EFFECT OF GRASS MATURITY AND WILTING ON THE NITROGEN FRACTIONS IN SILAGE , THEIR RATE AND EXTENT OF DEGRADATION IN THE RUMEN

Nathaniel Fungayi Makoni

B.Sc.(Agr.), University of Leeds, 1984

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

DEPARTMENT OF ANIMAL SCIENCE

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

October 1988

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of ANIMAL SCIENCE

The University of British Columbia
Vancouver, Canada

Date 12-10-88

0.1 ABSTRACT

The available literature indicates that, silages containing more undegraded protein will be better feeds for high levels of production from ruminants. Ensiling excessively wet herbage will increase degradation of both protein and energy. Ensiling wilted herbage will increase aerobic losses initially, during compaction and later, during removal which decreases digestibility. This study was carried out, to evaluate the effect of grass/legume maturity and wilting on the nitrogen fractions in silage, their rate and extent of degradation in the rumen and to determine the availability of undegraded silage protein and microbial protein to the animal.

At the beginning of this study, laboratory assessment of the nutritional composition of both grass/legume parent material, and their ensiled products was carried out. The results for both parent herbage and silage were then compared on DM basis. While, silage crude protein (CP) ($p<0.05$), water soluble carbohydrates (WSC) ($p<0.001$), ash ($p<0.001$), acid detergent fibre (ADF) ($p<0.05$), and grass/legume ammonia-N (NH_3N) ($p<0.001$), were significantly increased by wilting, silage hot water insoluble nitrogen (HWIN) was significantly reduced ($p<0.001$). Significantly lower pH ($p<0.05$), NH_3N ($p<0.05$), and ash ($p<0.01$) contents were observed in mature silage. The composition of silage nutrients was found to be governed by that of the standing crop, at the time of cutting and by modifications which take place during wilting and ensiling. Although, wilting increased WSC content, it had the undesirable effect of reducing silage hot water insoluble nitrogen. Maturity had desirable effects of reducing volatile NH_3N and silage pH. The fermented herbage contained lower levels of nutrients than the parent material.

In the second part of this study, the nylon bag technique was used to determine the rate and extent of protein degradation (in the rumen) of immature unwilted and

wilted, mature unwilted and wilted silages. Estimates of effective protein degradation as a function of rumen outflow rate and degradation rate were also evaluated. The study showed that wilting and maturity significantly reduced ($p<0.05$) the soluble nitrogen fraction of the grass/legume silage. DM disappearance was significantly reduced ($p<0.05$) by wilting. The insoluble protein fraction was increased ($p<0.05$) in the mature silages. At an estimated rumen outflow rate of 0.02%, effective DM and CP degradation were significantly reduced ($p<0.05$) by wilting. From these findings, it is likely that supplementation of both protein and energy is required most for the unwilted and immature silages. Also, when silages are wilted more undegraded protein leaves the rumen compared with unwilted silage.

In the final part of the study, the effect of wilting and maturity on rumen degradation of silage N fractions were estimated using four rumen and duodenal cannulated steers and heifers fed on grass/legume silage, at maintenance level. Solute, particulate, and RNA markers were used to determine digesta flow rate and microbial N respectively. Measurements of digestibility of DM, CP and ADF, proportions of duodenal bacterial N, amount of undegraded protein entering the duodenum, degradability of silage protein, rumen NH_3N and plasma urea nitrogen (PUN) were made. This showed that, there were no significant differences ($p<0.05$) in PUN and rumen NH_3N among silages. Significant increases ($p<0.05$) in duodenal and fecal CP and reduced ($p<0.01$) fecal ADF were observed in animals given wilted silages. The degradability and digestibility results were much lower than expected and no apparent differences ($p<0.05$) were observed among silages.

Table of Contents

List of Tables

List of Figures

0.0.1 ACKNOWLEDGEMENT

I would like to express my deep gratitude to my research supervisor Dr. J. A. Shelford for providing me with his invaluable assistance and guidance throughout the duration of the research period and the writing of this thesis.

I am most grateful to members of my graduate committe Dr. L.J. Fisher of Agriculture Canada, Agazziz, Professor B.D. Owen, Dr. A. Vera for reading and commenting on this thesis.

Sincere thanks to Mr. P. Willing for technical assistance and Mrs. M. Evans for laboratory assistance.

I am indebted to the Canadian Commonwealth Scholarship and Fellowship Administration for financially supporting my studies at U.B.C.

Finally, I would like to thank my family and colleagues, especially, I express thanks to my friend J. Onunga for encouragement and assistance in completing this thesis.

Chapter 1

EFFECT OF MATURITY AND WILTING ON THE NITROGEN FRACTIONS IN GRASS/LEGUME SILAGE:

1.1 INTRODUCTION:

The storing of green forage in silos and allowing it to ferment, goes back to antiquity [121]. In general, silage is the product derived from the controlled fermentation of a crop of high moisture [1], and [4]. The ensiled material passes through a sequential fermentation, the first stages of which cause cessation of plant tissue metabolism and a rapid exhaustion of trapped air, followed by reduction of pH through acid production [1], [90]. Further fermentation depends on the availability of water [90].

The purpose of ensilage is to preserve the digestible nutrients of the conserved parent material as efficiently as possible; to retain the feeding value, reduce the losses and finish with a product which is acceptable to the animal. The quantity of forage preserved as silage continues to increase and constitutes a major component of winter rations for ruminant animals [116], especially beef and dairy animals [2].

Unfortunately, ensiling often results in substantial reductions in voluntary feed intake and efficiency of crude protein utilization, thereby lowering performance of those animals fed silage as compared to fresh grass/legume forage. The major changes in chemical composition that result from ensiling are an increase in organic acids and non-protein nitrogen and a decrease in water soluble carbohydrates. There are two major stages in the breakdown of protein in the silo. Firstly, proteins are broken down

to amino acids through proteolysis; and secondly, by clostridia bacteria (clostridial fermentation), the decarboxylation and deamination of amino acids produces ammonia, amines and other simple nitrogenous compounds.

Poor protein supply represents the major nutritional limitation to the use of well preserved silage. Silages can be separated into several groups on the basis of moisture level: a) direct cut b) wilted silage and c) low moisture silage. The direct cut (high moisture) silages tend to undergo a more extensive fermentation, which results in a greater breakdown of the protein fraction [117]. Little information on the effect of inclement weather on silage protein quality has been published [105].

In evaluating productivity potential of silages, the fractionation of nitrogen into non-protein and protein is very important. The objective of this study is to determine the effect of maturity and wilting on the nitrogen fractions in silage, through a laboratory assessment of the nutritrional composition of the grass/legume parent material as compared to their ensiled products. The following review attempts to describe changes in nitrogen fractions as affected by ensiling, moisture content and maturity of the parent grass/legume material.

1.2 LITERATURE REVIEW:

1.2.1 NITROGENOUS COMPOUNDS IN HERBAGE:

There is little variation in the amino acid content of leaf protein among different plant species [80]. Examination of five grasses and six samples of lucerne *medicago sativa* harvested at different stages of maturity showed that protein composition were of closely similar amino acid content [116]. In all samples, arginine occurred in highest concentration, 12 to 14% N of total protein N, with amounts of lysine, aspartic acid and glutamic acid, alanine, leucine and glycine, each ranging from 6 to 8%. These seven

amino acids accounted for 63% of the total amino acid N recovered from hydrolysis of the herbage proteins. Nitrogenous compounds in fresh herbage can be split into two fractions, that is, true protein and non-protein nitrogen (NPN). About 75-90% of the total N (TN) is present as protein. The remaining 10-30% of TN consists mainly of free amino acids and amides (glutamine and asparagine), with smaller concentrations of ureides, amines, nucleotides, chlorophyll, low molecular weight peptides and amino acids bound in non-protein form [3], [119]. Nitrates may also be present as NPN, the concentration depending upon plant species, variety, moisture and fertilization [55]. However, the amount of free ammonia in fresh herbage is usually less than 1% of TN [118].

When leaves are detached from the stems of most plant species, a rapid breakdown of leaf protein occurs. This breakdown is non-selective since the residual protein has a similar amino acid composition to the original protein, and there appears to be no preferential release of amino acids [35]. The rate of breakdown in starving leaves varies from one plant species to another [8]. Moreover, since factors such as mineral nutrition, season, moisture and maturity alter the distribution of N between protein and NPN fractions in the growing plant, the effect of such factors on post-harvest protein breakdown warrants consideration. Previous work on proteolysis in the starving leaves of several grass species has defined the changes which occur in grass leaves treated in such a manner [7], [11], [13]. There is an initial rapid rise in the concentration of amino N, followed in the second and third days, at temperatures of $20 - 30^{o}C$, by a rise in amide N content. Then there is a fall in the concentration of these fractions, accompanied by a rise in the volatile N content [8]. Final distribution of N depends on the rapidity with which moisture is lost, with the major change being on hydrolysis of protein to amino acids [8]. Volatile N concentration may rise slightly while the rise in the amide N content is not great, unless the loss of moisture is particularly slow [11].

1.2.2 CHANGES IN NITROGENOUS FRACTIONS DURING ENSILING:

Proteolysis continues during ensilage. Its extent depends largely on the rapidity with which acid conditions are established, which, ultimately, has some bearing on the nature of fermentation [35]. Thus, the rate of acidification occurring in the silo is important for the conservation of protein. A sixfold increase in water soluble N was observed when proteolysis was studied during the first 10 days of ensilage of rye grass, subterranean clover and alfalfa silages [8]. Bergen et al. [5] reported similar large increases (13.2 to 40.4% of total N) within the first 10 days of ensiled corn. McPherson [11] revealed that N stability was not achieved until the pH of fermented silage had reached 4.3. Similarly, Capintero [12] showed that the addition of formic acid or cultures of lactic acid bacteria to herbage reduced the extent of proteolysis during ensilage; nevertheless, 45% of the original herbage protein was broken down. Whittenbury [6] also estimated a higher value of about 60%. From the foregoing, it is logical that high levels of WSC in a silage crop are conducive to protein stability [56].

During ensiling, considerable degradation of amino acids is known to occur, especially of the basic types, lysine, arginine and histidine, with appreciable loss of aspartic acid, threonine, tyrosine, proline, glutamine, methionine, serine, glutamic acid and cysteine but relatively little loss of valine, leucine [14], [15]. The products of amino acid breakdown include γ and Δ aminobutyric acids, ornithine, cadaverine, putrescine [7], together with propionic acid, isovaleric acid, histamine, tryptophan and tyramine.

Proteolysis prior to and following ensilage can be stemmed by the early and rapid establishment of anaerobic acid conditions or by the suppression of both plant and microbial activity in silage through the use of crop sterilants. Although proteins and amino acids reaching the animal's rumen undergo proteolysis, it is important that

amino acids do not undergo change during ensiling. Good ensiling conditions reduce chances of this happening. Furthermore, proteolysis in the rumen should be limited as far as possible, in the hope that dietary protein *per-se* will enter the duodenum, since, NPN will be utilized rapidly by the rumen micro-organisms.

1.2.3 FACTORS AFFECTING SILAGE FERMENTATION:

There are several factors reported to affect silage fermentation [118], [35]. In this section, only three factors pertinent to the intended study have been reviewed.

A. Moisture:

On one hand, moisture is essential for proliferation of desirable organisms, but on the other, excess moisture will encourage growth of undesirable organisms [35]. In forages with less than 15% DM, which can occur when harvesting is undertaken in wet weather or with an immature crop, the moisture invariably counteracts the preservative effects of the primary fermentation acids, and clostridia fermentation may not be suppressed at a pH as low as 4.0 [6], [4]. Also, butyric acid fermentation often results in such high moisture silages [35].

B. Prewilting:

Lactic acid bacteria have a greater tolerance to low moisture availability compared to clostridia [89]. In fact, the higher the DM of the crop the lower the bacterial activity and the role of fermentation acids in preservation [35]. Thus, the higher the DM content of forage, the greater will be the pH at which anaerobic stability is achieved due to reduced bacterial activity [17]. Similar trends of reduced fermentation acids and an increase in residual water soluble carbohydrates with increased silage DM have been reported [141]. Lanigan [18] demonstrated the high tolerance of lactic acid bacteria to wilting. From these findings, it was proposed that DM contents most favourable to silage fermentation are in the range 20-25% and that at levels above 33% no undesirable

microbial activity will occur. The relative intolerance of clostridia to low pH/high DM environments has been conclusively shown [10], [16]. However, in highly wilted silage (>45- 50%), high total losses of DM could result from oxidation and not fermentation, and for this reason no advantage could be seen in wilting to levels higher than 30-34% as a means for reducing DM losses in silage [141].

C. Maturity:

Throughout the growing season, grass/legume dry matter digestibility *in vitro* decreases at a rate of approximately 0.05% unit per day, and the decline is most rapid after heading [110]. Therefore, the stage of maturity of plants before cutting is the major determinant of forage nutritive value for, as grass plants mature, the proportion of cell walls increase while that of cell wall contents is reduced. Coupled to these changes, there is a change in composition and structure of both cell wall and cell contents. With increasing maturity there is a decrease in crude protein content, with soluble proteins of a relatively constant amino acid composition continuing to make up 75 to 85% of the C.P. [80]. The susceptibility of those soluble proteins to attack by proteolytic enzymes increases and this may have negative consequences during ensiling [150]. The ease with which crops can be satisfactorily ensiled depends in part on their contents of water soluble carbohydrates [132]. Species, stage of maturity, weather and plant density can all influence the amount of water soluble carbohydrates [35], [102]. Thus, the decision of forage harvesting for silage is a compromise between management for maximum vigour of the plants and maximum quality of the herbage.

1.3 MATERIALS AND METHODS:

The grass/legume forage conserved for this experiment was cut at Agazziz research station (field 1) from a sward of first-cut orchard grass and red clover mixture. The

weather was good through all the preparation period. Harvesting of the grass/legume forage occurred during the days August 18 and 19, for the immature cut and September 8 and 9 for the mature cut. The grass/legume forage was mown and harvested with a precision-chop harvester and then ensiled in 7ft. diameter by 7ft. high experimental silos. Silos were filled in duplicate for each treatment. A total of four treatments (2x2 factorial) were made as follows:

1. Immature direct ensiled.
2. Immature wilted ensiled, 24h post cutting.
3. Mature direct ensiled.
4. Mature wilted ensiled, 24h post cutting.

1.3.1 Grass/legume sampling:

Four to six grab samples were taken from each trailer load when it arrived at the silo. These were mixed, one subsample was taken and immediately stored in a tightly closed plastic bag at $-20°C$. These samples were later thawed and composited within treatment. Then, duplicate subsamples were taken and analysed in order to determine the chemical composition of the grass/legume forage entering the silo. The average duplicate values obtained were considered as being representative of the material entering the silo.

1.3.2 Silage sampling:

Similarly, grab samples were collected at the opening of each silo, over four experimental periods of the *in vivo* study. Four representative samples were collected per period and immediately frozen at $-20°C$, prior to analysis.

1.3.3 Analysis:

The DM content of silages was determined by distillation with toluene [149]. Grass/legume forage samples and silages analysed for acid detergent fiber (ADF), acid detergent insoluble nitrogen(ADIN), neutral detergent fiber(NDF), and ash were dried in a forced draught oven at 100°C for 24h. Dry matter(DM) and crude protein(CP) for both grass/legume forage and wet silage were analysed [22], NDF, ADF [104], ADIN, [44], total ash [112], pH [89], NH_3N in grass/legume forage and silage [19], hot water insoluble N [145].

All the statistical analyses were carried out using the general linear model from the SAS package [153]. The model used was;

$$Y_{ijk} = \mu + \beta_i + \tau_j + \gamma_k + \varepsilon_{ijk}$$

where Y_{ijk} = measured variable

μ = mean

β_i = period effect

τ_j = wilting effect

γ_k = maturity effect

ε_{ijk} = wilting x maturity (interaction) effect

1.4 RESULTS AND DISCUSSION:

Although the animal production must be the final arbiter, differences in chemical composition of silages made from unwilted, wilted, immature and mature herbage can be useful indicators of the feeding value. In this study the main focus was on the effects of the above treatments on nitrogenous fractions in silage, but their effects cannot be fully explained in isolation from other parameters which directly or indirectly affect them. This discussion will therefore include other parameters closely associated with nitrogenous fractions (see table 1.2).

The CP contents of the silages were less than those of corresponding herbages. This was very evident in the unwilted silage, indicating a relatively greater loss of protein than of dry matter in the effluent [105]. This would be expected since the high level of NPN in silage would be soluble. A report of Murphy [109], that protein content in wilted silage was less than in unwilted silage, with very little effluent loss, is contrary to our findings. An interaction effect between wilting and maturity in the parent material CP approached significance at ($p<0.05$). This interaction effect showed that, there were differences in CP content of all the treatments except for the immature wilted and mature unwilted silages (see table 1.1). Wilting had a significant effect ($p<0.05$) in increasing CP content of silage. Therefore, wilting appears to do little to prevent proteolysis [105], but can be effective in preventing deamination or breakdown of amino acids to simpler compounds.

Breakdown of proteins to amides and amino acids by plant enzymes can occur during wilting in the field [13], so that protein N may decrease from 80% of total N to 65-75% of total N, depending on the rate of drying [11]. Deamination of these amino acids yields volatile acids and NH_3 [114]. Wilkins [147] found that voluntary feed intake was negatively correlated with the content of acetic acid and NH_3 in silages.

Table 1.1: Chemical composition of the herbage ensiled

	Treatment				
	immature non-wilted	immature wilted	mature non-wilted	mature wilted	±s.e.m.
Herbage					
DM(%)oven	12.20	42.65	13.85	38.85	1.23
CP(%DM)	19.06[a]	17.97[b]	18.03[b]	17.13[c]	0.04
NH_3N(mgN/g)	0.12	0.41	0.12	0.39	0.02
ADF(%)	37.78	36.22	36.27	37.11	0.15
NDF(%)	49.45	46.29	48.39	47.38	0.74
Ash(%)	17.76	12.87	11.33	11.78	0.03
WSC(%)	27.40	20.75	26.86	23.58	0.95
Silage					
DM(%)oven	16.23	37.61	16.57	32.86	0.98
DM(%)toluene	21.32	40.72	21.54	36.42	1.35
Volatiles	5.09	3.12	4.97	3.56	0.62
pH	5.15	5.25	4.54	4.71	0.26
CP(%DM)	15.23	17.15	14.67	15.97	0.65
NH_3N(mgN/g)	0.33	0.71	0.27	0.51	0.05
HWIN(%CP)	60.28	28.91	60.31	36.58	3.28
WSC(%DM)	4.76	9.80	4.68	7.52	0.64
ADF(%DM)	49.44	42.61	47.74	43.16	0.92
NDF(%DM)	48.92	46.83	47.87	47.89	0.52
Ash(%DM)	12.19	14.24	10.37	12.54	0.58
ADIN(%DM)	0.88	0.82	0.95	0.95	0.06

Values with different letters are differnt at ($p<0.05$)

Table 1.2: Chemical composition of the herbage ensiled (main effects)

			Treatment		
	unwilted	wilted	immature	mature	±s.e.m.
Herbage					
DM(%)oven	13.03	40.75	27.43	26.35	1.65
CP(%DM)	18.55	17.55	18.52	17.58	0.02
NH_3N(mgN/g)	0.12	0.40**	0.26	0.25	0.01
ADF(%)	36.53	36.67	37.00	36.19	0.10
NDF(%)	57.16	55.44	57.46	55.14	0.51
Ash(%)	12.04	12.32	12.81	11.56	0.02
WSC(%)	27.13	32.16	27.07	35.22	0.67
Silage					
DM(%)oven	16.40	35.23	26.92	24.72	0.69
DM(%)toluene	21.43	38.57	31.02	28.98	0.95
Volatiles	5.03	3.34*	4.10	4.26	0.44
pH	4.85	4.98	5.20	4.62*	0.18
CP(%DM)	14.95	15.56*	16.19	15.32	0.46
NH_3N(mgN/g)	0.30	0.61**	0.52	0.39*	0.04
HWIN(%CP)	60.30	32.74**	44.60	48.44	2.32
WSC(%DM)	4.72	8.66**	7.28	6.09	0.45
ADF(%DM)	42.89	48.59*	46.03	45.45	0.65
NDF(%DM)	48.92	46.83	47.87	47.89	0.52
Ash(%DM)	11.28	13.39**	13.21	11.45†	0.41
ADIN(%DM)	0.91	0.88	0.85	0.95	0.04

** indicate values significantly different at ($p < 0.001$)

† indicate values significantly different at ($p < 0.01$)

* indicate values significantly different at ($p < 0.05$)

Results presented in (table 1.2) showed a marked increase in NH_3N concentration from parent herbage to the silages. While wilting alone had a very significant effect ($p<0.001$) in increasing NH_3N of the parent material, wilting and maturity significantly increased ($p<0.05$) and reduced ($p<0.001$) ($R^2 = 0.80$) NH_3N in silages respectively. These results are different from those of Durand [113] who compared wilting *vs* unwilting and found that NH_3N was higher in unwilted silage, indicating that there was less degradation of amino acids in the wilted silage. Generally, a lower NH_3N concentration associated with wilting indicates reduced clostridial activity rather than a reduction in production of amino acids from protein [116]. It is likely that NH_3N was higher because of wilting prior to ensiling. Also, the effect of wilting in increasing NH_3N content could be a consequence of unabated plant protease activity under the poor compaction usually observed for such silages. Under extended wilting conditions (as for haylage), a very large increase in NH_3N content in wilted *vs* unwilted is usually expected [102]. This was evidenced by Jackson [108] who reported wilted silage with a significantly higher NH_3N content than with the direct cut silage made from the same herbage. In 1983, Anderson further commented that a significant proportion of NH_3N in wilting can be derived from the deamination of amides accumulated during wilting. That maturity significantly reduced NH_3N content was expected since such silages normally have a high content of water soluble carbohydrates (WSC) which would ferment to reduce pH below the optimum range for clostridial activity. However, effect of moisture could be more important than maturity.

Again, wilting had a very significant effect ($p<0.001$) in decreasing the amount of hot water insoluble N (generally referred to as true protein). This could probably be a reflection of the extended effect of plant protease activity on the available protein fraction.

Work by Van Soest [93] showed that normal forage contains about 7% of its N as

acid detergent insoluble N. So, any feed having twice this amount could be used as an indication of heat damage [101]. The percentages ADIN reported in this study are far below the value reported by Van Soest and none of the treatments had a significant effect on silage ADIN. These low values could be a an indication of the level of fermentation in experimental silos as compared to farm silos. More heating of the forage is expected to occur in the latter silos. It can be safely concluded that there was no excessive heating of the silage prepared for this study.

Storage losses as effluent virtually cease above a forage DM concentration of 25% [105]. From a comparison of oven with toluene DM, it would appear that wilting caused lower contents of organic volatiles. The difference was significant at ($p<0.05$). Effluent loss from the unwilted silages probably explains the resultant increase in dry matter observed in silages compared to the parent material. DM higher than 20% and high WSC have been reported to favour lactic acid fermentation [89], and this type of fermentation has a positive effect on silage nitrogen fractions.

The presence of WSC in silage, although lower than that of the parent material, may result in a more efficient utilization of nitrogenous components [141] . When crops are ensiled, water soluble carbohydrates are fermented by lactobacilli under anaerobic conditions to produce organic acids, mainly lactic acid [116]. It has been established that wilting prior to ensiling restricts fermentation in the silo, thus resulting in increased level of WSC content of the silage when compared to unwilted material [105]. Our results confirm this effect of wilting increasing the WSC content, approaching significance at ($p<0.001$; $R^2 = 0.84$). Ensiling of high moisture grass often results in unsuccessful fermentation due to low concentrations of WSC available for fermentation [118].

Generally, pH values below 4.5 show that silages are well preserved. Maturity of the herbage had a significant effect ($p<0.05$) in reducing silage pH: 4.62 $\pm$0.18 in mature

silage *vs* 5.20 ±0.18 in immature silage. High pH values were anticipated from the wilted silages as was observed by Gordon [115],McDonald [141],Forbes [130], Murphy [109], who commented that pH in wilted silages compared to unwilted usually remain stable at a higher value, demonstrating the fact that bacterial growth (in particular Clostridia) decreases with increased forage cell osmotic pressure. However, significant effect of maturity on reducing pH can be partially explained by the usually low WSC observed in immature herbage leading to lack of substrate for lactobacilli fermentation. Also, the high moisture associated with these silages could play a role in diluting the acid end-products from the little carbohydrate fermented.

There were no visible changes in the ash content from parent material to the ensiled grass/legume forage which probably indicates little ash loss in ensiling. However, maturity significantly ($p<0.01$) reduced ash content of the silage while wilting significantly ($p<0.001$) increased ash content. The effect of wilting, in this case, can be attributed to a reduction in effluent [109]. The effect of maturity was expected since mineral content of plants decrease with maturity [110]. Reduced ash content could cause poor utilization of the prefermented diet since minerals such as sulphur are vital requirements for rumen microbes [52]. Since individual mineral analysis were not carried, the reduced ash content can be interpreted with caution.

It is well recognised that as plants mature, the yields of fiber increase [111]. This was not apparent for the grass/legume forage parent material, but ensiling had an effect of increasing this constituent in all treatments. A loss of soluble constituents relative to fiber could be a possibility. The ADF content of the silage was significantly increased by wilting which concurs with the results of Anderson [106] and could be attributed to a greater loss of other plant constituents relative to ADF. Levels of ADF in forages are good indicators of forage digestibility. High levels are often associated with poor digestibility of the roughage.

Laboratory analysis of silages offers a rapid method of determining nutritive value of silage, though results obtained cannot be totally accepted without further assessment of their utilization by the animal. In this study, the nitrogen fractions were not fully studied since our results do not say much about the type and extent of degradation of the crude protein. The term 'protein' is more or less synonymous with what is often referred to as 'true' protein. As mentioned by McPherson [7], 'Crude' protein, although a useful figure in practice, is chemically meaningless. In future, more attempts could be centred on fractionation of the silage crude protein into true protein and amino acids in the hope of assessing effects of such treatments on the true protein fed to the animal.

There is a problem in serial sampling. As has been shown by several workers Playne and Mcdonald [9], Anderson [106], the analytical figures and physical properties of the silage may vary considerably according to the position of the sample in the silo. For instance, with the type of silos used in this experiment, variations due to location of sampling could be expected.

1.5 SUMMARY AND CONCLUSIONS:

The study showed that the composition of silage is governed by that of the standing crop at the time of cutting and by changes which take place during field drying and ensiling. It is also evident that when grass/legume forage is conserved as silage the products of fermentation always have lower nutrients compared to that of the parent herbage. Wilting significantly increased CP in silage, NH_3N in grass/legume forage, WSC in silage, ash content in silage, ADF content of silage, but, also significantly decreased silage hot water insoluble nitrogen. Maturity of silage significantly reduced NH_3N content, ash content, and pH. In view of the reduced 'true protein', there is little advantage to be gained in reducing DM by wilting crops to DM greater than at least

30% as reported by Marsh [105]. Whether the extent of fermentation with consequent saving of WSC is of value to the animal is uncertain. Overall, wilting had undesirable effects of reducing true protein, and maturity had desirable effects of reducing volatile NH_3N and pH. The reduction of pH and ash content could be associated with the buffering capacity of mature herbages.

Future work could look into further separation and identification of nitrogenous fractions with relevance to production qualities and the examination of other products of protein breakdown that could directly influence animal intake of silages.

Chapter 2

DETERMINATION OF RUMEN DEGRADABILITY THROUGH THE USE OF NYLON BAG

2.1 INTRODUCTION:

The extent of protein degradation in the rumen is an important parameter in determining the protein supply for the rumen micro-organisms as well as the supply of amino acids to the ruminant animal postruminally. Therefore, the protein degradability of feedstuffs becomes an essential characteristic in determining the protein value of the feeds.

In the previous chapter, attempts were made to highlight the effect of ensiling grass/legume forage on the nutritive value of the fermented feed, with 'emphasis' on the nitrogen fractions. The effects of inclement weather, silage preparation procedures (such as wilting), and the time of cutting(crop maturity) on the nitrogen fraction, raises considerable concern about the utilization of these nitrogen fractions by ruminants . During fermentation in the silo, a considerable proportion of the nitrogen fraction is broken down into non-protein nitrogen(NPN). This NPN is rapidly degraded in the rumen and may not be utilized efficiently. It is felt that the two factors, maturity of the plant crop and wilting will affect the amount of NPN in the parent crop before ensiling and alter subsequently, the fermentation characteristics. From the literature values for degradation, there is a relatively wide range given for silages. It is postulated that some of these differences are due to species ensiled, maturity and moisture content

at ensiling time.

The objective of this study was to determine the rate and extent of protein degradation in immature, immature wilted, mature, and mature wilted grass silages. Also, estimates of their effective protein degradation as a function of the rumen outflow rate and degradation rate will be determined.

In this section, the effect of maturity and wilting on the rumen degradability of nitrogen fractions of grass silages will be reviewed. The main topics considered in the following literature review will be under the headings, plant nitrogen composition and degradation before ingestion, degradation of protein in the rumen, rumen degradability of silage nitrogen and the nylon bag technique as a tool in protein degradation studies. The first two sections will give background information on plant material or forage nitrogen degradation pre and postprandial. The nylon bag technique is discussed as a method most closely related to the environment in which protein degradation takes place.

2.2 LITERATURE REVIEW:

2.2.1 PLANT NITROGEN COMPOSITION AND DEGRADATION BEFORE INGESTION:

Plant proteins can be divided into two major classes, mainly leaf proteins and seed proteins [79]. About 75 % of total leaf protein is generally found in chloroplasts [81]. These leaf proteins are almost entirely functional proteins (enzymes) concerned with the growth and biochemical functions of leaf cells [80], [81]. The seed proteins, on the other hand, constitute part of the nutritive reserve for the embryo [80]. In fresh forage plants, the true protein content frequently accounts for 75 to 85% of the total crude protein [80]. The amount of non-protein nitrogen (NPN) varies from 9 to 25 % [81].

The soluble leaf proteins (up to 50% of the protein) contain two major classes of protein called fraction 1 and fraction 2. The former is a single homogenous fraction; while the latter is a complex mixture of different proteins which often account for up to 50% of the soluble protein, with a major part associated with the lipid material in cell wall membrane structures [80]. One insoluble protein fraction, extensin, is found in close association with the cellulose of the cell wall. This insoluble fraction is a glycoprotein, rich in arabinose, galactose and an unusual amino acid (in plants), hydroxy proline. Its degradation is probably a slow process [81].

Animal performance is directly related to the insoluble protein content of the forage, provided the forage is not overheated [102]. During dehydration (wilting) of the crop plant, proteins are degraded by plant enzymes to peptides, free amino acids and amides. The extent of proteolysis is influenced by the water content of the crop, the presence of oxygen, and the pH value [90]. When the crop is ensiled the proteolysis is often more extensive than it is during drying, owing to high water content. Plant proteases have been reported to be active down to pH 4.0 [118]. The extent of proteolysis during the ensiling process is thus, largely affected by the silage fermentation pattern, that is, lactic acid fermentation or clostridial fermentation [90]. Thus, the process of ensiling drastically changes solubility of proteins and their susceptibility to be hydrolysed by enzymes [119]. It is estimated that the amount of NPN can be as high 98% of total crude protein in silages [106]. In this section less attention was paid to seed proteins as there is little of this in grass silages. For further details, reference to Lyttleton [80] is given.

2.2.2 DEGRADATION OF PROTEIN IN THE RUMEN:

The quantity of protein degraded by micro-organisms in the rumen can greatly influence animal performance. In this chapter, the term degradation is mainly restricted to dry

matter and proteins and here rumen micro-organisms are involved in the process. The protein value of a feedstuff is determined by the amount and pattern of amino acids it provides for intestinal absorption. Chemically, feed nitrogen can be fractionated into several components, whose biological significance to ruminants cannot be judged unless their potential fate in the gastro-intestinal tract is known [96]. For example, it is known that nitrogen in the form of lignin and Maillard products is highly resistant to both microbial and mammalian enzymes and is, thus, largely unavailable for transformation [78]. However, available feed nitrogen can undergo transformation in the rumen, to a variable extent, through proteolysis, deamination, and the synthesis of microbial cells from simple nitrogen compounds. Thus, through microbial rumen fermentation, there can be increased losses of feed nitrogen as ammonia, and decreased amounts of undegraded feed protein reaching the duodenum [82]. Therefore, to evaluate the biological value of a given source of nitrogen for ruminants, it is necessary to understand its potential as a source of nitrogen for rumen microbes and its subsequent availability as a source of amino acids post-ruminally.

About 40 % of rumen bacteria have proteolytic activity [95]. The rumen bacterial proteases, although cell bound, are located on the cell surface and thus have free access to the substrate [78]. The protozoa present in the rumen are equipped with powerful intracellular proteases. All these microbial proteases operate at a pH range of 6-7 [92]. Plant materials entering the rumen are degraded by bacteria, protozoa and anaerobic fungi [83], [84] [85]. The thin primary cell walls of the mesophyll and phloem are degraded rapidly in the rumen by extracellular enzymes, without prior attachment of bacteria on to plant cell walls [85], [86]. The degradation of thick-walled bundle sheath and epidermal cells is a slower process and is preceded by bacterial attachment onto the plant particles by means of fibrous extracellular structures [87]. In addition, the degradation of cellulose in the rumen by anaerobic fungi is preceded by attachment of

the fungi onto the surface of plant material by rhizoids [84].

Breakdown of dietary nitrogenous material in the rumen occurs by the following steps:

Proteins–Proteases–>Peptides–Peptidases–>Amino Acids–Deamination–>Volatile fatty acids $+CO_2 + NH_3$

The subsequent end products (volatile fatty acids, carbon dioxide, methane, ammonia, heat and ATP) are absorbed and utilized by the host animal, some are lost as fermentation gases, urinary urea and heat. There are two types of anaerobic mechanisms for degradation of amino acids [62], viz.

a) Decarboxylation: amino acid to amine $+CO_2$. This pathway is a minor contributor in the rumen and it is associated with low pH, acidosis and putrefaction.

b) Non-oxidative deamination of which a number of degradation pathways for several amino acids are described below. Deamination of the amino-acids formed (in the rumen) is considerable, as for example, the formation of isobutyrate and ammonia from valine. Other branched- chain fatty acids, formed in the rumen by enzymatic breakdown of appropriate amino acids, are iso-valeric acids from leucine and 2-methyl-butyric acid from isoleucine [60]. The branched chain fatty acids in the rumen content are important as growth factors [26] for micro-organisms and as starting materials for the formation of long chain fatty acids [58]. A small amount of volatile fatty acids, such as acetic, propionic and butyric acid, present in the rumen content are formed by breakdown of amino acids, but the main quantity originates from carbohydrates [62]. Microbial breakdown of amino acids such as cysteine, glutamic acid and serine leads to pyruvic acid later transformed to VFAs.

About 90% of the total N content of the rumen exists in an insoluble form [78]. 60-90% of this insoluble N is microbial in origin; the remainder is undegraded dietary protein. N in the dissolved pool (about 10% of the total rumen N) consists mainly

of ammonia-N (NH_3N average 79%); the rest is a mixture of free amino acids and peptides [62].

Results obtained from *in vivo* and *in vitro* studies confirm that degradability of feed protein in the rumen varies largely, being 60-80% for most feeds [91], [66], even though feed protein can be manipulated (physically or chemically) to reduce microbial degradation in the rumen [103], [119] and [63]. Therefore, in the view of Storm and Orskov [65], which states that the amino acid composition of microbial protein is independent of diet fed, the quality and/or quantity of feed protein escaping rumen degradation is probably the main factor that causes variation in the amino acid composition of intestinal digesta.

A rapidly degraded protein in a low carbohydrate ration (such as silage diets) will result in high ammonia levels in rumen liquor due to uncoupled fermentation following a deficiency in energy (ATP). The speed and extent to which protein is broken down in the diet depends upon the surface area for microbial attack, the physical consistency, chemical nature and the solubility of the protein. In general, the degree of solubility of plant or animal protein is directly correlated with the rate at which ammonia is released into the rumen [100]. Tamminga [63], reported that the extent of degradation declines as total feed intake increases. This is contrary to Rohr [140], who found no such correlation.

2.2.3 FACTORS AFFECTING DEGRADATION OF PROTEIN IN THE RUMEN:

The degradation of dietary protein in the rumen varies considerably [90], [141], [20] and is largely dependent on the solubility of protein in rumen fluid [64], [28], [91], [101], [100], [103], on the protein intake [99], and on the rate of passage of digesta through the rumen [123].

Solubility of proteins in non-treated feedstuff is largely determined by the composition of protein classes present in it [61]. Albumins and globulins are the most soluble protein fraction; prolamins and glutelins constitute the more insoluble fraction of feedstuffs [100]. Therefore, feedstuffs composed largely of globulins and albumins are normally more soluble and of higher quality than those composed primarily of prolamins and glutelins [91]. Furthermore, the maturity of plants at harvest affects the type of protein present in the total plant and, thus, influences the solubility of the plant protein [102]. According to Clark [61], the soluble nitrogen content of forage is greatest at maturity since, as forage ages, the protein fractions such as albumin, globulin, prolamins and glutelin decrease and the non-protein nitrogen increases. Variations in protein solubility can range from 2.8 to 93.2 % because of the ratio of protein fractions composing the total protein in the feedstuff.

Harvesting procedures and storage conditions can also greatly affect the solubility of protein in silage. Wilting the crop before ensiling, for example, reduces the protein solubility of the forage. Also, forage overheated in storage often results in poorer animal performance, due to the Maillard reaction between aldehyde groups of sugars and the free amino groups of proteins. Through this reaction, the protein may be bound to the indigestible fraction of the plant (lignin) rendering it extremely insoluble and non-digestible. Overheating of protein is often related to temperature, duration of heat treatment and moisture content of the forage [145]. However, the protein is seldom damaged if moisture is evaporating when heat is applied.

Next, the degradation rate of protein as well as the cellulolytic activity is influenced by rumen pH [63]. Proteins are least soluble at their isoelectric pH. At this pH, the protein molecule has zero net charge and, thus, no electrostatic repulsions exist between neighboring protein molecules. Rumen pH range lies between 6.0-7.0 [92], which means that proteins with isoelectric pH within this range may be very insoluble

in rumen fluid. But, because feeds contain different mixtures of protein, it is difficult to determine the isoelectric pH of most feed proteins [61]. It is known that the pH optimum for cellulolysis is lower than 6.7-7.0, and that the lower limit of optimum proteolysis and deamination is 6 [82]. However, it has been demonstrated that pH alters the solubility of various proteins *in vitro* rumen fluid incubations [72], [91] , which suggests that pH of the rumen may affect the quantity of dietary protein that escapes rumen fermentation.

A shorter retention time in the rumen allows less time for microbial fermentation. Thus, factors that regulate the rate of flow of feed ingredients through the rumen directly determine the quantity of protein that bypasses fermentation. Frequent feeding [131], reduced particle size or increased dietary feed intake, increased protein intake [99], feeding diets with large quantities of salt and the rate of ruminal degradation will all increase rate of passage of digesta through the rumen [53]. Numerous processing procedures which alter physical forms have also been reported to affect nitrogen utilization in ruminants [102].

2.2.4 RUMEN DEGRADABILITY OF SILAGE NITROGEN:

The conversion of soluble forage components to fermentation acids may provide an energy substrate problem for rumen organisms forced to subsist on a prefermented diet [92].

The rate of degradation of silage NPN and soluble protein nitrogen in the rumen is characteristically high and the ingestion of silage is followed by a pronounced peak in rumen ammonia concentration [47]. Soluble protein here refers to that fraction which will dissolve in rumen fluid [100]. Generally, degradation values from silage N obtained using the nylon bag technique lie in the range 75-85% [52]. These figures are consistent with the high rumen ammonia concentrations observed with silage

diets. A comparison between the nylon bag incubations and various laboratory tests (based on solubility in buffers , *in vitro* incubation with rumen fluid, or incubation with acids or neutral proteases) revealed that the laboratory methods gave lower degradability values for silage N than those measured *in sacco* [143]. A literature survey by Thomas [59] estimated that the rumen degradability of total silage N *in vivo* was 78-86% for three silages made without additives;54-81% for seven formic acid silages; and 31-51% for four silages made with the addition of formaldehyde (35-60g formaldehyde/kg protein). In an experiment with four silages made from the same grass with formic acid applied at rates of 0, 2.3, 4.6 and 5.9 litres/tonne, Chamberlain [150] found that soluble proteins saved from hydrolysis in the silo were susceptible to attack in the rumen and protein degradability values were in the range 74-83%. Similarly, Rooke [75] working with formaldehyde-treated silages measured a protein degradability value of 80%. Beever [131] confirmed that many *in vivo* estimates for ruminal degradability of fresh forages similarly lie in the range 50-75%, though the herbages contain a high proportion of soluble protein which could be expected to be highly degradable.

2.2.5 THE NYLON BAG TECHNIQUE IN PROTEIN DEGRADATION STUDIES:

Cost and labor involved in conducting *in vivo* experiments stimulated search for alternative methods of determining degradability of feed protein. The nylon bag technique(*in situ or in sacco)* is one of these procedures that has the potential to aid in the assignment of protein degradability in the rumen.

Since the initial utilization of the *in situ* technique by Quin [144] the method has been widely used in studies of forage dry matter digestibility [151] and in studies of the release or disappearance of a range of plant constituents during fermentation in the rumen [9]. More recently, the technique has been promoted as a simple means of

estimating the rate and extent of food protein degradability in the rumen [99], [98]. Lately, efforts have been made to quantitate such results for estimating *in vivo* ruminal protein degradability [99], [27].

Estimation of rumen degradation of feeds, using the *in sacco* nylon bags measures disappearance of feed constituents. It is assumed that disappearance is synonymous with degradation [99]. This assumption holds in some cases, but is invalid in those cases where the substrates disappear rapidly. For example, water soluble materials and very fine particles which disappear are assumed to be degraded. According to Orskov [99], it is possible to obtain information on the extent to which fine particles are involved. But no simple laboratory method can be envisaged for the preparation of feed samples before incubation which takes into account processing of the feed by the animal which occurs before the feed is exposed to the rumen (such as mastication and mixing with saliva) [79]. Estimates of the extent of degradation or the degradation rate of various feedstuffs measured *in sacco* are, therefore, not necessarily directly comparable with *in vivo* estimates [79].

The simplest form of degradation will be that presented by Broderick [78] and described by this formula:

$$p = 100(1 - e^{-ct})$$

where p is the amount degraded at time(t) and c is the degradation rate for N disappearance. This assumes that the substrate starts to degrade as soon as it is incubated in the rumen, contains no water soluble fraction, and will in time degrade completely. However, few if any feeds degrade according to this very simple description, as most protein supplements have a fraction or several fractions which disappear very rapidly, and also a fraction or fractions where degradation, even after a long period, never reaches

100% [99]. Orskov [99] modified and described this by the following expression:

$$p = a + b(1 - e^{-ct}) \tag{2.1}$$

where p again is the amount degraded at time (t) but a, b, and c are constants in the exponential equation. Constant a is the rapidly soluble fraction ; b, the amount which in time will degrade ; and c, the fractional rate constant at which the fraction described by b will be degraded per hour.

Therefore, $100 - (a + b)$ gives that part which is totally undegradable in the rumen. Normally, however, in fitting the equation, the estimates of a and b are constrained so that their total does not exceed 100% [99]. There are also substrates where a will be negative. This occurs where there is little or no soluble material (such as straw) and where there is a lag phase before degradation proceeds [89]. There are other models in special cases. For instance, there could be a very soluble fraction and still a lag phase for the degradable part. All these combinations have been reviewed by Orskov [99].

On many occasions the nylon bag has been criticized for giving only relative, but not actual degradabilities for crude protein in feed. Attempts to avoid this claim were made by calculating the so-called effective protein degradability.

The effective percentage degradability (p) of protein supplements in the rumen is dependent not only on the course of degradation of the protein particles in the rumen, and will decrease if there is an increase in the rate of passage of particles [99]. Orskov [99] showed that if the percentage protein disappearance (p) from samples incubated for time t is described by equation (2.1), and if k is the fractional rate of passage from the rumen, then the effective degradability can be calculated as :

$$p = a + \frac{bc}{c + k}$$

The calculation depends on the equation for p remaining valid from $t = 0$ (time of ingestion) until a time when all the particles have passed beyond the rumen [99].

Where a is negative the final formula modifies to:

$$p = a\frac{b'c}{c+k}e^{-(c+k)t_0}$$

where $t_0 = \frac{1}{c}ln(\frac{b'}{a'+b'-a})$ and represents the intercept.

The modification makes little difference to the value of p, and in almost all instances, sufficient accuracy can be obtained using the simple expression above [118].

2.2.6 FACTORS AFFECTING ESTIMATES OF RUMEN DEGRADABILITY:

Factors affecting estimates of rumen degradability using the *in sacco* technique have been extensively reviewed [66], [67], [70], [79]. In brief, these factors are, bag pore size and sample weight; ratio between bag size and sample size [77], [71] , [76], [70], substrate particle size [68]; animal basal diet and species [69]; positioning of bags in the rumen; washing of bags; microbial colonization of the bag residue; variance in estimated rumen disappearance data; and sample size [67], [66], [94].

2.2.7 ERROR DUE TO MICROBIAL CONTAMINATION OF BAG RESIDUE:

There is no doubt that some of the microbes attached to feed particles cannot be washed away from the sample residue after incubation. Protein in microbial cells can affect both the degradability values and the quality (amino acids, digestibility) of the feed protein evaluated after incubation [94]. At present, there are no valid systems available to correct for this error, which obviously is relatively greater for poor quality roughage than for protein concentrates due to microbial attachment to fibrous constituents [73], [67] , [66].

2.3 MATERIALS AND METHODS:

The procedure used in this experiment was that described by Mehrez and Orskov [70]. One rumen-cannulated ayrshire steer was used. The steer was offered grass silage four times daily (0800hrs, 1400, 2000, 0200). This was done to reduce fluctuations in rumen environment, thus minimizing effects of time after feeding on in *situ* degradation.

The silage samples which had been stored at $-20°C$ were thawed and chopped with a pair of scissors [152]. The chopped silage (approximately 5gDM) was accurately weighed into nylon bags (size 5x11 cm with mean pore size of 40μm). Fourteen bags were prepared for each silo per period. The bags were secured at approximately 10cm intervals (with bags for each silo tied to same knot) on a weighted 50cm dacron string. The string was tied to the rumen cannula and all bags were placed into the ventral sac of the rumen for periods of 1, 2, 4, 6, 12, 18,and 24h. Samples to be exposed for 24h were placed (in duplicate) into the rumen first, 2h post prandial, followed by the introduction of the remaining samples in reverse order over time, such that, the last samples(1h incubation) were introduced 23h after introduction of the 24h samples. Thus, all bags were removed at the same time, 24h after entry of first set, with the hope of minimizing variation in washing. The bags were washed under gently falling tap water until rinsings were clear. The washing time averaged 2 minutes/bag. After gentle sqeezing to remove excess water, the bags were dried in a forced draught oven at 100°C for 24h. The bags were then weighed to determine DM disappearance followed by compositing of duplicate samples and analysis for CP [22].

The results were fitted to the following equation using a Eureka program which performs a Least Squares Fit to find the function (of the required form) that best matches the points x, f(x) where x is the %degradation values and f(x) is the incubation

time intervals:

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

where p is percentage disappearance at time t, a the soluble degradable fraction, b the insoluble degradable fraction, and c the degradation rate constant. When $t = 0$, p was fixed as the disappearance due to washing. All these constants were later used to calculate effective protein degradation using the formula:

$$p = a + \frac{bc}{c + k}$$

where k is the assumed rumen outflow rates (0.02, 0.04, 0.06, 0.08).

The statistical comparisons on all degradation variables were compared using the general linear model (least square means) of the SAS package [153]. The experimental model used was 4 x 4 Latin Square with 4 silage treatments replicated 4 times.

2.4 RESULTS AND DISCUSSION:

It was hypothesized that both protein and carbohydrate would be needed most when immature and unwilted silages are fed to dairy cattle, and that more degradation of N would occur in the immature and unwilted silages. Estimates of silage protein (N) degradability based on the nylon bag *(in situ, in sacco)* procedure are presented in table 2.3. It can be noted that N disappearance pattern indicates that a large proportion of silage N was susceptible to rumen microbial degradation. Fraction a is that part of silage protein going into solution in rumen fluid. The rate of degradation of this soluble fraction has been assumed to be so rapid that virtually none can escape degradation with normal rumen liquid turnover rate. This fraction is assigned degradability of 1.0 [52]. According to Orskov [99], the solubility of protein is related to rumen degradation and must be considered as a basis for recommendation of nitrogen supplementation. From the results obtained, silage protein solubility as denoted by the a fraction, increased from mature wilted silage (24.44% in period I) to immature unwilted silage (75.84% also in period I) (see table 2.3). In this case, both wilting and maturity had a significant effect ($p<0.05$) in reducing the soluble fraction a. With the exception of period I, these values fall in the range given by Waldo [102] of 35-75% soluble nitrogen for wilted silages, a percentage similar to that normally found for hay [102]. That the storing of unwilted silage increases solubility of the protein [101] is similar to our findings as illustrated in table 2.3. These authors showed the relationship between N of fresh forage and that of direct ensiled grass legume forage, the soluble N concentration doubled during ensiling. Soluble N in direct ensiled orchard grass increased 36% during ensiling [102]. In 1968, Waldo reported that this soluble N and volatile N can be 15 to 30% of the total N. Waldo [102] proposed that a ration should not contain more than one third of the total crude protein as soluble N. In this study, nearly all

silages had a soluble N fraction greater than one third. These high values probably indicate a low proportion of protein nitrogen in silage total N [67] , [139], and should probably be supplemented with energy and protein. Sniffen [100] reported that the degree of solubility of natural protein is directly correlated to the rate at which NH_3 is released in the rumen. Thus, a highly soluble protein can contribute greatly to the NH_3 concentration within minutes after feeding [100]. It becomes a question then how to best trap this ammonia for increased rumen microbial protein synthesis. From the above findings, it appears that supplementation is needed most for the unwilted silages. Similar trends for the *a* fraction were observed for DM disappearance (table 2.4).

The effect of wilting on reducing DM disappearance was significant ($p<0.05$). In 1986, Setala reported that protein degradation is dependent on the digestibility of DM, thus when digestibility of DM is correctly analysed, the degradability of CP can also be accurately determined. The effect of maturity on DM disappearance (degradation) was not significant. This was not expected since ADF increases with maturity thereby reducing digestibility. However, in this case this can be explained by the fact that the parent material harvested was not sufficiently mature to exhibit such effects. On the other hand, maturity of silage significantly ($p<0.05$) reduced fraction *a* for CP, 63.44% ($\pm$ 3.73) for immature forage and 49.52% ($\pm$ 3.48)for mature silage. This decline in solubility increasing with maturity is consonant to observations by Clark [21] and could be attributed to gradual dominance of stem tissue over leaf tissue.

The second fraction denoted by constant *b* is insoluble but potentially degradable by the rumen microbes according to first order kinetics [99]. Regarding the *b* fraction, CP values ranged from 8.79% in immature unwilted to 51.49% in mature wilted silage (see table 2.3). For CP, maturity significant increased the insoluble fraction *b* ($p<0.05$). The increased insoluble *b* fraction can be partially explained by increased stem:leaf ratio in wilted vs unwilted silages and also the unwilted silages might lose much of the

soluble fraction in effluent. Letter *c* denotes the rate at which the *b* fraction is degraded. It is therefore, logical that a low degradation rate for the *b* fraction, coupled to a high rumen outflow rate, will result in more of the true protein reaching the duodenum. For both CP and DM, maturity and wilting treatments did not have an effect on *c*, the rate of degradation of the *b* fraction. This could be attributed to the overall effect of ensiling on the resultant nature of the nitrogen fractions. However, if the more soluble and easily degraded material are broken down in the unwilted silage due to greater fermentation, one might expect the remaining material to be more resistant to attack and thus a slower rate of degradation. Theoretically, a higher rate of degradation of the *b* fraction was expected for unwilted and immature silages.

Table 2.3: Degradation of Crude Protein.

PERIOD	TREATMENT	CP DEGRADATION CONSTANTS		
		a(%)	*b*(%)	*c*(%)
I	Immature non-wilted	75.84	8.79	21.41
	Immature wilted	41.85	36.76	3.78
	Mature non-wilted	49.70	40.94	3.91
	Mature wilted	24.44	51.49	10.07
II	Immature non-wilted	62.98	27.33	14.42
	Immature wilted	58.24	28.25	3.38
	Mature non-wilted	64.13	22.29	3.04
	Mature wilted	50.32	33.01	6.06
III	Immature non-wilted	66.57	20.95	8.91
	Immature wilted	59.04	24.29	4.40
	Mature non-wilted	56.98	30.57	6.79
	Mature wilted	51.92	33.07	7.64
IV	Immature non-wilted	66.35	22.13	8.24
	Immature wilted	52.29	36.34	5.73
	Mature non-wilted	57.77	27.15	5.52
	Mature wilted	40.87	41.46	5.99

a= % solubility, b= potentially degradable fraction [%], c= rate of degradation of the 'b' fraction [%/hr].

Table 2.4: Degradation of silage Dry Matter

PERIOD	TREATMENT	DM DEGRADATION CONSTANTS		
		$a(\%)$	$b(\%)$	$c(\%)$
I	Immature non-wilted	69.44	5.76	19.02
	Immature wilted	30.26	35.54	4.42
	Mature non-wilted	37.30	27.63	3.71
	Mature wilted	19.00	48.16	4.76
II	Immature non-wilted	45.53	26.48	6.94
	Immature wilted	34.21	36.28	3.57
	Mature non-wilted	48.59	4.47	2.67
	Mature wilted	36.55	2.40	4.65
III	Immature non-wilted	49.75	3.56	4.25
	Immature wilted	33.97	0.87	3.47
	Mature non-wilted	38.19	6.58	6.04
	Mature wilted	33.44	35.34	2.18
IV	Immature non-wilted	48.63	23.42	4.79
	Immature wilted	34.49	28.10	3.68
	Mature non-wilted	50.12	23.95	2.21
	Mature wilted	36.29	29.88	3.08

a= % solubility, b= potentially degradable fraction [%], c= rate of degradation of the 'b' fraction [%/hr].

Table 2.5: Mean CP degradation values.

TREATMENT	MEAN CP DEGRADATION CONSTANTS		
	a (±s.e.m.)	b (±s.e.m.)	c (±s.e.m.)
Unwilted	62.57 ±4.11	25.45 ±3.58	6.44 ±2.00
Wilted	50.39*±3.15	33.21 ±2.75	8.06 ±1.53
Immature	63.44 ±3.73	23.66 ±3.24	8.38 ±1.81
Mature	49.52*±3.48	35.00* ±3.03	6.13 ±1.69

a= % solubility, b= potentially degradable fraction [%], c= rate of degradation of the 'b' fraction [%/hr].

* indicate values significantly different at ($p< 0.05$)

Table 2.6: % Effective CP degradation values.

TREATMENT	MEAN OUTFLOW RATE VARIABLES			
	K = 0.02±s.e.m.	0.04±s.e.m.	0.06±s.e.m.	0.08±s.e.m.
Unwilted	87.92±1.47	87.82±1.47	87.72±1.47	87.62±1.47
Wilted	83.49*±1.12	83.38±1.13	83.27±1.13	83.16±1.13
Immature	87.02±1.33	86.93±1.33	86.83±1.33	86.75±1.33
Mature	84.39±1.24	84.27±1.24	84.15±1.25	84.03±1.25

k= rumen digesta flow rate [%/hr].

Table 2.7: Mean DM degradation values.

TREATMENT	MEAN DM DEGRADATION CONSTANTS		
	a ±2.73	b ±2.24	c ±1.33
Unwilted	48.44	22.73	6.20
Wilted	34.27*	34.57	3.73
Immature	43.29	26.25	6.27
Mature	37.44*	31.05	3.66

* indicate values significantly different at ($p < 0.05$)

Table 2.8: % Effective DM degradation values.

TREATMENT	MEAN OUTFLOW RATE VARIABLES			
	k=0.02±s.e.m.	0.04±s.e.m.	0.06±s.e.m.	0.08±s.e.m.
Unwilted	71.06*±1.22	70.95±1.21	70.83±1.21	70.72±1.21
Wilted	66.65±1.22	66.46±1.21	66.27±1.21	66.09±1.21
Immature	69.41±1.21	69.29±1.21	69.17±1.21	69.05±1.21
Mature	68.30±1.21	68.11±1.21	67.94±1.21	67.75±1.21

k= rumen digesta flow rate [%/hr].

The sum of the two fractions $a + b$ will give the extent of potential degradation in the rumen, that is , total rumen degradability of any feed. Thus, $a + b$ cannot exceed 100% and 100 - $(a + b)$ will then account for, or represent the third fraction that is insoluble and undegradable. It is noted that this third fraction was very small in all silages. In forages, this third fraction is usually attached to the fiber fraction and part of it can be determined as ADIN [92]. According to Webster [135], this complete undegradable fraction tends to form a constant proportion of dry matter whilst degradable N varied with time (t), but in case of overheating in silos (as for wilted silages), ADIN concentration could be greatly increased [102].

The effective feed protein degradation 'P' in the rumen is determined not only by the rate of microbial degradation, but also by the residence time of feed in the rumen. Thus, it represents the amount of protein which is actually degraded in the rumen at a specific passage rate, and can be calculated from the constant rumen outflow rate. (k,(% /100)/h) and *in sacco* degradation values [99]. In evaluation of protein sources, it would be desirable to use one value for the outflow rate reflecting the most common feeding systems [136]. In this study, effective degradation was calculated for four outflow rates (viz. 0.02; 0.04; 0.06; 0.08), but for comparison purposes only the 0.02 value was adopted because animals used in the *in vivo* study were fed at maintainance level. The mean effective protein degradation value for all silages was 85%. Wilting significantly ($p< 0.05$) reduced silage effective protein degradation with, a value of 88% for the unwilted and 83% for the wilted silage. This finding suggests that when silages are wilted, more undegraded protein leaves the rumen than with unwilted silage, assuming an outflow rate of 0.02%. Although this does not explain much about the utilization of the undegraded protein in the lower gut, the assumption that protein not degraded within the rumen will have the same biological value as that of the original foodstuff [52] may not be valid at least for silages [75]. However, both

values obtained for effective degradation of protein in the rumen (88 and 83%) are close to those reported by Agricultural Research Council(ARC) [52], that is, 75 -85%, for silage degradability values obtained *in sacco*. That rumen degradability of silage protein is very high is confirmed by these findings. Effective DM degradability was significantly ($p<0.05$) reduced by wilting. This points out to the fact that when silages are wilted, there can be a reduction in digestibility or that wilted silages probably have less fermentable energy compared to the unwilted silages. This observation is similar to that of Donaldson [124].

The nylon bag technique provides a simple tool not only in determining the rumen degradability of feeds but also in evaluating the quality of rumen undegraded feed protein. This is supported by the agreement of these findings with those reported in literature. Variations in degradabilities of DM and CP occurs both between animals and incubation days and the effect of animals is greater even under standardized conditions [67]. It is recommended that only one animal be used [66].

The results and conclusions of this study are based on the means of residues of bags collected over four periods and analysis of residues from pooled duplicate bags, always using one animal. It thus seems likely that the variation between replications obtained from a single animal is low since in this study period effects were not significant. This is similar to the observations made by Rooke [75]. Theoretically, the length of incubation has to be increased until the contribution of the last interval is approaching zero [136]. It was decided to use incubations not longer than 24 hours. For protein sources with a rapid degradation rate such as silages, the contribution is approaching zero even well before 24 hours (1 - 6 hours). It is also important to have an even distribution of incubation times over the incubation period and these should be more closely represented where the degradation rate is fastest [79]. In this study, more measurements were taken between 1 and 6 hours as shown by the incubation times

1,2,4,6,12,18,24 hours.

That all forage sample would be contaminated by micro-organisms in the rumen leading to an underestimate of degradability Mathers [98] has been confirmed at least with silages [75]. In their experiment, degradability of silage N calculated from *in sacco* data when not corrected for microbial contamination was 87%; this value increased to 90% after correction. In this experiment, the data was not corrected for microbial contamination. Thus the results presented could be an underestimate of the real degradabilities. Finally, it is questionable whether rate of disappearance of proteins *in sacco* represents rate of degradation, because soluble protein may be washed out without actually being degraded [51].

2.5 SUMMARY AND CONCLUSIONS:

Wilting and maturity as silage treatments significantly reduced the N soluble fraction of grass-legume silage and the magnitude was within the range reported in the literature. The insoluble protein fraction was significantly increased by maturity and the DM insoluble fraction was significantly increased by wilting. Overall, there was no effect of both wilting and maturity on the rate of degradation of CP and DM. At an estimated rumen outflow rate of 0.02%, wilting significantly reduced the effective protein degradation. There were no significant differences among the four incubation periods.

There are pros and cons of the nylon bag technique: the technique offers a simple tool in determining the rate and extent of rumen degradability. However, the values obtained by this method are suspect because, microbial uncorrected effective protein degradation is seriously underestimated for slowly degrading fibrous feeds with a low content of insoluble N, and feeds rich in fast degrading soluble protein. Proper wilting before ensiling tends to reduce the solubilization of the protein.

Future research could be centered on identifying, and determining qualities of the discussed N fractions and identifying appropriate feeding systems for best utilization of silages differing in moisture content and maturity.

Chapter 3

DETERMINATION OF THE EFFECT OF WILTING AND MATURITY ON RUMEN DEGRADATION OF SILAGE NITROGEN FRACTIONS. *IN VIVO STUDIES*

3.1 INTRODUCTION:

When silage is eaten by ruminants there are the following processes involving nitrogen: a rapid release of N into the rumen, a breakdown of silage protein and NPN compounds by the rumen microbes, and an associated ruminal resynthesis of microbial protein. Consequently, the amount and composition of the mixture of proteins which passes from the rumen to furnish the animal's supply of amino acids in the small intestine depends on the amount of silage protein escaping degradation in the rumen and on the amount of microbial protein synthesized there.

From the previous chapters, it is evident that there are several factors influencing the composition of nitrogen fractions in grass/legume silages. Knowledge of the different effects of silage treatment on the degradability of nitrogen fractions is very important, in-order to recommend best ways of conservation, so as to achieve maximum production from such diets. One of the ways of determining this is through *in-vivo* studies which take into account most of the dynamic processes of digestion within the intact animal. These studies involve measurement of the protein in microbial (bacteria plus protozoa), endogenous and dietary components.

Measurements of the total protein flow through the digestive tract can be made with reasonable accuracy [48]. Information on rates of passage of digesta, and an accurate estimation of microbial N in duodenal digesta are, therefore, necessary prerequisites in determining a meaningful value. The former is achieved by use of dual phase markers [148], and the latter by use of microbial markers [28].

Much of the dynamics of degradation of feed protein (in particular, silage proteins) in the rumen has been reviewed in the foregoing chapters. For details regarding resynthesis of protein in the rumen, reference is given to excellent reviews [25], [26], [27], [63] and [99]. While the previous studies examined the effect of maturity and wilting on the *in situ* rate and extent of degradation, silage solubility, potentially degradable fractions and effective degradation of grass/legume silage in the rumen, this study will investigate effects of maturity and wilting on silage protein degradation *in-vivo*, availability of undegraded silage protein and microbial protein to the animal.

3.1.1 *IN-VIVO* ESTIMATION OF RUMEN DEGRADATION OF PROTEIN:

The extent of protein degradation in the rumen is a function not only of degradability by rumen micro-organisms, but also of the specific rumen retention time encountered by the protein [49]. These two factors are influenced by chemical and physical properties of feeds [103], fermentation of different feed components [92], microbial growth rates and yields [27], level of feed intake [40] and passage rates [30].

The amount of protein that escapes degradation is very variable [52], but an escape rate of around 40% can be found in most feeding conditions. The remaining 60% of dietary protein is almost entirely degraded [146]. It has also been estimated that of all protein reaching the duodenum, 40-80 % is of microbial origin, depending on several dietary factors [137]. Estimates of protein degradation may be made in a variety of ways [30]. One of these, the *in-vivo* method, is based on the fact that protein reaching

the duodenum can be either of dietary or rumen microbial origin. The ARC [52] report states that degradability of dietary protein is ideally defined as:

$$1 - \frac{\textit{dietary protein entering the duodenum}}{\textit{dietary protein consumed}}$$

However, in reality, estimation of dietary protein entering the duodenum *in vivo* must be made by a difference in calculation as:

Duodenal total protein flow - duodenal microbial protein flow - duodenal endogenous protein flow [135].

Faced with the limited amount of information available on endogenous protein secretions, the Agricultural Research Council group decided that, for their purposes, the proportion of dietary protein surviving rumen fermentation and thus 'undegraded' would be defined as:

$$1 - \frac{\textit{Duodenal N} - \textit{Microbial N}}{\textit{N intake}}$$

By the *in vivo* method, estimation of the duodenal microbial protein contribution is determined by the measurement of a microbial marker which can be related to microbial protein present. Microbial markers which have been employed include 2,6-diaminopimelic acid or total nucleic acids (DNA, RNA); other techniques use ^{15}N or ^{32}P labelling, or the amino acid profile of duodenal digesta [28]. Nucleic acid, and more particularly RNA, concentrations are suitable for estimating the contribution of total microbial N to rumen or duodenal digesta samples [34]. Error can be introduced by the presence of protozoa but this should be normally small [37], [57] and the existence of RNA from feed origin can overestimate the concentration measured.

3.1.2 MEASUREMENT OF DIGESTA FLOW BY REFERENCE TO MARKER SUBSTANCES:

The methodology of this technique was extensively reviewed by Faichney [148]. The theory underlying the technique is that if a marker, an inert unabsorbed substance with

properties similar to that of a component of digesta, is pumped (or fed) into one section of the digestive tract continuously for a sufficient time to reach equilibrium, then, on average, 100 % of the daily dose of the marker will pass each subsequent section of the tract each day. Hence, if samples of digesta are collected from a point distal to the infusion point, the flow of digesta may be estimated mathematically.

In practice, in many sections of the tract, particles are retained longer than solutes; in addition, separation between these phases of digesta may occur during sampling. To overcome these difficulties, dual phase markers are used, one of which travels with the liquid phase, the other with the solid phase [31], [30]. Given that equilibrium has been achieved and is maintained by continuous infusion of the two markers, the concentration of those markers (expressed as a fraction of the daily dose per unit of digesta) in digesta flowing past any point must be equal [148]. If a subsample of fluid is prepared by straining or centrifugation of a sample digesta so that fluid and digesta contain different proportions of liquid and particulate matter, the true digesta can be reconstituted by combining fluid and digesta either mathematically or physically, so that the concentrations of the two markers are the same [148]. For example:

If x = a quantity of digesta (D)

y = a quantity of fluid (F) which, when added to or removed from x, reconstitutes true digesta (TD)

S_D, S_F, S_{TD} , = concentrations of the solute marker

P_D, P_F, P_{TD} , = concentrations of the particle marker

then $x.S_D + y.S_F = xP_D + yP_F$

so that

$$\frac{y}{x} = \frac{P_D - S_D}{S_F - P_F} = R$$

where R is the reconstitution factor that is, the number of units fluid that must be added to (or removed from) one unit of digesta to obtain true digesta. Then

$$\frac{S_D + R.S_F}{1+R} = S_{TD} = \frac{P_D + R.S_F}{1+R} = P_{TD}$$

and flow of

$$TD = \frac{1}{S_{TD}} = \frac{1}{P_{TD}}$$

Liquid markers used to determine rates of passage include Chromium ethylene diaminetetra-acetic acid (Cr EDTA) [29] and EDTA complexes of Co, Fe and Yb [33]. Markers for the solid phase include the forage material lignin, and substances which are adsorbed by particulate material, including various rare earths such as cerium [43] samarium and lanthanum [24], and the phenanthroline complex of ruthenium [42]. Chromium sesquioxide has frequently been used but its specific gravity is very much greater than that of the digesta thus it cannot be used to give reliable estimates of digesta flows [45]. For properties of these markers, extensive reports have been presented by Faichney [148], Hogan and Weston [30], Faichney and Black [39]. Recent reports, Teeter and Owens [33] and Hartnell and Satter [24] confirmed the suitability of Co and samarium as digesta flow rate markers. The markers selected for this study were Co EDTA for liquid phase and samarium chloride ($SmCl_3$) for the solid phase.

3.2 NITROGEN METABOLISM IN ANIMALS GIVEN SILAGE DIETS:

The degree of protein hydrolysis and amino acid breakdown varies with the conditions and type of fermentation in the silo, and is restricted by rapid attainment of low pH [35]. Depending on the method of silage making, therefore, a variable but inevitable large proportion of silage N is present in non-protein form (see previous chapters).

When silage is eaten by ruminants, there is a rapid release of N into the rumen. The average NH_3 values during the day are around 200 mg NH_3/l [47], a breakdown of silage protein and NPN compounds by the rumen microbes, and an associated ruminal resynthesis of microbial protein occurs [147]. Consequently, the amount and composition of the mixture of proteins, which passes from the rumen to furnish the animal's supply of amino acids in the lower gut, depends on the amount of silage protein escaping rumen degradation and the amounts of microbial proteins synthesized in the rumen.

An extensive examination of quantitative aspects of silage N used in the rumen has been undertaken over the past years [30], [54], [52], [58], [63], [147], [79] and [47]. For formic acid silages degradability of silage N varies between 50% and 80%. Experiments in which the proportion of NPN in silage has been reduced, through additions of formic acid additive at ensilage, have shown that soluble protein which is spared from breakdown in the silo is readily degraded in the rumen [47]. For other silages, the amount of undegraded protein passing to the duodenum corresponds approximately to the intake of true protein fraction in the silage, although this appears to reflect principally the fact that, with these forages, the true protein represents mainly enzyme resistant plant fractions [118].

The rate of microbial protein synthesis in the rumen with silage diets is characteristically low [36]. For example rates of synthesis on hay diets are usually 32g microbial N per kg organic matter apparently digested in the rumen [135], whilst rates for silages are usually 65% to 75% of that figure [26]. The reason for this is partly attributable to the fact that although silage organic matter contains a substantial component of silage fermentation products that are later fermented by rumen microbes, these products have a relatively low yield of energy ATP to support microbial growth [39]. Also, examination of the rumen contents of sheep given silage diets have shown substantial

ruminal populations of protozoa; the activity of these organisms will also reduce the efficiency of total microbial protein synthesis because of predation [37].

3.3 MATERIALS AND METHODS:

A total of 4 animals (two Ayrshire steers and two Friesian heifers) weighing between 465 and 486 kg were used in this experiment. The animals were each prepared with permanent cannulae in the rumen and in the duodenum (about 12 cm from the pylorus) and were maintained in a tie-stall experimental area. Preparation of the silages fed in this experiment was as outlined in the materials and methods of the first part of this study. The animals were fed the silages for a 10 day adaptation period four times a day (0800,1400,2000 and 0200 h). The silages were then fed for a further 8 day period after having samarium chloride and CoEDTA mixed with the silage.

3.3.1 Application of markers:

The Co complex of EDTA [33] and samarium chloride [24] are used routinely in this laboratory as, solute and particulate markers, respectively. Silage was removed from the silos prior to the start of each week of each period and the required amount for an 8 day feeding period was marked with samarium and cobalt. The marking procedure was as follows: The silage to be marked was weighed in a Calan data ranger (about 100 kgs on wet basis) and required amounts of markers were sprayed using an atomiser hand sprayer. Samarium at 15,000 mg(Sm)/kg was sprayed on at a rate of 0.5 ml per/kgDM fresh silage. CoEDTA at 15,000 mg(Co)/kg was sprayed at a rate of 5 ml/kgDM fresh silage. Silage DM for this purpose was determined by oven drying. The data ranger was run for 10 minutes after spraying to ensure uniform mix. The mixed silage was then off-loaded into air tight plastic bags, compacted, tightly sealed and stored in a

cool place until feeding.

3.3.2 Sample collection:

During the last three days of an eight day period, duodenal and fecal samples were collected every six hours in a staggered fashion such that twelve samples per animal per period were collected every even hour. At the same time, a rumen sample was also obtained by means of a large bore perspex tube [32] for the isolation of microbes for RNA and protein measurement. Feed samples collected each day of the collection trial were eventually composited and subsampled to yield a 1 kg silage sample for each period. Of the 50 ml of rumen sample collected at a time, 30 ml were frozen for VFA analysis. To the remaining 20 ml, 7.5 ml of a 0.9% NaCl in 37% formaldehyde solution was added, and the mix was stored at 5°C until centrifugation (that evening or the following morning) was carried out following compositing.

For the duodenal samples collected and composited, half were freeze-dried for the determination of RNA concentration and the other half was centrifuged to obtain liquid and solid phases for marker analysis. Grab fecal samples collected were dried to a constant weight in a 100°C forced draught oven, ground, composited and stored for analysis.

3.3.3 Blood sampling:

At least four blood samples were collected from each animal, on the third day of sampling of each period. Blood taken from the jugular vein, was collected into heparinized centrifuge tubes, plasma was separated and stored at −20°C for subsequent analysis.

3.3.4 Parameters:

Measures made included; rumen ammonia and plasma urea N [19], protein degradation [52], RNA concentration on rumen microbial pellet for microbial cell production [40], protein and DM digestibility. Analysis for CP, DM [22], ADF, NDF [104], ADIN [44] and total ash [112] were carried out on feed, duodenal and fecal samples. Duodenal DM contents were determined by oven drying. Dried feed, duodenal and fecal samples were accurately weighed into small plastic vials (approximately 0.5g) and sent to TRIUMF research institute for Co and Sm analysis by neutron activation.

3.3.5 Calculations:

Calculations for %DM and %CP digestibilities were from the formulae:

$$\% \text{ DM digestibility} = 100 - \frac{marker\ in\ feed(Sm)}{marker\ in\ faeces(Sm)} \text{x} 100;$$

$$\% \text{ CP digestibility} = 100 - \frac{marker\ in\ feed(Sm)}{marker\ in\ faeces(Sm)} \text{x} \frac{\text{CP in faeces}}{\text{CP in feed}} \text{x} 100.$$

Calculations formulae for rates of passage were as outlined on page 44-45. Animal liveweights were measured at the beginning of the trial and at the end of each period. The experimental design was a 4x4 latin square with 4 animals and 4 silage treatments replicated four times. The silages were randomly allocated to the animals during the experimental periods. Results were analysed by general linear model of the SAS package. The model used was as in the previous section with the addition of animal and interaction effects.

3.4 RESULTS AND DISCUSSION:

Digestibility of silage is largely governed by the digestibility of the parent material which in turn is affected by plant species and maturity at harvest [126]. Ensilage does not directly affect the digestibility of herbage [130]. However other workers have found the effect of ensiling on digestibility to be more variable, with a tendency for digestibility to be reduced by ensiling [105]. In this experiment, the mean dry matter digestibility (DMD) coefficient was 57.59% for all treatments. The values in table 3.9 are much lower than the values of 70 and 69% reported by Marsh [105] for unwilted and wilted silages respectively. No significant differences were observed for the four treatments studied (viz: unwilted, wilted, immature, and mature). There was a large standard error ($\pm$5.36) associated with these values thus, opening to question the accuracy of the presented estimates. Generally, lower DMD values were expected for wilted and mature silages than for the direct cut silages. Forages with low DMD values are usually associated with low voluntary feed intake. If the results presented are true, this would suggest that all the silages had low concentrations of digestible organic matter. Similar results were reported by Castle and Watson [129]. Other factors known to alter digestibility *in vivo,* in the manner above, include cold stress, increased rate of passage, reduced feed particle size, lag time, and reduced rumen pH [123].

CP digestibility was similar to that of DM with a mean digestibility coefficient of 56.53%. Although no significant differences were observed between treatments, immature and wilted silages had lower coefficients (see table 3.9). Marsh [105] reported results from twelve experiments which showed a mean fall of 2.9 %units due to wilting (65.8% *vs* 62.9%). Higher CP digestibility values (compared to those obtained) were also expected with lower values in immature and wilted silages. The reduction in efficiency of CP utilization in all silages may have resulted from a lower level of

Table 3.9: Grass/legume silage digestibility *in vivo*

	TREATMENT					
	mean	unwilted	wilted	immature	mature	±s.e.m.
%DM digestibility	57.59	57.51	57.67	57.88	57.30	5.36
%CP digestibility	56.53	57.16	55.90	54.34	58.72	4.59
%Intestinal CP digestibility	59.75	55.40	64.10	60.11	59.39	3.64
%ADF digestibility	49.67	51.61	47.72	50.92	48.41	6.70
%NDF digestibility	45.84	45.28	46.40	47.57	44.11	8.08
Proportion bacterial N	0.45	0.47	0.43	0.42	0.48	0.03
Bacterial N yield(kg)	0.71	0.75	0.68	0.64	0.79	0.15
Undegraded dietary protein(kg)	0.85	0.81	0.90	0.86	0.85	0.16
%CP Degradability	43.00	45.00	41.00	41.00	45.00	0.09

microbial protein synthesis in the rumen in the absence of a readily available source of fermentable carbohydrates. Such low values demonstrate the need to supplement energy and protein for silage diets in order to meet the nutrient requirements for high producing and growing animals.

Coefficients of digestibility of total amino acids in the small intestines of animals receiving silage diets normally lie in the range 65-75% [47].For the intestinal CP digestibility, the values obtained in this experiment are lower than the range above. The obtained mean CP value for all silage treatments was 59.75%. There were no significant differences. Beever [131] reported three experiments in which amino acid absorption from the small intestine was lower with wilted than with direct cut silages.

From the work presented in chapter 2, the sum of the two constants for the soluble fraction a and the potentially degradable fraction b, (that is $a + b$) will give the asymptote (extent of degradation), thus, these values can be compared with the *in vivo* digestibility coefficients reported in this section. The CP values from table 2.5

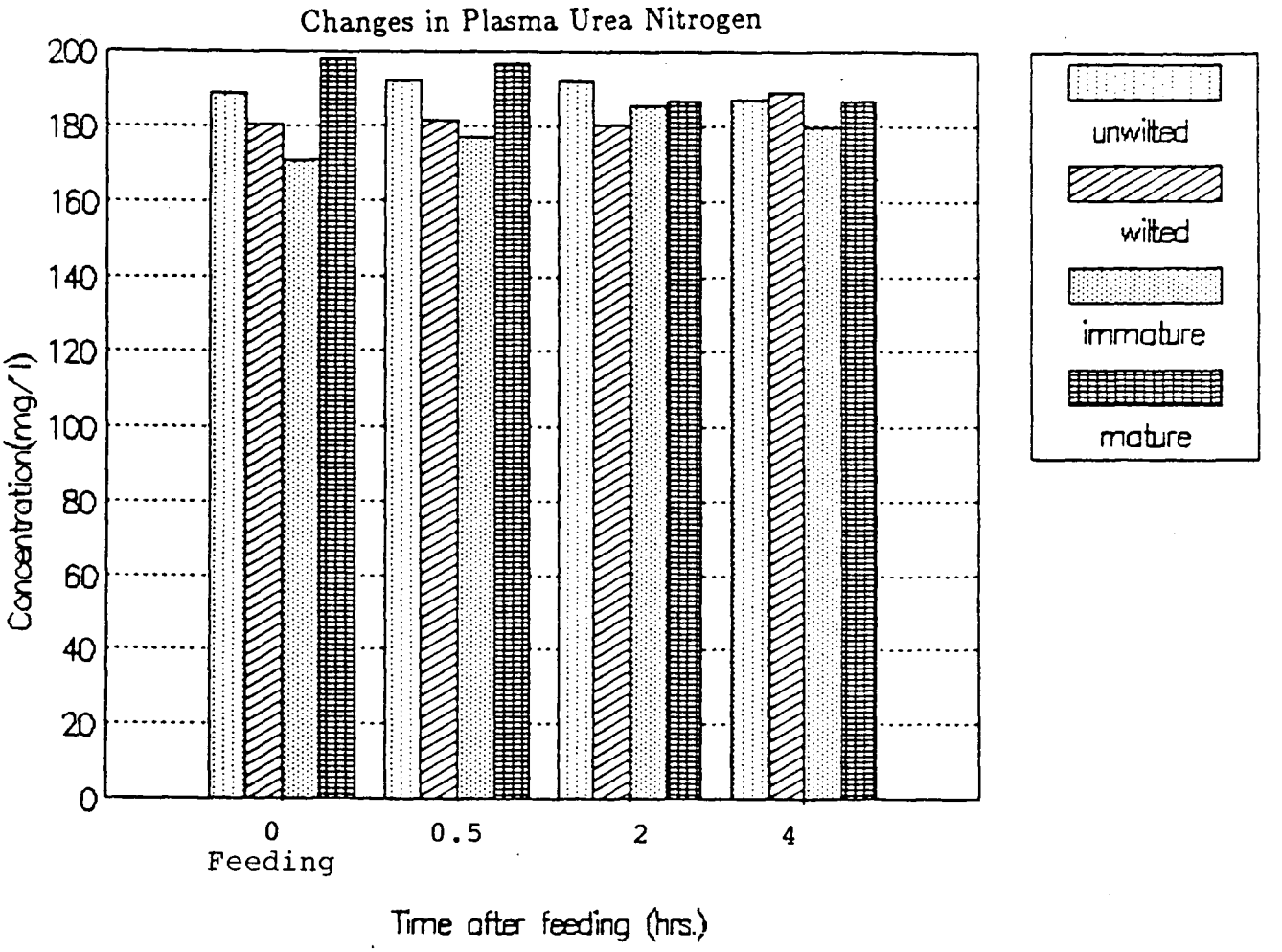

Figure 3.1: Changes in Plasma Urea Nitrogen (mg/l)

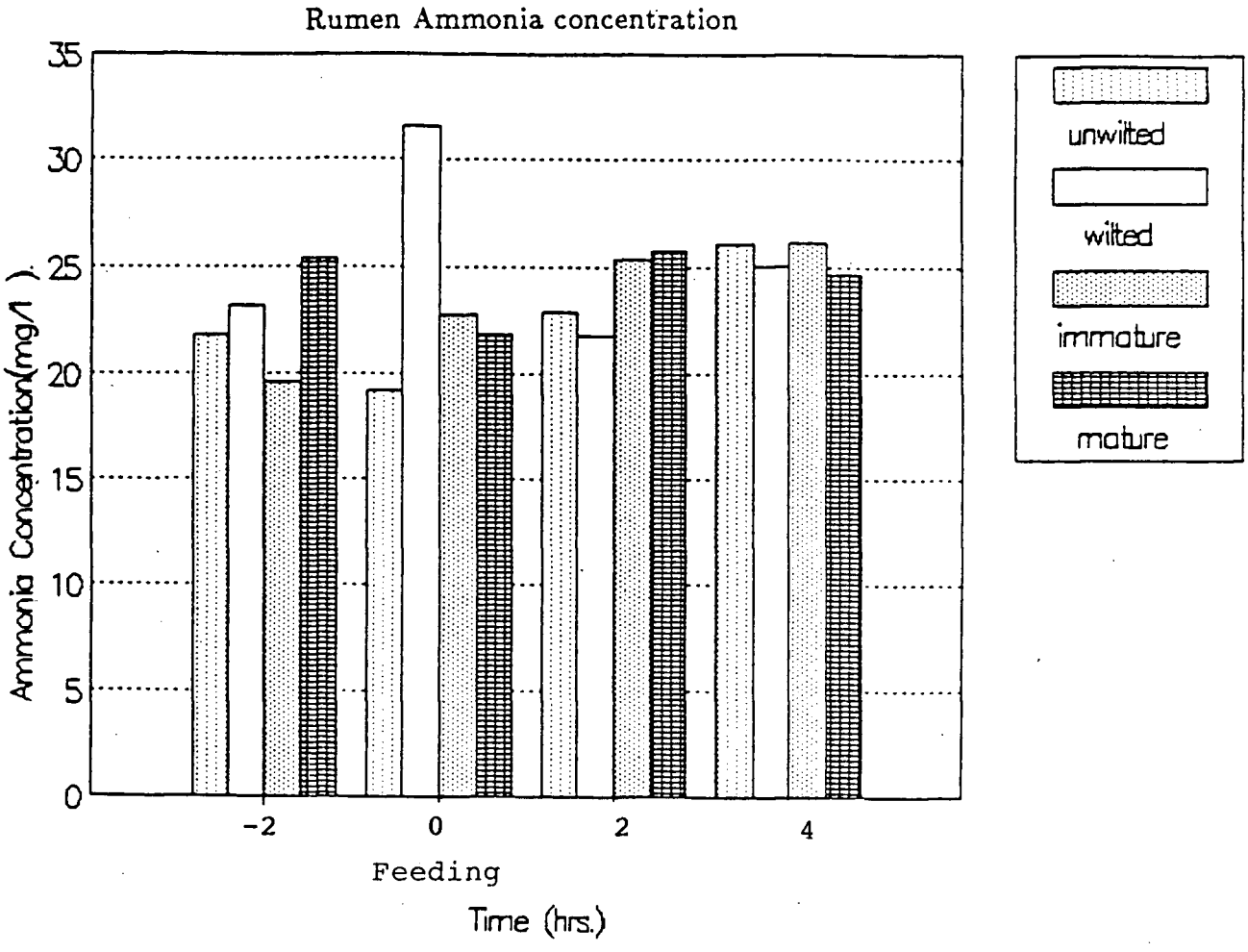

Figure 3.2: Rumen Ammonia concentration (mg/l)

Table 3.10: CP and ADF in silage, duodenum and feces.

	TREATMENT					
	mean	unwilted	wilted	immature	mature	±s.e.m.
%CP feed	15.47	14.93	16.00	15.18	15.75	0.49
%CP duodenum	22.65	22.08	23.23*	23.03	22.27	0.04
%CP feces	16.13	15.13	17.14*	16.81	15.45	0.54
%ADF feed	45.74	48.59	42.89	46.03	45.45	0.66
%ADF duodenum	51.58	48.72	54.43	55.46	47.69	0.39
%ADF feces	53.95	55.41	52.49†	53.60	54.30	0.47

* indicate values significantly different at ($p < 0.05$)

† indicate values significantly different at ($p < 0.01$)

are 88, 84, 87, and 84% for unwilted, wilted, immature, and mature silages respectively. Similarly, following the same treatment order, those for DM values from table 2.7 are 71, 69, 70, 68%. It can be concluded that the *in situ* study(nylon bag trial) *vs* this *in vivo* study, overestimated the digestibility of silage nutrients by mean of 28 and 11 %units for CP and DM respectively.

High degradability of silage N in the rumen (often associated with high concentrations of NPN), coupled with low contents of water soluble carbohydrates, may result in high rumen ammonia, substantial losses of N in urine and low levels of N retention by the animal [134]. Also, because of this high degradability in the rumen, the proportion of dietary N which escapes rumen degradation may be as low as 15% [131] with the consequence that the animal fed silage is particularly dependent on microbial protein for its supply of amino acids. This gives problems in satisfying N requirements of high producing animals. A mean degradability value of 43% was obtained in this study. Overall, there were no significant differences among treatments. It appears that higher degradation of silage protein occurred in the unwilted and mature silages, thus, 45% *vs* 41% for mature and unwilted *vs* immature and wilted respectively. The higher values observed for the former could be a reflection of the amount of NPN substances present

as was evident with silages of high moisture [117]. Lower values for wilted and mature silages could be a result of a reduction in moisture content restricting the amount of fermentation which occurs with clostridial bacteria being particularly suppressed. Several experiments suggest that the feed conversion efficiency of animals given wilted silages may be lower than that with well preserved wetter silages [131]. This could be an indication of the utilization and retention of the N from such wilted silages.

The degradability results obtained can be compared with the effective degradation values in table 2.6 of the previous chapter. Higher values were obtained by the *in situ* technique thus, 87, 82, 87, 84% for unwilted, wilted, immature, and mature silages respectively. Although a similar trend was observed, values from the *in vivo* study were reduced by almost half compared with the *in situ* study. The reliability of these *in vivo* estimates is determined by precise estimation of rates of passage thus, it can be speculated that, the low values obtained in this study could be a consequence of errors associated with determination of rates of passage. Generally, the *in vivo* method is considered to be more accurate when compared to the *in situ* nylon bag technique since, the former method involves nearly all the dynamic events associated with digestion in an intact animal. Thomas [134] estimated that the rumen degradability of silage N *in vivo* was 78-86% for three silages made without additives. High proportions of soluble protein which were observed in the previous chapter would be expected to be highly degradable.

The extent to which protein is degraded in the rumen will depend upon microbial proteolytic activity, microbial access to the protein, and rumen turnover. There were no significant differences observed for the proportion of bacterial N, bacterial N mass and amount of undegraded protein reaching the duodenum. Notable features of N digestion observed in animals receiving silage are the relatively low proportion of microbial N in the protein of the duodenal digesta [127].This is confirmed in this study by the

proportion of bacterial N with a mean value of 45%. Silage organic matter contains a substantial component of silage fermentation products that are fermented by the rumen micro-organisms but will have a relatively low yield of energy (ATP) to support microbial growth. However, examination of rumen contents in sheep given silage diets showed substantial ruminal proportions of protozoa and the activity of these organisms will also reduce the efficiency of total microbial protein synthesis [131].

The ruminal concentrations of ammonia reflects the relative rates with which ammonia enters and is removed from the rumen 'ammonia pool'. Ammonia enters the pool through degradation of nitrogenous constituents of dietary and endogenous origins and through the intraruminal recycling of N that results from the engulfment of bacteria by protozoa and from bacteria and protozoa lysis [128]. While Lewis (cited by Pisulewski et al. [154]), reported toxic rumen NH_3 levels of 1020-1700mg/l, Pisulewski et al. [154] reported an optimal rumen NH_3 concentration range for microbial protein production of 107.1 to 161.5mg/l. Typically, in animals given grass silage diets ruminal concentrations of ammonia are high [147]; often the average values during the day are around 200mgNH_3N/l [150]. Rumen ammonia values obtained in this study were very low ranging from 19.3 to 31.6 mg/l. This could be attributed to loss of NH_3 from the samples during storage since mercuric chloride was not added to the samples during the collection period. Rumen NH_3 patterns are illustrated in figure 3.2. Generally, a marked increase in rumen NH_3 concentration was expected 1 to 2 hours post prandial as evidenced by Donaldson and Edwards [124]. Higher concentration of NH_3 were expected for the immature and unwilted silages due to their lower content of WSC (see table 1.2).

The relationship between plasma urea nitrogen (PUN) and N intake is affected by intake of water, NPN and the variable response both between animals and within animals to any particular diet. There were no significant differences in PUN and the values

obtained lie within the range of 150mg/l and 250mg/l reported by McIntyre [125] and Donaldson and Edwards [124]. A similar pattern as for rumen ammonia concentration was expected for PUN, the results are illustrated in figure 3.1.

Wilting significantly increased ($p<0.05$) fecal CP, increased duodenal CP ($p<0.05$) and decreased ($p<0.01$) fecal ADF. Nitrogenous substances lost in feces include both undigested protein residues and microbial protein. A significant synthesis of the latter may occur in the caecum as a consequence of the fermentation of mainly fibrous residues that have survived digestion earlier in the gut. The higher fecal CP in the animals eating wilted silage could be an indication of poor utilization of the N in such silages. Reduced fecal ADF could be a result of hindgut fermentation. There was no significant difference in ADF entering the duodenum.

The *in vivo* method is essential for determining ruminal digestion, rate of passage and other animal production parameters. Although such a method is expensive, laborious and certainly not free of problems, it is the yardstick against which any alternative method should be compared.

With *in vivo* studies, precise estimates of rumen degradability of feed protein are difficult to obtain since they must be made indirectly by subtracting from the duodenal flow of endogenous secreted N and microbial N. The errors of estimation of both these fractions reduce the accuracy of the degradability values.

Measurement of digesta flow to the duodenum is subject to considerable error. Digesta markers used are not ideal and do not always reflect the solid and liquid phase that they are intended to represent [45]. Thus, the calculation of degradability *in vivo* is very sensitive to a correct estimate of flow to the duodenum. Calculations of the results presented showed unrealistically low values.

Estimations of bacterial CP based upon digesta or microbial marker techniques are subject to errors inherent in those techniques. RNA as a marker does not include

protozoa protein and use of such values to estimate microbial N can seriously underestimate amounts of microbial N reaching the duodenum. Recent work by Arambel and associates [122], revealed that RNA is relatively unstable and appreciable losses of RNA can occur when samples are stored.

Because of difficulties in estimation, accuracy of *in vivo* values for dietary protein degradation remains uncertain.

3.5 SUMMARY AND CONCLUSION:

This study, evaluated digestibility and degradability *in vivo* of silage N as affected by wilting and maturity of the parent herbage. The results obtained showed no significant differences in DM, CP, and ADF, digestibilities, proportions of duodenal bacterial N, undegraded protein entering the duodenum, degradability of silage protein, rumen NH_3N and PUN. However significant effects of wilting were observed as increased duodenal CP, increased fecal CP and reduced fecal ADF.

The results obtained by this method are suspect and errors could have been introduced in calculating digesta flow to the duodenum. A comparison of the *in vivo* results and the *in situ* results showed an underestimation of both degradability and digestibility by the former method.

Ensilage can markedly affect the nature of N moieties in the resulting herbage, and in previous studies, these have been shown to have a considerable influence on the digestive and synthetic processes occurring within the reticulo-rumen. The results of this study did not show any apparent differences in degradability and digestibility among different treatments.

Bibliography

[1] Watson, S.J. and M.J. Nash, 1960. The conservation of grass and forage crops. 2^{nd} ed. Oliver and Boyd Ltd. Edinburgh.

[2] McCullough,1978. Fermentation of silage. A Review National Feeds Ingredients Association.

[3] Hergarty, M.P. and P.J. Peterson, 1973. Free amino acids, bound amino acids and ureides. In chemistry and biochemistry of herbage. Vol.1 (G.W. Butter and R.W. Bailey, eds.) AP. NY.

[4] McDonald, P. and R. Whittenbury, 1973. Ensilage. In the chemistry and biochemistry of herbage (eds. G.W. Buttler and R.W. Bailey) AP. NY.

[5] Bergen, W.G., E.H. Cash and H.E. Henderson, 1974. Changes in nitrogenous compounds of the whole corn plant during ensiling and subsequent effects on dry matter intake by sheep. J. Anim. Sci. 39:629-637.

[6] Whittenbury, R. 1968. Microbiology of grass silage. Proc. Biochem. 3:27.

[7] McPherson, H.T. and P.J. Violante, 1966 a. Ornithine, putrescine and cadaverine in farm silage. J. Sci. Fd. Agric. 17:124-127.

[8] Brady, C.J. 1960. Redistribution of N in grass and leguminous fodder plants during wilting and ensilage J. Sci. Fd. Agric. 11:267-284.

[9] Playne, M.J. and McDonald, P. 1966. The buffering constituents of herbage and of silage. J. Sci. Fd. Agric. 17:264-268.

[10] Wieringa, G.W. 1969. Influence of moisture and nutrient content of forage plants on fermentation processes. Proc. 3rd General Meeting of EGF p 133-137.

[11] McPherson, H.T. and Wall, R.A. 1970. Automated amino acid analysis of grass and silage. J. Sci. Fd. Agric., 21:129-130.

[12] Capintero, C.M., Henderson, A.R. and McDonald, P. 1979. The effects of some pretreatments on proteolysis during the ensiling of herbage. J. Br. Grassld. Soc. 34:311-315.

[13] Kemble, A.R. and McPherson, H.T. 1952. Liberation of amino acids in perenial rye grass during wilting. Biochem. J. 58:46-49.

[14] DeVuyst, A., Vervack, W. and Arnould, R. 1969. Changes in nitrogenous material in alfalfa during ensilage. Zootechnica Agricoltura Veterinaria, 7:61-67.

[15] Barry, T.N., Mundell, D.C., Wilkins, R.J. and Beever, D.E., 1978. The influence of formic acid and formaldehyde additives and type of harvesting machine on the utilization of N in lucerne silages. 2. Changes in amino acid composition during ensilage and their influence on nutritive value. J. Agric. Sci. Camb., 91:717-725.

[16] Wieringa G.W. 1960. Some factors affecting the silage fermentation. Proc. 8th Int Grassld. congress. Reading p.497-502.

[17] Woolford, M.K., Honig, H., and Fenlon, J.S. 1979. In the silage fermentation. Microbiology series, Volume 14. (by M.K. Woolford) Marcel Dekker, Inc. N.Y. and Basel. 1984 p 156.

[18] Lanigan, 1963. Silage bacteriology. Water activity and temperature relationships of silage strains of lactobacillus plantarum, lactobacillus forevis, Pedicoccus cerevisiae. Aust. J. Biol. Sci. 16:606-615.

[19] Fawcett, J.K. and Scott, J.E. 1960. A rapid and precise method for the determination of urea. J. Clin. Path. 13:156-163.

[20] Hume, J.D. 1970. Synthesis of microbial protein in the rumen. A response to higher volatile fatty acids. Aust. J. Agric. Res. 21:305-314.

[21] Clark, J.M. 1975. Nitrogen metabolism in ruminants. Protein solubility and rumen bypass of protein and amino acids. In protein nutritional quality of foods and feeds. (II) ed. Friedman, M. Mercel Dekker Inc. NY. p 261.

[22] Parkinson, J.A. and Allen. 1975. A wet oxidation procedure suitable for the determination of N and mineral nutrients in biological materials. Comm. Soil Sci. Plant Anal. 6:1.

[23] Faichney, F.J. 1975. In digestion and metabolism in the ruminant (eds. I.W. McDonald and A.C.I. Warner) Univ. of New England, publ. Unit Armidale, N.S.W. p 277-291.

[24] Hartnell, G.F. and Satter, L.D. 1979. Extent of particulate marker (samarium, lanthanum and cerium) movement from on digesta particle to another. J. Anim. Sci. 48:375-380.

[25] Buttery, P.J. and Lewis, D.1982. Nitrogen metabolism in the rumen. Forage protein in ruminant. Animal Prod. Occ. Pupl. No. 6 BSAP(ed. Thomson, D.J., D.E. Beever and R.G. Gunn) p 1-11.

[26] Shirley, R.L. 1986. In nitrogen and energy nutrition of ruminants A.P. Inc. London p 144.

[27] Stern, M.D. and Hoover, W.J, 1979. Methods for determining and factors affecting microbial protein synthesis. A Review. J. Anim. Sci. 49:1590-1603.

[28] Smith, R.H. 1975. Nitrogen metabolism in the rumen and the composition and nutritive value of nitrogen compounds entering the duodenum. In digestion and metabolism in the ruminant (ed. I.W. McDonald and A.C.I. Warner). p 399- 415.

[29] Downes, A.M. and McDonald, I.W. 1964. The chromium-51 complex of EDTA as a soluble rumen marker. Br. J. Nutr. 18:153-162.

[30] Hogan, J.P. and Weston, R.H. 1967. The digestion of chopped ground roughages by sheep. (II) Digestion of nitrogen and some carbohydrate fractions in the stomach and intestines. Aust. J. Agric. Res. 18:803-819.

[31] Weston, R.H. and Hogan, J.P. 1967. The digestion of chopped ground roughages by sheep. Movement of digesta through the stomach. Aust. J. Agric. Res. 18:789-801.

[32] Faichney, G.J. 1980. Measurement in sheep of the quantity and composition of rumen digesta and of fractional outflow rates of digesta constituents. Aust. J. Agric. Res. 31:1129-37.

[33] Teeter, R.G. and Owens, F.N. 1983. Characteristics of water soluble markers for measuring rumen liquid volume and dilution rate. J. Anim. Sci. 56:717-734.

[34] McAllan, A.B. and R.H. Smith, 1969. Nucleic acid metabolism in the ruminant. Determination of nucleic acids in digesta. Brit. J. Nutr. 23:671-682.

[35] Woolford, M.K. 1984. In the silage fermentation (ed. Al. Laskin and R.I. Mateles.) Marcel Dekker, Inc. NY. and Basel.

[36] Harstad, O.M. and Vik-Mo, L. 1985. Estimation of microbial and undegraded protein in sheep on grass silage based diets. Protein evaluation for ruminants. Acta Agric. Scand. Suppl. 25:37-48.

[37] Weller, R.A. and Pilgrim, A.F. 1974. Passage of protozoa and VFA from the rumen of sheep and from a continous in-vitro fermentation system. Br. J. Nutr. 32:341-351.

[38] Kaufmann, W. and Lupping, W. 1982. Protected proteins and protected amino acids for ruminants. In Protein contribution of feedstuffs for ruminants: Application to feed formulation, 36-75(ed. E.L. Miller, I.H. Pike and A.J.H. Van Es). Butterworth.

[39] Faichney, G.J. and Black, J.L. 1979. In physiological and enviromental limitations to wool growth, (ed. J.L. Black and P. J. Reis.) p 179.

[40] Zinn, R.A. and Owens, F.N. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66:157-166.

[41] Hogan J. P. and Weston, R.H. 1980. Laboratory methods for protein evaluation. In forage evaluation (ed. J. L. Wheeler and R.D. Mochrie) AFGC p 75- 88.

[42] Tan, T.N., Weston, R.H. and Hogan, J.P. 1971. Use of ^{103}Ru labelled Tris(1,10-phenanthroline) ruthenium (II) chloride as a marker in digestion studies with sheep. Int. J. App. Rad. Isot. 22:301-308.

[43] Ellis, W.C. and Huston, J.E. 1968. Ce and Pr as particulate flow markers in ruminants. J. Nutr. 95:67-78.

[44] Shelford, J.A., Cathcart, E.B., France, R.T. and Dobbs, J.L. 1980. A survey of composition and heat damage in dehydrated alfalfa products over the growing season from two B.C. processing plants. Can. J. Anim. Sci. 60:197-200.

[45] Faichney, G.J. 1972. An assessement of chromic oxide as an indigestible marker for digestion studies in sheep J. Agric. Sci. 79:493-499.

[46] Drennan, M.J., Holmes, J.W.G. and Garret, W.N. 1970. A comparison of markers for estimating magnitude of rumen digestion. Br. J. Nutr. 24:961-970.

[47] Thomas, C. and Thomas, P.C. 1985. Factors affecting the nutritive value of grass silages. in Rec. Adv. Anim. Nutr. (ed. Haresign, W. and Cole, D.J.A.) p 223-256.

[48] Thomas, P.C. 1978. Annual Report of the Hannah Research Institute p 108- 117.

[49] Zinn, R.A. and Owens, F.N. 1980. Bypass values vary with dietary conditions. Feedstuffs 52 (6):28-29.

[50] Zinn, R.A., Bull, L.S. and Hemken, R.W. 1978. Influence of level of intake on degradation of supplemental protein in the rumen. Joint meeting ADSA and ASAS.

[51] Mohamed, O.E. and Smith, R.H. 1977. Measurement of protein degradation in the rumen. Proc. Nutr. Soc. 36:152A(abstr.)

[52] ARC, 1980; 1984. Nutrient requirements of ruminant livestock. CAB.

[53] Balch, C.C. and Campling, R.C. 1965. Rate of passage of digesta through the ruminant digestive tract. In physiology of digestion in the ruminant (ed. R.W. Dougherty), p 108-123. Butterworth. Washington.

[54] AFRC,1987. Nutrition abstracts and reviews (series B). CAB Int. Vol. 57 no.12.

[55] Ohshima, M. and McDonald, P. 1978. Review of changes in nitrogenous compounds of herbage during ensilage. J. Sci. Fd. and Agric. 29:497-505.

[56] Voss, N. 1966. Amines and ammonia as products of protein decomposition in silage. Proceedings of the 10^{th} Int. Grassld. Congr. Helsinki. 540-546.

[57] Harrison, D.G. and McAllan, A.B. 1980. Factors affecting microbial growth yields in the reticulo-rumen. In digestive physiol. and met. in ruminants. (ed. Y. Ruckebusch and P. Thivend), p 205-226.

[58] Baldwin, R.L. and Denman, S.C. 1979. Quantitative and dynamic aspects of Nitrogen metabolism in the rumen: a modelling analysis. J. Anim. Sci. 49:1631-1639.

[59] Thomas, P.C., Kelly, N.C., Chamberlain, D.G. and Wait, M.K.1980. Nutritive value of silages. Digestion of organic matter, gross energy and carbohydrate constituents in the rumen and intestines of sheep receiving diets of grass silage and barley. Br. J. Nutr. 43:481-489.

[60] Demeyer, D.I. and Van Nevel, C.J. 1980. Nitrogen exchanges in the rumen. Proc. Nutr. Soc. 39:89-95.

[61] Clark, B.J. 1974. Biochemical and physiological changes occurring during wilting and the early stages of ensilage. PhD Thesis, Univ. Edinburgh.

[62] Bondi, A. 1981. Metabolism of protein in ruminant animals. A review. Nutr. Rep. Int . 23:993-1003.

[63] Tamminga, S. 1979. Protein degradation in the forestomachs of ruminants. J. Anim. Sci. 49:1615-1630.

[64] Henderickx, H. and Martin, J. 1963. In vitro study of the nitrogen metabolism in the rumen. IRSIA. comptes rendus de recherches 31.

[65] Storm, E. and Orskov, E.R. 1983. The nutritive value of rumen microorganisms in ruminants. 1. Large scale isolation and chemical composition of rumen microorganisms. Br. J. Nutr. 50: 463-470.

[66] Varvikko, T. 1986. Evaluation of rumen-undegraded feed protein. Helsinki. Acad. Dissertation.

[67] Setala, J. 1983. The nylon bag technique in the determination of ruminal feed protein degradation. Helsinki Acad. Dissertation.

[68] Figroid, W., Hall, W.H. and Theurer, B. 1972. An evaluation of the nylon bag technique for estimating rumen utilization of grains. J. Anim. Sci. 35:113- 120.

[69] Ganev, G., Orskov, E.R. and Smart, R. 1979. The effect of roughage or concentrate feeding and rumen retention time on total degradation of protein in the rumen. J. Agric. Sci. (Camb.) 93:651-656.

[70] Mehrez, A.Z. and Orskov, E.R. 1977. A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. Agric. Sci. (Camb.) 88:645-650.

[71] Uden, P., Parra, R. and Soest, P. Van. 1974. Factors influencing reliability of the nylon bag technique. J. Dairy Sci. 57:622(abstr.).

[72] Isaacs, J. and Owens, F.N. 1972. Protein soluble in rumen fluid. J. Anim. Sci. 25:855-875.

[73] Kennedy, P.M., Hazlewood, G.P. and Milligan, L.P. 1984. A comparison of methods for the estimation of the proportion of microbial nitrogen in duodenal digesta, and the correction for microbial contamination in nylon bags incubated in the rumen of sheep. Br.J. Nutr. 52:403-417.

[74] Setala, J. and Syrjala-Qvist, L. 1984. Degradation of crude protein and quality of undegradable protein in untreated or formaldehyde-treated rapeseed meal. Anim. Feed Sci. Technol. 12:19-27.

[75] Rooke, J.A., Greife, H.A. and Armstrong, D.G. 1984. The effect of in sacco rumen incubation of a grass silage upon the total and D amino acid composition of the residual silage dry matter. J. Agric. Sci. (Camb.) 102:695-702.

[76] Playne, M.J., Khumualthong, W. and Echevarria, M.G. 1978. Factors affecting the digestion of oesophageal fistula samples and hay samples in nylon bags in the rumen of cattle. J. Agric. Sci. (Camb.) 90:193-204.

[77] Van Hellen, R.W. and Ellis, W.C. 1977. Sample container porosities for rumen in situ studies. J. Anim. Sci. 44:141-146.

[78] Broderick, G.A. 1978. In vitro procedures for estimating rates of ruminal protein degradation and proportions of protein escaping the rumen undegraded. J. Nutr. 108:181-190.

[79] Lindberg, J.E. 1985. Estimation of rumen degradability of feed proteins with the in sacco technique and various in vitro methods. A Review. Acta. Agric. Scand. Suppl. 25: 64-97.

[80] Lyttleton, J.W. 1973. Proteins and nucleic acids. In chemistry and biochemistry of herbage. (ed. G.W. Butter and R.W. Bailey), p 63-100. Academic press, London and New York.

[81] Mangan, J.L. 1982. The nitrogenous constituents of fresh forages. In forage protein in ruminant animal production (ed. D.J. Thomson, D.E. Beever and R.G. Gunn), p 25-40. Occ. Publ. No. 6 BSAP.

[82] Moller, P.D. 1985. Results of grass silage based rations on the N absorption in the gastro-intestinal tract of dairy cows applied in Nordic protein evaluation system. Acta Agric. Scand. Suppl. 25:49-63.

[83] Bauchop, T.1979. The rumen ciliate epidemium in primary degradation of plant tissues. Appl. Environ. Microbiol. 37:1217-1223.

[84] Bauchop, T. 1981. The anaerobic fungi in rumen fibre digestion. Agric. Environ. 6:339-348.

[85] Akin, D.E., Burdick, D. and Michaels, G.E. 1974. Rumen bacterial interrelationships with plant tissue during degradation revealed by transmission electron microscopy. Appl. Microbiol. 27:1149-1156.

[86] Akin, D.E. and Amos, H.E. 1975. Rumen bacterial degradation of forage cell walls investigated by electron microscopy. Appl. Microbiol. 29:692-701.

[87] Cheng, K.J., Akin, D.E. and Costerton, J.W. 1977. Rumen bacteria interaction with particulate dietary components and response to dietary variation. Fed. Proc. 36:193-197.

[88] Ferguson, K.A. 1975. The protection of dietary protein and amino acids against microbial fermentation in the rumen. In digestion and metabolism in the ruminant (ed. I.W. McDonald and A.C.I. Warner), p 448-464.

[89] McDonald, I. 1981. A revised model for the estimation of protein degradability in the rumen. J. Agric. Sci. (Camb.) 96:251-252.

[90] McDonald, P. 1982. The effect of conservation processes on the nitrogenous components of forages. In forage protein in ruminant animal production. (ed. D.J. Thomson, D.E. Beever and R.G. Gunn), p 4-49. Occ. Publ. No. 6 BSAP.

[91] Wohlt, J.E., Sniffen, J.C. and Hoover, W.H. 1973. Measurement of protein solubility in common feedstuffs. J. Dairy Sci. 8:1052-1057.

[92] Van Soest, P.J. 1982. Nutritional ecology of the ruminant. O and B Books Inc. Corrallis, Oregon.

[93] Van Soest, P.J. 1965. Use of detergents in analysis of fibrous feeds (III). Study of the effects of heating and drying on yield of fibre and lignin in forages. J.A.O.A.C. 48:785-790.

[94] Weakley, D.C., Stern, M.D. and Satter, L.D. 1983. Factors affecting dissappearance of feedstuffs from bags suspended in the rumen. J. Anim. Sci. 56:493-507.

[95] Hungate, R.E. 1966. The rumen and its microbes. Academic Press. New York, San Francisco, London.

[96] Krishnamoorthy, U., Sniffen, C.J. and Van Soest, P.J. 1982. Nitrogen fractionation in ruminant feedstuffs for feed evaluation. Proc. Cornell Nutr. Conf.,p 95-102.

[97] ,Broderick G.A. 1982. Estimating rates of ruminal protein degradation and proportions of protein escaping the rumen undegraded. J. Nutr. 108:181-190.

[98] Mathers, J.C. and Aitchison, E.M. 1981. Direct estimation of the extent of contamination of food residues by microbial matter after incubation within fibre bags in the rumen. J. Agric. Sci. (Camb.) 96:691-693.

[99] Orskov, E.R. 1982. In protein nutrition in ruminants. AP Inc. London Ltd.

[100] Sniffen, C.J. 1974. Nitrogen utilization as related to solubility of NPN and protein in feeds. Proc. Cornell Nutr. Conf. p 12-18.

[101] Goering, H.K. and Waldo, D.R. 1974. Processing effects on protein utilization by ruminants. Proc. Cornell Nutr. Conf. p 25-36.

[102] Waldo, D.R. 1968. Symposium:Nitrogen utilization by the ruminant, nitrogen metabolism in the ruminant. J. Dairy Sci. 51:265.

[103] Chalupa, W. 1975. Rumen bypass and protection of proteins and amino acids. J. Dairy Sci. 58:1198-1218.

[104] Waldern, D.E. 1971. A rapid micro-digestion procedure for neutral and acid detergent fibre. Can. J. Anim. Sci. 51:67.

[105] Marsh, R. 1979. The effect of wilting on fermentation in the silo and on the nutritive value of silage. Grass and Forage Science 34:1-9.

[106] Anderson, R. 1983. The effect of extended moist wilting and formic acid additive on the conservation as silage of two grasses differing in total N content. J. Sci. Fd. Agric. 34:808.

[107] Jackson, N. and Anderson, B.K. 1968. Conservation of fresh and wilted grass in air tight metal containers. J. Sci. Fd. Agric. 19:1-6.

[108] Jackson, N. and Forbes,T.J. 1970. The voluntary intake by cattle of form silages differing in dry matter content. Anim. Prd. 12:591.

[109] Murphy, J.J. and Gleeson, P.A. 1984. Effect of wilting and formic acid treatment on silage preservation and performance of autumn-calving cows.Ir. J. Agric. Res. 23:105.

[110] Fliport, P., Mason, W. and Lalande, G.1984. Chemical composition and animal performance of grass forage of varying maturity stored as hay or silage. Anim. Feed Sci. and Technol. 11:35.

[111] Gervais, P. and St-Pierre, J.C. 1979.Influence du stade croissance a la premiere recolte sur le rendement, la composition chimique et les reserves nutritives de la fleole des pres. Can. J. Plant Sci. 59:177-186.

[112] Chapman, H.D. and Pratt, P.F. 1961. Methods of analysis for soils, plants and waters. Univ. of California.

[113] Durand, M., Zelter, S.Z., Tisserand, J.L. 1968. Annls.Biol. Anim. Biochim. Biophys. 8:45. Cited by Wilkinson Outlook Agric (1976) 9:3-8.

[114] Neumark, H. Bondi, A. Volcani, R. 1964. Amines, Aldehydes and keto acids in silages and their effects on food intake by ruminants. J. Sci. Fd. Agric. 15:487-492.

[115] Gordon, C.H., Derbyshire,J.C. and Humphrey, J.L. 1965. Inhibition of aerobic spoilage in low moisture silage. J. Dairy Sci. 48:811(abstr.P102).

[116] Wilkinson, J.M. Wilson, R.F. and Barry, T.N. 1976. Factors affecting the nutritive value of silage. Outl. Agric. 9:3-8.

[117] Merchen, N.R. and Satter, L.D. 1983. Changes in nitrogenous compounds and sites of digestion of alfalfa harvested at different moisture content. J. Dairy Sci. 66:789-801.

[118] McDonald, I. 1981. The biochemistry of silage. John Wiley and Sons, Chichester.

[119] Ferguson, W.S. and Terry, R.A. 1953. Purines in grassland herbage. Nature, 172:346-354.

[120] Ely, D.G., Little, C.O., Woolfork, P.G. and Mitchell, G.E. Jr. 1967. The estimation of the extent of conversion of dietary zein to microbial protein in the rumen of lambs. J.Nutr. 91:314-318.

[121] Noller, C.H. and Thomas, J.W. 1985. Hay-crop silage. In Forages. The Sci. Grassld. Agric. (ed. Heath, M.E., Barnes, R.F. and Metcalfe.) 4^{th} ed. Ames. Iowa. U.S.A.

[122] Arambel, M.J., Bartley, E.E., Dennis, S.M., Dufva, G.S., Nagaraja, T.G., Nuzback, D.E., Riddell, D.O., Dayton, A.D. and Galitzer, S.J. 1987. Evaluation of several methods for estimating microbial nitrogen concentration in the rumen. Nutr. Rep. Int. 25:25-39.

[123] Owens, F.N. and Goetsch, A.L. 1984. Digesta passage and microbial protein synthesis. In control of digestion and metabolism in ruminants. (ed. Milligan, L.P., Grovum, W.L. and Dobson, A.) Proc., (VI) Int. Symp.Rum. Physiol. Banff. Canada. p 196-223.

[124] Donaldson, E. and Edwards, R.A. 1976. Feeding value of silage: Silages made from freshly cut grass, wilted grass and formic acid treated wilted grass. J. Sci. Fd. Agric. 27:536-544.

[125] McIntyre, K.H.1970. Effects of increased N intakes on plasma urea N and rumen ammonia levels in sheep. Aust. J. Agric. Res. 21:501-507.

[126] Castle, M.E.1975. Silage and milk production. Agric. Progress. 50:53-60.

[127] Hannah Research Institute report. 1981 p 29.

[128] Nolan, J.V.1975. Quantitative models of N metabolism in sheep. In digestion and metabolism in the ruminant. (ed. I.W. McDonald and A.C.I. Warner), p 416-431.

[129] Castle, M.E. and Watson,S.J. 1984. The Voluntary Food Intake in farm animals. (by J.M. Forbes 1986. Butterworths and Co. Ltd. p 130-143.

[130] Forbes, J.M. 1986. Voluntary food intake in farm animals. Butterworths and Co. Ltd. p 130-143.

[131] Beever, D.E. 1980. The utilization of protein in conserved forage. Occ. Symp. No. 11. Br. Grassld. Soc. p 131-143.

[132] Waite, R. 1958. The water soluble carbohydrates of grasses. J. Sci. Fd. Agric. 9:39-43.

[133] Dermaquilly, C. 1973. Chemical composition, fermentation characteristics, digestibility and voluntary and intake of forage silages: changes compared to green forage. Annales de Zootechnie, 22:1-35.

[134] Thomas, P.C. 1982. Utilization of conserved forages. In forage protein in ruminant animal production. Occ. Publ. 6 BSAP. (ed. Thomson, D.J., Beever, D.E. and Gunn, R.G.) p 67-76.

[135] Webster, A.J.F., Simmons, I.P. and Kitcherside, M.A. Forage protein and the performance and health of the dairy cow. Occ. Publ. 6. BSAP. p 89-95.

[136] Kristensen, E.S., Moller, P.D. and Hvelplund, T. 1982. Estimation of the effective protein degradability in the rumen of cows using the nylon bag technique combined with the outflow rate. Acta. Agric. Scand. 32:123-127.

[137] Owens, F.N. and Bergen, W.G. 1983. Nitrogen metabolism of ruminant animals: Historical perspective, current understanding and future implications. J. Anim. Sci. 57 (Suppl. 2), 498-518.

[138] Andersen, P.E. and Sorensen, M. 1963. Cited by Moller, P.D. 1985. Acta. Agric. Scand. Suppl. 25 p 49-63.

[139] Prigge, E.C., Baker, M.J. and Varga, G.A. 1984. Comparative digestion, rumen fermentation and kinetics of forage diets by steers and wethers. J. Anim. Sci. 59:237-245.

[140] Rohr, K. 1979. Cited by Thomas, C. and Thomas, P.C. 1985. In Rec. Adv. Anim. Nutr. (ed. Haresign, W. and Cole, D.J.A. Butterworths. p 223-256.

[141] McDonald, P. 1968. Chemical changes and losses during the ensilage of wilted grass. J. Sci. Fd. Agric. 19:125.

[142] Beever, D.E., Prescott, J.H., and Armstrong, D.G. 1972. Sites of digestion of organic matter, energy and carbohydrate. Br. J. Nutr.28:347-356

[143] Beever, D.E. Siddons, R.C. and Kaiser, G. 1982. Evaluation of the effect of formic acid and level of formaldehyde application before ensiling on silage protein degradability. J. Sci. Fd. Agric. 33:609-613.

[144] Quin, J.L., Van Der Wath, J.G. and Myburgh, S. 1938. cited by Weakley 1983. J. Anim. Sci. 56:493-507.

[145] Goering, H.k. and VanSoest, P.J. 1970. Forage fiber analysis. Agric hanbook No. 379 p.12

[146] Satter, L.D. and Roffler, R.E. 1981. cited by Satter, L.D. Protein supply from undegraded dietary protein. J. Dairy Sci. 69:2734-2734

[147] Wilkins, R.J.1980. progress in silage production and utilization. J. Roy. agric. Soc. England 141:127-141

[148] Faichney, G. J. 1984. Control of digestion and metabolism in ruminants(ed Milligan, L.P., Grovum, W.L.and Dobson, A.)Proc. VI Int. Symp. Rum. Physiol.

Banff. p173-195.

[149] Dewar, W.A. and McDonald, P 1961. Determination of dry matter in silage by distillation with toluene. J Sci. Fd. Agric. 12:790-795.

[150] Chamberlain, D.G., Thomas, P.C. and Kelly, N.C. Wait, M.K. 1980.Nutritive value of silages. Digestion of Nitrogenous constituents in sheep receiving diets of grass silage and grass silage and barley. Br. J. Nutr. 43:469-479.

[151] Van Keuren, R. W. and Heinemann, W.W. 1962. Study of nylon bag technique in vivo estimated of forage digestibility. J. Anim. Sci. 21:340-345

[152] Mcdonald, P., Proven, M.J. and Henderson, A.R. 1983 The effect of some pre-ensiling treatments on silage composition and nitrogen disappearance in the rumen. Anim. Fd. Sci. and Technol. 8:259-269.

[153] SAS/STAT Software.1985. General linear model procedure. SAS Institute Inc. N. Carolina.

[154] Pisulewski, P.M., Okorie, Al. U., Buttery, P.J., Haresign, W. and Lewis, D. 1981. Ammonia concentration and protein synthesis in the rumen. J. Sci. Fd. Agric.32:759-766.