinaltrakt resorbiert. Die Variationsbreite der Resorptionskoeffizienten für 16 einzelne Aminosäuren lag zwischen 79 bis 90 % nach Mais-Eiweiß-Fütterung. Für die Fütterung mit Soja-Eiweiß lagen diese Zahlen bei 83 % bzw. 76 bis 89 %.

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4. The influence of the method of preservation of forages on the digestion in dairy cows. 1. Composition of the forages and digestibility of dry matter, organic matter and nitrogen

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Summary

Grass silages with formic acid, with a mixture of formic acid and formaldehyde, and without additive were made from the same sward. Another part of the grass was dried artificially and pelleted. The roughages preserved in these four different ways were included in rations for dairy cows, and the chemical composition and digestibilities of the preserved roughages and the rations they were included in, were studied.

Because of conservation, nitrogen solubility decreased in the silage with a mixture of formic acid and formaldehyde, and in the grass pellets, but increased in the silage with formic acid and increased even more in the silage without additive. The apparently digested nitrogen in the different rations showed a tendency to decrease with a decreasing nitrogen solubility, but the differences were not significant.

Good agreement was found between the calculated digestibility of the organic matter in vitro, based on the in vitro digestibilities of the rational components, and on the apparent digestibility determined in vivo for the rations containing silages. The calculated in vitro digestibility of the ration containing grass pellets tended to be rather high.

Introduction

After the growing season, roughage has to be conserved as feed for ruminants. Preservation of green forages as silage often causes considerable protein and energy losses, particularly if it is a high moisture silage. To prevent these losses, additives can be used, such as AIV acid (Virtanen, 1947), formic acid (Saue & Breirem, 1969; Castle & Watson, 1970a, 1970b; Wilson & Wilkins, 1972) and formaldehyde (Waldo et al., 1972; Barry & Fennessy, 1972, 1973; Brown & Valentine, 1972; Valentine & Brown, 1973). It is possible to conserve green forages by drying them, either artificially or in the field. Especially in the field, losses are considerable.

During research on nitrogen metabolism in the ruminant at our Institute, dairy

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Table 1. Chemical composition and digestibilities of artificially dried and pelleted grass and of grass silages.

Factors[1]	Artificially dried grass pellets	Silage with formic acid	Silage with formic acid and formaldehyde	Silage without additive	Fresh grass
DM (%)	86.6	21.5	23.7	17.7	18.5
OM/DM (%)	88.0	89.7	90.3	89.1	88.5
N/DM (%)	2.65	2.49	2.79	2.57	2.59
NH_3-N/DM (%)		0.25	0.07	0.52	
pH		4.1	4.0	5.1	
VFA/DM (%)					
L		4.1	1.0	0.2	
A		1.0	1.5	1.9	
B		—	0.2	1.3	
Soluble N (%)	34	51	40	64	42
NH_3 formation in rumen fluid (% NH_3-N/DM)	0.07	0.59	0.31	0.94	
DOM/DM (vitro) (%)	65.0	59.4	59.4	58.2	

[1] DM = dry matter; OM = organic matter; VFA = volatile fatty acids; L = lactic acid; A = acetic acid; B = butyric acid; DOM = apparently digested organic matter.

decreased due to the artificial drying, remained unchanged in the silage treated with a mixture of formic acid and formaldehyde, but increased in the silage treated with formic acid alone and increased even more in the silage without additive. Thus fermentation losses due to protein breakdown were prevented by artificial drying and by treatment with a mixture of formic acid and formaldehyde. These results are in good agreement with the differences in the amounts of ammonia which were produced in the medium after incubation with rumen fluid.

Intake and digestion of dry matter, organic matter and nitrogen

In Table 2 a survey is given of the mean intake after feeding the different rations and of the apparent digestibilities in the entire ration of dry matter, organic matter and nitrogen, together with the in vitro digestibility of the organic matter. This in vitro digestibility was calculated from the in vitro digestibilities and the intakes of organic matter from the different components of the ration.

Between the different rations no significant differences ($P > 0.05$) were found in the apparent digestibilities of dry matter organic matter, and nitrogen. Except for the ration containing artificially dried and pelleted grass, good agreement was found between the apparent digestibility of the organic matter in the ration and the calculated in vitro digestibility of the organic matter in the same ration. The difference between both digestibilities after feeding the ration containing grass pellets was not significant ($P > 0.05$).

Table 2. Intake and digestibilities of rations containing artificially dried and pelleted grass (rations GP), grass silage with formic acid (ration GSF), grass silage with a mixture of formic acid and formaldehyde (ration GSFF), and grass silage without additive (ration GS), and given to dairy cows.

Factors		Ration GP	Ration GSF	Ration GSFF	Ration GS
Intake (kg/day)	DM[1]	15.2	15.6	15.6	14.6
	OM[1]	13.7	14.1	14.3	13.3
	N	0.38	0.38	0.40	0.36
Apparent digestibility (% of intake)	DM[1]	69.0±0.89	69.7±1.16	70.9±1.04	71.1±0.83
	OM[1]	71.4±0.95	72.6±1.39	73.8±1.09	74.3±0.94
	N	61.1±1.20	62.7±2.16	60.8±1.60	63.5±1.45
Digestibility in vitro (% of intake)	OM[1]	74.8±0.68	72.7±0.49	73.2±0.54	74.2±1.13

[1] DM = dry matter; OM = organic matter.

Discussion

Chemical composition of the silages

From the results of Table 1 it is concluded that the untreated silage is of poor quality and that the addition of chemical agents like formic acid and formaldehyde seems to be a good practice in making high-moisture grass silages.

Addition of formic acid usually lowers the pH in the grass silage to a level which is favourable for bacteria that produce lactic acid and harmful for undesirable silage bacteria (Saue & Breirem, 1969). Formaldehyde, however, is often used as a bactericidal agent. Addition of formaldehyde to freshly cut grass inhibits the fermentation, and therefore the amount of organic acids produced is much lower. Due to the bactericidal effect of formaldehyde, the protein in the grass silage is protected against microbial breakdown during conservation. In general, a chemical bond is formed between formaldehyde and protein and this usually decreases the solubility of the protein. Because this bond is stable at a neutral pH, as found in the rumen, but weak at a low pH, as is found in the abomasum, formaldehyde has been used as a protecting agent against microbial protein breakdown in the rumen (Ferguson et al., 1967). Compared with fresh grass, only a small decrease was found in protein solubility in our experiments, possible because the forage was ensiled without cutting or macerating. The formation of a chemical bond between formaldehyde and the soluble protein in the cytoplasma may have been prevented by the intact cell walls in the long material.

It is also possible to reduce the solubility of protein by heat treatment (Chalmers et al., 1954). Therefore the low protein solubility in the artificially dried and pelleted grass is considered to be the result of a high temperature during drying and during the passage through the die in the press.

Digestion of the rations

To maintain rumination and digestion in the forestomachs, even when grass pellets and concentrates were given, a minimum quantity of 4 kg long meadow hay was included in all rations. The intake of silage was about ad libitum, but to maintain the level of milk production, it was necessary to include a considerable amount of concentrates in the rations. Hence only about 30–35 % of the dry matter intake originated from artificially dried grass pellets or from silage. The remaining 65–70 % of the dry matter intake was the same in all rations, and this of course limits the differences to be expected between the rations. The slightly reduced apparent digestibility of the organic matter in the ration containing grass pellets compared with the in vitro digestibility, has probably to be ascribed to a decreased ruminal fermentation due to an increased flow of digesta from the rumen to the lower parts of the digestive tract. A reduction in the particle size of the ration, as with grass pellets, increases the rate of passage of digesta through the forestomachs of ruminants (Balch, 1961; Thomson, 1972).

No significant differences in apparent nitrogen digestibilities were found between the different rations in our experiments, but, compared with the ration containing grass silage without additive, the apparent nitrogen digestibility seems to be slightly depressed in the rations containing grass pellets or formaldehyde-treated silage.

In literature it was mentioned in some experiments, in which formaldehyde-treated protein was compared with untreated protein, that the apparent nitrogen digestibility was decreased after feeding the treated protein (Coetzee, 1970; Faichney, 1971; Nishimuta et al., 1973). This may be explained by decreased ammonia losses from the rumen due to the protection against microbial breakdown. On the other hand it is possible that the protein was not only protected against microbial breakdown in the rumen, but also against enzymic digestion in the intestines. This problem is further discussed in a subsequent paper (Tamminga, 1975).

The practical application of formaldehyde as a silage additive for high-moisture silages seems to be limited, as a result of the secondary fermentation and mould growth that took place in some experiments after the silo was opened (Barry & Fennessy, 1972; Brown & Valentine, 1972). Addition of formic acid may overcome this problem (Waldo et al., 1972; Brown & Valentine, 1973), but in our experiments this was not sufficient to protect the silage against secondary fermentation. However, the development of this fermentation could be influenced by the slow rate of removing the silage, due to the limited number of experimental animals involved. Under practical circumstances the rate of feeding may be fast enough to prevent the development of this secondary fermentation.

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5. The influence of the method of preservation of forages on the digestion in dairy cows. 2. Digestion of organic matter, energy and amino acids in forestomachs and intestines

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Summary

Two re-entrant cannulated dairy cows were given rations containing artificially dried and pelleted grass (GP), grass silage treated with formic acid (GSF), grass silage treated with a mixture of formic acid and formaldehyde (GSFF) and grass silage without additive (GS). Dry matter intake ranged from 14.8 to 16.0 kg per day and about 30 % of the nitrogen in the rations originated from grass pellets or silages. Between 45 and 57 % of the apparently digested organic matter and between 26 and 41 % of the apparently digested energy disappeared before the intestines. The higher values were found after feeding the rations GSF and GS.

Total amino acid N reaching the duodenum was between 104 and 134 % of the intake, with highest values after feeding ration GP and ration GSFF. The amount of individual amino acids reaching the small intestine ranged from 75 to 270 % of the amounts ingested. High values ($>$ 150 %) were found for glycine, lysine, methionine and tyrosine; low values ($<$ 100 %) were found for arginine, glutamic acid and proline. The amount of amino acids containing sulphur reaching the intestine was between 123 and 159 % of the intake, with lowest values for the ration GS.

Total N disappearing from the intestines was 66.1 $\pm$ 0.50 % of the amount entering the small intestine and 76.8 $\pm$ 2.22 % of the amount ingested. For amino acid N these values were 73.2 $\pm$ 0.91 % and 91.6 $\pm$ 2.01 %, respectively. Between 71 and 107 % of the ingested amount of sulphur-containing amino acids disappeared from the intestines, with the lowest values after feeding ration GS. The different digestion of the rations GP and GSFF, compared with the rations GSF and GS, was ascribed to an increased flow due to the reduced particle size and protein protection by heat treatment for ration GP, and due to protein protection by formaldehyde treatment for ration GSFF.

The possibilities of further research with high-yielding dairy cows and the use of computer modelling techniques in this area are discussed.

Introduction

It is assumed that in the forestomachs of ruminants food protein is broken down to a certain extent, due to the action of the ruminal microbial population. Often this leads to a loss of protein nitrogen from the diet, without contributing to the amino acid supply of the host-animal. In recent years, however, methods have been developed to protect food protein against microbial breakdown in the rumen. Some of these methods are based on influencing physiological mechanisms, like increasing the flow through the stomachs by reducing the particle size of the ration (Balch, 1961), or by-passing the rumen due to stimulation of the oesophageal groove reflex (Ørskov & Frazer, 1969; Ørskov, 1972). Other methods are based on reducing the protein solubility by heat treatment (Chalmers et al., 1954; Tagari et al., 1962), or by treatment with chemical agents such as formaldehyde (Ferguson et al., 1967) or tannins (Zelter & Leroy, 1966; Zelter et al., 1970).

Artificial drying and pelleting of green forage is suggested to reduce the microbial protein breakdown in the rumen (Beever et al., 1969; Coelho da Silva et al., Thomson, Beever and Armstrong, 1972a, 1972b; Thomson, 1972; Harrison et al., 1973; Armstrong, 1973; Hartmann, 1973). In addition, it was shown that casein can be protected against microbial breakdown in the rumen by treatment with formaldehyde in sheep (Ferguson et al., 1967; Offer et al., 1971; Faichney & Weston, 1971; McRae et al., 1972) and in dairy cows (Hagemeister & Pfeffer, 1973). In sheep it appeared also possible to protect forage protein against microbial protein breakdown in the rumen by treatment with formaldehyde (Hemsley et al., 1970).

These results suggested that treatment of the roughage part of the ration of dairy cows might provide some protection against microbial protein breakdown in the forestomachs. Since comparable research with dairy cows is very limited, it was decided to study the influence of heat treatment and reducing the particle size and of treatment with some chemical agents as a way of preserving grass, on the digestion in the dairy cow. Therefore, experiments were carried out in which rations containing artificially dried and pelleted grass (ration GP), grass silage treated with formic acid (ration GSF), grass silage treated with a mixture of formic acid and formaldehyde (ration GSFF), and grass silage without additive (ration GS) were given to re-entrant cannulated dairy cows. The digestion of compounds containing energy and nitrogen in forestomachs and intestines was studied; this paper deals with the results.

Materials and methods

Experimental animals and rations

Four experiments were conducted with two lactating Friesian cows (cows 93 and 94) fitted with a rumen fistula and a re-entrant cannula at the proximal part of the duodenum, just beyond the pancreatic and biliary duct. Surgery was performed within one week after parturition, and the first experiment started after eight weeks. Milk production decreased from 22.0 (cow 93) and 19.3 (cow 94) kg/day to 19.7

and 16.3 kg/day, respectively, and live-weight increased from 520 kg (cow 93) and 560 kg (cow 94) to 535 and 579 kg, respectively, over a period of 20 weeks.

The animals were given rations containing long meadow hay, grass pellets or grass silage, and concentrates. Grass pellets and silages accounted for between 27 and 34 % of the crude protein (CP) in the ration. For more details about the rations see Tamminga & van der Koelen (1975).

Experimental procedure

Each experimental period lasted 5 weeks and consisted of a transition and adaptation period of 30 days and a collection period of 5 days. Twice daily the cows were given equal amounts of feed, at 07h30 and 16h00. Sequence of feeding was long meadow hay (1), concentrates (2) and silage or grass pellets (3).

From at least 10 days before the start to the end of each collection period, 25 g of paper impregnated with chromic oxide was administered twice daily by the rumen fistula.

In the collection period the duodenal flow was measured continuously for 96 hours. During the same time faeces were collected, weighed and sampled daily. Faecal samples were preserved with formalin and stored at 4 °C, and bulked samples were made for each animal.

Collecting and sampling the duodenal digesta

Duodenal content flowed from the proximal part of the re-entrant cannula into a receiving barrel with a capacity of about 10 litres. From this barrel amounts of about 4.5 kg were weighed and a proportional sample of 1.65 % was taken by means of a sampling bucket. The remainder was poured into another barrel from where it was pumped upwards by a peristaltic pump to a funnel which was connected with the distal part of the cannula. For a detailed description of the measuring and sampling procedure and the equipment used, see Tamminga et al. (1973). In this way a proportional sample of the duodenal digesta flowing through the cannula was collected each day. The samples were preserved with toluene and stored at 4 °C. After terminating the 96-hour collection, composite samples were made from the daily samples for each animal.

Laboratory analysis

Silages, hay, grass pellets and concentrates were dried at 60 °C for 24 hours and milled. The air-dry samples were analysed for dry matter (DM), ash, energy, and nitrogen content. Corrections were made for losses of organic acids and ammonia from the silages during drying. Wet samples of faeces were analysed for dry matter (DM) and nitrogen content, and the air-dry faecal samples for DM, ash, energy and chromic oxide content. Composite and daily samples of duodenal content were analysed for DM content by drying at 103 °C to a constant weight. Parts of these samples were freeze-dried. Freeze-dried daily samples were analysed for DM and chromic oxide content, and freeze-dried composite samples were analysed for DM, ash, energy, nitrogen and chromic oxide content. Chromic oxide analyses were done according to the method of Williams et al. (1962).

Amino acid analyses were performed in fresh samples of faeces and silages, in air dry samples of grass pellets, hay and concentrates and in freeze-dried samples of duodenal content. The amino acid compositions were determined by column chromatography on a Carlo Erba amino acid analyser (two-column system).

Results

The digestion of dry matter, organic matter and energy in stomachs and intestines
The duodenal flow and the quantity of faeces produced were corrected for a 100 % recovery of chromic oxide. The mean daily recoveries of chromic oxide in the duodenal flow increased from 83 % during the first day up to 99 % during the fourth day of the collection periods. Recoveries for the entire collection period ranged from 83 to 102 % with a mean recovery in all experimental periods of 93.3 ± 3.57 %. The corrected values were used in all calculations.

The measured daily duodenal flow ranged between 177 and 331 kg per day, with a dry matter content ranging from 3.43 to 4.33 %. Correction of the flow for 100 % recovery of Cr_2O_3 reduced the variation substantially and the corrected daily duodenal flow was calculated to range between 255 and 374 kg per day.

Table 1 shows the mean digestion of DM, organic matter (OM) and energy in stomachs and intestines, after feeding the different rations. Although the results showed a considerable variation between animals, both cows showed the same tendency in digestion of the different rations. When artificially dried and pelleted grass or a silage treated with a mixture of formic acid and formaldehyde are in-

Table 1. Mean intake and digestion of dry matter (DM), organic matter (OM) and energy after feeding two dairy cows rations containing artificially dried and pelleted grass (ration GP), grass silage treated with formic acid (ration GSF), grass silage treated with a mixture of formic acid and formaldehyde (ration GSFF) or grass silage without additive (ration GS).

	Ration GP	Ration GSF	Ration GSFF	Ration GS
Intake				
DM (kg/day)	15.2	15.1	15.7	14.8
OM (kg/day)	13.8	13.7	14.3	13.5
Energy (Mcal/day)	64.3	63.8	68.1	63.1
Apparently digested				
DM (% of intake)	67.5	68.6	69.2	70.7
OM (% of intake)	69.8	71.2	72.1	73.1
Energy (% of intake)	63.7	65.0	66.4	66.8
Disappeared from the stomachs				
DM (% of app. digested)	32.4	38.6	34.2	40.8
OM (% of app. digested)	46.5	52.7	49.6	54.4
Energy (% of app. digested)	28.2	35.1	31.7	37.8

Table 2. Mean intake, duodenal flow and apparent absorption of total N (TN), amino acid N (AAN), essential amino acid N (EAAN), nonessential amino acid N (NEAAN) and sulphur-containing amino acid N (SAAN) after feeding rations containing artificially dried and pelleted grass (ration GP), grass silage treated with formic acid (ration GSF), grass silage treated with a mixture of formic acid and formaldehyde (ration GSFF) and grass silage without additive (ration GS), to two dairy cows.

		Ration GP	Ration GSF	Ration GSFF	Ration GS
Intake (g/day)	TN	397	372	402	370
	AAN	266	267	266	240
	EAAN	130	132	134	119
	NEAAN	137	130	132	121
	SAAN	7.2	7.2	7.2	6.9
Duodenal content (% of intake)	TN	116	112	125	114
	AAN	128	114	130	120
	EAAN	131	110	125	113
	NEAAN	125	117	135	126
	SAAN	150	138	151	128
Apparently absorbed from the intestines (% of duodenal flow)	TN	66	65	67	66
	AAN	77	73	69	72
	EAAN	77	72	68	70
	NEAAN	76	74	70	74
	SAAN	66	64	62	60
Apparently absorbed from the intestines (% of intake)	TN	76	74	84	76
	AAN	98	82	90	86
	EAAN	100	79	86	78
	NEAAN	96	86	94	94
	SAAN	96	88	95	76
Apparently digested (% of intake)	TN	60	61	58	62

cluded in the ration, the digestion of organic matter and energy seems to move from the stomachs towards the intestines.

The fate of amino acids in stomachs and intestines

In Table 2 a survey is given of mean total nitrogen (TN), amino acid N (AAN), essential amino acid N (EAAN), non-essential amino acid N (NEAAN) and sulphur-containing amino acid N (SAAN) in intake and duodenal flow. In the same table the mean quantities that disappeared from the intestines are given as a percentage of the quantity entering the duodenum and as a percentage of the intake. From these results it is concluded that amino acid N is absorbed from the intestine to a larger extent than non amino acid N.

Table 3 shows the intake of the individual amino acids and only minor differences were found between the different rations. The amounts of individual amino

Table 3. The mean intake of total amino acid N (AAN) (g/day) and the mean amino acid composition of the intake (individual amino acid N as a percentage of total amino acid N), after feeding rations containing artificially dried and pelleted grass (ration GP), grass silage treated with formic acid (ration GSF), grass silage treated with a mixture of formic acid and formaldehyde (ration GSFF) and grass silage without additive (ration GS) to 2 dairy cows.

	Ration GP	Ration GSF	Ration GSFF	Ration GS
AAN intake (g/day)	266	267	266	240
Lysine	6.7	7.4	7.1	7.0
Histidine	4.2	4.7	4.4	4.3
Arginine	14.2	14.6	14.8	14.0
Threonine	3.8	3.8	3.9	3.5
Valine	5.4	5.1	5.3	5.4
Methionine	1.2	1.3	1.2	1.3
Isoleucine	3.4	3.5	3.5	3.6
Leucine	6.8	6.8	6.9	7.1
Phenylalanine	3.0	3.3	3.3	3.4
Essential AAN (% of total AAN)	*48.6*	*50.4*	*50.4*	*49.6*
Aspartic acid	8.6	7.6	8.2	7.3
Serine	5.0	5.0	5.0	4.8
Glutamic acid	13.2	12.2	12.2	12.3
Proline	6.4	6.2	6.1	6.2
Glycine	7.6	7.6	7.4	7.8
Alanine	7.4	8.0	7.7	8.8
Tyrosine	1.6	1.8	1.6	1.7
Cystine + cysteïne	1.6	1.4	1.4	1.5
Non-essential AAN (% of total AAN)	*51.4*	*49.6*	*49.6*	*50.4*

acid N entering the duodenum were compared with the intakes, and the results are given in Table 4. For all rations a substantial increase was found for lysine, threonine, methionine, glycine and tyrosine. Smaller increases were found for valine, isoleucine, leucine, phenylalanine, aspartic acid, serine, alanine and cystine + cysteïne. Arginine and histidine remained about the same and decreases were found for glutamic acid and proline. Only sometimes could these differences be ascribed to the ration. Higher increases in quantities of lysine, histidine and arginine were found after feeding the ration containing grass pellets. The amount of methionine seems to be less increased after feeding the ration containing grass silage without additive.

The mean apparent absorptions of the individual amino acids from the intestines are shown in Tables 5 and 6. Table 5 shows the mean apparent absorptions as a percentage of the amount present at the beginning of the small intestine, which were calculated as follows:

apparent absorption = 100 (duodenal AAN – faecal AAN)/duodenal AAN.

Only minor differences were found between the different rations. Sometimes no tyrosine could be detected in the faecal samples, so that apparent absorption was

Table 4. The mean amount of amino acid N, flowing through the duodenum of two dairy cows, given as a percentage of the amount ingested, after feeding rations containing artificially dried and pelleted grass (ration GP), grass silage treated with formic acid (rations GSF), grass silage treated with a mixture of formic acid and formaldehyde (ration GSFF), and grass silage without additive (ration GS).

	Ration GP	Ration GSF	Ration GSFF	Ration GS
Lysine	188	152	176	158
Histidine	121	92	112	102
Arginine	104	84	96	90
Threonine	138	125	148	146
Valine	125	116	123	115
Methionine	191	168	183	140
Isoleucine	128	114	138	114
Leucine	132	109	116	101
Phenylalanine	124	104	116	101
Aspartic acid	118	119	131	136
Serine	122	107	123	116
Glutamic acid	86	82	96	88
Proline	81	74	85	78
Glycine	226	216	257	242
Alanine	128	105	121	100
Tyrosine	290	157	192	177
Cystine + cysteïne	218	108	124	114

100 %. The apparent absorption of the amino acids from the intestines, after feeding silage treated with a mixture of formic acid and formaldehyde, showed a tendency of being slightly reduced. Differences in the apparent absorption were higher for some individual amino acids. Basic amino acids seem to be absorbed slightly better than the remaining essential amino acids. Of the non-essential amino acids glycine and tyrosine showed the highest apparent absorption. Relatively low apparent absorptions were found for the sulphur-containing amino acids and alanine.

In Table 6, a comparison is made between the mean amount of individual amino acids apparently absorbed from the intestines and the amounts ingested, which were calculated as follows:

absorption = 100 (duodenal AAN – faecal AAN)/ingested AAN.

The results show striking differences between the individual amino acids. The differences between the different rations are less pronounced, partly due to the large variation between cows given the same rations. Compared with the other rations, the amounts of sulphur-containing amino acids, after feeding the ration containing silage without additive, were absorbed in smaller quantities.

Table 5. The apparent absorption of amino acids from the intestines of two dairy cows (expressed as a percentage of the amount entering the duodenum), fed with rations containing artificially dried and pelleted grass (ration GP), grass silage treated with formic acid (ration GSF), grass silage treated with a mixture of formic acid and formaldehyde (ration GSFF), and grass silage without additive.

	Ration GP	Ration GSF	Ration GSFF	Ration GS	Mean
Lysine	80	75	72	72	74.7 ± 1.41
Histidine	82	80	76	74	77.7 ± 1.59
Arginine	81	74	75	72	75.6 ± 1.24
Threonine	71	66	64	67	67.0 ± 1.22
Valine	73	68	61	67	67.3 ± 1.81
Methionine	68	66	63	62	64.7 ± 1.55
Isoleucine	72	67	64	68	67.3 ± 1.33
Leucine	76	71	67	68	70.2 + 1.61
Phenylalanine	70	69	63	68	67.5 ± 1.17
Aspartic acid	74	68	64	70	69.1 ± 1.19
Serine	76	72	68	70	71.2 ± 1.33
Glutamic acid	74	69	65	70	69.5 ± 1.17
Proline	71	70	68	71	69.8 ± 0.77
Glycine	84	82	80	84	82.7 ± 0.67
Alanine	70	66	58	64	64.2 ± 1.67
Tyrosine	93	94	79	98	90.9 ± 3.21
Cystine + cysteïne	64	62	62	56	61.2 ± 1.47

Discussion

Digestion of organic matter and energy in stomachs and intestines

In the experiments reported here the portion of the apparently digested organic matter and energy disappearing before the intestine is low compared with most of the results in cows (van 't Klooster & Rogers, 1969; Pfeffer et al., 1972; Watson et al., 1972; Tamminga, 1973). So far results were obtained in cows with a daily intake of between 6 and 10 kg DM, which is considered to be a low or a medium level of intake for the dairy cow. In the experiments reported here DM intakes ranged between 14 and 16 kg/day. Increases in the feeding level may increase the flow of digesta through the stomachs (Balch, 1961), and thereby reduce the portion of the digestion of OM in this part of the digestive tract. Moreover about half of the DM in all rations consisted of ground and pelleted concentrates. Changes in the physical form of forage diets, by reducing the particle size, resulted in sheep in a shift of the digestion of OM and energy from the stomachs towards the intestines (Thomson, 1972). Reduction of the portion of roughage in the ration of dairy cows, provided with rations consisting of roughage and concentrates, had the same effect (Kaufmann & Hagemeister, 1973).

Compared with the other rations, the portion of the organic matter and the energy disappearing before the intestines is reduced after feeding a ration con-

Table 6. The mean apparent absorption of amino acids from the intestines of two dairy cows (expressed as a percentage of the intake), fed with rations containing artificially dried and pelleted grass (ration GP), grass silage treated with formic acid (ration GSF), grass silage treated with a mixture of formic acid and formaldehyde (ration GSFF), and grass silage without additive.

	Ration GP	Ration GSF	Ration GSFF	Ration GS
Lysine	150	116	126	114
Histidine	99	74	184	76
Arginine	84	62	72	64
Threonine	98	82	94	98
Valine	91	80	75	76
Methionine	130	110	116	88
Isoleucine	92	77	89	76
Leucine	100	73	77	68
Phenylalanine	88	72	73	68
Aspartic acid	86	82	85	95
Serine	93	77	83	80
Glutamic acid	64	57	62	62
Proline	58	52	58	56
Glycine	190	184	206	204
Alanine	88	68	70	64
Tyrosine	175	147	152	173
Cystine + cysteïne	76	68	77	64

taining artificially dried and pelleted grass (Table 1). In this ration the portion of long roughage was further reduced by replacing silage with artificially dried and pelleted grass. These results are in agreement with the results of other research workers as is discussed above. Feeding a ration containing grass silage treated with a mixture of formic acid and formaldehyde also seems to reduce the OM digestion in the stomachs compared with the other rations containing silage. This decrease is thought to be the result of the protein-protecting properties of formaldehyde. The same was found in dairy cows after feeding formaldehyde-treated casein (Hagemeister & Pfeffer, 1973).

If it is assumed that the OM disappearing from the forestomachs consists entirely of carbohydrates, the percentages of energy expected to disappear from the forestomachs can be calculated. It appeared, however, that only 60.4 ± 1.80 % of these calculated values were in fact disappearing between the intake and the reentrant cannula. This difference can at least partly be explained by the input of endogenous energy containing substances in digestive enzymes and bile, which have a higher energy content than carbohydrates. However, the possible contribution of the microbial fermentation in the forestomachs should not be overlooked. Due to the microbial fermentation in the forestomachs of the ruminant, carbohydrates and amino acids are broken down into volatile fatty acids, methane, carbon dioxide, water and heat. Volatile fatty acids are absorbed, mainly from the

stomachs and due to the disappearance of carbohydrates, the remaining organic matter contains higher percentages of protein and lipids. The energy content of protein and of lipids exceeds the energy content of carbohydrates, and this partly explains the difference between the energy content of the OM in the ration and the OM in duodenal digesta. The anaerobic fermentation in the rumen means the generation of a limited amount of energy as adenosine triphosphate (ATP), which subsequently can be used in microbial synthesis. Due to the absence of oxidative phosphorylation in anaerobic micro-organisms the anaerobic generation of ATP is coupled with the formation of 'metabolic hydrogen', such as reduced co-factors and phosphoroclastic hydrogen. Several possibilities have been suggested for the way the micro-organisms in the rumen get rid of their surplus of 'metabolic hydrogen', like reducing carbon dioxide to methane, biohydrogenation of unsaturated long-chain fatty acids (Dawson & Kemp, 1970), changing the fermentation pattern towards propionic acid (Demeyer et al., 1972; Czerkawski, 1973a), synthesis of palmitate from acetate (Czerkawski, 1973a) and probably the formation of water from oxygen and hydrogen (Czerkawski, 1972). It has been suggested that phosphoroclastic hydrogen is used te reduce carbon dioxide to methane (Demeyer et al., 1970; Wolfe, 1971).

Reduced co-factors, however, are suggested to be used at least partly in the microbial synthesizing processes (Demeyer et al., 1972). Synthesis of microbial material from carbohydrates means a net uptake of hydrogen (Demeyer et al., 1972), since proteins as well as lipids are CHO-containing organic compounds which are more reduced than carbohydrates. It is suggested therefore that, as a result of the microbial action in the rumen, a net synthesis of protein and lipids takes place, also causing an increased energy content of the OM in duodenal digesta, compared with the energy content of the OM in the ration.

Amino acid supply in the intestines

In sheep given artificially dried and chopped forages, the amount of non-ammonia nitrogen (NAN) reaching the duodenum was calculated to exceed the nitrogen intake, if the ingested nitrogen was less than 4 % of the apparently digested organic matter (DOM) (Hogan & Weston, 1970). This was also found to be true in dairy cows, given different rations with a varying nitrogen content (van 't Klooster & Boekholt, 1972). The amounts of nitrogen ingested with the rations of the experiments reported here, ranged from 3.7 to 4.2 % of the DOM; so sometimes the amount of NAN reaching the small intestine was expected to be less than the amount of nitrogen ingested. However, the amount of NAN reaching the duodenum always ranged between 100 and 123 % of it the nitrogen intake. Most divergence was found after feeding the rations containing grass pellets and grass silage treated with a mixture of formic acid and formaldehyde. In sheep it was also found that after feeding processed rations the amount of NAN entering the duodenum was larger than was expected from the nitrogen content and the amount of DOM in the ration (Coelho da Silva et al., 1972a, 1972b). It is suggested, therefore, that the relationship mentioned before may be influenced by grinding, pelleting or treating the ration with the chemical agents.

Apparent absorption of amino acids from the intestines

The findings in sheep showing a larger apparent absorption of essential amino acids from the small intestine compared with non-essential amino acids (Coelho da Silva et al., 1972a, 1972b), which was also found in cows (van 't Klooster & Boekholt, 1972), do not seem to be valid for the entire intestine in dairy cows, as can be seen from the results in Table 2.

Earlier results, indicating that the percentage of amino acids entering the small intestine of cows that is apparently absorbed ranges between narrow limits (van 't Klooster & Boekholt, 1972; Hagemeister & Pfeffer, 1973), were confirmed in our experiments. The results agreed fairly well with the findings of Hagemeister & Pfeffer (1973). In sheep about the same percentage was found to disappear from the small intestine only (Coelho da Silva et al., 1972a; 1972b; Harrison et al., 1973), and an additional disappearance of 5-15 % from the large intestine was shown (Coelho da Silva et al., 1972a; 1972b). Results on the disappearance of amino acid N from the large intestine of dairy cows are not available yet, but nitrogen losses of between 5 and 10 % of the nitrogen intake have been reported (van 't Klooster & Rogers, 1969). If it is assumed that the losses of amino acid N from the large intestine of dairy cows do not differ much from the losses found in sheep, the apparent absorption of amino acids from the small intestine of dairy cows is about 10 % less than the apparent absorption of amino acids from the small intestine of sheep. This difference has probably to be ascribed to the difference in relative length of the small intestine. In small ruminants like sheep and goats, the length of the small intestine is about 22 times the body length, in larger ruminants about 16 times (Hill, 1970).

The slightly reduced apparent absorption of amino acids from the intestines after feeding formaldehyde-treated silage (ration GSFF), is in agreement with the findings in cows given formaldehyde-treated casein (Hagemeister & Pfeffer, 1973) and also with the reduced apparently digested nitrogen found in sheep several times after feeding formaldehyde-treated protein (Coetzee, 1971; Faichney, 1971; Nishimuta et al., 1973).

Special attention has been paid to the supply of sulphur-containing amino acids from fresh and conserved forages. The suggestion that the supply of sulphur-containing amino acids in ruminants may be deficient after feeding grass products which are not conserved in the right way (Armstrong, 1973), seems to be confirmed by the results reported here, as is shown in Table 2.

Possibilities of digestion studies in high yielding dairy cows

During the last decades many experimental results about the influence of rumen fermention on the digestive processes in the ruminant have become available. The research about this subject in the high-yielding dairy cow, however, is very limited. From a nutritional point of view, however, research about sites of digestion and the influence of rumen fermentation on digestive processes in the high-yielding dairy cow are urgently needed. One of the possibilities of increasing the productivity in animal husbandry is stimulating the production per animal. Milk yields of 6000 kg and more are therefore expected to be normal in the future. These levels

of production can only be achieved if a balanced ration is supplied, with a high level of intake, and if concentrates based on grains, grain by-products and vegetable proteins are fed. Grains as well as protein are expected to become scarce in the future, due to the increasing demand for human consumption. So, more attention will have to be paid to the efficiency with which the food is used, and that needs a better understanding of the digestive processes in stomachs and intestines in high-yielding cows.

Unfortunately, it is far from easy to perform complicated nutritional experiments in such animals for various reasons. In this type of research the experimental animals usually have to be prepared surgically which often reduces their high production. Maintaining a high production is only possible if mixed rations of roughage and (rather high amounts) of concentrates are provided. This limits the possible differences between the different experimental rations and also the possible influence on ruminal fermentation. The variation between animals appears to be considerable, so that the results could be easily misunderstood. Because of the substantial amount of labour involved in this type of research, the number of experiments and the number of animals in each experiment has to be limited. For these reasons experimental results about the digestive processes in high-yielding dairy cows are expected to remain rather rare, and progress in this field will be made slowly.

To gain a better understanding of the digestive processes in these animals, simulation techniques may be very helpful in estimating the influence of alterations of the various physiological mechanisms and biochemical pathways within the digestive tract on the digestive processes. One should realize, however, that in doing so, the accuracy and reliability of the results of simulation techniques depend on the accuracy of the data used in the computer programme. Therefore, the results will always have to be controlled by physiological and nutritional experiments.

Attempts were made to simulate the ruminal fermentation processes (Baldwin et al., 1970) and to simulate the rate of passage of digesta in sheep (Grovum & Phillips, 1973). A more predicting model of ruminal fermentation was suggested by Czerkawski (1973). In the same area it is possible to make forecasts about protein digestion and amino acid supply in the dairy cow. Recently, attempts to do so have been made (Kaufmann & Hagemeister, 1973; Van Es & Boekholt, 1974). It was concluded from their results that more research is needed about the influence of various biochemical transactions and physiological mechanisms in the ruminant on its digestive processes, such as the influence of the physical form of the ration and the level of feeding on the rate of passage of digesta and the rate and proportion of ruminal fermentation, the efficiency of microbial protein synthesis in the rumen and the extent of ruminal breakdown of food protein in the forestomachs.

With a better understanding of these problems it should be possible to improve the simulation in order to trace the most important parameters determining the digestive processes in the high-yielding dairy cow and to concentrate research on those particular topics.

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6. EFFECT OF THE ROUGHAGE/CONCENTRATE RATIO ON NITROGEN ENTERING THE SMALL INTESTINE OF DAIRY COWS

SUMMARY

Experiments were conducted with dairy cows in which the effect of a varying roughage/concentrate ratio on N entering the small intestine was studied at various levels of feed intake. Experimental animals were equipped with a rumen fistula and re-entrant cannulae at the beginning of the small intestine. In three series a total of 10 treatments were applied with 2 to 5 animals per treatment. Dry matter intake ranged between 3.8 and 15.7 kg per day; the proportion of long roughage in the diet varied from 0.29 to 0.81.

The apparent digestibility of organic matter (O), crude fibre (XF) and N-free extractives (XX) were 0.76 ± 0.007; 0.69 ± 0.027 and 0.81 ± 0.012 respectively. The proportion of the digestion taking place in the stomachs was 0.59 ± 0.008; 0.94 ± 0.012 and 0.76 ± 0.007 for O; XF and XX respectively. A tendency was found that a larger proportion of the apparently digestible organic matter was digested in the stomachs with a larger proportion of long roughage in the diet.

The contribution of microbial N to the intestinal N was estimated, using DAPA as a microbial marker. From this the efficiency of microbial protein synthesis was estimated and related to the amount of carbohydrates (XF + XX) fermented in the stomachs. Per kg of carbohydrates fermented 32 ± 1.5 g microbial N was produced. The percentage of dietary N not degraded in the stomachs was also estimated and averaged 30 ± 1.5.

Varying the ratio between long roughage and pelleted concentrates appeared to have little effect on the degradation of dietary protein or on the efficiency of microbial protein synthesis. Hence with a proportion of long roughage in the diet between 0.29 and 0.81 there seems to be little effect of varying the roughage/concentrate ratio on the protein supply in dairy cows.

INTRODUCTION

Lactating dairy cows are generally fed with a basal diet of long roughage (meadow hay, grass silage) supplemented with ground and often pelleted concentrates, the amount of which varies with the level of production. At high levels of production it may be necessary to replace part of the roughage by concentrates in order to maximize energy intake. This increase in energy intake is achieved because more dry matter can be ingested from ground feeds (Van der Honing, 1975) and because in general the energy density of concentrates is higher than of roughages.

Changing the ratio between long roughage and ground and pelleted concentrates in the diet of dairy cows may change rumen fermentation, including degradation and synthesis of protein, in various ways. Degradation of dietary ingredients depends on the rate of degradation and on the rate of passage of that ingredient through the forestomachs. Rate of passage differs between long hay particles and ground and pelleted concentrates (Hartnell & Satter, 1979; Uden, 1981). Rate of passage of particulate matter is higher with ground and pelleted concentrates. The rate of degradation may also differ. Rate of protein degradation is lower with high concentrate diets (Ganev et al., 1979). Rate of carbohydrate degradation on the other hand is higher with concentrate rich diets than with roughage rich diets, which probably influences microbial protein synthesis (Tamminga, 1979).

In a number of experiments it was therefore investigated if the duodenal protein supply in dairy cows was affected by changes in the ratio between long roughage and ground and pelleted concentrates.

MATERIALS AND METHODS

For the experiments a total of 7 dairy cows were used. All animals were fitted with a rumen fistula according to the method of Van 't Klooster & Rogers (1970). Polythene tubing with an internal diameter of 37 mm, normally applied as waste pipe of a wash bowel (vulcathene, Eriks, Alkmaar) was used for this purpose. The animals were also fitted with a re-entrant cannula in the beginning of the small intestine, just beyond the pancreatic and biliary duct. Cannulae were of the same type as those used by Ash (1962) in sheep, except that they were made of polyvinylchloride and had an internal diameter of 19 mm. Cannulation was performed according to the method described by

Hogan & Phillipson (1960) in sheep.

The animals were fed with various amounts of long meadow hay and various amounts of ground and pelleted concentrates. No specific composition was requested for the concentrates, they were bought from a local feed manufacturer.

Three series of experiments were performed. The first series consisted of 3 treatments with 3 animals per treatment. In the second series again 3 experiments were done, but only 2 animals per treatment were available. In the third series of experiments, the animals were fed depending on their stage of lactation, resulting in 11 experiments which were grouped in 4 groups of experiments. A summary of the experiments is given in Table 1.

Table 1. Animals and experimental treatments.

	diet offered		
treatment	kg hay	kg concentrates	animals
I-A	4	10	66, 78, 81
I-B	6	9	66, 78, 93
I-C1	8	8	66, 78
I-C2	6	6	93
II-A	3	7	35, 78
II-B	6	4.5	35, 78
II-C	9	2	35, 78
III-A1	6.5	12	93
III-A2	8.5	10	93
III-B1	10	2	78
III-B2	8	3	2R
III-B3	8	1	128
III-B4	7	1	35
III-B5	6	2	93
III-C1	3	4	78
III-C2	2.5	3.5	93
III-D1	4	2	93
III-D2	3	0	35

MEASUREMENTS

Measuring and sampling feed intake, duodenal flow and faecal output

Of each part of the diet (roughage, concentrates) a sample was prepared, while weighing the daily portion for an entire collection period, before the collection of duodenal digesta and faeces started. Feed refusals were collected during an entire measuring period and sampled at the end of each period.

The measuring and sampling procedure of duodenal flow was described in detail by Tamminga et al. (1973). At the start of an experiment the re-

entrant cannulae were disconnected. The proximal cannulae was fitted to a polyethylene tube, through which the digesta flowed into a container which contents were kept at a constant temperature of 38°C. From this container amounts of approximately 4.5 litres were removed and transferred to a sampling bucket. The contents of this bucket were weighed in grammes, and a proportional sample of 1.65% was taken. The remainder of the digesta was transferred to a second container from where it was gradually pumped upwards by means of a peristaltic pump into a funnel, which was connected with the distal cannula. For each period of 24 hours the small samples were bulked, cooled immediately to 8-10 °C and kept at that temperature. Preservation of the samples was done with 10 ml of toluene. At the end of each 24 hour period the samples were split in daily samples and composite samples, frozen and kept at -20 °C until freeze-drying or further analysis. Measuring and sampling of the duodenal flow was continued for a period of 96 hours.

During the same period faeces were also collected, immediately after being dropped, thus avoiding contamination with urine. This procedure was chosen because wearing harnesses for a separate collection of faeces and urine was considered less appropriate for such surgically modified animals. The collected faeces were weighed daily and proportional daily samples were bulked. The samples were kept at 4 °C until the end of the collection period and were then prepared further.

Isolation of mixed rumen bacteria

Mixed rumen bacteria were isolated according to the method of Meyer et al. (1967). Approximately 1 litre of ruminal fluid was collected by sucktion, using a 100 ml syringe fitted with a polythene tube which was placed in a stainless steel rod of which the lower end was perforated. The rod was placed in the rumen through the rumen fistula. Rumen fluid was cooled immediately by placing it in ice, and subsequently centrifuged at 550xg to remove feed particles and protozoa. The supernatant was subsequently centrifuged at 70 000xg. The resulting supernatant was discarded and the residue taken up in buffer, homogenised in a Potter-Elvehjem homogeniser and recentrifuged. This procedure was repeated and finally the suspension was frozen and kept at -20 °C until freeze-drying.

Preparation of samples and chemical analysis

Fresh samples of faeces and duodenal content were analysed for their dry matter content and N content. Otherwise samples were dried at 60 °C for 24 hours and ground in a hammermill through a sieve of 1 mm and this material was used for further analysis. The bulked samples of duodenal digesta were freeze-dried and also ground.

Most analytical procedures were according to the instructions of the Netherlands Normalisation Institute (NEN-instructions).

Dry matter in airdry samples was determined by drying a small sample (approx. 3 g) for a period of 4 hours at a temperature of between 103 and 105 °C (NEN 3332). Dry matter in wet samples (faeces and duodenal digesta) was determined by drying a small sample (10 to 15 g for faeces and 20 to 30 g for duodenal digesta) for a period of 15 hours at a temperature of between 103 and 105 °C. The ash content of airdry samples was determined by ashing a small sample (approx. 3 g) in a furnace at 550 °C for a period of 3.5 hours (NEN 3329). Crude protein (N x 6.25) in part of the samples was determined according to the Kjeldahl method (NEN 3145). In the remaining samples crude protein was determined according to the Dumas method, using an automatic N analyser (Mikro-rapid-N, Heraeus, W-Germany). Crude fibre in airdry samples was determined by boiling a small sample (approx. 3 g) with an aquous solution of 1.25% H_2SO_4 for 30 minutes, followed by boiling the residue with an aquous solution of 1.25% NaOH for 30 minutes. The residue was washed with acetone, dried at 140 °C for 2 hours and ashed in a furnace at 550 °C for 1 hour. The difference in weight after drying and ashing is a measure for the crude fibre content (NEN 3326). Crude fat in airdry samples was determined by extracting a small sample (approx. 3 g) in a Soxhlet apparatus with peroxide free diethyl-ether for a period of 16 hours (NEN 3148). Diaminopimelic acid was determined according to the method of Mason & Anderson (1976) in which hydrolysis by boiling the sample under reflux for 22 hours with 400 ml of 6N HCl is preceded by oxidation with performic acid.

RESULTS

The composition of the hay and concentrates fed in the various experiments is shown in Table 2.

In Table 3 the digestion results of Experiment I are shown for dry matter (T), organic matter (O), crude protein (XP), crude fibre (XF) and NfE (XX)

Table 2. Chemical composition of hay and concentrates fed in the different experiments.

exp.	feed	%T	O/T	XP/T	XF/T	XX/T
I	hay	83.2	0.900	0.177	0.303	0.396
	conc.	85.7	0.919	0.193	0.072	0.613
II	hay	82.5	0.915	0.131	0.302	0.462
	conc.	86.2	0.933	0.190	0.070	0.631
III-A	hay	82.3	0.914	0.128	0.301	0.464
	conc.	85.9	0.931	0.193	0.053	0.628
III-B	hay	85.2	0.900	0.130	0.291	0.453
	conc.	86.0	0.916	0.168	0.084	0.627
III-C	hay	84.8	0.912	0.145	0.335	0.410
	conc.	86.0	0.930	0.195	0.090	0.601
III-D	hay	85.1	0.902	0.154	0.294	0.427
	conc.	85.3	0.924	0.204	0.082	0.595

Table 3. Intake (I_X), total apparent digestibility (d_X) and apparent digestibility in the forestomachs, as a percentage of d_X ($d_{X,S}$) in Experiment 1.

	treatment		
	I-A	I-B	I-C
number of animals	3	3	3
DM from roughage (%)	29±0.5	40±0.2	48±1.3
XP from roughage (%)	27±0.4	27±0.7	46±0.5
I_T (kg/day)	11.8±0.18	12.3±0.51	12.1±0.96
I_O "	10.8±0.16	11.2±0.44	11.0±0.88
I_{XP} "	2.20±0.04	2.29±0.14	2.23±0.15
I_{XF} "	1.67±0.04	2.06±0.11	2.26±0.14
I_{XX} "	6.45±0.18	6.45±0.18	6.15±0.56
d_T (%)	74±2.2	75±1.1	76±0.9
d_O "	76±2.2	77±1.1	78±1.0
d_{XP} "	70±4.6	70±0.3	71±1.5
d_{XF} "	62±4.1	69±2.3	72±2.0
d_{XX} "	82±1.4	83±1.3	83±0.7
$d_{T,S}$ "	44±1.8	44±3.4	49±2.8
$d_{O,S}$ "	57±1.7	57±2.8	62±2.8
$d_{XP,S}$ "	3±5.5	1±2.8	10±7.4
$d_{XF,S}$ "	90±0.8	88±6.8	96±2.4
$d_{XX,S}$ "	77±3.9	74±2.0	74±3.5

in terms of intake (I_X), apparently digested (d_X) and apparently digested in the stomachs ($d_{X,S}$), the latter as percentage of d_X.

The proportion of duodenal N of bacterial origin was estimated using DAPA as a marker. Since carbohydrates are by far the most important energy donating substrate for rumen microorganisms and since hardly any interfering endogenous carbohydrates are involved, the energetic efficiency of microbial protein synthesis was related to the amount of carbohydrates (XF + XX) disappeared between intake and duodenal flow (Tamminga, 1978; Chapter 2). The results are shown in Table 4. Duodenal flow was corrected for NH_3-N; of the remaining NAN, 15% was considered to be of endogenous origin (Kaufmann & Hagemeister, 1976). This corrected duodenal N-flow was considered as the total of microbial N and undegraded feed-N. The undegraded feed-N was calculated as proportion of the N ingested and the results are also shown in Table 4.

Table 4. Efficiency of microbial protein synthesis and extent of degradation of feed-N in Experiment I.

	treatment		
	I-A	I-B	I-C
(XF + XX) disappeared (kg/day)	5.1±0.19	5.3±0.30	5.5±0.46
microbial N in duodenal content (g/day)	176±3.2	198±10.4	150±19.5
micr. N/(XF + XX) ferm. (g/kg)	34±1.4	38±4.3	28±5.0
undegraded feed N (% of I_N)	28±7.2	24±2.9	29±9.4

In Table 5 the digestion results of Experiment II are shown for dry matter (T), organic matter (O), crude protein (XP), crude fibre (XF) and NfE (XX) in terms of intake (I_X), apparently digested (d_X) and apparently digested in the stomachs ($d_{X,S}$).

Similar as in Experiment I, the energetic efficiency of the synthesis of bacterial N was calculated. The results are shown in Table 6. The results of the estimated proportions of feed N escaping degradation in the rumen are also shown in Table 6.

Table 5. Intake (I_X), total apparent digestibility (d_X) and apparent digestibility in the forestomachs, as a percentage of d_X ($d_{X,S}$) in Experiment II.

	treatment		
	II-A	II-B	II-C
number of animals	2	2	2
DM from roughage (%)	29±0.2	56±0.1	81±0.1
XP from roughage (%)	21±0.4	48±1.0	75±1.3
I_T (kg/day)	8.5±0.01	8.8±0.04	9.2±0.02
I_O "	7.9±0.01	8.2±0.06	8.5±0.03
I_{XP} "	1.48±0.02	1.41±0.01	1.30±0.05
I_{XF} "	1.19±0.02	1.82±0.02	2.40±0.03
I_{XX} "	4.92±0.03	4.71±0.04	4.55±0.05
d_T (%)	73±2.2	74±0.6	70±1.2
d_O "	75±2.1	76±0.5	72±0.8
d_{XP} "	68±2.6	66±0.2	63±1.4
d_{XF} "	51±2.0	70±0.7	71±0.7
d_{XX} "	83±2.3	83±0.6	72±0.8
$d_{T,S}$ "	42±4.7	44±1.7	42±0.8
$d_{O,S}$ "	55±3.0	58±1.4	61±0.3
$d_{XP,S}$ "	-7±12.3	-15±2.1	-32±1.6
$d_{XF,S}$ "	97±0.2	90±1.6	99±1.4
$d_{XX,S}$ "	73±1.8	75±2.7	79±3.7

Table 6. Efficiency of microbial protein synthesis and extent of degradation of feed N in Experiment II.

	treatment		
	II-A	II-B	II-C
(XF + XX) disappeared (kg/day)	3.6±0.26	4.0±0.13	4.1±0.01
microbial N in duodenal content (g/day)	139±6.5	119±10.4	116±20.0
micr. N/(XF + XX) ferm. (g/kg)	38±1.0	30±3.6	28±5.0
undegraded feed N (% of I_N)	24±10.3	33±6.4	39±7.8

The experiments in Series III were grouped in 4 treatments with 1 to 5 animals per treatment. Treatments were a low roughage to concentrate ratio at a high level of feed intake (treatments A1 and A2), a high roughage to concentrate ratio at a medium level of feed intake (treatments B1, B2, B3, B4 and B5), a low roughage to concentrate ratio at a low level of feed intake (treatments C1 and C2) and a high roughage to concentrate ratio at a low level of feed intake (treatments D1 and D2).

Mean digestion results of the various treatments expressed in a similar way as for the Experiments I and II are shown in Table 7.

Table 7. Intake (I_X), total apparent digestibility (d_X) and apparent digestibility in the stomachs, as percentage of d_X ($d_{X,S}$) in Experiment III.

	treatment			
	III-A	III-B	III-C	III-D
number of animals	2	5	2	2
DM from roughage (%)	40±6.6	81±3.1	42±0.30	83±16.6
XP from roughage (%)	30±4.2	77±4.0	35±2.6	82±18.4
I_T (kg/day)	15.7±0.03	7.9±0.53	5.6±0.45	3.8±1.30
I_O "	14.5±0.05	7.1±0.20	5.2±0.44	3.5±1.20
I_{XP} "	2.61±0.10	1.07±0.07	0.96±0.01	0.64±0.30
I_{XF} "	2.54±0.16	2.01±0.15	1.10±0.00	0.98±0.29
I_{XX} "	8.81±0.09	3.82±0.30	2.91±0.43	1.76±0.57
d_T (%)	73±0.4	77±2.8	77±1.8	73±0.5
d_O "	74±0.4	78±2.7	80±1.6	75±0.1
d_{XP} "	66±0.0	67±3.0	70±5.3	66±4.7
d_{XF} "	62±0.8	78±2.3	74±3.7	79±0.2
d_{XX} "	81±0.5	83±3.4	85±0.4	77±1.2
$d_{T,S}$ "	43±1.1	49±3.1	47±4.3	42±1.2
$d_{O,S}$ "	56±0.7	62±2.6	61±3.2	60±1.1
$d_{XP,S}$ "	-20±5.7	-28±7.2	-3±19.1	-19±14.1
$d_{XF,S}$ "	97±2.7	95±2.0	95±4.0	93±0.7
$d_{XX,S}$ "	79±1.3	77±1.7	75±1.5	75±1.9

Similar as in Experiments I and II were the energetic efficiencies of bacterial protein synthesis in the forestomachs calculated. The results are shown in Table 8. In this table are also shown the estimated proportions of feed N escaping degradation in the rumen.

Table 8. Efficiency of microbial protein synthesis and extent of degradation of feed N in Experiment III.

	treatment			
	III-A	III-B	III-C	III-D
(XF + XX) disappeared (kg/day)	7.3±0.10	4.0±0.32	2.7±0.15	1.8±0.58
microbial N in duodenal content (g/day)	261±37.4	109±11.7	69±22.6	52±5.9
micr. N/(XF+XX) ferm. (g/kg)	36±4.6	27±1.4	25±7.0	32±6.9
undegraded feed N (% of I_N)	30±11.4	30±5.2	33±3.1	26±18.2

DISCUSSION

Digestion and site of digestion Increasing the proportion of long roughage in the diet tends to increase the total apparent digestion of crude fibre in all three series of experiments. It has often been observed that with concentrate rich diets the degradation of cell wall constituents is inhibited, mainly as result of a decreased ruminal pH with such diets. This inhibition may be caused by a specific inhibition of cellulose digesting strains of rumen bacteria (Stewart, 1977) or protozoa (Demeyer, 1980).

Our results show a tendency of a larger proportion of the apparently digestible organic matter (ADOM) being digested in the stomachs with a larger proportion of long roughage in the diet. Little information has been published on the effect of changing the roughage/concentrate ratio on site of digestion. In experiments with sheep Chamberlain & Thomas (1979) found that a gradual replacement of chopped hay by ground concentrates increased the total apparent digestibility of the dietary organic matter; the proportion of this ADOM digested in the stomachs was not clearly affected. The shift in site of digestion observed in our experiments must undoubtedly be related to changes in the rate of passage of different pools of feed particles. An increasing proportion of long roughage increases the passage rate of rumen fluid (Tamminga et al., 1978; Bull et al., 1979) and probably also of small feed particles. Passage rate of the long particles themselves is lower than of fluid and small feed particles (Hartnell & Satter, 1979), so, replacing ground and pelleted concentrates by long roughage will probably increase the average retention time of feed particles in the forestomachs and thus enable the

microorganisms to degrade a larger part of the apparently digestible organic matter in the diet. From the results of the experiments reported here it remains inconclusive which part of the diet (cell wall constituents or cell contents is affected most.

Protein degradation and microbial protein synthesis The results on protein degradation show a large variation and it becomes therefore difficult to draw conclusions or to discover significant effects resulting from the shifts in level of intake or the ratio between long roughage and ground and pelleted concentrates. It should be realised however that the extent of protein degradation is calculated rather than measured. What remains after subtracting the total duodenal protein flow with the estimated endogenous contribution and the measured flow of microbial protein is assumed to be undegraded dietary protein. In this fraction however all inaccuracies of estimating or measuring the other fractions are accumulated.

In the experiments of Chamberlain & Thomas (1979) referred to earlier of the 5 diets fed, only diets consisting of either pure roughage or diets consisting of over 85% ground concentrates, showed a decreased efficiency of microbial protein synthesis. In our experiments no such diets were fed, except in one case where the diet consisted of only 3 kg of long hay (treatment III-D2). In this case a rather high efficiency of microbial protein synthesis was found of 38 g of microbial N per kg carbohydrates fermented. As a result the proportion of feed N escaping degradation in this case became extremely low (8%). Although not detectable, an analytical failure cannot be excluded. It seems therefore justified to conclude that if extreme roughage to concentrate ratios are avoided the best way of estimating the contribution of microbial protein to the intestinal protein supply of ruminants is to use a constant factor related to some measure of dietary energy.

The average figure of 32.2 g of microbial N produced per kg of carbohydrates fermented agrees well with figures in literature. In new protein evaluating systems microbial protein synthesis is often related to digestible organic matter (DOM). From our results an average value of 132 g of microbial crude protein per kg DOM was calculated, which agrees very well with the values used in the different new protein evaluating systems for ruminants (Vérité et al., 1979).

Extent of degradation of dietary protein in ruminant diets results from the rate of degradation and the rate of passage of both the protein in the roughage part of the diet and the protein in the concentrate part. Rate of

passage of long roughage is much slower than that of ground and pelleted concentrates. The rate of passage of small particles may even be increased by the inclusion of long roughage in the diet, because the latter stimulates salivary production and the rate of passage of fluid (Tamminga et al., 1978). Part of the small particles will be suspended in the fluid and an increased outflow rate of fluid may therefore also increase the outflow rate of the small particles suspended in it. Rate of degradation also differs between long roughage and concentrates and this is probably also true for protein in both dietary ingredients. Besides rate of degradation of protein in one part of the diet, for instance the concentrate part may be influenced by the presence of the other part. It was found in sheep (Ganev et al., 1979) that the rate of degradation of protein supplements in sheep fed a roughage based diet was higher than in sheep fed a concentrate based diet. Because of all these interactions it becomes difficult if not impossible to give a reliable prediction of the extent of degradation of dietary protein in the forestomachs of ruminants.

Our results show that if mixed diets, consisting of long roughage and ground concentrates are fed, with no special measures taken to alter protein degradation, some 30% of the dietary protein escapes microbial degradation in the forestomachs. This figure agrees well with the figures proposed by Kaufmann (1979). That does however not exclude the possibility of altering the extent of degradation either by increasing the rate of passage, or by decreasing the rate of degradation.

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7. EFFECT OF THE LEVEL OF FEED INTAKE ON NITROGEN ENTERING THE SMALL INTESTINE OF DAIRY COWS

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ABSTRACT

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The effect of the level of feed intake on the quantity of protein entering the small intestine was studied in re-entrant cannulated dairy cows fed mixed diets with varying N contents. The degradation of dietary protein in the forestomachs was estimated by two methods, one based on the use of diaminopimelic acid (DAPA) as a microbial marker, the other based on regression techniques.

At the higher level of intake, the flow of N into the small intestine as a proportion of the N ingested was higher than at the lower level of intake. This difference could be explained by a decreased degradation of dietary N at the higher level of feed intake. This was thought to be the result of an increased rate of passage of digesta through the forestomachs at a higher level of feed intake.

INTRODUCTION

Protein supply in ruminants is mainly determined by the amount of protein entering the small intestine, which is the result of feed protein escaping microbial degradation, microbial protein synthesized in the forestomachs, and endogenous protein. The quantity of protein entering the small intestine of ruminants can be measured in experiments with animals equipped with re-entrant cannulae in the small intestine. By using analytical or mathematical techniques, the proportions of microbial protein and other protein can be estimated. The main part of the latter is usually formed by undegraded dietary protein.

It has been found that differences in degradability exist between the various protein sources applied in ruminant nutrition, varying from almost completely degradable to more than half of it resistant against microbial degradation in the rumen (Miller, 1973; Burroughs et al., 1975; Kaufmann, 1977). The amount of microbial protein synthesized per kg of feed ingested is also variable, but the nature of this variation is poorly understood (Tamminga, 1978).

So it seems useful to study the influence of dietary factors on the total nitrogen (N) entering the small intestine. One of the factors most likely to influence the flow of N into the small intestine is the level of feed intake. Therefore, the effect of a difference in level of feed intake on the flow of N into the small intestine was studied in dairy cows.

EXPERIMENTAL PROCEDURE

Six experiments were conducted with three dairy cows equipped with a rumen fistula and a re-entrant cannula in the small intestine. The animals were fed mixed diets, consisting of long meadow hay, and ground and pelleted concentrates. Three diets, in which the concentrate part contained varying protein levels were fed at two levels of feed intake. The protein content of the mixed concentrates, mixtures I, II and III, was altered by replacing corn, wheat, artificially dried sugarbeet pulp and tapioca with maize gluten feed, solvent extracted soybean meal, linseed expeller and coconut expeller. The composition of the concentrate mixtures is shown in Table I. At the high level of intake the animals received 5 kg of long meadow hay and 10—12 kg of concentrates per day. This diet was fed to the animals from early lactation to beyond mid-lactation. During late lactation and a dry period the animals were fed at a lower level of intake and received a diet consisting of 4 kg of hay and 6 kg of concentrates.

Duodenal flow was measured and sampled continuously for 96 h according to a method described earlier (Tamminga, 1975). Although recoveries

TABLE I

Composition of the concentrates (g/kg)

Ingredient	Mixture I	Mixture II	Mixture III
Maize	175	100	25
Wheat	200	110	20
Dried beetpulp	200	110	20
Tapioca meal	200	110	20
Maize gluten feed	25	100	175
Soyabean meal	25	170	315
Rapeseed meal	25	25	25
Linseed expeller	25	100	175
Coconut expeller	25	100	175
Cane molasses	75	50	25
Minerals	23	23	23
Vit. AD_3	2	2	2
N	16	32	48
Soluble N[1]	4	8	12

[1] Calculated from figures of Mertens (1977).

of Cr_2O_3 in both duodenal flow and faecal output were incomplete and ranged from 78 to 92%, no corrections were made for this incomplete recovery. Overall apparent digestibilities in re-entrant cannulated animals did agree better with those in normal animals fed comparable diets if no corrections were made for an incomplete recovery of Cr_2O_3 (Zijlstra, 1977) in the fistulated animals.

Undegraded dietary protein and microbial protein reaching the small intestine were estimated by two methods. In one method, microbial protein was estimated with diaminopimelic acid (DAPA) as a marker. In each experiment bacteria were isolated from the rumen 3 days before and 3 days after measuring the duodenal flow. The ratio DAPA/N in duodenal digesta was compared with the mean ratio in the two bacterial samples for each animal in each experiment. The ratio between the two was then used as a measure for the proportion of microbial N in duodenal digesta. The difference of total N and microbial N was corrected for ammonia-N. A further correction of 4 g of N per kg of DM ingested was made for endogenous contributions, originating from secretions in abomasal juice, pancreatic juice, bile and epithelial cells. This figure was based on observations by Kaufmann and Hagemeister (1976) who estimated an endogenous contribution of 15% in duodenal protein in dairy cows and on observations by Schwarting and Kaufmann (1978) who estimated an endogenous contribution of 26 g of crude protein per kg of dry matter ingested. The remaining non-ammonia-N (NAN) was taken as a measure of undegraded dietary N. The second method was based on the regression technique of Hvelplund et al. (1976). In this method, the proportions of undegraded dietary N and microbial N entering the small intestine can be estimated from the relation between the ratio duodenal NAN/N ingested and the N content of the diet. Before applying this method the NAN entering the small intestine was also corrected for endogenous N.

RESULTS AND DISCUSSION

Results on the total apparent digestibility of organic matter (OM) as a proportion of the intake and the proportion of this digestion taking place in the stomachs are summarized in Table II.

In Table III the quantities of N ingested and of N entering the small intestine are summarized. The results show an increased amount of N entering the small intestine with an increased dietary protein content, particularly at the higher level of intake. This is in agreement with the results of Miller (1973) and Ørskov et al. (1974), who also observed in sheep that the duodenal flow of N increased if the dietary protein content increased. From the results in Table III, it can also be seen that the ammonia-N represented 3 to 8% of the total N entering the small intestine and that this contribution tends to increase with an increasing dietary N content.

After correcting the duodenal N for ammonia N and an endogenous contribution of 4 g of N per kg of DM ingested (Kaufmann and Hagemeister, 1976;

TABLE II

Apparent digestibilities of organic matter (OM) (proportion of intake) and the proportion of this digested in the stomachs of dairy cows fed diets differing in N-content (figures are means of three animals)

Level of intake	OM ingested (kg/day)	Proportion apparently digested	Proportion digested in stomachs
'Low'	7.73	0.80	0.60
'Low'	7.86	0.81	0.59
'Low'	7.05	0.82	0.59
'High'	11.62	0.79	0.57
'High'	11.53	0.80	0.56
'High'	12.17	0.80	0.52

TABLE III

N intake and duodenal N flow in dairy cows fed diets differing in N content (figures are means of three animals)

Nitrogen content of diet (g N/kg DM)	Intake 8.2 kg of DM/day			Intake 12.9 kg of DM/day		
	N-intake (g/day)	Duodenal N-flow		N-intake (g/day)	Duodenal N-flow	
		(g N/day)	(g NAN/day)		(g N/day)	(g NAN/day)
21.7	185	230	223	272	377	366
31.0	264[1]	260	246	399	433	411
39.2	292	247	234	521	508	466

[1] Mean of two animals.

Schwarting and Kaufmann, 1978), the relationship between duodenal N flow (as a proportion of N ingested) and the dietary N content (g of N per kg of DM) was calculated for both levels of intake. The results are shown in Fig. 1.

A significant difference ($P < 0.05$) was found between the two levels of feed intake. At the higher level of feed intake (12.9 kg of DM/day) relatively more NAN entered the small intestine compared with the lower level of feed intake (8.2 kg of DM/day).

The difference may be the result of an increased flow of microbial N, an increased flow of undegraded dietary N or both. In the regression equations which are of the form:

$$Y = A + \frac{b}{X}$$

A represents the proportion of dietary N entering the small intestine undegraded and b the amount of microbial N synthesized per kg of DM ingested

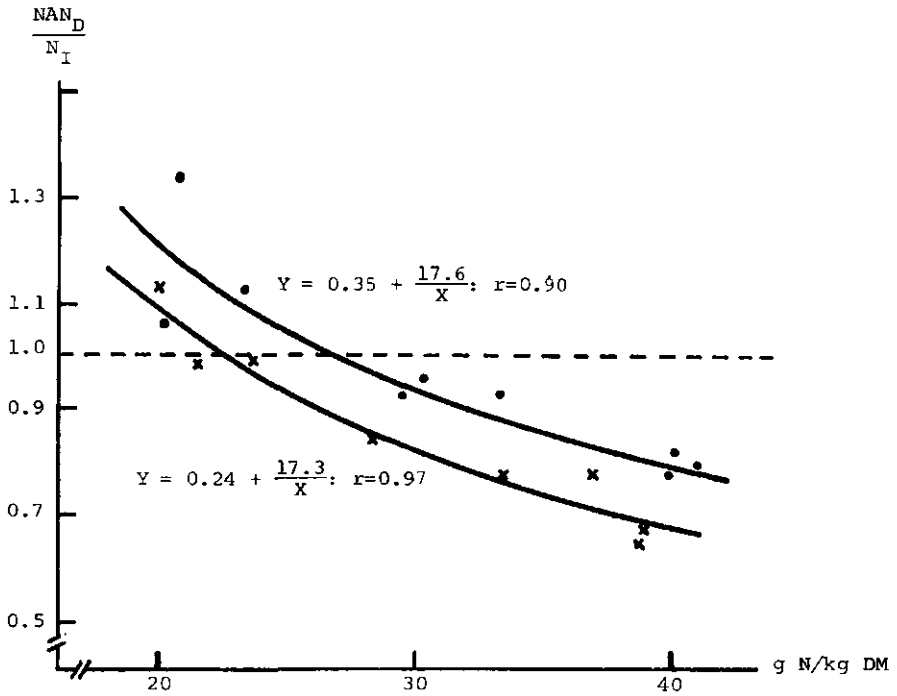

Fig.1. Relation between the ratio of NAN entering the small intestine/N intake (NAN_D/N_I) and the dietary N content (g N/kg DM) in dairy cows fed at a lower (x) and a higher (·) level of feed intake.

(Hvelplund et al., 1976). The results of the regression equations indicate that the increased flow of N into the small intestine was mainly the result of a decreased breakdown of dietary N in the stomachs. Of the dietary N ingested a proportion of 0.24 ± 0.020 entered the small intestine at the lower level of feed intake; at the higher level of feed intake this figure was 0.35 ± 0.040.

Estimating undegraded dietary N from the difference between the flow of total NAN and microbial N entering the small intestine, the latter estimated with DAPA as a marker, showed a similar tendency (Table IV). Using this method a proportion of 0.29 ± 0.020 of the dietary N appeared undegraded in the small intestine at the lower level of intake; at the higher level of intake this figure was 0.45 ± 0.021.

The figures based on DAPA show a slightly lower degradation of the dietary protein than the figures based on the regression technique. The latter is expected to estimate both bacterial-N and protozoal N, whereas the DAPA method estimates only bacterial N. If the difference between the two methods is the result of protozoal N, the contribution of protozoa to the N supply of dairy cows is rather small. This would confirm findings of Weller and Pilgrim (1974) in sheep.

The results of both methods show a lower degradation of dietary protein at

TABLE IV

Proportion of undegraded dietary N* reaching the small intestine of dairy cows, fed mixed diets at two levels of feed intake

Nitrogen content of diet (g N/kg DM)	Intake 8.2 kg of DM/day			Intake 12.9 kg of DM/day		
	Cow 93	Cow 2R	Cow 35	Cow 93	Cow 2R	Cow 128
21.5	0.32	0.37	0.20	0.53	0.41	0.40
30.6	—	0.30	0.30	0.49	0.50	0.47
38.9	0.27	0.33	0.22	0.32	0.49	0.47
Mean ± S.E.		0.29 ± 0.020			0.45 ± 0.021	

* Estimated with DAPA as a marker for microbial N.

a higher level of intake. At the lower level of intake a proportion of 0.40 of the DM intake originated from long roughage; at the higher level of intake this proportion was 0.32. As a result, the proportion of the total protein ingested, which originated from long roughage was on average some 0.06 higher with the lower level of intake. However, at this level of intake only a proportion of between 0.25 (high dietary N) and 0.40 (low dietary N) of the total protein originated from roughage protein. The degradation of roughage protein may have differed from the concentrate protein in these diets, and thus a shift in the ratio between roughage and concentrates would have an effect on the degradation of the total dietary protein. It was calculated however that this shift could only explain the difference in protein degradation between both levels of intake if the degradation of the protein in the concentrates was zero or less and the degradation of the roughage protein 150% or more. So the shift in roughage/concentrates ratio cannot explain the difference in protein degradation between the two levels of feed intake.

Theoretically one would expect a more efficient synthesis of microbial protein at a higher level of feed intake, because of a higher rumen liquor dilution rate and possibly a higher growth rate of the microbes. However, no evidence was found for an increased flow of microbial protein into the small intestine and the efficiency of microbial protein synthesis (expressed as g of microbial N synthesized per 100 g of carbohydrates apparently fermented in the forestomachs) did not differ significantly between the two levels of food intake (Tamminga, 1978). But a tendency was found that the efficiency of microbial protein synthesis was slightly lower at the higher level of intake.

Also a tendency was found that a smaller proportion of the apparently digestible organic matter (ADOM) disappeared before the small intestine at the higher level of feed intake (Table II). At the lower level of feed intake a proportion of 0.59 ± 0.014 of the ADOM disappeared before the small intestine; at the higher level of intake this figure was 0.55 ± 0.12. An increased

level of feed intake seems therefore slightly to decrease the total amount of microbial protein entering the small intestine.

The results in Table IV show no marked differences in protein degradation between the diets with a differing protein content. This does suggest that in diets containing substantial proportions of mixed concentrates, the degradation of the mixture of proteins is fairly constant.

The most likely explanation for the difference in flow of N into the small intestine between both levels of feed intake remains, therefore, a decreased degradation of dietary protein due to an increased rate of passage of digesta through the forestomachs. This is different from observations of Miller (1973). When supplementing a basal diet with sunflower-seed meal and feeding the latter diet at two levels of intake, 1.25 and 2.5 times maintenance, respectively, no decrease in the degradation of the sunflower-seed meal was observed.

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RESUME

Tamminga, S., Van der Koelen, C.J. et Van Vuuren, A.M., 1979. Influence du niveau d'ingestion de la ration sur le flux d'azote entrant dans l'intestin grêle des vaches laitières. *Livest. Prod. Sci.*, 6: 255—262 (en anglais).

L'influence du niveau d'ingestion de la ration sur la quantité de protéines entrant dans l'intestin grêle a été étudiée chez les vaches laitières porteuses de canules réentrantes, qui recevaient des rations mixtes de teneur en azote différente. On a estimé la dégradation des matières azotées alimentaires à l'aide de deux méthodes: l'une utilisant l'acide diaminopimélique (DAPA) comme marquer microbien, l'autre basée sur des techniques de régression.

La quantité d'azote entrant dans l'intestin grêle, exprimée en pourcentage de la quantité d'azote ingérée, a été supérieure pour le niveau d'ingestion le plus élevé. Cela s'explique par une dégradation plus faible de l'azote alimentaire résultant d'une vitesse de passage plus rapide du content digestif à travers les préestomacs.

KURZFASSUNG

Tamminga, S., Van der Koelen, C.J. und Van Vuuren, A.M., 1979. Einfluss der Höhe der Futteraufname auf den Stikstoffluss in den Dunndarm von Milchkühen. *Livest. Prod. Sci.*, 6: 255—262 (in Englisch).

Der Einfluss der Höhe der Futteraufnahme auf die Eiweissmenge, welche in den Dünndarm eintritt, wurde an Milchkühen untersucht, die mit wiedereintretender Kanüle versehen waren und ein Futtergemisch mit variierendem Stickstoffanteil erhielten. Der Abbau des Eiweiss in den Vormägen wurde durch zwei Methoden geschätzt. Einerseits wurde Diaminpimelinsäure (DAPA) als mikrobieller Marker angewendet, andererseits ein Regressionsverfahren durchgeführt.

Bei höherem Aufnahmeniveau war der Stickstoffluss in den Dünndarm als Anteil des aufgenommenen Stickstoff höher als bei dem niedrigeren Aufnahmeniveau. Dies könnte auf einen abnehmenden Stickstoffabbau bei höherem Fütterungsniveau hindeuten, verursacht durch eine erhöhte Passagerate der Nährstoffe durch die Vormägen.

8. EFFECT OF FREQUENCY OF FEEDING CONCENTRATE DIETS ON N ENTERING THE SMALL INTESTINE OF DAIRY COWS

SUMMARY

Dairy cows with duodenal re-entrant cannulae were fed 5 kg of long hay and 6 kg of concentrates. Protein content of the diet was 11.8% in the dry matter in Experiment I and 18.5% in Experiment II. Concentrates were fed two (treatment A) or six (treatment B) times per day. In Experiment I duodenal N flow was 21% higher than N intake with treatment A, but 57% higher with treatment B. In Experiment II duodenal flow was 5% lower than N intake with treatment A, but 2% higher with treatment B. Treatment B decreased the proportion of feed N escaping degradation in the rumen, but this was more than compensated for by a more efficient microbial protein synthesis. Treatment B stabilised rumen fermentation.

INTRODUCTION

Increased productivity of dairy cows requires a high intake of feed with a high nutritive value. To achieve this the roughage part of the diet is increasingly replaced by concentrate feeds with a higher nutritive value. If these highly digestible concentrates are fed in large quantities, microbial fermentation in the rumen will initially proceed very rapidly, resulting in high concentrations of volatile fatty acids (VFA) and a rapid drop in pH is enhanced by the decreased salivary production with such a feeding regime (Kaufmann, 1976). The low pH in the rumen inhibits microbial fermentation and microbial growth. This may have an effect on microbial protein production and ultimately on the protein supply of the host animal.

Feeding the animal more frequently will stabilize rumen fermentation and reduce diurnal variation in ruminal pH and VFA concentrations (Kaufmann, 1976). This may stimulate microbial growth and microbial protein synthesis. To test this hypothesis the effect of feeding the concentrate part of the diet more frequently on N-metabolism in dairy cows was studied.

MATERIALS AND METHODS

Two series of experiments were carried out with 3 dairy cows equipped with a rumen fistula and with re-entrant cannulae at the beginning of the small intestine. The animals were fed diets consisting of long meadow hay and concentrates in a ratio 40/60. The concentrates had a low (8-10% CP/DM) or a higher (18-20% CP/DM) protein content and they were fed in two or six equal portions per day. Average protein contents of the diets were 11.8 and 18.5% in the dry matter.

After an adaptation period of at least 21 days, the duodenal flow was measured continuously for a period of 96 hours according to a procedure described earlier (Tamminga, 1975) or by using fully automated equipment. A proportional sample of 1.6% of the total flow was collected. Part of the fresh sample was analysed for its dry matter (DM) content; about 4 kg of fresh material was freeze-dried and used for Weende analysis. Feeds and feed refusals were weighed and also analysed according to the Weende method. Rumen samples were taken at 7.00; 8.00; 10.00; 12.00 and 14.00 hours for pH, VFA and NH_3 content, on day 4 before the beginning and on day 6 after the end of the duodenal collection period. On these days rumen samples were also taken at 3, 4, 5, 6, 9, 18, 20, 22 and 24 hours after the introduction of 1 litre of a Cr-EDTA solution, containing 2.8 g of Cr, in the rumen. These samples were analysed for their Cr-content and from the results the ruminal liquor clearance rate (D) was calculated.

RESULTS

Intake and duodenal flow of organic matter (OM) and nitrogen (N) are shown in Table 1. In brackets the duodenal flow of OM and N expressed as percentage of the intake is given. From the results it appears that with more frequent feeding a higher proportion of the organic matter ingested appears in the small intestine.

The results on N flow were subjected to the calculating procedure developed by Hvelplund et al. (1976) from which an estimate can be obtained of the contribution of feed N and microbial N to the duodenal N by relating the ratio duodenal N/feed N (N_D/N_I) to the %N in the dry matter of the feed:

$$N_D/N_I = A + \frac{b}{\%N/DM_I}$$

Table 1. Intake and duodenal flow of organic matter and N.

	feeding	low N OM (kg/day)	low N N (g/day)	high N OM (kg/day)	high N N (g/day)
intake	2 x	6.47	140	7.75	255
	6 x	6.36	138	7.84	250
duodenal flow	2 x	2.98(46)	170(121)	3.71(48)	242(95)
	6 x	3.49(55)	216(157)	4.12(53)	256(102)

In this equation A represents the proportion of dietary N escaping degradation in the forestomachs and b represents the microbial protein production (gN/kg DM ingested). The results of our duodenal flow measurements were first corrected for an endogenous contribution of 4.2 g of N per kg DM ingested (Schwarting & Kaufmann, 1978). The results of the two feeding regimes were then subjected to the calculation procedure mentioned above. This resulted in values for A of 0.41 $\pm$ 0.015 and -0.01 $\pm$ 0.035 for feeding the concentrates twice daily or 6 times per day respectively. Values found for b were 1.19 $\pm$ 0.035 and 2.59 $\pm$ 0.079 for both treatments respectively.

Microbial protein synthesis largely depends on the energy extracted from the conversion of carbohydrates into VFA. The calculated average figures for microbial protein synthesis were therefore related to the amounts of carbohydrates (total of crude fibre and N-free extracts) disappeared between intake and the beginning of the small intestine. The results are shown in Table 2.

Table 2. The efficiency of microbial protein synthesis.

feeding	low N 2x	low N 6x	high N 2x	high N 6x
carbohydrates fermented (kg/day)	3.78	3.62	4.02	3.86
microbial N (g/day)	84	187	104	220
microbial N / kg CB fermented	22	52	26	57

Increasing the N content of the diet tends to increase the efficiency of microbial N production, but increasing the frequency of feeding seems to have much larger effect. Feeding the concentrate part of the diet six times per day more than doubled the efficiency of microbial N synthesis as compared with feeding two times per day.

In Table 3 the results of ruminal measurements of the cows fed with the high protein diets are summarised. The results of the low protein diets were lost in a fire.

Table 3. Rumen characteristics of cows fed twice daily or more frequently.

feeding	2x		6x	
pH	6.4	(0.20)[1]	6.4	(0.08)
NH_3 (mg/100 ml)	22.4	(9.1)	20.1	(6.0)
NGR[2] of VFA	4.1	(0.41)	5.0	(0.18)
dilution rate (hr^{-1})	0.131		0.130	

1) s_d in brackets

2) Non-glucogenic Glucogenic Ratio = $\frac{HAc + 2HB + HVal}{HVal + HP}$

DISCUSSION

Experiments in which the effect of frequent feeding on duodenal N flow was studied are very limited. Comparing hourly feeding with once daily feeding in sheep fed dried grass or dried grass supplemented with formaldehyde treated casein (MacRae et al., 1972) showed no favourable effect on the duodenal flow of amino acids with frequent feeding. Feeding a concentrate rich diet more frequently to dairy cows did not show any effect on N entering the small intestine (Oldham, unpublished results). Experiments in which the effect of frequent feeding on the degradation of feed protein and the synthesis of microbial protein was studied are even more limited. It was found in sheep that frequent feeding substantially increased the efficiency of microbial protein synthesis (Al Attar, Evans & Axford, 1976). Our results show also that the efficiency of microbial protein synthesis is substantially enhanced with more frequent feeding, but so is the extent of degradation of dietary protein. More microbial synthesis with more frequent feeding not only means more microbial protein, but also more protein free microbial organic matter. This can explain why a slightly higher proportion of the ingested organic matter appears in the small intestine with more frequent feeding.

From the results in Table 3 it may be concluded that feeding the concentrates more frequently decreases the diurnal variation of several rumen characteristics, suggesting a more stable microbial activity in the rumen. The NH_3 content becomes lower which agrees with more N being captured for incorpora-

tion in microbial protein.

Further research may prove that feeding the concentrate part of the diet more frequently is advisable under conditions when large amounts of concentrates are fed, particularly if the diet is rather poor in protein. It will prevent ruminal disturbance and promote the flow of N to the small intestine.

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9. AMINO ACID UTILIZATION BY DAIRY COWS. I. METHODS OF VARYING AMINO ACID SUPPLY

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ABSTRACT

Oldham, J.D. and Tamminga, S., 1980. Amino acid utilization by dairy cows. I. Methods of varying amino acid supply. *Livest. Prod. Sci.*, 7: 437--452.

The point of amino acid supply must be defined — duodenal amino acid supply (DAAS) is now seen as crucially important in new systems for rationing protein, but metabolism of amino acids during passage across the gut wall and in the body affect supply to the liver and peripheral tissues.

Measurement of DAAS can be reliable, provided that animal replication is adequate, but partition into amino acids of microbial or food material is unreliable because of deficiencies in available techniques. Comments on methods for varying microbial or food amino acid supply are still little more than qualitative.

There appears to be a limit to duodenal non-ammonia N (NAN) supply/MJ ME intake which does not change with level of food intake (MJ ME/$kg^{0.75}$). The limit is close to 2.5—3 g NAN/MJ ME intake for both "concentrate" and "forage" diets. Cows may need 2.6 g NAN/MJ ME at peak production so methods for varying DAAS should be evaluated against this. Heat treatment, particularly of forages, and formaldehyde treatment of proteins, are methods which can be used to achieve high levels of duodenal NAN supply.

INTRODUCTION

The protein, or amino acid, economy of the dairy cow can be described as the balance between supply and demand. In the lactating animal much of the demand comes from the mammary gland which maintains very high rates of net protein synthesis. No less important, though usually quantitatively smaller, are the demands made on amino acids by those processes encompassed by the term 'maintenance', by growth (of maternal and/or foetal tissue) and by processes associated with milk production, especially those concerned with glucose metabolism.

To match amino acid supply with demand, at particular rates of animal production (milk yield in the case of the dairy cow) is the aim of the various fledgling systems which have been proposed for redefining protein feeding standards for ruminants (Kaufmann and Hagemeister, 1975; Roy et al., 1977;

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Vérité et al., 1979). These systems have rightly identified amino acid supply to the intestines (duodenal amino acid supply; DAAS) as a critical point in the chain of metabolic events that links intake of food to output of milk. This paper will concentrate on methods of varying DAAS although it will be realised that variation in supply at this point is of practical interest only if it results in a change in amino acid supply to the tissues (TAAS) and a change in animal performance. The ultimate test of any method for varying amino acid supply to the duodenum is that it produces an economic return in enhanced milk yield (or growth). There is little to be gained by increasing supply beyond the level at which the cow has the capacity to respond (the requirement). As the aim is usually to increase DAAS to meet a particular need, potential supply has to be looked at in relation to likely demand. It is therefore necessary to be able to describe requirements for amino acids at the tissue level and to examine the relationship between these requirements and the amounts of amino acids which must be supplied to the intestines to meet them. The succeeding paper (Tamminga and Oldham, 1980, this issue) attempts to do this.

The task of both of these papers is made difficult by the relative lack of quantitative data from experiments with lactating dairy cows. This often makes it necessary to extrapolate from results obtained with other ruminant species, usually fed a low level of intake relative to the high-yielding cow. For describing amino acid requirements a degree of speculation is needed which requires experimental verification. It is therefore difficult with present knowledge to make reliable quantitative statements about the relationship between amino acid supply and requirement.

The qualitative basis of variation in supply is, however, long-established. A third of a century ago Phillipson (1946) pointed out that "The ruminant lives not only on . . . microorganisms but also on food . . . protein that leaves the rumen . . . The final answer must depend on quantitative measurements of the amount of these materials that becomes available to the animal". Methods to vary duodenal amino acid supply may therefore alter either food or microbial amino acid supply or both. The supply of endogenous secretion to the duodenum may also vary but as a consequence of, rather than as a deliberate aim of manipulation of diets. Observations on variation in each of these are subject to the accuracy (and precision) of techniques used to measure them. It is therefore relevant to begin discussion of methods for varying DAAS by consideration of the adequacy of currently available techniques for making appropriate measurements.

TECHNICAL PROBLEMS

Reliable techniques for measuring DAAS are needed, in order to evaluate methods for varying it. DAAS measurements are only as accurate as measurements of duodenal digesta flow. Provided that animal replication is adequate, which has not always been the case in experiments with cows, the coefficient

of variation of duodenal DM flow (based on between-animal variation) is close to 10% (Sutton and Oldham, 1977). DAAS measurements will have an error of similar magnitude. The consequence of this is that to identify significant differences (at $P < 0.05$) in DAAS between treatments in two experiments out of three; four animal replicates (4 × 4 Latin Square) are needed if the difference is 20% between treatments, and six animal replicates (6 × 6 Latin Square) if the difference is 15%. This degree of animal replication (and hence reliability of quantitative results) has rarely if ever been achieved in studies with dairy cows.

If DAAS is to be partitioned into amino acids from microbial or food material, the error of each part will be proportionately greater. Of the various techniques which have been used for identifying microbial material (Ling and Buttery, 1978; Smith et al., 1978; Siddons et al., 1979), those which employ radioisotopes are unsuitable for routine use with dairy cows because of the practical difficulties (appropriate housing, waste disposal, etc.) involved. This is unfortunate because these are perhaps the best of available methods.

There can, however, be big differences in estimates of duodenal microbial supply, depending on the microbial marker used (Siddons et al., 1979) so that the quantitative reliability of all measurements of "microbial N" obtained thus far must be questionable.

Diamino pimelic acid (DAPA) (a bacterial marker) has been used most often with cows (Hagemeister et al., 1976; Hvelplund and Møller, 1976) but there is evidence that this may underestimate the true microbial contribution (Tamminga, 1978; Smith et al., 1978). This might suggest a substantial protozoal protein supply to the duodenum of cows. It certainly means that published estimates of microbial contributions to DAAS in cows have a larger (possibly much larger) error associated with them than measurements of total amino acid supply.

Food amino acid supply, and subsequently the "degradability" of food protein, is calculated as: duodenal amino acids − (microbial amino acids + amino acids of endogenous secretions). Calculation by difference compounds errors, so that methods to vary supply of food amino acids may be described qualitatively but, to be reliable, not quantitatively. This is particularly so because there are no good estimates of endogenous contributions to DAAS in cows.

In sheep, endogenous N contributions have been found to be variable and comprise as much as 20% of duodenal N (Harrop, 1974; Nolan and MacRae, 1976).

The intellectual appeal of describing methods to vary either food or microbial amino acid supply or both to the duodenum of dairy cows is therefore tempered by our present inability to measure each of these accurately. As total amino acid flow is both the critical nutritional parameter, and the measurement which can be made accurately, it is appropriate to consider factors which affect this first, then to suggest methods for manipulating it and to qualify the observations with comments on possible variation in microbial or non-microbial components.

But first, some comments are appropriate on the distribution of N between the various components of duodenal total N and their relative rates of absorption.

N FRACTIONS IN DUODENAL N

Non-ammonia-N (NAN) is frequently accepted as a measure of protein entering the small intestine. Part of this NAN is of microbial origin and some 20% of this microbial NAN is usually assumed to be present in nucleic acids. So, with a varying proportion of microbial NAN, theoretically between 0 and 100%, duodenal NAN will contain between 0 and 20% NAN in nucleic acids.

In most newly-developed protein evaluating systems for ruminants (Kaufmann, 1977; Roy et al., 1977; Vérité et al., 1979), it is accepted that intestinal N not present in ammonia or in nucleic acids is present in amino acids. This assumption must be questioned, as shown by the results in Table I, where the distribution of N over various N-containing compounds in the small intestine of dairy cows is shown (Tamminga, unpublished results). A significant part of the total N appears to be of unknown origin. No doubt part of this N is in tryptophane, because this amino acid is usually not included in the amino acid analysis. Also N may be present in intermediates of the urea-cycle, such as ornithine and citrulline. Little is known of their contribution to duodenal N, but Tagari and Bergmann (1978) found significant amounts of ornithine in the small intestine of sheep. Part of the unknown fraction may be N in taurine from bile, since results in Table I were from cows with re-entrant cannulae situated posterior to the pancreatic and biliary duct and substantial amounts of endogenous N secretions may be expected in digesta collected at this point. More research is needed on the N of unknown origin.

Table I also shows estimates of the apparent absorption of N in the various classes of N-containing compounds. Amino acids, entering the small intestine in protein, become hydrolysed in the abomasum and small intestine due to the action of digestive proteolytic enzymes (Armstrong and Hutton, 1975). The resulting peptides and amino acids are transported across the gut wall to

TABLE I

Distribution and apparent absorption of N in duodenal digesta of cows

N-source	Percentage present	Percentage apparently absorbed
Essential amino acids	35	75—80
Non-essential amino acids	30	70—75
Amides	4	?
Nucleic acids	11	80—90
Ammonia	6	?
Unknown	14	60

the portal blood. Peptides are hydrolysed further to amino acids, possibly as part of the transport process (Matthews, 1975). In the gut wall significant amounts of amino acids are metabolized (Bergmann and Heitmann, 1978). Apparent absorption is rather high (70—80%) and only small differences have been observed between essential and non-essential amino acids in this respect.

Nucleic acids, RNA and DNA, are largely degraded in the small intestine, mainly due to the action of pancreatic nucleases (Armstrong and Hutton, 1975). RNA and DNA are presumably degraded to their component nucleosides and absorbed to a high degree as is shown in Table I.

It also appears from Table I that N present in compounds other than amino acids or nucleic acids has a low apparent absorption. This suggests that at least part of the unknown N is present in compounds other than tryptophane, ornithine, citrulline and taurine, because these compounds are expected to behave very similarly to the other amino acids. The contribution of these compounds to apparently absorbed N seems therefore to be of minor importance.

Bearing these comments in mind, and to facilitate comparison of data from different published experiments, we have chosen for the remainder of this paper to adopt NAN as an index of amino acid supply.

LIMITS TO DUODENAL SUPPLY AND LIKELY DEMAND

It is convenient to divide rations into those which contain predominantly (>70%) forages or predominantly (>50%) concentrates.

Figures 1 and 2 have been produced by assembling results from a variety of sources of which A—H refer to cows, I—M to calves or steers and N—W to sheep or lambs. So that affects of level of food intake can be compared between different classes of stock, intake is expressed as MJ ME/day kg body-weight $^{0.75}$. NAN flow to the duodenum is expressed as g NAN/day/MJ ME intake. No correction has been applied for increased endogenous N flow where cannulas were sited posterior to the entry of the bile and pancreatic ducts.

The "limiting value" for NAN/MJ ME appears to be close to 2.5—3 for both "concentrate" and "forage" diets. These establish target values against which the success of various methods for varying amino acid supply (NAN) can be judged. There is no indication that these limiting values for NAN/MJ ME change with level of intake for either "concentrate" or "forage" diets. ME intake is therefore a major determinant of optimal NAN supply which is not surprising in view of the recognized relationships between microbial N production and energy intake. A good correlation between duodenal amino acid N flow (y g/day) and DOM intake (x kg/day) in cows has been reported by Tamminga and van Hellemond (1977) such that

$$y = 32.3x - 8.53$$

The intake range was from 3.5 to 11 kg DOM/day (0.55—1.7 MJ ME/kg$^{0.75}$/day). The relationship suggests a constant duodenal NAN supply of about

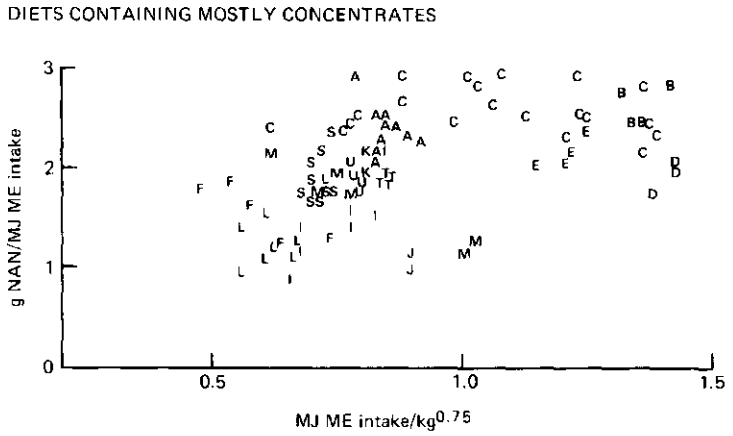

Fig. 1. Variation in duodenal non-ammonia nitrogen per MJ ME intake (NAN/MJ ME) with level of ME intake (MJ ME/day/kg bodyweight$^{0.75}$), for diets which contained predominantly concentrates. Data sources (for Figs. 1 and 2) were as follows. For cows: A, Hagemeister et al. (1976); B, Tamminga (1975); C, Tamminga, van der Koelan and van Vuuren (1979 and unpublished); D, Vérité (1978); E, Oldham et al. (1979); F, Teller et al. (1978); G, van 't Klooster and Boekholt (1972); H, Hvelplund and Møller (1976). For calves and steers: I, Williams and Smith (1976); J, Young et al. (1975); K, Redd et al. (1975); L, Cole et al. (1976); M, Smith et al. (1978). For sheep and lambs: N, Coelha da Silva et al. (1972a); O, Coelha da Silva et al. (1972b); P. MacRae et al. (1972); Q, Hogan and Weston (1967); R, Weston and Hogan (1968); S, Ørskov et al. (1971); T, Ørskov et al. (1972); U, Ørskov et al. (1974); V, MacRae et al. (1972); W, Beever et al. (1977).

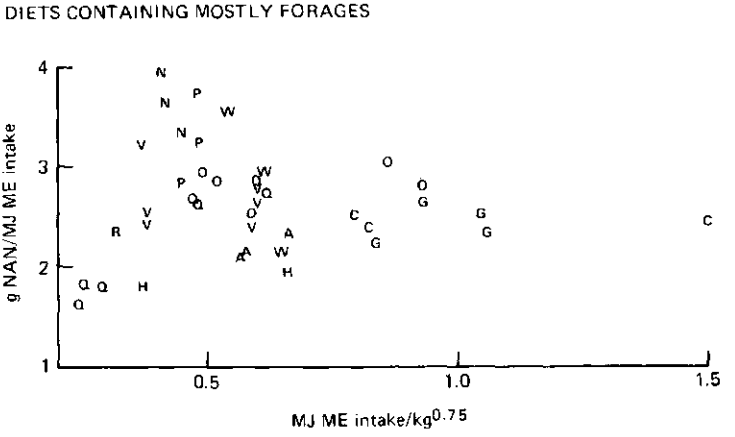

Fig. 2. As for Fig. 1, but for diets which contained predominantly forages.

3.0 g NAN/MJ ME which represents good agreement with the limiting values quoted here.

Journet and Vérité (1979) have given another relationship between duodenal NAN and DOM intake for cows such that

Duodenal NAN (g/day) = 23.85 DOM (kg/day) + 0.60 Non-degradable N intake (g/day) + 8.6

In this case DOM intake ranged from 4.3 to 12.2 kg/day and the average ratio of non-degradable N/DOM in food was 17.3 non-degradable N/kg DOM. Applying this value for non-degradable N/DOM to their equation suggests an average flow of NAN/DOM of 42.8 or about 2.8 g NAN/MJ ME intake, again very close to the value of 3.0 g NAN/MJ ME. In fact the Journet and Vérité (1979) equation would suggest values for NAN/MJ ME above 3.0 only when non-degradable N (as defined by Journet and Vérité) exceeded 23 g/kg DOM intake or about 10% non-degradable crude protein in ration dry matter.

It is clear from Figs. 1 and 2 that wide variation in NAN/MJ ME has been found at low levels of food intake. This suggests considerable scope for finding methods to vary NAN/MJ ME. The variation at higher intakes (>1 MJ ME/$kg^{0.75}$) is not so great but it is not clear if this is due to decreased variation in NAN/MJ ME at high intakes or to the scarcity of published data for animals at high intakes. It is relevant to point out that high-yielding dairy cows are likely to consume about 2 MJ ME/$kg^{0.75}$, but no data have been published with intakes greater than 1.7 (Tamminga and van Hellemond, 1977). Extrapolation of apparently "established" principles to the dairy cow is obviously somewhat speculative.

The cow's demand for duodenal NAN must be taken into account in evaluating the significance of these limits to supply. Amino acid N (AAN) is normally 0.6—0.7 of duodenal NAN (Table I). Duodenal needs for NAN/MJ ME can therefore be calculated from requirements for AAN using the approach described by Oldham (1979). For Friesian cows yielding 40, 30, 20 and 10 kg milk per day, these will be close to 2.6, 2.4, 2.1 and 1.9 g NAN/MJ ME intake.

Limitations to the intake of forage diets restricts use of these to meet ME requirements for very high yields, but at lower yields optimum NAN/MJ ME for "forage" diets easily satisfies requirements. Where "concentrate" diets are needed to boost ME intake it is clearly important that NAN/MJ ME is optimal. Methods for varying NAN/MJ ME with "concentrate" diets are consequently particularly important.

MANIPULATION OF NAN/MJ ME

Variation in forage/concentrate ratio

Figures 1 and 2 do not establish any relationship between NAN/MJ ME and forage : concentrate ratio; but they hint at the possibility that inclusion of forages may be beneficial.

Work at Shinfield (Table II; Oldham, Sutton and McAllan, 1979 and unpublished) has examined the effect of varying hay : concentrate ratios and form of cereal on DAAS in lactating cows fed at one level of energy and N intake.

An increase in the ratio concentrates : hay increased EAAN and NAN/MJ ME with barley concentrates, but had no effect with maize. The direction of this change is contrary to that suggested from Figs. 1 and 2. A high rate of rumen fermentation leading to efficient capture of "rumen degraded N" (Table III) was perhaps the dominating factor. Increases in NAN/MJ ME

TABLE II

Flow of non-ammonia-N (g/day) and essential amino acid N (EAAN) to the duodenum of cows fed rations containing rolled barley (B) or ground maize (M) in the concentrates and fed in the ratios, concentrates : hay, of either 60:40 (60) or 90:10 (90). Values are means for 3 cows

Diet	60B	60M	90B	90M
N intake (g/day)	286	291	269	281
ME intake (MJ/day)	129	128	132	122
Duodenal flow of:				
NAN (g/day)	277	263	308	244
EAAN (g/day)	93	88	103	83
NAN (g/day/MJ ME)	2.14	2.05	2.34	2.0
EAAN (mg/day/MJ ME)	720	688	784	678

TABLE III

Fractional outflow of PEG (FOM/PEG) from the rumen, microbial (measured using DAPA) and food non-ammonia N supply to the duodenum and the efficiencies of microbial N production in cows fed the diets described in Table I. Values are means for 3 cows

Diet	60B	60M	90B	90M
FOM (PEG) (h^{-1})	0.12—0.24	0.11—0.18	0.07—0.14	0.07—0.15
Microbial NAN (g/day)	216	136	194	70
Food NAN (g/day)	60	128	114	174
"Degradability" of food N	0.84	0.61	0.64	0.38
Energetic efficiency of microbial N production (g N/kg OM apparently fermented in the rumen)	35	22	31	13
Nitrogen efficiency of microbial N production (microbial N per g food N degraded)	0.90	0.77	1.14	0.59

have been achieved in beef steers by substituting cotton seed hulls for maize grain, but the maximum NAN/MJ ME achieved was only 2.1 (Cole et al., 1976).

Form and processing of cereals

Table II shows an advantage in NAN/MJ ME in feeding rolled barley rather than ground maize in concentrates. The effect is particularly marked at high levels of concentrate inclusion. Steam flaking of barley has been found to increase DAAS in sheep and cattle. Provided that urea is included in the ration, the same effect can be achieved with maize. Micronizing, rather than steam-flaking the grain, exaggerates these effects and it appears that the benefit in TAAS is at least as great as that for DAAS (Papasolomontos and Wilkinson, 1976). This means that the intestinal digestibility of amino acids was not impaired even by heat processing of the cereal. Papasolomontos and Wilkinson suggested that increases in DAAS were the result of an increase both in undegraded food and microbial protein supply. The latter is undoubtedly linked to more efficient N capture in the rumen with more rapidly fermented diets.

Heat treatments

Heat treatment of cereals was considered in the preceding section. Heat applied to forages during pelleting increases NAN/MJ ME in sheep (Coelha da Silva et al., 1972a,b). In cows an increase in NAN/MJ ME from 2.5 to 2.8 was achieved by drying and pelleting grass rather than ensiling it (Tamminga, 1975). In view of likely demands for NAN/MJ ME this is significant. High-temperature dehydration has been found to increase NAN/MJ ME from grass silage treated with formaldehyde in addition to the effect of formaldehyde (Beever et al., 1977). Heat treatment of forages appears to increase both DAAS and TAAS. This is important as overheating of proteins can have severe effects on amino acid absorption (Ford, 1975).

Heat treatment of protein supplements during processing could obviously have important consequences. Smith et al. (1978) have found that heating of soya bean meal increases NAN/MJ ME in calves, but only from 1.8 to 2.0 g/day. Apart from this comparison the folklore about the effect of heating protein meals on passage of amino acids to the duodenum largely rests on the known effects of heating on solubility of proteins. But it is not correct to assume that solubility is a good index of amino acid supply to the duodenum (Smith et al., 1978) although it seems reasonable to say that those proteins which are heated during processing are most likely to enhance NAN/MJ ME.

Chemical treatment

The best established is formaldehyde treatment (see Hagemeister, 1977). An increase of duodenal NAN/MJ ME intake from 2.4 to 2.7 has been achieved by formaldehyde "protection" of soya bean meal (Vérité et al.,

1977) and from 2.1 to 2.9 by formaldehyde "protection" of casein (Hagemeister et al., 1976). It is interesting that a similar increase (0.8 units) but from a higher "basal" level (2.9 g NAN/MJ ME) was produced in sheep fed dried grass diets supplemented with casein or "formaldehyde-protected" casein (MacRae et al., 1972). The results with soya bean meal and casein are obviously significant in relation to the needs of the high yielding cow.

Preservation of silage with formaldehyde has successfully increased NAN/MJ ME in sheep (Beever et al., 1977). Formaldehyde and formic acid applied together to silage did increase NAN/MJ ME in cows — from 2.5 to 2.8 g/day (Tamminga, 1975).

Other chemical agents have not been evaluated in these terms although production responses have been found. For example, lamb growth rate was increased by glyoxal treatment of food protein (Peter et al., 1971).

Yet other materials, e.g. N-methylolacrylamide, have shown promise with *in vitro* trials, but have not been tested *in vivo* (Friedman and Broderick, 1977).

In all cases care must be exercised in ensuring that both DAAS and TAAS are increased, since chemical agents, like heat, can reduce amino acid absorption.

Urea addition

This can only be expected to increase NAN/MJ ME when microbial needs for N have not been met. Urea addition to maize silage and concentrate rations fed to cows, or barley concentrates fed to sheep, has increased NAN/MJ ME up to a maximum of 2 g/day (Ørskov et al., 1971; Vérité, 1978) but this seems to be the limit of supply which can be achieved by this means. The reason that this rate of supply exceeds that which has been suggested for "microbial" N/MJ ME (for example, 1.25 g/day by Roy et al., 1977) is that with practical rations there will always be some "undegraded" food protein, and endogenous protein, passing to the duodenum.

Other methods

(a) The natural properties of different food proteins are likely to make them resistant to rumen degradation to different extents and so may influence NAN/MJ ME. Exploitation of these properties is difficult because processing may mask or confound differences found by experimentation. Results have been equivocal. For example, Smith et al. (1978), using calves, found that supplementation of a hay/flaked maize basal ration with fishmeal, heated soya bean meal or groundnut meal increased duodenal NAN flow from 1.2 to 2.2, 2.0 and 1.7 g/day/MJ ME intake, but similar protein supplements fed to cows all supplied 2.4—2.6 g NAN/MJ ME (Hagemeister et al., 1976). Although the position of cannulas in relation to the entry of bile and pancreatic secretions into the gut might explain differences in the magnitude of NAN flow between these two reports, relative values between protein sources

should not be affected. Since rumen degradability of food protein (the proportion of food-N eaten which passes direct to the duodenum) is as much dependent on the way the food is processed and fed as on its natural properties, methods to vary NAN/MJ ME might make use of natural properties, but could not rely on them totally.

(b) Frequent feeding has been used to moderate the rate of rumen fermentation and to try to improve nutrient utilisation (e.g. urea). Information on effects on amino acid supply is virtually nil, although MacRae et al. (1972) noted no change in DAAS on feeding dried grass 24 rather than 2 times daily. NAN/MJ ME was not changed, at 2 g/day, when a 75% concentrate, 25% hay ration was fed to cows twice or 24 times daily (Sutton, Youssef and Oldham, unpublished results). These data suggest no advantage in more frequent feeding as a method to manipulate NAN/MJ ME with these feeds but this does not exclude the possibility that there may be an advantage when rapidly fermentable N is fed.

(c) Considerable interest has been shown in using "protected lipids" (lipids coated with protein, e.g. soya, and treated with formaldehyde) to maximise ME intake in high yielding cows. It is possible that these products may also affect NAN/MJ ME. The few data available suggest that unprotected lipids have no effect but that, at a relatively high level of inclusion in sheep diets, protected lipids may reduce NAN/MJ ME (from 2.6 to 2—2.3 g/day; Knight et al., 1978). This might explain the drop in milk protein often found at high levels of protected lipid feeding (see Oldham and Sutton, 1979).

RUMEN TURNOVER, AMINO ACID SUPPLY AND "MICROBIAL SYNTHESIS"

Rumen turnover has been given a lot of attention because a high turnover rate may not only reduce food protein breakdown, by reducing retention time in the rumen, but also increase the energetic efficiency of microbial growth.

Here again we encounter technical problems. There is good evidence that the pattern of removal of different sized particles and of soluble materials from the rumen is very complex (Faichney and Griffiths, 1978). The usual approach has been to use the fractional outflow of PEG or Cr-EDTA to measure "rumen fluid dilution rate". It is perhaps better to use the term "fractional outflow of marker" (FOM) since this is what is measured.

FOM has been correlated with food intake, but at the same time has been found to give poor correlation with microbial growth efficiency in cows (Tamminga, 1978). Oldham et al. (1979 and unpublished) (Table III) have found that even at one level of intake both diet, and animals-within diet, show wide variations in FOM measured using PEG. Correlation of FOM with microbial N yield/kg OM digested in the rumen was poor (Table II). Despite the wide variation in FOM with diets, duodenal amino acid supply (Table II) was relatively constant both between diets and cows. It appears that FOM, using PEG, does not measure a parameter which has a large effect on DAAS. Methods which increase FOM cannot be relied upon to increase NAN/MJ ME.

PARTITION OF DUODENAL AMINO ACIDS INTO "MICROBIAL" AND "FOOD" AND EFFECTS ON THEIR RELATIVE CONTRIBUTIONS TO SUPPLY

Bearing in mind the comments made earlier, duodenal supply can be partitioned into its component parts. The result of doing this for four diets is shown in Table III.

Replacement of barley by ground maize in concentrates decreased microbial supply and both the "energetic" and "nitrogen" efficiencies of microbial N capture, and increased food N supply. The net effect of these dramatic changes in partition of supply was a small decrease in total duodenal supply (Table II). Beever et al. (1977) and Knight et al. (1978) produced equally dramatic shifts in the proportion of duodenal amino acids identified as "microbial" or "food" by feeding untreated, formaldehyde-treated or formaldehyde-treated and dehydrated grass silages or lipid supplement to sheep. The effects on duodenal amino acid supply were much smaller, and on the supply of "absorbed" amino acids smaller still. Are these real effects or artifacts of the techniques used to partition amino acid supply? This is an important question to answer if mean values for such things as microbial N yield per kg rumen-digested OM are to be used in feeding systems.

Consequent on the measurements of duodenal microbial supply are measurements *in vivo* of food protein "degradability" or the amount of "bypass protein" reaching the duodenum. Roy et al. (1977) have tentatively suggested values for the proportion of dietary N likely to pass "undegraded" from the rumen for a range of protein supplements. If the assumption is made that microbial protein supply to the intestines remains constant when the dietary N source changes (all currently published "metabolisable protein" systems do assume this), then DAAS should be capable of manipulation by changing the dietary N source.

All of the preceding discussions show how relatively difficult it is, at present, to ascribe variation in DAAS to such a simple mechanism as variation of undegraded food protein in excess of microbial supply. However, the future success of systems designed to predict variation in DAAS by describing "microbial" and "food" protein components entering the intestines will depend on improvements in both the precision and accuracy of methods for partitioning duodenal amino acids into "microbial", "food" and "endogenous" contributions. If these protein requirement systems are to be adopted into practice, then these technical improvements will be an important aim of future research.

CONCLUSIONS

Clear statements on methods to vary duodenal amino acid supply to dairy cows are difficult to make because of inadequacies in available information. Firstly, there are very few data from experiments with milking cows fed at high levels of intake. An important question to answer is to what extent principles established with ruminants fed at low levels of intake can be used

to make quantitative predictions for dairy cows. For example, Armstrong et al. (1977) have suggested that intestinal absorption of amino acids may be reduced at high levels of intake – this has clear implications for the dairy cow.

Secondly, animal replication in experiments to measure DAAS has usually been inadequate to establish quantitative relationships reliably. This is particularly so for work with dairy cows. Reasonable guidelines are however available to improve experimental designs in the future.

Thirdly, available methods to partition DAAS into amino acids of microbial or food origin are highly suspect, and so quantitative description of variation in these components is limited.

Limits to variation in supply of non-ammonia N to the duodenum can be identified and appear to be 2.5—3 g NAN/MJ ME intake. Energy intake is therefore an important determinant of DAAS. Heat and formaldehyde treatment of proteins are, perhaps, the most reliable ways of increasing DAAS within this limit. These methods will only be successful in affecting animal performance if (1) the proteins are not denatured to the extent that intestinal absorption of amino acids is reduced so that the net effect on amino acid supply to the tissues is a reduction in supply, and (2) the animal has the metabolic capacity to respond to an increase in amino acid supply, that is, the requirements for amino acids have not already been met. Amino acids supplied in excess of requirements would be useful only as energy-yielding substrates. The concept of amino acid requirements for the dairy cow is examined in the following paper.

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RESUME

Oldham, J.D. et Tamminga, S., 1980. Utilisation des acides aminés par les vaches laitières. I. Moyens de faire varier l'apport d'acides aminés. *Livest. Prod. Sci.*, 7: 437—452 (en anglais).

La quantité d'acides aminés apportée dans le duodénum est un point crucial des nouveaux systèmes d'alimentation azotée, mais le métabolisme des acides aminés au cours de leur passage dans la paroi digestive et dans l'organisme modifie les quantités apportées au foie et aux tissus périphériques.

La mesure de la quantité totale d'acides aminés entrant dans le duodénum (DAAS) peut être sûre si elle est reproduite sur suffisamment d'animaux, mais la séparation entre acides aminés d'origine alimentaire et acides aminés d'origine microbienne n'est pas sûre en raison des insuffisances des techniques disponibles. Les commentaires sur les moyens de faire varier les apports alimentaires et microbiens ne sont encore guère plus que qualitatifs.

Il semble y avoir une limite à l'apport d'azote non ammoniacal (NAN) dans le duodénum par MJ d'énergie métabolisable (ME) ingérée, qui ne change pas avec le niveau d'ingestion énergétique (MJ ME/$kg^{0.75}$). Cette limite est voisine de 2.5—3 g de NAN/MJ d'ME à la fois pour la ration de "concentrés" et de "fourrages". Les vaches pouvant avoir besoin de 2.6 g de NAN/MJ d'ME, à la pointe de leur lactation, il faut évaluer les méthodes d'apport d'acides aminés dans le duodénum par rapport à cet objectif. Pour atteindre des apports élevés, on peut utiliser les traitements à la chaleur, particulièrement pour les fourrages, et le traitement des protéines par le formol.

KURZFASSUNG

Oldham, J.D. und Tamminga, S., 1980. Aminosäureverwertung bei Milchkühen. I. Methoden wechselnder Aminosäureversorgung. *Livest. Prod. Sci.*, 7: 437—452 (in Englisch).

Die Bezeichnung Aminosäureversorgung bedarf der näheren Erläuterung — die duodenale Aminosäureversorgung (DAAS) wird heute als überaus bedeutend bei neuen Systemen der Proteinzuteilung angesehen, ausserdem beeinflusst der Aminosäurestoffwechsel während der Passage durch die Darmwand und im Körper die Versorgung der Leber und der peripheren Gewebe.

Die Messung der DAAS kann zuverlässig sein, vorausgesetzt eine vergleichbare Reaktion der Einzeltiere ist gegeben; eine Untergliederung jedoch in Aminosäuren mikrobieller Herkunft oder aus Futterstoffen ist unzuverlässig, da es an brauchbaren Techniken fehlt. Berichte über Methoden, die Versorgung mit mikrobiellen oder Futter-Aminosäuren zu variieren, haben mehr qualitativen Bezug.

Es scheint eine Grenze für die duodenale Nicht-Ammonium-Stickstoff (NAN) Versorgung/MJ ME-Aufnahme zu geben, die von der Höhe der Futteraufnahme (MJ ME/$kg^{0.75}$) unbeeinflusst bleibt. Diese Grenze liegt nahe bei 2.5—3.0 g NAN/MJ ME-Aufnahme und zwar sowohl für "Kraftfutter-" als auch für "Rauhfutter-" Rationen. Kühe benötigen wahrscheinlich 2.6 g NAN/MJ ME während der Höchstleistungsphase, deshalb sollten Methoden einer Veränderung der DAAS daraufhin untersucht werden. Hitzebehandlung besonders von Rauhfutter sowie Formaldehydbehandlung von Proteinen sind einsetzbare Methoden, um hohe Werte einer duodenalen NAN-Versorgung zu gewährleisten.

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0. AMINO ACID UTILISATION BY DAIRY COWS. II. CONCEPT OF AMINO ACID REQUIREMENTS

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ABSTRACT

Tamminga, S. and Oldham, J.D., 1980. Amino acid utilisation by dairy cows. II. Concept of amino acid requirements. *Livest. Prod. Sci.*, 7: 453—463.

In dairy cows amino acids may be required for four processes: maintenance; as precursors for the synthesis of glucose (gluconeogenesis); for protein deposition in muscle or associated with foetal growth; and for the synthesis of milk protein.

Estimates of the protein requirements for maintenance are often conflicting and seem far from accurate. Estimates of the ratio in which essential amino acids are required for maintenance are not yet available.

Requirements of amino acids for gluconeogenesis are difficult to estimate. It seems rather unlikely that essential amino acids will be used in significant quantities for gluconeogenesis, even at high milk yields.

Protein requirements for pregnancy and muscle growth in dairy cows are relatively low. The ratio in which essential amino acids are supplied in the blood seems adequate for both processes.

Amino acid requirements for milk protein synthesis are somewhat higher than the net protein output. This is particularly true for essential amino acids of which, in the mammary gland, a surplus of some 50% needs to be extracted from the blood. The ratio in which essential amino acids are supplied to the mammary gland seems reasonably adequate for milk protein synthesis.

INTRODUCTION

In Part I (Oldham and Tamminga, 1980, this issue), factors influencing the quantity of amino acids entering and subsequently passing through the wall of the small intestine into the blood were discussed. After entering the blood, the amino acids can be used for various purposes.

In a lactating dairy cow the mammary gland is the site where a major part of the absorbed amino acids are required for the synthesis of milk protein. Apart from this the animal has a maintenance requirement which will be met first. A third requirement is that for amino acids needed as precursors in gluconeogenesis. Finally, the requirement for protein retention (i.e. muscle growth, pregnancy) has to be considered.

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Ruminants as well as non-ruminants need to be supplied at the tissue level with sufficient pre-formed essential amino acids or their C-skeletons. This group contains arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine. Sometimes cyst(e)ine and tyrosine are also considered essential for milk production (Clark et al., 1978).

Protein requirements of animals at the tissue level can therefore be defined as requirements for essential amino acids and for precursors with a suitable N source to synthesize the necessary non-essential amino acids. Three major questions with respect to these requirements arise.

(1) What quantities of amino acids are required for a given milk production including a requirement for maintenance, gluconeogenesis and protein retention?

(2) What proportion of these amino acids must be essential amino acids?

(3) In what ratio are these essential amino acids required?

PROTEIN AND AMINO ACIDS REQUIRED FOR MAINTENANCE

With respect to protein requirements for maintenance, these are difficult to estimate. One approach is to estimate the total of inescapable losses in urine (endogenous urinary nitrogen = EUN), in faeces (metabolic faecal nitrogen = MFN) and in hair and skin (Agricultural Research Council, 1965).

Based on a large number of data from the literature, Boekholt (1976) concluded that, above 200 kg liveweight, EUN becomes a constant per kg $W^{0.75}$ and amounts to 0.10 g N/KG $W^{0.75}$/day. Estimating MFN with linear regression resulted in a value of 4.9 g N/kg dry matter ingested (Boekholt, 1976). Inescapable losses in hair and skin have been estimated to be 0.02 g of N/kg $W^{0.73}$ (ARC, 1965).

Because of acceptability problems with such diets, estimates of inescapable losses are rarely based on results obtained with animals fed N-free diets. The validity of extrapolating these results to animals in full production may also be questioned.

A more frequently used approach is to estimate N requirements for maintenance from N-balance data, extrapolated to N-retained + N-output in milk = zero. This also results in a rather inaccurate estimate, because extrapolation often takes place to a point far away from the data on which the regression calculations were based (Boekholt, 1976; van Es and Boekholt, 1976).

Estimates of N-requirements for maintenance are not very accurate and often conflicting. This is illustrated by comparing the maintenance protein requirements for a 600 kg lactating cow in the various new protein evaluating systems (Vérité et al., 1979); values range between 100 and 395 g α-amino N × 6.25 absorbed daily from the intestine. Part of these differences arise because "α-amino N absorbed from the intestine" is not defined in exactly the same way in all systems. In some systems the faecal loss of endogenous protein is included in the protein requirement, in others in the protein value of the feed. Besides, the faecal loss of endogenous protein is not clearly de-

fined. According to Hogan (1975), only about one third of the MFN is of endogenous origin, the remaining two thirds being of microbial origin, part of which may however originate from endogenous secretions in the abomasum and small intestine due to conversion to microbial protein in the hind gut. Even if these aspects are taken into account, substantial differences remain in maintenance requirements for protein between the different new protein evaluating systems for ruminants.

Part of the amino acids required for maintenance are those metabolized in the gut wall and liver. An estimate of amino acids metabolized in the gut wall can be obtained by comparing amino acids disappearing from the small intestine and those appearing in the portal blood. Only very few such data are available from experiments in the same animals and these are restricted to sheep. For dairy cows such data are entirely lacking. Such measurements require complicated surgical techniques and the results do not have a high degree of precision. Besides, the results for individual amino acids are often invalidated by interconversions in the gut wall. Part of the glutamate taken up by the gut wall is converted and subsequently released in the portal blood as alanine, whereas arginine may be converted to ornithine and/or citrulline (Tagari and Bergmann, 1978). Also, amino acids disappeared from the small intestine, but not appearing in the portal blood, may be utilised for the synthesis of enzymes.

From the results of experiments in which the apparent absorption from the small intestine was measured in one group of sheep, and the appearance of amino acids in the portal blood measured in another group of sheep fed comparable diets, MacRae (1978) found evidence that a substantial part of most amino acids apparently absorbed from the small intestine were metabolized in the gut wall in metabolic processes associated with absorption.

In sheep fed two different diets, 800 g/day of a high (19.8% crude protein) or 650 g/day of a medium (15.6% crude protein) protein content, both measurements were made in the same animals (Tagari and Bergmann, 1978). Their results largely confirmed MacRae's suggestions, in that a large part of the absorbed amino acids were metabolized in the gut wall. No preference appeared for either essential or non-essential amino acids. When the medium protein diet was fed, 67 and 71% of essential and non-essential amino acids respectively were metabolized in the intestinal wall. After feeding the high protein diet, these figures were 55 and 57% respectively.

Most of the experiments referred to were performed with animals kept at or slightly above maintenance, and under such conditions the gut wall seems to metabolise a major proportion of the amino acids absorbed from the small intestine. From the data of Tagari and Bergmann (1978) we calculated that increasing the amount of amino acids absorbed from the small intestine of sheep from 2.8 to 4.5 g/kg $W^{0.75}$ per day decreased the proportion which was not released in the portal blood from 71 to 52%. This suggest that, in a high-producing dairy cow in which, per kg $W^{0.75}$, two to three times more amino acids are absorbed from the small intestine than in the sheep referred to

above, a much smaller proportion of the absorbed amino acids are metabolized in the gut wall.

The next major organ with respect to amino acid metabolism is the liver. From arterio-venous differences, estimates can be made of the amino acids metabolized by the liver. Recalculation of the data of Wolff et al. (1972), as presented by Bergmann and Heitmann (1978), shows that the liver seems to have a preference to metabolize non-essential amino acids.

If the uncertainties mentioned above are taken into account, it becomes almost entirely speculative to assess the preferable proportion of essential to non-essential amino acids required for maintenance. Hogan (1975) assumed that for maintenance the amino acids are required in the same ratio as they are found in meat protein. He therefore assumed that 56% of the N required for maintenance should be present as essential amino acids. The high content of sulphur-containing amino acids in hair and skin suggests a high requirement for these amino acids, but the contribution of the requirements of hair and skin in large animals to the total maintenance requirement is only small.

PROTEIN AND AMINO ACID UTILISATION FOR GLUCONEOGENESIS

Like other animals, ruminants need glucose for various metabolic processes. The role of amino acids in glucose metabolism in ruminants depends on the balance between demand and supply of glucose or glycogenic precursors.

High-producing dairy cows have a high demand for glucose, because they release lactose in the milk in large quantities, for the synthesis of which glucose is needed as a precursor. Glucose is also believed to be used as a source of NADPH, needed in the synthesis of milk fatty acids.

The majority of dietary carbohydrates from which glucose could be derived to meet the high requirement is degraded in the forestomachs to volatile fatty acids of which only propionic acid is glycogenic. Occasionally, a significant amount of lactic acid is formed, which is also glycogenic. If diets containing large quantities of maize or sorghum are fed, significant amounts of α-glucose polymers may escape degradation in the rumen (Waldo, 1973). At least part of this is expected to be hydrolysed in the small intestine and subsequently absorbed as glucose. A further supply of glycogenic precursors is possible from glycogenic amino acids, either in the case of a shortage of glycogenic precursors from other sources, or because of a surplus of glycogenic amino acids which are not required or cannot be utilised as precursors for protein synthesis. The latter situation may occur if less amino acids are required than are absorbed from the small intestine, or if amino acids are required in a ratio differing from that in which they are absorbed.

The utilisation of amino acids as precursors for gluconeogenesis is only competitive in meeting the protein needs of animal tissues if carbon skeletons of limiting essential amino acids are used, or if it causes a shortage of precursors for the synthesis of non-essential amino acids.

Only part of the amino acids can be utilised as precursors for gluconeo-

genesis, because some amino acids are degraded into ketogenic intermediates or end products which are unsuitable as precursors for gluconeogenesis. Of the essential amino acids, lysine and leucine are completely ketogenic. Only arginine, valine, histidine, methionine and cyst(e)ine are glucogenic. Depending on its degradation pathway, threonine is glucogenic or only partly glucogenic. The remaining essential amino acids, isoleucine, phenylalanine, tyrosine and tryptophane are partly ketogenic and partly glucogenic (Boekholt, 1976).

In Table I the distribution of amino acids present in duodenal digesta (Tamminga, 1975) is shown. From these results it can be seen that 16% of the amino acids are essential and glucogenic, while 15% are essential and partly glucogenic. Not all amino acids are absorbed to the same extent, but differences seem relatively small (Armstrong and Hutton, 1975) and so a similar ratio may be expected for the total of absorbed amino acids.

TABLE I

Distribution of amino acids in duodenal digesta of dairy cows

Type of amino acid		Percentage
Essential:	ketogenic	15.4
	ketogenic + glucogenic	14.7
	glucogenic	16.4
Non-essential:	ketogenic	0
	ketogenic + glucogenic	4.6
	glucogenic	48.9
Total		100

If it is assumed that the glycogenic and ketogenic part of partly glycogenic amino acids are equally important and that no preference exists for certain amino acids to be used as precursors for gluconeogenesis, then no more than about one quarter of the amino acids used for gluconeogenesis could be essential amino acids.

Results on the fate of labelled amino acids injected in the blood of various species, including lactating dairy cows, were reviewed by Lindsay (1976). Only small amounts of activity appeared in glucose, but relatively high proportions of the amino acids were oxidised and the labelling appeared in CO_2, particularly in the case of non-essential amino acids. Oldham (1978) presented evidence that in dairy cows protein is utilised for protein production with an efficiency between 65 and 85%, leaving 15 to 35% of the absorbed protein as a possible precursor for gluconeogenesis. Taking into account that a major proportion of the "surplus" protein is oxidised and that only 55 g of glucose can be synthesised from 100 of protein, he concluded that in high yielding

dairy cows less than 2% of the required glucose could come from protein.

If essential amino acids were extensively used for gluconeogenesis, post-ruminal infusions of glucose should have a sparing effect on protein, resulting in an increased output in milk. Of 9 experiments reviewed by Clark (1975) in which glucose or mixtures of mono-, di- or higher saccharides were infused postruminally, only one showed a significantly increased output of milk protein. Similar results were obtained by Boekholt (1976). It should be realised however that glucose infusions may affect the endocrine balance, resulting in changes in glucose and/or amino acid utilisation by the animal tissues.

PROTEIN AND AMINO ACID REQUIREMENTS FOR PROTEIN RETENTION

In a young dairy cow some protein is needed for growth. In a lactating dairy cow the liveweight loss often observed in the first two months after parturition may include the mobilisation of some protein, whereas the liveweight gain normally observed in the second half of the lactation may include the retention of some protein. However, in the young growing dairy animal and in the lactating animal, protein retention is small compared with protein output in milk during lactation. The protein requirement for retention will therefore not be discussed in detail.

It has been estimated (ARC, 1965) that pregnancy causes the deposition of some 10 kg of protein in foetus, placenta, placental fluid and uterus, of which some 75% is present in the foetus. Compared to milk protein output during a lactation cycle (almost 200 kg of protein for a 6000 kg yielding animal) this is a quantity of minor importance, even if it is accepted that an important part of this deposition will take place in the last month of gestation. During this period it will result in a deposition of 0.12 to 0.15 kg/day (Jarrige et al., 1978), the protein equivalent of a milk production of some 4 kg/day. Protein turnover in the growing foetus is probably higher than with milk protein synthesis (Mepham, 1976; van Es and Tamminga, 1978), so the net utilisation for foetal growth will be lower. Even if this is taken into account the protein requirement for pregnancy is still small, even compared with the requirement for maintenance.

For protein deposition in muscle or tissue associated with pregnancy, the amino acids are likely to be required in a ratio similar to that in meat protein, so some 56% of the N in this protein should be present in essential amino acids (Hogan, 1975). The ratio in which amino acids are absorbed from the small intestine in dairy cows resembles very much the ratio in meat protein, so the composition of absorbed amino acids is likely to be adequate for protein deposition.

PROTEIN AND AMINO ACID REQUIREMENTS FOR MILK PROTEIN PRODUCTION

Finally let us consider what quantity of amino acids are required for a given protein output in milk.

Satter and Rofler (1975) assume that metabolisable protein, defined as the amount of protein absorbed from the gastrointestinal tract, is utilised above maintenance needs for milk protein production with an efficiency of 60%. A similar figure was suggested by van Es and Boekholt (1976), based on regression calculations. More recent estimates in recently developed protein evaluating systems for ruminants use slightly different values. In the ARC-system (Roy et al., 1977), a value of 75% has been accepted, Kaufmann (1977) uses 70%, whereas in the French PDI-system (Vérité et al., 1979) a value of 67% was calculated from feeding trials.

Oldham (1978) estimated the efficiency of protein utilisation (EPU) for milk production, based on measured intestinal protein supply (total of amino acids) and (partly estimated) milk productions in lactating dairy cows fitted with re-entrant cannulae in the small intestine. His estimate resulted in values between 27 and 59%, but no allowance was made for protein deposition in the body. Applying estimates of absorbed protein (Oldham, 1978) to animals in feeding trials and taking into account body weight changes, values ranging between 73 and 83% resulted. Efficiency tended to increase with a higher energy supply, suggesting that energy supply affects the efficiency of protein utilisation (Oldham, 1978).

A slightly different approach involves the use of the ratio between N output in milk and the output of N in milk plus that part of the plasma urea-N production resulting from amino acid catabolism. The latter was estimated to be approximately one third of the total plasma urea-N production (Oldham, 1978). This approach led to an estimate for EPU ranging from 0.62 to 0.72 (Oldham, 1979).

Estimates for the efficiency of utilisation of individual amino acids were derived from data on the oxidation of amino acids. This resulted in figures ranging from 0.61 to 0.84 for the different amino acids (Oldham, 1979).

From results of input—output studies in the mammary gland (Bickerstaffe et al., 1974), it was calculated (Clark et al., 1978) that amino acid N was utilised by the mammary gland with an efficiency of 91%. We did similar calculations on the data of Spires et al. (1975) and Clark et al. (1977) which gave slightly lower efficiencies, on average 83%. Values in new protein evaluating systems for the efficiency of utilisation of absorbed amino acids for milk protein synthesis, i.e. after making an allowance for maintenance of which amino acid metabolism in gut wall and liver are considered part, are all lower. Two explanations are possible. Absorbed protein in the new protein evaluating systems may contain larger amounts of N, not present in amino acids. A second explanation is that the results of arteriovenous differences yield overestimates of the efficiency, because they only take into account the uptake of free amino acids from the plasma and ignore the possibility of uptake of amino acids from blood cells or blood protein.

From the data of Bickerstaffe et al. (1974), Spires et al. (1975) and Clark et al. (1977), it appears that part of the non-essential amino acids excreted in milk protein are synthesized de novo in the mammary gland and the major

part of the N required for this synthesis seems to be provided by essential amino acids, particularly arginine, which is extracted by the mammary gland in quantities far in excess of the amount secreted in milk protein (Clark et al., 1975). The carbon-skeletons of essential amino acids, extracted from the blood in excess, may be used for the synthesis of non-essential amino acids or as a source of energy (Clark et al., 1975; Wohlt et al., 1977). It was calculated (Clark et al., 1978) that in milk protein excreted in the experiments of Bickerstaffe et al. (1974), 60% of the N was present in essential amino acids. Of the total of amino acids taken up by the mammary gland in the same experiments, 71% of the N was present in essential amino acids. A similar increase was found in the experiments of Spires et al. (1975) and Clark et al. (1977). Hence, essential amino acids extracted from the blood are utilised by the mammary gland for milk protein synthesis with a lower efficiency than the total of extracted amino acids. Combining the data of Bickerstaffe et al. (1974), Derrig et al. (1974), Spires et al. (1975) and Clark et al. (1977) we calculated a mean value of 67%, suggesting that for a given output of essential amino acids in milk protein a surplus of some 50% must be extracted from the blood.

With respect to the ratio in which essential amino acids are required for milk protein synthesis from the arteriovenous differences, it appears that except for arginine, essential amino acids are required in a rather similar ratio to that in which they are excreted in milk. In studying apparent absorption of amino acids in dairy cows, it was found (Tamminga and van Hellemond, 1977) that the proportion of arginine in apparently absorbed essential amino acids was considerably higher than its proportion in the essential amino acids of milk protein. Arginine does not therefore appear to become a limiting amino acid for milk production. From the various research papers in the literature dealing with this subject, it appears that methionine is most often regarded as the first limiting amino acid for milk protein production, followed by lysine. Comparing the ratio in which essential amino acids are present in duodenal digesta (Hogan, 1975), rumen bacteria (Hagemeister and Kaufmann, 1978) or apparently absorbed protein (Tamminga and van Hellemond, 1977) with the ratio in which they are excreted in milk protein, indicates that there is not much evidence for one of the essential amino acids being limiting for milk protein synthesis per se. This conclusion is in agreement with the often observed lack of response to postruminal infusions of individual essential amino acids as reviewed by Clark (1975). Better responses were obtained after infusions of mixtures of essential amino acids or casein.

If, under certain conditions, one essential amino acid seems limiting for milk protein synthesis, this may be caused by other requirements such as those of maintenance or gluconeogenesis.

CONCLUSIONS

One can envisage that in the future we may expect protein requirements

for ruminants to be expressed in terms of a requirement for essential amino acids and a requirement for precursors and a suitable N source for the synthesis of non-essential amino acids.

A lack of data on N transactions in the digestive tract and tissues of high-producing dairy cows still exists. Consequently little is known on the amino acid requirements for maintenance, pregnancy and gluconeogenesis. It seems likely that net glucose synthesis from amino acids makes only a small contribution to the glucose requirement of the lactating cow.

The supply of amino acids for the high yielding dairy cow sometimes seems inadequate. No single amino acid appears clearly limiting for milk protein synthesis under most conditions; occasionally methionine may become limiting. The net transfer of absorbed amino acids into milk protein seems generally to fall in the range 65—70%. Within the mammary gland the efficiency of utilisation of amino acids is higher, with 85—90% of the extracted amino acids appearing in milk protein. For essential amino acids these figures are somewhat lower, while for non-essential amino acids it is somewhat higher.

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RESUME

Tamminga, S. et Oldham, J.D., 1980. Utilisation des acides aminés par les vaches laitières. II. Concept des besoins en acides aminés. *Livest. Prod. Sci.*, 7, 437—452 (en anglais).

Les vaches laitières ont besoin d'acides aminés pour quatre processus: l'entretien, la néoglucogénèse, le dépôt des protéines dans les muscles ou les produits de la conception et la synthèse des protéines du lait.

Les estimations des besoins azotés d'entretien sont souvent divergentes et semblent loin d'être précises. On ne connaît pas encore le rapport dans lequel les acides aminés indispensables sont nécessaires pour l'entretien.

Il est difficile d'estimer les besoins en acides aminés pour la néoglucogénèse. Il est peu probable que les acides aminés indispensables y soient utilisés en quantités significatives, même lorsque la production laitière est élevée.

Les besoins azotés pour la gestation et la croissance musculaire des vaches laitières sont relativement faibles. Le rapport dans lequel les acides aminés indispensables sont fournis dans le sang semble adéquat pour ces deux processus.

Les besoins en acides aminés pour la synthèse des protéines du lait sont supérieurs à la quantité exportée. Cela est particulièrement le cas pour les acides aminés indispensables dont un excès de quelques 50% semble nécessaire. Le rapport dans lequel les acides aminés indispensables sont fournis semble être à peu près adéquat pour la synthèse des protéines du lait.

KURZFASSUNG

Tamminga, S. und Oldham, J.D., 1980. Aminosäureverwertung bei Milchkühen. II. Zum Konzept des Aminosäurenbedarfs. *Livest. Prod. Sci.*, 7: 437—452 (in English).

Milchkühe benötigen Aminosäuren für 4 Vorgänge, nämlich für die Erhaltung, als Vorstufen der Glukosesynthese (Glukoneogenese), zur Proteinbildung im Muskel oder beim foetalen Wachstum und zur Synthese des Milchproteins.

Schätzungen des Proteinbedarfs für die Erhaltung sind oft widersprüchlich und ungenau. Schätzungen des Anteiles der essentiellen Aminosäuren am Erhaltungsbedarf sind noch nicht verfügbar.

Der Bedarf an Aminosäuren für die Glukoneogenese ist schwierig abzuschätzen. Es scheint ziemlich unwahrscheinlich, dass essentielle Aminosäuren in signifikanten Mengen zur Glukoneogenese herangezogen werden, nicht einmal bei hohen Milchleistungen.

Der Proteinbedarf für Trächtigkeit und Muskelwachstum ist bei Milchkühen relativ niedrig. Der Anteil, in dem essentielle Aminosäuren im Blut zur Verfügung stehen, scheint für beide Vorgänge ausreichend.

Der Aminosäurenbedarf für die Milchproteinsynthese liegt etwas über dem Nettoproteinertrag. Dies gilt besonders für essentielle Aminosäuren, an denen ein Überschuss von 50% notwendig erscheint. Der Anteil, in dem essentielle Aminosäuren bereitgestellt werden, scheint für die Milchproteinsynthese ausreichend.

11. FEEDING PRINCIPLES OF THE HIGH YIELDING DAIRY COW WITH SPECIAL REFERENCE TO ENERGY/PROTEIN RELATIONSHIPS. A GENERAL DISCUSSION.

INTRODUCTION

The genetic potential of dairy cows to produce milk has improved tremendously during the last decades and is still improving. In order to respond to this potential the dairy cow requires large amounts of nutrients, particularly in early lactation. Unfortunately feed intake is often insufficient during this period. Milk production in such animals may become limited by an inadequate supply of nutrients rather than by genetic limits. In order to fully exploit the high genetic potential of such animals new feeding strategies are needed. In the following chapter an attempt will be made to outline the principles of such feeding strategies, taking into account as much as possible the findings reported in the preceding chapters of this thesis.

NUTRIENT REQUIREMENTS AND SUPPLY IN HIGH PRODUCING DAIRY COWS

Discussion will be restricted to the two major classes of nutrients, energy yielding nutrients and protein or amino acid yielding nutrients. The pools of energy yielding and amino acid yielding nutrients are very much interrelated. Amino acid yielding nutrients or proteins contribute to the pool of energy yielding nutrients. Part of the pool of amino acid yielding nutrients has a rather linear dependance on part of the pool of energy yielding nutrients. As explained in chapters 2 and 9, the quantity of microbial amino acids synthesized in the forestomachs is related to the amount of organic matter degraded in the forestomachs. Particularly the conversion of carbohydrates into volatile fatty acids is important in this respect (Tamminga, 1979a).

Because of the relationship between energy and protein supply, the nutrient requirements of dairy cows are best considered as a requirement for energy, of which a proportion has to be supplied as protein. This proportion varies for different processes. In the high yielding dairy cow two energy requiring processes are important. First of all energy is required for maintenance. The requirement of metabolisable energy (ME) for maintenance of dairy cows

equals about 488 kJ ME/$W^{\frac{3}{4}}$ (Van Es, 1978). If we want to calculate this back to absorbed nutrients a correction of about 10% for the heat of fermentation lost from the forestomachs for which the ME figure is not corrected is necessary. The requirement in terms of absorbed nutrients becomes then 439 kJ/$W^{\frac{3}{4}}$. According to the recently developed French PDI-system (Vérité et al., 1979) a new protein evaluating systems for ruminants, 3.25 g of absorbed protein is required per kg $W^{\frac{3}{4}}$ for maintenance of cattle. The energy content of this amount of protein equals 78 kJ. So for maintenance a proportion of approximately 78/439 = 0.18 of the energy in absorbed nutrients must be supplied as protein.

A similar calculation can be made for the energy and protein required for milk production. The energy content of milk with 4% fat equals 3054 kJ/kg (Van Es, 1978). The efficiency of utilisation of ME for milk production equals 0.60, but again this should be corrected for the 10% heat of fermentation lost from the forestomachs, which is counted as ME. Expressed as absorbed nutrients the efficiency of utilisation becomes therefore 0.67 and the requirement for milk production 4558 kJ absorbed nutrients per kg of milk. The protein content of milk with 4% fat averages 33 g/kg. The efficiency of utilisation of absorbed protein was estimated to be 0.67 (Vérité et al., 1979) so some 50 g of absorbed protein is required for the production of 1 kg of milk. The energy content of this protein equals 1175 kJ, so a proportion close to 0.26 of the energy in absorbed nutrients required for milk production should be supplied as protein. This proportion may increase somewhat if significant amounts of amino acids are needed for gluconeogenesis (see Chapter 10).

The high producing dairy cow can meet her energy requirements for maintenance and milk production from two sources. The main part comes from absorbed nutrients, but if this supply is inadequate to meet the requirements an additional supply is possible from energy stored in the body. With regard to the N requirement the results in chapter 9 show that in dairy cows the maximum flow of ammonia-free N (NAN) into the duodenum seems to be about 3.0 g NAN/MJ ME intake. However these upper limits were largely based on results found in animals equipped with cannulae beyond pancreatic and biliary duct where some 15% of the NAN may be expected to be of endogenous origin. So the true maximum is probably close to 2.5 g of NAN/MJ ME intake. From the results in chapter 9 (Table 3) it appears that 11% of the N in duodenal digesta is found in nucleic acids. The maximum flow of true protein N becomes then 2.2 g/MJ ME intake. If it is assumed that a proportion of 0.75 of this true protein N is being absorbed (Tamminga, 1980) and that the absorbed true protein contains 16% of

N, then 10.3 g of absorbed true protein will be supplied per MJ ME ingested. Correcting the ME for 10% heat of fermentation, changes this to 10.3 g of absorbed true protein per 0.9 MJ in absorbed nutrients. The energy content of the absorbed protein equals 246 kJ, so a maximum proportion of 0.27 of the energy in absorbed nutrients may be supplied as protein. This is very close to what is required for milk production.

It is generally assumed that mobilisation of body reserves can only contribute significantly to the pool of amino acids in the very beginning of the lactational period. The contribution of protein to the energy mobilised after the first few weeks after calving is small and does usually not exceed 8 to 10% (Van Es, pers. comm.).

In Table 1 a summary is given of the proportion of the energy being required for or being present in the various processes which are important in high producing dairy cows.

Table 1. Proportion of energy required as or supplied by protein in various processes.

process	proportion of energy in protein
maintenance	0.18
milk production	0.26
absorption of nutrients	max. 0.27
mobilisation of reserves	0.08

From these figures it can be calculated under what conditions protein becomes limiting for milk production. This depends on the level of milk production and on the contribution of mobilised fat to the energy supply of the animal as is shown in Table 2.

Table 2. Proportion of energy to be supplied as protein in absorbed nutrients under different conditions in dairy cows.

production level kg/day	x maint.	contribution of fat mobilisation 0	0.2M	0.4M	0.6M	0.8M	1.0M
0	1	0.18	0.21	0.35	0.33	0.58	
10	2	0.22	0.24	0.26	0.28	0.31	0.36
20	3	0.233	0.24	0.26	0.27	0.29	0.31
30	4	0.240	0.25	0.26	0.27	0.28	0.29
40	5	0.244	0.25	0.26	0.27	0.28	0.28
50	6	0.247	0.25	0.26	0.27	0.27	0.28

The figures seem to indicate that protein supply at high levels of milk production tends to become inadequate only if the intestinal protein supply per MJ ME ingested is lower than what is maximally possible or if feed intake is inadequate and energy (fat) is mobilised from within the body. It should also be realised that in the latter case part of the protein may be used to meet the energy requirement rather than the protein requirement.

Maintaining the intestinal protein supply at a maximum level as related to energy supply can be achieved in various ways as was shown in various chapters of this thesis. Two major possibilities which could be exploited simultaneously appear to exist. First of all microbial protein synthesis should be maximised. To achieve this rumen fermentation should be as close to steady state as possible, preferably occurring at a ruminal pH of between 6.5 and 7.0. Possible ways to achieve this may be frequent feeding (Chapter 8), the inclusion of buffers in the diet (Erdman, 1980) or the inclusion of less easily degradable carbohydrates in the diet (de Visser & de Groot, 1981). Another way by which the intestinal protein supply can be increased is the inclusion of protected proteins in the diet, as was shown or discussed in the Chapters 2, 5 and 9.

EVALUATION OF RUMINANT FEEDS FOR THEIR PROTEIN VALUE

A major question at present is what kind of protein evaluating system should be used in ruminant feeding. At present the digestible crude protein (dcp) system is still being used in the Netherlands (Anonymus, 1977). Under certain conditions the dcp system has severe limitations (Tamminga, 1979b), but in the Netherlands it is still considered to be a workable system. However in surrounding countries the dcp system has already been abandoned and new systems were introduced in France (Anonymus, 1978) and more recently in Great Britain (Anonymus, 1980). A key question in these new systems is how feed compounds should be evaluated for their protein value. In the French system two values are attributed to each feedstuff, a value expressing the amount of absorbable feed protein escaping microbial degradation in the rumen and a value representing the amount of absorbable microbial protein which can be synthesized from a given feedstuff. In the British system feeds are divided in classes of degradability and for the whole diet the potential to produce microbial protein is estimated.

It is beyond doubt that two components, both of which contribute to the

protein supply of the host animal should be taken into account. They are the amount of digestible dietary protein which escapes microbial degradation in the forestomachs and the amount of microbial protein which can be synthesized in the forestomachs from a given feedstuff. A severe drawback is that the reliability and accuracy of the measuring methods developed to measure undegraded dietary protein and microbial protein are as yet inadequate. This was discussed in Chapters 1 and 2. Recently, advantages, disadvantages and limitations of the various methods were summarized (Tamminga, 1980). This led to the following conclusions: The best simulation of the situation in practice seems to study the whole process of digestion in the living animal. However this requires surgical modifications, often resulting in a compromise between an attempt to modify the experimental animal as little as possible from normal and the need to get access to the digestive tract at the right sampling site. Although two pairs of re-entrant cannulae, one at the beginning and one at the end of the small intestine, would be desirable, more simplified procedures are often applied. A drawback of re-entrant cannulae is that the gut needs to be dissected which may affect the intestinal flow pattern (Wenham, 1979). However this does not seem to have much influence on the digestion (Zijlstra, 1977). With only one pair of re-entrant cannulae, microbial fermentation in the hindgut may interfere with a correct interpretation of the results. This may be important in sheep fed low quality diets (Ulyatt et al., 1975), but probably very little in dairy cows fed highly digestible diets. More simplified procedures like inserting T-piece cannulae in small intestine or abomasum have the advantage that maintaining the animal is easier and that interference with the intestinal flow pattern is less severe, but the disadvantage that sampling is less reliable and flow measurements have to be based on the use of indigestible markers.

Duodenal flow measurement techniques are very laborious, reason why very often the length of the measuring period is restricted, sometimes to 24 hours or less. The number of animals used in one experiment is for the same reason often restricted. With dairy cows more than 2 or 3 animals per treatment are rarely used, despite the fact that variation, both between and within animals is rather high. A way to overcome these problems seems to fully automatize the measuring and sampling techniques. However this creates a number of complicated technical problems, partly associated with the (physical) nature of duodenal digesta resulting in blocking the tubes and partly associated with the fact that the behaviour of a living animal is rather unpredictable.

The analytical procedures applied to discriminate between microbial protein, undegraded feed protein and endogenous protein also create problems. Different markers in the estimation of microbial protein may give different results (Siddons et al., 1979) and direct measurements of undegraded feed protein and endogenous protein are as yet not available for this type of experiments.

Finally it is difficult if not impossible to feed single feedstuffs to ruminants, certainly to dairy cows, except perhaps certain roughages.

Because of all these difficulties more simple methods were developed. At present the method in which feed samples in dacron bags are incubated in the rumen and removed after different times becomes increasingly popular. From this method information can be obtained on the rate of degradation of the feed protein (Mehrez & Ørskov, 1977). Combining the rate of degradation with the rate of passage through the rumen can yield estimates on the actual extent of degradation of protein in the rumen (Ørskov & McDonald, 1979). The main limitation of the method is that as yet too little is known on the passage rate of feed particles, especially in the high yielding cow.

Estimating the possible protein value of a given feedstuff in terms of its capacity to yield microbial protein is also difficult. One approach is to assume that the amount of microbial protein which can be synthesized is related to the amount of energy (ATP) which can be extracted by the microbes from the given feedstuff under anaërobic conditions. For this purpose in the feed a distinction is required between undegraded protein, protein degraded in the rumen, carbohydrates degraded in the rumen and fat, because they yield different amounts of ATP (Chapter 3). Within carbohydrates a further distinction is possible in that easily degradable carbohydrates such as free sugars seem to yield less microbial protein than carbohydrates of which the degradation is more slowly (Tamminga, 1979a).

Just to demonstrate how these new concepts may affect the protein value of feeds used in ruminant feeding, the following exercise was done:

Five ingredients of diets for dairy cows were subjected to the incubation technique with dacron bags. The ingredients used were maizegluten feed, soyabeanmeal, fishmeal, hay and barley. It was assumed that the rate of disappearance of the N from the dacron bags combined with an adopted rate of passage of 0.05 hr^{-1} for maizegluten, soyabeanmeal, fishmeal and barley and 0.02 hr^{-1} for long hay could give an estimate of the extent of degradation (Tamminga & Rijpkema, 1981). Further assumptions were:

- Of the apparently digestible crude fibre 90% is fermented in the rumen and of the apparently digestible N-free extracts 80% is fermented in the rumen (Tamminga, 1979a). These carbohydrates fermented in the rumen will yield 3.2 g of microbial N per 100 g fermented carbohydrates.
- The dietary protein degraded in the rumen will yield only 1.6 g microbial N per 100 g fermented protein (Chapter 3; Demeijer & Van Nevel, 1979).
- Rumen undegradable protein was assumed to contain true protein only and to be absorbed with an efficiency of 0.75.
- Microbial protein was assumed to contain 80% true protein and to be absorbed with an efficiency of 0.75.
- It was assumed that the supply of rumen degradable protein was sufficient to maintain maximum microbial activity and microbial protein synthesis.

Then a comparison can be made between the digestible crude protein content and the protein value in terms of absorbed protein calculated based on the assumptions mentioned above.
Such a comparison for the five feedstuffs mentioned earlier and the protein free maize starch is given in Table 3. To the latter in brackets a negative dcp value of -25 g/kg was given because it causes the excretion of about that amount of crude protein in faeces which is counted as undigestible crude protein.

Table 3. A comparison between the protein value estimated in different ways, for six feedstuffs in ruminant diets.

feedstuff	degraded protein (g/kg)	undegraded protein (g/kg)	fermented carb. (g/kg)	dcp (g/kg)	absorbed protein (g/kg)
maizegluten	110	530	142	567	421
soyabeanmeal	325	155	248	427	166
fishmeal	320	360	-	583	290
hay	120	80	395	126	115
barley	52	63	490	82	114
maize starch	0	0	675	0(-25)	81

Except barley and maize starch, protein values calculated for absorbed protein are lower than the dcp values of the feeds. However requirements for milk production expressed as absorbed protein are also lower than those expressed as dcp. Assuming that absorbed protein will be utilised for milk production with an efficiency of 0.67 results in a requirement of 50 g of absorbed protein per kg of milk (Vérité et al., 1979), whereas according to the Dutch feeding standards 63 g dcp is needed per kg of milk.

Expressing the protein value of a feedstuff in its milk production potential, it becomes possible to compare the protein value calculated as dcp or calculated as absorbed protein. Such a comparison is made in Table 4.

Table 4. Protein value in terms of milk production potential (kg milk/kg feed) of various feedstuffs.

feedstuff	digestible crude protein	absorbed protein
maizegluten	9.00	8.42
soyabeanmeal	6.78	3.32
fishmeal	9.25	5.80
hay	2.00	2.30
barley	1.30	2.28
maize starch	0(-0.40)	1.62

The results show that estimating the protein value of feedstuffs according to new procedures would change the protein value of most feedstuffs considerably. Particularly protein rich feedstuffs which are not very resistant against ruminal degradation would get a substantially lower protein value. On the other hand those feeds low in N but high in digestible carbohydrates would benefit from this degradable N as it would be an excellent source for microbial protein synthesis.

However feedstuffs as mentioned in Table 4 are almost never fed as a single feed to dairy cows. Therefore diets were composed from these 6 ingredients and their protein value in terms of milk production potential calculated according to the dcp system and as absorbed protein. The results are shown in Table 5.

Table 5. Protein value in terms of milk production potential (kg milk/kg feed) of various diets.

	I	II	III	IV	V
hay (%)	40	40	40	40	40
maizegluten (%)	3.6	6.3	9.0	18	-
soyabeanmeal (%)	3.6	6.3	9.0	-	24
fishmeal (%)	3.6	6.3	9.0	-	-
barley (%)	3.6	6.3	9.0	-	-
maize starch (%)	45.6	34.8	24.0	42	36
% crude protein	15	20	25	20	20
kg milk/kg feed dcp system	1.75(1.57)	2.46(2.32)	3.17(3.07)	2.42(2.25)	2.43(2.29)
kg milk/kg feed absorbed protein	2.37	2.73	3.09	3.12	2.30

Substantial differences only occur if diets with a very low protein content (less than 15% crude protein) are composed or if diets are based on a very

small number of ingredients which have a very high (diet IV) resistance against protein degradation in the rumen.

The introduction of a new protein evaluating system cannot be restricted to putting a protein value to each individual feedstuff, but by composing diets from such feedstuffs, certain constraints will have to be put on the whole diet. In order to ensure that the full potential of microbial protein synthesis is used, a minimum amount of rumen degradable protein in the diet is necessary. A percentage of 10% rumen degradable protein seems adequate, part of which could be supplied by non-protein-N under certain conditions. A further constraint is that the diet should not contain too much free sugars or easily fermentable starch as such diets would not allow maximal microbial growth. Because of differences in rate of degradation and hence in rate of VFA production it is difficult to put a maximum value on this. Restricting the amount of free sugars and starch to some 25% in the concentrate part of the diet however seems fairly safe (de Visser, pers. communication).

CONCLUDING REMARKS

It becomes clear from the foregoing discussion and from the results reported in this thesis that protein metabolism in ruminants is a complicated affair, which cannot easily be manipulated. Besides protein and energy metabolism are highly related. The best way to ensure an adequate protein supply seems to maximize energy intake and this in such a way that microbial protein synthesis is maximal. Ways to achieve this were discussed.
If under some conditions an adequate feed intake cannot be achieved, then there is some scope for the use of proteins selected for their low degradability in the rumen. Even under such conditions one should aim at a maximum microbial protein synthesis and make sure that sufficient rumen degradable protein is available for the microbes. Besides the intestinal digestibility of the "resistant" feed protein should be high and its amino acid composition should resemble the amino acid composition of the protein needed for milk and maintenance.

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SUMMARY

For the process of milk production, the dairy cow requires nutrients of which energy supplying nutrients and protein or amino acid supplying nutrients are the most important. Amino acid supplying nutrients have to be absorbed from the small intestine and the research reported in this thesis mainly concentrates on the study of methods by which the intestinal supply of amino acids in dairy cows can be manipulated. Information of this sort can only be obtained if one can get access to the small intestine of normally functioning animals. For this purpose the small intestine of mature dairy cows was cannulated in such a way that all the digesta flowing through the small intestine had to pass an "artificial intestine" just on the outside of the animal. This "artificial intestine" consisted of two pieces of PVC tubing inserted through the body wall into the two blind sacs of the dissected and sewed up small intestine and a piece of soft tubing connecting both PVC cannulae. By removing the connecting tube one gets access to the interior of the small intestine and the intestinal flow of digesta can be measured and sampled.

The intestinal flow of protein is governed by the microbial degradation of dietary protein in the forestomachs of the ruminant animal on the one hand and by the microbial protein synthesis in the same forestomachs on the other. Both processes occur simultaneously. A detailed discussion of the process of microbial degradation of dietary protein in ruminants is given in chapter 1. It appears that dietary protein is degraded to a varying degree, depending on nature and solubility of the dietary protein, rate of passage through the forestomachs and level of feed intake. A further quantification of the differences in degradation and factors which influence these differences is hampered by the lack of accuracy or reliability of the measuring techniques. The process of microbial protein synthesis and measuring techniques was studied and discussed in chapter 2. Measuring the contribution of microbial protein to the intestinal protein supply is usually based on the use of marking substances which are present in the microbes, but not in the feed. For this purpose nucleic acids (RNA in particular), 2.6-diaminopimelic acid (DAPA) and various radio-isotopes such as ^{15}N, ^{32}P or ^{35}S can be used. The efficiency of microbial protein synthesis (microbial N/100 g of carbohydrates apparently fermented in

the forestomachs) was compared using 3 different methods. The methods were the use of the microbial markers RNA and DAPA and a regression method. All methods showed considerable variation and the results of the different methods differed considerably from each other.

Despite an observed close relationship between energy intake and duodenal protein flow it seems reasonable to assume that apart from energy intake some other factors may have an influence on the intestinal protein flow. Some of these possible factors were investigated and the results of these investigations are reported in the following chapters.

Comparing two protein sources of a widely different nature viz. maizegluten feed and soyabean meal showed that after feeding maizegluten feed substantially more protein entered the small intestine of a dairy cow than after feeding soyabean meal. The difference was explained by a difference in extent of degradation in the forestomachs between both protein sources. The value of this experiment is somewhat limited because only one animal was involved in it. However some provisional studies on the degradation of different protein sources in the rumen by using the so-called dacron bag technique seems to confirm the findings presented in chapter 3 as can be concluded from the results presented in chapter 11 (table 3).

A further question is if the degradation of the dietary protein in the forestomachs of ruminants can be reduced by treatments like heating or the application of chemicals. This aspect was also studied and the results in chapter 5 do suggest that heat treatment (achieved by artificial drying) as well as treatment with certain chemicals (formaldehyde in particular) of roughages can improve the intestinal protein flow. Some indications were found however that treatment with formaldehyde may slightly decrease the efficiency of absorption of amino acids from the small intestine, but the increase in duodenal protein flow appeared higher than the decrease in absorbed protein. Heat treatment and formaldehyde treatment can easily lead to overprotection. Under such conditions the decrease in absorption may exceed the increase in intestinal flow and the total result is negative. Such treatments must therefore be applied with great care.

Because of differences in their nature, protein degradation may differ between long roughage and ground concentrates. Diets were therefore fed in which the roughage to concentrates ratio varied widely. A tendency was found that a larger proportion of the apparently digestible organic matter was digested in the stomachs with a larger proportion of long roughage in the diet.

Despite the wide variation in the roughage to concentrates ratio no detectable changes took place in the degradation of total feed protein or in the synthesis of microbial protein. Approximately 70% of the ingested feed protein became degraded in the stomachs whereas for each kg of carbohydrates fermented some 32 g of microbial N was produced.

Factors influencing duodenal protein flow discussed sofar were largely associated with differences in rate of degradation of protein in the forestomachs. It was realised that decreasing the time during which the feed proteins were subjected to microbial degradation might also reduce the extent of degradation. Reducing the time that feed particles spend in the rumen can be achieved by increasing the level of feed intake. The effect of the level of feed intake on N entering the small intestine was therefore studied and the results are reported in chapter 7. Increasing the level of feed intake appeared to increase the duodenal flow of non-ammonia-nitrogen (NAN), not only the absolute quantity, but also the quantity per kg feed ingested, which could entirely be explained by a reduction in extent of protein degradation in the forestomachs.

From the preceding chapters it became evident that there are ways to reduce the extent of degradation of dietary protein in the forestomachs of ruminants. The question arises what measures can be taken to improve microbial protein synthesis or to prevent that microbial protein synthesis becomes reduced. Frequent feeding, particularly of concentrate rich diets, is thought to have a stabilising effect on rumen fermentation and this might be advantageous for microbial growth and hence for the microbial protein production. The effect on duodenal N flow of feeding the concentrate part of the diet in smaller portions but more frequently per day was further investigated. The results are shown in chapter 8. Feeding the concentrate part of a low N diet twice daily resulted in a relatively low N flow which could be increased to more normal values if the concentrates were administered in 6 portions per day. Repeating the experiment with diets containing more normal protein levels showed only a small positive effect of more frequent feeding on the duodenal N flow. Evidence was obtained from regression calculations that frequent feeding decreased the proportion of the dietary protein escaping degradation in the forestomachs but that this decrease was more than compensated for by an increased production of microbial protein.

In the last three chapters attempts are being made to evaluate the practical significance of the findings reported or discussed in previous chapters.

Chapter 9 shows that it seems more promising to study and develop methods by which the total duodenal protein flow is enhanced rather than to concentrate on methods which try to improve either the intestinal flow of undegraded feed protein or the intestinal flow of microbial protein. The relationship between energy intake and intestinal protein flow is emphasized again. Evidence is obtained that the maximum flow of intestinal protein if related to energy intake is likely to be in the order of 3 g of NAN/MJ ME ingested.

The ultimate aim of improving the intestinal supply of protein is to provide the dairy cow with more amino acids in the blood so that more milk can be produced, provided the genetic capacity potential and physiological state of the animal will allow such an improvement. After entering the blood the amino acids can be used for various processes. In chapter 10 an attempt is made to get some information, mainly based on data from literature, on the amino acid requirements for maintenance, gluconeogenesis, protein retention in the body and protein output in milk. Because of a lack of precise quantitative data the result remains rather descriptive. Evidence is presented however that amino acids do not play an important role as precursors for gluconeogenesis in dairy cows, not even at high levels of milk production. Evidence is also presented that if milk output is limited because of an inadequate supply of amino acids, this is often because of the total supply of amino acids is limiting. Under some conditions one single amino acid may be limiting and then methionine appears more often limiting than one of the other essential amino acids.

The final chapter 11 deals with a general discussion of the feeding principles of the high yielding dairy cow. Following the concepts developed in the previous chapters some guidelines for the protein feeding of high yielding dairy cows are given. It is stressed that maintaining optimal conditions for fermentation, microbial activity, microbial growth and protein production in the forestomachs are the best guarantee for an adequate intestinal protein supply. In this respect the significance of protein/energy interactions is emphasized. Only if an optimal rumen fermentation cannot be maintained there seems scope for the feeding of protein with a high resistance against degradation in the rumen, provided the intestinal digestibility of this protein is high and it has an amino acid composition resembling the composition which is required by the animal.

SAMENVATTING

Voor het produceren van melk heeft een koe voedingsstoffen nodig. De belangrijkste zijn energie leverende voedingsstoffen en voedingsstoffen die eiwit of liever aminozuren leveren. Aminozuren moet de koe absorberen in haar bloed vanuit de dunne darm en het onderzoek waarover in dit proefschrift gerapporteerd wordt houdt zich voornamelijk bezig met het zoeken van methoden waardoor het aanbod van aminozuren in de dunne darm kan worden beïnvloed. Deze methoden kunnen alleen bestudeerd worden als men toegang heeft tot de dunne darm van een fysiologisch normaal functionerend dier. Voor dit doel werd in een aantal koeien langs operatieve weg een stuk kunstdarm aan de buitenkant van het dier aangebracht. Dit stukje kunstdarm omvatte twee stukjes PVC buis die door de huid uitmondden in twee blinde zakken van de doormidden gesneden en daarna dichtgenaaide dunne darm en een korte flexibele buis die de beide stukken PVC buis buiten het dier met elkaar verbond. Alle darminhoud die in deze koeien door de dunne darm stroomde moest deze kunstdarm passeren. Door het verbindingsstuk te verwijderen werd toegang gekregen tot het inwendige van de dunne darm en kon de passagesnelheid van de voedselbrij worden gemeten en de inhoud bemonsterd.

De toevoer van eiwit naar de dunne darm van herkauwers wordt bepaald door enerzijds de mate van microbiële afbraak van opgenomen voereiwit en anderzijds de synthese van microbiëel eiwit. Beide processen spelen zich tegelijkertijd af in de voormagen. De microbiële afbraak van voereiwit in de voormagen van herkauwers wordt diepgaand besproken in hoofdstuk 1. Daarbij blijkt dat er tussen verschillende voedermiddelen verschillen bestaan in mate van afbraak. De mate van afbraak blijkt beïnvloed te worden door de aard van het eiwit, de oplosbaarheid van het eiwit, de passagesnelheid van het eiwit door de voormagen en van het opnameniveau van het dier. Als gevolg van gebrek aan nauwkeurigheid en betrouwbaarheid van de gebruikte meetmethoden bleek het niet mogelijk deze verschillen in getallen weer te geven of aan te geven hoe groot de invloed van de verschillende factoren is.

Het mechanisme van de synthese van microbiëel eiwit en de meetmethoden die gebruikt worden om dit te meten worden besproken in hoofdstuk 2. Voor het meten van het aandeel van microbiëel eiwit in de toevoer van eiwit naar de

dunne darm wordt meestal gebruik gemaakt van merkstoffen die wel in microben, maar niet in het voer aanwezig zijn. Voor dit doel worden nucleinezuren (met name RNA), 2,6-diaminopimelinezuur (DAPA) en diverse radio-isotopen zoals ^{15}N, ^{32}P of ^{35}S gebruikt. De efficiëntie waarmee in de voormagen microbiëel eiwit werd gemaakt (microbiëel N/100 g in de voormagen schijnbaar verteerde koolhydraten) werd gemeten door het vergelijken van 3 verschillende meetmethoden. Die methoden waren het gebruik van RNA als microbiële merkstof, het gebruik van DAPA als microbiële merkstof en het gebruik van regressieberekeningen. Alle drie methoden vertoonden een aanzienlijke spreiding en de verschillende methoden gaven uitkomsten die nogal van elkaar verschilden.

Niettegenstaande een aangetoond nauw verband tussen de opname van energie en de toevoer van eiwit naar de dunne darm, lijkt het aannemelijk dat er nog andere factoren zijn die een invloed hebben op de toevoer van eiwit naar de dunne darm. Een aantal van deze factoren werd nader onderzocht en de resultaten van deze onderzoekingen zijn weergegeven in de hoofdstukken 3 t/m 8.

Eerst werd de soort eiwit als mogelijke beïnvloedende factor onderzocht. In dit onderzoek (hoofdstuk 3) werden twee eiwitbronnen van zeer uiteenlopende aard met elkaar vergeleken, nl. maisglutenmeel en sojaschroot. Na het voeren van maisglutenmeel bleek de eiwittoevoer naar de dunne darm aanzienlijk groter dan na het voeren van sojaschroot. Het verschil werd toegeschreven aan een verschil in microbiële afbraak in de voormagen. De waarde van deze proef is ietwat beperkt, omdat het een proef met slechts 1 dier betreft. Echter uit voorlopige onderzoekingen over de afbraak in de voormagen van eiwithoudende veevoedergrondstoffen m.b.v. de zogenaamde dacron bag techniek komt ook naar voren dat maisglutenmeel veel minder snel wordt afgebroken dan sojaschroot, wat kan worden afgeleid uit de resultaten die in hoofdstuk 11 worden gegeven (tabel 3).

De vraag rees in hoeverre de afbraak van voereiwit in de voormagen van herkauwers kan worden verminderd door behandelingen zoals verhitting of het toepassen van bepaalde chemicaliën. Uit de proefresultaten in hoofdstuk 5 komt de tendens naar voren dat een hittebehandeling, die bereikt werd door kunstmatig drogen, zowel als het behandelen met bepaalde chemicaliën (in het bijzonder formaldehyde), van ruwvoeders de toevoer van eiwit naar de dunne darm kan verbeteren. Er werden echter ook aanwijzingen gevonden dat behandelen met formaldehyde de mate van absorptie van aminozuren uit de dunne darm iets vermindert. Echter de toename in de toevoer van eiwit naar de dunne darm bleef groter dan de afname van de absorptie daaruit van de aminozuren.

Uiteraard zal bij te sterke behandeling het netto effect negatief zijn.

Verschillen in structuur en deeltjesgrootte tussen lang ruwvoer en gemalen krachtvoer zou de eiwitafbraak en microbiële eiwitsynthese van beide typen voedermiddelen ongelijk kunnen beïnvloeden. Er werd daarom een aantal rantsoenen gevoerd waarin de verhouding tussen lang ruwvoer en krachtvoer sterk uiteenliep. De resultaten van deze proeven worden beschreven in hoofdstuk 6. Hierbij bleek een tendens dat naarmate de gevoerde rantsoenen een hoger percentage lang ruwvoer bevatten er een groter deel van de schijnbaar verteerde organische stof in de magen werd verteerd. Ondanks de grote verschillen in ruwvoer/krachtvoer verhouding werden er geen merkbare veranderingen waargenomen in de mate van afbraak van het totaal aan voereiwit of de synthese van microbiëel eiwit. Gemiddeld werd er zo'n 70% van de met het voer opgenomen eiwit in de pens afgebroken, terwijl er voor iedere kg gefermenteerde koolhydraten (de som van ruwe celstof en overige koolhydraten) gemiddeld 32 g microbiëel eiwit werd geproduceerd.

De tot nu toe besproken factoren die van invloed zijn op de toevoer van eiwit naar de dunne darm van koeien hadden vooral te maken met verschillen in afbraaksnelheid tussen verschillende veevoedergrondstoffen of als gevolg van zekere behandelingen. Verkorten van de tijd gedurende welke de voerdeeltjes in de voormagen verblijven, hetgeen bereikt kan worden door het verhogen van het voeropnameniveau, zou ook de mate van eiwitafbraak in de voormagen kunnen verminderen. De invloed van het verhogen van de voeropname op de toevoer van N naar de dunne darm werd daarom onderzocht en de resultaten worden gegeven in hoofdstuk 7. Verhoging van het voederopnameniveau bleek niet alleen in absolute hoeveelheden, maar ook per kg opgenomen N de toevoer van niet-ammoniak-stikstof (NAN) naar de dunne darm te verhogen. Deze verhoging kon geheel worden toegeschreven aan een vermindering in de eiwitafbraak in de voormagen.

Uit de voorgaande hoofdstukken werd duidelijk dat er mogelijkheden bestaan om de afbraak van voereiwit in de voormagen van herkauwers te verminderen. De vraag rees welke maatregelen genomen kunnen worden om de synthese van de hoeveelheid microbiëel eiwit te doen toenemen, of om te voorkomen dat de hoeveelheid microbiëel eiwit die geproduceerd wordt gaat verminderen. Het verstrekken van het krachtvoer in meerdere porties per dag, met name wanneer zeer krachtvoerrijke rantsoenen worden gevoerd, wordt geacht een stabiliserend effect te hebben op de microbiële fermentatie in de pens. Dit zou de microbiële groei en daarmee de produktie van microbiëel eiwit gunstig kunnen beïnvloeden. De invloed van het verstrekken van het krachtvoerdeel van het rantsoen in

meerdere porties per dag op de toevoer van N naar de dunne darm werd daarom onderzocht bij koeien. De resultaten worden gegeven in hoofdstuk 8. Het voeren van het krachtvoergedeelte van een rantsoen met een laag eiwitgehalte in twee porties per dag had tot gevolg dat de toevoer van N naar de dunne darm relatief laag was. Deze toevoer bereikte meer normale waarden door het krachtvoer in 6 porties per dag te verstrekken. De proef werd vervolgens herhaald met rantsoenen met een meer normaal eiwitgehalte. Het positief effect van het vaker voeren op de toevoer van N naar de dunne darm was nu veel kleiner. Er waren aanwijzingen (gebaseerd op de resultaten van regressieberekeningen) dat het voeren van krachtvoer in meerdere porties per dag het percentage rantsoeneiwit dat aan afbraak in de voormagen ontsnapt, verlaagt, maar dat deze verlaging meer dan teniet wordt gedaan doordat er meer microbiëel eiwit wordt gemaakt.

In de laatste drie hoofdstukken wordt een poging gedaan de praktische betekenis van de onderzoekresultaten in de voorgaande hoofdstukken nader uit te werken. In hoofdstuk 9 wordt aangetoond dat het zinvoller is te zoeken naar methoden waardoor de totale toevoer van eiwit naar de dunne darm wordt verbeterd in plaats van zich te concentreren op methoden die òf de toevoer van rantsoeneiwit òf de toevoer van microbiëel eiwit naar de dunne darm verbeteren. De relatie tussen energie-opname en toevoer van eiwit naar de dunne darm wordt opnieuw benadrukt. Aangetoond wordt dat de maximale toevoer van eiwit naar de dunne darm, als die afhankelijk wordt gesteld van de energie-opname in de orde van 3 g NAN/MJ opgenomen ME ligt.

Het uiteindelijke doel van het verbeteren van de toevoer van eiwit naar de dunne darm is het verhogen van de toevoer van aminozuren naar het bloed van het gastheerdier, zodat er meer melk kan worden geproduceerd, aangenomen dat de erfelijke aanleg van het dier zo'n verhoging toestaat. Na toevoer naar het bloed kunnen de aminozuren voor diverse processen worden gebruikt. In hoofdstuk 10 wordt een poging gedaan, vooral gebaseerd op literatuurgegevens, iets te zeggen over de aminozuurbehoefte voor onderhoud, voor gluconeogenese, voor eiwitaanzet in het lichaam en voor eindproduktie in de melk. Bij gebrek aan kwantitatieve gegevens blijft het een nogal beschrijvend geheel. Wel wordt vastgesteld dat aminozuren in de meeste situaties niet een belangrijke rol spelen als bouwstof voor de gluconeogenese, zelfs niet bij hoge melkprodukties. Ook blijkt dat als de melkproduktie beperkt wordt door een onvoldoende aanbod van aminozuren in het bloed, dit veelal niet het gevolg is van een tekort aan één of enkele soorten aminozuren, maar van een te gering aanbod van het totale

aminozurenmengsel. In bepaalde situaties kan het echter ook voorkomen dat er een tekort is aan één enkel aminozuur. Dit lijkt vaker voor te komen met methionine dan met één van de andere essentiële aminozuren.

In het laatste hoofdstuk 11 wordt getracht door middel van een algemene discussie richtlijnen te geven voor de eiwitvoeding van de hoogproduktieve melkkoe. Benadrukt wordt dat een goede eiwitvoorziening in de dunne darm van de koe het best wordt gediend met optimale condities voor de fermentatie in de voormagen, d.w.z. optimale condities voor microbiële activiteit, microbiële groei en eiwitproduktie. In dit verband wordt vooral gewezen op de betekenis van eiwit/energie interacties. Slechts als een optimale fermentatie in de voormagen niet kan worden gerealiseerd of gehandhaafd lijkt het zinvol om eiwit te verstrekken dat een hoge weerstand bezit tegen afbraak in de pens, mits de vertering in de dunne darm van dat eiwit hoog is en de aminozuursamenstelling in overeenstemming is met de behoefte van het dier.

Appendix. Intake of dry matter (I_T in kg/day), organic matter (I_O in kg/day) and Nitrogen (I_N in g/day), total apparent digestibility of organic matter (d_O) and Nitrogen (d_N) and apparent digestibility in the stomachs (as proportion of total apparent digestibility) of organic matter ($d_{O,S}$) and Nitrogen ($d_{N,S}$).

exp.	animal	I_T	% long roughage	I_O	d_O	$d_{O,S}$	I_N	d_N	$d_{N,S}$	chapter(s)
1	ZS	7.2	25	6.80	0.73	0.47	173	0.65	-0.85	3
1	ZS	7.1	25	6.64	0.79	0.51	201	0.77	-0.09	3
2	93	16.4	21	14.76	0.68	0.50	415	0.58	-0.26	4,5,9
2	94	15.0	14	13.67	0.77	0.66	379	0.70	0.13	4,5
2	93	14.9	48	13.59	0.73	0.54	368	0.66	-0.13	4,5,9
2	94	14.9	48	13.44	0.76	0.57	365	0.66	-0.15	4,5,9
2	93	16.0	52	14.59	0.73	0.57	405	0.62	-0.21	4,5,9
2	94	15.5	51	14.14	0.78	0.57	390	0.65	-0.17	4,5,9
2	93	14.8	48	13.52	0.75	0.58	363	0.66	-0.07	4,5,9
2	94	14.8	48	13.52	0.77	0.53	363	0.67	-0.22	4,5,9
3	93	12.9	49	11.71	0.68	0.58	322	0.61	-0.18	9
3	94	13.2	48	11.97	0.71	0.59	331	0.67	-0.06	9
3	93	12.0	43	10.95	0.73	0.54	287	0.61	-0.41	9
3	94	12.0	43	10.95	0.73	0.58	287	0.62	-0.38	9
4	94	12.8	33	11.73	0.79	0.60	405	0.72	0.04	9
4	93	12.9	34	11.82	0.79	0.64	419	0.74	0.14	9
4	93	12.5	34	11.40	0.76	0.59	385	0.66	-0.14	9
4	93	12.7	35	11.52	0.79	0.64	410	0.73	0.06	9
4	66	9.2	47	8.67	0.73	0.63	320	0.73	0.37	9
4	66	10.2	42	9.64	0.76	0.69	327	0.72	0.35	9
4	78	10.0	43	9.47	0.77	0.53	304	0.68	0.03	9
4	78	10.2	42	9.63	0.70	0.55	299	0.59	-0.05	9
4	78	10.3	42	9.78	0.72	0.54	339	0.65	-0.02	9
4	66	10.3	41	9.71	0.76	0.59	322	0.68	0.08	9
4	93	9.5	37	8.55	0.80	0.56	263	0.70	-0.21	9
4	35	9.4	36	8.48	0.81	0.61	254	0.69	-0.16	9
4	35	9.8	36	8.87	0.80	0.50	281	0.70	-0.31	9
4	93	9.4	36	8.52	0.80	0.59	261	0.67	-0.23	9
4	93	9.4	36	8.54	0.79	0.58	262	0.65	-0.19	9
5	66	11.4	30	10.44	0.80	0.57	352	0.79	0.11	3,6,9
5	78	11.9	28	10.93	0.73	0.60	343	0.63	-0.08	3,6,9
5	81	12.0	28	10.88	0.75	0.54	363	0.70	0.05	3,6,9
5	66	12.8	40	11.62	0.75	0.63	386	0.69	0.17	3,6,9
5	78	12.9	40	11.73	0.78	0.56	391	0.69	0.01	3,6,9
5	93	11.3	40	10.35	0.78	0.53	322	0.70	-0.14	3,6,9
5	66	13.5	49	12.31	0.77	0.60	381	0.69	0.02	3,6,9
5	78	12.6	46	11.49	0.77	0.67	383	0.71	0.24	3,6,9
5	93	10.3	50	9.34	0.80	0.58	308	0.74	0.02	3,6,9
5	78	8.6	80	7.71	0.74	0.68	187	0.65	-0.09	3,6,9
5	93	6.8	75	6.21	0.77	0.56	168	0.68	-0.32	3,6,9
5	93	5.1	67	4.69	0.75	0.59	151	0.71	-0.05	3,6,9
5	93	5.1	42	4.73	0.81	0.64	154	0.75	0.16	6,9
5	2	9.6	73	8.57	0.84	0.59	207	0.75	-0.24	6,9
5	128	7.6	89	6.89	0.85	0.70	158	0.71	-0.23	6,9

exp.	animal	I_T	% long roughage	I_O	d_O	$d_{O,S}$	I_N	d_N	$d_{N,S}$	chapter(s)
5	35	2.6	100	2.30	0.75	0.61	54	0.61	-0.33	6,9
5	35	8.5	30	7.89	0.73	0.52	233	0.65	-0.20	3,6,9
5	78	8.5	29	7.90	0.77	0.58	240	0.70	0.05	3,6,9
5	35	8.8	56	8.14	0.76	0.56	225	0.66	-0.12	3,6,9
5	78	8.9	56	8.26	0.77	0.59	225	0.66	-0.17	3,6,9
5	35	9.2	81	8.50	0.72	0.61	216	0.62	-0.30	3,6,9
5	78	9.2	81	8.45	0.73	0.61	200	0.64	-0.33	3,6,9
5	35	6.8	87	6.28	0.71	0.60	140	0.57	-0.53	3,6,9
5	78	6.1	43	5.61	0.78	0.58	152	0.65	-0.23	3,6,9
5	93	15.6	45	14.44	0.74	0.55	402	0.66	-0.26	3,6,9
5	93	15.7	34	14.53	0.75	0.56	434	0.66	-0.14	3,6,9
6	93	15.4	43	14.17	0.72	0.53	432	0.67	-0.06	9
6	93	15.3	44	14.20	0.76	0.56	422	0.67	0.01	9
7	93	8.5	39	7.66	0.80	0.57	169	0.59	-0.62	2,7,9
7	2	8.3	37	7.59	0.81	0.59	201	0.64	-0.25	2,7,9
7	35	8.6	39	7.93	0.79	0.65	185	0.56	-0.38	2,7,9
7	2	8.6	40	7.77	0.81	0.56	285	0.75	0.07	2,7,9
7	35	8.6	40	7.94	0.81	0.62	244	0.68	-0.03	2,7,9
7	2	8.5	39	7.69	0.82	0.53	315	0.79	0.08	2,7,9
7	35	8.7	40	7.94	0.81	0.64	333	0.78	0.27	2,7,9
7	93	5.9	52	5.35	0.83	0.59	228	0.80	0.24	2,7,9
7	128	14.3	26	13.10	0.79	0.59	332	0.63	-0.54	2,7,9
7	2	12.8	33	11.85	0.82	0.57	259	0.67	-0.43	2,7,9
7	93	10.8	40	9.92	0.77	0.55	224	0.58	-0.99	2,7,9
7	128	12.8	32	11.64	0.81	0.54	426	0.74	-0.11	2,7,9
7	2	13.0	33	11.97	0.7[illegible]	0.56	390	0.75	-0.17	2,7,9
7	93	12.0	35	10.99	0.80	0.57	348	0.71	-0.20	2,7,9
7	128	14.5	27	13.11	0.78	0.53	575	0.74	0.06	2,7,9
7	93	12.7	34	11.53	0.81	0.54	496	0.74	0.05	2,7,9
7	2	12.9	32	11.86	0.80	0.50	530	0.79	0.08	2,7,9
8	93	5.4	33	4.80	0.84	0.68	92	0.60	-0.55	8
8	35	5.5	33	4.86	0.79	0.60	96	0.53	-0.62	8
8	2	10.7	25	9.64	0.82	0.67	231	0.66	-0.20	8
8	93	5.4	33	4.86	0.81	0.66	96	0.46	-1.25	8
8	35	5.4	33	4.63	0.79	0.57	89	0.50	-1.57	8
8	2	10.6	33	9.58	0.81	0.51	229	0.64	-0.76	8
8	432	5.5	40	5.03	0.80	0.68	182	0.65	0.18	8 *
8	2	9.6	45	8.76	0.80	0.65	290	0.65	0.11	8 *
8	2	9.4	44	8.56	0.80	0.69	276	0.65	0.12	8 *
8	411	9.6	45	8.66	0.78	0.63	274	0.68	-0.06	8
8	432	6.1	52	5.53	0.80	0.66	187	0.65	0.17	8 *
8	2	9.3	45	8.48	0.80	0.54	268	0.65	-0.17	8 *
8	2	9.6	45	8.77	0.80	0.58	279	0.65	-0.06	8 *
8	411	9.4	46	8.57	0.77	0.64	267	0.68	-0.02	8

* Because of the use of fully automated duodenal flow measuring equipment, no faeces were collected and d_O and d_N were assumed to be 0.80 and 0.65 respectively.

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 24 juli 1942 te Midlum (gemeente Franekeradeel). Hij behaalde in 1960 het einddiploma HBS-B aan de Christelijke Hogere Burgerschool te Leeuwarden. In 1963 werd de studie aan de Landbouwhogeschool te Wageningen aangevangen, welke in 1970 werd afgesloten met de ingenieursvakken Veevoeding, Fysiologie der Dieren en Biochemie. Sinds 1 september 1970 is de auteur als wetenschappelijk onderzoeker werkzaam bij het Instituut voor Veevoedingsonderzoek, van 1970 t/m 1975 te Hoorn, van 1976 tot heden te Lelystad. De onderzoek-opdracht bij genoemd instituut is de eiwithuishouding bij herkauwers, in het bijzonder bij melkvee, in het kader waarvan het in dit proefschrift beschreven onderzoek werd verricht. Van 1 oktober 1975 tot 1 november 1976 was de auteur werkzaam in het Department of Agricultural Biochemistry van de universiteit van Newcastle upon Tyne. Gedurende deze periode werd onderzoek verricht naar de mogelijkheden de methaanvorming bij herkauwers te remmen. Deze periode werd afgesloten met het behalen van het diploma Master of Science in 1977.