THE EFFECT OF SUPPLEMENTATION OF ALFALFA HAY OR UREA ON INTAKE DIGESTIBILITY AND RUMEN FERMENTATION OF SHEEP FED TIMOTHY HAY

by

ANNICK DELAQUIS

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THE EFFECT OF SUPPLEMENTATION OF ALFALFA HAY OR UREA ON INTAKE DIGESTIBILITY AND RUMEN FERMENTATION OF SHEEP FED TIMOTHY HAY

Abstract

Annick Delaquis M.Sc. Animal Science.

The supplementation of low quality forages with a crude protein supplement or a legume hay has been shown to improve animal performance and dry matter intake. The effects of protein supplementation are attributed to an improved nitrogen (N) status of rumen microbes and/ or of the animal. The causative agents in legume playing a role in increased dry matter intake (DMI) are still unclear but because of its high N content it may act by supplying extra ammonia or amino acids to the microbes or the host. The main objectives of the present research were to investigate if the effects of alfalfa supplementation on DMI of sheep fed timothy hay were due only to the N content of the alfalfa or to some other nutritive factor(s), to determine if the palatability of alfalfa plays a role in the increased DMI, and to examine the effects of different levels of alfalfa supplementation. In experiment I six rations were randomly assigned to 6 mature rams over 6 time periods. The rations were offered once a day and were as follows: 1- 10% alfalfa hay 90% timothy hay mixed offered ad libitum.; 2- same as 1 but hays were offered separately; 3- timothy hay ad lib and a urea solution isonitrogeneous (iso-N) to 10% alfalfa hay infused in the rumen at feeding; 4- same as 3 except that the total daily urea solution was divided in 4 equal

portions and infused at 4 times during the day 0600, 1200, 1800, 2400. 5- same as 2 except that the alfalfa hay was placed into the rumen; 6- same as 5 except that the alfalfa was placed in the rumen 4 times a day. Total DMI (TDMI) on a metabolic body weight basis were significantly higher when alfalfa supplements were fed compared with iso-N urea supplements. However the improved TDMI was attributed to the alfalfa DMI since no difference between diets was obtained in terms of timothy DMI. The increase in DMI was not always parallelled by faster rates of passage nor higher rumen ammonia suggesting that the increased TDMI observed when alfalfa was supplemented was not only mediated by a higher supply of ammonia . Palatability was also eliminated as a causative agent. To investigate further the respective effects of urea and alfalfa as well as a potential benefit of supplementing both simultaneously a second experiment was conducted having the same design as experiment I. For all 6 diets, sheep were offered timothy hay ad libitum plus one of the following supplements: 1- no supplement; 2- 150 g(as fed) alfalfa hay; 3- urea iso-N to 150g alfalfa; 4- 300g alfalfa; 5- 150g alfalfa plus iso-N urea; 6- 450g alfalfa. All alfalfa supplements resulted in higher TDMI $g/W^{.75}$ compared to diet 1 specifically due to the alfalfa intake since no difference in timothy hay intake was observed. Dry matter, crude protein and acid detergent fiber digestibilities were not affected in spite of the higher TDMI. Digestible energy intakes were increased by alfalfa

supplements and N retention although not significant tended to be improved. The lack of difference between diet 2 and 1 in terms of rumen ammonia suggested again that the effect of alfalfa on intake was mediated by factors other than N per se. Rumen concentration of valeric and isovaleric acids, essential growth factors for rumen microbes, were increased by alfalfa and may have played a role. Although non significant replacement of alfalfa by iso-N quantities of urea tended to cause decreases in TDMI as for trial I. Urea supplementation of timothy hay did not improve TDMI over diet I suggesting that the alfalfa effect is not due to its N content, No benefit of supplementing urea and alfalfa simultaneously were found. Thus the results of both trials indicated that alfalfa effect on grass DMI was mediated by nutrients other than N per se. In addition the second trial demonstrated that there was no benefit in terms of DMI by supplementing urea to timothy hay.

EFFET D'UN SUPPLEMENT DE FOIN DE LUZERNE ET D'UREE SUR LA CONSOMMATION ET DIGESTIBILITE DE LA MATIERE SECHE ET SUR LA FERMENTATION DANS LE RUMEN DE MOUTONS OFFERST UN FOIN DE FLEOLE.

Annick Delaquis **M.Sc.** **Zootechnie**

Résumé

Suppléments protéiques et suppléments de foin de légumineuses peuvent tous deux améliorer la performance des animaux offert des fourrages de basse qualité principalement en causant une augmentation de leur consommation de matière sèche. Les effets des suppléments protéiques sont attribués à une amélioration du statut en azote des microorganismes du rumen et/ ou de l'animal. Les causes de l'augmentation de la consommation observée lorsqu'un foin de légumineuse est offert sont encore obscures mais vue la quantité d'azote qu'il contient, il pourrait aussi agir via un apport supplémentaire d'ammoniaque ou d'acides aminés pour les microorganismes et/ ou l'animal.Les objectifs principaux de la présente recherche étaient donc de déterminer si les effets d'un supplément de foin de luzerne sur la consommation chez les moutons offerts un foin de fléole sont dûs seulement à son contenu d'azote ou à d'autres nutriments de la luzerne, déterminer si l'appétence de la luzerne est un facteur, et examiner les effets de différents niveaux de supplémentation. Au cours de la première expérience six diètes ont été distribuées au hazard à six béliers au cours de six périodes. Les rations fûrent offertes une fois par

jour et consistaient de: 1- mélange de 10% de foin de luzerne et 90% de foin de fléole 2-même que 1 sauf que les deux foins fûrent offerts séparément 3- foin de fléole à volonté et une solution d'urée infusée dans le rumen à un taux apportant une quantité d'azote équivalente à la consommation de 10% de luzerne 4- même que 3 sauf que la solution quotidienne d'urée fût divisée en 4 fractions isovolumétriques et infusées à 0600, 1200, 1800, 2400 5- même que 2 sauf que la luzerne fût placée dans le rumen 6- même que 5 sauf que la luzerne fût placée dans le rumen en 4 portions égales à 0600, 1200, 1800, 2400. Les consommations totales de matière sèche fûrent significativement plus élevées avec un supplément de luzerne versus un supplément d'urée. Toutefois cette plus forte consommation fût attribuée à la consommation de luzerne comme telle puisqu'aucune différence en terme de consommation de fléole ne fût notée. Une plus haute consommation ne fût pas toujours accompagnée par un passage plus rapide de la phase solide ni par une plus forte concentration d'azote dans le rumen suggérant que la plus forte consommation observée lorsque la luzerne fût offerte ne fût pas causée seulement par un apport supplémentaire d'ammoniaque. L'appétence fût aussi éliminée comme facteur causatif puisqu'aucune différence en terme de consommation totale ou de fléole ne fût obtenue entre les diètes 1 et 2 vs 5 et 6. Pour étudier plus en détail les effets de l'urée et de divers niveaux de supplémentation de luzerne de même qu'un bénéfice potentiel

de supplémenter les 2 ensemble, une deuxième expérience a été conduite suivant le même modèle que la première. Les six diètes cette fois consistaient du foin de fléole à volonté et d'un des 6 suppléments suivants: 1- aucun 2- 150g de foin de luzerne 3- quantité d'urée apportant une même quantité d'azote que 150g de luzerne 4- 300g de luzerne 5- 150g de luzerne et le supplément d'urée de la diète 2 6- 450g de luzerne. Tous les suppléments de luzerne ont causé une amélioration de la consommation totale de matière sèche vs la diète 1 dû à la consommation de la luzerne puisqu'aucune différence quant à la consommation de foin de fléole ne fût notée. La consommation d'énergie digestible fût augmentée par les suppléments de luzerne et une tendence vers une augmentation de la rétention d'azote fût aussi notée. La similarité entre les concentrations d'ammoniaque pour les diètes 2 et 1 a suggére que l'effet de la luzerne fût causé par des facteurs autres que son seul contenu d'azote. Les proportions molaires des acides valériques et isovalériques, facteurs de croissance essentiels pour les microorganismes fûrent augmentées par la luzerne et pourraient avoir joués un role. Même si non significatif, la substitution de la luzerne par l'urée a causé une diminution de la consommation. Un supplément d'urée (diète 3) n'a pû causer une augmentation de la consommation et aucun bénéfice ne fût observé lorsque urée et luzerne fûrent offerts simultanément. Les résutats des deux expériences ont donc indiqué que l'effet de la luzerne sur la consommation

totale de matière sèche est dû, du moins en partie à des facteurs autres que son seul contenu d'azote. De plus, la deuxième expérience à démontré qu'il n'y a aucun avantage en terme de consommation à donner de l'urée à des béliers consommant un foin de fléole.

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LIST OF TABLES

LIST OF APPENDIX TABLES

Tables **Page**

TABLE OF CONTENTS

I. INTRODUCTION

In developed industrialized countries as well as in the developing world, the conceptualization of the fact that natural resources are limited and, in some cases non renewable has, increased scientific research in areas such as development of techniques maximizing the efficiency of utilization of raw products; developments of uses for wastes from industries; and maximal and efficient uses of by-products. A large variety of examples can be drawn specially from the integrative sector of agriculture-food production; research in maximizing efficiency of production of cereal grains , by controlling weeds, insects, determining optimal planting time,etc, and research to maximize efficiency of use of the by-products for human consumption and or animal feeding and to maximize utilization of wastes such as straws, corn stovers and animal wastes.

Focusing more on the animal production industry, this attempt to reduce the amount of wastage has renewed the interest in maximizing the use of low quality forages such as cereal straws, corn stovers, corn cobs, soybean hulls and low quality grass hays. The animals best-suited to consume these fibre-rich products are the ruminants because of their pregastric fermentation. Maximizing forage feeding would also reduce some of this competition between human and animals for products such as barley, wheat, and corn... It also allows the utilization of grazing of lands that are

not suitable for any other cultivation.

Early work on forages was an attempt to characterize their nutritive value for ruminants using *in vivo* experiments and to determine the chemical(s) element(s) of feeds by laboratory quantification that could allow the prediction of animal performance. Having extensively analyzed these feeds it became evident that grass hays and crop residues have a very high content of fiber and a low content of crude protein, both of which could play a role in limiting maximal animal performance by potentially affecting intake and digestibility of dry matter. Subsequently, research on low quality forage feeding took one of two major orientations: first, try to change the fibrous components of these feeds by altering physically or chemically their structure or composition, to maximize their nutritive value and animal performance; and second, was to try to improve the performance of animals fed these diets by supplying extra nitrogen (N) to compensate for the inherently low content of N in these roughages.

It is also well recognized that supplementation of a good quality legume hay to a basal low quality forage diet results in improved animal performance mainly via an increased dry matter intake. The mechanisms triggering this higher voluntary consumption are still not understood and further scientific research as to what controls the dry matter intake of low quality forages would be of great value

in order to maximize the inclusion of these products in ruminants rations.

Although very often the greater crude protein (CP) content of legumes is assumed to be a major factor involved in increased intake when supplemented to low quality forages it has never been demonstrated. Plant composition, animal requirements, rumen microbial requirements must all be integrated in order to elucidate the mechanisms involved.

A better understanding of how legume supplementation results in increased dry matter intake was the main objective of this study.

variations in the number of protozoa as well as types are common, and this effect on the nutrition of the host is unknown.

The complexity of the population of rumen microorganisms due to the diversity, number and interactions among microorganisms for substrates as well as the interdependency of certain strains upon end products of others is now evident. Because of this, it is very difficult when studying the host-microbe system to predict the effects of varying the characteristics of a diet such as composition and/or physical form, and/or feeding frequency on the rumen microorganisms to subsequently predict the changes in end products of fermentation available to the host. Thus, most of the time researchers directly measure the end products of fermentation such as VFA's, NH_3 and microbial cell yield that are the main determinants of animal performance.

II LITERATURE REVIEW

2.1 NUTRITIVE VALUE OF FORAGES

2.1.1 Chemical Analysis

The chemical composition of a forage or any feed is the first limiting factor determining the overall availability of nutrients to the animal (Van Soest 1982). Knowing the anatomical components and their respective chemical composition different laboratory methods have been developed that can be used routinely to evaluate the general composition of specific feed ingredients or rations. Such analyses have been designed to meet several objectives, among which there are two important ones; the formulation of rations according to animal requirements, and development of methods and equations to predict animal performance based on the composition of the feeds offered. The proximate analysis and detergent analysis systems and infrared analysis are the three major analytical systems presently used.

2.1.1.a Proximate Analysis

The Weende system of proximate analysis has been developed more then 100 years ago (Van Soest 1967) and divides the total components of a forage into six main categories (Van Soest 1982):

- dry matter content (DM) determined by drying at 100 C
- crude protein (CP) obtained from the quantitative determination of nitrogen (N) multiplied by 6.25
- ash content determined by ignition
- lipid fraction (EE) determined by ether extraction of

e originate from the assumptions made in the development of the system. First, the determination of the CP content by the measurement of total N considers nucleic acid-N and water soluble non-protein N (NPN) (eg ammonia as true protein-N when it has been estimated that in forages only 60-80% of the N is present as protein-N (Van Soest 1982)). In addition, multiplying N content by the factor 6.25 assumes that all feeds including forage proteins contain 16% N. Part of the carbohydrate contained in the sample are contained in the CF fraction. This analysis is intended to recover the cell wall components (lignin, hemicellulose, cellulose); the remaining carbohydrate is contained in the NFE fraction, which should include the cell contents or water soluble carbohydrate and starch. The attempt to develop a system that would allow the quantification of the different types of carbohydrates present in structural or cell wall tissues the intracellular carbohydrateis based on the fact that most of the components or nutrients unavailable to both animal enzymes and bacterial enzymes are located in the cell walls of plants (Van Soest 1977). However, in analyzing for CF some cellulose, hemicellulose and lignin are solubilized resulting in an overestimation of the NFE fraction. The problem of partitioning plant carbohydrates between CF and NFE had been recognized by Crampton et al. (1938). This partial solubilization of certain cell wall components is

not uniform among various plant species partly due to the fact that CF is not a chemically uniform substance. For instance, when comparing grasses and legumes 53 to 90% of the total lignin in grass vs 8 to 62% of the lignin in legume and 5 to 29% of the cellulose in grass vs 12 to 30% of the cellulose in legumes are lost in the CF determination (Van Soest 1977), resulting in a much greater error associated with the NFE for grasses vs legumes. Thus, the CF method of analysis does not meet its goal of evaluating the amount of indigestible carbohydrates The inaccuracy of the method is clearly shown by experiments in which the digestibility figure obtained for CF exceeds the NFE digestibility value (Van Soest 1977). Not only are losses of lignin, hemicellulose and cellulose variable among plant species but so are their digestibilities (Van Soest 1977, 1967) rendering the evaluation of the nutritive value of forages from such results even more difficult. Note that in addition, because NFE is obtained by substraction its accuracy is affected by all the analytical errors associated with the CF, CP, ash, DM and EE determinations.

2.1.1.b Detergent System

Van Soest attempts to minimize the problem of CF analysis developed the detergent system of analysis, which divides components of a forage into 2 or 3 major fractions depending on wether or not water is considered as a fraction. The two major fractions are cell contents that

have a very high digestibility and include lipids, sugars, organic acids, water soluble compounds, pectin, starch, NPN (Van Soest 1982), and cell wall constituents whose digestibility is much lower and are affected by lignification (Van Soest 1982, Cullison 1982) and include hemicellulose, fiber-bound proteins, cellulose, lignin, lignified N (Van Soest 1982), silica (Cullison 1982).

These 2 major fractions are isolated by boiling a sample in a neutral detergent solution composed of 3% lauryl sulfate and EDTA, in which the cell contents are soluble. The neutral detergent insoluble fraction is commonly referred to as neutral detergent fiber (NDF). This portion of a forage can be subdivided further into acid detergent fiber (ADF) containing cellulose, lignin, lignified-N and silica, and fiber soluble in acid detergent solution, which includes hemicellulose and fiber-bound protein. This separation is accomplished by boiling a sample in a solution of 49.04% sulfuric acid and 20% cetyl trimethylammonium bromide (Cullison 1982). It is often recommended that a sample be treated with a neutral detergent prior to ADF determination in order to minimize the error introduced by pectins, tannins (Van Soest and Robertson 1977, 1980) and biogenic silica (Van Soest 1977).

Since the degree of lignification affects the digestibility of cell walls, the quantification of lignin is often accomplished by digesting the ADF in 72% sulfuric acid and ashing the resulting residue (acid detergent lignin or

acid insoluble lignin) or, by oxidation of ADF with acetic acid buffered potassium permanganate solution (permanganate lignin). The hemicellulose content can be evaluated by substracting ADF from NDF.

The laboratory method used in this case to quantitate the cell wall components (NDF) is more precise than the CF methodology of the proximate analysis; less lignin and hemicellulose are lost resulting in a higher NDF value vs CF value for a forage (Cullison 1982).

2.1.1.c Infrared Analysis

This system offers several advantages over the previous ones namely: rapidity, automation, non destruction of the sample (Brown 1987, Van Soest 1982, Shenk et al 1979, Norris et al 1976) and often less sample preparation as only drying and grinding are required (Shenk et al 1979). The principle behind this system is as for any spectrophotometric method based on the fact that different compounds such as starch or proteins will absorb different amounts of light at different wavelenghts (Isaac et al 1984), thus each one has a specific absorption band, the intensity of which being related to the concentration. However the system does not eliminate the need for wet chemistry analytical methods because to determine the chemical entities and their concentrations in unknown samples standard curves have to be constructed using samples of kncwn composition (Van Soest 1982). The samples used as standards must be similar to the unknown samples in order to

obtain good precision because factors such as particle size, method of preservation of the samples, and grinding time can interfere with the analysis (Norris et al 1976, Shenk et al 1979, Hymowitz et al 1974, Barnes and Marten 1979). In addition, different proportions of nutrients present may alter the spectra of the compound studied, for example, in forages the large absorption peak of cellulose tends to mask the peak of protein, in other circumstances the peak could also be shifted (Shenk et al 1979). Another example to underline the importance of the correct choice of standards resides in the fact that individual amino acids have different specific spectra and the spectra obtained from a dipeptide or a protein is completely different from the sum of the spectra of its individual amino acids (Shenk et al 1979). However, despite the high selectivity required to construct the standard curves researchers have generally obtained good correlation between wet chemistry analysis and infrared reflectance analysis (Brown 1987, Isaac et al 1984, Marten et al 1984). Hymowitz et al (1974) obtained a correlation of .98 between CP values determined by both methods for a variety of feeds.

Thus, although Near Infrared analysis can be useful to determine the chemical composition of large numbers of samples it does not eliminate or improve wet chemistry methods of analysis.

All the analytical systems presented so far have had as only purpose the qualification and quantification of

nutrients present in forages. This is obviuosly the first step in the evaluation of the nutritive value of a forage since as said by Van Soest et al (1978) "The nutritive value of a forage is limited by composition".

2.1.2 Evaluating the nutritive value of forages

Not eliminating the importance of the chemical composition as the first limiting factor to the nutritive value of a forage, the presence of a nutrient does not automatically imply that it is available to the animal consuming it, the proof being that many forage constituents have digestibility figures below 100%. Thus, when trying to establish the nutritive value the other factors to consider are voluntary intake and digestibility, not excluding any interactions among these two, and chemical composition. In fact, the links between nutritive value, intake and digestibilty have been recognized in the late 1950's early 60' by Crampton, Donefer and Lloyd who developed the concept of the nutritive value index (NVI) for forages (Crampton et al 1960). At that time the researchers focused more specifically on the importance of the energy digestibility rather than on proteins or other nutrients assuming that if a forage was consumed in amounts adequate to meet the energy requirements of the animals the requirements for other nutrients would also be met (Crampton 1957, Crampton et al 1960). It is now recognized that this concept of meeting

digestible energy requirements may not always apply even for forages since Seoane et al (1981) demonstrated that Bounty timothy was adequate in energy but not in CP. By knowing the nutritive value of forages, an estimation of their intake and digestibility would be of great benefit to animal producers trying to design feeding programs to attain maximal production efficiency. Evaluating the nutritive value of forages by in vivo feeding and digestibility trials for every forage is an enormous, if not impossible, task, as such experiments require much time, labour and important feed quantities (Aerts 1977). Thus, to simplify the task researchers have first tried to develop in vitro and in situ laboratory methods that could reduce the time needed to determine digestibility and that would produce results similar to in vivo measurements and second, to establish or determine chemical components of a forage that would be highly correlated with voluntary intake and/or digestibility and thus be used as predictors of nutritive value, eliminating the need for in vivo digestibility trials.

2.1.2.a In Vitro techniques

Although a variety of in vitro techniques have been developed to try to predict digestibility (eg solubility of cellulose and DM in cupriethylene diamine (Dehority and Johnson 1963), treatment of cell walls with cellulolytic enzymes (Hartley et al 1974)), one is commonly

used presently in studies related to ruminant nutrition and will be presented: in vitro rumen fermentation.

The principle of this method is basically the incubation of forage samples or feed in general with some rumen contents obtained from a fistulated sheep or steer under laboratory controlled conditions (Czerkawski 1976). A sample of forage ground and of known weight is incubated with a rumen inoculum in a defined buffer-nutrient medium of composition similar to ruminant saliva for different periods of time (Van Soest 1982, Marten and Barnes 1980, Donefer et al 1960). To establish fermentation over time different samples of a same forage are prepared, incubated and the fermentation is terminated at different times: 3, 6, 12, 24...hours after initiation (Donefer et al 1960). With this method one can also establish the lag time before the initiation of fermentation of any of the forage components. One of the first in vitro rumen fermentation methods was developed at the Ohio Agricultural Experimental Station (Donefer et al 1960) and was subsequently modified by several researchers such that today different systems are available. These can be divided into three general categories; 1- bulk incubation, 2- continuous flow system, and 3- semipermeable type (Czerkawski 1976). Depending on which measurement needs to be made, each of these systems can be opened or closed; closed ones allowing gas exchanges to be monitored. The continuous flow system simulates more closely in vivo rumen conditions since end-products of

fermentation can be removed, some of which, particularly volatile fatty acids (VFA), causing a reduction in the rate of fermentation when allowed to accumulate (Ewart 1974). The semipermeable type simulates the permeability of the rumen wall thus also allow removal of VFA, however with no change in the microbial population. For all these systems anaerobic conditions, temperature, redox potential, pH must be controlled (Czerkawski 1976). The source of inoculum or rumen extract can represent a large source of variation in the results obtained depending on the breed of the donor animal, the animal inherent characteristics as well as the ration it is consuming (Marten and Barnes 1980). However, it is common practice to obtain rumen content from a fistulated sheep or steer fed a good quality alfalfa hay twice a day specially when the objectives to be met are to compare the digestibility of different forages (Donefer et al 1960, Hensberger et al 1959). Depending on the goals the inoculum could be obtained from an animal fed a different diet or different animals fed different diets as pointed out by Orskov et al (1980) for <u>in situ</u> incubations. The rumen sample obtained is filtered; the solids collected are resuspended in a phosphate buffer solution, repressed, and refiltered with the resulting liquid being used as the inoculum (Johnson et al 1958). More recently, researchers suggested that the inoculum be enriched with particle-associated microorganisms (Craig et al 1984). Sometimes glucose and urea are supplemented (Marten and Barnes 1980).

After fermentation is completed, centrifugation is carried out and different chemical analyses can be performed on the residue: DM, organic matter (OM), cellulose, CP, etc, and on the supernatant: carbon dioxide (CO_2), VFA, ammonia (NH_3),etc. The digestibility of different nutrients can be calculated as well as rates of production of different end-products and intermediate metabolites be identified.

In 1963 Tilley and Terry suggested that a classical rumen fermentation be followed by a 48 hours digestion of the residue in an acid-pepsin solution to try to more closely simulate an in vivo "whole tract" digestion condition.

Realizing that in vitro fermentation could not exactly reproduce all characteristics of in vivo rumen conditions (Orskov 1980), especially the dynamics of such a biological system and that the maintenance of anaerobic conditions and appropriate temperature requires very careful monitoring when manipulating the inoculum as well as during the fermentation per se (Marten and Barnes 1980, Van Soest 1982), researchers developed a more simple method of estimating digestibility that does not require an extensive feeding trial and simulates in vivo conditions better than in vitro fermentation (Nocek and Hall 1983) namely: incubation of feed samples in nylon bags suspended in the rumen of fistulated animals.

2.1.2.b In situ incubation

Orskov et al (1980) pointed out that the in situ incubation method also has some limitations. The samples by-pass two steps of the digestion process of a normally ingested feed, namely chewing and rumination. The sample, once broken down or digested, cannot escape the rumen before the particles are small enough to leave the bags. This does not imply that at this point digestion is completed and since the results are obtained from analysis of the residue in the bags, interpretation should be done with care (Orskov 1980). Similarly, the soluble components of the forage or the feed incubated freely flow in and out of the bags but are definitly washed out during post incubation manipulations. Consequently, nylon bags cannot be used to study digestion of the soluble components and could potentially affect results obtained for the digestibility of the other parameters studied (Nocek 1985). It was suggested that to remove this confounding factor, bags containing the samples ready for incubation be soaked or washed (Orskov 1980, Nocek 1985, Nocek and Hall 1984). Although it has been designed to eliminate a source of variation presoaking introduces another one; the non uniform removal of soluble entities across sample weights and particle sizes (Nocek 1985). In situ incubations of different feeds can be done under similar rumen conditions with animals used consuming a standard ration, allowing a comparison of the rates of disappearance of different feeds or, samples of a specific feed can be incubated under different rumen conditions with

animals consuming different diets thus allowing the study of different fermentation environment on the digestion of a certain feed (Orskov et al 1980). The choice depends on the objectives of the experiment. As for in vitro methods, the times of incubation can be varied and different chemical analysis performed on the residue. Since the washing step after removal of the bags from the rumen has been shown to cause much of the variation in the results (Nocek 1985, Weakley et al 1983, De Boer et al 1987) two alternatives have been proposed to reduce this effect. Placing the bags in the rumen in a sequential pattern so as to remove them all at the same time and consequently wash them all at once (Nocek 1985), and second, washing the bags in an automatic machine (De Boer et al 1987).

Other factors have been identified that affect results obtained from nylon bag incubation: pore size of the bags, which should be large enough to allow entry of organisms but small enough to prevent excessive losses of feed particles, sample particle size, sample weight/bag surface ratio, site of incubation in the rumen, and diet of the animals (Nocek 1985, Weakley 1983, Orskov 1980)

With the methodology and limitations of these techniques being known it is now possible to examine some of the results obtained in an attempt to estimate the nutritive value of forages as well as results obtained from simple correlation with chemical components and see how they relate to in vivo feeding and digestibility trials.

2.1.2.c Prediction of the nutritive value

Crampton (1957) studied the voluntary intake of sheep fed five different types of hay an attempt to find which chemical component(s) of the proximate analysis system would predict voluntary intake (VI). The only significant correlations he could establish were between forage VI and % lignin in the forage, and, between digestible energy (DE) and % CP, with no correlation between VI and DM digestibility (DMD), or % CF and VI. In contrast Dulphy et al (1980) studied six grasses and demonstrated that for a 60 Kg sheep, a 1% increase in CF leads to a decrease of 38gd^{-1} in VI. Seoane et al (1981), working with varieties of alfalfa, bromegrass and timothy could not establish any correlation between any of the chemical components measured (CF, CP, EE, NFE, NDF, ADF, cellulose) and VI, however, subsequent experiments yielded significant correlation between DM intake (DMI) and % cell wall. No definite conclusion could be made.

The lack of correlation between intake and apparent digestibility of DM observed by Crampton has been supported by Van Soest (1982, 1977) and Hovell et al. (1986). Van Soest and Mertens (1977) and Van soest (1977) reported large variations in correlations between chemical components of the detergent system and VI and /or digestibility. Van Soest (1977), however observed tendencies for parameters

such as NDF, cell wall and cellulose to be better correlated with DMI vs DMD whereas lignin and ADF were better correlated with DMD, suggesting that different parameters affect DMD and DMI. It is therefore possible that no correlation exists between DMD and DMI latter in every cases. It must be mentioned that in certain circumstances positive correlations between digestibility and intake of forages have been established (Baile and Forbes 1974, Balch and Campling 1962). The question is still not resolved and no assumption about digestibility and VI correlation should be made.

Regarding cellulose, Van Soest et al (1978) demonstrated that cellulose intake was correlated to digestibility only when simultaneously correlated with lignin, which is not always the case. A good example being the tropical grasses. The lack of association between lignin and cellulose in aftermath forages also explains the lack of correlation between ADF, NDF and digestibility in each case (Van Soest et al 1978). It can therefore be concluded in trying to evaluate digestibility and/ or intake of a forage that not only must the overall chemical composition be considered but also do their relative structural arrangement. This fact is very well illustrated comparing grass and legume species where, for a similar digestibility, grasses will have higher cell wall and lower lignin content compared to legumes (Van Soest 1977, 1982) indicating that lignin, which is indigestible, does not always reduce

digestibility. Rather, its effect depends upon its association with the other structural carbohydrates. In legumes lignin is more highly concentrated in cell walls of the stems. As stated by Crampton (1957) to emphasize the importance of structural arrangement of chemical constituents:

> "Extensive but slight lignification may hamper the rate of digestion of the total forage more than heavy lignification of restricted portions"

More recent histochemical studies revealed that in fact, lignin in grasses was not only confined to the schlerenchyma cell walls but also present in cell walls of certain living cells and that none of these lignified cells could be degraded by rumen microorganisms, and consequently were indigestible. This raises the whole problem of antiquality factors or refractory or inhibitory substances that may be present in forages but are not specifically detected by proximate analysis or detergent system of analysis, thus confounding the interprtation of correlation or non-correlation between chemical composition and nutritive value (Van Soest 1982).

These antiquality factors include such compounds as phenylpropanoid (lignin, flavenoids, coumarins, tannins, isoflavones), terpenes (oils, saponins, steroids), cutins and suberins, and alkaloids. The first class is the most important in forages and includes phenolic acids such as benzoic and hydroxycinnamic acids that are thought to be crosslinking agents between lignin and structural

carbohydrates (Burritt et al 1984). In fact, these authors obtained a significant correlation between the p-coumaric acid/ ferulic acid ratio (PCA/FA), and in vitro DMD (IVDMD) and, a significant negative correlation between PCA and IVDMD when studying three grasses: lignin was positively correlated to the PCA/FA. Jung (1985) using pure cellulose and hemicellulose demonstrated different effects of various benzoic and cinnamic acids on in vitro digestibility of these compounds by mixed rumen bacteria.

Lignin is part of these antiquality factors with however no standard method of analysis. It is as mentioned before associated with lower digestibility in certain circumstances. The IVDMD of different tissues of grasses can be improved by delignification but the response is not consistent, suggesting that cell wall polysaccharides could somehow be inherently resistant to degradation (Barton and Akin 1977, Akin and Burdick 1981). A potential effect of other phenolic compound has not been examined and thus cannot be eliminated. The authors also reemphasized Crampton's proposal that that when studying digestibility the type, site and extent of lignification are not to be neglected. Van Soest and Moore (1965) suggested that ratios of lignin to cell wall or CF or ADF be established. Such ratios have been shown to be highly correlated to cell wall digestibility by the same authors.

In addition to the presence of lignin or other antiquality factors in plants and to the importance of their

relative structural arrangement the nonuniformity of the basic chemical makeup of the gross entities (lignin, cell wall, CF measured in the laboratory) also contributes to the variability of results observed trying to correlate digestibility to chemical composition (Van Soest 1982, Van Soest and Robertson 1977,Reeves 1985). Reeves (1985) demonstrated that lignin composition best predicted DMD and cell wall digestibility vs fiber components .

All these results lead to the following conclusions:

1- the interrelationship between intake, digestibility and chemical composition are highly species-oriented (Van Soest 1965)

2- estimating nutritive value from a simple proximate analysis or detergent analysis component is too variable to be dependable across a variety of conditions and forage species. It is thus logical that fermentation procedures result in more reliable estimates and it will remain as such until all the factors involved in determining digestibility and intake are known.

Researchers working on the estimation of nutritive value from in vitro fermentation have focused mainly on the correlation between in vitro cellulose digestion and in vivo cellulose digestibility or DMD. In vitro cellulose digestibility has been shown to be similar to in vivo cellulose digestibility (Hershberger 1959, Johnson et al 1962, Donefer et al 1960, Quicke et al 1959). It was observed however that legume yielded highly variable

results. In vitro cellulose digestibility also appears to be highly correlated with in vivo energy digestibility in grasses (Hershberger 1959, Johnson et al 1962, Donefer et al 1960), with in vivo DMD in grasses (Johnson et al 1962), VI and NVI in grasses (Johnson et al 1962, Donefer et al 1960). Note that Johnson et al (1962) also examined such correlations between in vitro cellulose digestion and in vivo DMD, DE, NVI for alfalfa and obtained lower coefficients than for grasses specially for NVI . Vogel et al (1984) obtained similar figures for DMD of three strains of Switchgrass in vivo vs in vitro. Good prediction of organic matter digestibility (OMD) of grass hays is also possible from in vitro results of OMD (Aerts et al 1977). In fact these authors obtained higher correlation between in vivo and in vitro then when trying to establish correlation with components of the proximate analysis or detergent system. The correlation was however further improved by using in situ nylon bag incubation. The introduction of this nylon bag technique has permitted the demonstration that hay degradation in the rumen was better related to VI than in vivo digestibility (Hovell 1986, Chenost 1970).

The variability and non-consistency of the results trying to predict nutritive value of forages, and especially the intake component , from chemical composition or in vitro or in situ techniques indicate that not all factors playing a role in ultimately determining NVI have yet been

identified, and/or that interactions among them may be as important as their individual presence.

Trying to integrate various chemical components in order to predict animal performance or the nutritive value of a forage some researchers developed summative equations. The earliest ones have been established by Van Soest, Wines and Jones (1967, 1968) to calculate OMD from NDF, lignin, ADF and sometimes SiO_2 and in vitro digestibility of NDF; these equations are summarized in Aerts et al (1977). However, ten years later Aerts et al (1977)working with 42 grass hays demonstrated that in vitro and in situ technique yielded much better estimates of in vivo OMD than Van Soest summative equations, in situ being superior to in vitro. The same ranking applied when 56 silage samples and 39 pellets samples were studied. More recently, Seoane et al (1981) established a summative equation based as previous ones on chemical analysis of cell wall components that could predict DMD of alfalfa, 3 varieties of timothy and 1 of Bromegrass grown under similar management conditions. In a subsequent experiment Seoane (1982) formulated regression equations able to predict digestible energy and CP intakes for similar forages. However, as Seone and coworkers themselves emphasized that these equations are applicable to the forages studied and not necessarily to others grown under different conditions or of very different chemical compositions.

Seoane et al. (1981) while constructing summative

equations also observed significant correlation between physical characteristics of the hays namely packed cell volume and water retention and VI. In addition, the development of the in situ technique and its marked superiority to the others, the fact that particle size of samples incubated and the diet of the animal used affect the results obtained all these observations indicate that rumen environment in terms of its microorganisms and fermentation products and its dynamic characteristics: rate of breakdown, rate of passage as well as interactions with the host are to be considered in evaluating DMI and/ or DMD of a forage (Mertens and ELY 1979). As stated by Mertens and Ely (1982):

"...both digestibility and intake of a forage are the result of the dynamic interaction of the plant, microbe and animal ".

Mathematical modeling of all these processes and interactions may become useful tools to predict nutritive value of forages as long as they include all factors involved; for now, they help to conceptualize and verify the theories proposed for the control of intake and digestibility. Under most feeding conditions and especially for low quality forages, DMI is the key factor determining animal performance (Waldo and Jorgensen 1981).

In fact DMI accounts for 70% of the nutritive value index developed by Crampton et al (1960). As stated by Worrell et al (1986):

" Before intake can be predicted from these" referring to physical characteristics of forages "and other ruminal models, individual parameters that influence intake must be identified and estimated".

Over the years several strategies have been adopted to improve DMI and animal performance of ruminants on high forage diets ; mechanically treating the forages (grinding and pelleting) treating the material with chemicals (sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), sodium bicarbonate ($NaHCO_3$) or ammonia (NH_3)) , and supplementing the forage with a CP source. The two first points mainly "attack" the fiber components of the forage whereas the last one deals with the N status or protein status of the animal-rumen microflora complex. The CP content of the grass hays and crop residues is usually very low and often below National Rsearch Council Requirements for growing or lactating animals. Note that the CP aspect has never been considered in the development of summative equations nor in vitro method, nor chemical composition correlations in trying to predict the nutritive value of forages.

Although the main objective of most of these studies was to develop feeding regimes and/ or processing methods of forages that would lead to improved animal performance, the changes they provoke in rumen-host metabolism parallelling changes in intake are a mean of trying to determine the parameters that are of importance in controlling the voluntary consumption of ruminants. It should also be understood that nutrition is a critical and primary factor involved in intake control, whereas sciences

such as endocrinology, neurology should eventually be integrated.

Before the effects of chemical and physical treatments, CP supplementation can be discussed, a general understanding of rumen metabolism and how it relates to its host is necessary. Since forages are being studied the brief overview of rumen metabolism to be presented will focus mainly on carbohydrates and protein fermentation and digestion.

2.2 RUMEN METABOLISM IN RELATION TO FORAGE FEEDING

2.2.a Rumen microorganisms

The rumen offers a site of fermentation prior to the true gastric and intestinal digestion and absorption distinguishing ruminants anatomically from non-ruminant herbivores and monogastric animals (Hungate 1966). Despite the almost continuous input of a variety of feeds and their acid producing fermentation, acids, do not accumulate in the rumen because of the buffering effect of saliva, acid absorption through the rumen wall, passage to the lower gastrointestinal tract (GI) (Van Soest 1982), and interactions between rumen microorganisms where one microbe utilizes end-products of others (Hungate 1966). The rumen microorganisms include principally a mixture of small bacteria (Selenomonads and Oscillospora) , ciliate protozoa

and fungi (Van Soest 1982). More then half of the metabolic work accomplished in the rumen can be attributed to the rumen bacteria emphasizing their importance eventhough they represent about half of the total rumen biomass (Van Soest 1982). The rumen bacteria are often classified according to the substrate they attack, albeit some may be able to use several different substrates depending on availability. Hungate (1966) had divided rumen bacteria into the cellulose digesters including rod shaped cellulolytic bacteria (eg. Bacteroides succinogenes, B fibrisolvens), the cellulolytic cocci (eg. Ruminococcus albus, R flaveciens), the starch digesters or amylolitic bacteria (eg. Steptococcus bovis, B ruminocola Selenomonas ruminantium) the hemicellulose digesters, most of which are cellulolytic bacteria, the sugar fermenters, the bacteria using acids, the methanogenic bacteria, the proteolytic bacteria of which only a few are non saccharoclastic, and the lipolytic bacteria.

The nutritional requirements of these bacteria have not been clearly defined yet, the task being complicated by the fact that some bacteria can fulfill their requirements from compounds from the diet but some use fermentation products of other microbes. However, it is well known that carbohydrates are the major energy source of rumen microbes. As noted by Hungate (1966) the intermediate state of oxidation of the carbon renders carbohydrates the most efficient energy source for anaerobic organisms. Although

some microorganisms are facultative anaerobes, the majority are obligate anaerobes (Van Soest 1982). In some instances proteins or amino acids can be used and will be used as sources of energy, but, very few bacteria are totally dependent on protein. (Hungate 1966). Energy derived from carbohydrate fermentation as adenosine triphosphate (ATP) is required for growth and maintenance of the bacteria. As ATP is generated from carbohydrates, so are volatile fatty acids (VFA) such as acetic, propionic, formic, butyric, isovaleric and succinic acids. Some of these compounds become an energy source for the host (Orskov 1982) while others are reused and transformed by other bacteria. The energy from synthesis of acetic, propionic and butyric acids is thought to be 2 ATP $mole^{-1}$ of acetic acid, 3 ATP $mole^{-1}$ of propionic acid produced via the succinate pathway, 3 ATP $mole^{-1}$ of butyric acid and 1 ATP $mole^{-1}$ methane (Van Soest 1982).

The interdependence of rumen bacteria has long been recognized; 60% of the rumen microbes utilize as energy sources end-products of other microbes (Van Soest 1982). A good example of bacterial interdependency is the decarboxylation of succinate to propionate and CO_2 such that succinate is not often found in rumen fluid (Wolin 1983) . In addition to energy, rumen bacteria also require a N source in order to synthesize proteins. The N sources available are non-protein nitrogen (NPN) from the forage itself, ammonia (NH_3) from urea in the diet or saliva, amino acids from endogeneous sources or from the feed,

protein from the feed or endogeneous sources.

Cellulolytic, methanogenic and some amylolytic bacteria use NH_3-N preferentially and in fact some bacteria are unable to survive if given N as amino acids-N only (Van Soest 1982, Hungate 1966). However, amino acids can be assimilated by Streptococcus bovis, Bacteroides succinogenes, Butyvibrio ruminicola, B. fibrisolvens . Some strains of B. ruminocola, Butyvibrio fibrisolvens and succinivibrio appear to require preformed amino acids (Hungate 1966). Thus, the fact that ruminants can survive on a diet containing only urea does not imply that microbial production will be optimal (Orskov 1982). The presence of amino acids can increase the efficiency with which energy sources are used for microbial protein synthesis (Cotta and Russell 1982).

Branched-chain VFA (BC-VFA) (isovaleric, isobutyric and 2-methylbutyric acids) are also required by rumen bacteria especially cellulolytic bacteria, for maximal growth (Hungate 1966, Orskov 1982, Gorosito et al 1985). The presence of these BC-VFA in an in vitro culture system have been shown to increase cell wall digestion and ammonia utilization by mixed rumen bacteria (Gorosito et al 1985). The growth promoting effect of BC-VFA for cellulolytic bacteria has been demonstrated by Mir et al. (1986) who obtained higher digestibility for barley straws when supplemented with BC-VFA. Supplementation with the corresponding BC-amino acids yielded variable results

suggesting that the requirements are for the BC-VFA's.

For ammonia to be used for microbial protein (MCP) synthesis, a source of sulphur must also be available (Hungate 1966, Orskov 1982). For ammonia to be efficiently utilized energy must be simultaneously available, enhancing the importance of adequate timing of energy release for protein synthesis (Hungate 1966). In addition to energy, N, and BC-VFA, bacteria also need minerals that, if not present in adequate amounts may limit growth and/ or efficiency (Orskov 1982). Heme protein and B vitamins may also be limiting in some instances (Van Soest 1982).

All rumen bacteria can be subdivided further according to their physical location: the predominant bacteria are found free in the rumen environment, some are attached to feed particles, about 38%, and, a third population is found attached to the epithelium of the rumen (Baldwin and Allison 1983, Cheng and Costerton 1981). The attachment of bacteria to the feed particles, which is thought to be via the glycocalyx, is the major reason for the lag time of digestion observed when forages are studied <u>in vitro</u> or incubated in nylon bags. The requirement for attachment before any digestion can occur could be related to the fact that some of the enzymes required are located in the polysaccharide glycocalyx or that they need this association in order to remain stable (Cheng and Costerton 1981). Akin and Barton (1983) also observed that different "attaching bacteria" have different affinities for various plant cell

wall tissues. Also hypothesized was that some bacteria may first break down cell wall allowing other ones to come in and digest the material.

The bacteria adhering to the rumen epithelium, in contrast to the rest of the rumen microflora, do not seem to be affected by variations in diets (Cheng and Costerton 1981). Between 25 and 50% of these are facultative anaerobes and may play a role in maintaining anaerobic conditions by scavenging oxygen that could diffuse in from the blood (Cheng and Costerton 1981). These epithelium adhering bacteria have another critical role especially on poor quality forage diet as they produce urease, the enzyme required to split urea into ammonia and CO_2.

The general location of the rumen bacteria is of primary importance in determining their rate and extent of passage to the lower GI tract as well as if they will leave the rumen with the solid or liquid phase, the liquid phase moving much faster than the solid phase (Prins and Clarke 19 , Cheng and Costerton 1981). Note that the tissue adhering bacteria will eventually leave the rumen since they will be released upon sloughing off of the rumen epithelium.

Aside from the bacteria, another important group of rumen microorganisms in terms of its biomass is the ciliate protozoa population (Veira 1986) of which two major categories exist; the Holotrichs that assimilate soluble sugars very rapidly and, the Entodiniomorphs able to ingest starch especially from small grains and digest cellulose

(Hungate 1966). Fermentation products by protozoa results in compounds similar to fermentation by bacteria: CO_2, hydrogen (H_2) and the VFA's (mainly acetate and butyrate) (Van Soest 1982). The protozoa have also been demonstrated to have high proteolytic activity and are able to utilize bacteria as their protein source. They may also use proteins to obtain energy subsequently releasing some NH_3 (Hungate 1966, Van Soest 1982). The fact that protozoa recycle bacterial-N may cause a reduction in the amount of bacterial-N leaving the rumen, thus of potential use to the host. Note that protozoa do not leave the rumen as extensively as bacteria (Veira 1986), and their higher retention time may be a condition for their survival (Orskov 1982). Bacterial-N recycling also reduces the efficiency of microbial cell production since this assimilation of bacterial-N is an energy requiring process (Van Soest 1982). A major problem is encountered when trying to understand better the role of protozoa in the rumen: their <u>in vitro</u> culture without any bacteria is very difficult (Van Soest 1982). Consequently, their roles are assessed from comparing faunated animals vs defaunated ones. Their possible functions in rumen metabolism have been extensively reviewed by Veira (1986): uptake of rapidly fermentable sugars preventing drastic drops in rumen pH, regulation of lactate metabolism, digestion of complex CP and carbohydrates. As mentioned by the author care must be taken in interpreting the results from faunated vs defaunated animals since in naturally faunated animals large

variations in the number of protozoa as well as types are common, and this effect on the nutrition of the host is unknown.

The complexity of the population of rumen microorganisms due to the diversity, number and interactions among microorganisms for substrates as well as the interdependency of certain strains upon end products of others is now evident. Because of this, it is very difficult when studying the host-microbe system to predict the effects of varying the characteristics of a diet such as composition and/or physical form, and/or feeding frequency on the rumen microorganisms to subsequently predict the changes in end products of fermentation available to the host. Thus, most of the time researchers directly measure the end products of fermentation such as VFA's, NH_3 and microbial cell yield that are the main determinants of animal performance.

2.2.b Relationship between fermentation products and host metabolism

The digestive patterns in the rumen have been reviewed in details by Baldwin and Allison (1983) and Russel and Hespell (1981) and will not be discussed here. Carbohydrates, which are the major fraction of forages and the major source of energy, are ultimately transformed to VFA methane and CO_2. Methane is a major "H_2 sink" preventing accumulation of H_2 produced from intermediary

metabolism. Increased acetate production leads to increased methane production. The VFA's are absorbed through the rumen wall, some of them being metabolized by rumen epithelium before entering the blood circulation, especially butyrate which is transformed to acetoacetate and DB-hydroxybutyrate (Annison and Armstrong 1975). Acetate can enter the Kreb cycle and furnish some ATP's or be channelled into fat synthesis. The importance of propionate resides in the fact that it is the major precursor of glucose in ruminants (Annison and Armstrong 1975). Propionate can also be used as an energy source or for fat synthesis.

Since the fermentation in the rumen is not complete the indigestible residues will pass to the lower GI tract where some post gastric fermentation in the large intestine and colon can occur. Some unabsorbed monosaccharides may also be absorbed at the duodenal level as in monogastric animals as well as some carbohydrates derived from the digestion of microbial cells passing out of the rumen.

NPN entering the rumen can lead to the formation of NH_3, CO_2 and acetate. The NH_3 can subsequently be incorporated into microbial cells as proteins or other N compounds provided energy, BC-VFA and S are available (Baldwin and Allison 1983) or diffuse in the blood. Proteins entering the rumen can either first be cleaved to amino acids that can directly be incorporated into microbial cells or be deaminated with the NH_3 entering the rumen NH_3 pool and the carbon skeleton used as energy or,

directly pass out of the rumen for potential absorption in the duodenum. Alternatively, the amino acids can remain undegraded and by-pass the rumen (Chalupa 1984). When amino acids are used as energy sources some VFA's and CO_2 are also generated. Thus, the host is supplied by various sources of N: ammonia, which may be absorbed from the rumen or post ruminal fermentation and be used for subsequent synthesis of non-essential amino acids or be detoxified in the liver to urea; some protein and/ or amino acids from the diet reaching the duodenum intact; some microbial proteins which especially appear to be the major N source for ruminants on forage diets, reaching the duodenum and undergoing normal gastric digestion and duodenal absortion; N absorbed from the lower GI tract as end product of fermentation.

The amino acids absorbed from the diet or via the microbes enters the host's metabolism as in monogastric animals and can be used for protein synthesis, energy production and when biochemically possible gluconeogenesis (Owens and Bergen 1983). Thus,the essential amino acids can be of microbial or dietary origin and the overall supply from these two sources will determine if the host's requirements are met. The biological value of microbial proteins does not seem to be altered much by differences in diets varying between 66 and 87 as reviewed by Owens and Bergen (1983). Restriction of N, however, appears to cause increased polysaccharide synthesis vs protein (Owens and Bergen 1983). Another point for protein of microbial or

dietary origin is that they not only have to reach the duodenum and have the correct amino acid profile the protein must also be available to the host.

When forages are fed the ability of ruminants to recycle N becomes an important factor in protein nutrition. The rumen NH_3 that arises after a meal can be absorbed by microbes, flushed to the omasum or absorbed through the rumen wall as previously mentioned. It can be converted to urea in the liver that can then enter the blood urea pool. This blood urea can be eliminated through the urine, however, depending on rumen NH_3 concentration, on blood concentration, and on the amount of OM digested in the rumen and VFA produced, it can also be recycled to the rumen via the rumen wall or saliva, where it is hydrolyzed to NH_3 (Owens and Bergen 1983). Recycling via the saliva is the most important route when diets high in forage are fed. Note that NH_3 can also diffuse from the gut into the blood or urea can be recycled to the GI tract postruminalyl where fermentation also exists. It has been estimated that between 23 and 92% of plasma urea can be recycled to the gut (Owens and Bergen 1983). The N recycling ability of the ruminant is an excellent example of the close association and symbiosis present between its own "mammaliam metabolism" and its "rumen microorganisms metabolism". This close relationship, however, complicates the researcher's task when trying to understand the mechanisms by which dietary changes bring about changes in types and amounts of nutrients ultimately

available to the host as well as the time when they are available to ultimately determine how they integrate to control feed intake. Factors such as efficiency of microbial protein synthesis, efficiency of growth , outflow of microbial cells from the rumen, microbial cell composition will eventually affect the host, can be influenced by a variety of factors such as; feeding frequency, overall chemical composition of a diet, ruminal pH, ruminal dilution rate, nutrient solubility, degradability, timing of release of different nutrients. The study of all these factors independently of factors directly affecting the host would not be very relevant when trying to determine how ruminants control their forage intake and what dietary factors are of importance. They will thus be included in the subsequent discussion with other parameters altered by mechanical or chemical treatments or CP supplementation of forages.

2.3 METHODS TO IMPROVE DRY MATTER INTAKE OF FORAGES

2.3.1 Mechanical treatment

As mentioned earlier one of the early step in the digestion of forages , is the attachment of rumen microorganisms, specifically cellulolytic bacteria to the plant cell wall, which assists in the colonization by other types of bacteria (Wolin and Miller 1983). Under high - forage feeding conditions physical breakdown of feed

particles is an integral step of the digestive process and if accomplished by mechanical means greatly eases the task of the rumen microorganisms. The natural "mechanical means" a ruminant has are mastication, rumination, microbial fermentation and rumen contractions (Kerley et al 1985).

The development of mechanical processes, breaking down plant particles to facilitate and enhance all these steps, has been the objective of several experiments. This reduction in plant particle size prior feeding would increase the surface area available to microorganisms (Thomson and Beever 1982, Jaster and Murphy 1983, Kerley et al 1985) and thus increase rate of fermentation (Blaxter 1962). However, pelleting and chopping of grass hays have been shown to have a variety of other effects on the digestive parameters in most instances ultimately resulting in higher DMI by ruminants (Thomson and Beever 1980, Laredo and Minson 1975, Minson 1967). Grinding and pelleting results in increased rate of passage through the rumen or decreased rumen retention time of the particulate fraction (Laredo and Minson 1975, Minson 1967, Thomson and Beever 1980, Hodgson 1973), usually parallelled by a decreased DMD (Laredo and Minson 1975, Minson 1967), and digestibility of cell wall components (Laredo and Minson 1975) with the effect being greater for the forages of lowest quality. To eliminate a potential confounding factor of intake on the digestibility measurements, pair feeding studies have also been conducted comparing chopped <u>vs</u> ground and pelleted

grass hays. These studies confirmed a reduction in apparent digestibility of DM (Beever et al 1981) and NDF (Firkins et al 1986). The increased rate of passage could in fact explain the lower digestibility of fiber. Pelleting and grinding has also been shown to cause a shift in sites of digestion from the reticulorumen to the duodenum particularly for the proteins and carbohydrates, energy and OM (Beever et al 1972, Coelho da Silva et al 1972). The greater flow of N to the duodenum (Coelho da Silva et al 1972, Beever et al 1981) is due to an increase in bypass protein from the feed not to an increase in microbial protein flow (Beever et al 1981). Overall an improvement in N retention is observed when grass hay is ground and pelleted.

All these effects seem to relate back to the increased rate of passage through the rumen caused by pelleting and grinding of hays; the intermediate agent between the two being reduced particle size of forages entering the rumen. This is supported by results obtained by another group of researchers studying the effect of feeding forages ground to precise and graded sizes prior to feeding on digestion (Kerley et al 1985, Kinser et al 1985) observing different rates of passage for different sizes with the effect often being quadratic. Reid et al (1977) concluded that in order for the ruminal solid fraction to be able to leave the rumen the particles must be reduced to 1 millimeter (mm) or less. Smith et al (1967) opted for the .84mm. The potential

optimal density or specific gravity favoring ruminal escape (Ehle 1984, Welch 1986, DesBordes and Welch 1984) is also an important factor. Although the optimal particle size or density favoring the fastest escape from the rumen has not been determined and could vary across different diets (Troelson and Campbell 1968) the threshold size for the legume particles to leave the rumen is greater than for grass particles.

There is not much doubt that there are important parameters affecting rate of passage that may play a role when hays are ground and pelleted. This increase rate of passage of solid material observed upon mechanical processing of hays could in turn be a key factor in explaining the concomittant improved DMI.

The concept of bulk or reticuloruminal distension being a limiting factor to intake of low quality forages is not new. Increasing the rate of passage, keeping the reticulorumen distension constant could allow for greater intake. Although the concept has been challenged by Mowatt (as reported by Grovum 1987) stating the "role of rumen in physiologically inhibiting forage intake is of much less importance than that currently attributed to it" and by Grovum (1987) who suggested that distension of the reticulum and cranial sacs were of greater importance it cannot be refuted nor proven by the above results. The evidence for the existence of some distension receptors in the rumen or reticulorumen came from studies of Grovum (1979) who

observed reductions in intake as balloons filled with water were placed in the reticulum and studied of Egan (1972) who observed decreases in intake when balloons were placed in the rumen. Laredo and Minson (1973) observed that rumen contents of sheep fed stems or leaves of different grasses would not differ despite higher intakes of leaves. Leaves however caused shorter retention time. A significant negative correlation has been established between DMI of Panicum grass and rumen retention time (Thornton and Minson 1972) further suggesting that the rate of passage through the reticulorumen is affected by particle size reduction and could, under similar levels of reticulorumen distension, physically allow different daily intakes. The animal would then be able to eat more to try and improve its energy and/or N status which are often not ideal on a low quality roughage diet. The improved N balance due to greater ruminally undegraded proteins could inherently affect DMI as will be elucidated in subsequent discussion.

The rate of passage or liquid dilution rate has also been shown to be an important factor for microbial growth, microbial synthetic efficiency and microbial mass reaching the duodenum. Higher passage rates increase the efficiency of yield by reducing the proportion of energy used for maintenance by reducing recycling protozoa being particularly sensitive to high turnover rates (Van Soest 1982). Microbes, being an important source of protein for the host as well as energy from fermentation product,

could also affect host metabolism and intake by alteration in their fermentation patterns.

Aside from mechanical processing to enhance intake and digestibility, forages can be chemically treated with alkali solutions including or not some N.

2.3.2 Chemical treatment

2.3.2.a Treatment with sodium hydroxide

Among the alkali solutions used, a major one is NaOH whose use has been investigated for the past century (Nolte et al 1987).

Treating low quality forages with NaOH solutions alters their chemical composition by decreasing the relative proportion of NDF (Levy et al 1977, Nolte et al 1987) and ash (Nolte et al 1987). several studies demonstrated that treating cereal straws with NaOH greatly improves its DM and OM digestibilities to levels of 55-60% (Nolte et al 1987, Shriskandarajah and Kellaway 1984, Levy et al 1977, Lin et al 1986, Carmona and Greenhalgh 1972), as well as NDF digestibility (Lin et al 1986, Nolte et al 1987). DMI and OMI are also positively affected by NaOH treatment (Nolte et al 1987, Shriskandarajah and Kellaway 1984, with up to 100% increases in OMI observed. However if very high levels of NaOH applied, some palatability problems are encountered (Levy et al 1977).

It was suggested that NaOH can improve digestibility of lignin, silica and crystalline cellulose closely associated with lignin (Levy et al 1977, Owens 1978, Lin et al 1986, Akin and Barton 1983) by splitting ester linkages thus solubilizing hemicellulose (Lin et al 1986) resulting in a better exposure of the slowly and readily digested tissues, and enhancing the attack by bacteria (Akin and Barton 1983).

While NaOH has on a few occasions been reported to affect dilution rate or rate of passage as pelleting and grinding (Shriskandarajah and Kellaway 1984, Berger et al 1980), the main changes appear in rumen fermentation as indicated by increased total N per se , microbial-N/total-N reaching the duodenum (Shriskandarajah et al 1984), increased rumen total VFA production with no changes in molar proportions (Lin et al 1986, Levy et al 1977, Shriskandarajah et al 1984), and increased OM digested in the rumen. These changes could however be confounded with the improvements in DMI and DMD. A better energy and/or protein status of the microorganisms or the host caused by improved availability of substrates could be involved in determining DMI.

Treating low quality forages with alkaline solutions prepared from $NaCO_3$ (Shriskandarajah and Kellaway 1984) or from wood ashes (Nolte et al 1987) yielded similar results as NaOH and appears to act in a similar manner.

Though the exact mechanisms by which these treatments affect DMI have not been established yet, results obtained to this point certainly demonstrated that rumen metabolism and its end-products cannot be overlooked when studying intake. The importance of the rumen had been recognized in 1962 by Balch and Campling stating that "the rate at which rumen fermentation proceeds has a great influence on both total and digestible feed intakes."

The recognition of the needs of rumen microbes for N and the low inherent content of poor quality forages as well as the limitations on fermentation imposed by certain cell wall or fiber components all led to the development of NH_3 containing solutions to chemically treat the forages.

2.3.2.b Ammoniation of forages

As demonstrated earlier, alkali treatment acts on the cell wall or fiber component improving the overall DMD and, it may well be that under these circumstances the requirements by the microbes for N can be increased (Orskov et al 1983) and, consequently, supplying extra N by ammoniating forages could be beneficial. Treating cereal straws and low quality grasses with ammonia increases their N content by 2 and 3 times their original value (Brown et al 1987, Paterson et al 1981, Graham and Aman 1984, Horton 1978). Lower levels of NDF (Brown et al 1987, Paterson 1981, Chestnut et al 1987, Buettner et al 1982), ADF (Brown et al

1987), and phenolic acid (Chestnut 1987) are also observed with NH_3 treatment. Ammoniation causes, as does NaOH a solubilization of cell wall components as well as substances inhibiting bacterial attack probably responsible for the highest DMD, OMD, NDF digestibility (NDFD), and ADF digestibility (ADFD) observed *in vitro* and *in vivo* by the same authors. Kolankaya et al (1985) studying the effect of NH_3 treatment on the degradation of straw by cellulolytic bacteria observed a much higher colonization of the ammoniated straw vs untreated straw, a faster solubilization of the treated material and an improved growth and greater accumulation of protein in bacteria grown on ammoniated straw. The authors also noted higher acetate production by *R. flavefaciens* and *B. succinogenes* when grown on ammoniated straw. This enhanced acetate production was accompanied by a higher acetate/propionate ratio *in vivo* when steers are fed a 3% ammoniated tall fescue (Chestnut et al 1987). The role of propionate in the control of intake has been suggested by Elliot (1984) where a drop in intake was observed by infusion of propionate into the mesenteric vein of cattle. the lower propionate here may suggest a lower inhibition of intake.

In vivo NH_3 treatment also resulted in higher rumen ammonia (Chestnut et al 1987, Horton 1978) which is a required N source for cellulolytic bacteria that could also play a role in better utilization of the CF fraction. In all studies reviewed, NH_3 treatment caused important increases

in DMI and OMI. Horton (1978) obtained an increase in DMI of 41% with ammoniation of cereal straws and Buettner et al. (1982) an increase of 32% for tall fescue. Since the effects of NH_3 treatments on the kinetics of rumen fermentation and rates of passage when studied were nil (Nelson et al 1984) and since carbohydrate and protein intakes and metabolism in the rumen are altered, it is difficult to make inferences as to what primarily affected DMI: improved energy status of the host because of higher VFA, higher rates of degradation reflected by higher VFA which could lead to faster rates of passage and altered flow and form of N reaching the duodenum as for NaOH, higher VFA due to improved microbial efficiency due to altered ruminal ammonia concentration.

Although the optimal level of rumen NH_3 for microbial growth and/ or efficiency is still controversial there is no doubt that N can be a limiting nutrient when ruminants are fed high forage diets as demonstrated by DMI reponses to urea supplementation (Neutze et al 1986).

In fact, when a supplement containing some soybean meal (SBM) and rapeseed meal (RPM) was given to steers with ammoniated or non treated cereal straws, the beneficial effect of NH_3 in terms of DMD and DMI were eliminated (Horton 1978). In this case however the supply of preformed protein and amino acids in addition to NPN does not suggest that both types of N sources are required to promote maximal animal response, introducing the importance of the N nutrition of the rumen microorganisms and host in the

control of feed intake.

The variations in intake observed when a low quality forage is fed with an extra protein or NPN source will thus be reviewed. Note that much of the work has also been done on protein supplementation of grain containing or silage containing rations but those will not be considered because of different initial rumen fermentation caused by the basal diet composition which is completely different from a basal forage diet. Comparisons are therefore difficult eventhough concepts such as ruminally degradable and undegradable, N vs amino acids requirements of the microorganisms and the host, altered flow of nutrients to the duodenum are still valid in all cases.

2.4 SUPPLEMENTATION OF FORAGES

2.4.1 Crude protein supplementation of low quality forages

As mentioned earlier low quality forages are inherently low in N and thus cannot optimally supply the N requirements of the rumen microorganisms and/ or the host. The N status of the ruminant that ultimately results from a combination of dietary N and microbial N has been suggested to be a potentially important factor in controlling intake, in addition to the rate of passage. In fact, in 1965 Egan demonstrated that feeding N supplement improved N balance of sheep on a low protein roughage diet parallelled with higher

dry matter intakes which cannot be accounted for only by the increase in rate of passage. The author obtained positive response in DMI and DEI with urea and casein infused into the duodenum of the sheep. With ruminants, it is difficult to determined if the DMI effect of an extra N source occurs via an increased availability of NH_3 to microorganisms leading to an increased microbial protein synthesis leading to more proteins available to the host or if similar beneficial effects on microbial metabolism are brought about by increased dietary amino acids available in the rumen or, if the extra N source supplies more ruminally undegradable protein that would be directly available to the host at the duodenal level. The integration of all these sources of N for the host will at the end determine the overall N supply available for absorption and retention and thus directly affect the N status of the host.

Intraruminal infusion of urea and inclusion of urea in the diet have been demonstrated to improve DMI of sheep and steers fed low quality forages such as oat hay (Egan and Doyle 1985), oat chaff (Redman et al 1980), oat hulls and ~~solka~~ flock (Kempton and Leng 1979, Kempton et al 1979). The addition of urea (11.5gN kg^{-1} DM) to a diet of oat hay increased the fractional outflow rates of dietary and microbial digesta fractions and, notably the flow of microbial non ammonia N (NAN) to the duodenum (Egan and Doyle 1985). However this could be confounded with the higher DMI. Kempton et al (1979) did not observe any changes

in microbial NAN/OM digested when supplementing 25g urea kg^{-1} DM of a oathulls solka floc basal diet. Consequently, the improved DMI could have resulted from an enhanced bacterial fermentation activity caused by added NH_3 resulting in faster breakdown and passage as well as more microbial protein, in absolute terms, reaching the duodenum. Increased fermentation activity is supported by a increased VFA production in moles metabolizable energy $intake^{-1}$ (MEI) when urea is supplemented with no alteration in the molar proportions (Kempton and Leng 1979).

Supplementing casein to an oat hull solka floc ration (Kempton and Leng 1979, Redman et al 1980), or supplementing blood meal corn gluten meal to corn cobs (Nelson 1985), or supplementing cottonseed meal to prarie hay (Krysl et al 1987) resulted in higher dry matter intakes. These effects may be mediated by their N or amino acid supply to the microbes and/ or host.

Kempton and Leng (1979) and Kempton et al. (1979) demonstrated the importance of meeting microbial N needs as well as host amino acid requirements to obtain maximal performance or maximal intake by sheep. Sheep being offered a supplement of 25g urea kg^{-1} DM in a basal oat hull solka floc diet, or 75g casein kg^{-1} of the basal diet or, 25 g urea and 75g casein all showed similar improvements in DMI over the basal diet (+27%) suggesting that the urea was sufficient to meet N requirements of the microbes with no other benefit of supplying amino acids to the rumen.

However, when 25g urea and 75g formaldehyde treated casein (HCHO-casein) which is not degraded in the rumen, produced a 67% increase in DMI demonstrating that supplying duodenally required amino acids is also of critical importance in addition supplying N to the rumen. Only supplying HCHO-casein resulted in a similar response as normal casein or urea. Both are required; N in the rumen and amino acids for the host. A subsequent experiment further supported this fact. When a 150g kg^{-1} DM casein supplement was given or 100g casein and 50g HCHO casein kg^{-1} the liveweight gain of lambs were lower than when the proportion of HCHO casein was increased to 100g. Even if casein and/or urea had caused an increase in abomasal microbial N flow it was still not enough to match the effects of the HCHO casein. Both are thus important although each can individually increase intake if deficient.

Supplying N to the rumen could also cause greater amounts of protein reaching the duodenum and in turn supply amino acids to the host as mentioned and demonstrated earlier. However, the profile of amino acids supplied must also be adequate to meet the animal requirements. Thus, duodenal amino acid flow and profile either arising from the diet or microbes can be considered as important factors playing a role in determining intake.

The microbial requirements for N or amino acids and how deficiencies can affect DMI are still unclear since some studies did not show any beneficial effect of supplying

preformed protein to a urea containing diet (Howell et al 1983); However, DMD seemed to be improved as well as liveweight gain of the animals. The form in which N is supplied, the rate of passage and of degradation of feed particles, the extent of particle breakdown and the availability of cell wall components as the main energy source of forages, and microbial activity and resulting fermentation conditions (end-products and kinetics) have all been shown to affect DMI of low quality forages. These conclusions come from experiments in which known dietary parameters have been altered and consequently the effects observed can be related back to these.

The situation however, is more complicated when studying mixtures; the supplementation of low quality forages with legume hays such as alfalfa or clover hays. Such a practice is also commonly used to improve DMI amd performance of animals fed low quality forages, in many instances less expensive than mechanical processing or protein supplementation and less hazardous than chemical treatments. The reasons behind the observed increase in DMI however remains unclear.

2.4.2 Supplementation of low quality forages with legume

A positive response in terms of liveweight gain of

animals offered legume or a mixture of low quality forages (eg: corn cobs, grass hays, corn stovers) and legume over the low quality forage fed alone has been demonstrated on several occasions (Brandt and Klopfenstein 1986a, Klopfenstein and Owen 1981, Moseley 1974, Cruickshank et al 1985, Paterson 1982). Although the reasons behind this improved utilization of low quality forage when a good quality legume is supplemented are still unclear (Ndlovu and Buchanan-Smith 1985) as the increased DMI almost invariably observed could be the origin of all the positive effects described alone. However, the magnitude of the response is highly variable. DMI of lambs offered different ratios ad libitum of alfalfa/ tall fescue namely 0, 25, 50, 75 and 100% alfalfa linearly increased as the level of alfalfa increased with no effect on DMD (Hunt et al 1985). When the intake was restricted to 450g DM an increased DMD was observed as alfalfa increased while NDFD decreased suggesting that NDF from alfalfa was more resistant to degradation then NDF of fescue or that passage rates were higher for alfalfa allowing less time for digestion in the rumen. Similarly Soofi et al (1982) observed a linear increase in DMI and OMI when soybean stover/ alfalfa ratio was varied from 1:0, 2:1, 1:2, 0:1, and an increase in DMD with increases in alfalfa when intake was restricted. Moseley (1974) studying ryegrass-red clover combinations, Reid et al (1987) with alfalfa-ryegrass, Baker (1969) with afalfa and timothy all observed DMI, DMD, NDFD for mixtures

of grass and legumes higher than those expected from the weighted mean intake of each of the forages included, demonstrating a true associative effect, especially at lower levels of supplementation (Reid et al 1987). The fact that in certain cases some linear responses in DMI and DMD are observed as legume incorporated increases and in other cases the response is quadratic seems to depend on which legumes and grasses are combined. Brandt and Klopfenstein (1986a) observed linear increases in DMI as alfalfa was added to non-treated corn cobs and a quadratic response if the corn cobs were treated with ammonia. Reid et al. (1987) observed quadratic responses for alfalfa-ryegrass combinations, but linear responses for alfalfa-orchardgrass, red clover-orchardgrass, red clover-ryegrass. The DMD might not even show same response as DMI (Reid et al 1987). Different mixtures will have different initial nutrient combinations and nutrient availability making a comparison based on grass legume difficult.

Although its magnitude may vary there is no doubt that a positive intake response is observed when a legume-grass mixture is fed vs grass alone. The understanding of the factors responsible for it would certainly help explaining and even predicting the increase to be expected. Simply comparing digestion characteristics of legumes (alfalfa and clover) and grasses or low quality forages (wheat straw and wheat hay) Egan et al. (1975) demonstrated higher rumen NH_3 levels for legumes as well as grater apparent digestion of

(NAN)/OM digested post abomasally, and greater amino acid-N /OM flowing to the duodenum. All of these observations had previously been observed when protein supplements were offered and/ or rates of passage and fiber digestion were enhanced.

Ndlovu and Buchanan-Smith (1985) feeding bromegrass or barley straw with or without 30% alfalfa to sheep under pair feeding conditions observed higher rumen NH_3-N and BC-VFA, no difference in total VFA nor in the amount rapidly solubilized DM or NDF when alfalfa was present, rates of passage of liquid and solid fractions through the rumen were not altered but <u>in situ</u> rates of DM and NDF disappearance were increased. These results suggest that rates of degradation of alfalfa being higher than grass could supply some amino acids and /or peptides available for deamination thus the increased NH_3 and formation of BC-VFA essential factors for maximum microbial activity. Varga and Prigge (1982) comparing alfalfa and orchardgrass did not obtain any difference in total VFA nor passage rates but higher rumen NH_3 for alfalfa supporting Ndlovu and Buchanan-Smith (1985) results. The fact that rates of passage were not altered do not infer that the total amino acid-N flowing to the duodenum was not improved. A better N status of the microbes and potentially host cannot be eliminated. In fact comparing N status of steers fed legume (clover) or a grass (perennial ryegrass) Beever et al. (1986) demonstrated an improvement in N retention when the animals were fed legumes despite

greater urinary losses under pair feeding conditions. The same authors observed higher NAN flow to the duodenum when clover was fed vs ryegrass with a higher % NAN reaching the duodenum from non microbial origin on the clover diet. This might have resulted from greater amounts of dietary-N reaching the duodenum when clover was fed. Moseley (1979) also showed an augmentation of the flow of N when clover was fed with ryegrass.

The legume effect could also be related to an increased disappearance or faster breakdown of feed particles in the rumen. This is supported by a study of Moseley (1979) who noticed a greater proportion of smaller particles in the rumen when a legume was added. In a subsequent study Moseley and Jones (1984) also demonstrated a faster rate of passage of large particles when clover was fed. This supports the suggestion made much earlier that the threshold size at which particles can leave the rumen may be altered by dietary conditions. The authors concluded that the beneficial effect of clover on DMI was related to improved rates of passage. However Beever et al (1986) also working with clover and ryegrass suggested that the beneficial effect of the legume may relate more to the rate of digestion as well as greater facility for undigestible particles to leave the rumen when originating from clover. Such a conclusion was based on a faster OM and N disappearance from nylon bags for clover but no demonstration of a greater passage out of the rumen of

undigested potentially digestible particles supporting the conclusions of Ndlovu and Buchanan-Smith (1985).

Overall when legumes are fed alone or in combination with a grass: rumen NH_3-N, BC-VFA, N flow to the duodenum, N retention by the animals are increased, rate of passage is inconsistently affected but disappearance rate is increased, and total VFA concentration is not altered nor are the molar proportion of the major ones. The components of the legumes that cause all these changes that ultimately result in improved animal performance are not known. Not much work has been conducted to this point to define the key factors involved. However, from the review of the effects of supplementing legumes on rumen parameters altered N metabolism (higher NH_3, BC-VFA, flow of NAN to the duodenum) of the rumen microorganisms and host seems to be the focal point. This is further supported by previously presented beneficial effects of extra-N supplementation for low quality forage diets as urea or protein. Although the extra protein supplied by an alfalfa or legume hay may not be the only factor involved, strong evidence suggests that it plays an important role. Note that higher rumen NH_3 observed when legume is supplemented could be of benefit to cellulolytic bacteria which could in turn play a role in the enhanced rates of DM disappearance noted without neglecting the inherent characteristic of the legumes - concentrated non extensive lignification easing in itself the task of the bacteria.

With crude protein as an important factor, it remains to be determined how it acts and if its effects are related to its N supply only and or to its amino acid supply fulfiling some deficiencies of the microbes and/ or the host. Alfalfa has been shown to be composed of ruminally degradable as well as undegradable proteins, 75-80% being potentially degradable (Merchen and Satter 1983).

Research in this area is still limited. However if alfalfa protein was acting only via an extra supply of N then an NPN source could be used. However work by Brandt et al (1986b) with alfalfa supplementation of NH_3 or NAN treated corn cobs suggested a role for the protein per se (peptides or amino acids) since greater responses in animal performance were observed when 15-30% alfalfa were supplemented to a basal ammonia treated corn cobs diet vs non treated ones. This study, plus earlier work of Kempton et al (1979) supports the fact that amino acids supplied to the animal or to the rumen may result in increased DMI and animal performance. However more work is needed to clarify the mode of action of alfalfa-N per se: via extra N as ammonia only or via peptides or amino acids or, via a combination of all these.

III.EFFECT OF FEEDING FREQUENCY, PALATABILITY AND SOURCE OF N SUPPLEMENTATION ON INTAKE BY SHEEP FED LOW QUALITY GRASS HAY.

3.0 INTRODUCTION

A major advantage of ruminants over other domestic animals resides in their ability to convert forages into high quality products for human consumption. Recycling of by-products as well as maximal utilization of low quality forages such as cereal straws, corn stover, oat hulls and grass hays is receiving much attention. However, it has long been recognized that the major limitation to increased forage utilization in ruminant rations is voluntary intake. Attempts to establish correlation between voluntary intake and several chemical constituents of the forages (Van Soest 1978, 1977, 1982, Crampton 1957), intake and in vitro digestibility (Donefer et al 1960, Quicke 1959 Johnson et al 1962, Hershberger 1959) have not always been succesfull. The establishment of summative equations (Seoane et al 1981, 1982, Van Soest 1967, 1968 as reported by Aerts et al 1977) or mathematical models (Mertens and Ely 1979, 1982) to predict DMI of several forages can be useful under very restricted conditions and for selected groups of forages. The lack of success in developping methods of predicting DMI under a variety of conditions regardless of the forage species is due to the lack of knowledge of all the factors controlling DMI of forages.

Trying to elucidate the mechanisms of control researchers have studied the effects on DMI of varying

several physical and chemical aspects of forages . Mechanical treatment as grinding and pelleting before feeding (Thompson and Beever 1980, Laredo and Minson 1975, Minson 1967) or treating forages with alkaline solutions (Nolte et al 1987, Shrikandarajah and Kellaway 1984) or ammoniation of forages (Chestnut et al 1987, Paterson et al 1981, Buettner et al 1982, Horton 1978) have all been shown to improve performance and DMI of animals fed such forages vs untreated forages.

Aside from changes in DMI, parameters such as rate of passage through the rumen, rates of particle breakdown, the optimal size that feed particles must reach to leave the rumen, rumen NH_3, sites of digestion and flow of NAN to the duodenum have been shown to be altered. A potential role has been attributed to these in the control of forage intake.

DMI of grass hays and low quality forages can also be improved by a protein or NPN supplement (Egan 1965, Egan and Doyle 1985, Redman et al 1980, Kempton et al 1979) DMI parallelled by increased flow of NAN to the duodenum (Egan and Doyle 1985) and enhanced microbial activity due to increased NH_3 availability reflected in higher VFA (Kempton and Leng 1979) have lead to the confounding effect of N per se vs preformed amino acid supply. Kempton and Leng (1979), Kempton et al (1979) clearly demonstrated that the DMI of animals can be improved either by a supply of NH_3 in the rumen or by amino acids in the rumen or an increased supply to the duodenum but that the combination of the latter two

yields the best results. Thus the N and amino acid status of the rumen microbes are of importance in determining DMI of low quality forages.

The positive effects of a legume supplement added to a basal grass hay or cereal straw diet on DMI and animal performance are well recognized (Klopfenstein and Owen 1981, Moseley 1974, Hunt et al 1985, Soofi et al 1982). Changes in digestive characteristics such as faster rates of passage and rates of breakdown (Soofi et al 1982, Moseley 1974), higher rumen NH_3 and BC-VFA concentrations (Ndlovu and Buchanan-Smith 1985, Varga and Prigge 1982), enhanced NAN flow to the duodenum (Egan 1975, Beever et al 1986) with no changes in total VFA (Varga and Prigge 1982) and improved N retention (Beever et al 1986) have all been noticed. The cause and effect relationship is difficult to determine. Compelling evidence from the improvements in DMI observed when forages are treated with ammonia, doubling their N content, or when protein supplements are added, plus the facts that low quality forages are limited in N and that alfalfa contains some ruminally degradable and undegradable protein, all suggest that the legume beneficial effect is mediated by their N content and most likely by their preformed amino acid supply.

Since no work has been conducted to determine exactly how legume supplementation causes increase in DMI, an elimination process will be adopted and the first step is to determine if in fact the alfalfa effect is due only to its N

content or to some other specific protein or amino acid or mineral effect.

In addition to trials where alfalfa and timothy were both offered free choice to sheep the animals consistently chose larger quantities of alfalfa vs timothy hay suggesting a potential palatability effect of the alfalfa (Baker 1969). These studies are supported by observations of oesophageally fistulated sheep grazing swards composed of clover and ryegrass in which situation the proportion of clover ingested was always greater than the proportion in the sward (Milne et al 1982). Thus, it could well be that alfalfa increases DMI by improving palatability of the ration.

The first experiment to be presented was designed to compare DMI of sheep fed a basal grass hay diet supplemented with either NPN or alfalfa at isonitrogeneous (iso-N) levels, as well as to determine if palatability was a factor involved in the DMI of the grass-legume mixture. Note that since previous work has shown the responses to legume supplementation at levels of 25% and more (Hunt et al 1985, Paterson 1985) and not much work has been done at lower levels the proposed work will study effects of supplementing alfalfa at lower levels ranging from 10% to 25%. Since iso-N supplement of urea will be used to try to determine if in fact the alfalfa effect is related only to its N content low levels will allow avoiding any risk of ammonia toxicity. Thus for the first trial a 10% legume 90% grass (as fed)

model will be adopted. A potential benefit of supplying N sources to the rumen at several time intervals rather than all at once will also be investigated.

3.1 OBJECTIVES

Experiment I was designed to meet the following four objectives:

1- to determine if palatability is involved in controlling DMI when alfalfa hay is supplemented to grass hay.

2- to determine if alfalfa and urea have the same effect on total DMI and grass DMI when added to grass hay in iso-N amounts.

3- to determine if both N sources have the same effects on rumen digestive characteristics when supplemented to grass hay.

4- to determine if different feeding schedules of iso-N supplements (Once per day vs 4 times a day) would affect DMI and rumen digestive characteristics differently.

3.2 MATERIALS AND METHODS

3.2.a Experimental Design

The experimental design was a 6 by 6 Latin Square Design consisting of 6 periods, 6 diets and 6 animals. The six animals were randomly allocated to the 6 rations. Each experimental period lasted 10 days : 5 days allowed for adaptation of the animals to the diet and 5 days for sample collection and intake measurements.

3.2.b Animals and Housing

Six ruminally cannulated mature crossbred rams (averaging 50 kg body weight), were used. The rams were dewormed 2 weeks before the beginning of the trial using Tramisol at a rate of 0.05 ml per kg of body weight. After these 2 weeks the animals were weighed again, put in individual metabolic cages and allowed 4 days adaptation to their new environment before the trial started. The temperature of the room was maintained between 18 and 20 C. Animals were subjected to an artificial lighting program : lights were turned on at 0530 h and off at 1730 h. Each cage was equipped with a feeder divided in 2 individual compartments allowing separate but simultaneous feeding of 2 different types of feed. Each cage also had an individual water supply (plastic bucket) as well as trace mineral salt block holder. Animals were weighed at the beginning and end

of the trial.

3.2.c Diets and feeding schedule

The sheep were fed once daily between 0600 h and 06:30h and offered one of the following ration:

-Diet 1. A mixture of 10% (as fed) alfalfa hay and 90% (as fed) timothy hay ad libitum. The two forages were mixed prior feeding. The total daily quantity to be offered was calculated to be 10% more then the intake of the previous day.

-Diet 2. 10% alfalfa hay and 90% timothy hay, as diet 1, except that each hay was offered separately. The total amount offered was also calculated based on the intake of the previous day.

-Diet 3. 90% timothy hay was offered in the feeder and a solution of urea (10% w/v) isonitrogeneous with 10% alfalfa hay was infused into the rumen via the fistula immediately after the grass hay was offered. The total of both feeds was offered ad libitum.

-Diet 4. This ration was the same as diet 3 in terms of types and proportions of ingredients included. However, the total urea solution to be infused daily was divided in 4 equal amounts and infused at 4 different times during the day at 0600, 1200, 1800, 2400h.

-Diet 5. The diet was the same as diet 2 except that the 10% alfalfa hay was placed directly in the rumen during

the morning feeding instead of being offered in the feeder.

-Diet 6. This treatment was the same as 5 in terms of total amounts offered except that the daily quantity of alfalfa was divided into 4 equal amounts and placed in the rumen at 4 different times during the day at 0600, 1200, 1800, 2400.

It is important to note that all diets were isonitrogeneous. Regardless of the diet all forages were chopped to a length of 2 cm. Water and a trace mineral salt block were available at all times.

3.2.d Sampling schedule and procedure

Feeds and Refusals

The collection period started on day 6 of the experimental period and ended on day 10. From day 6 through day 10 the voluntary intakes of the animals were recorded by measuring feed offered and feed refused on a daily basis. Samples of refusals were also collected daily during these 5 days and pooled by diet at the end of the collection period. Pooled samples of feed and refusals were dried for 48 h in a forced air oven at 60 C, then stored in plastic bags at room temperature until further analysis.

Rumen Samples

On the first day of the collection period immediately prior to feeding, a sample of rumen fluid (150 ml) and rumen solid (100g wet weight) were taken. The animals were then

given a dose of chromic oxide (0.05% of the average daily intake recorded during adaptation period) and polyethylene glycol (25g of PEG Sigma Chemical Co MW 3350-4000 in 100 ml of distilled water as by Clarke et al 1972). These 2 markers were directly infused in the rumen of the animals via the fistula five minutes prior feeding. Rumen samples, both solid and liquid fractions were then collected at 3, 6, 12, 24, 48 and 72h post-feeding. Note that the 24, 48, 72 h samples, corresponding to mornings of day 2, 3, and 4 of collection, were always taken prior feeding. The whole rumen digesta samples were collected in a 1 l plastic Erlenmeyer connected to a vacuum pump (Gast Manufacturing Co Model No: 0211v46N G8CX). The Erlenmeyer and tubing were rinsed with water between each sample. After collection the solid and liquid fractions were separated by filtration through 4 layers of cheesecloth. The solid fractions were dried in a forced air oven 48 hrs at 60 C and stored in plastic bags.

The pH of the liquid fraction was immediately measured using an Acumet pH meter after which 50 ml of the sample were transferred to a plastic bottle containing 4 or 5 drops of concentrated hydrochloric acid (37.3% Baker Analyzed reagent); the other 100 ml were transferred to a bottle containing 15 ml of a 25% trimetaphosphoric acid solution. All rumen samples were then stored in a freezer at -20 C.

Blood Samples

On days 4 and 5 of the collection period 31 ml of blood

were taken by puncture of the jugular vein, prior feeding (and prior rumen sample collection on day 4) and at 4 and 8 h post feeding. 20 G 1 1/2 inch needles were used. For each blood sampling time 21 ml of blood were collected in lithium heparin coated vacutainers and 10 ml in vacutainer containing potassium oxalate and sodium fluoride. Whole blood samples were centrifuged at 2500 r.p.m. at 4 C for 15 minutes immediately after collection using a Sorvall RC-3 refrigerated centrifuge. Plasma was then extracted and transferred to disposable test tubes and stored at -20 C.

3.2.e Analytical Procedures

Feeds and Refusals

All samples of feeds and refusals were ground through a 1mm screen in a hammer mill. Their absolute dry matter and ash contents were then determined by drying 1 g of sample in a vacuum oven (National Appliance Co) at 100 C overnight and then ignited in a furnace (Thermolyne furnatrol II, Sybron) for 8 hours at 600 C. The crude protein content of the samples was determined using a Kjel-Foss macroautomatic analyzer (Foss electric, Hillerod, Denmark), and acid detergent fiber (ADF) content obtained according to Van Soest procedure (1973).

Rumen Samples

1. Solid fraction

Solid rumen samples were ground through a 1mm screen in a hammer mill and analyzed for absolute dry matter with the same procedure as for feeds. A wet digestion was performed using 15 ml of nitric acid and 2 ml of perchloric acid per g of sample. The digested samples were then diluted with deionized distilled water in a 1:100 ratio. A 1 in 10 dilution was then done using an 8% NH_4Cl solution. The chromium content of these digested and diluted samples were then determined by atomic absorption spectrophotometry using a Perkin Elmer Model 2380 atomic absorption spectrophotometer with an air-acetylene flame.

2. liquid fraction

The rumen fluid samples to which hydrochloric acid had been added were thawed, centrifuged at 20 000 G for 20 min at 4 C using a Sorvall centrifuge. Subsequently, their ammonia content was determined using a Fisher acumet pH/ion meter (model 750) and an Orion ammonia electrode (model 95-12).

Fluid samples to which trimetaphosphoric acid was added were also thawed and centrifuged and then analyzed for volatile fatty acids by gas liquid chromatography. The apparatus used was a Hewlett Packard Gas Chromatograph model 5710 A. A glass column was used packed with 20% neopentylglycolsuccinate (NPGS) and 2% H_3PO_4 as liquid phase on 60/80 chromosorb P. The column was conditioned overnight before any analysis was done. After samples that had been collected during one experimental period were

analyzed the column was removed and cleaned. External standards were used in the machine after each 15 analyses. The septum at the injector port was changed every day corresponding to about 40 injections. The column temperature was set at 150 C, detector and injector temperatures at 200 C. Fluid samples were also analyzed for their polyethylene glycol content following Mallawer et al procedure (1967).

Blood Samples

Plasma samples containing lithium heparin were thawed and analyzed for plasma urea nitrogen using a laboratory kit from Sigma Chemical Cie., kit #535-A, and samples containing potassium oxalate were analyzed for plasma glucose using a kit from Sigma (kit # 16-50). For both determinations a Beckman spectrophotometer (model 35) and 4ml 1cm lightpath acrylic cuvettes (Sarsted 67-738) were used.

Prior to statistical analysis results of the 2 days collection were pooled.

3.2.f Statistical analysis

The intake and rate of passage results were analyzed according to the analysis of variance of Steel and Torrie (1980) for a Latin Square design. Main effects were sheep, diet and period. When diet effect was found to be significant ($P<.05$), least square means were generated to locate differences between diets. For blood and rumen parameters the same model was used with the data sorted by

time of sampling . Time effect and time diet interaction were also tested.

3.3 RESULTS

3.3.a Forage composition

The composition of the grass and alfalfa hays are presented in Table 1. The grass hay had slightly higher DM and ADF content but lower CP then the alfalfa hay. The low content of ADF-N in both forages indicated that neither hay had been subjected to significant heat damage (Goering et al 1972). Alfalfa had higher ash than timothy hay and thus lower OM.

3.3.b Dry matter intakes

Daily DMI was obtained by averaging the intakes for each sheep for the 5 days of the collection period and expressed as daily DMI as g day^{-1} then corrected for body weight (kg) and metabolic body weights ($W^{.75}$). Table 2 presents the least square means (LS-means) generated to compare the diets.

DMI expressed in g consumed per day was 13% lower ($P<.065$) for diets 3 and 4 (urea supplement) compared with diets 1 and 2 when alfalfa was offered to the sheep. The difference did not, however, reach statistical significance. The effect of diet became significant when DMI was corrected for kg of body weight ($P<.025$) or by metabolic body weight .

The DMI was not affected by the method of alfalfa

TABLE 1 COMPOSITION OF THE TIMOTHY AND ALFALFA HAYS[1] (Exp.I)

	Timothy hay (Phleum pratense)	Alfalfa hay (Medicago sativa)
Dry matter (%)	94.47	91.87
Crude protein (%)	9.26	13.80
Ash (%)	6.64	7.73
Acid Detergent Fiber (ADF %)	46.44	46.32
ADF-Nitrogen	.65	.74
Digestible energy Mcal/kg[2]	2.65	2.34

1 All values except dry matter are presented on a dry matter basis.

2From NRC (1985).

TABLE 2 Least square means of average total daily dry matter intakes (TDMI) expressed as g, g kg^{-1} body weight (g kg^{-1} BW, g kg^{-1} of metabolic body weight (g kg^{-1} $W^{.75}$). (Exp.1)

	TDMI		
Diets[1]	g	g kg^{-1} BW	g kg^{-1} $W^{.75}$
1	1571.4	29.8[a]	79.9[a]
2	1589.0	29.8[a]	80.1[a]
3	1378.9	25.9[a]	69.6[b]
4	1359.8	25.9[b]	69.5[b]
5	1475.3	28.3[a b]	75.8[a b]
6	1500.0	28.5[a b]	80.7[a]
SE[2]	60.20	0.97	3.27

[1] Diet 1= 10% alfalfa (A) 90% timothy (T) mixed; 2=10% A 90% T non- mixed; 3= 90% T, urea isonitrogeneous to 10% A infused in the morning; 4= same as 3 except that urea was divided in four isovolumetric portions and infused four times a day; 5= 10% A placed in the rumen in the rumen 90% T; 6= same as 5 except that the alfalfa was divided in four equal portions placed in the rumen four times a day.

[2] Standard error of the mean.

[a b] Means in a column with different superscripts differ ($P<.05$).

feeding (per os or directly in the rumen) since no significant difference was observed between diets 1 and 6 nor 2 and 5. The feeding schedule of the N supplement (the daily allowance being given all at once in the morning in diets 2, 3 and 5 or, in subportions through the day in diets , 4 and 6) did not affect DMI regardless of the units of expression. Note in the case of diet 1, the alfalfa was mixed with the grass hay prior feeding and assuming that the sheep consumed several meals through the day the final alfalfa entry through the rumen should have related closely to the 4 times a day feeding as for diet 6.

DMI of diets 5 and 6 were not significantly different from the DMI of the urea supplemented diets when expressed in g day^{-1} or g kg^{-1}. However when DMI was corrected for metabolic body weight the DMI of diet 6 was significantly greater than both urea-containing diets. Placing the daily allowance of alfalfa in the rumen during the morning feeding (diet 5), did not result in different DMI $kg^{-1}W^{.75}$ compared to the urea containing diets.

Regarding grass DMI, diet had no significant effect on timothy hay DMI as can be seen from Table 3.

3.3.c Crude protein intakes (CPI)

The CPI were calculated similarly as DMI and LS means were generated to compare the diets (table 4) and expressed

TABLE 3 Least square means of average daily timothy DMI expressed as g, g kg^{-1} of body weight (g kg^{-1} BW) and g kg^{-1} of metabolic body weight (g kg^{-1} $W^{.75}$). (Exp. I).

	Grass DMI		
Diets[1]	g	g kg^{-1} BW	g kg^{-1} $W^{.75}$
1	1339.45	25.28	67.90
2	1437.40	26.96	72.52
3	1370.47	25.74	69.21
4	1351.98	25.77	69.06
5	1385.02	26.58	71.08
6	1370.33	25.95	69.64
SE[2]	57.04	0.90	2.54

[1] Diet 1= 10% alfalfa (A) 90% timothy (T) mixed; 2=10% A 90% T non- mixed; 3= 90% T, urea isonitrogeneous to 10% A infused in the morning; 4= same as 3 except that urea was divided in four isovolumetric portions and infused four times a day; 5= 10% A placed in the rumen in the rumen 90% T; 6= same as 5 except that the alfalfa was divided in four equal portions placed in the rumen four times a day.

[2] Standard errors of the means.

TABLE 4 Least square means of average crude protein intakes (CPI) expressed as g, g kg^{-1} body weight (g kg^{-1} BW) and g kg^{-1} metabolic body weight (g kg^{-1} $W^{.75}$). (Exp.I).

	CPI		
Diets[1]	g	g kg^{-1} BW	g kg^{-1} $W^{.75}$
1	161.30	3.04	8.16
2	160.03	2.95	7.96
3	150.57	2.81	7.57
4	153.06	2.94	7.87
5	151.75	2.92	7.81
6	147.99	2.91	7.58
SE [2]	7.84	0.14	0.36

[1] Diet 1= 10% alfalfa (A) 90% timothy (T) mixed; 2=10% A 90% T non- mixed; 3= 90% T, urea isonitrogeneous to 10% A infused in the morning; 4= same as 3 except that urea was divided in four isovolumetric portions and infused four times a day; 5= 10% A placed in the rumen in the rumen 90% T; 6= same as 5 except that the alfalfa was divided in four equal portions placed in the rumen four times a day.

[2] standard errors of the means.

as average daily CPI in g, g kg^{-1} and g $kg^{-1}W^{.75}$. Regardless of the units no difference was observed between diets with animals consuming an average of 7.83g CP $kg^{-1}W^{.75}$ or 2.93g kg^{-1} day^{-1}.

3.3.d Acid detergent fiber intakes (ADFI) and estimated digestible energy intakes (DEI)

The ADFI were calculated according to the same mathematical steps as CPI or DMI. The LS means generated are presented in Table 5. Feeding alfalfa per os or via the fistula did not significantly affect ADFI regardless of the units of expression as can be seen by comparing diets 1, 2, 5 and 6. The feeding schedule of N supplement as once daily or 4 times daily also did not result in difference in ADFI as can be seen by comparing ADFI for diet 1 vs 2, 3 vs 4, 5 vs 6. Comparing the results obtained when urea supplements were given vs alfalfa supplements, results demonstrated that as for DMI, ADFI were significantly lower, (16.29 % and 18.73%) if expressed in g kg^{-1} or g $kg^{-1}W^{.75}$ for the urea containing diets vs the alfalfa containing diets. There were no difference, however, in ADFI g $kg^{-1}W^{.75}$ between diets containing urea or diets where the alfalfa supplements were placed in the rumen ($P>.05$).

Diet had no significant effect on estimated DEI (Table 6).

3.3.e Rumen parameters

TABLE 5 Least square means of the average daily acid detergent fiber intakes (ADFI) expressed as g, g kg^{-1} body weight (g kg^{-1} BW) and g kg^{-1} metabolic weight (g kg^{-1} $W^{.75}$) (Exp.1).

	ADFI		
Diets[1]	g	g kg^{-1}	g kg^{-1} $W^{.75}$
1	759.79[a]	14.44[a]	38.73[a]
2	719.29[ac]	13.67[a]	39.10[a]
3	631.80[bc]	11.89[bc]	31.95[b]
4	617.94[b]	11.65[b]	31.31[b]
5	702.24[abc]	13.32[ac]	35.74[ab]
6	699.08[abc]	13.22[ac]	35.48[ab]
SE[2]	31.22	0.52	1.85

[1] Diet 1= 10% alfalfa (A) 90% timothy (T) mixed; 2=10% A 90% T non- mixed; 3= 90% T, urea isonitrogeneous to 10% A infused in the morning; 4= same as 3 except that urea was divided in four isovolumetric portions and infused four times a day; 5= 10% A placed in the rumen in the rumen 90% T; 6= same as 5 except that the alfalfa was divided in four equal portions placed in the rumen four times a day.

[2] Standard errors of the means.

abc Means in a column with similar superscripts are not significantly different P>.05.

TABLE 6 Least square means of the estimated average daily digestible energy intake (DEI) expressed as Mcal, Mcal kg^{-1} body weight (Mcal kg^{-1} BW) and Mcal kg^{-1} metabolic body weight (g kg^{-1} $W^{.75}$). (Exp.1).

	DEI		
Diets[1]	Mcal	Mcal kg^{-1}	Mcal kg^{-1} $W^{.75}$
1	3.85	0.072	0.194
2	4.10	0.077	0.207
3	3.80	0.072	0.193
4	3.58	0.068	0.183
5	4.01	0.077	0.206
6	3.91	0.074	0.199
SE[2]	0.177	0.029	0.008

[1] Diet 1= 10% alfalfa (A) 90% timothy (T) mixed; 2=10% A 90% T non- mixed; 3= 90% T, urea isonitrogeneous to 10% A infused in the morning; 4= same as 3 except that urea was divided in four isovolumetric portions and infused four times a day; 5= 10% A placed in the rumen in the rumen 90% T; 6= same as 5 except that the alfalfa was divided in four equal portions placed in the rumen four times a day.

[2] Standard errors of the means.

Rumen VFA concentration

The total concentration of VFA (mmol/l) was obtained by adding the concentrations of the measured individual VFA (acetic, propionic, butyric, isobutyric, valeric and isovaleric). Subsequently, the molar proportions of the acids were calculated and expressed as a percentage of total VFA concentration. The acetic to propionic ratio (A/P) was also computed. All these calculations were performed for individual samples and the means per time of collection and diet were calculated and LS means generated to compare diets. Results are shown in Tables 7 to 10 . No significant effect of diet was observed at any time in terms of total VFA concentration or molar proportions. For none of the VFA was there a significant time diet interaction, however, significant time effects were observed (Appendix A).

Rumen pH

LS means generated to compare diets are presented in Table 11 . No sifnificant difference in terms of rumen pH was observed between diets at any time pre or post feeding. As for VFA no diet time interaction was noted but the effect of time was significant ($P<.0001$) (Appendix A). Rumen pH was lower at any time post-feeding vs prefeeding .

Rumen ammonia

The effect of diet on rumen ammonia was significant at all times studied. the LS means generated are presented in

TABLE 7 Least square means of the total volatile fatty acid concentration (mmol L^{-1}), molar proportion(%) of acetic, propionic, butyric, isobutyric, valeric and isovaleric acids, and acetic/propionic ratio(A/P) prefeeding. (Exp.I)

	Diets[1]						
	1	2	3	4	5	6	SE[2]
Total	90.93	91.02	85.54	97.39	87.24	93.83	2.98
Acetic	70.84	70.69	71.81	71.55	71.50	72.88	0.89
Propionic	18.88	19.08	18.38	18.57	18.53	17.34	0.52
A/P	3.78	3.74	3.93	3.92	3.93	4.23	0.16
Isobutyric	0.83	1.03	0.75	0.71	0.80	0.73	0.13
Butyric	8.04	7.79	7.68	7.86	7.75	7.73	0.30
Isovaleric	0.97	0.96	0.91	0.84	0.91	0.85	0.08
Valeric	0.50	0.47	0.46	0.46	0.51	0.47	0.03

[1] Diet 1= 10% alfalfa (A) 90% timothy (T) mixed; 2=10% A 90% T non- mixed; 3= 90% T, urea isonitrogeneous to 10% A infused in the morning; 4= same as 3 except that urea was divided in four isovolumetric portions and infused four times a day; 5= 10% A placed in the rumen in the rumen 90% T; 6= same as 5 except that the alfalfa was divided in four equal portions placed in the rumen four times a day.

[2] Standard errors of the means.

TABLE 8 Least square means of the total volatile fatty acid concentration (mmol L^{-1}), molar proportion(%) of acetic, propionic, butyric, isobutyric, valeric and isovaleric acids, and acetic/propionic ratio (A/P) three hours, post feeding. (Exp.I).

	Diets[1]						
	1	2	3	4	5	6	SE[2]
Total	84.59	91.84	83.56	87.19	91.81	87.02	3.31
Acetic	68.90	69.69	69.25	68.37	69.03	69.55	0.87
Propionic	19.72	19.34	20.08	20.47	19.83	19.16	0.53
A/P	3.57	3.62	3.50	3.40	3.52	3.64	0.12
Isobutyric	0.68	0.67	0.70	0.70	0.68	0.73	0.03
Butyric	9.36	8.96	8.63	9.17	9.07	9.16	0.47
Isovaleric	0.68	0.65	0.71	0.71	0.70	0.76	0.05
Valeric	0.67	0.69	0.61	0.58	0.68	0.64	0.03

[1] Diet 1= 10% alfalfa (A) 90% timothy (T) mixed; 2=10% A 90% T non-mixed; 3= 90% T, urea isonitrogeneous to 10% A infused in the morning; 4= same as 3 except that urea was divided in four isovolumetric portions and infused four times a day; 5= 10% A placed in the rumen in the rumen 90% T; 6= same as 5 except that the alfalfa was divided in four equal portions placed in the rumen four times a day.

[2] Standard errors of the means.

TABLE 9 Least square means of the total volatile fatty acid concentration (mmol L^{-1}), molar proportion(%) of acetic, propionic, butyric, isobutyric, valeric and isovaleric acids, and acetic/propionic ratio (A/P) six hours post feeding. (Exp.I).

	Diets[1]						
	1	2	3	4	5	6	SE[2]
Total	89.91	88.31	84.66	81.89	86.78	83.04	4.06
Acetic	70.06	69.43	70.32	68.19	68.95	69.12	0.70
Propionic	19.09	19.14	19.02	20.58	19.96	19.53	0.50
A/P	3.73	3.74	3.76	3.39	3.54	3.62	0.11
Isobutyric	0.66	0.57	0.56	0.62	0.60	0.61	0.07
Butyric	9.16	9.78	9.06	9.47	9.37	9.53	0.35
Isovaleric	0.48	0.48	0.50	0.58	0.54	0.65	0.08
Valeric	0.55	0.60	0.53	0.56	0.57	0.55	0.03

[1] Diet 1= 10% alfalfa (A) 90% timothy (T) mixed; 2=10% A 90% T non- mixed; 3= 90% T, urea isonitrogeneous to 10% A infused in the morning; 4= same as 3 except that urea was divided in four isovolumetric portions and infused four times a day; 5= 10% A placed in the rumen in the rumen 90% T; 6= same as 5 except that the alfalfa was divided in four equal portions placed in the rumen four times a day.

[2] Standard errors of the means.

TABLE 10 Least square means of the total volatile fatty acid concentration (mmol L^{-1}), molar proportion(%) of acetic, propionic, butyric, isobutyric, valeric and isovaleric acids, and acetic/propionic ratio (A/P) twelve hours post feeding. (Exp.I).

	Diets[1]						
	1	2	3	4	5	6	SE[2]
Total	83.09	93.72	87.35	83.45	88.42	92.89	3.23
Acetic	69.87	69.49	69.54	69.26	69.85	72.37	0.77
Propionic	19.37	20.03	19.96	20.48	19.63	17.87	0.57
A/P	3.57	3.49	3.52	3.41	3.62	4.09	0.15
Isobutyric	0.42	0.45	0.43	0.50	0.46	0.51	0.08
Butyric	9.05	9.18	9.15	8.93	9.13	8.33	0.28
Isovaleric	0.40	0.32	0.40	0.34	0.40	0.43	0.06
Valeric	0.53	0.54	0.52	0.49	0.52	0.50	0.03

[1] Diet 1= 10% alfalfa (A) 90% timothy (T) mixed; 2=10% A 90% T non- mixed; 3= 90% T, urea isonitrogeneous to 10% A infused in the morning; 4= same as 3 except that urea was divided in four isovolumetric portions and infused four times a day; 5= 10% A placed in the rumen in the rumen 90% T; 6= same as 5 except that the alfalfa was divided in four equal portions placed in the rumen four times a day.

[2] Standard errors of the means.

TABLE 11 Least square means of the average rumen ammonia (mg dL^{-1}) and rumen pH at different times post feeding.(Exp.I)

	Prefeeding values						
	Diets						SE*
	1	2	3	4	5	6	
Ammonia	9.66^{ab}	8.37^{a}	9.63^{ab}	11.22^{b}	8.07^{a}	9.30^{ab}	0.69
pH	6.50	6.51	6.46	6.43	6.53	6.45	0.05
	Three hours post feeding						
	Diets						SE*
	1	2	3	4	5	6	
Ammonia	10.46^{a}	11.62^{a}	21.55^{b}	12.77^{a}	11.54^{a}	12.70^{a}	1.32
pH	6.38	6.38	6.48	6.39	6.40	6.43	0.07
	Six hours post feeding						
	Diets						SE*
	1	2	3	4	5	6	
Ammonia	7.30^{a}	8.03^{a}	13.09^{b}	9.24^{a}	8.43^{a}	7.87^{a}	1.01
pH	6.23	6.26	6.36	6.33	6.28	6.38	0.06
	Twelve hours post feeding						
	Diets						SE*
	1	2	3	4	5	6	
Ammonia	5.46^{ac}	4.58^{a}	8.34^{b}	7.73^{bc}	4.93^{a}	6.43^{ab}	0.94
pH	6.06	5.96	6.08	6.14	6.04	6.09	0.07

^{abc} Means of a line having different superscripts differ $P<.05$

Table 11 . Prefeeding, no difference in rumen ammonia was observed due to the feeding schedule of the N supplement as seen comparing diets 1 *vs* 2, 3 *vs* 4 and 5 *vs* 6. No difference was caused by *per os vs* directly in the rumen method of feeding of the alfalfa supplement. Diet 4, for which urea was infused 4 times a day, resulted in higher rumen ammonia *vs* diets 2 and 5, for which alfalfa was given once a day in the morning. No difference, however, was obtained between 4 times a day alfalfa feeding *vs* 4 times a day urea infusion.

3.3.f *Blood parameters*

Plasma urea nitrogen (PUN)

LS means generated to compare differences between diets are in Table 12 . At all times diet had a significant effect on PUN. Prefeeding PUN was higher for diet 4 where urea was infused 4 times a day compared to all other diets. No difference was observed between any other diet. At 4 h post feeding, both diets containing urea resulted in greater PUN compared to all other diets. PUN for urea diets were similar and averaged 13.9 mg dl^{-1} and PUN for all other diets were also similar averaging 9.62 mg dl^{-1}. No effect of method of feeding *per os* or in the rumen nor feeding schedule of the N supplement was noted comparing diets 1 *vs* 2, 3 *vs* 4 and 5 *vs* 6. At 8 h post feeding, urea supplemented diets caused a greater PUN compared to all the other diets with , the once

TABLE 12 Least Square means of the average plasma urea N (PUN) (mg dL^{-1}) and plasma glucose (mg ml^{-1}) at different times post feeding. (Exp.1).

Prefeeding

	Diets						
	1	2	3	4	5	6	SE*
PUN	8.30[a]	7.89[a]	8.61[a]	11.12[b]	8.61[a]	7.54[a]	0.55
Glucose	76.59	80.46	78.53	77.25	77.52	76.51	2.00

Four hours post feeding

	Diets						
	1	2	3	4	5	6	SE*
PUN	10.26[a]	9.55[a]	14.47[b]	13.35[b]	9.87[a]	8.81[a]	0.67
Glucose	71.67	78.42	77.50	72.20	73.51	75.33	2.38

Eight hours post feeding

	Diets						
	1	2	3	4	5	6	SE*
PUN	9.06[a]	8.86[a]	14.23[b]	11.93[c]	9.13[a]	8.18[a]	0.55
Glucose	80.00	77.92	76.18	75.75	78.87	2.07	2.07

*Standard errors of the means.
[abc] Means in one line with different letter $P<.05$.

a day infusion having the greater value. Note that at 8h post feeding, also meant 2 hrs after the second urea infusion or alfalfa introduction in the rumen. For the alfalfa diets, however, no effect of feeding schedule of the N supplement was noticed. The route of feeding effect was also nil.

Plasma glucose

LS means computed to compare diets are in Table 12 . No effect of diet on plasma glucose at any time was noted.

3.3.g Rate of passage of the solid fraction

The rate of passage or rate of outflow from the rumen of the solid fraction was obtained from the regression of the natural log values of the decreasing concentration of chromium (Cr) in the rumen solid samples vs time (Aitchison et al 1986). Note that data for one sheep on diet 1 was eliminated from the AOV since a very poor regression coefficient was obtained. Average R^2e for all other regressions was .92. The concentrations of Cr preinfusion as well as 72 h later were nil meaning that within the 5 collection period essentially all the marker had left the rumen eliminating the need for correction because of residual Cr from the previous period infusion. The effect of diet on rate of passage was significant and LS means are in Table 13.

For the alfalfa diets, the feeding schedule affected rate of passage, being significantly greater for diets 5 vs

TABLE 13 **Least square means of the average rates of passage out of the rumen of the solid fraction. (Exp.1).**

	Diets					
	1	2	3	4	5	6
% per hour	4.36[a]	3.37[b]	3.78[c]	3.92[c]	3.90[c]	3.26[b]
SE[x]	.082	.072	.082	.072	.072	.072

[x] Standard errors of the means.

[abc] figures with different superscripts differ P<.05.

6 and 1 *vs* 2. Thus when the alfalfa was fed in diets 1 and 2, mixing it with the grass and allowing intake throughout the day resulted in higher rate of passage *vs* feeding the supplement once in the morning. However, when alfalfa was placed directly in the rumen the rate of passage was increased by a once a day allowance vs 4 times a day. The rate of passage of diet 2 was significantly lower than the rate of passage for diet 5 but similar to the rate of diet 6. No effect of feeding schedule of the urea supplement was obtained. Rates of passage for diets 3 and 4 were similar. The latter were different but intermediate to rates of passage obtained when alfalfa was fed mixed with the grass or once in the morning. Urea containing diets resulted in rates of passage greater than diet 6 where alfalfa was placed 4 times a day in the rumen, but, similar to rate obtained for diet 5.

The concentration of Cr at time 0 was obtained from the y intercept of the regression line; the initial dose given divided by the Cr concentration at time 0 allowed the determination of the rumen pool size. The effect of diet was significant and LS means are presented in Table14. The feeding schedule had no effect for diets 1 and 2 but did for diets 3, 4, 5 and 6. Rumen pool size was greater when urea was infused once a day *vs* 4 times a day, or when alfalfa was placed in the rumen 4 times a day *vs* once. Once daily alfalfa feeding resulted in similar pool size across method of feeding. The 4 times a day alfalfa introduction in the

TABLE 14 Least square means of the estimated average rumen pool size. (Exp1).

	Diets						
	1	2	3	4	5	6	SE[x]
Pool size kg	3.63[a]	3.58[a]	3.49[a]	3.03[b]	3.65[a]	4.22[c]	0.11

[x] Standard error of the mean.
[a b c] Figures with different letter differ P<.05

rumen resulted in the greatest pool size vs all other diets and the 4 times a day urea infusion the lowest.

3.3.h Rate of passage of the liquid fraction

Results obtained gave concentrations of PEG consistently lower than those obtained by Froestschel et al (1987) for over 3 times the amount infused and thus extrapolating the data to calculate rumen volume figures in the 30 L range were obtained. In addition, at several occasions increasing concentrations of PEG were measured. Because of these reasons the results obtained for this analysis will not be presented. Several explanations can be proposed for the difficulties encountered :

- non adequate mixing of the marker with rumen fluid at the time of infusion.

- unadequate sample handling.

- marker moving with the solid fraction and not only with the liquid as suggested by Neudoerffer et al (1973) and McRae (1974).

3.4 DISCUSSION

3.4.a Forage Composition

The DM and CP content of timothy and alfalfa hays (Table 1) were similar to values reported in NRC (1985) feed composition tables for suncured midbloom timothy hay and suncured full bloom alfalfa hay with DM figures being slightly greater. The CP content of the alfalfa hay was lower than in most of the studies thus far presented, among which, those of Ndlovu and Buchanan-Smith (1985) and Brandt and Klopfenstein (1986a) probably because of a later stage of maturity. The ADF content of the alfalfa and timothy hays were similar to the values obtained by Baker (1969) ranging from 43.8% to 45.2% of DM for alfalfa and 44-47.6% for timothy. The ADF of alfalfa was also close to the 43% figure reported by Brandt and Klopfenstein (1986a). The ADF content of the alfalfa hay was elevated compared to earlier cut alfalfa hay having higher CP content as reported by Reeves (1985) at 39.2% ADF, Soofi et al (1982) at 35.4% and Hunt et al (1985) at 34,3%. Similarly, the ADF content of the timothy hay used in this trial was higher than several reported values of other low quality forages such as corn cobs (46.6% ADF) (Brandt and Klopfenstein 1986a), tall fescue (48.4% ADF) (Hunt et al 1985).

Alfalfa %OM was similar to previously reported figures of 92.7% OM (Hunt et al 1985), and 91.7% (Soofi et al 1982).

In general, the range of DMI in terms of g kg^{-1} or g

$kg^{-1}W^{.75}$ observed in this trial of 25.9 to 29.8 and 69.5 to 80.7 are in the same range than figures reported by Seoane et al. (1981) for timothy and alfalfa fed alone, by Cruickshank et al (1985) for ryegrass and prairie grass, by Corbett and Pickering (1979) for alfalfa and by Reid et al. (1987) for alfalfa and ryegrass. The DMI can thus be considered normal for sheep consuming these forages but are greater than figures reported for intakes of other low quality forages such as cereal straws (Horton 1978) and tall fescue (Buettner 1982).

ADF intakes (ADFI) were affected by diet and the differences observed reflected the differences obtained in DMI. Values for ADFI obtained in this trial are also comparable with intakes of cell walls observed by Seoane et al (1981) for sheep fed alfalfa or timothy. Our ADFI was slightly lower because ADF does not include hemicellulose The lack of difference in CPI indicates that all animals consumed similar quantities confirming that all diets were iso-N.

3.4.b Palatability

The lack of difference in DMI between the methods of feeding of alfalfa (per os vs placed directly in the rumen) indicates that palatability was not a factor in determining DMI kg^{-1} when a mixture of alfalfa and timothy was fed. These results might appear to conflict with those obtained

previously from conventional palatability trials (Baker 1969) or from choice of animals under grazing situations (Milne et al 1982). However, it must be recognized that in the present trial the alfalfa hay timothy hay mixture or combination was offered ad libitum , not the alfalfa only. Thus, in order for the animals to be offered more alfalfa they also had to consume more of the suggested less palatable timothy hay. At this level of supplementation the better palatability of the alfalfa hay was not enough to cause improved total DMI (TDMI) by the sheep. Palatability trials where both feeds are offered ad libitum and free choice undoubtedly demonstrated that sheep selected more alfalfa when fed fresh or dried, early or full bloom (Baker 1969); heifers also opted for alfalfa vs timothy (Pratt et al 1950 as reported by Baker 1969). Such palatability trials do not demonstrate, however, that only gustative factors are involved in causing higher DMI of alfalfa vs timothy; the animals might be responding to some other physiological signals resulting from the ingestion of these forages. This is supported by the fact that although offered alfalfa free choice (Baker 1969) or clover free choice (Milne et al 1982) the animals still consumed in all cases some smaller amounts of the grass forage. The results of the present trial also supported the involvement of signals other than purely "gustative " at this level of supplementation since for a given amount of alfalfa sheep consumed similar quantities of timothy hay even when the animals were not allowed to chew

the alfalfa. This is in agreement with results of Weston (1966) who noted a reduction in oral intake of DM when feed was fed intragastrically. The magnitude of the reduction corresponded to the amount of food introduced intragastrically.

The lack of effect of method of alfalfa feeding also demonstrated that when alfalfa constituted 10%(as fed) of the diet its mastication and thus initial particle breakdown was not a limiting factor for TDMI. As mentioned before the animals have four inherent mechanisms to physically reduce particle size of forages ingested: mastication, rumination, microbial fermentation and rumen contractions (Kerley et al 1985). In the present experiment the first step mastication and ensalivation was by-passed but might have been compensated by the 3 other ones.This cannot, however, be directly demonstrated from the present results since time spent eating, ruminating, degree of rumen contractions and particle sizes in the rumen were not monitored. The similarity in rumen fermentation parameters would tend to eliminate mastication as an important factor. There were no differences between diets 1, 2, 5 and 6 in VFA concentration nor molar proportion, nor rumen pH and NH_3 at any time post feeding, nor was there any significant interaction of diet and time post feeding for VFA profiles nor pH. From the similarity in rumen fermentation characteristics and the lack of difference in TDMI it can be concluded that the mastication of the alfalfa was not an important factor in

the control of intake not eliminating potential effects on the kinetics of ruminal fermentation and subsequent nutrient flow to the duodenum.

In fact, the rates of passage of the solid fraction out of the rumen were found to be lower when alfalfa was introduced directly in the rumen vs when it was fed mixed with the timothy hay.

A slower rate of passage of non-masticated feed is also suggested by results of Bailey and Balch (1961) who found that feeding 6.4 kg day^{-1} of a similar ration to cows via a fistula or by mouth resulted in greater dry matter accumulation in the rumen postfeeding thus slower rates of passage. Reduced rates of passage parallelled by larger rumen pool size (diet 6) at least did not cause any reduction in TDMI. This certainly casts doubt on the previously mentioned concept of rumen distension and even reticulorumen distension playing an important role in the feed intake control of forages by ruminant. Eventhough Grovum (1987) suggested that distension threshold points causing a reduction in intake may vary among diets, it is not likely to be the case here since diet compositions were identical.

Bailey (1962) incubated swallowed and unswallowed dried grass thus masticated vs non masticated dried grass in nylon bags suspended in the rumen of steers and observed faster rates of fiber digestion between the fourth and fifteenth hours, and of protein, DM, and ash during the first hour of

incubation for the swallowed dried grass. The author thus emphasized the importance of crushing and ensalivation in regards of rate of degradation in the rumen. A slower rate of degradation when alfalfa was introduced in the rumen instead of being ingested by mouth might thus be the cause of the reduced rate of passage observed since it has been demonstrated that particles must reach a specific size before being able to leave the rumen (Reid et al 1977, Smith et al 1967). In certain instances, however, no link has been found between rates of degradation and rates of passage (Ndlovu and Buchanan-Smith 1985); the reduced rate of degradation could in turn be explained by smaller surface area exposed to the rumen microbes, which is thought to be an important factor determining microbial attachment and ease of fermentation of feed particles (Thomson and Beever 1982, Jaster and Murphy 1983, Kerley et al 1985). From the lack of difference in TDMI, ADFI as well as digestibilities I can conclude that the rate of particle breakdown was not affected by fistula feeding due to the small amount given, or, that rate of breakdown was affected but was not critical for a 10% alfalfa 90% timothy mixture.

The possibility of particles being reduced to sizes too small or of non-optimal specific gravity (a factor also suggested to be important in determining rate of passage (Ehle 1984, Welch 1986)) due to more time spent ruminating over compensating for the lack of mastication when the feed was introduced directly in the rumen. No definite conclusion

can be drawn from the parameters monitored as to what caused the lower rates of passage when the alfalfa hay was placed in the rumen instead of being fed. Some authors have suggested that high rumen ammonia could reduce saliva flow (Oltjen et al 1969) and the activity of the rumen wall (Bueno et al 1977) which would in turn cause a decrease in rumen turnover rate (Nikolic et al 1980). However, this possibility must also be eliminated in the attempt to explain the lower passage rates for diets 5 and 6 vs 1 since no difference in rumen ammonia was observed at none of the sampling time.

Although differences in rates of outflow of feed particles from the rumen were noticed among diets, the range of values obtained are similar to figures reported for bromegrass and barley straw with or without 30% alfalfa (Ndlovu and Buchanan-Smith 1985). The latter authors obtained rates of passage varying from 2.54% h^{-1} for barley straw without alfalfa to 3.07% h^{-1} for bromegrass without alfalfa. The tendency for the rates of passage of the present trial to be slightly greater may relate to differences in initial composition of the ration. Hunt et al (1985) working with tall fescue alfalfa combinations, obtained rates of disappearance of DM varying between 3.6% and 5.1% h^{-1} depending on the percentage of alfalfa included. Aitchison et al. (1986) reported figures of 2.98% to 3.32% h^{-1} for ryegrass at different stages of maturity. Krysl et al. (1987) reported particulate matter

passage rate of 3.72% h^{-1} when ewes were fed prairie hay.

For these 4 diets rumen total VFA concentration averaged 90.56mmol L^{-1} prefeeding, 88.82 mmol L^{-1} 3h post feeding, 87.01 mmol L^{-1} 6 post feeding and, 89.53 mmol L^{-1} at 12 h post feeding with percentage acetic between 68.7 and 72.4, percentage propionic between 17.3 and 20.0, and percentage butyric between 7.7 and 9.8 with no difference between diets. These concentrations are similar to figures reported by Ndlovu and Buchanan-Smith (1985) who fed bromegrass with or without 30% alfalfa to sheep at restricted intakes, however, they observed an average VFA voncentration of 92.66 and 103.58 mmol L^{-1}. Beever et al (1986) reported VFA concentrations ranging from 97 to 110mmol L^{-1} in the rumen of steers fed perennial ryegrass at different stages of maturity and at different levels of intake with molar proportion of acetate ranging from 66 to 68%, propionate from 20 to 30% and butyrate from 10 to 12%. Panditharatme et al (1986) feeding orchradgrass to sheep fertilized with different rates of N and S, with or without clover observed acetic acid ranging from 63.56 to 75.01%, propionic acid from 15.04 to 16.77%, and butyric acid from 5.91 to 7.84%. Therefore results obtained in the present trial were characteristic of rumen VFA concentration observed on high forage diets both in terms of total VFA and molar proportions. The VFA's corresponded well with the classical 69:17:14 or 70:20:10 ratios of acetic: propionic: butyric acids reported for high forage diets (Harrison and

Mc Allan 1980, Bergman 1973). Rumen NH_3 in mg dL^{-1} has been reported by the same authors to range from 5.1 to 21.8 on perennial ryegrass, from 13.02 to 24.12 for barley straw or bromegrass with or without 30% alfalfa, and 3.26 to 30.88 for orchardgrass plus or minus clover and different N and S fertilization rates . Varga and Prigge (1982) fed alfalfa or orchradgrass and reported rumen ammonia varying between 13.5 mg dL^{-1} for orchardgrass and 21.2-22.6 mg dL^{-1} for alfalfa. Regardless of the method of alfalfa feeding in the present trial figures for diet 1, 2, 5 and 6 were within the lower spectrum of the range reported above and varied from 4.58 to 12.70 mg dL^{-1}. Thus, figures were normal for high forage diets and far below the critical level for toxicity (100mg dL^{-1}) (Owens and Bergen 1983).

The question of ideal ammonia concentration for maximal microbial yield, for maximum efficiency of microbial growth, efficiency of microbial protein synthesis and/ or for maximum rate of fermentation is still controversial, especially because the ideal concentration needed to maximize any one of the above parameters may not be the same (Mehrez et al 1977) and may vary depending on the basal diet studied (Mehrez et al 1977, Slyter et al 1979). Using continuous culture fermenters,and three different basal diets (purified, concentrate, forage concentrate) with and different rates of urea infusion, Satter and Slyter (1974) demonstrated that an NH_3 concentration of 5 mg dL^{-1} was enough to support maximum growth rate of rumen bacteria. The

authors also observed that excessively high concentrations that were above figures usually encountered in rumen fluid 80 mg dL^{-1} did not have any deleterious effect on bacterial growth. NH_3 began to accumulate when the CP equivalent of the substrate reached 11 to 14% . Orskov et al. (1972) supporting the optimal value of 5mg/dl in a study where they observed maximal growth rate of lambs when abomasal NH_3-N ranged between 4-8 mg dL^{-1}. Abomasal NH_3-N corresponds to ruminal NH_3-N (Hume 1970). However, Mehrez et al (1977) suggested that in order to obtain maximal DM disappearance as measured with nylon bags optimal concentration of rumen NH_3 must be between 20 and 27mg dL^{-1}, which is substantially higher than previously reported figures required for maximal microbial synthesis.

Also important to note is that for ammonia to be used efficiently energy sources must be available and consequently, for a high forage diet that is likely to be less digestible than all diets used in the previously mentioned studies, the rumen ammonia concentration required to reach maximal microbial synthesis is likely to be less (Roffler and Satter 1975). In the present trial, values for rumen ammonia on diets 1, 2, 5, 6 that ranged from 4.58 to 12.70 mg dL^{-1} correspond to optimal NH_3 concentration for maximal microbial synthesis but are lower than concentrations required for maximal rates of fermentation. The non-significant effect of diet at any sampling time on rumen NH_3 may be linked to the lack of effect of diet on

VFA, with microbes being supplied with similar quantities of N. The similarity in DEI as well as ADFI also indicated that for all these diets, similar quantities and types of energy yielding substrates were available again leading to similar VFA production.

The non-significant effect of diet on rumen pH at any times for diets 1, 2, 5 and 6 further supports the similar patterns of fermentation. Values for pH varied between 5.96 and 6.58, which are below rumen pH values reported by Ndlovu and Buchanan-Smith (1985) whose diets, however, were fed at restricted levels, all containing a basal urea level. In addition when supplemented 30% alfalfa was added as 10% (as fed) in the present trial. It is well recognized that alfalfa has a high rumen buffering capacity and if supplemented in larger amounts may help to maintain higher pH (Sniffen and Robinson 1987). This may have been the case in the present trial. As well, and urea hydrolysis to the alkali ammonia could may have also a role. However, the rumen pH reported in this study is low for an all forage diet in which pH is normaly maintained close to neutrality (Erfle et al. 1982). But several studies suggest that bacterial growth rates are decreased by pH values of 5.5 and lower (Russell and Dombrowski 1980, Prins and Clarke 1980) 5 is lower than 5.5 (Erfle et al. 1981). Russell and Dombrowski (1980) observed a decrease in the number of cellulolytic bacteria when rumen pH was decreased to between 5.7 and 6.2. Thus, it is unlikely that the microbial

population had been severely affected in the present trial and if so , it was affected uniformily since no differences in rumen pH were found across diets in nor other rumen parameters for diets 1, 2, 5 and 6.

Plasma urea N (PUN) for diets 1, 2, 5 and 6 did not differ at any sampling time. Because NH_3 absorption from the rumen, is related to the concentration of rumen NH_3 (Chalmers et al. 1976, Leng and Nolan 1984) and to rumen pH the lack of dietary effect on pH and NH_3 explained the lack of difference observed between the 4 diets considered. The absolute values corresponded well to figures reported for sheep consuming 9.2-11% CP, for which blood urea N (BUN) concentrations were 7.8 mg dL^{-1} and 10.2 mg dL^{-1} (Preston et al 1965). Cows fed a 8.9%CP or 11.3%CP diet had BUN levels of 9.60 and 11.27 mg/dl, respectively, (Prewitt et al 1971). In the present trial the average CP for diets 1, 2, 5 and 6 was 9.92%.

Although the reasons remain unclear plasma glucose concentrations were elevated compared to reported normal values for adult ruminants usually ranging between 40-60 mg dL^{-1} vs 80 and 100 mg dL^{-1} for newborn ruminants or other adult mammals (Pergman 19). Bassett (1975) reported plasma glucose ranging from 57.7 to 59.8 mg dL^{-1} when adult wethers were offered a lucerne chaff plus wheat chaff ration. Although alfalfa hay contains more soluble carbohydrates, the amount fed is unlikely to have increased the amount of glucose supplied to the intestines to explain the high

plasma levels. The blood glucose values elevated for all diets and the lack of difference between the diets indicates that most probably gluconeogenic precursors were never limited.

3.4.c Urea vs Alfalfa

The supplementation of the basal timothy hay diet with an iso-N quantity of urea instead of alfalfa significantly lowered the daily DMI as can be seen by comparing diets 1 and 2 and 6 to diets 3 and 4 (Table 2). DMI for diet 5 also tended to be higher compared to urea supplemented diets. This reduction in DMI was also accompanied by lower intakes of ADF for diets 3 and 4 compared to diets 1 and 2 at similar CP and DE intakes. Thus the iso-N urea supplement was unable to support DMI similar to the alfalfa supplement. These results support the hypothesis that the beneficial effect of alfalfa DMI when added to a basal grass hay diet is not entirely due to its N content per se and that some other factors are involved. However, looking more closely at the results urea-N and alfalfa -N can be attributed similar effects in terms of grass intake. As can be seen in Table 3, no diet effect was obtained for grass DM intake (GDMI) regardless of the units in which it was expressed. Consequently, the higher TDMI observed when alfalfa was supplemented instead of urea resulted from the alfalfa intake. It can be concluded that alfalfa and urea

supplemented at iso-N levels resulted in similar DMI of a basal timothy hay diet. The animals eating the alfalfa supplemented rations showed higher TDMI because of the lowered proportion of CP in 1 g of alfalfa vs 1 g of urea. In addition, the results shown here shade further doubts on the reticulorumen distension theory of control of forage intake. Urea containing diets had the lowest TDMI but also had slower rates of passage but similar pool sizes which would tend to support the theory. However, controversy is raised when comparing urea containing diets to diet 2 and 6 in which cases the rates of outflow of the particulate fraction was higher for the diets having the lowest intakes (diets 3 and 4). This non consistency of rate of passage and intake may well indicate that, as for particle size and particle density effects reported by Reid et al. (1977) and Ehle (1984), there is a threshold point at which rate of passage or rumen distension will affect intake or vice versa, and at values above or below act similarly. Thus the higher TDMI observed when alfalfa is fed vs urea cannot be explained by faster rates of passge suggesting that the initial grass intake was not limited by bulk but may be by a N deficiency. All animals ate to a constant CP level. However, this cannot be proven from the present results since no unsupplemented timothy hay diet was included in the design.

Similar effects of iso-N supplementation of urea or preformed proteins on DMI of a low quality forage diet had

been demonstrated previously. Wohlt et al (1978) demonstrated that N supplied as urea or soybean meal in rations for dairy cows containing 11 to 12% CP were used as efficiently for both sources in terms of milk production. However, such was not the case when the CP level of the ration was increased between 13 to 14.5%. At this point milk production of cows receiving soybean meal was greater than for those receiving urea. The authors also observed that at the 11-12% CP level rumen NH_3 concentrations were below 5 mg dL^{-1} whereas with the 13-14.5% level values were well above 5 mg dL^{-1}. It could be that at the 11-12% CP level animals or rumen microbes were suffering from a N deficiency that could be supplied by urea or soybean meal as well. Once this N deficiency is eliminated, at the 13-14% CP level then benefits of preformed proteins can be observed via supply to the microbes or increased by-pass of preformed proteins. Shriskadarajah et al (1982) observed similar intakes of wheat straw when heifers were supplemented with either urea alone, urea and casein or urea and a 70% casein 30% HCHO-casein supplement. Again, supplementing preformed protein could not result in higher intakes. However, it must be noted that increasing the proportion of HCHO-casein resulted in higher intakes. As pointed out by the authors as well as by Van Soest (1982), the improved intake due to post ruminal supply of amino acids could also have resulted, in part, from increased N recycling to the rumen. The suggestion that NH_3 and post ruminal amino acids

requirements must be fulfilled to obtain maximal DMI could also explain the beneficial effect of increasing HCHO casein in the diet. These results agree with observations previously reported by Kempton et al (1979) and Kempton and Leng (1979) who fed to sheep a diet based on cellulose with supplements of urea, casein or HCHO-casein and obtained similar increases in intake when these were fed individually, but greater responses were obtained when both HCHO casein and urea were combined. Regarding alfalfa supplementation, it is unlikely that its effect was all mediated via post ruminal supply of N and then recycled to the rumen since Neutze and Kellaway (1986) demonstrated a limited capacity for this mechanism, because in the present trial PUN values were not elevated when alfalfa diets were fed. Beever et al. (1981) suggested that greater amounts of N were digested in the cecum when dried legumes vs grasses are fed. This would suggest a potential benefit of N recycled to the rumen from legumes. Alfalfa could partly act by supplying recycled NH_3.

Orskov and Gribb (1979, 1977) could not show any benefit of replacing urea by fishmeal in cereal based diets for lambs. The necessity to meet the ammonia requirements of the microbes before any further benefit of supplying preformed protein can be observed is further supported by the greater response in DMI by lambs when alfalfa was supplemented to NH_3 treated corn cobs vs untreated ones (Brandt and Klopfeinstein 1986a). The fact that urea

supplementation is only beneficial up to a limit in term of N retention and that passed that point further improvements can only be achieved by increasing the true protein content of the diet is also well documented (Coombe and Tribe 1963, Swift et al 1947). Thus the similar DMI for grass hay observed when urea or alfalfa were fed in the present trial could be due to their supply of N as rumen NH_3. Note that rumen NH_3 reported herein were found to be in the lower range of previously reported figures for different forage diets. Rumen NH_3 never exceeded 21 mg dL^{-1} which was at 3 h post feeding on a once a day urea infusion and was never below 4.6 mg dL^{-1}, thus, was always higher than the 5 mg dL^{-1} proposed to be required for maximal microbial growth but always lower then the 20-27 mg dL^{-1} required for maximal DM disappearance. Requirements for NH_3 may have just been met with the alfalfa supplement and no benefit of the preformed protein could be observed. If NH_3 requirement was met by lower supplementation then animals may still eat more alfalfa to benefit from other nutrients present without affecting grass intake per se. At 3 h and 6 h post feeding, rumen NH_3 were higher on diet 3 vs 1 and 2, which was probably a reflection of the higher dose of hydrolyzable urea given in the morning compared to the other diets. Although non-significant, rumen NH_3 for diet 4 also tended to be higher vs others probably due to the urea infusion itself or enhanced N recycling to the rumen. Even if the magnitude of the recycling of N is still controversial

(Kennedey and Milligan 1978a, b, Neutze and Kellaway 1986) the fact it does occur leaves no doubt. Neutze and Kellaway (1986) suggested that the capacity of this mechanism was limited and that that was the reason why animals respond to increased urea supplementation. However, in the present case it could be argued that basal urea secretion was too low initially and that at any level of supplementation the absolute quantity of N recycled was the same for different diets.

The increased rumen NH_3 concentrations were reflected in the levels of PUN 4 and 8 h post feeding, being greater on diets 3 and 4 with no difference among them at 4 h but some at 8 h, following the pattern of rumen NH_3. Diet 4 had lower rumen NH_3 at 6 h post feeding and lower PUN 8 h post feeding. Note that rumen pH, not being affected by urea or alfalfa supplement, could not have played a role in influencing absortion of NH_3 across the rumen wall.

The non-significant effect of diet on total and molar proportion of VFA demonstrated similar fermentation patterns which further supports the similarity between alfalfa and urea supplemnts. Information on the effects of legume supplementation on rumen parameters is limited. Hunt et al (1985) working with alfalfa-tall fescue combinations did not report any effect on VFA or NH_3, nor did Soofi et al (1982) for alfalfa corn stover combinations, nor Moseley and Jones (1979) with ryegrass clover mixtures. Varga and Prigge (1982) reported no difference in total VFA between

orchardgrass and alfalfa . None of the authors reported effect of urea vs alfalfa. Ndlovu and Buchanan-Smith (1985) found higher BC-VFA with and alfalfa supplement, however, their level of supplementation was higher than in the present experiment.

Note that at all times, values obtained for diets 3 and 4 for VFA total concentrations and molar proportions, rumen ammonia, rumen pH, PUN, plasma glucose and rates of particulate passage related to reported values from other studies in a similar way as did values obtained for diets 1, 2, 5 and 6, and thus were considered normal, except for glucose which was slightly elevated.

None of the studies mentioned when reviewing the effect of legume supplementation had compared iso-N sources of legume and urea on respective low quality forage intake. For some of these studies the N content of the basal diet was lower than the 9.26% of the timothy hay used in the present trial and the beneficial effects of the supplemented legumes might well have been due to N only. Generalizations across basal diet can be risky because the protein solubility and degradability may affect the NH_3 levels generated the possibility cannot be eliminated. Similarly, it must also be stated that not all levels of supplementation even of similar feeds act via the same mechanisms to increase TDMI explaining the sometimes quadratic instead of linear response in DMI and DMD seen (Brandt and Klopfenstein 1986a, Hunt et al 1985). It could also be that within one level of

N supplementation different sources act differently especially if several nutrients are deficient in the diet resulting at the end in similar DMI of the basal diet. In the present trial for axample if both NH_3 and preformed amino acids had been deficient in the timothy hay then either supplement, urea or alfalfa hay would fulfill one of the deficienciesy, ultimately supporting similar grass DMI.

It could also be that alfalfa supplied part of each deficient nutrient. This hypothesis would not contradict the results obtained previously by Kempton et al (1979) and Kempton and Leng (1979). However from the parameters measured and because only one level of supplementation was used and an unsupplemented grass diet was not included in the present trial, no defenite conclusion can be made as to how urea and alfalfa resulted in similar timothy DMI.

Another possible explanation would be that the urea supplement caused a certain grass DMI even though too much NH_3 was being supplied; the animal could not reduce the urea intake. This would be supported by concentrations of rumen ammonia over 5 mg dL^{-1} at all times, the non deleterious effect of higher levels having been suggested before. Levels indicating ammonia toxicity were not reached either. Sheep may have responded to the fulfillement of their N requirement from alfalfa by consuming similar grass as when urea was fed but since more protein was supplied than was deaminated the remainder could have been used as preformed

amino acids explaining the superior TDMI observed when alfalfa was fed.

These are all suggested mechanisms but the exact one cannot be determined fron these results. Trial 2 to be presented herein will persue the research in this area.

3.4.d Feeding Schedule effect

Regarding the feeding schedule of the N supplements no effect on grass DMI, TDMI, ADFI nor CPI were observed comparing diets 1 vs 2, 3 vs 4, 5 vs 6. Plasma glucose, VFA and rumen pH were not affected either. Some differences were noted, however, between the two urea-containing diets in terms of rumen NH_3 concentrations at 3 and 6 h post feeding and for PUN prefeeding and 8 hours after. Although the difference did not reach statistical significance PUN tended to be higher 4 h post feeding for diet 3.

In 1965 Raleigh and Wallace showed that calves consuming diet of grass hay diet performed better if given a urea- containing supplement three times a day instead of once daily. This is contrary to the results obtained in the present trial. The difference may relate to the level of supplementation. At no time did sheep given the urea once a day show any sign of NH_3 toxicity. If the amount infused had been enough to physiologically affect the animals then some benefits of more frequent feeding would have been observed. Intake was not affected by once a day infusion , which

indicated that animals were able to conserve the N given by absorbing it into the blood and recycling it to the rumen threafter, or, that the animals could not conserve the N which was not deficient. The first suggestion is supported by the elevated PUN for diets 3 vs 4. Owens et al (1980) suggested the use of slow release urea compounds and demonstrated its benefits vs prilled urea when incorporated at 5 to 10% in concentrates for sheep fed cottonseed hulls ad libitum in terms of DMI and reduced signs of toxicity in steers. However, the levels used were much greater then in the present trial . Mizwicki et al (1980) used 3 different patterns of urea infusion: continuous 3.5g h^{-1} for 24 hrs, 14.2g h^{-1} for 6 h, or, 85g h^{-1} for 1 h for steers fed prairie hay. The authors found significant beneficial effect of urea feeding on DMD and N retention but no difference was observed among the rates of infusion even though rumen NH_3 was stabilized by continuous infusion. These agree with our results. It also doubtfull that NH_3 produced was badly utilized due non availability of energy sources in view of the high production of VFA observed. The lack of effect of feeding of the alfalfa supplement may have been caused by a more inherent slow release of N from alfalfa vs urea and thus, spreading the alfalfa feeding throughout the day does not affect NH_3 release as can be seen from rumen NH_3 concentration or that recycling compensated for faster release on a once a day feeding.

The lack of effect on VFA for any diet of feeding

schedule further supports that no benefits were gained by more frequent delivery of N to the rumen at this level of supplementation. The lack of difference between rumen NH_3 on diets 4 vs 1 or, 6 vs 1 also indicated that the 4 times infusion of N as alfalfa or urea were succesfull in mimicking NH_3 release from a 10% alfalfa 90% grass mixture and that this release was, in fact gradual. No difference between diet 1 and 2 further support this since alfalfa N was released faster than post feeding rumen NH_3 and should have been greater for diets 2 vs 1 which was not the case.

3.5 CONCLUSION

The results of this experiment clearly demonstrated that for a hay mixture composed of 10% alfalfa 90% timothy hay the resulting TDMI and timothy DMI were not influenced by a greater palatability of the alfalfa hay.

The TDMI of sheep was increased significantly when an alfalfa supplement was given instead of an iso-N supplement of urea directly infused in the rumen. However, this higher TDMI was due to the DM of the alfafa supplement *per se* since no diet effect was observed on timothy DMI. The non-consistent effect of the alfalfa supplement *vs* urea on rates of passage strongly suggested that the grass DMI was not controlled by bulk since the animals could ingest more DM, which was not always parallelled by increases in rates of particulate outflow from the rumen. Thus, the animals must have responded to some physiological signal(s) further supported by the lack of difference in DMI when alfalfa was placed in the rumen instead of being fed *per os*.

The urea and alfalfa supplements resulting in similar grass DMI was strong evidence for N or rumen NH_3 to be the signal, however, it cannot be clearly confirmed from the results of this trial. The only conclusion that can be made is that alfalfa and urea given in iso-N amounts supported similar intakes of grass hay, but alfalfa improved TDMI. Alfalfa could have acted via its N content, amino acids or other nutrients.

The two different feeding shedules of the supplements

had similar effects on grass DMI, TDMI, ADFI, DEI and CPI. Thus at this level of supplementation no benefit was gained from more frequent feeding of urea in terms of intake.

Although three of the initial objectives were met, results of the present trial did not allow us to clearly state that alfalfa-N and urea-N were equivalent since each supplement may have acted via different mechanisms, indicating the need for more research to try to elucidate these mechanisms of action. The experiment presented herein concentrated on the effects of the supplements on intakes but their effects on nutrient digestibility and N retention were not studied and could be of importance in determining the benefit of these supplements in terms of overall animal performance.

IV. EFFECT OF DIFFERENT LEVELS OF ALFALFA SUPPLEMENTATION AND UREA SUPPLEMENTATION ON INTAKES BY SHEEP FED A BASAL GRASS HAY DIET.

4.0 INTRODUCTION

The improved voluntary consumption of grass hays and other low quality forages by supplementation of a protein or NPN source is well documented (Egan 1965, Egan and Doyle 1985, Redman et al 1980, Kempton et al 1979). The fact that the DMI of such forages can be increased by a simple supplementation of a legume hay is also well recognized (Soofi et al 1982, Hunt et al 1985, Moseley 1974, Klopfenstein and Owen 1981).

However, the mechanisms by which these improved DMI are brought about when NPN, preformed protein or legume supplements are fed are unclear and could also differ among levels of supplementation and basal diets. Several studies suggest that maximal DMI responses are observed only when microorganisms requirements for NH_3 and/ or amino acids and host requirements for essential amino acids are met (Kempton et al 1979, Kempton and Leng 1979). Thus, at low levels of N supplementation the beneficial effects of increased crude protein supplements could simply be mediated by increased availability of ammonia since iso-N supplements of urea or protein result in similar responses (Orskov and Grubb 1979, 1977, Shriskandarajah et al 1982, Wohlt et al 1978, and results of Experiment I).

Similarly, the increased reponse observed by previously mentioned authors in DMI as legume supplementation increased

could also be mediated by N only until NH_3 starts accumulating in the rumen, and, then further benefit of higher levels could result from preformed proteins and or other nutrients.

Thus this second experiment was designed to gain more knowledge about the effects of alfalfa and urea on grass intake, their respective mechanisms of action by comparing different levels of supplementation to an all grass diet.

Since beneficial effects of increased DMI on animal performance could be negated by a decrease in digestibility of nutrients or an altered N balance these parameters will also be monitored.

4.1 OBJECTIVES

The second experiment was designed to meet the following objectives:

1- to determine the effects of urea supplementation on grass DMI and rumen parameters;

2- to determine if urea and alfalfa at iso-N levels have the same effects on grass DMI and TDMI , rumen parameters, digestibility and N balance;

3- to determine if there is any benefit of supplementing both N sources simultaneously on intake, rumen parameters, N balance;

4- to determine the effects of increasing the level of alfalfa supplementation on grass intake, TDMI, rumen parameters, N balance.

4.2 MATERIALS AND METHODS

4.2.a Experimental Design

This trial was designed as a 6x6 latin square design consisting of 6 periods, 6 diets and 6 animals with the six diets randomly assigned to the 6 animals. Each experimental period was of 17 days: 8 days of adaptation to the diet and 9 days of sample collection and measurement.

4.2.b Animals and housing

Six mature crossbred rams (35-70 kg) that were ruminally fistulated were used. Metabolic cages, lighting regimes, temperature, deworming procedures were as in Trial I. Animals were weighed at the beginning and end of each collection period.

4.2.c Diets and feeding schedule

For this trial all animals were offered a basal diet of timothy hay and one of the following supplements:

1- no supplement;

2- 150g (as fed) of alfalfa hay;

3- a 10% (w/v) urea solution infused in the rumen. The total amount given was calculated to be isonitrogeneous to 150g of alfalfa hay;

4- 300g (as fed) alfalfa hay;

5- 150g (as fed) alfalfa hay and a 10% (w/v) urea solution isonitrogeneous to 150 g alfalfa infused in the rumen;

6- 450g (as fed) alfalfa hay.

All hays were chopped to a length of 2 cm before being fed. The timothy hay was offered twice daily: 2/3 of the total daily amount in the morning (0700-0730 h), and 1/3 at 1500-1530 h. Although offered twice daily, grass hay was always present in the feeder to fulfill the ad libitum feeding criteria. For diets 2, 4, 5, 6 the alfalfa supplement was offered during the morning feeding separately from the grass hay (feeders were compartmented as in Trial I) .

The urea solution in diet 3 and 5 was infused in the morning together with a mineral mix prepared to supply the calcium, phosphorous, magnesium and sulphur contained in 150g alfalfa. To determine these quantities, a sample of alfalfa was taken from the whole supply to be used during the experiment and analyzed by atomic absorption spectrophotometry except for sulphur. For this element, a value was taken from an NRC table. The same alfalfa sample was used to determine the amount of urea to be infused in order to supply the same amount on nitrogen as 150g alfalfa. The mineral mix was prepared from calcium phosphate dibasic (Anachemia AC-1997), calcium carbonate (Anachemia AC- 1940) and magnesium sulfate (Anachemia AC_ 5567). Water and trace

mineral salt blocks were available at all times.

4.2.d Sampling schedule and procedure

Rumen samples

Rumen digesta samples were collected on day 9 of each experimental period. Whole digesta samples were taken at different times through the day: within 15 min before feeding and at 1, 3, 4, 5, 6 and 12 h postfeeding. The digesta collected was filtered as in Trial I the solid fraction was discarded, pH was measured on the liquid fraction . Subsequently, samples were processed as in Trial I.

Blood samples

Whole blood was collected from the jugular vein of each sheep on day 10 of the experimental period prefeeding and at 1, 3, 4, 5, 6 and 12 h postfeeding. For each sampling time 10ml were collected in a vacutainer coated with potassium oxalate and sodium fluoride and 14 ml in 2 vacutainers coated with lithium heparin. Samples were then processed as in Trial I and frozen.

Feeds and refusals

Voluntary intakes of the sheep were recorded daily from day 11 to day 15 of the experimental period. Samples of feed were taken every day, pooled at the end of the 5 days and dried in a forced air oven for 48 hours at 60 C. Refusals were weighed and sampled every morning; samples were pooled by diet at the end of the 5 days and dried. Samples of feeds

and refusals were stored as in Trial I.

Feces and urine

During the same 5 days as intake was being recorded digestibility and Nitrogen (N) balance studies were conducted.

Digestibility was measured by total feces collection. To collect feces plastic bags were glued onto the sheep using Lepage cement the day before collection was to be started. Note that the bags were closed only after the morning feeding of day 11. These bags were removed after the 5th day of collection and new ones were installed for each collection period. The bags were emptied 3 times a day in a plastic bucket immediately after the morning feeding, after the afternoon feeding and between 2200 and 2300 h. The total wet weight excreted was recorded daily in the morning at which time a 100g sample was taken and dried in a forced air oven at 60 C for 48 h. Samples were pooled by diet at the end of the 5 d collection and stored in plastic bags.

Urine was collected in a plastic bottle using the metabolic cage itself. The cages were washed down with water just after feeding on the morning of day 11. The urine was collected in a plastic bottle to which 20 ml of concentrated sulfuric acid were added daily. Every morning after feeding and feces collection, the total urine excreted on the previous day was filtered through 4 layers of cheesecloth and the volume of the filtrate was recorded. Then a sample was taken (100ml) and stored in a refrigerator

in a plastic bottle. At the end of the 5 d collection, samples were pooled by diet and only 100 ml were kept.

4.2.e Analytical Procedure

Rumen samples

Samples preserved with hydrochloric acid were analyzed for NH_3 content and those containing trimetaphosphoric acid were analyzed for their volatile fatty acids content; both analysis performed according to the same procedures followed in Trial I.

Blood samples

Following same procedures as in Trial I the plasma collected was analyzed for plasma glucose and urea-N.

Feeds, refusals and feces.

All these samples were ground through a 1mm screen in a hammer mill. They were then analyzed for absolute dry matter, ash, crude protein and ADF (same methods as in Trial I). In addition the N was determined on the ADF residue.

Urine

Urine samples were analyzed for CP for N retention calculated by substracting CP excreted in feces and urine from CP intake.

4.2.f Statistical analysis

Results of this experiment were analyzed according to the same procedure as for results of the first experiment.

4.3 RESULTS

4.3.a Forage composition

The composition of the two hays are presented in table15. Alfalfa and timothy hays had similar DM content and OM content. The CP content of the alfalfa was much higher then the CP content of the timothy hay whereas the ADF content followed the reverse pattern. Alfalfa was analyzed for calcium, magnesium and phosphorous and the results are also included in Table 15.

4.3.b Intakes and apparent digestibilities of DM

Intakes were calculated for each day and diet of each collection period , and averages per diet over one collection period calculated. Chemical analyses were performed on the pooled samples and means per diet over the whole experiment were then calculated. LS means were generated to compare differences between diets and results are shown in Tables 16 to 18 for legume grass and total DM intakes. Alfalfa intake was significantly affected by diet ($P<.0001$) regardless of the units in which it was expressed as were grass DMI ($P<.02$) and TDMI ($P<.025$).

The alfalfa intakes for diets 1 and 3 were 0 as expected. Intakes of alfalfa for diets 2 and 5 were similar, intake on diet 4 was higher vs diets 2 and 5 and lower than

TABLE 15 Composition of the alfalfa and timothy hays[1] (Exp.II)

	Timothy hay	Alfalfa hay
Dry matter (%)	87.77	87.35
Crude protein	9.95	17.60
Acid detergent fiber (ADF)	44.60	34.46
Ash	6.11	9.17
ADF-Nitrogen	0.59	0.72
Calcium		1.34
Phosphorous		0.20
Magnesium		0.25
Digestible Energy Mcal/kg[2]	2.47	2.65

[1] All values except dry matter are presented on a dry matter basis.

[2] From NRC (1985)

TABLE 16 Least square means of the average daily dry matter intakes (DMI) of alfalfa hay expressed as g, g kg^{-1} body weight (g kg^{-1} BW), g kg^{-1} of metabolic body weight (g kg^{-1} $W^{.75}$) (Exp.II).

	Alfalfa DMI		
Diets[1]	g	g kg^{-1} BW	g kg^{-1} $W^{.75}$
1	0[a]	0[a]	0[a]
2	131.03[b]	2.16[b]	6.01[b]
3	0[a]	0[a]	0[a]
4	262.06[c]	4.36[c]	11.92[c]
5	131.03[b]	2.20[b]	6.10[b]
6	393.09[d]	6.66[d]	18.24[d]
SE[2]	.40	.18	.38

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means

abcdMeans in one column with same letter do not differ P>.05.

TABLE 17 Least square means of the average daily dry matter intake (DMI) of timothy hay expressed as g, g kg^{-1} body weight, (g kg^{-1} BW) and g kg^{-1} of metabolic body weight (g kg^{-1} $W^{.75}$). (Exp.II).

	Timothy DMI		
Diets[1]	g	g kg^{-1} BW	g kg^{-1} $W^{.75}$
1	1450.28^{ab}	22.89^{ab}	64.49^{abc}
2	1503.77^{a}	24.38^{ab}	68.23^{ab}
3	1480.40^{ab}	25.10^{a}	69.46^{a}
4	1355.64^{bc}	22.23^{bc}	61.99^{bc}
5	1338.11^{bc}	22.19^{bc}	61.74^{c}
6	1255.33^{c}	21.23^{c}	58.72^{c}
SE[2]	48.70	0.78	2.13

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[abc] Means in one column with same superscripts do not differ P>.05

TABLE 18 Least square means of the average daily total dry matter intakes (TDMI) expressed as g, g kg^{-1} body weight (g kg^{-1} BW) and g kg^{-1} of metabolic body weight (g kg^{-1} $W^{.75}$). (Exp.II)

	TDMI		
Diets[1]	g	g kg^{-1} BW	g kg^{-1} $W^{.75}$
1	1450.28[a]	22.89[a]	64.49[a]
2	1634.80[b]	26.53[bc]	74.33[bd]
3	1487.85[ac]	25.22[b]	69.81[abc]
4	1617.70[bc]	26.59[bc]	74.10[bcd]
5	1476.60[ac]	24.51[ab]	68.18[ab]
6	1648.42[b]	27.88[c]	77.14[d]
SE[2]	48.76	0.78	2.13

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[abcd] Means in one column with same superscripts do not differ P>.05.

diet 6.

Grass DMI showed that none of the levels of alfalfa supplementation (150, 300 or 450 g d^{-1}) improved grass DMI ($P>.05$). However, a trend can be seen toward an improved DMI at the lowest level of supplementation. A trend toward a reduced intake vs grass given alone when alfalfa supplement was increased was also observed. The 450 g d^{-1} supplement significantly reduced grass DMI/$W^{.75}$ compared to the 150g supplement. Supplementing the basal timothy hay diet with urea (diet 3) did not alter grass DMI regardless of the units of expression, and there was no difference in grass DMI between the 150 g d^{-1} alfalfa supplement and an iso-N supplement of urea. Similarly, replacing half of the alfalfa supplement by an iso-N source of urea did not alter DMI of grass (diets 4 vs 5) both yielding results similar to the control and 450 g d^{-1} supplement. Grass DMI (g kg^{-1} $W^{.75}$) was significantly reduced by adding some alfalfa to urea as can be seen by comparing diets 5 and 3, or urea to the alfalfa as can be seen by comparing diets 5 and 2.

Although grass DMI (g kg^{-1} $W^{.75}$) of diets 2 and 4 were not significantly different there appear to be a trend for lower N supplement as alfalfa or urea to result in highest grass DMI.

Regarding TDMI, regardless of the units of expression diet had a significant effect. TDMI expressed in any unit was improved by any level of alfalfa supplementation with no difference among them, although the 450 g d^{-1} level tended

to have a greater effect then the 2 others. Urea supplementation of diet 3 did not cause any increase in TDMI vs the non supplemented diet 1 and although it did not reach significance ($P<.15$) an iso-N supplement as alfalfa tended to result in higher TDMI g kg^{-1} $W^{.75}$. Adding an iso-N amount of urea to 150g alfalfa supplement (diet 5) tended to depress DMI vs the alfalfa supplement given alone diet 2 or 300g alfalfa diet 4. The TDMI of diets containing urea were similar with or without 150 g alfalfa and a tendency was observed for a slight improvement in TDMI vs the unsupplemented diet but lower TDMI vs any alfalfa supplement.

Regarding apparent dry matter digestibility (DMD) no effect of diet was observed and similar digestible DMI (DDMI) resulted for all diets. LS means generated are in Table 19. A tendency ($P<.20$), however, was observed for improved DDMI with a urea supplement vs unsupplemented diet and further improvement with an alfalfa supplementation.

4.3.c Crude Protein intake (CPI) and apparent digestibility (CPD)

Total CPI was significantly affected by diet ($P<.0001$). LS means computed to locate differences among diets are shown in Table 20 . CPI expressed as g kg^{-1} or gkg^{-1} $W^{.75}$ was increased by all supplement vs diet 1. Diet 6 had the highest CPI being significantly different from all the others. CPI g kg^{-1} $W^{.75}$ was not changed by replacing

TABLE 19 Least square means of the average dry matter digestibility (DMD) and average daily digestible dry matter intake (DDMI) expressed as g, g kg^{-1} body weight (g kg^{-1} BW) and k kg^{-1} of metabolic body weight (g kg^{-1} $W^{.75}$). (Exp.II)

	Diets[1]						SE[2]
	1	2	3	4	5	6	
DMD (%)	48.54	52.01	52.33	52.39	49.84	49.95	2.25
DDMI							
g	701.12	849.62	777.30	842.80	729.28	805.19	50.8
g kg^{-1} BW	11.14	13.81	13.20	13.85	12.10	13.33	0.77
g kg^{-1} $W^{.75}$	31.32	38.62	36.52	38.87	34.56	37.08	2.25

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

TABLE 20 **Least square means of the average daily crude protein intake (CPI), crude protein digestibility (CPD) and digestible crude protein intake (DCPI) expressed as g, g kg^{-1} body weight (g kg^{-1} BW) and g kg^{-1} metabolic body weight (g kg^{-1} W$^{.75}$). (Exp. II)**

	Diets[1]						SE[2]
	1	2	3	4	5	6	
CPI							
g	143.49^{a}	172.43^{b}	172.38^{b}	181.38^{bc}	182.50^{bc}	193.92^{c}	4.80
g kg^{-1}	2.29^{a}	2.80^{b}	2.87^{bc}	2.97^{bc}	3.04^{c}	3.28^{d}	0.07
g kg^{-1}W$^{.75}$	6.43^{a}	7.84^{b}	7.98^{bc}	8.29^{bc}	8.45^{c}	9.03^{d}	0.20
CPD (%)	52.92	58.77	62.95	59.87	62.54	59.67	2.32
DCPI							
g	76.31^{a}	102.92^{b}	108.92^{b}	108.45^{b}	111.58^{b}	114.13^{b}	5.90
g kg^{-1}	1.24^{a}	1.67^{b}	1.80^{b}	1.77^{b}	1.90^{b}	1.89^{b}	0.09
g kg^{-1}W$^{.75}$	3.46^{a}	4.68^{b}	5.02^{b}	4.94^{b}	5.27^{b}	5.25^{b}	0.25

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[a b c] Means in one line with same superscripts do not differ P>.05.

alfalfa with an iso-N source of urea, nor was it affected by replacing half of the 300g alfalfa by an iso-N source (diets 4 and 5). However, adding a urea supplement to 150g alfalfa (diet 5) caused an increase in CPI over the alfalfa supplement alone (diet 2) but diet 5 did not result in higher CPI over the same amount of urea given alone (diet 3). As can be seen in Table 20, CPD was not affected by diet. Table 20 also presents the digestible CPI (DCPI), which shows that all of the N supplements resulted in higher DCPI vs the control with no difference among them. However, urea containing diet tended to result in higher DCPI vs their iso-N alfalfa supplemented counterpart.

4.3.d Estimated digestible energy intake (DEI)

Diet had a significant effect on estimated DEI regardless of the units of expression . LS means computed are presented in Table 21. All levels of alfalfa supplementation resulted in a higher DEI with no difference among the levels. The urea containing diets (3 and 5) had similar DEI g kg^{-1} $W^{.75}$ as their iso-N counterparts and the DEI for diet 3 did not differ from the unsupplemented diet. There was no difference between the combination of alfalfa and urea and alfalfa or urea given alone.

4.3.e ADF intake and apparent digestibility (ADFI and ADFD)

TABLE 21 Least square means of the estimated daily digestible energy intakes (DEI) in Mcal, Mcal kg^{-1} body weight and Mcal kg^{-1} metabolic body weight ($g\ W^{.75}$). (Exp.2).

	Diets[1]						SE[2]
	1	2	3	4	5	6	
DEI							
Mcal	3.74[a]	4.22[b]	3.82[a]	4.21[bc]	3.94[abc]	4.24[b]	0.18
Mcal kg^{-1}	.059[a]	.069[bc]	.064[ab]	.069[bc]	.065[b]	.071[c]	.002
Mcal $kg^{-1}W^{.75}$	.166[a]	.192[bc]	.178[ab]	.192[bc]	.182[b]	.197[c]	.005

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[abc] Figures with the same letter in one line do not differ P>.05

Diet had a significant effect on ADFI g kg^{-1} and g kg^{-1} $W^{.75}$ ($p<.02$ and .04). LS means are shown in Table 22 and indicate that all supplemented diets resulted in significantly higher ADFI g kg^{-1} $W^{.75}$ compared to the unsupplemented diet with no difference among the supplements. ADFD was not affected by treatment and thus, overall there was no effect on DADFI (Table 22). A tendency, however, for higher values was observed as a supplement was fed.

4.3.f Crude protein retention

Table 23 shows the LS means generated to compare the CP retention of the different diets. Diet had no significant effect on CP excreted in the urine or CP retention. The lack of significance could be related to the high coefficient of variation. As can be seen all supplements tended to improve CP retention vs diet 1.

4.3.g Rumen parameters

Rumen VFA

The molar proportion of acetic, propionic, isobutyric and butyric acids were not affected by diet at any time as shown in Tables 24 to 30. Diet effect was significant for total VFA concentration prefeeding and at 3, 5 and 6 h postfeeding, for isovaleric prefeeding and for valeric at 1, 3, 4, 5 and 6 h postfeeding. At 12 h none of the parameters

TABLE 22 Least square means of the average acid detergent fiber intakes (ADFI), acid detergent fiber digestibility (ADFD) and digestible acid detergent fiber intake (DADFI) in g, g kg^{-1} body weight (g kg^{-1} BW) and g kg^{-1} metabolic body weight (g kg^{-1} $W^{.75}$). (Exp. II).

	Diets[1]						SE[2]
	1	2	3	4	5	6	
ADFI							
g	632.08	696.77	648.58	682.79	646.18	687.41	20.71
g kg^{-1}	10.00[a]	11.33[b]	10.84[a b]	11.16[b]	10.76[a b]	11.50[a b]	0.29
g kg^{-1} $W^{.75}$	25.44[a]	31.69[b]	30.10[b]	32.15[b]	29.90[b]	31.95[b]	1.51
ADFD (%)	42.98	42.11	44.82	42.79	42.05	38.46	2.87
DADFI							
g	263.94	292.34	289.53	291.15	266.86	258.09	23.99
g kg^{-1}	4.24	4.77	4.83	4.72	4.45	4.11	0.39
g kg^{-1} $W^{.75}$	10.71	13.34	13.42	13.59	12.37	11.55	1.19

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[a b] Means in one line with same superscripts do not differ P>.05.

TABLE 23 Least square means of the average N protein equivalent excretion in the urine and retention in g, g kg^{-1} g body weight (g kg^{-1} BW) and g kg^{-1} metabolic body weight (g kg^{-1} $W^{.75}$). (Exp.II).

	Diets[1]						SE[2]
	1	2	3	4	5	6	
urine CP g/day	71.01	62.13	66.41	79.78	64.32	91.06	8.34
CP Retention g	5.30	38.72	44.26	28.67	38.18	23.08	12.00
g kg^{-1}	0.11	0.64	0.70	0.46	0.65	0.33	0.21
g kg^{-1} $W^{.75}$	0.28	1.79	1.98	1.28	1.79	0.96	0.57

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

TABLE 24 **Least square means of total volatile fatty acid concentration (mmol L^{-1}), molar proportion of acetic, propionic, butyric, isobutyric, isovaleric, valeric acids (%), and ratio of acetic/propionic (A/P) prefeeding. (Exp.II).**

	Diets[1]						SE[2]
	1	2	3	4	5	6	
Total	85.98^{ab}	91.06^{a}	79.77^{bc}	75.59^{c}	76.74^{bc}	72.80^{c}	3.3
Acetic	70.89	71.53	70.00	70.00	69.52	69.35	0.9
Propionic	17.35	17.09	18.12	17.72	17.97	17.60	0.6
A/P	4.10	4.22	3.99	4.03	3.93	4.02	0.1
Isobutyric	1.07	0.91	1.17	1.11	1.50	1.49	0.2
Butyric	8.93	8.65	8.81	9.01	8.89	9.32	0.4
Isovaleric	1.13^{a}	1.13^{a}	1.30^{ab}	1.47^{b}	1.50^{b}	1.55^{b}	0.1
Valeric	0.62	0.68	0.60	0.69	0.62	0.69	0.0

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

^{abc} Means in one line with same superscripts do not differ P>.05.

TABLE 25 **Least square means of total volatile fatty acid concentration (mmol L^{-1}), molar proportion of acetic, propionic, butyric, isobutyric, isovaleric, valeric acids (%), and ratio of acetic/propionic (A/P) 1 h post feeding. (Exp.II).**

	Diets[1]						SE[2]
	1	2	3	4	5	6	
Total	88.48	91.79	82.41	86.26	86.60	92.36	4.44
Acetic	67.69	69.29	69.71	67.23	68.27	66.00	1.43
Propionic	19.51	18.96	18.47	20.20	19.73	21.73	0.94
A/P	3.51	3.69	3.94	3.44	3.50	3.07	0.24
Isobutyric	1.40	1.08	1.23	1.66	1.42	1.72	0.27
Butyric	9.64	8.76	8.75	8.56	8.33	8.24	0.37
Isovaleric	1.07	1.07	1.20	1.46	1.35	1.39	0.12
Valeric	0.69_{ab}	0.84^{ac}	0.65^{b}	0.89^{c}	0.90^{c}	0.86^{c}	0.06

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[abc] Means in one line with same superscripts do not differ P>.05.

TABLE 26 Least square means of total volatile fatty acid concentration (mmol L^{-1}), molar proportion of acetic, propionic, butyric, isobutyric, isovaleric, valeric acids (%), and ratio of acetic/propionic (A/P) 3 h post feeding. (Exp. II).

	Diets[1]						SE[2]
	1	2	3	4	5	6	
Total	79.81[a]	96.72[b]	79.24[a]	95.36[b]	89.61[a b]	90.15[a b]	4.55
Acetic	66.49	69.68	69.94	68.55	71.48	66.91	1.74
Propionic	20.46	18.84	20.42	19.32	17.24	20.84	1.19
A/P	3.33	3.78	3.43	3.62	4.27	3.29	0.30
Isobutyric	1.27	0.91	1.27	1.27	0.88	1.27	0.23
Butyric	10.09	8.82	9.69	8.46	8.49	8.65	0.49
Isovaleric	0.93	0.83	0.99	1.14	0.95	1.28	0.07
Valeric	0.76[a b]	0.92[a c]	0.69[b]	1.26[d]	0.96[c]	1.28[d]	0.06

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[a b c d] Means in one line with same superscripts do not differ P>.05.

TABLE 27 **Least square means of total volatile fatty acid concentration (mmol L^{-1}), molar proportion of acetic, propionic, butyric, isobutyric, isovaleric, valeric acids (%), and ratio of acetic/propionic (A/P) 5 h post feeding. (Exp.II).**

	Diets [1]						SE[2]
	1	2	3	4	5	6	
Total	83.98^{ab}	92.67^{d}	78.87^{b}	91.61^{a}	93.97^{a}	90.89^{a}	3.70
Acetic	69.46	69.14	67.39	68.32	70.01	67.73	1.36
Propionic	17.82	19.42	20.33	19.56	18.73	20.06	0.96
A/P	4.74	3.56	3.41	3.53	3.76	3.45	0.50
Isobutyric	1.14	0.79	1.11	1.22	0.82	1.11	0.20
Butyric	9.94	8.92	9.58	8.82	8.68	9.10	0.40
Isovaleric	0.86	0.79	0.88	1.01	0.86	0.89	0.09
Valeric	0.78^{bd}	0.94^{acd}	0.71^{b}	1.07^{ac}	0.90^{bc}	1.11^{a}	0.07

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

^{abcd} Means in one line with same superscripts do not differ P>.05.

TABLE 28 **Least square means of total volatile fatty acid concentration (mmol L^{-1}), molar proportion of acetic, propionic, butyric, isobutyric, isovaleric, valeric acids (%), and ratio of acetic/propionic (A/P) 5 h post feeding. (Exp.II).**

	Diets[1]						SE[2]
	1	2	3	4	5	6	
Total	79.20^{ab}	88.18^{c}	76.26^{ad}	86.51bc	84.11^{bcd}	88.72^{c}	2.80
Acetic	68.65	69.41	69.27	69.94	70.38	67.79	0.92
Propionic	19.52	18.40	19.21	18.15	18.36	19.84	0.52
A/P	3.59	3.78	3.70	3.87	3.84	3.46	0.11
Isobutyric	0.96	0.78	1.00	0.87	0.84	0.97	0.14
Butyric	9.56	9.74	9.15	9.20	8.77	9.58	0.52
Isovaleric	0.70	0.80	0.76	0.86	0.82	0.81	0.09
Valeric	0.65$_{a}$	0.86^{bc}	0.62^{a}	0.97^{bc}	0.82^{b}	1.00^{c}	0.06

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

^{abc} Means in one line with the same letter do not differ P>.05

TABLE 29 **Least square means of total volatile fatty acid concentration (mmol L^{-1}), molar proportion of acetic, propionic, butyric, isobutyric, isovaleric, valeric acids (%), and ratio of acetic/propionic (A/P) 6 h post feeding. (Exp.II).**

	Diets[1]						SE[2]
	1	2	3	4	5	6	
Total	79.98^{ab}	86.84^{c}	74.78^{a}	83.46^{bc}	82.17^{bc}	85.39^{bc}	2.16
Acetic	69.18	69.53	69.01	70.36	70.05	69.07	0.70
Propionic	19.20	19.01	19.29	18.33	18.59	19.01	0.42
A/P	3.66	3.67	3.66	3.85	3.78	3.64	0.09
Isobutyric	0.75	0.75	0.97	0.82	0.80	0.76	0.11
Butyric	9.63	9.24	9.44	8.87	9.02	9.53	0.32
Isovaleric	0.61	0.70	0.67	0.77	0.77	0.74	0.08
Valeric	0.63^{ab}	0.78^{c}	0.62^{a}	0.84^{c}	0.76^{bc}	0.89^{c}	0.05

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[abc] Means in one line with same superscripts do not differ P>.05.

TABLE 30 Least square means of total volatile fatty acid concentration (mmol L^{-1}), molar proportion of acetic, propionic, butyric, isobutyric, isovaleric, valeric acids (%), and ratio of acetic/propionic (A/P) 12 h post feeding. (Exp.II).

	Diets[1]						SE[2]
	1	2	3	4	5	6	
Total	86.56	94.48	81.68	89.25	86.86	85.10	2.81
Acetic	69.05	69.00	70.01	70.04	70.46	69.17	0.84
Propionic	19.47	19.38	18.99	18.59	18.60	19.06	0.54
A/P	3.66	3.57	3.72	3.80	3.80	3.64	0.12
Isobutyric	0.78	0.66	0.62	0.68	0.64	0.70	0.08
Butyric	9.52	9.63	9.17	9.36	8.98	9.65	0.41
Isovaleric	0.52	0.61	0.57	0.62	0.64	0.68	0.06
Valeric	0.66	0.72	0.64	0.71	0.68	0.75	0.04

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

were affected by diet. Total VFA levels prefeeding tended to be higher for the 150g alfalfa supplement vs the unsupplemented diet and was significantly higher vs all other diets. The other alfalfa supplemented diets (4 and 6) resulted in higher total VFA concentration vs diet 1 with no difference among them. Replacing half of the 300g alfalfa by a iso-N source of urea did not affect prefeeding total VFA as demonstrated by the lack of difference between diet 4 and 5. However, substitution of the 150g alfalfa by urea significantly reduced total VFA prefeeding . Prefeeding, the percentage of isovaleric acid was significantly higher for diets 4, 5 and 6 which were the higher N supplements . When 150g alfalfa was replaced by iso-N urea or when half the 300g alfalfa was replaced by urea no difference was observed. At 1 h post feeding, diet effect on total VFA concentration and isovaleric acid was eliminated. The percentage of valeric acid was significantly higher for diets 4, 5 and 6 vs diet 1 with no difference among them. replacing 150g alfalfa by urea was accompanied by a decrease in percentage of valeric acid.

At 3 h post feeding total VFA showed a significant diet effect with values for diets 1 and 3 lower than values for diets 5 and 6. Thus 150g and 300g alfalfa supplemented diets had the highest total VFA concentration at 3 h post feeding compared to the unsupplemented diet. Replacing 150g alfalfa by urea significantly reduced total VFA, however, no effect was observed by replacing half the 300g alfalfa. The

alfalfa addition to urea (diets 5 vs 3) did not significantly affect total VFA, additionnally a simple urea supplement (diet 3), could not alter total VFA concentration vs timothy hay given alone. The percentage of valeric acid at 3 h post feeding was higher when an alfalfa supplement of 150g was given compared to urea alone. The urea infusion tended to decrease the percentage of valeric acid vs the unsupplemented diet while alfalfa tended to increase it. The highest percentage of valeric acid was obtained when diets 4 and 6 were fed (higher level of alfalfa supplementation) with no difference among them. Adding some urea to 150g alfalfa did not alter the percentage of valeric acid, but the percentage of valeric acid was increased when alfalfa was added to urea diets 5 vs 3.

At 4 h post feeding the effect of diet on total VFA concentration was eliminated ($P<.06$), with diets supplemented with alfalfa tending to have greater total VFA and the urea supplement of diets 2 and 5 tending to decrease the value vs the unsupplemented basal diet. The percentage of valeric acid was increased by alfafa supplemented diets with no difference among them. Replacing alfalfa by urea did result in lower valeric acid in diet 2 vs 3 with the urea containing diet tending to be lower compared to diet 1. Adding urea to 150g alfalfa (diet 5) did not alter the percentage of valeric acid compared to only alfalfa supplementation.

At 5 h post feeding total VFA were highest for 150g and

450g alfalfa vs diet 1. The 300g supplement also tended to be accompanied by higher total VFA. Again, urea instead of alfalfa caused a decrease in total VFA while the urea supplemented diet tended to result in lower VFA vs the control. No effect of substituing half of the 300g alfalfa nor adding alfalfa to urea or urea to alfalfa was observed. The percentage of valeric acid was increased by alfalfa supplementation compared to no supplement or to urea. No difference between diets 4 and 5 was found.

At 6 h post feeding alfalfa supplemented diets resulted in higher total VFA with no difference among them. Replacing 150g alfalfa by urea caused a decrease in the VFA concentration with diet 3 tending to be lower than diet 1. Combining alfalfa and urea yielded similar results as alfalfa alone but higher then urea alone. No effect of replacing half the 300g alfalfa by urea on total VFA at 6 h post feeding was observed. Diet had a significant effect on percentage ofvaleric acid 6 h post feeding, with alfalfa supplement resulting in significantly greater values than no supplement or urea alone with no difference among the levels.

No significant diet time interaction was found for any of the acids. Time effects are in Appendix B.

Rumen pH

Diet effect on rumen pH was significant only prefeeding and at 1 and 12 h post feeding. LS means generated are presented in Table 31. Prefeeding rumen pH values were lower

TABLE 31 Least square means of the average rumen pH at different times prefeeding and post feeding. (Exp.II).

	Diets [1]						SE[2]
	1	2	3	4	5	6	
Prefeeding pH	6.54[a]	6.46[a]	6.73[b]	6.75[b]	6.87[b]	6.77[b]	0.06
1 hour post feeding pH	6.46[a]	6.45[a]	6.87[b]	6.58[a]	6.88[b]	6.59[a]	0.07
Three hours post feeding pH	6.45	6.35	6.56	6.51	6.61	6.51	0.06
Four hours post feeding pH	6.38	6.33	6.56	6.46	6.57	6.44	0.06
Five hours post feeding pH	6.43	6.34	6.40	6.47	6.48	6.45	0.06
Six hours post feeding pH	6.35	6.27	6.45	6.49	6.46	6.43	0.06
Twelve hours post feeding pH	6.18[ab]	6.07[a]	6.27[bc]	6.43[c]	6.33[bc]	6.42[c]	0.06

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[abc] Means in one line with same letter do not differ P>.05

for diets 3, 4, 5 and 6 vs 1 and 2 with no difference among diets 3, 4, 5 and 6 nor 1 and 2.

At 1 h post feeding, pH was greater ($P<.0005$) for diets 3 and 5 compared to all others with no difference between diets 3 and 5 or between diets 1, 2, 4 and 6.

At 12 h post feeding diet effect on rumen pH was significant ($P<.004$). The two highest alfalfa supplements resulted in significantly higher pH compared to the unsupplemented diet. Supplementing 150g alfalfa vs urea was parallelled by lower pH. The 150g alfalfa supplemented diet had lower pH compared to all other supplemented diets with no difference among the others. Rumen pH for diet 2 was not different then pH for diet 1 at 12 h post feeding.

Rumen ammonia

The effect of diet on rumen NH_3 was significant prefeeding and at 1, 3, 4 and 12 h after the morning feeding. LS means are in Table 32. Prefeeding and at 1 h postfeeding rumen NH_3 concentration was greater for diets 3 and 5 containing urea vs all others. No difference between diets 3 and 5 or between others were found.

At 3 h post feeding the highest rumen ammonia concentrations were still obtained for diets 3 and 5 with no difference between them. Diets 4 and 6 (300 and 450g alfalfa) showed higher NH_3 concentration vs the control with no difference between them. The 150g alfalfa supplement tended to increase rumen NH_3 compared to the unsupplemented diet. Iso-N supplementation of urea instead of alfalfa

TABLE 32 Least square means of rumen ammonia concentrations (mg dL^{-1}) at different times prefeeding and post feeding. (Exp.II).

	Diets[1]						SE[2]
	1	2	3	4	5	6	
Prefeeding Ammonia	11.49[a]	13.20[a]	20.44[b]	14.19[a]	20.28[b]	12.41[a]	1.90
1 hour post feeding Ammonia	12.73[a]	15.09[a]	33.43[b]	17.37[a]	34.98[b]	16.86[a]	2.69
Three hours post feeding Ammonia	11.97[a]	14.77[a b]	23.44[c]	17.07[b]	23.59[c]	17.53[b]	15.02
Four hours post feeding Ammonia	9.56[a]	12.43[a b]	16.89[b c]	15.62[b]	22.00[c]	16.16[b c]	2.05
Five hours post feeding Ammonia	9.96	12.45	13.48	18.20	15.13	12.63	2.48
Six hours post feeding Ammonia	8.75	10.64	12.18	10.87	12.72	12.32	11.02
Twelve hours post feeding Ammonia	9.25[a b]	10.24[a]	9.93[a]	10.43[b c]	11.15[c]	10.18[a b c]	5.86

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[a b c d] means in one line with same letter do not differ P>.05

significantly increased the rumen NH_3 concentration at 3 h feeding as can be seen by comparing diets 2 and 3 and, 4and 5. At 4 h post feeding for all supplemented diets except diet 2, NH_3 concentration was still higher then the unsupplemented diet.

Both urea containing diets resulted in greater concentration of rumen NH_3 compared to diet 1. When timothy was supplemented with urea alone rumen ammonia was not different vs an iso-N supplement of alfalfa at 4 h post feeding nor were the iso-N diets (2 and 3) different from the higher N containing diets (4 and 6). The combination of 150g alfalfa and iso-N quantity of urea did alter NH_3 concentration compared to urea alone . Rumen NH_3 concentration was higher for the 300 and 450g alfalfa containing treatments vs the unsupplemented diet.

At 5 and 5 h post feeding the effect of diet on rumen NH_3 was nil. However, 12 h post feeding diet effect again became significant. No difference between iso-N diets were noted. Diets 4 and 5 both resulted in higher rumen NH_3 vs the lower N supplement of diets 2 and 3. Rumen NH_3 concentration of any of the alfalfa containing diets were similar to the unsupplemented diet. Level for diet 6 was similar to the concentration for all other diets at 12 h post feeding.

4.3.h Blood parameters

TABLE 33 Least square means of average plasma urea nitrogen (PUN) (mg dL^{-1}) at different times prefeeding and postfeeding. (Exp.II).

	Diets[1]						SE[2]
	1	2	3	4	5	6	
prefeeding	9.63	10.50	11.68	12.27	12.32	12.35	0.90
One hour postfeeding	11.49[a]	11.89[a b]	14.06[b c]	14.65[c]	14.16[b c]	15.77[c]	0.87
Three hours postfeeding	12.23	12.55	14.32	15.45	16.59	14.34	1.19
Four hours postfeeding	12.66[a]	12.91[a b]	16.61[b c]	15.65[a b c]	18.13[c]	14.19[a b]	1.31
Five hours postfeeding	13.43	13.58	14.58	18.26	17.75	14.64	1.51
Six hours postfeeding	14.49[a]	11.65[a]	13.67[a]	14.92[a]	18.49[b]	13.25[a]	1.28
Twelve hours postfeeding	9.62	9.35	9.35	12.25	12.87	12.05	0.97

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[a b c] Figures of a line with the same letter do not differ P>.05

Plasma urea N (PUN)

LS means are presented in Table 33. The effect of diet was not significant prefeeding or at 3 or 5 h postfeeding. Only trends were noted at these times.

At 1 h postfeeding, PUN was greater for all supplemented diets except diet 2 vs the unsupplemented timothy hay with no difference between diets 3, 4, 5 and 6. PUN was not affected by urea supplementation compared to iso-N amount of alfalfa.

At 4 h post feeding PUN was significantly elevated for urea containing diets compared to diet 1. However, for none of the alfalfa containing diets was PUN higher than for diet 1 and no difference among these treatments were noticed. PUN was not different among iso-N diets. However PUN for diet 5 was greater than diet 2 indicating that the combination of alfalfa and urea resulted in higher PUN vs alfalfa alone but the similarity of values for diets 5 and 3 indicated that the combination was same as urea alone. A tendency for PUN to be elevated for diets 4 and 5 was noted at 5 h post feeding. At 6 h post feeding PUN was significantly higher for diet 5 compared to all others with no difference between the latter.

No significant interaction of diet and time was observed for PUN. The time effect was significant and LS means are in Appendix C.

Plasma glucose

Plasma glucose was measured at the same times as PUN.

The effect of diet was not significant at any times nor was the diet time interaction (Table 34) . The time effect was significant and LS means are in Appendix C.

4.3.i Rates of DM disappearance

The rates of DM disappearance for timothy hay incubated ruminally in nylon bags were calculated from the regression of the natural logarithm of the percentage loss of DM with time. The effect of the host diet being highly significant ($P<.0001$). LS means were generated to compare the differences between diets and results are presented in Table 35. Rate of DM disappearance was significantly higher when the sheep were fed diet 6 compared to any other one. Diets 3 and 5 resulted in similar rates of DM disappearance which were significantly lower than rates for diets 1, 2, 4 and 6. No difference was noted between diets 1, 2 and 4. Thus among the diets supplemented with alfalfa and no urea, only the 450g alflfa resulted in higher rate of DM disappearance compared to diet 1. It can also be seen that replacing 150g alfalfa by iso-N quantities of urea resulted in lower rates of DM disappearance. The combination of alfalfa and urea (diet 5) significantly reduced the rate of disappearance vs alfalfa alone but was similar to urea alone.

TABLE 34 Least square means of average plasma glucose (mg ml^{-1}) at different times prefeeding and postfeeding.(Exp.II).

	Diets[1]						SE[2]
	1	2	3	4	5	6	
Prefeeding	70.04	69.06	68.85	66.98	68.87	66.56	3.20
One hour postfeeding	64.43	65.02	60.65	68.40	59.23	58.81	2.79
Three hours postfeeding	70.19	72.23	66.89	64.51	68.17	65.06	2.68
Four hours postfeeding	75.64	75.99	67.04	62.14	65.46	72.06	4.8
Five hours postfeeding	65.05	70.08	66.80	64.38	74.02	76.42	3.19
Six hours postfeeding	72.24	62.32	68.62	63.51	69.41	67.21	3.79
Twelve hours postfeeding	70.81	66.39	69.08	70.19	64.59	57.03	3.79

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

TABLE 35 Least square means of the rates of dry matter disappearance from nylon bags. (Exp.2).

	Diets [1]					
	1	2	3	4	5	6
%/hour	2.0[a]	2.1[a]	1.9[b]	2.1[a]	1.8[b]	2.6[c]
SE[2]	.05	.05	.05	.05	.07	.08

[1] diet 1=grass ad libitum; 2= grass ad libitum and 150g alfalfa; 3= grass ad libitum and urea iso-N to 150g alfalfa; 4= grass ad libitum and 300 g ad libitum; 5= grass ad libitum and 150g alfalfa and urea iso-N to 150g alfalfa; grass ad libitum and 450g alfalfa.

[2] Standard errors of the means.

[abc] Figures with the same letter do not differ P>.05

4.4 DISCUSSION

4.4.a Forage composition

The composition of the forages are presented in Table 15. The forages DM contents were slighly lower than the 89-90% figures reported by NRC (1985) for alfalfa and timothy hays but comparable to the 88.3 and 88.5% DM of fescue and alfalfa hay shown by Hunt et al (1985). The CP content of the timothy hay (9.95%) corresponded to the CP of suncured midbloom timothy hay (NRC 1985) and was higher than the CP of the timothy hay reported by Baker (1969) but lower than for Champ timothy and Climax timothy hay (10.9 and 11.4%) reported by Seoane et al (1981). Regarding the alfalfa hay the CP content (17.6%) was higher than the hay used in the first trial indicating an earlier stage of maturity and related to suncured hay between late vegetative and early bloom stage. The figure was similar to the early bloom alfalfa hay of Baker (1969) at 17.0%, and Saranac alfalfa (17%) reported by Seoane et al. (1981). The % ADF of timothy hay (44.6%) was similar to values of Baker (1969). The % ADF of the alfalfa hay (34.6%) was about 10% lower then the alfalfa of Trial 1 again due to earlier maturity but corresponded to values of Hunt et al (1985) and Varga and Prigge (1982). Alfalfa content of ash corresponded to percentage ash in Saranac alfalfa (Seoane et al.) (1981), as did the percentage ash in timothy hay.

As for Trial I, ADF-N were low indicating that no heat

damage had occurred for either forage.

The alfalfa hay was also analyzed for calcium (Ca), phosphorous (P) and magnesium (Mg).

4.4.b Legume intakes

The intakes of alfalfa differed between diets as intented in the design of the experiment. The similarity between alfalfa hay intake of diets 2 and 5 confirmed that the same supplementation was given. When intakes were corrected for body weights alfalfa intake of diets 4 and 6 were twice and three times greater than intakes of diets 2 and 5.

4.4.c Increasing levels of alfalfa supplementation

Grass DMI in g kg^{-1} as well as g kg^{-1} $W^{.75}$ were significantly affected by diet. Note that absolute numbers for grass DMI and TDMI were within the same range as reported for Trial 1 and thus similarly compared with figures found in the litterature for intakes of alfalfa and grass forages. Comparable ranges of intakes were shown by Reid et al (1987), Corbett et al (1979), Cruickshank et al (1985).

None of the supplement caused any significant increase or decrease in grass DMI when compared to the unsupplemented diet thus, the subsequent effects on TDMI were due to, as i

Trial I to the intakes of the supplements per se. This indicated that the animals did not substitute the legume for the grass hay entirely. This non-substitution phenomenon had been demonstrated earlier.

Moseley and Jones (1979) feeding clover or ryegrass to sheep ad libitum, or ryegrass ad lib or a 2:1 mixture of ryegrass clover (approximatively 33% clover) observed a 50% increase in TDMI when clover was fed vs ryegrass and a 67% increase (624g vs 931g) when the mixture was fed vs grass alone. By simple calculation, and assuming that the percentage of legume consumed by the animals was the same as the percentage in the ration, it can be seen that sheep consumed 621g of grass when given the mixture indicated no substitution. Similarly Hunt et al. (1985) feeding alfalfa tall fescue combinations to sheep observed increases in TDMI from 537g when animals were offered fescue alone vs 695g when a 25% alfalfa fescue mix was fed. Thus on the mixture animals still consumed 521g of fescue indicating no substitution. Note however that as the percentage of legume increased past 25% grass intake decreased. In fact, the results of this trial showed a tendency for sheep to consume less timothy hay as the amount of alfalfa hay supplemented increased from 150g to 300g (as fed) and consumed significantly less grass hay when given the 450g alfalfa compared to 150g. Overall, grass DMI tended to be increased by the lowest N supplementation level (diets 2 and 3) and decreased by the two highest (diets 4, 5 and 6) vs the

control (diet 1).

Compared to the unsupplemented diet all levels of alfalfa supplementation resulted in significantly higher TDMI in g kg^{-1} $W^{.75}$. As previously before, the positive effect of supplemnting alfalfa on TDMI is well recognized (Hunt et al. 1985, Moseley and Jones 1979, Paterson et al. 1982) However, in the present trial no significant difference was observed between levels of supplementation indicating that although non significant, the trend toward decreased grass DMI was of importance supporting the increased substitutuion shown by Hunt et al. (1985) as alfalfa level increased. The phenomenum however occurred at lower percentage of alfalfa vs the previous study. This could be due to the lower CP of the fescue of Hunt et al (1985) compared to timothy hay and lower intakes observed for their lambs (16.3 g kg^{-1} for fescue alone and 21 g kg^{-1} for 25% alfalfa). Interestingly, none of the supplements altered the digestibility of DM or ADF vs the unsupplemented diet. Again, this was contradictory to previous results of Hunt et al (1985) who observed decreased NDFD as alfalfa increased from 0 to 25%, Moseley and Jones (1979) who observed lower cellulose and hemicellulose digestibilities when clover and grass were mixed vs grass alone under ad lib feeding conditions. However, intakes differed tremendously across experiments as well as level of supplementations being respectively lower and higher in the mentioned studies. These could be counfounding factors.

Despite higher ADFI and similar digestibility for supplemented vs unsupplemented diets digestible ADFI (DADFI) only tended to increase as alfalfa supplementation increased. The increased ADFI parallelled by greater TDMI and similar DADFI indicated that ADF content of the grass was not limiting voluntary consumption. It cannot either be stated that it was the major driving force for the increased DMI since DADFI was not affected. Similar patterns were observed for DMD and digestible DMI (DDMI). Animals fed an all grass diet vs supplemented diet had similar amounts of digestible DM and ADF available, and thus some other nutrient must have caused the greater intake of alfalfa.

The palatability effect has been eliminated in Trial I for the lowest supplementation level so the animals must have responded to another physiological signal. As in Trial I reticulorumen distension seemed an unlikely cause since rates of DMD measured from nylon bags were not similar for supplemented diets resulting in similar TDMI (ie. diets 2 vs 6 and 4 vs 6. Rather then causing increased TDMI the altered rates of disappearance may have resulted from the altered digestive conditions caused by the differences in intake, which may not be equivalent for all intakes. Note that the increased DM disappearance observed with the highest supplement compared to the unsupplemented diet or other levels of alfalfa supplementation agreed with the results of Ndlovu and Buchanan-Smith (1985) who observed faster DM disappearance of barley straw and bromegrass

measured with *in sacco* when supplemented with 30% alfalfa. Rate of DM disappearance was also increased by alfalfa supplementation of fescue (Hunt et al 1985). However none of these studies considered supplementation levels between 0 and 25 or 30% as in the present trial, at which points the supplements may have had beneficial effects on TDMI without causing or requiring altered rates of disappearance or passage. Thus, improved TDMI caused by alfalfa supplementation cannot be explained by increased ADFD nor DMD nor rates of DM disappearance at least not for 150, 300g supplements.

Alfalfa supplementation regardless of its level caused significant increases in CPI as well as digestible CPI (DCPI) over the unsupplemented diet. CPI in g kg^{-1} or g kg^{-1} $W^{.75}$ were significantly higher with no difference in grass intake, thus, indicating that the extra CP eaten was supplied by the alfalfa. The trend, however, for decreased grass intake between diets 2 and 4 may explain the non-significant difference among these diets in CPI. The greater increase in CPI was observed for diet 6 being superior to all others and was also the diet that tended to have the greatest TDMI. The diet effect on CPD was not significant ($P<.07$) but the values tended to be increased by alfalfa supplementation resulting in significantly higher DCPI for the alfalfa supplemented diets *vs* the unsupplemented diet. Apparent digestibility of CP was not studied by previously mentioned authors, therefore it is difficult to compare the

results obtained. Since apparent digestibility of CP can be affected by endogeneous N secretion as well as different N recycling under different dietary conditions and different degrees of bacterial contamination being especially important here after the description of how CP supplements can affect N bacterial flow to the intestines in previous sections. It is difficult to discuss the effects of diets on DMI based on DCPI.

Note that although non significant, all levels of alfalfa tended to increase CP retention indicating the the increased CPI was not negated by an increased excretion. Alfalfa could have caused higher TDMI via an improved CP status of the host.

The total VFA concentration as well as molar proportions of acetic, propionic, butyric, isobutyric acids did not differ between diets containing alfalfa at any time. Total VFA concentrations were greater for diets 4 and 6 vs 1 and for diets 2 and 4 vs 1 at 3 h post feeding, for diets 2 and 6 vs 1 at 5 h post feeding, for diet 2 vs 1 at 6 h post feeding. These differences in total VFA concentrations indicated enhanced fermentation for alfalfa containing diets vs the unsupplemented diet with the time pattern being different between levels of supplementation suggesting that alfalfa effect on DMI may have been partly mediated by a more efficient use of the fiber resulting in more energy available to the host. The magnitude of the increased fiber digestion appeared highest for the lowest suplementation

level. In all cases no major alteration in bacterial population types seemed to have occurred since molar proportions of the major VFA were the same at all times. But it is important to remember that this apparently enhanced fiber utilization could be an artefact of a parallel increased TDMI.

From these results it cannot be ensured that fiber was fermented more efficiently as alfalfa level increased. It would be contradictory to the lack of increase in DDMI and DADFI observed despite greater DM and ADF intakes. Results of Ndlovu and Buchanan-Smith (1985) using restricted feeding did not observed any increase in total VFA concentrations when alfalfa was supplemented. The results of Brandt and Klopfenstein (1986 c), however, did indicate higher VFA when 20% alfalfa was supplemented to ammonia cobs at equal intakes. The ammonia concentration in rumen post feeding reached levels much greater than in our study or Ndlovu and Buchanan-Smith (1985) study and may be a confounding factor since ammonia is required for cellulolytic bacteria.

The latter authors observed no difference in the molar proportions of individual VFA when alfalfa was supplemented except for the BC-VFA. In the present study only valeric and isovaleric acids were found to be altered. Isovaleric acid (%) was significantly higher for diets 4 and 6 vs 1 and 2 at 1 h post feeding, percentage of valeric was higher for diets 4 and 6 vs 1 prefeeding and at 3 and 4 h post feeding, and for diets 2, 4 and 6 vs 1 at 5 and 6hrs post feeding. Thus

for all levels of alfalfa supplemented, increases in percentage of valeric and for diets 4 and 6 in percentage of isovaleric vs unsupplemented diet were noted at some point in time. The higher % BC-VFA and its precursor indicated that alfalfa supplementation caused an increased production due to its own composition or that it enhanced the release and production from the grass forage. These compounds are essential growth factors for certain rumen bacteria and could have improved growth of these bacteria, their efficiency at utilizing fiber although controversial here, or their efficiency at synthesising proteins. The issue cannot be closed from the present results.

BC-VFA could be one mode of action of alfalfa supplement although their mechanisms of action cannot be determined from the present results. Note that higher percentage of BC-VFA could also be indicative of an accumulation vs other acids and a reduced efficiency of microbes or requirements of microbes for these acids potentially because of more preformed amino acids supplied by the alfalfa.

Rumen pH showed some effects of diet prefeeding and at 12 h post feeding being in both cases higher for diets 4 and 6 vs 1 and 2 and were not different at any other times. Rumen pH values were always between 6 and 7 therefore it is unlikely that this parameter had affected the pattern of fermentation. It could also be stated that diets were not different enough to alter rumen pH again supporting similar

fermentation patterns. Ndlovu and Buchanan-Smith (1985) did not observe any variation in pH due to alfalfa, with their figures ranging from 6.54 to 6.88.

Rumen NH_3 concentrations were significantly higher for diets 4 and 6 vs 1 at 1, 3, 4 h post feeding and consistently tended to be greater on diet 2 vs 1 indicating a greater NH_3 supply resulting from alfalfa supplementation. The greater concentration could have been caused by higher intake due to alfalfa since grass intake was similar, alternatively other factors in alfalfa may have enhanced NH_3 production from grass proteins or, from greater N recycling. The source cannot be determined from the results and may also be a combination of all these. However the important factor is that rumen NH_3 was effectively increased by alfalfa supplementation. As BC-VFA are an important factor for cellulolytic bacteria activity and protein synthesis, alfalfa could have played a role in the increased TDMI. The level of NAN in the rumen was not monitored, thus, the effect of alfalfa supplementation on amino acid availability cannot be discussed. However, it can be hypothesized that alfalfa caused a greater microbial synthesis and a greater total NAN flow to the duodenum improving the N status of the host as suggested from results of the N balance study. Note also that for diets 1, 2, 4 and 6 rumen NH_3 never reached the 21 to 30 mg dL^{-1} required for maximal microbial synthesis and never was below the 5 mg dL^{-1} which is the minimum NH_3 required for maximal growth. If the latter was

achieved then it was most probable that beneficial effects were mediated via an increased protein synthesis. Since rumen NH_3 only tended to be higher for diet 2 vs 1 but was never significantly different, the higher TDMI observed for diets 2 vs 1 must have been mediated by some other nutrient supplied by the alfalfa with the NH_3 requirement of microbes in face of other nutrient availability being met. It also indicated that meeting rumen NH_3 requirements may be important but not the only factor involved in obtaining maximal DMI. Brandt and Klopfenstein (1986 b,c) demonstrated that several fractions or several nutrients in alfalfa were able to increase DMI of ammoniated cobs to different extents. Among the alfalfa fractions that caused higher DMI were alfalfa fiber, juice containing all solubles as well as deproteinized juice. The protein coagulum could not improve DMI which could be related to a denaturation of the proteins occurring upon heating. These results demonstrated that in fact other fractions than the N could play a role in increasing DMI.

The importance of the CP/Energy ratio has been emphasized by Oldham and Smith (1982) and Oldham (1984) as well as the importance of supplying readily available carbohydrates to ensure efficient use of NH_3 Johnson 1976). The animals consuming only grass could have had enough NH_3 available but were lacking other nutrients as amino acids, minerals or soluble carbohydrates to use it efficiently. These nutrients were supplied by the alfalfa

supplements.

Although no difference in TDMI nor grass DMI among supplements was found grass intake tended to decrease probably because alfalfa had better balanced nutrients for the animals.

4.4.d Iso-N supplementation of urea vs alfalfa

Replacing the 150g (as fed) alfalfa by iso-N amounts of urea diet 2 vs 3 and 4 vs 5 did not cause any decrease in grass DMI. As for Trial I, although not significant here, TDMI of diets containing urea tended to be lower vs their iso-N alfalfa containing diet probably only because of their lack of alfalfa DM. Grass intakes were not increased to compensate because the absence of alfalfa also meant the absence of stimulatory compounds. Therefore N was not enough, further supporting results of Brandt and Klopfenstein (1986b, c) as well as conclusions from comparisons among different levels of alfalfa. Note that the requirement of some alfalfa nutrients other then N in order to increase TDMI was illustrated by the lack of difference in TDMI between diets 1 and 3, as well as between diets 5 and 2. The latter comparison indicated that increasing N supplementation by adding some urea could not cause increases in DMI greater than the alfalfa alone. The non effect of urea on TDMI supported the earlier conclusion as to ammonia not being the limiting factor. The DMD of the unsupplemented diet was not affected in any way by urea

supplementation nor was the resulting DDMI. Since intake and digestibility remained unchanged it is normal that no effect was observed on DDMI.

ADFI was increased by urea supplementation reflecting the trend in TDMI but no difference in digestibility nor DADFI was noted. Total VFA concentration was not higher at any time postfeeding for urea containing diets vs unsupplemented diet and, trends toward lower concentrations specially when urea was the only supplement were observed prefeeding and at 1 and 4 h post feeding suggesting some potential adverse effects of urea on rumen fermentation. These depressing effects were reflected in lower rates of DM disappearance for urea containing diets vs all others even their iso-N alfalfa containing counterparts.

A relation between the above parameters and higher rumen ammonia prefeeding and at 1, 3 and 4 h post feeding exists since Brandt and Kopfenstein (1986a) observed in some instances significant negative correlation between rumen NH_3 and NDF and DM digestibilities. High ammonia levels were also shown to negatively affect rumen motility (Bueno et al 1977). In the present trial, DM disappearance was depressed when the diet contained urea. Lower rates of DM disappearance could in turn affect rates of passage. Although this parameter was not monitored here and showed variable results in Trial I, it has been demonstrated that decreased rates of passage have deleterious effects on rumen bacteria efficiency and favor growth of protozoa (Van Soest

1982). More protozoa would cause more bacterial-N to be recycled thus less available to the host. More protozoa could also mean less carbohydrates for efficient use of ammonia since protozoa are good carbohydrate scavengers (Veira 1986). This could explain why adding some alfalfa to urea could alleviate depressing effects of urea.

An increased N recycling via protozoa or other mechanisms would be supported but the fact that CP retention tended to be higher with urea containing diets compared to their iso-N alfalfa containing counterparts. The greater N retention could also be an artefact of lower endogeneous secretion or bacterial contamination.

The higher NH_3 concentrations in the rumen were parallelled in some instances by higher PUN and these two were not related to any beneficial effects on DMI nor DMD.

Although comparing iso-N supplements also eliminated NH_3 as the mode of action of alfalfa since in both cases diets 2 vs 3, diets 4 vs 5, rumen NH_3 concentrations were significantly higher prefeeding and at 1 and 3 h post feeding and did not result in increased DMI a reverse trend was indeed observed.

Trial 1 demonstrated that there was no benefit of spreading N supplementation through the day, this conclusion plus the additional observation that rumen NH_3 concentration never approached toxic levels suggested that the tendency for urea to have depressing effects vs alfalfa was not caused by an inappropriate release of N in the rumen.

DEI were not different between urea and alfalfa supplements and thus similar quantities of energy were available for rumen fermentation. The patterns of release of N and DE, however, may have been different and not always optimal for urea containing diets. Sheep could not have corrected the problem by increasing their intake of grass hay explaining the lack of improvement in DMI when urea was supplemented.

A role for BC-VFA as suggested earlier and by Ndlovu Buchanan-Smith (1985) cannot be eliminated since for diet 3 vs diet 1 at any times there was no difference in percentage of valeric acid, percentage of isovaleric, percentage of isobutyric parallelled by similar intakes. However, comparing iso-N diets (2 vs 3), percentage of valeric acid was lower at 1, 3, 4, 5 and 6 h post feeding for diet 3, and comparing 4 and 5 lower at 5 hrs post feeding. In both cases trends for lower DMI were observed. The effect appeared greater for the lower supplement perhaps indicating that at 300g (as fed) alfalfa requirements for these were met explaining the previously reported similarity between intake on 150 and 300g supplemented diets.

The combination of alfalfa and urea (diet 5), not improving DMI over the urea alone or unsupplemented diet suggested again a deleterious effect of too much NH_3 since alfalfa alone had caused increased TDMI. This effect could be due to an inappropriate CP/E ratio or inadequate supply of carbohydrates or negative N balance. It certainly

suggested that alfalfa effect on intakes was not mediated only by its amino acid perhaps because microbes requirements were fulfilled, or not enough by passed rumen fermentation to be beneficial to the host. The amino acid profile of the alfalfa may not be adequate to improve the N status.

Conclusion

A urea supplementation iso-N to 150g (as fed) of alfalfa did not cause any improvement in TDMI of mature sheep offered timothy hay ad libitum, despite higher ruminal NH_3 concentrations. These results allow to conclude that with such a basal diet, NH_3 was not a limiting factor for DMI. It may in fact have had deleterious effects on fiber digestion as indicated by the tendency of VFA concentration to be lower when urea replaced iso-N amounts of alfalfa. The depressing effect of urea could also relate to its lack of supply of BC-VFA vs alfalfa, with the percentage of BC-VFA being significantly lower at several times post feeding. The depressing effect of urea was also observed when alfalfa was supplemented simultaneously, the latter probably being unable to establish proper carbohydrate/N ratio or amino acids or minerals imbalances created or to alleviate the deleterious effect of NH_3 on OM digestion. The exact reason remains unclear.

TDMI of mature sheep could, however, be improved by supplementation of alfalfa with no difference among levels supplemented contrary to previous research. This contradiction could be due to the fact that in the present trial, levels of alfalfa supplemented were much lower then in stusies of Soofi et al. (1982) or Hunt et al. (1985).

V GENERAL CONCLUSIONS

The combined results of both trials demonstrated that when alfalfa hay was supplemented to a basal timothy hay diet to represent 0-20% of intake, the improvements seen in TDMI vs unsupplemented or urea supplemented diets were due to the intake of the alfalfa over the already consumed timothy hay. This increased consumption was not the result of higher rates of passage and not due entirely either to higher rumen ammonia concentrations. BC-VFA could have been of importance since they were increased by all levels of alfalfa supplementation.

In this range of supplementation it was also demonstrated that alfalfa effect did not result from improved appetence.

The supplementation of urea to the basal diet for mature rams did not improve DMI although trends toward improved N retention were observed.

Different feeding schedules of the urea or alfalfa supplements did not show any effect on final TDMI.

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VII APPENDIX TABLES

Appendix A Least square means of the average molar proportions of VFA and rumen pH across diets at different times post feeding. (Exp.1).

	Times				SE*
	0	3	6	12	
Acetic	71.54[a]	69.13[b]	69.35[b]	70.06[b]	0.42
Propionic	18.46[a]	19.77[b]	19.55[b]	19.62[b]	0.28
Butyric	7.81[a]	9.06[bc]	9.40[b]	8.96[c]	0.15
Isobutyric	0.81[a]	0.69[b]	0.60[c]	0.46[d]	0.03
Isovaleric	0.91[a]	0.70[b]	0.54[c]	0.38[d]	0.02
Valeric	0.47[a]	0.65[b]	0.56[c]	0.52[d]	0.01
pH	6.48[a]	6.41[b]	6.31[c]	6.06[d]	0.02

*Standard errors of the means.
[abcd] Figures with the same letter do not differ P>.05

Appendix B. Least square means of the average molar proportions of acetic (A), propionic (P), isobutyric (IB), butyric (B) and isovaleric (IV), and total VFA concentration across diets at different times post feeding. (Exp2).

	VFA					
Times	A	P	IB	B	IV	Total
Prefeeding	70.21^{a}	17.64^{a}	1.21$^{a b}$	8.93$^{a b}$	1.35^{a}	80.32^{a}
1hour after	68.04^{d}	19.77^{b}	1.42^{a}	8.71^{a}	1.26^{b}	88.32$^{b c}$
3hrs after	68.34$^{c d}$	19.52$^{b c}$	1.15^{b}	9.04$^{a b c}$	0.98^{c}	88.48^{b}
4hrs after	68.67$^{b c}$	19.32$^{b c}$	1.03$^{b c}$	9.18$^{b c}$	0.88^{d}	88.66^{b}
5hrs after	69.24$^{a b c d}$	18.91^{c}	0.90$^{c d}$	9.33$^{b c}$	0.79$^{d e}$	83.83$^{a c}$
6hrs after	69.53$^{a b c}$	18.91^{c}	0.81$^{d c}$	9.29$^{b c}$	0.71^{e}	82.40^{a}
12hrs after	69.62$^{a b}$	19.01$^{b c}$	0.68^{c}	9.39^{c}	0.61^{f}	87.32$^{b c}$
SE*	0.45	0.30	0.07	0.14	0.03	1.63

$^{a b c d e f}$ Figures in a column with the same letter do not differ P>.05
*Standard errors of the means.

Appendix C. Least square means of the average PUN and plasma glucose (mg/dl, mg/ml) across diets at different times post feeding. (Exp.2).

Times	PUN	Glucose
prefeeding	11.46[a]	68.39[a]
1hour after	13.67[b]	62.76[b]
3hrs after	14.25[bc]	67.84[a]
4hrs after	15.02[c]	69.72[a]
5hrs after	15.37[c]	69.46[a]
6hrs after	14.41[bc]	67.22[a]
12hrs after	10.92[a]	66.35[a]
SE[x]	0.40	1.52

[abc] Figures in a column with the same letter do not differ P>.05
[x] Standard errors of the means.