# MODELS FOR LUCERNE QUALITY GRADING

by

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I, the undersigned, declare that the dissertation hereby submitted by me for the degree M.Sc. Agric. at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I further cede copyright of the dissertation in favour of the University of the Free State.

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**CHAPTER 1**

**The nutritive value of South African lucerne hay *(Medicago sativa)***

## LIST OF ABBREVIATIONS

| | |
|---|---|
| a | an intercept representing soluble protein |
| AA | amino acid |
| ad libitum | free access |
| ACP | adjusted crude protein |
| ADF | acid detergent fibre |
| ADF-CP | acid detergent fibre-crude protein |
| ADF-N | acid detergent fibre-nitrogen |
| ADF-N[a] | acid detergent fibre-nitrogen expressed as a percentage of total nitrogen |
| ADIN | acid detergent insoluble nitrogen |
| AP | absorbed protein |
| Arg | arginine |
| ADS | acid detergent solution |
| ATFI | adjusted total forage index |
| b | insoluble but potential degradable protein fraction |
| BCP | bacterial crude protein |
| c | degradation rate of the b fraction |
| Ca | calcium |
| CF | crude fibre |
| °C | degrees centigrade of Celsius (temperature) |
| cm | centimeter |
| CP | crude protein |
| CV | coefficient of variation |
| DCP | digestible crude protein |
| DDM | digestible dry matter |
| DE | digestible energy |
| DIP | degradable intake protein |
| DM | dry matter |
| DMI | dry matter intake |
| DMP | digestible microbial protein |
| DUP | digestible undegraded true protein |

| | |
|---|---|
| EAA | essential amino acid |
| ECP | endogenous crude protein |
| ERDMD | effective ruminal dry mater degradability |
| ERDP | effective rumen degradable protein |
| ERPD | effective ruminal protein degradability (*in sacco* degradability of protein) |
| FME | fermentable metabolizable energy |
| g | gram |
| ha | hectare |
| His | histidine |
| ICP | insoluble crude protein |
| Ile | isoleucine |
| IVDMD | *in vitro* dry matter digestibility |
| IVOMD | *in vitro* organic matter digestibility |
| K | potassium |
| kg | kilogram |
| Leu | leucine |
| ℓ | Litter |
| LQI | lucerne quality index |
| Lys | lysine |
| MADF | modified acid detergent fibre |
| Max | maximum |
| MCF | modified crude fibre |
| MCP | microbial crude protein |
| ME | metabolizable energy |
| Min | minimum |
| MJ | megajoules |
| mm | millimeter |
| MP | metabolizable protein |
| mℓ | milliliter |
| N | nitrogen |
| n | number |
| NDF | neutral detergent fibre |

| | |
|---|---|
| NDP | non degradable protein |
| NDS | neutral detergent solution |
| NE | netto energy |
| NFC | non-fibre carbohydrates |
| NIRS | near infrared reflectance spectroscopy |
| NPN | non-protein nitrogen |
| NSC | non-structural carbohydrates |
| OM | organic matter |
| P | phosphorus |
| P<0.0001 | significant at 0.01% level of significance |
| P<0.01 | significant at 1% level of significance |
| P<0.05 | significant at 5% level of significance |
| par. | paragraph |
| Phe | phenylalanine |
| r | correlation coefficient |
| $r^1$ | fractional outflow rate |
| $r^2$ | coefficient of determination |
| RDP | rumen degradable protein |
| RFV | relative feed value |
| RUP | rumen undegradable protein |
| SEC | standard error of calibration |
| SEC-V | standard error of cross validation |
| SD | standard deviation |
| TDN | total digestible nutrients |
| TFI | total forage index |
| Thr | threonine |
| Try | tryptophan |
| UDP | undegraded feed protein |
| Val | valine |

## GENERAL INTRODUCTION

Lucerne (*Medicago sativa*) is the most important hay crop in South Africa. According to Grönum *et al.* (2000) the current area planted with lucerne for hay production in South Africa is estimated as being between 208 000 ha and 240 000 ha. The average annual lucerne hay production in South Africa is approximately 3.8 million tons. Approximately 90% of the lucerne hay produced in South Africa is under irrigation. Grönum *et al.* (2000) mentioned that the estimated area planted with lucerne has remained more or less constant over the last few years.

One of the most important characteristics of lucerne is its high nutritional quality as animal feed. Jagusch *et al.* (1970) are of the opinion that lucerne is equal to, or better than most concentrates. Lucerne hay is an important roughage source for dairy cattle, and according to Grönum *et al.* (2000) the viability of the lucerne industry in certain regions depends to a large extent on the dairy and ostrich industry. The animal feed manufacturing industry also recognizes lucerne as one of the important protein sources for animal feeds in South Africa. Hanson *et al.* (1988) reported that lucerne contains between 15 and 22% crude protein on a dry matter basis as well as all of the macro- and trace minerals and all the fat- and water soluble vitamins.

Van Soest (1987) described five features of lucerne which makes it superior to grasses, namely, it incurs only a small depression in digestibility with higher intake; it has a moderate neutral detergent fibre content; higher cell wall density leads to higher intakes; it has a high buffering capacity and a moderately fast rate of fermentation. Another reason lucerne may be superior to grasses is that it contains a higher concentration of pectin. Although a component of cell walls, pectin has some very desirable nutritional characteristics. Hall (1994) noted that it is a highly digestible, fermentable carbohydrate energy source. During its fermentations it appears not to produce lactic acid, tends not to depress ruminal pH, and it barely ferments during silage fermentation. Ward *et al.* (1957) confirmed earlier studies which showed that lucerne ash stimulated the digestibility of low quality roughage in sheep. Compared to grasses, lucerne has a rich mineral profile.

Van der Merwe & Smith (1991) mentioned that dry matter losses of sun dried lucerne hay under good weather conditions could amount to 25%. When dry matter is lost, quality (nutritive value) is also generally reduced because of leaf losses. It is well known that lucerne leaves contain more nutrients than stems. Factors influencing the quality of lucerne have been studied intensively since as early as 1903 (Snyder *et al.*, 1903 as cited by Hanson, 1972). Several factors can influence the quality of lucerne, namely locality, climate, soil, fertilisation, water, harvest schedule, moisture content, loss of leaves, storage, disease, insects, weeds and cultivar (Wedin *et al.*, 1956; Gordon *et al.*, 1962; Anderson & Thacker, 1970; Hanson, 1972; Temme *et al.*, 1979; Hanson *et al.*, 1988; Smith *et al.*, 1996; Cherney & Hall, 1997; Grönum *et al.*, 2000).

Most of the factors influencing lucerne quality can be controlled to some extent through proper management. For example, adjusting harvest dates can control maturity. Soil testing can identify optimum lime and fertilizer requirements. The highest quality species that suit the available soil resources could be chosen. Drying agents and preservatives may help to avoid rain-damaged forage. Although variety selection is very important for yield and persistence, it has relatively little effect on forage quality (Hanson *et al.*, 1988).

Attempts have been made to modify lucerne plant composition and the leaf-to-stem ratio through hybridization, as with the multi-leaf lucerne, using chemical analyses as a selection criterion. According to Cherney & Hall (1997) several lucerne trials throughout the United States now include forage quality in their evaluations of new varieties.

According to Erasmus (2000) roughage quality of a feed refers to the voluntary intake and the efficiency of utilisation of the relevant nutrients in the specific feed. Linn (1992) is of the opinion that high quality feeds should have a consistent nutrient content, high nutrient availability, absence of mold or other toxic substances, adequate physical characteristics as in the case of roughage to stimulate rumination, readily consumed by animals, and result in animal production that meet or exceed expectations. The quality of lucerne hay can vary considerably in accordance with the many factors influencing it. This variation in quality hampers the efficient utilisation of lucerne hay in animal diets. One of the major problems in the lucerne industry is the current grading system. This and many other factors, have led

to the stagnation of prices paid for lucerne and could mean that the value of lucerne is underestimated. In a survey by Grönum *et al.* (2000) producers indicated that a grading system, which could be implemented effectively in terms of application and cost, needs to be in place. This could lead to the price of lucerne reflecting its true value. Various methods are available for the evaluation of lucerne hay quality.

## 1. Lucerne hay quality testing

### 1.1 Samples

#### 1.1.1 Hay sampling methods

Forage quality can vary greatly and, as in soil testing, a proper sampling technique is essential. Taylor (1997) has assumed that even a good representative sample provides only an estimate of the average quality of a hay lot.

Obtaining a random but representative sample for a batch of hay for testing, is extremely important. Data on quality results will be useful only if the sample represents what the animal will eat. Sampling should be done for each batch of hay. According to Taylor (1997) a batch of hay is defined as hay taken from the same harvest and the same field and having the same species (pure or mixed) and variety, same type of harvest conditions, same method of baling, same method of storage, and same weather conditions during harvest (Taylor, 1997). An individual lot should not exceed 200 tons.

Taylor (1997) also mentioned that a chemical analysis is valid only to the extent that the sample truly represents the stack or lot under consideration. If the lot is uniform, collect and pool 15 to 20 cores (one per bale) from the lot by using a bale probe. It is impossible to get a representative sample by using bale slices. Bales should be selected at "random" from the hay lot. Hannaway & Ballerstedt, (1988) define random as no pre-chosen reason for selecting or rejecting a specific bale to sample (location, colour, leafiness, etc.). Hannaway & Ballerstedt (1988) described two ways to guard against pre-selection: (1) sample every fourth or fifth bale going around the stack (or truck) or down the row in the field; (2) take at least five random samples from each of the four sides of the stack. Taylor (1997) has

suggested that the hay probe should have a minimum diameter of 13 mm, a minimum internal diameter of 25 mm, a minimum length of 300 mm, and a sharp tip to cut through the bale or hay package. Cores should be taken from the centre of the butt end of each selected bale. The probe should be inserted into the bale to about 2/3 to 3/4 of its length. Taylor (1997) recommended that when using a probe with an electric drill, the drill should be set for slow speed to avoid "grab" samples or flakes of hay.

### 1.1.2 Handling of a sample

Proper handling of the sample between the time it is taken and the time of testing is of utmost importance. According to Hannaway & Ballerstedt (1988) the cores should be bulked and thoroughly mixed before the pooled sample is placed in an airtight bag for dispatching to the lab. Twenty cores with a relatively "large-barreled" probe will produce a large volume of sample. Stems and leaves will separate and settle if the sample is divided into smaller samples before dispatch it to a lab. When a sample is stored for any length of time before laboratory analysis, it should be placed in a cool place. Airtight bags prevent changes in sample moisture content between sampling and analysis.

## 1.2 Objective evaluation

### 1.2.1 Analytical methods

Two methods are used to analyse hay quality namely wet chemistry which includes a complex series of chemical analysis and near infrared spectroscopy (NIRS) which is a more rapid method of analysis.

#### 1.2.1.1 Wet chemistry

The Van Soest Fibre Analyses System separates feed into distinct fractions that relate to their nutritive value (Cherney & Hall, 1997). The chemical analyses necessary for completing this standardised lucerne hay test include dry matter (DM), acid detergent fibre (ADF), neutral detergent fibre (NDF) and crude protein (CP).

(a) *Dry matter (DM)*

At the time of baling, moisture content may range from 10 to 25% (90 to 75% dry matter). Moisture is usually lost from newly harvested hay and can account to a total weight loss of 5% or more. Depending on conditions, hay can lose or gain moisture during storage. Hanson (1972) emphasized that storage of hay at a moisture content higher than the critical level results in continued plant respiration, mold growth, and the development of a great deal of associated heat. This critical value of moisture is variable depending on the type and condition of forage stored, ambient temperature in the storage area, density of the hay, and air circulation, but it is usually in the 20 to 25% range (Hanson, 1972).

The U.S. lucerne hay test requires chemical analysis on a 100% DM basis. Thus all hay is evaluated on a common basis for comparing feed values (Hannaway & Ballerstedt, 1988). Stored hay or "as-fed" moisture varies according to conditions of storage.

(b) *Acid detergent fibre (ADF)*

Alternative procedures for fibre analysis have been developed by Van Soest (1963). The ADF is the residue after refluxing with an acid detergent and represent essentially the crude lignin and cellulose fractions of plant material but also include silica and insoluble protein and ash, which are the least digestible part of the plant. It is closely related to the indigestibility of forages and is the most important factor in the calculation of available energy.

(c) *Neutral detergent fibre (NDF)*

NDF which is the residue after extraction with boiling neutral detergent solutions, consists mainly of lignin, cellulose and hemicellulose and can be regarded as a measure of the plant cell wall material (Van Soest & Wine, 1967). It represents substances in plants that are difficult to digest and break down to small particle size (Mertens, 1992). According to Mertens (1992) NDF gives "bulk" or "fill" to the diet and as a result limits intake. Because NDF can be used to predict intake, it is one of the most valuable analyses to have conducted on roughage for dairy diets, and can be

useful for beef diets that rely primarily on roughages. As NDF increases, dry matter intake (DMI) generally decreases. Putnam *et al.* (1997) reported that nutritionists in California increasingly use NDF measurements to evaluate forages.

*(d) Crude protein (CP)*

According to McDonald *et al.* (1995) proteins are complex organic compounds of high molecular weight. In common with carbohydrates and fats they contain carbon, hydrogen and oxygen, but in addition they all contain nitrogen (N) and generally sulphur. Dietary protein generally refers to crude protein (CP), which is defined for feedstuffs as the N content × 6.25. The definition is based on the assumption that the average N content of feedstuffs is 16 g per 100 g of protein (NRC, 2001). The calculated CP content includes both true protein and non-protein N (NPN). According to Putnam *et al.* (1997) the true protein value would be determined by separately measuring all amino acids (most of which have a different N%). Feedstuffs vary widely in their relative proportions of true protein and NPN, as well as the rate and extent of ruminally undegraded feed protein. Protein for ruminants is divided into rumen degradable protein (RDP) and rumen undegradable protein (RUP). To be of nutritional value the RDP must be reformed into microbial protein. The RUP, or bypass protein is the protein that escapes digestion in the rumen but may be digestible in the small intestine (Van der Merwe & Smith, 1991). Taylor (1997) emphasized that CP gives no indication of heat damage that can alter protein availability.

The quality of lucerne hays is closely related to their CP content, because of its relationship to stage of maturity and leafiness. Hay high in protein reduces the need for supplemental high protein concentrates in the ration.

*(e) Adjusted crude protein (ACP)*

Insoluble crude protein (ICP), acid detergent fibre-nitrogen (ADF-N), acid detergent insoluble nitrogen (ADIN), unavailable nitrogen or protein all refer to the same nitrogen or protein fraction that has become chemically linked to carbohydrates to form an indigestible compound (Taylor, 1997). ADF-N gives an indication as to

whether excessive heating has occurred. This might reduce protein digestibility. Some forage may have up to 12% ADF-N. Adjusted crude protein (ACP) is a calculated protein value corrected for heat damage. Demjanec *et al.* (1995) is of the opinion that it should be used in place of crude protein to balance diets whenever ADF-N/CP exceeds 0.1.

#### 1.2.1.2 Near infrared reflectance spectroscopy (NIRS)

As emphasised by Snyman & Joubert (1992) the estimation of forage quality from published tables, although of great value, is inaccurate and may lead to over- or underfeeding with respect to production needs. NIRS is a rapid, computerised method to analyse feeds for their nutrient content. Advantages of this technique include rapid turnaround and less complicated laboratory processes, elimination of reagent chemicals used in wet chemistry, and the ability to determine multiple values (e.g. ADF, NDF and CP) in a single analytical procedure. The principle on which NIRS works is that when a light source strikes a sample, a "fingerprint" of a reflected spectra from that particular sample shows a relationship with a measured laboratory value, such as ADF (Putnam *et al.,* 1997). When a large enough number of wet chemistry analyses are collected, NIRS can predict the quality of that sample based upon the values of known samples in the database.

McDonald *et al.* (1995) mentioned that this technique might provide a solution to the problem of determining degradability. They also stressed the significant relationships that have recently been demonstrated between degradability characteristics, determined *in sacco*, and reflectance. According to McDonald *et al.* (1995) this method is capable of predicting effective rumen degradable protein (ERDP), soluble and slowly degradable crude protein fractions with reasonable accuracy. Effective degradability of crude protein could then be estimated at any desired outflow rate.

Reliable wet chemistry results and updating calibrations are important when using NIRS in predicting forage quality. The process of upgrading the calibration involves selecting previously scanned samples that are significantly different spectrally from others in the calibration (Putnam *et al.*, 1997). These samples are analysed for forage quality using wet chemistry analyses techniques. The new wet chemistry values and spectra are included in

the calibration population, and a new population is created (Putnam *et al.,* 1997), as illustrated in Table 1.

**Table 1 Statistics for NIRS calibration used for quality analysis**

| Variable | n[1)] | Average[2)] | SEC[3)] | r[4)] | SEC-V[5)] |
|---|---|---|---|---|---|
| Crude protein | 414 | 22.71 | 0.58 | 0.96 | 0.63 |
| Acid detergent fibre | 349 | 29.8 | 0.84 | 0.94 | 0.91 |
| Neutral detergent fibre | 90 | 38.94 | 0.81 | 0.76 | 0.93 |

[1)] Number of samples from calibration population used to create equation

[2)] Average of values n

[3)] Standard Error of Calibration

[4)] Proportion of variation in spectra data which is explained by the equation

[5)] Standard Error of Cross Validation (an estimate of prediction accuracy) (Putnam *et al.*, 1997)

The accuracy of NIRS results is highly dependent on the quality parameter being measured. The general ranking of accuracy according to Rankin (1997) is presented in Table 2.

**Table 2 Accuracy of NIRS in predicting forage quality parameters (Rankin, 1997)**

| Parameter | Relative Accuracy |
|---|---|
| Dry matter | Excellent |
| Crude Protein | Excellent |
| Acid detergent fibre | Excellent |
| Neutral detergent fibre | Excellent |
| Rumen undegradable protein | Good |
| Soluble crude protein | Good |
| Calcium | Good |
| Acid detergent fibre-crude protein | Fair |
| Neutral detergent fibre-crude protein | Fair |
| Phosphorus | Poor |
| Potassium | Poor |
| Magnesium | Poor |

According to Rankin (1997) NIRS readings for heat-damaged protein, phosphorus, potassium, and magnesium are not reliable and should not be included in the analyses.

### 1.2.2 Energy evaluation methods

#### 1.2.2.1 Total Digestible Nutrients (TDN)

Two important criteria of lucerne hay grading would be protein and energy contents. In contrast with protein no simple method exists to determine digestible energy in the laboratory. According to Putnam *et al.* (1997) TDN has been the most extensively used measure of lucerne hay quality in the United States. Total digestible nutrients represent the total of the digestible components of crude fibre, protein, fat (×2.25) and nitrogen free extract in the diet. This can be calculated from the ADF laboratory value using the following equation: TDN% (dry matter basis) = 82.38 - (.7515 × ADF%) (Bath & Marble, 1989 as cited by Putnam *et al.*, 1997).

TDN has the approximate value of Digestible Energy (DE), but several faults exist of which the most important is that TDN overestimates the DE contents of roughages. However TDN data on various feeds ensures its continued use.

Putnam *et al.* (1997) reported that some laboratories used to estimate TDN by doing a modified crude fibre (MCF) test. This test was developed in California as a more rapid one than the crude fibre (CF). A negative correlation exists between MCF and digestibility (Putnam *et al.*, 1997).

According to Putnam *et al.* (1997) it would be simpler to use ADF value itself, rather than TDN, since TDN is just a calculation from ADF.

#### 1.2.2.2 *In vitro* organic matter digestibility (IVOMD)

*In vitro* rumen fermentation is the universally preferred procedure for estimating digestibility, and it is often expressed as *in vitro* digestible dry matter (McDonald *et al.*, 1995). According to Hanson *et al.* (1988) the *in vitro* digestibility procedure is not recommended for routine, commercial hay-quality testing as it is difficult to standardise and expensive to conduct in commercial laboratories.

A preliminary study at the UFS (Van der Merwe & Fair, 1999-unpublished data) indicated that the NDF content of lucerne hay could possibly be used with a high degree of accuracy ($r^2 = 0.8$) to predict its IVOMD.

### 1.2.2.3 Relative feed value (RFV)

Relative feed value (RFV) as indicated in Table 3 combines the important nutritional factors of intake and digestibility and expresses it as an index (Rohweder *et al.*, 1976). It has no units, but allows comparisons of legume, grass, and legume-grass forages (Hannaway & Ballerstedt, 1988). RFV, which is an estimate of overall forage quality (Table 3), is calculated from estimates of intake [dry matter intake (DMI) from NDF] and digestibility or energy level [digestible dry matter (DDM) from ADF] of forages on a dry-matter basis. RFV increases as percentage ADF and NDF decrease.

Dairy farmers can use RFV to decide which hay should be fed to various groups of cattle. It is thus an index used to allocate forages to the proper livestock class with a given level of production performance. Coppock (1997) proposed the following guidelines in this regard:

| *Relative feed value* | *Use* |
|---|---|
| Over 170 | Excellent forage but should be limited to half of the forage dry matter. Maize silage is an excellent diluting forage. |
| 140 to 170 | Forage for high producing cows as sole forage |
| 120 to 140 | Forage for lower-producing cows, young heifers, or diluted with high-quality roughage for high producers |
| 100 to 120 | Dry cows (check calcium levels) and other heifer feed (add energy such as maize silage or grain). |
| Under 100 | Older heifer forage if supplemented properly. One alternative is to sell to a beef cow operation |

**Table 3 Proposed quality standards for legume, grass, and legume-grass mixed hays (Coppock, 1997)**

| Quality standard [1] | CP[2] | ADF%[2] | NDF[2] | DDM[3] | DMI % of BW[4] | RFV[5] |
|---|---|---|---|---|---|---|
| Prime | >19 | <31 | <40 | >65 | >3.0 | >151 |
| 1 | 17-19 | 31-35 | 40-46 | 62-65 | 3.0-2.6 | 151-125 |
| 2 | 14-16 | 36-40 | 47-53 | 58-61 | 2.5-2.3 | 124-103 |
| 3 | 11-13 | 41-42 | 54-60 | 56-57 | 2.2-2.0 | 102-87 |
| 4 | 8-10 | 43-45 | 61-65 | 53-55 | 1.9-1.8 | 86-75 |
| 5 | <8 | >45 | >65 | <53 | <1.8 | <75 |

[1] Standard assigned by Hay Market Task Force of the American Forage and Grassland Council (Rohweder *et al.*,1976)

[2] Analyses associated with each standard: CP = Crude protein; ADF = Acid detergent fibre; NDF = Neutral detergent fibre

[3] Digestible dry matter (DDM%) = 88.9 - 0.779 ADF (% of DM)

[4] Dry matter intake (DMI, % of body weight) = 120 / forage NDF (% of DM)

[5] Relative feed value (RFV) = [(DDM X DMI)/1.29] (Rohweder *et al.*, 1976)

Relative feed value is most valuable for animals using high roughage diets such as dairy cows and growing animals, because the RFV provides an index to rank roughage according to its digestible energy intake potential (Coppock, 1997). However, according to Grant (1994) it is important to note that RFV is only an energy intake index and does not take into account either protein, which is more expensive, or minerals in roughage. Protein level has to be evaluated separately from RFV as indicated in Table 3.

### 1.2.3 Protein evaluation methods

#### 1.2.3.1 Total forage Index (TFI)

The RFV system does not use protein in calculating its value, and forages, higher in protein, may be undervalued by this system. One alternative is to add a protein index to RFV when evaluating forage.

According to Hutjens (1998) a protein index value can be calculated in two ways. The first method is based on the value of the protein in the forage based on soya bean meal or another protein feed source. The second method described by Hutjens (1998) is more subjective because the user assigns a protein multiplier (from 1-6) based on the importance of protein in the diet of the animal. A lower multiplier (1-2) would apply to heifers' rations when protein needs are lower or when rations are based on high quality hay (some protein may not be used effectively). A high multiplier (5-6) would reflect rations where supplemental protein is needed or protein is relatively expensive.

Method two has the advantage that the user determines the importance of protein needed in the ration independent of based protein prices. Once a multiplier has been selected, it is multiplied with the percentage of crude protein in the forage to calculate a protein index. After developing a protein index, it is added to the RFV, which represents the TFI value of the forage. The formula can be modified depending on the protein value, the importance of energy value versus protein, and other ration factors. The comparable values of hay according to the RFV and TFI evaluation systems are shown in Table 4.

**Table 4 Price of hay based on different evaluation systems (models)**

| Quality standard of forage | | Relative feed value | TFI value |
|---|---|---|---|
| Prime | (24% CP, RFV 170) | R 600,00 | R 613.30 |
| Prime | (24% CP, RFV 190) | R 670,60 | R 657,10 |
| Prime | (20% CP, RFV 160) | R 564,70 | R 551,18 |
| Prime | (22% CP, RFV 160) | R 564,70 | R 571,30 |
| One | (18% CP, RFV 140) | R 494,10 | R 487,30 |
| One | (16% CP, RFV 140) | R 494,10 | R 467,20 |

The addition of a protein index to the RFV brings a more complete expression of nutrient value to the forage (Hutjens, 1995). However, TFI does not include measures of protein quality for ruminant animals. In this regard McDonald *et al.* (1995) emphasised that the crude protein fraction contains variable amounts of non-protein nitrogen. This led to the use of true protein instead of crude protein but this was unsatisfactory since no allowance was made for the nutritive value of the non-protein nitrogen fraction.

### 1.2.3.2 Digestible crude protein (DCP)

McDonald *et al.* (1995) emphasized that the crude protein content provides a measure of the nitrogen present in the food but gives little indication of its value to the animal. Before the food becomes available to the animal it must undergo digestion, during which process it is broken down into simpler substances which are then absorbed into the body. In the situation where large numbers of foods have to be evaluated on a routine basis, determination of digestible crude protein (DCP) by means of digestibility trials is impracticable. For many years there has been considerable dissatisfaction concerning the use of DCP for evaluating food proteins. This has its roots in the extensive degradative and synthetic activities of the micro-organisms of the rumen. These degradative and synthetic processes are of major importance in the nitrogen economy of the host animal since they determine the nature of the amino acid mix made available for protein synthesis at tissue level.

### 1.2.3.3 Protein requirement systems

According to AFRC (1992) a total of eight new international protein requirement systems have been published since 1977. Currently six of them are being used for dairy cattle, namely, absorbed protein (AP, United States), metabolic protein (MP, United Kingdom), amino acids (AA) absorbed in the small intestine and protein balance in the rumen (AAT - PBV, Nordic countries), crude protein (CP) in the duodenum (duodenal CP, Germany) and true protein digestible in the small intestine (PDI, France) of which the first two (AP and MP) are the most regularly used (Mallo, 1997).

Cruywagen (2000) emphasised that the main sources of variation between systems are due to microbial CP production, digestible AA content of undegraded feed protein (UDP) (originating from the same diet), and protein requirement in terms of absorbed protein. The absorption of essential amino acids (EEA) from digestible protein is vital to the maintenance, reproduction, growth and lactation of dairy cattle (NRC, 1989). NRC (1989) further described the dietary protein input as the undegradable intake (crude) protein and the degradable intake (crude) protein (DIP) needed to supply this requirement expressed as absorbed protein (AP).

The key components of the NRC (1989) protein system are the calculation of microbial protein production in the rumen and the need for non-degradable protein (NDP) for a certain production level. According to MacRae *et al.* (1993) this system discredited the importance of absorption of specific amino acids and the net requirements of end products like meat, wool and milk.

The AFRC (1992) proposed the metabolisable protein system (MP) for the quantitative nutrition of ruminant animals. This requires that factors such as degradability, efficiency of nitrogen capture, microbial yield, digestibility of microbial protein, digestibility of dietary undegraded protein and the true biological value of the absorbed nitrogen be quantified. Prediction of such dietary values is extremely difficult since the biological values of the individual proteins are no guide to their value in combinations.

#### 1.2.3.4 Ideal protein

Cole & Van Lunen (1994) have suggested that the most important single factor affecting the efficiency of protein utilisation for production of meat and other products is the balance of absorbed amino acids. According to Chen & Ørskov (1994) the concept of an ideal protein has been used to refer to the protein that provides absorbed amino acids in the proportion that gives maximum efficiency of utilization. Therefore the ideal protein can be theoretically defined as one in which the composition of the EAA absorbed from the small intestine matches exactly the amino acid requirement of the animal for production purposes (Ferreira, 1998). The net EAA requirements can be calculated as a sum of those deposits in tissue and fetus, secreted in milk, plus those used for maintenance.

The relative feed value (RFV) quality standard for roughage as proposed by Rohweder *et al.* (1976) has the disadvantage of not taking into account either protein or minerals in roughage analyses. TFI calculates a value from RFV and the protein content of the hay. However, this roughage quality standard does not take into account the quality of protein. Although ACP takes unavailable protein into account, the most important protein quality measures as proposed inter alia by AFRC (1992) are ignored. Further research is needed to develop a model for lucerne quality grading based on the most important and latest energy value and protein quality measures for ruminants. As lucerne hay is mostly used in dairy cattle diets

(Grönum *et al.*, 2000) a model to predict lucerne quality specially for dairy cattle should be developed. This model can be used to rank lucerne according to its digestible energy intake potential and protein quality.

Grönum *et al.* (2000) conducted a study in which both players, producers and consumers, in the lucerne industry concluded that the existence of timely and relevant market information would play an important role in the sustainability of the lucerne industry. This automatically includes the development of an objective grading system that could help establish a basis for the true reflection of value in prices. The purpose of this study was to develop and identify an evaluation system (model) to predict lucerne hay quality.

In Chapter 1 the variation in nutritive value of South African lucerne hay was investigated.

In Chapter 2 a study was conducted to include protein quality according to the United Kingdom metabolizable protein system (AFRC, 1992) into the TFI system to determine more accurately the quality of lucerne hay. This model was compared to existing models for the determination of lucerne hay quality.

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# CHAPTER 1

# THE NUTRITIVE VALUE OF SOUTH AFRICAN LUCERNE HAY *(MEDICAGO SATIVA)*

## 1. INTRODUCTION

Lucerne hay is a very important roughage source for livestock in South Africa (Van der Merwe & Smith, 1991). Grönum *et al.* (2000) estimated that about 3.8 million ton of lucerne hay is produced annually in South Africa. A large proportion of this lucerne hay crop is utilized by the dairy feed industry. Consequently its nutritive value for especially dairy cattle is important.

According to Blaxter (1964) nutritive value is the result of chemical composition, digestibility and intake per unit time by an animal. Linn (1992) mentioned that high quality feeds should have in addition to a high nutrient content, a consistent nutrient content, high nutrient availability, absence of mold and other substances, adequate physical characteristics as in the case with roughages to stimulate rumination, readily consumed by animals and result in production that meet or exceed expectations. It is obvious that quality and nutritive value of feeds can be regarded as synonymous. The quality of lucerne hay (nutritive value) could however vary considerably and is influenced by factors such as, harvesting at specific phenological stages, climatic factors, edaphic factors such as soil conditions, leaf losses during haymaking, storage and feeding, diseases and insects, weeds, lucerne cultivar, moisture content during storage, sites, water supply, fertilization and environmental exposure (Bezeau & Sonmor, 1964; Cords, 1973; Hanson *et al.*, 1988; Loper, 1968; Putnam *et al.*, 1997; Rankin, 1997; Van Wyk *et al.*, 1955; Woodman & Evans, 1935).

The variation in quality of lucerne hay hampers the accurate formulation of ruminant diets especially for dairy cattle. Van Wyk *et al.* (1955) published the first nutritive values for lucerne in South Africa, based on only 38 samples. This was followed by Van der Merwe (1970) who classified the quality of lucerne according to stage of harvesting (bloom stage). Van der Merwe (1970) emphasised that the nutritive value of roughages can vary

considerably. Therefore their values can serve at best as a general guide and should be verified by actual analysis. These values also do not include analysis for acid detergent fibre (ADF), neutral detergent fibre (NDF) and the extent of protein degradation. Mertens (1992), McDonald *et al.* (1995) and NRC (2001) reported values in this regard. The only South African particulars in this regard in the available literature is that of Erasmus *et al.* (1990) who obtained only three samples from various locations and pooled it to obtain an average sample. This clearly illustrates the urgent need for more reliable data on the nutritive value of South African lucerne hay.

The object of this study was to evaluate the variation- and expand the existing nutritive value database of lucerne hay for use in lactating dairy cattle diets.

## 2. MATERIALS AND METHODS

### 2.1 Sampling

Two hundred and ten lucerne hay samples for chemical analyses and *in vitro* digestibility were obtained from different cuttings during two seasons (100 samples for 1998/1999 and 110 for 1999/2000), at different times in a season and from different lucerne producing areas (sites) in the Northern Cape, South Africa. Hundred and eighteen of these samples were used for essential amino acid analysis. Thirty of the 210 samples originated from Douglas and an additional 42 lucerne hay samples were obtained during one season (1999/2000) to estimate protein degradation (*in sacco*). The origin of lucerne hay samples for chemical analysis and estimates of protein degradation are shown in Table 1 and Figure 1. It is evident that the samples were from different locations in the Northern Cape province.

Representative samples of different lucerne cuttings were obtained from bales with a 1.5 cm (in diameter) probe, mounted on an electric drill. At least 15 bales taken at random in a lot were sampled to ensure a statistically valid sample. A lot included the same species, variety, cutting, land and time.

**Table 1** **The distribution of lucerne hay samples used for chemical analysis and estimates of effective degradability.**

| LOCALITIES | SEASON | NUMBER OF SAMPLES | SCREEN SIZE (mm) |
|---|---|---|---|
| **Chemical analyses** | | | |
| Hartswater | 1998/1999 | 20 | 0.8 |
| | 1999/2000 | 10 | 1 |
| Magogong | 1998/1999 | 20 | 0.8 |
| | 1999/2000 | 10 | 1 |
| Tadcaster | 1998/1999 | 20 | 0.8 |
| | 1999/2000 | 5 | 1 |
| Jan Kempdorp | 1998/1999 | 20 | 0.8 |
| | 1999/2000 | 5 | 1 |
| Bull Hill | 1998/1999 | 10 | 0.8 |
| | 1999/2000 | 15 | 1 |
| Hartsvallei | 1998/1999 | 10 | 0.8 |
| | 1999/2000 | 5 | 1 |
| Douglas | 1999/2000 | 40 | 0.5 |
| Prieska | 1999/2000 | 9 | 0.5 |
| Modderrivier | 1999/2000 | 6 | 0.5 |
| Barkly West | 1999/2000 | 5 | 0.5 |
| ***In sacco* degradability** | | | |
| Hartswater | 1999/2000 | 10 | 10 |
| Magogong | 1999/2000 | 15 | 10 |
| Jan Kempdorp | 1999/2000 | 2 | 10 |
| Bull Hill | 1999/2000 | 11 | 10 |
| Hartsvallei | 1999/2000 | 4 | 10 |
| Douglas | 1999/2000 | 30 | 10 |

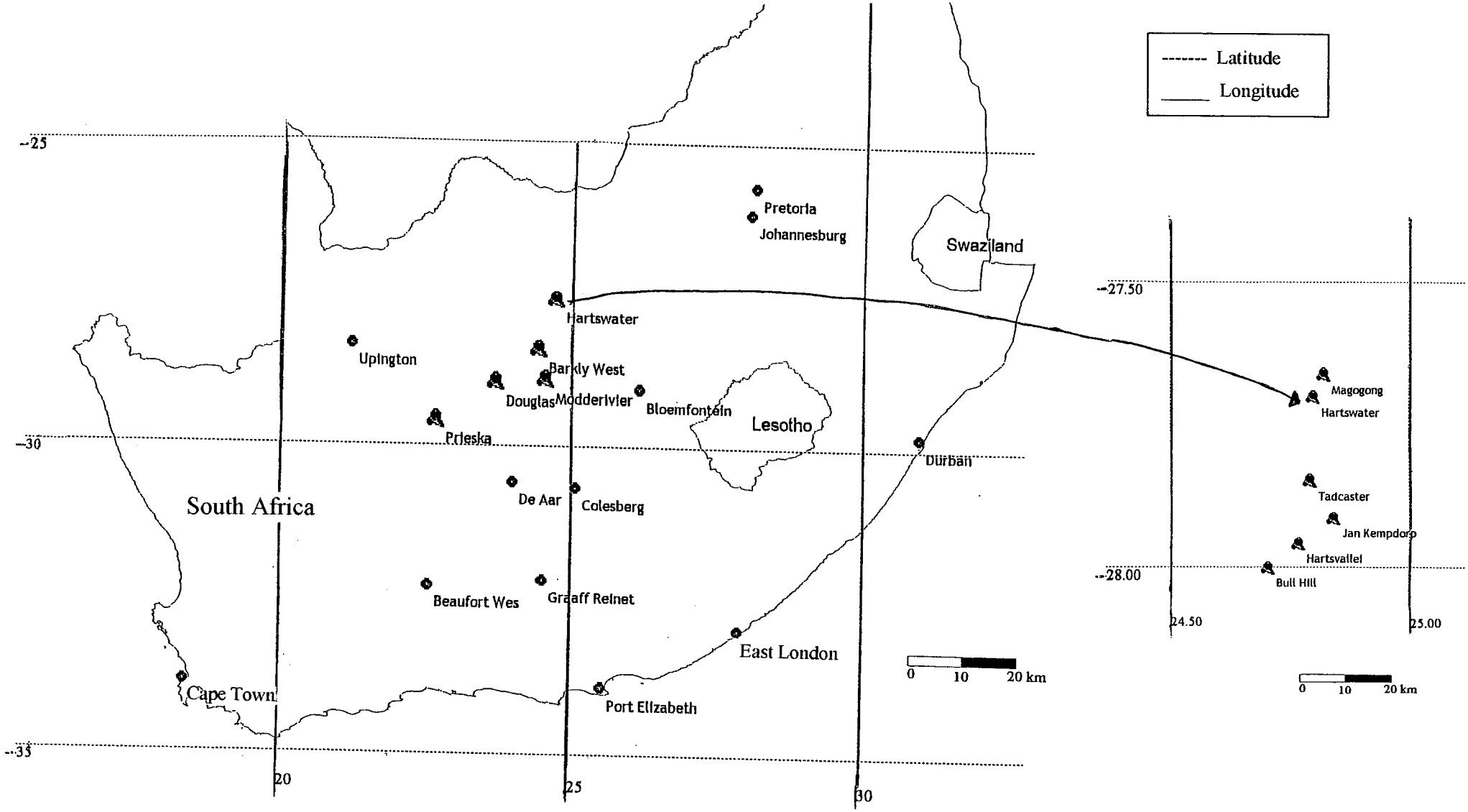


**Fig. 1. Origin ( ) of lucerne hay samples**

The 15 samples of each lot were thoroughly mixed and stored in sealed, clean plastic bags. Before chemical analysis the samples were milled in a Wiley mill to pass through screens with varying screen sizes as indicated in Table 1. The 72 lucerne hay samples used for the *in sacco* technique were milled through a 10 mm screen to ensure a homogeneous sample (De Waal, 1994). This particle size is also more representative of course roughage used in dairy diets. A representative sample was obtained in duplicate using the quartering method.

## 2.2 Chemical analyses

All chemical analysis was carried out in duplicate.

### 2.2.1 Dry matter and Moisture content

Method:

Approximately 2 g of each lucerne hay sample was weighed accurately into a dry 30 mℓ porcelain crucible and dried in an oven at 100°C for a minimum of 16 hours (overnight) to a constant mass (AOAC, 1984).

Calculation:

$$\% \text{ Dry matter (DM)} = \frac{\text{MCDS} - \text{CM}}{\text{MOCS} - \text{CM}} \times \frac{100}{1}$$

Where CM = crucible mass

MOCS = mass of crucible plus sample

MCDS = mass of crucible plus dried sample

% Moisture = 100 - %DM

All samples for further analysis were dried in an oven as described above.

### 2.2.2 Ash and Organic matter (OM)

Method:

The same procedure was followed as described for dry matter (DM). After determining the DM content the crucible with contents was placed in a cool muffle furnace and ashed at 550°C for a minimum of four hours (AOAC, 1984).

Calculation:

$$\% \text{ Ash} = \frac{\text{MCAS - CM}}{\text{MCOS - CM}} \times \frac{100}{1}$$

Where CM = mass of crucible

MCOS = mass of crucible plus sample

MCOS = mass of crucible plus ash

% Organic matter = 100 - %Ash

### 2.2.3 Crude fibre (CF)

Crude fibre was determined according to the method of AOAC (1984).

Reagents:

1. 0.128 M Sulphuric acid solution: 6.96 mℓ of 98% $H_2SO_4$ was added to distilled water and made up to one litre with distilled water.
2. 0.313 M Sodium hydroxide solution: 12.5 g NaOH dissolved in distilled water and made up to one litre.
3. N-Octanol.
4. Acetone.

Method:

Approximately 1 g of the lucerne hay sample was weighed accurately into a clean, dry and weighed sintered glass crucible (porosity two) and placed in a hot extraction unit of a Tecator Fibretec System. To each crucible 150 mℓ of boiling sulphuric acid solution and three drops of octanol were added. The solution was boiled for exactly 30 minutes and then filtered. Thereafter it was washed three times with hot distilled water. Then 150 mℓ sodium hydroxide (NaOH) solution and 3 drops of octanol were added to each crucible. The solution was boiled again for 30 minutes, filtered and washed three times with hot water before rinsing twice with acetone.

The contents were then dried overnight at 100°C in a forced draught oven, cooled for 30 minutes in a desiccator, weighed and ashed in a furnace at 550°C for a minimum of four hours.

Calculation:

$$\% \text{ Crude fibre} = \frac{\text{RCD} - \text{RCA}}{\text{Dry sample mass}} \times \frac{100}{1}$$

Where RCD = residue in crucible after drying

RCA = residue in crucible after ashing

### 2.2.4 Acid detergent fibre

ADF was determined by the method of Goering & Van Soest (1970).

Reagents:

1. Acid Detergent Solution (ADS): 20 g cetyl trimethyl ammonium bromide dissolved in one litre 1N sulfuric acid ($H_2SO_4$).
2. Acetone

Method:

Approximately 1 g aliquot of the lucerne hay sample was weighed into a clean, dry and weighed sintered glass crucible and placed in a hot extraction unit of a Tecator Fibertec System. To each crucible 100 ml of cold ADS was added.

The solution was boiled for exactly 60 minutes and then filtered. Thereafter it was washed three times with hot water before being rinsed twice with acetone.

The contents were then dried overnight at 100°C in a forced draught oven, cooled for 30 minutes in a desiccator, weighed and ashed at 550°C for a minimum of four hours.

Calculation:

$$\% \text{ ADF} = \frac{\text{RCD} - \text{RCA}}{\text{Dry sample mass}} \times \frac{100}{1}$$

Where RCD = residue in crucible after drying

RCA = residue in crucible after ashing

### 2.2.5 Neutral detergent fibre (NDF)

NDF was determined according to the method described by Robertson & Van Soest (1981).

Reagents:

1. Neutral detergent solution (NDS):
   -30 g sodium laurel sulphite
   -18.61 g EDTA-sodium salt ($Na_2EDTA.2H_2O$)
   -16.81 g sodium borate decahydrate
   -4.56 g disodium hydrogen phosphate anhydrous
   -10m$\ell$ 2-etohoxy ethanol - purified
   -1 $\ell$ distilled water
2. Acetone

Method:

The same as described for ADF except that 100 m$\ell$ cold NDS was added to each crucible.

Calculation:

$$\% \text{ NDF} = \frac{\text{RCD} - \text{RCA}}{\text{Dry sample mass}} \times \frac{100}{1}$$

Where RCD = residue in crucible after drying

RCA = residue in crucible after ashing

### 2.2.6 Non-fibre carbohydrates (NFC)

Non fibre carbohydrate (NFC) was calculated by difference (Mertens, 1988; Mertens, 1992; NRC, 2001; Sarwar *et al.*, 1992; Varga & Kononoff, 1999) as follows:

$$\% \text{ NFC} = 100 - (\% \text{ Neutral detergent fibre} + \% \text{ Crude protein} + \% \text{ Fat} + \% \text{ Ash})$$

### 2.2.7 Fat

Fat was analysed according to the method described by the Official and Tentative Methods of the American Chemist Society (1985).

Reagents:

1. Hexane

Method:

A lucerne hay sample of approximately 2 g was accurately weighed on a Whatman no 1 filter paper and placed in an extraction thimble. The thimble was placed in a Tecator Fibertec System extraction unit and extraction was maintained for at least four hours. Then the flat bottomed flask was removed from the heating elements just prior to complete evaporation of the hexane. The flasks were dried in an oven at 105°C and weighed. A blank extraction was also carried out (thimble, filter paper and wadding, but no sample).

Calculation:

$$\% \text{ Fat} = (D - E - C) \times 100$$

Where D = the mass of sample flask after extraction and
E = mass of sample flask before extraction.
C = (a – b)

Where a = was the mass of the blank flask after extraction and
b = the mass of the blank flask before extraction

### 2.2.8 *In vitro* organic matter digestibility (IVOMD)

IVOMD was determined according to the two–phase technique described by Tilley & Terry (1963) as modified by Engels & Van der Merwe (1967).

Reagents:

1. McDougall's artificial saliva: dissolve 49 g $NaHCO_3$, 46.5 g $Na_2HPO_4.12H_20$, 2.85 g KCl, 2.35 g NaCl and 0.6 g $MgSO_4.7H_2O$ dissolved in 4.5 ℓ of distilled water in a 5 litre volumetric flask. 5 mℓ of a 4% m/v $CaCl_2$ was added, mixed and made up to volume with distilled water.
2. Urea solution: 8.6 g urea dissolved in 1 ℓ distilled water.
3. Hydrochloric acid solution: 200 mℓ HCl added to 800 mℓ distilled water.

4. Pepsin solution: 8 g pepsin dissolved in 1 ℓ distilled water.

Sample and rumen fluid:

1. The samples were prepared as described (par. 2.1).
2. Twelve rumen fistulated sheep was fed a good quality lucerne diet *ad libitum* for at least 7 days prior to collection of rumen fluid.

Method:

Approximately 0.5 g of a lucerne sample was weighed accurately in duplicate into test tubes. The saliva solution was placed in a waterbath at 39°C and carbon dioxide gas ($CO_2$) was bubbled through the solution while the solution was thoroughly mixed at the same time. The urea solution (3.5 mℓ) was added to each of the test tubes (blanks, standards and samples) and the solution mixed with the feed sample by swirling it gently. The test tubes were then placed in the waterbath at 39°C. Rumen fluid was taken from the rumen fistulated sheep, filtered through cheesecloth, added to the saliva mixture and mixed continuously throughout the period of addition to the test tubes. The rumen–saliva mixture, (50 mℓ) was then added to each test tube including the blanks and standards. Immediately after adding the rumen-saliva mixture, each tube was flushed for about 15 seconds with carbon dioxide before the tubes were sealed firmly with a slit rubber stopper to allow excess gas to escape. This helped to establish an aerobic environment in the test tube.

A standard fodder sample with known digestibility, e.g. Panicum was also included in the test run to serve as a check in the test conditions. The tube contents were mixed gently by swirling 3 times a day and kept at 39°C. After 48 hours incubation a 7 mℓ HCl solution was added to each tube. Pepsin solution 11.5 mℓ was then added, the tubes swirled, and the sides of the tubes rinsed with warm water (about 45°C). The tubes were then incubated for a further 48 hours while the contents were swirled twice a day.

At the end of this period the contents of each tube were filtered through a Gooch crucible packed with asbestos fibre and the residue rinsed three times with hot water. All the matter from the test tubes was quantitatively transferred to the Gooch crucibles. The Gooch crucibles plus contents were dried at 100°C for 24 hours and then weighed.

After the mass of crucibles was recorded they were placed in a muffle furnace and ashed at 550°C for 5 hours whereafter they were allowed to cool in a desiccator for 30 minutes and weighed again. The dry - and organic matter content was determined on a separate set of samples.

Calculations:

$$\%IVOMD = \frac{\text{Sample OM content} - \text{undigested sample OM}}{\text{Sample OM content}} \times \frac{100}{1}$$

Where OM = Organic matter (par. 2.2.2)

### 2.2.9 Metabolizable energy (ME)

ME was calculated as follows (McDonald *et al.*, 1995)

$$ME\ (MJ/kg\ DM) = 0.016\ IVOMD$$

Where IVOMD = g *in vitro* organic matter digestibility per kg dry matter

### 2.2.10 Crude protein (CP)

Crude protein was determined using the Dumas method of combustion with a LECO FP2000. The combustion method relies on the Dumas principle (Randall, 1993). A sample is combusted in an atmosphere of oxygen to give oxides of nitrogen and other gases.

Method:

An oven dried lucerne sample of approximately 1 g was accurately weighed into a reusable ceramic boat and placed into a purge chamber of the horizontal furnace. The boat was pushed into the furnace, oxygen was allowed to flow directly onto the sample and combustion initiated at 950°C. The resulting gaseous products were passed through a thermoelectric cooler, removing most of the moisture. Gases were collected in a ballast chamber. Nitrogen (N) was measured using a thermal conductivity detector against a background of pure helium. The detector signal was transmitted to the computer via a microprocessor and the data was analysed to obtain the nitrogen content of the sample. Crude protein (CP, g/100 g DM) was calculated as N (g/100 g DM) × 6.25.

### 2.2.11 Acid detergent fibre–nitrogen (ADF-N)

ADF–N was determined by the method of Goering *et al.* (1972)

Method:

The same procedure was followed as described for ADF, but instead 2 g original sample was used and the contents in the crucibles were not ashed. The total content was transformed in crucibles for N-analyses.

Calculation:

$$\% \text{ ADF} - \text{N} = \frac{\text{g N} \times 100}{\text{Dry sample mass}}$$

### 2.2.12 Degradability

#### 2.2.12.1 Animals

The *in sacco* technique described by Erasmus *et al.* (1988) and Erasmus *et al.* (1990), with some alterations was used. Twelve Dorper lambs with a mean (empty stomach) mass of 49.8 kg (SD ± 7.1) were individually housed indoors in metabolism crates under continuous illumination of urine and manure throughout the trial.

The lambs were fitted with rumen cannula (40 mm internal diameter) which facilitate manual placement of bags in the ventral portion of the rumen. They were dosed with a wide spectrum worm killer (Valbantal®) against internal parasites two weeks before the degradability determinations were begun.

#### 2.2.12.2 Basal diet

The 12 Dorper lambs with an average DM intake of 1.54 kg/d were fed a complete dairy cattle diet on *ad libitum* basis. No species differences were observed (P>0.05) by Huntington & Givens (1997) with overall mean degradability of 55.8% and 55.6% for lucerne hay in sheep and dairy cows respectively. Similarly Nandra *et al.* (2000) found no significant species differences for protein degradation of lucerne hay between sheep and dairy cattle.

During the degradation study the experimental animals received 10% more feed that they consumed the previous day. A basal diet was formulated (Table 2) to provide in the nutrient

**Table 2 Physical and chemical composition of the basal diet on an air dry basis**

| ITEM | CONTENT |
|---|---|
| **Physical composition** | |
| Lucerne hay (%) | 50.00 |
| Maize meal (%) | 36.50 |
| Molasse (kalori 3000) (%) | 4.00 |
| Wheat bran (%) | 0.19 |
| Hominy chop (%) | 1.00 |
| Cotton seed oil cake (%) | 0.80 |
| Gluten feed (%) | 2.40 |
| Gluten prime (%) | 0.35 |
| Soya oil cake (%) | 1.11 |
| Sunflower oil cake (%) | 2.10 |
| Fishmeal (%) | 0.60 |
| Limestone (%) | 0.58 |
| Salt (%) | 0.12 |
| Monocalcium phosphate (%) | 0.07 |
| Urea (%) | 0.16 |
| Premix[1)] (g/t) | 200.00 |
| **Chemical composition** [2)] | |
| Dry matter (%) | 89.64 |
| Crude protein (%) | 14.22 |
| Crude fibre (%) | 15.58 |
| Acid detergent fibre (%) | 19.68 |
| Neutral detergent fibre (%) | 24.72 |
| Fat (%) | 2.89 |
| Ca (%) | 1.02 |
| P (%) | 0.37 |
| Metabolizable energy (MJ/kg) | 9.79 |

[1)] Minerals and vitamins; grams per ton

[2)] Values Van der Merwe & Smith (1991)

requirements of lactating dairy cows with a 600 kg live weight and 25 kg daily milk production (NRC, 1989). Allotments were offered twice daily at 12–hour intervals, namely at 08h00 and 20h00.

### 2.2.12.3 Preparation of samples

Seventy-two samples were prepared as described (par. 2.1) and subjected to *in sacco* degradability measurements.

*(a) Bags*

Dacron bags measuring 150 x 80 mm and with a pore size of 53 ± 10-µm were used for *in sacco* incubation. The artificial fibre bags were made according to the specifications described by Cronje (1992). Bags were made with double seams sown using a lockswitch (16 stitches/cm) with a No. 10 fine needle and polyester thread (Huntington & Givens, 1997), and were closed by means of a 250 mm length of braided fishing line.

*(b) Incubation*

A lucerne hay sample of mass approximately 5 g, moisture free, which had been milled to pass through a 10 mm screen (see sampling) was accurately weighed into each bag (≈ 15 mg DM $cm^{-2}$ bag surface area). In order to avoid period effects all samples were incubated simultaneously (complete exchange method; Paine *et al.*, 1981) for each of the following durations:

Day 1 – 1, 4 and 12 hours

Day 2 – 2, 8 and 24 hours

According to Meyer (1985) the N in lucerne hay is highly soluble and calculation of degradation over a 12-hour incubation period is adequate.

Dry matter and N disappearance were measured in duplicate with each of three sheep allocated per sample as recommended by Mehrez & Ørskov (1977), giving a total of six estimates per sample. The experimental design is shown in Table 3.

**Table 3 Experimental design for the degradability study**

| | | Period (days) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Day 1 | Day 2 | Day 1 | Day 2 | Day 1 | Day 2 |
| | | | | Incubation time (h) | | | |
| **Animals no.** | **Lucerne sample no.** | 1 h | 2 h | 4 h | 8 h | 12 h | 24 h |
| 1 | 1 | # | # | # | # | # | # |
| 2 | 1 | # | # | # | # | # | # |
| 3 | 1 | # | # | # | # | # | # |
| 4 | 2 | # | # | # | # | # | # |
| 5 | 2 | # | # | # | # | # | # |
| 6 | 2 | # | # | # | # | # | # |
| 7 | 3 | # | # | # | # | # | # |
| 8 | 3 | # | # | # | # | # | # |
| 9 | 3 | # | # | # | # | # | # |
| 10 | 4 | # | # | # | # | # | # |
| 11 | 4 | # | # | # | # | # | # |
| 12 | 4 | # | # | # | # | # | # |

# = each representing a sample in duplicate

After removal from the rumen, bags were washed in running water until the fluid squeezed from the bags was clear, and dried for 24 hours at 60°C. The contents of bags were removed and milled in a Wiley mill to pass through a 0.8 mm sieve. Milled samples were stored in polyethylene vials for later analyses of nitrogen by LECO procedures (par. 2.2.10). Incubations were repeated when DM disappearance varied more than 10% from established disappearance curves (Erasmus *et al.*, 1990). However the differences between days and sheep were minimal.

The percentage DM and N disappearance at each incubation time was calculated from the proportion remaining after rumen incubation:

$$\text{Degradability} = \frac{\text{Initial N} - \text{N after incubation}}{\text{Initial N}}.$$

The degradation rate was adapted to the equation as suggested by Ørskov & McDonald (1979):

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

Where p = proportion degraded at time t

a, b and c = non–linear parameters estimated by an iterative least square procedure (Du Toit & Herbst, 1981)

The degradation rate of the b fraction is described by c, the fractional rate constant/h and a + b represent the maximum extent of degradation or the asymptote of the equation. By introducing the fractional outflow rate, $r^1 = 0.08$ for dairy cattle (McDonald *et al.*, 1995), the effective protein degradation (P) was calculated as follows (Ørskov & McDonald, 1979):

$$P = a + bc/(c + r^1)$$

Where a = an intercept representing soluble protein.

b = insoluble but potentially degradable fraction.

c = degradation rate of the b fraction.

$r^1$ = fractional outflow rate

### 2.2.13 Metabolizable Protein (MP)

MP was determined according to the United Kingdom (UK) system as described by AFRC (1992).

#### 2.2.13.1 Effective rumen degradable protein (ERDP)

The microbial demand for protein or effective rumen degradable protein (ERDP) for each tested lucerne hay sample was calculated as proposed by the AFRC (1992):

$$ERDP = CP \times [0.8a + bc/(c + r^1)]$$

Where CP = Crude protein of the tested lucerne hay sample
a, b and c = Fitted parameters as described in par. 2.2.12.3

### 2.2.13.2 Fermentable metabolizable energy (FME)

The energy available to the rumen micro-organisms in terms of fermentable metabolizable energy (FME) from lucerne hay was calculated as:

$$FME\ (MJ/kg\ DM) = ME - ME_{fat}$$

Where ME (MJ/kg DM) = ME of the lucerne hay sample
$ME_{fat}$ (MJ/kg DM) = 35 MJ/kg

### 2.2.13.3 Digestible microbial protein (DMP)

The contribution of microbial protein (DMP) to the truly absorbed amino acids of lucerne hay was calculated as:

$$DMP\ (g/kg\ DM) = FME\ (y \times 0.75 \times 0.85) = 0.6375(FME\ y)$$

Where y = 11 for lactation

### 2.2.13.4 Digestible undegraded true protein (DUP)

The truly digestible undegraded true protein (DUP) of lucerne hay was calculated as:

$$DUP\ (g/kg\ DM) = 0.9[CP\ (1 - a - bc/(c+r^1)) - 6.25ADF\text{-}N]$$

Where a, b and c = The non–linear parameters as described in degradability by Ørskov & McDonald (1979).
ADF-N = Acid detergent fibre nitrogen of the tested lucerne hay sample

### 2.2.13.5 Metabolizable protein (MP)

The MP supplied by lucerne hay was calculated as:

MP (g/kg DM) = DMP + DUP.

### 2.2.14 Essential amino acids (EAA)

Reagents:

1. Internal standard: 0.05 g Norvaline was dissolved in 0.2 N HCI and topped up to 50 mℓ with distilled water.
2. 6 N HCl (1 l HCI + 650 mℓ $H_2O$)+ 1.6 g Fenol.

Method:

The acid hydrolysis technique described by AOAC (1984) was used. The weight of the sample was calculated from the protein content as follows:

$$\text{Sample weight} = \frac{0.08}{\text{protein value} \times 100}.$$

The calculated lucerne sample mass was accurately weighed into a Schott glass bottle (with stopper) and 1 mℓ internal standard and 40 mℓ of solution two was added. The bottle with contents was placed on an ultrasonic bath and the air removed with a vacuum pump until all foam disappeared. Then the bottle was filled with nitrogen, sealed, and placed on a heating block for 24 hours at 110°C. Thereafter the contents were poured into a 50 mℓ volumetric flask, topped up to 50 mℓ with distilled water and stored in a plastic container for further analysis. Essential amino acid (EAA) analysis was determined with a BECKMAN SYSTEM 7300 high performance analyser after 24 hours of acid hydrolysis described above.

Tryptophan was not quantified. However, tryptophan does not appear to be a limiting amino acid in the available literature cited by Ferreira (1998) and is thus apparently not such an important factor in ruminant nutrition.

## 2.3 Statistical analysis

Standard deviation and coefficients of variation were calculated using Microsoft Map program (1997). Maximum and minimum values were calculated using standard deviations (Berenson & Levine, 1986)

# 3. RESULTS AND DISCUSSION

## 3.1 Energy

### 3.1.1 Dry matter

The moisture content of lucerne hay must be within a specific range for effective storage. Hay stored too wet will undergo pronounced fermentation with the production of heat. The feeding value may be greatly decreased because of mould or the loss of nutrients that occurs during extensive fermentation.

According to Table 4 the average dry matter content of lucerne hay in the present study was slightly higher than the approximately 90% reported by Morrison (1961), Van der Merwe & Smith (1991) and NRC (1996). According to Morrison (1961) the primary object in haymaking is to dry the green plants to such an extent that the hay can be safely stored without heating unduly or becoming mouldy. Hanson *et al.* (1988) mentioned that high-moisture lucerne hay (above 20% moisture) causes damage like heating, molding and respiration losses of soluble carbohydrates and recommended a safe upper limit moisture content of 15%. However Smith *et al.* (1996) are of the opinion that the upper moisture level depends on the type of hay, density and size of bale, drying conditions after baling and other factors and recommended 18% for lucerne hay. In the present study the highest moisture content recorded (Table 4) was safely below the critical moisture level. Therefore heat and mould damage were unlikely to occur.

### 3.1.2 Ash

Ash is an approximate measure of the mineral content and other inorganic matter which remains as residue after the combustion of organic matter.

The mean ash content of lucerne hay in Table 4 was similar to the value reported by NRC (1989). Various researchers (Van Wyk *et al.*1955; Morrison, 1961; Van der Merwe & Smith, 1991 and Mertens, 1992) reported slightly lower values ranging from 7.5–9.5% ash.

**Table 4 Energy composition and utilization of lucerne hay on a dry matter basis**

| CHEMICAL ANALYSIS | n | Min[2)] | Average | Max[3)] | SD[4)] | CV[5)] |
|---|---|---|---|---|---|---|
| Dry matter (%) | 210 | 90.65 | 93.55 | 96.44 | 0.97 | 1.03 |
| Ash (%) | 110 | 3.55 | 10.54 | 17.54 | 2.33 | 22.11 |
| Organic matter (%) | 110 | 82.46 | 89.46 | 96.45 | 2.33 | 2.61 |
| Crude fibre (%) | 209 | 18.24 | 35.40 | 52.56 | 5.72 | 16.16 |
| Acid detergent fibre (%) | 208 | 21.89 | 39.98 | 58.08 | 6.03 | 15.08 |
| Neutral detergent fibre (%) | 209 | 26.70 | 48.26 | 69.82 | 7.19 | 14.89 |
| Non fibre carbohydrates (%) | 109 | 4.17 | 19.14 | 37.07 | 6.51 | 34.02 |
| Fat (%) | 210 | 0.07 | 1.66 | 3.25 | 0.53 | 31.98 |
| *In vitro* organic matter digestibility (%) | 210 | 56.65 | 67.58 | 78.51 | 3.64 | 5.39 |
| Metabolizable energy (MJ/kg DM) | 30 | 7.76 | 10.64 | 13.51 | 0.96 | 9.02 |
| Metabolizable $energy_{Fat}$ (MJ/kg DM) | 30 | -0.03 | 0.44 | 0.91 | 0.16 | 35.50 |
| Fermentable metabolizable (MJ/kg DM) | 30 | 7.66 | 10.19 | 12.73 | 0.85 | 8.29 |
| a[1)] (%) | 72 | -16.90 | 17.98 | 52.86 | 0.12 | 64.67 |
| b[1)] (%) | 72 | -123.98 | 73.90 | 271.77 | 0.66 | 89.26 |
| c[1)] (%) | 72 | -57.62 | 18.43 | 94.47 | 0.25 | 137.56 |
| Effective ruminal dry matter degradability (%) | 72 | 17.52 | 41.28 | 65.04 | 0.08 | 19.19 |

[1)] Fitted parameters a, b and c derived from the *in sacco* determination of effective ruminal dry matter degradability (ERDMD)

[2)] Average minus 3 x Standard deviation (Berenson & Levine, 1986)

[3)] Average plus 3 x Standard deviation (Berenson & Levine, 1986)

[4)] Standard deviation

[5)] Coefficient of variation

In the current study the ash content of lucerne hay varied from 3.6% to 17.6% (CV = 22%). Morrison (1961) suggested that lucerne hay has a higher mineral content than grains like maize and wheat. This is because of the accumulation of minerals in the leaves during

growth, soil washed onto the growing plants by rain, and dust settling on the roughage before it is stored. Ward *et al.* (1957) confirmed earlier studies, which showed that lucerne ash stimulated the ability of sheep to digest low quality roughage. Compared to grasses, lucerne hay has a rich mineral profile. According to Hanson *et al.* (1988) calcium and magnesium concentrations in lucerne are greater than for grasses at equivalent stages of maturity. The high calcium and potassium content of lucerne hay could play a mayor role in the dietary anion-cation balance of dairy diets. In addition to the 14 mineral elements for which requirements are defined by the NRC (1989) for dairy cattle, there are at least 12 more which have been shown to be required by some animals (McDowell, 1992). Coppock (1997) is of the opinion that lucerne hay is a richer source of these exotic 12 minerals than grasses.

### 3.1.3 Organic Matter (OM)

Organic matter is the material that is available for the digestion process. Compared to grasses, lucerne has a higher % ash (Ward *et al.*, 1957) and thus a lower % organic matter content. Many organic compounds contain mineral elements as structural components. Protein, for example, contains sulphur, and many lipids, and carbohydrates contain phosphorus. The average OM value of lucerne hay in this study is because of its low CV (Table 4), a reliable guideline.

### 3.1.4 Structural Carbohydrates

According to Mertens (1992) structural carbohydrates are located in cell walls and provide the structural support needed for plants to grow upright. Plant cell walls consist of pectin, hemicellulose, cellulose and a non-carbohydrate polymer, lignin. These compounds are listed in the descending order of their degradability. Pectin is so digestible that it is best considered as a non-fibre carbohydrate similar to sugar.

The traditional measure for fibre according to the Weende analysis is crude fibre (CF) as described by Van der Merwe & Smith (1991). CF is the residue remaining after successive boiling in dilate acid and alkali (NRC, 1989). CF measures the cellulose and part of the lignin. A modified crude fibre (MCF), which includes the ash or mineral fraction, was used in some stages, notably in California, to evaluate lucerne (Baker & Ball, 2001). This test

was developed in California as a more rapid test than the CF test. Hannaway & Ballerstedt (1988), proposed that MCF has a negative correlation with animal digestibility (as MCF increases, digestibility decreases). However, according to several researchers (Van Soest, 1968; Lofgren & Warner, 1970 and Mertens, 1982) CF is an incomplete measure of the less digestible part of feeds as it fails to measure hemicellulose and most of the lignin. They suggest that acid detergent fibre and neutral detergent fibre are much more useful and accurate measures of the fibre component of feeds than are values for crude fibre. However, the Canadian Feeds Act and Regulations continues to require that CF levels be included in the guaranteed analysis of manufactured feeds (Baker & Ball, 2001).

Smith (1992) also proposed that the most commonly used fibre measurements in dairy cattle diets are ADF and NDF, which equates to digestibility and intake respectively. Of the two, NDF seems to be more suitable for determining fibre requirements (Allen, 1991a). According to Hannaway & Ballerstedt (1988) hay quality is also related to its energy content. The ADF test evaluates the available energy in lucerne hay. ADF is the plant fibre that remains after acid detergent removes part of the digestible cell wall material and the cell contents, and consists of cellulose, lignin, heat damaged proteins (ADF-N), and acid insoluble ash (Morse & Sedivec, 1990; Ball & Lauriault, 1999). Hannaway & Ballerstedt (1988) and Cherney & Hall (1997) found that ADF has a negative correlation with estimated digestible energy (DE). In the United Kingdom the ADF method has been modified slightly (MADF) by increasing the duration of boiling and acid strength. Morse & Sedivec (1990) concluded that since both total digestible nutrients (TDN) and netto energy (NE), values can be estimated from ADF, it is critical that ADF is determined as precisely as possible. According to McDonald *et al.* (1995) available data suggests that ADF is the measure of fibre which is the most highly correlated with the fat content of milk.

McDonald *et al.* (1995) noted that NDF is the chemical component of foods that determines their rate of digestion the best. NDF is the plant fibre that remains after the removal of cell contents by neutral detergent. According to Mertens (1992) NDF is not a chemically pure entity, but represents substances (ADF fraction plus hemicellulose) in plants that are difficult to digest and break down into small particle sizes. Accordingly there is a negative relationship between the NDF content of feeds and the rate at which they are digested. One

consequence of this relationship is that feeds that are equal in digestibility but differ in NDF content will promote different intakes. McDonald *et al.* (1995) illustrated this phenomenon by means of two strains of pasture plants, namely grasses and legumes. At equal digestibility, legumes (e.g. lucerne) contain less NDF and are consumed in quantities about 20 percent greater than grasses. NDF can be used to predict intake and is therefore one of the most valuable analyses to do when lucerne hay is used in dairy rations. It can also be useful when lucerne is included in beef rations that rely primarily on roughages. Estimating NDF values too high or too low can have tremendous implication on intake, animal performance, and health. Mertens (1992) is of opinion that although rate of digestion and intake are related to the concentration of NDF in ruminant foods, the physical form of the cell walls also affects intake. The optimal fibre level resulting in maximum fat corrected milk, has also been shown to be more consistent across forages for NDF than for ADF (Allen & Mertens, 1988). According to Welch & Smith (1969) rumination activity in cattle is highly correlated with NDF intake ($r = 0.94$). Mertens (1988) proposed the NDF-Energy Intake System for formulating dairy rations. The objective of the NDF-Energy Intake System is to identify the optimal NDF content that will maximize forage and fibre intake, while meeting energy requirements for target milk yield and body weight changes.

Mean, minimum, maximum, standard deviation and coefficient of variation values for energy related analyses are shown in Table 4. The mean CF content of lucerne hay in the current study (Table 4) was higher than the 30.4% and 28.4% reported by Erasmus *et al.* (1990) and Van der Merwe & Smith (1991) respectively for lucerne hay in South Africa. This also applies for overseas mean values given by Mertens (1992) and McDonald *et al.* (1995), namely 27.8% and 30.2% respectively.

In the present study CF varied more (Table 4) than the SA values reported by Van der Merwe & Smith (1991), ranging from 23.6% pre bloom to 31.6% for post bloom cut hay. Mertens (1992) reported corresponding CF values of 21.9% for lucerne hay cut at an early vegetative stage compared to 33.7% for hay cut at full bloom. The larger variation of CF values found in the current study could possibly be attributed to the influence of other factors than the cutting stage per se.

RFV is an index that combines ADF (digestibility) and NDF (intake) nutritional factors to arrive at one number to measure and compare forage quality (Linn *et al.*, 1987). Therefore ADF and NDF are important in evaluating lucerne hay quality for dairy rations. In the current study the mean ADF value (Table 4) was almost identical to the value published by Erasmus *et al* (1990) and higher than the 33.8% and 34.0% reported by NRC (1996) and Mertens (1992) respectively. The variations in ADF values in Table 4 were larger than those found in the available literature. According to NRC (1996), ADF values ranged from 31.9% for lucerne hay at an early bloom stage to 38.7% at full bloom. Mertens (1992) published ADF values that ranged from 28.0% for early vegetative stage to 40.0% for full bloom. McDonald *et al.* (1995) is of opinion that the ADF content of dairy cow diets should be maintained above 190g/kg DM. This might not be possible if energy requirements at times of highest yield must be met. The variations that exist in ADF content of lucerne hay emphasise the importance of the ADF analysis for accurate diet formulation.

Erasmus *et al.* (1990) found a mean NDF content of 42.8% for lucerne hay produced in South Africa. According to the results in Table 4 the mean NDF content of lucerne hay in SA is higher. The mean NDF value in the current study (Table 4) was also slightly higher than the 45.3% value and 43.9% observed by Mertens (1992) and NRC (1996) respectively. In comparison with findings by Mertens (1992) the NDF content of lucerne hay of the present study (Table 4) varied considerably more. Results obtained by Mertens (1992) indicated that NDF values ranged from 38.4% for hay cut at an early vegetative stage to 52.1% at full bloom. The NRC (1996) reported an even lower variation of 31.9% at early bloom and 48.8% at full bloom. Allen (1991a) recommended NDF levels between 25% and 30% in diet DM for high producing dairy cows. The high NDF values of lucerne hay found in the present study could restrict the inclusion level thereof in high yielding dairy cow diets.

### 3.1.5 Non-fibre carbohydrates (NFC)

According to Mertens (1992) nonstructural and structural carbohydrates refer to their function in plants. Sugars, starches, organic acids, and other reserve carbohydrates such as fructans, make up the non-structural carbohydrates (NSC) fraction and are major sources of energy for ruminants such as high producing dairy cattle (NRC, 2001). NSC and pectin are

highly digestible and are generally increased in the diet at the expense of NDF to meet the energy demands of lactating dairy cows. Although the structural/non-structural classification of carbohydrates is appropriate for describing plants, a slightly different classification of carbohydrates is needed to describe their nutritional characteristics (Mertens, 1992). They recommended that classification of carbohydrates into fibre or non-fibre fractions should be based on nutritional characteristics rather than on chemical composition or plant function. Non-fibre carbohydrates represent the more rapidly digested fraction that includes pectins, starch and sugars.

Mertens (1992) suggested a convenient method of calculating NSC, which he termed non-fibre carbohydrates (NFC). NFC is calculated by difference (par. 2.2.6). NSC is measured by enzymatic methods (Smith, 1969; Smith, 1981; Sarvar *et al.*, 1992) and is a distinct fraction. In some earlier studies by Van Soest *et al.* (1991) and Sniffen *et al.* (1992) no distinction between NSC and NFC was recognized. Smith (1992) proposed that NSC and NFC are interchangeable terms. This finding was in agreement with that of Sniffen *et al.* (1992) which found that the calculated NFC is usually in close agreement with direct measured NSC. However several researchers (Mertens, 1988; Hoover, 1996 as cited by Bethard, 1997; Varga & Kononoff, 1999) reported that the concentrations of NFC and NSC are not equal for many feeds and the terms should not be used interchangeably. According to Bethard (1997) NFC and NSC for lucerne hay were not significantly ($P>0.05$) correlated. Varga & Kononoff (1999) found a significant difference between NFC (22% DM) and NSC (12.5% DM) for lucerne hay. Similar results for lucerne hay have been previously reported by Casper *et al.* (1990). Much of the difference is caused by the contribution of pectin and organic acids (Sniffen *et al.*, 1992; NRC, 2001). Pectin is included in NFC but not in NSC. According to Van Soest (1982) legume forages (eg. lucerne hay) contain significant amounts of pectin. Pectins are rapidly fermented in the rumen (Van Soest, 1982). Therefore it was important to determine NFC in the current study. Russell *et al.* (1992) reported that all NFC fractions are fermented by ruminal bacteria that can utilize either ammonia or peptide as a nitrogen source.

The mathematical approach (NFC) is similar to the proximate analysis to determine nitrogen-free-extract (NFE), which was also intended to be an estimate of digestible

carbohydrates in the Weende analysis described by Van der Merwe & Smith (1991). However, according to Mertens (1992), hemicellulose and lignin that are dissolved by the crude fibre method contaminate NFE. Mertens (1992) mentioned that contamination of NDF also exists which can cause inaccuracies, e.g., NDF usually contains some protein and ash that causes a slight underestimate of true NFC. A variation in the calculation of NFC was proposed by Van Soest & Sniffen (1984) and Van Soest *et al.* (1991) namely NFC = 100-[CP+Fat+(NDF-NDF protein)+Ash]. Van Soest & Sniffen (1984) stated that the insoluble protein in NDF is the slowest to be degraded and should therefore be excluded. In the current study NDF-protein has not been determined and will be conducted in a further study.

Another variation of NFC was proposed by Wang *et al.* (2001) where NFC was calculated as follows: NFC = 100-[(NDF-NDF protein)+CP+Ash+(fatty acids/0.9)]. Total fatty acid concentration of feed samples is needed for this equation, which was not done in the current study.

Currently, there is a very limited database of sugar and starch (NSC) analysis for lucerne hay to base recommendations upon. Alternatively, there is a comparative large database of NFC values because of the ease of calculation. The mean NFC content of lucerne hay (Table 4) in the present study was lower than the 24.4% reported by Mertens (1992). The NFC data of the current study varied widely while Mertens (1992) reported values that ranged from 25.4% for lucerne hay cut at an early vegetative stage to 23.2% fot hay in full bloom. According to Bethard (1997) widely divergent protein levels in lucerne hay may substantially increase the variation in NFC, as it is used in the calculation. Highly digestible lucerne hay is usually used in diets of high yielding dairy cows. Shaver (1991) emphasised the danger of low NFC values approaching 32% (minimum) of the diet DM of high producing dairy cows. A certain amount of NSC must be provided to encourage microbial digestion and protein synthesis in the rumen. Van Soest *et al.* (1991) is of the opinion that balancing rations for NFC becomes important for high producing dairy cows when the roughage content increases beyond 50%. According to Smith (1992) the optimum NFC levels for high producing cows are under investigation and different views on recommendations are found in the literature. Bethard (1997) concluded that using NFC

values in diet formulation should enable more precision in balancing carbohydrates in the diet. According to the results of the present study a high variation occurred in the NFC content of lucerne hay. It is evident that an average value would be an unreliable measure to use in ration formulation. However Van Soest *et al.* (1991) suggested that this problem is greater with grass- or maize silage-based diets than with lucerne hay.

### 3.1.6 Crude fat

Crude fat is the amount of fat or oil extracted by hexane (Official and Tentative Methods of the American Chemists Society, 1985). The hexane soluble compound may include true fats and oils, fatty acid esters, compound lipids and fat soluble vitamins or pro-vitamins such as the carotenoids, all of which may have nutritional value (Mason, 2000-unpublished data; Van der Merwe & Smith, 1991). However, (Mason 2000-unpublished data) suggested that hexane extract may also contain a significant concentration of indigestible waxes, resins and essential oils. On the other hand Van der Merwe & Smith (1991) concluded from the composition of ether extraction fraction, that it could be misleading and does not represent the true fat or oil content of a feed. According to McDonald *et al.* (1995) crude fat contains 2.25 times the energy (caloric) value of carbohydrates and proteins. The estimated crude fat content of a feed is also used to formulate total dietary fat level (Smith, 1992) and calculate non-fibre carbohydrates (NFC) by difference (par. 2.2.6).

The mean crude fat content (Table 4) of lucerne hay in the current study corresponds with the 1,8% reported by Van der Merwe & Smith (1991) for lucerne hay in South Africa. These values were however lower than the 2.3% and 2.4%, values published by Mertens (1992) and the NRC (1996) respectively. The NRC (1996) reported values that ranged from 2.9% at early bloom, to 2.6% at mid bloom and 3.4% at full bloom. However, Mertens (1992) observed that early vegetative hay contains 3.1%, early bloom 2.4% midbloom 2.1% and full bloom 1.5 % crude fat. These values suggested that crude fat content decreased with maturity. From some of the literature cited it seems as if maturity has no definite influence on the crude fat content of lucerne hay. Van der Merwe & Smith (1991) also reported no particular pattern: pre-bloom 2.9%, early bloom 1.8%, full bloom 2.1% and past bloom 2.3%. According to Van der Merwe & Smith (1991) the fat content of feeds is the unstable part. However, the crude fat content of lucerne hay is probably too low to cause

any problems during storage (constituent and losses could occur due to the oxidation of fats).

### 3.1.7 *In vitro* organic matter digestibility (IVOMD)

Since digestibility trials are laborious to perform, there have been numerous attempts made to determine the digestibility of foods by reproducing in the laboratory the reactions which take place in the alimentary track of the animal (McDonald *et al.*, 1995). The *in vitro* technique of Tilley and Terry (1963), as modified by Engels & Van der Merwe (1967), is currently probably the most widely used method. According to McDonald *et al.* (1995) *in vitro* fermentation techniques make it possible to determine the digestibility of large amounts of small samples, as is the case in the present study. Hvelplund *et al.* (1997) are of the opinion that organic matter digestibility is the determinant factor of the energy value of a feed. McDonald *et al.* (1995) describe an alternative to chemical analysis which is the assessment of digestibility by fermentation *in vitro* and the prediction of metabolizable energy (ME) value from the digestible organic matter digestibility of the food. For roughage given to ruminants, McDonald *et al.* (1995) proposed a formula to calculate ME, which is commonly used (par. 2.2.9).

The *in vitro* digestibility and calculated ME content of lucerne are presented in Table 4. Although the maximum value of lucerne IVOMD was ± 26% higher than the minimum value, the CV was surprisingly low. This corresponded with ME and FME that showed a fairly low variation. The ME of a food is the digestible energy less the energy loss in urine and combustible gases. The mean ME content of lucerne hay in the current study was higher than the 8.2 MJ/kg value given by McDonald *et al.* (1995) and the 9.1 MJ/kg measured by MAFA (1990) as cited by the AFRC (1992). Van der Merwe & Smith (1991) also reported a lower value of 8.25 MJ/kg for South African lucerne hay.

### 3.1.8 ME from Fat

From (Table 4) it is evident that ME from fat had a small (± 4%) contribution to the total average ME content of lucerne. This contribution tends to increase as the ME content of lucerne increases (± 2% for minimum to 6% for maximum). This is in agreement with the

increasing difference between ME and FME as the ME and/or fat content of the lucerne hay increases.

### 3.1.9 Fermentable Metabolizable Energy (FME)

According to McDonald *et al.* (1995) the yield of microbial crude protein is related to the energy available to the rumen micro-organisms in terms of fermentable metabolizable energy (FME). FME is expressed as MJ/kg DM, and calculated from the ME of feed or diet, minus the ME contributed from dairy fat. Allowances were made for fat content in both the ARC and INRA system (Parker, 2001). The mean FME value (Table 4) of lucerne hay in the current study was higher than the 7.4 MJ/kg DM value published by McDonald *et al.* (1995). The minimum FME value corresponds to that of ME. Differences between ME and FME tend to increase as ME value rises. However, the difference between FME and ME values were small.

### 3.1.10 Effective ruminal dry matter degradability (ERDMD)

The mean soluble and slowly degradable dry matter (DM) fractions in lucerne hay, ERDMD a and ERDMD b respectively, showed a high coefficient of variation (Table 4). Variability in degradation rates of the a fraction was the lowest but still high. The highest variability was found for the degradation rate c. The mean ERDMD was similar to the 37% reported by Mathison *et al.* (1999) for barley straw. In contrast with its fractions a, b and c, ERDMD showed a low CV.

## 3.2 Protein

### 3.2.1 Crude Protein

Proteins are complex organic compounds of high molecular weight (McDonald *et al.*, 1995). Traditionally, proteins in food for ruminant animals have been evaluated in terms of crude protein (CP) or digestible crude protein (McDonald *et al.*, 1995). According to Ball & Lauriault (1999) crude protein represents the nitrogen (nitrogen content x 6.25) fraction of the forage and measures true protein (Amino acids), non-protein nitrogen (NPN) and also insoluble CP or acid detergent fibre nitrogen (ADF-N). Foodstuffs contain numerous different proteins and several types of NPN compounds. According to the NRC (2001)

proteins are large molecules that differ in size, shape, function, solubility and amino acid (AA) composition. Proteins have been classified on the basis of their 3-dimensional structure and solubility characteristics (NRC, 2001). Amino acids (AA) are the building blocks of proteins (NRC, 1989). According to Polan (1992) it should be noted that AA values of feeds are expressed relative to crude protein. The absorption of essential amino acids from digested protein is vital to the maintenance, reproduction, growth and lactation of dairy cattle (NRC, 1989). The NRC (1996) indicates four different protein constituents, namely degradable and undegradable protein as well as soluble protein and non-protein nitrogen constituents. Polan (1992) stressed that CP is of utmost importance in dairy rations and is often the first limiting and usually the highest priced nutrient.

Hanson *et al.* (1988) proposed that the quality of lucerne is closely related to its CP content, since it is related to stage of maturity and leafiness. Roughage is bulky and, because of presumed limitations of ruminal fermentation capacity and microbial growth, Polan (1992) suggested that the quality of especially the CP should be high.

In the present study the average CP content was higher (Table 5) than the 16.7% on a moisture free basis, indicated by Van der Merwe & Smith (1991) for South-African lucerne hay. Morrison (1961) reported a corresponding mean value of 16.9, while McCullough (1994) reported a mean value of 18.0 which was slightly lower than the value indicated in the present study.

The variation of CP in Table 5 was higher than the values given by Van der Merwe & Smith (1991) for South African lucerne hay. According to Van der Merwe & Smith (1991) crude protein values for SA lucerne hay ranged from 20.0% for lucerne hay cut before bloom to 14.4% cut past bloom. This is almost identical to the before bloom and past bloom values of Morrison (1961) of 20.6% and 14.6% respectively. As mentioned before, not only the physiological stage, but also various other factors could influence the quality and consequently the protein content of lucerne hay.

**Table 5 Protein composition and utilization of lucerne hay on a dry matter basis**

| CHEMICAL ANALYSIS | n | Min[3] | Average | Max[4] | SD[5] | CV[6] |
|---|---|---|---|---|---|---|
| Crude protein (%) | 209 | 12.27 | 18.83 | 25.38 | 2.18 | 11.60 |
| a[1] (%) | 72 | -26.24 | 11.90 | 50.03 | 0.13 | 106.84 |
| b[1] (%) | 72 | -1559.70 | 190.40 | 1940.49 | 5.83 | 306.39 |
| c[1] (%) | 72 | -31.98 | 11.45 | 54.89 | 0.14 | 126.41 |
| Effective ruminal protein degradability (%) | 72 | 20.51 | 47.37 | 74.23 | 0.09 | 18.90 |
| Effective rumen degradable protein (g/kg) | 30 | 2.74 | 87.88 | 173.01 | 28.38 | 32.29 |
| ERDP/FME[2] | 30 | 2.114 | 8.51 | 14.89 | 2.13 | 25.05 |
| Digestible microbial protein (g/kg DM) | 30 | 53.71 | 71.48 | 89.25 | 5.29 | 8.29 |
| Acid detergent fibre-Nitrogen (%) | 205 | -0.07 | 0.22 | 0.51 | 0.09 | 0.43 |
| Acid detergent fibre-Nitrogen[a] (%) | 204 | -2.02 | 7.34 | 16.71 | 3.12 | 42.51 |
| Digestible undegraded true protein (g/kg) | 30 | 23.60 | 66.28 | 108.95 | 14.22 | 21.46 |
| Metabolizable protein (g/kg) | 30 | 96.81 | 137.76 | 178.70 | 13.65 | 9.91 |
| Arginine (g/100g CP) | 118 | 2.55 | 3.64 | 6.01 | 0.62 | 17.05 |
| Histidine (g/100g CP) | 118 | 1.85 | 2.45 | 7.12 | 0.66 | 27.07 |
| Isoleucine (g/100g CP) | 118 | 0.41 | 3.63 | 5.76 | 0.66 | 18.20 |
| Leucine (g/100g CP) | 118 | 5.08 | 7.20 | 11.17 | 1.04 | 14.52 |
| Lysine (g/100g CP) | 118 | 1.58 | 4.55 | 7.28 | 0.79 | 17.36 |
| Methionine (g/100g CP) | 118 | 0.14 | 0.44 | 0.76 | 0.12 | 26.36 |
| Phenylalanine (g/100g CP) | 118 | 2.82 | 3.74 | 8.33 | 0.95 | 25.46 |
| Threonine (g/100g CP) | 117 | 1.46 | 3.32 | 6.26 | 0.86 | 25.77 |
| Valine (g/100g CP) | 117 | 4.27 | 5.49 | 9.26 | 0.96 | 17.47 |

[a] Acid detergent fibre – Nitrogen expressed as a percentage of total nitrogen

[1] Fitted parameters a, b and c derived from the *in sacco* determination of effective ruminal protein degradability.

[2] Effective rumen degradable protein/Fermentable metabolizable energy

[3] Average minus 3 x Standard deviation (Berenson & Levine, 1986)

[4] Average plus 3 x Standard deviation (Berenson & Levine, 1986)

[5] Standard deviation

[6] Coefficient of variation

### 3.2.2 Degradability

According to McDonald *et al.* (1995) nitrogen fractions within the diet will vary in their susceptibility to breakdown, from immediately degraded to undegradable, and from 0 to 1 in the extent to which they are degraded. Degradability will depend upon such factors as the surface area available for microbial attack, the physical and chemical nature of the protein and the protective action of other constituents. (McDonald *et al.,* 1995; NRC, 2001; Polan, 1992). It is therefore a characteristic of the protein itself and should be measurable. It has been suggested by McDonald *et al.* (1995) that a major factor affecting degradability is the amino acid sequence within the protein molecule. If this is so then the nature of the microbial produced rumen peptidases is of considerable importance and it seems doubtful whether any simple laboratory test for degradability is possible. The *in sacco* procedure has emerged as the most widely used approach for estimating rumen undegradable protein (RUP) (NRC, 2001; Stern *et al.,* 1997). Consequently it is used in the present study. However, testing feed for rumen degradability is time-consuming and is influenced by various factors such as bag porosity, particle size, sample size to bag surface ratio, method of washing bags, position in the rumen, dietary effects, animal effect, bag incubation sequence and microbial contamination. (Mehrez & Orskov, 1977; Moseley & Johnes, 1984; Uden & Van Soest, 1984; Nocek, 1988; Nocek & Kohn, 1988; Erasmus *et al.*, 1990; Stritzler *et al.*, 1990; AFRC, 1992; Huntington & Givens, 1997; Nandra *et al.,* 2000; NRC, 2001).

Determination of degradability *in sacco* entails placing a weighed sample of feed in synthetic fibre bags suspended in the rumen, as described in the procedures (par. 2.2.12). The non-linear parameters a, b and c are estimated by an iterative least square procedure (Ørskov & McDonald, 1979) and the effective degradability of crude protein (CP) is presented in Table 5. The a value may be interpreted as a measure of the rapidly soluble nitrogen fraction. The b value represents that fraction which will degrade in time; while c represents the rate at which the b fraction degrades (Nocek & English, 1986; McDonald *et al.*, 1995; NRC, 2001). The soluble fraction a represents the intercept of the degradation curve at time zero hours (0-h). Erasmus *et al.* (1990) found a close agreement between 0-h (wash only) values and the degradation curve intercept values. Therefore the intercept

values of the degradation curve were used in the current study as an indication of the soluble value.

According to McDonald *et al.* (1995) the efficiency with which nitrogen is captured by the micro-organisms of the rumen depends not only upon the speed and extent of breakdown but also upon the synchronous provision of a readily available, utilizable source of energy to fuel the synthesis of microbial protein. Failure to achieve this balance can result in too rapid and extensive breakdown, and the synthetic powers of the rumen micro-organisms may be overwhelmed. Wastage may then occur since the excess ammonia is absorbed and largely excreted as urea; some, though, is recycled via the rumen wall and contributes further to the nitrogen economy of the rumen. That part of the food crude protein that is immediately degradable, is therefore unlikely to be as a effective source of nitrogen for the micro-organisms as that which is more slowly degraded. According to AFRC (1992) the slowly degraded nitrogen fraction is incorporated into microbial protein with an efficiency of 1.0, whereas the immediately degraded is less efficiently used. Estimates of the efficiency with which immediately degraded protein is incorporated vary, but 0.8 is a commonly used figure (McDonald *et al.*, 1995).

In the available literature the a fraction for lucerne hay ranged from 29.4% (Janicki & Stallings, 1988) to 42.3% (Erasmus *et al.*,1990). The mean a value in Table 5 was remarkably lower than those found in the literature. In accordance with this variation NRC (2001) suggests that grasses and legume forages (eg. lucerne) contain the highest and most variable concentration of non-protein nitrogen (NPN). NPN is rapidly soluble in the rumen and is exclusively used by the micro-organisms. NRC (2001) further postulated that hays contain higher amounts of NPN than the same feeds when fresh, because of the proteolysis that occurs during wilting. The proteolysis that occurs in forages during wilting is a result of plant and microbial proteases and peptidases. Plant proteases and peptidases are active in cut forages and are considered to be the principal enzyme responsible for the conversion of true protein to NPN in hays (Fairbairn *et al.*, 1988; Van Soest, 1994). Rapid wilting of cut forages slows down proteolysis and reduces the conversion of true protein to NPN (Garcia *et al.*, 1989; Van Soest, 1994). This phenomenon possibly contributed to the variation of the a fraction in lucerne hay as found in the current study.

Theoretically the sum of fraction a and b should not exceed 100%. However, an average value of more than 100% was observed for lucerne in the present study (Table 5). According to Erasmus *et al.* (1990), the large b value could be due to bacterial contamination causing a disproportionate apparent N disappearance at certain time intervals. Ha & Kennelly (1984) reported similar results when evaluating soya bean meal. Several other researchers also reported b values exceeding 100% (Hughes-Jones, 1979; Ørskov *et al.*, 1980). These findings suggested that the exponential model may not be entirely appropriate (Huntington & Givens, 1997; Lopez *et al.*, 1999). Accordingly minimum and maximum values also seems unrealistic.

The extent of protein breakdown (effective degradability) will depend upon the length of time which the protein remains in the rumen, thus upon its rate of passage through the rumen. As the time of incubation increases, the fraction of the protein remaining in the rumen falls to zero, as does the rate of breakdown. McDonald *et al.*, (1995) recommended a rumen outflow rate of 0.08/h for dairy cows yielding more than 15 kg milk. Accordingly this outflow rate was used in the present study to calculate effective degradability. Effective protein degradation in this study (Table 5) was lower than the values reported by various researchers ranging from 72% (Allen, 1991a), to 75% (Erasmus *et al.*, 1990; Van der Merwe & Smith, 1991). In this regard Snyman & Joubert (1992) pointed out that although of great value, the estimation of forage degradability from published tables (Allen, 1991b; Van der Merwe & Smith, 1991) is inaccurate and may lead to over or underfeeding with respect to production needs.

The large variation in effective degradability of crude protein stressed the need for degradability determination of individual cuttings. A possible explanation for the variation of effective ruminal protein degradation can be a lack of standardization of the *in sacco* artificial fibre bag technique. Dewhurst *et al.* (1995) suggested that the *in sacco* technique might not be as precise with forages as with concentrates or protein supplements because of the high proportion of water-soluble materials that can leave the bag unfermented. Stern *et al.* (1997) is of the opinion that the extent of protein disappearance from the bag at specific intervals of rumen exposure does not provide a correct estimate or degradation because it

does not account for protein contained in feed particles that leave the rumen with digesta flow. The lower effective ruminal protein degradation values in this study could possibly be explained by the larger particle size used (ground through a 10 mm screen.), compared to other researchers. Huntington & Givens (1997) used a 1 mm screen, Erasmus *et al.* (1990) a 2 mm screen, Nandra *et al.* (2000) a 4 mm screen, and Cronje (1983 and 1992) and Nocek (1988) a 5 mm screen, for hay and other roughage. Because the bags used in *in sacco* digestion studies are not masticated or ruminated, microbial fermentation and detraction by ruminal activity are the only means by which particle reduction occurs (Nocek, 1988). However, there is much controversy as to the degree of particle breakdown associated with microbial digestion (Moseley & Jones, 1984; Murphy & Nicoletti, 1984; Nocek & Kohn, 1988). It is difficult to establish which particle size is the most appropriate for use in *in sacco* study, since no studies have been conducted with the specific objective of developing correlation between *in vivo* and *in sacco* digestion as it relates to particle size (Nocek, 1988). According to Nocek (1988) it is therefore debatable as to whether prepared material for *in sacco* study should parallel that which is fed or parallel that after mastication and presentation to the rumen. In the present study the lucerne samples (through a 10 mm screen) represent to a certain extent those in the rumen after mastication.

Generally larger and coarser materials (as used in the present study) are associated with slower rates of digestion and greater variation. However, finely ground materials are subjected to greater mechanical losses from the bags (assumed to be soluble protein, resulting sometimes in unrealistically rapid rates of digestion), but according to Nocek (1988) variation is more controlled.

Thus the explanation for the higher degradation values reported by other researchers, as mentioned before, is probably due to the smaller particle size of the samples used. Grinding, particularly of forages, increases surface area per unit weight of sample and the surface area accessible for microbial attachment. This generally results in increased digestion rate (Nocek, 1988). Solaiman *et al.* (1982) showed shorter lag times and less indigestible cell wall for lucerne hay and orchard grass ground at 1 mm than that ground at 8 mm. A logical approach would appear to be to establish some degree of uniformity in size

within major categories of foodstuffs. An appropriate particle size or form would seem to be that which is masticated and presented to the rumen.

Currently much controversy exists around the specie factor. Several researchers (Siddons & Paradine 1983; Priggle *et al.,* 1984; Uden & Van Soest, 1984; Sebek & Everts, 1999; NRC, 2001) are of the opinion that rumen degradation kinetics have been shown to differ between sheep and dairy cows. However, the Technical Committee on response to nutrients (AFRC, 1992) made no definitive recommendation regarding the species of animal to be used for protein degradability. The results of Nandra *et al.* (2000) are in accordance with the findings of Huntington & Givens (1997) who found no differences between host species on *in sacco* DM disappearance of hay. Uden & Van Soest (1984) also found that mature ruminant species degrade the fibre fraction of feeds similarly. The findings of Nandra *et al.* (2000) quantified no difference between sheep and cattle in the *in sacco* degradability of DM specifically for lucerne hay.

Priggle *et al.* (1984) suggested that differences in rumen retention time might have accounted for some of the differences in apparent whole track digestibility between sheep and cows. Since adjustments were made for outflow rates (par. 2.12.13) in the current study, it may therefore be prudent to extend comparisons of the *in sacco* technique to the high yielding dairy cow (Huntington & Givens, 1997).

According to the NRC (2001) the *in sacco* procedure has been modified and adopted in several countries. Adherence to guidelines for standardizing factors known to affect the results (Nocek, 1988; Stern *et al.*, 1997) have increased considerably the reproducibility of the measurement within and among laboratories.

#### 3.2.2.1 Effective rumen degradable protein (ERDP)

The yield of microbial protein that becomes available for digestion and absorption post-ruminate by the host has been related to the energy of the diet and the supply of protein to the microorganisms. McDonald *et al.* (1995) concluded that if the supply of protein to the micro-organisms is limiting the yield of microbial protein would depend upon the supply of protein available to the rumen micro-organisms. According to the AFRC (1992) the

microbial demand for protein is stated in terms of Effective Rumen Degradable Protein (ERDP) and foods should be evaluated in the same terms (Table 5). McDonald *et al.* (1995) reported an ERDP value of 151 g/kg DM for lucerne hay, at an identical fractional outflow rate (0.08/h). It is nearly double compared to the value found in the present study. This reflects that protein available to the microorganisms might be limiting. The high CV for ERDP in Table 5 again stresses the need for routine analysis of different cuttings.

#### 3.2.2.2 Ratio of effective rumen degradable protein (ERDP) to fermentable metabolizable energy (FME)

The AFRC (1992) proposed that the yield of microbial crude protein is related to the energy available to the rumen micro-organisms as previously mentioned. When FME is limiting, then $\sum$ERDP / $\sum$FME is greater than 11 for lactation. On the other hand when ERDP is limiting this ratio is less than 11. From Table 5 it is evident that the average ERDP value of lucerne hay was limiting.

### 3.2.3 Digestible microbial protein (DMP)

The microbial protein synthesized in the rumen may be protozoal or bacterial, the relative proportions depending upon conditions within that organ (McDonald *et al.* 1995). Van der Merwe & Smith (1991) proposed that rumen pH tends to reduce protozoal activity and stimulate that of certain bacteria. The mixture of bacterial and protozoal protein along with dietary protein not degraded in the rumen passes to the abomasum and small intestine. Here they are broken down to amino acids, which are then absorbed into the body. According to AFRC (1992) the digestibility of bacterial protein is lower (about 0.75) than that of protozoal (about 0.90) and the overall digestibility of microbial protein will depend to some extent upon the rumen environment. However protozoal protein constitutes only some 5 to 15 percent of the total microbial protein flow from the rumen and its influence on the overall digestibility of microbial protein will be small. According to McDonald *et al.* (1995) there is considerable evidence that the true digestibility of microbial protein is in the order of 0.85. The proportion of microbial crude protein present as true protein is assumed to be 0.75 (par. 2.2.13.3). NRC (2001) suggesting that microbial crude protein (MCP) as provided by bacteria and protozoa is considered to contain 80% true protein. The remaining 20% of MCP is considered to be provided by nucleic acids (NRC, 1989).

The contribution of microbial protein (DMP) from lucerne hay in the diet to the truly absorbed amino acids is shown in Table 5. DMP made a 52% contribution to the average metabolizable protein (MP) content of lucerne hay. The rest was from DUP.

### 3.2.4 Digestible undegradable protein (DUP)

The digestibility of the undegraded dietary protein is a characteristic of the protein mix in the food (McDonald *et al.*, 1995). Recent work has shown that the digestibility is inversely related to the content of acid detergent fibre-nitrogen (ADF-N), which reflects the part of the food nitrogen which is closely bound to insoluble fibre.

According to Van Soest (1984) ADF-N as proposed by Van Soest (1984) is the nitrogen remaining in the acid detergent fibre residue. While some occur naturally in all plant material (Mertens, 1979), ADF-N is generally considered to be an estimate of heat damage occurring during storage (high moisture or processing millins or pelletins). Nitrogen in excessive heated samples is highly resistant to microbial and mammalian enzymes (Krishnamoorthy *et al.*, 1982; Van Soest, 1984). Several researchers (Goering *et al.*, 1972; Mertens, 1979) suggested that the non-heat-damaged ADF-N is related to the lignin (protein irreversibly bound to lignin) and to a fraction of protein in forages. ADF-N can be converted to acid detergent fibre crude protein (ADF-CP) by multiplying it by 6.25 (Linn & Martin, 1999). ADF-N, acid detergent insoluble nitrogen (ADIN), insoluble crude protein (ICP), unavailable protein and bound protein, all refer to the same fraction (Erasmus *et al.*, 1990; Undersander *et al.*, 1993; Linn & Martin, 1999).

According to the results in Table 5 the average nitrogen concentration of ADF (ADF-N) in lucerne hay was higher than the 0.2% mean reported by McDonald *et al.* (1995) and lower than the 0.4% and 0.6% found by Erasmus *et al.* (1990) and NRC (2001) respectively. Mertens (1979) has shown that ADF-N also exists in forages that have not been heated and observed that this non-heat-damage ADF-N is related to the lignin and to a fraction of protein in forages. His work suggest that 5-12% of the nitrogen in non-heat-damaged forages is isolated as ADF-N (lower values for grasses than lucerne). This researcher also mentioned that the heterogeneity of ADF-N might explain the difficulty in measuring it

(especially with near infrared reflectance spectroscopy). The lack of a constant biological availability value for ADF-N is also a problem. Undersander *et al.* (1993) stressed the fact that heating can have both positive and negative effects on protein utilization by the animal. Heating generally results in lowered digestibility of protein. Digestibility of ADF-N varies from 0 to 60% depending on the feed ingredient and the time and intensity of heating. Mason (2000-unpublished data) suggested that a fixed proportion (e.g. 70%) of ADF-N is unavailable. Undersander *et al.* (1993) further proposed that reducing the solubility of proteins at low heat inputs could compensate for the negative effect of heating by making them less degradable in the rumen. Therefore if feeds are slightly heated it reduces the potential loss of protein in the rumen (as ammonia) and actually increases protein utilization efficiency in the small intestines.

Unavailable N (ADF-N), when expressed as a percentage of total N (ADF-N$^a$) was slightly less (Table 5) than the 7.6% measured by Erasmus *et al.* (1990) and higher than the 7.0% value reported by Coblentz *et al.* (1998). A high CV was calculated for ADF-N$^a$. ADF-N$^a$ values ranging from 3.5 to 13.6 and 8.3 to 14% in some roughages have been reported by Krishnamoorthy *et al.* (1982) and Janicki & Stallings (1988) respectively. This is much higher than the ADF-N content found in the current study (Table 5) and those reported in the literature for lucerne hay (Erasmus *et al.* 1990). Therefore the influence of ADF-N on the DUP of lucerne hay seems to be small. According to Mason (2001-unpublished data) most feed labs report an adjusted crude protein (ACP) value if heat damaged protein (ADF-N) is higher than about 9% of total crude protein. However Undersander *et al.* (1993) suggested no adjustment of CP when ADF-N$^a$% is less than 14%, partial adjustment when ADF-N$^a$ is between 14 and 20%, and complete adjustment of CP for ADF-N$^a$ values above 20% of total CP. Linn & Martin (1999) recommended an adjustment for CP availability when the CP% in the ADF fraction increases above 12% of total CP.

In the present study 96.6% of the lucerne samples showed an ADF-N$^a$ content of less than 14%; 2.4% were between 14% and 20%, and 1.09% higher than 20%. It is obvious from these results that heat damage possibly occurred in only a small percentage (3.5%) of the samples. This is in agreement with the low moisture content found in the lucerne hay of the present study (Table 5).

AFRC (1992) calculated the true digestibility of DUP on the assumption that the ADF-N content is indigestible and that the remainder had a true digestibility of 0.9. Erasmus *et al.* (1990) reported that the available DUP is over-estimated if not corrected for ADF-N. However although ADF-N is assumed to be an indigestible fraction (AFRC, 1992; AOAC, 1979) it could be degraded slightly in the ruminal compartment. It was also evident in this study that there was no need for correction for ADF-N.

At an identical fractional rumen outflow rate (0,08/h), average DUP values determined in the present study were almost double that of the 36.5 g/kg DM reported by Erasmus *et al.* (1990). McDonald *et al.* (1995) also reported a lower value of 55 g/kg DM at the same rumen outflow rate. In literature cited by AFRC (1992), MAFF (1990) reported a lower DUP value of 44 g/kg DM. However, Van Straalen & Tamminga (1990) measured a value of 64 g/kg DM, slightly less than the DUP value presented in Table 5. This dissimilarity in DUP could possibly be attributed to factors like species, rations, number of samples used and differences in laboratory techniques.

The NRC (2001) used a different approach to estimate rumen undegradable protein. This approach will be implemented in a further study.

### 3.2.5 Metabolizable protein (MP)

Formulation of rations to meet the protein and amino acid (AA) requirements of ruminant livestock has developed from systems, which were based on digestible crude protein (ARC, 1980; NRC, 1984) to those which set out to express protein requirements in terms of metabolizable protein (AFRC, 1992; NRC, 1996; NRC, 2001). The NRC (2001) defined metabolizable protein (MP) as the true protein that is digested post-ruminally and the component AA absorbed by the intestine.

The proposed MP system of the AFRC (1992) adopts the principles of ARC (1980) and (1984), but with some amendments to the magnitude of factors in the system, in the light of subsequent published research and the results of specially designed experiments to test the original ARC recommendations. The main factors modified were rumen microbial needs

for N and the digestibility of the efficiency of utilization of absorbed AA to meet tissue amino acid needs.

The relative low CV of MP is in agreement with that of its component, DMP. As previously mentioned, DMP made a 52% contribution to the average MP content of lucerne hay in the present study.

The NRC (2001) proposed a somewhat different approach from the one used in this current study (AFRC, 1992). Apart from the contribution of microbial crude protein (MCP) and RUP as mentioned earlier, the NRC (2001) also emphasised the contribution (although to a much lesser extent) of endogenous crude protein (ECP) to MP in the small intestine.

### 3.2.6 Amino Acids (AA)

Amino acids (AA) are the building blocks of proteins. Each unique protein is distinguishable by the sequence of 20 different AA found in its peptide–linked chains and by its AA profile (NRC, 2001). According to McDonald *et al.* (1995) plants and many micro-organisms are able to synthesise protein from simple nitrogenous compounds such as nitrates. Animals cannot synthesise the amino group, and in order to build up body proteins they must have a dietary source of AA. Certain AA can be produced from others by a process known as transamination, but the carbon skeleton of a number of AA cannot be synthesised in the animal body and these are referred to as indispensable or essential AA (EAA). Of the 20 AA commonly found in feed proteins, 10 cannot be manufactured by the dairy cow (NRC, 2001). According to the NRC (2001) these EAA must appear in the small intestine in either microbial protein (MCP), rumen undegradable protein (RUP) or endogenous crude protein (ECP). Here they are absorbed into the blood stream.

McDonald *et al.* (1995) mentioned that an important feature of the formation of microbial protein is that bacteria are capable of synthesising essential as well as non essential AA, thus rendering their host independent of dietary supplies of the former, once the rumen micro-organisms have become established. AA composition of the microbial protein has been reported to be quite constant and not significantly affected by the diet (Meyer *et al.*, 1967; Burris *et al.*, 1974). However, a summary by Clark *et al.* (1992) of the AA composition of

441 bacterial samples from animals fed 61 dietary treatments in 35 experiments, indicates significant differences in AA composition. Mason (1998-unpublished data) is of the opinion that microbial protein contains a blend of EAA, which is far from ideal, relative to the requirements of the high producing cow. According to Erasmus (2000) the objective is to develop a rumen microbial protein and RUP combination that presents a more desirable array of AA to the lower digestive track. NRC (2001) noted that absorbed AA used principally as building blocks for syntheses of proteins, are vital to the maintenance, growth, reproduction and lactation of dairy cattle. The efficiency of this process, which depends upon the composition of the mix relative to that of the protein to be synthesised, is best presented by its true biological value (McDonald *et al.*, 1995). This will in turn depend upon the biological values of the digested RUP and the digested microbial protein, and upon the relative proportions of each contributing to the mix. In addition it will vary with the primary function for which it is required and an ideal pattern of absorbed AA exists for each of the mentioned physiologic functions. Parker (2001) is of the opinion that the AA profile of protein entering the duodenum as a mixture of microbial and RUP, may not be ideal for the efficient syntheses of muscle and milk. Several researchers (Ferreira, 1998; Erasmus, 2000; NRC, 2001) demonstrated that if the EAA profile of the absorbed protein is not in balance with the needs of the animal, both the productivity and protein efficiency will decrease leading to poor results (e.g. milk production). Therefore the "ideal" protein can be theoretically defined as one in which the composition of the EAA absorbed from the small intestine matches exactly the AA requirement of the animal for productive purposes.

Erasmus (2000) indicated that the UK's metabolizable system (AFRC, 1992) used in the current study, does not consider EAA content of protein. Therefore information on EAA composition of lucerne hay is important in formulating modern dairy diets for high producing cows. The EAA composition of lucerne hay in the current study is presented in Table 5. The highest average value was found for valine and for leucine.

As previously mentioned, lysine and methionine are the two most limiting AA for milk synthesis (Schwab *et al.*, 1992; Rulquin & Deluby, 1994; NRC, 1996; NRC, 2001; Parker, 2001). The estimated requirement for optimal milk protein syntheses is 15% lysine and

5.3% methionine of total EAA in duodenal digesta (Schwab, 1996). From the results in Table 5 it is evident that lucerne hay is relatively high in lysine and low in methionine.

Erasmus (2000) and NRC (2001) illustrated that microbial protein is one of the best available sources of protein for milk synthesis, and histidine is the first limiting AA in microbial protein. This finding corresponds with that of Polan (1992). Sloan *et al.* (1998) have suggested that after lysine and methionine, histidine is the next limiting AA for milk production which needs urgent attention in research. In this regard Erasmus *et al.* (1994) and Shroeder *et al.* (1996) mentioned that RUP, in the small intestine varies in AA composition according to the nature of and within feeds. According to Polan (1992) it is possible to select combinations of feed sources in an effort to meet the AA requirement for milk protein syntheses. The EAA contribution of lucerne hay, though small in some instances, could contribute to a more accurate AA profile in dairy diets for high producers. However, the high variation in the individual EAA-content of lucerne hay (Table 5) hampers accurate diet formulation for EAA. NRC (2001) also mentioned that the EAA-composition of the RUP varies from that of crude protein.

In Table 6 the EAA contents of body tissue, milk and ruminal bacteria are compared with that of lucerne hay in the present study. Chandler (1989) and Schingoethe (1991) predicted limiting AA by assuming that the AA composition of milk protein is indicative of AA requirement for milk production. When protein requirements are calculated for lactating cows that are still growing, requirements for tissue accumulation probably account for less than 10% of metabolic protein (Polan, 1992).

Based on EAA content (Table 6) methionine, lysine and isoleucine were apparently the least abundant in lucerne hay for milk synthesis. This finding concurs with that of Polan (1992). According to Polan (1992) the branched-chain AA leucine, isoleucine, and valine tend to be higher in milk protein than bacterial crude protein (BCP) and most individual feed sources that potentially meet requirements for milk synthesis. However, it is evident from Table 6 that lucerne hay can potentially fulfil some of these requirements, especially with regard to valine. The approach followed by Erasmus (2000) in which AA scores were defined as the proportions of the first limiting AA in that protein relative to milk protein, was used in this

**Table 6 Essential amino acid profiles of body tissue, milk, ruminal bacteria and lucerne hay of the present study**

| ITEM | Arg | His | Ile | Leu | Lys | Met | Phe | Thr | Try | Val |
|---|---|---|---|---|---|---|---|---|---|---|
| Lean tissue[1)] (% of total EAA) | 16.8 | 6.3 | 7.1 | 17 | 16.3 | 5.1 | 8.9 | 9.9 | 5.5 | 10.1 |
| Milk[2)] (% of total EAA) | 7.2 | 5.5 | 11.5 | 19.5 | 16 | 5.4 | 10.1 | 8.9 | 2.9 | 13.0 |
| Rumen bacteria[3)] (% of total EAA) | 10.6 | 4.3 | 11.6 | 15.5 | 17.3 | 4.9 | 10.0 | 11 | 2.6 | 12.2 |
| Lucern hay[4)] | 9.5 | 6.6 | 9.5 | 19.1 | 12.2 | 1.1 | 9.8 | 7.7 | 3.4 | 14.6 |
| Lucern hay as % of milk | 132 | 120 | 83 | 98 | 76 | 20 | 97 | 87 | 117 | 112 |
| Lucern hay as % of microbes | 90 | 154 | 82 | 123 | 71 | 22 | 98 | 70 | 130 | 119 |

[1)] From Ainslie *et al.* (1993)

[2)] Each value is an average of 4 observations from Jacobson *et al.* (1970), McCance & Widdowson (1978), Waghorn & Baldwin (1984) all cited by NRC (2001) and Mantysaari *et al.* (1978) as cited by Polan (1992).

[3)] From Storm & Ørskov (1983); average from 62 literature reports.

[4)] Average values from current study except for Tryptophan (Try) from Allen (1991b)

current study (Table 6). The apparent limiting AA in lucerne hay in decending order was methionine, lysine and isoleucine. Since methionine is the first limiting AA of lucerne hay, it has a milk protein score of 0.20 (Table 6). According to Erasmus (2000) these "milk protein scores" of feed ingredients are possible indicators of protein quality for milk protein production. The EAA arginine, histidine, tryptophan and valine are all abundant in lucerne hay for milk production. Several researchers (Schwab, 1996; Erasmus, 2000; NRC, 2001) are of the opinion that microbial protein is one of the best available sources of protein for milk synthesis and that histidine is the first limiting AA in MCP. Where lucerne hay protein was expressed relative to MCP (Table 6) it is evident that the limitation of histidine could be overcome.

In many countries, previously popular feedstuffs with high rumen undegradable protein content, such as blood-, meat-, and bone meal can no longer be used in feeds for ruminants because of the threat of bovine spongiform encephalopathy (Erasmus, 2000). Furthermore the ever-increasing fish meal prices and occasional unavailability thereof, might in future

force the dairy industry into relying only on plant protein that is strategically supplemented with rumen protected AA to fulfil the AA requirements of high producing dairy cows. Lucerne could play a significant role in this regard.

## 4. CONCLUSIONS

The moisture content of lucerne hay in the present study, was within the desired range for effective storage. Therefore heat and mould damage were unlikely to occur. This was confirmed by the low ADF-N content of the lucerne hay. The relative high dry matter content of lucerne hay in the present study, could possibly be attributed to moisture losses during storage. This aspect is of great importance when buying and selling hay and needs further investigation.

The high ash content of lucerne hay emphasis its potential mineral contribution (especially Ca and K) to the diet. On the other hand the high ash content could be an indication of soil and dust settling on the lucerne before storage. The high NDF values of some lucerne hay samples in the present study could restrict the inclusion level thereof in high yielding dairy diets. In this regard, guessing NDF values too high or too low can have tremendous implication on intake, animal performance and health. The variation that exist in ADF and NDF content of lucerne hay emphasise the importance of analysis to ensure accurate dairy diet formulation. This also applies for CP, ash, NFC, ME, ERPD and EAA. However the maximum IVOMD value of lucerne hay was ±21.9% units higher than the minimum. It showed a surprisingly low CV. Therefore the mean IVOMD value in the current study seems to be representative of the IVOMD for the lucerne hay population.

Results from the present study revealed that the mean crude protein and energy (ME) content of lucerne hay was higher than those found in the literature (Morrison, 1961; Van der Merwe & Smith, 1991; McCullough, 1994). CP is the highest priced and often the first limiting nutrient in dairy diets. The variation in CP content of lucerne hay however hampers the use of a general mean value.

According to results of the present study the effective protein degradation parameters a, b and c showed the highest CV. The average effective degradability of lucerne hay determined in the present study, differ substantially from those in the literature (Erasmus *et al.* 1990; Allen, 1991a; Van der Merwe & Smith, 1991). These lower effective degradability protein values of the present study are possibly due to the larger particle size of samples (ground through a 10mm screen) used in the *in sacco* study. This larger particle size of lucerne hay was probably more representative of those found in the rumen of dairy cows after mastication. The results of the present study thus do not support the view in the literature that lucerne hay CP has a fairly high RDP fraction. According to the results of the present study lucerne hay is an under utilised source of RUP supplement in the local dairy industry.

It is obvious from the results of the present study that the influence of ADF-N on RUP of lucerne hay was negligible. Due to the low mean ADF-N content together with the low CV the practical implication of ACP is therefore debatable concerning lucerne hay. According to the results of the present study heat damage possibly occurred only to a small percentage (3.5%) of the samples. This is in agreements with the low moisture content found in the tested samples.

Although a low CV was detected for MP the small number of hay samples (n = 30) might influence the significance of these data. Therefore these results should be interpreted and used with care. Information on EAA composition of lucerne hay is important to formulate modern dairy diets for high producing dairy cattle. From the present study it is evident that lucerne hay has a high lysine and low methionine content. These are the two most limiting EAA for milk syntheses (Schwab *et al.*, 1992; Rulquin & Deluby, 1994). The high variation in individual EAA content of lucerne hay hampers however accurate diet formulation from average EAA values.

According to the available literature (Schwab, 1996; Erasmus, 2000; NRC, 2001) MCP is one of the best available sources of protein for milk syntheses. Histidine is however the first limiting EAA in MCP. From the results of the present study it seems as if this limitation could be partly overcome by using lucerne hay. In the present study methionine, lysine and

isoleucine appeared to be in a descending order the least abundant in lucerne hay for milk synthesis. Especially methionine is limiting in lucerne hay for milk syntheses. However the EAA contribution of lucerne hay, though small in some instances, could contribute to the ideal protein formulation of dairy cow diets.

From the results of the present study the variation in energy and protein composition as well as utilisation of nutrients in lucerne hay is evident. Accordingly, using average values with the exception of IVOMD, in diet formulation are inaccurate. This emphasises the need for a rapid and accurate quality evaluation- and grading system for lucerne hay in practice.

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# CHAPTER 2

# EVALUATION OF DIFFERENT MODELS FOR LUCERNE HAY QUALITY GRADING

## 1. INTRODUCTION

Lucerne hay is an important roughage source in dairy cattle diets. According to Smith (2000) roughage quality is by far the single most important factor influencing the efficiency of milk production in South Africa. Roughage quality refers inter alia to voluntary feed intake and the efficiency of nutrient utilization in the roughage for production (Linn, 1992; Erasmus, 2000). As stated in Chapter 1 feed quality and nutritive value could be regarded as synonymous. The results in Chapter 1 emphasized the variation in quality of South African lucerne hay and the need for an objective method to evaluate the quality of lucerne hay. Any objective method for estimating or predicting lucerne hay quality must be fast, simple and reasonably accurate. Marble (1986) noted that visual examination does not accurately reflect the acceptability and digestibility of lucerne hay.

Two important nutritive standards used in feed formulation are energy (digestible energy; metabolisable energy) and protein (rumen degradability). A method which allows rapid and accurate estimates of energy value and degradability of the nitrogen fraction of lucerne is needed. Any determination method involving surgically treated animals is not acceptable owing to problems of labour, housing, technical resources, expense and speed. The assessment of energy value can be made more precisely if the feed is subjected to a chemical analysis (McDonald *et al.*, 1995). The analytical data can either be used to "type" the feed more accurately, or it could be fitted into equations that have been devised to predict energy value from chemical composition. McDonald *et al.* (1995) mentioned that the determination of ADF is particularly useful for forages as there is a good statistical correlation between it and the extent to which the food is digested (digestibility). In addition neutral detergent fibre (NDF) has been related to intake regulation, digestibility, and chewing activity in various dairy cow experiments (Mertens, 1982). McDonald *et al.* (1995) proposed an alternative to chemical analysis which is the assessment of digestibility

by fermentation *in vitro* and the prediction of metabolizable energy value from the digestible organic matter content of the food. According to McDonald *et al.* (1995) a number of researchers have shown significant correlation between crude protein (CP) content and protein degradability of grasses. The author is of the opinion that more complete models are needed to integrate chemical information collected in the laboratory with animal and feeding situation characteristics to predict intake and digestibility more accurately. Such models do not need to be sophisticated mathematically, but must be realistic in terms of their dependence on, and correspondence to, biological principles and theories. This information would provide the basis for more efficient forage utilization by ruminants through more accurate determination of feed nutritive value and optimum diet characteristics.

Relative feed value (RFV) as proposed by Rohweder *et al.* (1976b) is an index of the most relevant quality factors and includes the intake (NDF) and digestibility (ADF) of a feed. RFV is most valuable for animals using high-roughage diets, such as dairy cows and growing animals, because the RFV provides an index to rank a roughage according to its digestible energy intake potential. However, according to Grant (1994) it is important to note that RFV is only an energy intake index and does not take into account either protein (concentration) minerals or the physical characteristics of roughage. It is therefore important to evaluate protein and physical characteristics along with RFV for a complete assessment of forage quality.

Hutjens (1995) used the term Total Forage Index (TFI) to describe an index which builds on RFV, but adds a protein and physical value. This addition to the RFV results in a more complete expression of the nutritive value of the forage. However, TFI has the shortcoming that it does not include measures to establish protein quality of forages for ruminants.

Sometimes proteins are exposed to heat damage due to high moisture levels during storage. The amount of damaged or unavailable protein can be determined by the amount of nitrogen in the acid detergent fibre-nitrogen (ADF-N), acid detergent insoluble nitrogen (ADIN) or bound protein analysis (Goering & Van Soest, 1970). According to McDonald *et al.* (1995) recent work has shown that the digestibility is inversely related to the content of ADF-N,

which indicates that part of the food nitrogen is closely bound to insoluble fibre. Linn & Martin (1999) used ADF-N to calculate the adjusted crude protein content of lucerne hay.

The demand for amino acids at tissue level is quantified in terms of truly digestible protein required to be absorbed from the small intestine and designated 'Metabolizable Protein' (MP) (AFRC, 1992). Microbial protein and rumen undegradable protein contribute towards satisfying this demand. This requires that factors such as degradability, efficiency of nitrogen capture, microbial yield, digestibility of microbial protein, digestibility of dietary undegraded protein and true biological value of the absorbed nitrogen be quantified. Several countries have developed systems similar to the United Kingdom metabolizable protein system in so far as they rely on estimation of the amino acids truly absorbed as the baseline. As presently constituted, the proposals in all the systems rely heavily on crude protein degradability values (McDonald *et al.*, 1995). Accordingly any evaluation system (model) of lucerne hay quality should include rumen crude protein degradability.

A study was conducted to include protein quality according to the United Kingdom MP system (AFRC, 1992), into the TFI system to determine the quality of lucerne hay more accurately. This model (lucerne quality index) was compared to chemical analysis and existing models for the determination of lucerne hay quality.

## 2. MATERIALS AND METHODS

### 2.1 Chemical analysis

Lucerne hay samples obtained from different seasons, sites and time of year for chemical, *in vitro* digestibility and degradability analysis (Chapter 1) were used in this study. The number of samples analysed are shown in Tables 1 to 5.

### 2.2 Model calculation

Several models to determine the quality of lucerne were evaluated.

### 2.2.1 Relative feed value (RFV)

Relative feed value (RFV) was estimated from digestible dry matter (DDM) and dry matter intake (DMI) as follows:

RFV = (%DDM) × (DMI as % of body weight) × (0.775) (Rohweder *et al.*, 1976b).

DDM and DMI were derived from ADF and NDF respectively. The formula used for estimating digestible dry matter (DDM) was as follows:

%DDM = 88.9 – (0.779 × % ADF).

The following formula was used to estimate DMI, as a % of animal body weight:

$$\%DMI = \frac{120}{\%NDF}$$

### 2.2.2 Total Forage Index (TFI)

TFI was calculated from the following formula (Hutjens, 1995):

TFI = RFV + (%crude protein × $x$)

Where $x$ = protein multiplier of 6. According to Hutjens (1998) a high multiplier (5 or 6) would reflect rations in which supplemental protein is needed or protein is relatively expensive.

### 2.2.3 Adjusted Total Forage Index (ATFI)

The effect of heat damage on protein availability was calculated from the crude protein (CP) and acid detergent fibre-nitrogen (ADF-N) content (Erasmus, 2000). This adjusted crude protein (ACP) was used in the TFI formula:

ATFI = RFV + (ACP × $x$)

Where $x$ = a multiplier of 6

### 2.2.4 Lucerne Quality Index (LQI)

LQI was calculated from the following formula and expressed as an index:

LQI = (IVOMD) × (DMI as % of body weight) + (MP × $x$)

Where MP = metabolizable protein (AFRC, 1992).
$x$ = protein multiplier of 6
IVOMD = *In vitro* organic matter digestibility
DMI = Dry matter intake

Several equations were used to calculate MP as described in chapter 1 (par. 2.2.13).

## 2.3 Statistical analysis

Simple and multiple linear regressions and correlation coefficients were calculated for the data using PC SAS 6.04 (Cary NC: SAS institute Inc.). Guidelines were followed from the SAS Procedure Guide (1988) and the second edition of the SAS system for regression (1991). Expressions of the proportion of the sum of squares for the dependent variable explained by these regressions were calculated as $r^2$.

# 3. RESULTS AND DISCUSSION

Selected linear regressions and coefficients of statistical significant ($P<0.0001$) determinations between chemical, calculated and degradability parameters are given in Table 1. All the varieties included in the regression equation have a significant ($P<0.05$) contribution to the coefficients of determination ($r^2$).

## 3.1 Chemical analysis

### 3.1.1 Energy related parameters

The high correlation observed in Table 1 between ADF and NDF was expected. The hemicellulose fraction in NDF is the only difference between these two fibre analysis. On the other hand ADF and NDF respectively were significant ($P<0.0001$) but less related to CF. Van Soest (1968) and Mertens (1982) emphasised that CF is an incomplete measure of

the less digestible part of feeds, as it fails to measure hemicellulose and most of the lignin. This probably explains the poorer prediction of ADF and NDF from CF. Van Soest (1968) mentioned that ADF or NDF are much more useful and accurate measures of the fibre component of feeds than are values for CF.

**Table 1 Simple linear regressions equations and coefficients of determination of energy related parameters in lucerne hay**

| Independent variate ($X_1$) | Dependent variate (Y) | $r^2$ | n | Regression equation $Y = b_0 + b_1X_1$ |
|---|---|---|---|---|
| Neutral detergent fibre | Acid detergent fibre | 0.74 | 209 | $Y = 4.26 + 0.74X_1$ |
| Crude fibre | Acid detergent fibre | 0.57 | 209 | $Y = 11.42 + 0.81X_1$ |
| Crude fibre | Neutral detergent fibre | 0.61 | 209 | $Y = 13.39 + 0.89X_1$ |
| Acid detergent fibre | Non-fibre carbohydrate | 0.56 | 110 | $Y = 46.05 - 0.65X_1$ |
| Neutral detergent fibre | Non-fibre carbohydrate | 0.76 | 110 | $Y = 52.12 - 0.67X_1$ |
| *In vitro* organic matter digestibility | Non-fibre carbohydrate | 0.66 | 110 | $Y = -62.53 + 1.20X_1$ |
| Dry matter intake | Non-fibre carbohydrate | 0.74 | 110 | $Y = -10.52 + 11.86X_1$ |
| Acid detergent fibre | *In vitro* organic matter digestibility | 0.47 | 209 | $Y = 83.54 - 0.39X_1$ |
| Neutral detergent fibre | *In vitro* organic matter digestibility | 0.57 | 209 | $Y = 86.00 - 0.38X_1$ |
| ERDMD c[2)] | ERDMD a[1)] | 0.72 | 30 | $Y = 0.25 - 0.32X_1$ |

[1)] Soluble fraction of the effective rumen dry matter degradability

[2)] Degradation rate of the slowly degradable fraction of dry matter

Van Soest (1964), described a highly significant ($P<0.0001$) negative relationship between ADF content and the extent to which legumes are digested. This is in agreement with the findings of Cilliers & Van der Merwe (1993) ($P<0.0001$; $r^2 = 0.74$) using veld herbage. According to Hvelplund *et al.* (1997) *in vitro* organic matter digestibility (IVOMD) is a laboratory method on which the calculation of energy value of a feed is based. As ADF content also evaluates the available energy in a feed, it should correlate highly with IVOMD. This is confirmed by Rohweder *et al.* (1976a) who stated that ADF is the chemical assay of choice to estimate the *in vitro* dry matter digestibility (IVDMD) of a wide

range of forages, including lucerne hay. In contrast with these results, ADF predicted IVOMD less accurately ($r^2$ = 0.47) in the present study. In fact a better prediction was obtained with NDF ($r^2$ = 0.57) than with ADF. Accordingly Mertens (1980) proposed the use of NDF to estimate the fibre content and energy value of feeds as well as diet bulk density and rumen fill (Mertens, 1985). Therefore it would seem that, compared to NDF, ADF is a less accurate estimator of energy value or IVOMD. According to these results the best predictor of the energy content of forages could differ among various roughages. A preliminary study done by Van der Merwe & Fair (1999-unpublished data) with 50 lucerne hay samples indicated that the NDF content can be used to predict IVOMD with a significantly (P<0.0001) high degree of accuracy ($r^2$ = 0.80). In the present study the NDF content of 208 lucerne hay samples (Table 1) tended to be a poorer predictor of IVOMD than that found in the preliminary study. These results suggest that correlation coefficients calculated from a small number of hay samples should be interpreted and used with care.

The correlation coefficients in Table 1 also revealed that non-fibre carbohydrate (NFC) were significantly (P<0.0001) correlated with ADF, NDF and IVOMD respectively. The high correlation coefficient between NFC and NDF suggested that NDF is adequate to estimate readily available carbohydrates (NFC) in lucerne hay. An even stronger (P<0.01) relationship (r = 0.99) was reported by Bethard (1997) between NFC and NDF. However, the small number of hay samples (n = 3) used by Bethard (1997) should be interpreted with care. In contrast with the present study, Bethard (1997) found a non-significant (P>0.05) relationship between ADF and NFC.

The highest correlation between effective ruminal dry matter degradability (ERDMD) parameters (a, b and c) occurred between ERDMD a and ERDMD c (Table 1). The total amount of dry matter (DM) estimated to be degraded in the rumen (ERDMD) was positively related ($r^2$ = 0.37) to ERDMD a (P<0.0001). However it was not significantly (P>0.05) related to either ERDMD b ($r^2$ = 0.0001) or ERDMD c ($r^2$ = 0.05). In contrast with these results Mathison *et al.* (1999) reported that ERDMD c of barley straw was significantly (P<0.05) negatively correlated (r = -0.63) to ERDMD b. They found a significant (P<0.05) correlation (r = 0.85) between ERDMD and ERDMD for barley straw at a fractional outflow rate of 0.02/h. According to Mathison *et al.* (1999) the amount of DM estimated to be

degraded in the rumen was positively related to the protein content of the straw (r = 0.70) and negatively related to ADF (r = -0.89) and NDF (r = -0.84). For lucerne hay in the present study these correlations were non-significant (P>0.05).

### 3.1.2 Protein related parameters

The prediction of protein related parameters adjusted crude protein (ACP), effective ruminal protein degradability (ERPD) and effective rumen degradable protein (ERDP) are shown in Table 2. A significant positive relationship was found between ACP and CP. In accordance with the low acid detergent fibre-nitrogen (ADF-N) content of lucerne hay (Chapter 2), this high relationship was to be expected. From these results it seems that there is little to gain from determining ACP in lucerne hay.

In the present study no reliable coefficient of determination ($r^2$ = 0.29) between CP and IVOMD was found. Contrary to this, Lema (1972) found a positive relationship (r = 0.82) between CP content (of lucerne) and *in vitro* dry matter digestibility (IVDMD).

From the coefficients of determination in Table 2 it seems that effective ruminal protein degradability (ERPD) could be predicted fairly accurately from chemical analyses and IVOMD showed the highest relationship ($r^2$ = 0.56) with ERPD. According to McDonald *et al.* (1995) a significant correlation exists between CP and degradability, reflecting the decreased proportion of the nitrogen fraction bound to fibre with increasing nitrogen content. In the present study a significant (P<0.0001) but moderate ($r^2$ = 0.50) relationship was found. Metabolizable energy (ME), fermentable metabolizable energy (FME) and digestible microbial protein (DMP) which were calculated from IVOMD, showed the same relationship ($r^2$ = 0.56) with ERPD. As expected, a significantly high correlation was observed between effective rumen degradable protein (ERDP) and effective ruminal protein degradability (ERPD).

**Table 2 Simple linear regression equations and coefficients of determination of protein related parameters in lucerne hay**

| Independent variate ($X_1$) | Dependent variate (Y) | $r^2$ | n | Regression equation $Y = b_0 + b_1X_1$ |
|---|---|---|---|---|
| Crude protein | Adjusted crude protein | 0.93 | 194 | $Y = -0.52 + 0.95X_1$ |
| Acid detergent fibre | ERPD[1)] | 0.50 | 30 | $Y = 0.84 - 0.01X_1$ |
| Neutral detergent fibre | ERPD[1)] | 0.50 | 30 | $Y = 0.85 - 0.01X_1$ |
| *In vitro* organic matter digestibility | ERPD[1)] | 0.56 | 30 | $Y = -0.43 + 0.01X_1$ |
| Crude protein | ERPD[1)] | 0.50 | 30 | $Y = -0.03 + 0.03X_1$ |
| Relative feed value | ERPD[1)] | 0.51 | 30 | $Y = 0.32 + 0.01X_1$ |
| Total forage index | ERPD[1)] | 0.56 | 30 | $Y = 0.19 + 0.01X_1$ |
| Adjusted total forage index | ERPD[1)] | 0.55 | 30 | $Y = 0.22 + 0.01X_1$ |
| Lucerne quality index | ERPD[1)] | 0.10 | 30 | $Y = 0.39 + 0.01X_1$ |
| ERPD a[2)] | ERPD[1)] | 0.48 | 30 | $Y = 0.42 + 0.54X_1$ |
| Effective rumen degradable protein | ERPD[1)] | 0.86 | 30 | $Y = 0.18 + 0.01X_1$ |
| Metabolizable energy | ERPD[1)] | 0.56 | 30 | $Y = -0.43 + 0.01X_1$ |
| Fermentable metabolizable energy | ERPD[1)] | 0.57 | 30 | $Y = -0.45 + 0.01X_1$ |
| Digestible microbial protein | ERPD[1)] | 0.56 | 30 | $Y = 0.21 + 0.01X_1$ |
| Crude protein | Effective rumen degradable protein | 0.75 | 30 | $Y = 83.23 + 9.34X_1$ |
| Acid detergent fibre | Effective rumen degradable protein | 0.63 | 30 | $Y = 185.53 - 2.27X_1$ |
| Neutral detergent fibre | Effective rumen degradable protein | 0.58 | 30 | $Y = 191.28 - 1.95X_1$ |
| Crude fibre | Effective rumen degradable protein | 0.61 | 30 | $Y = 190.45 - 2.64X_1$ |
| *In vitro* organic matter digestibility | Effective rumen degradable protein | 0.68 | 30 | $Y = -0.42 + 0.01X_1$ |
| Digestible dry matter | Effective rumen degradable protein | 0.62 | 30 | $Y = -74.00 + 2.92X_1$ |
| Dry matter intake | Effective rumen degradable protein | 0.66 | 30 | $Y = 5.31 + 34.39X_1$ |
| Relative feed value | Effective rumen degradable protein | 0.68 | 30 | $Y = 35.56 + 0.49X_1$ |
| Total forage index | Effective rumen degradable protein | 0.78 | 30 | $Y = -2.43 + 0.42X_1$ |
| Adjusted crude protein | Effective rumen degradable protein | 0.76 | 30 | $Y = -61.34 + 8.92X_1$ |
| Adjusted total forage index | Effective rumen degradable protein | 0.76 | 30 | $Y = 4.17 + 0.40X_1$ |
| Metabolizable energy | Effective rumen degradable protein | 0.68 | 30 | $Y = -170.91 + 24.35X_1$ |
| Fermentable metabolizable energy | Effective rumen degradable protein | 0.68 | 30 | $Y = -191.89 + 27.45X_1$ |
| Digestible microbial protein | Effective rumen degradable protein | 0.67 | 30 | $Y = -180.78 + 25.23X_1$ |

[1)] Effective ruminal protein degradability

[2)] Soluble fraction of the effective rumenal protein degradability

ERPD was significantly ($P<0.0001$) related to ERPD a. However, no significant ($P>0.05$) relationship ($r^2 = 0.10$) exists between ERPD and ERPD b. Janicki & Stallings (1988) did find a statistically significant ($P<0.001$) correlation ($r = -0.89$) between ERPD and ERPD b, and a non significant relationship with ERPD a ($P>0.05$). All ERPD parameters (a, b and c) were poorly ($r^2<0.25$) but significantly ($P<0.01$) predicted by CP. IVOMD showed the same relationship ($r^2 = \pm 0.50$) with these parameters as did CP. Krishnamoorthy *et al.* (1983) emphasized that a small number of hay samples may be partly responsible for some loss of significance in correlation, especially with a large variation among samples. Correlation with ERPD and its parameters in this present study includes only 30 samples and this should therefore be interpreted with care.

Microbial demand for protein (ERDP) could be predicted moderately to highly accurately from chemical analysis, components of evaluation models and evaluation models in Table 2. CP showed the highest relationship ($r^2 = 0.75$) with ERDP. CP is a component in the calculation of ERDP, and explained 75% of the variation of the ERDP value. Thus the results of the present study suggested that the variation in ERDP were due mainly to large differences in the CP content which represented the main portion of ERDP.

### 3.2 Chemical analysis and models

Simple correlations between different models for lucerne hay quality determination and chemical variables are shown in Table 3. Analysis of correlation between relative feeding value (RFV) and ADF, NDF, CF and NFC showed a strong significant ($P<0.0001$) relationship. CF showed the lowest ($r^2 = 0.55$) and NDF the highest ($r^2 = 0.86$) coefficient of determination to predict RFV. From the coefficient of determination it seems that an even more reliable function as independent factor in a simple regression to predict RFV, was DMI (which was calculated from NDF). RFV does not account for the crude protein content of the forage. It is based only on fibre levels and is therefore an index of forage digestibility and an estimate of energy value or energy intake potential (Taylor, 1997). IVOMD, which is also a predictor of energy content (Hvelplund *et al.* 1997), related

**Table 3 Simple linear regressions equations and coefficients of determination of quality evaluation models for lucerne hay**

| Independent variate ($X_1$) | Dependent variate (Y) | $r^2$ | n | Regression equation $Y = b_0 + b_1X_1$ |
|---|---|---|---|---|
| Acid detergent fibre | Relative feed value | 0.81 | 209 | $Y = 275.93 - 4.02X_1$ |
| Neutral detergent fibre | Relative feed value | 0.86 | 209 | $Y = 282.29 - 3.46X_1$ |
| Crude fibre | Relative feed value | 0.55 | 209 | $Y = 238.66 - 3.84X_1$ |
| Non fibre carbohydrate | Relative feed value | 0.67 | 110 | $Y = 34.37 + 4.07X_1$ |
| Digestible dry matter | Relative feed value | 0.67 | 209 | $Y = -130.29 + 4.24X_1$ |
| Dry matter intake | Relative feed value | 0.94 | 209 | $Y = -47.59 + 64.01X_1$ |
| Crude protein | Total forage index | 0.61 | 209 | $Y = -21.92 + 13.28X_1$ |
| Acid detergent fibre | Total forage index | 0.80 | 208 | $Y = 440.68 - 5.31X_1$ |
| Neutral detergent fibre | Total forage index | 0.82 | 209 | $Y = 448.28 - 4.56X_1$ |
| Crude fibre | Total forage index | 0.57 | 209 | $Y = 399.96 - 4.85X_1$ |
| Non fibre carbohydrate | Total forage index | 0.52 | 110 | $Y = 131.72 + 4.93X_1$ |
| Digestible dry matter | Total forage index | 0.60 | 209 | $Y = -85.13 + 5.40X_1$ |
| Dry matter intake | Total forage index | 0.85 | 209 | $Y = 17.51 + 82.81X_1$ |
| Relative feed value | Total forage index | 0.90 | 209 | $Y = 79.44 + 1.29X_1$ |
| Adjusted crude protein | Total forage index | 0.66 | 194 | $Y = -11.94 + 13.80X_1$ |
| Crude protein | Adjusted total forage index | 0.59 | 117 | $Y = -23.01 + 12.93X_1$ |
| Acid detergent fibre | Adjusted total forage index | 0.79 | 116 | $Y = 440.04 - 5.51X_1$ |
| Neutral detergent fibre | Adjusted total forage index | 0.84 | 117 | $Y = 446.05 - 4.68X_1$ |
| Crude fibre | Adjusted total forage index | 0.57 | 117 | $Y = 392.78 - 4.88X_1$ |
| Non-fibre carbohydrate | Adjusted total forage index | 0.58 | 110 | $Y = 120.13 + 5.16X_1$ |
| *In vitro* organic matter digestibility | Adjusted total forage index | 0.51 | 117 | $Y = -262.47 + 7.15X_1$ |
| Digestible dry matter | Adjusted total forage index | 0.57 | 209 | $Y = -93.77 + 5.41X_1$ |
| Dry matter intake | Adjusted total forage index | 0.88 | 209 | $Y = 6.88 + 83.45X_1$ |
| Relative feed value | Adjusted total forage index | 0.93 | 209 | $Y = 69.25 + 1.31X_1$ |
| Total forage index | Adjusted total forage index | 0.97 | 209 | $Y = -10.18 + 1.01X_1$ |
| Adjusted crude protein | Adjusted total forage index | 0.66 | 194 | $Y = -23.49 + 13.99X_1$ |
| Acid detergent fibre | Lucerne quality index | 0.50 | 30 | $Y = 1319.46 - 7.67X_1$ |
| Neutral detergent fibre | Lucerne quality index | 0.50 | 30 | $Y = 1352.19 - 6.85X_1$ |
| Relative feed value | Lucerne quality index | 0.43 | 30 | $Y = 832.56 + 1.47X_1$ |
| Total forage index | Lucerne quality index | 0.48 | 30 | $Y = 723.46 + 1.23X_1$ |
| Adjusted total forage index | Lucerne quality index | 0.49 | 30 | $Y = 737.61 + 1.22X_1$ |
| Metabolizable protein | Lucerne quality index | 0.50 | 30 | $Y = 116.70 + 6.34X_1$ |

moderately ($r^2$ = 0.48) but significant (P<0.0001) with RFV. A significant positive relationship (P<0.0001) was noted between RFV and CP ($r^2$ = 0.35). Similar results (P<0.0001) were obtained by correlating CP with DMI ($r^2$ = 0.35). Cilliers & Van der Merwe (1993) accordingly described a highly significant (P<0.01) correlation ($r^2$ = 0.85) between DMI and CP of summer veld herbage. The $r^2$ and significance of estimating RFV from NDF, suggest that the advantages to be gained from the use of RFV are small. However, as ADF and NDF are used to calculate RFV these results were expected. Therefore the potential energy intake (RFV) can be determined ($r^2$ = 0.86) only from the NDF content of lucerne hay.

Hutjens (1995) suggested that protein has considerable value and should be incorporated into the RFV system to reflect more completely the nutritive value of the forage. From Table 3 it is evident that the CP content of lucerne hay showed a significant (P<0.0001) correlation with the total forage index (TFI) (Table 3). The same was found for ADF, NDF, DMI, CF and NFC. However, the contribution of TFI as a practical model to determine lucerne hay quality was still small compared to the use of an individual chemical parameter like NDF.

Acid detergent fibre-nitrogen (ADF-N) was incorporated into the TFI system of Hutjens (1995) (par. 2.2.3) to reflect more completely the true nutritive value of lucerne hay. The term adjusted total forage index (ATFI) describes an index which incorporates RFV and adds the true adjusted crude protein value as well as a physical value (subjective). The chemical contents ADF, NDF, CP and ADF-N were used to estimate this ATFI of lucerne hay. In terms of chemical analysis NDF ($r^2$ = 0.84) and the calculated value from NDF, DMI gave the best estimations of ATFI. The poorer prediction of ATFI from CF was expected, as ADF and NDF were used to calculate ATFI. IVOMD, which is an accurate determination of the energy value (digestibility) of a feed (Hvelplund *et al.* 1997), predicted ATFI less accurately ($r^2$ = 0.51) than the other variables.

DMI predicted RFV, TFI and ATFI with almost the same accuracy. Failure to obtain improved predictions ($r^2$) using RFV, TFI and ATFI values rather than their components

ADF and NDF, may indicate that they have similar effects with regard to evaluating lucerne hay quality.

A significant ($P<0.0001$) relationship occurred between LQI and variables like ADF, NDF and metabolizable protein (MP) respectively. ADF and NDF, however, did not show the same high $r^2$ with LQI compared to RFV, TFI and ATFI respectively. In contrast with the other models no significant ($P>0.05$) correlation between LQI and CP was detected. A possible explanation for the lower prediction of LQI from simple chemical parameters is the complexity of the model. This model probably shows the importance of analysing the proper combination of energy and protein quality parameters to calculate the quality of lucerne hay. However a significant positive correlation ($P<0.0001$) was observed between LQI and MP. This observation probably indicated that LQI is a better calculation of lucerne hay quality than the other models. In the first place LQI includes IVOMD which is a better estimation of energy value (digestibility) than ADF. Secondly MP is a better value for protein quality compared to CP or ACP.

### 3.3 Correlations between lucerne hay quality models

In Table 3 coefficients of determination between the various lucerne hay quality models are given. A significant ($P<0.0001$) positive relationship was found between RFV and TFI (Table 3). An even stronger relationship was noted between RFV and ATFI (Table 3). The highest correlation was observed between TFI and ATFI. Thus the relative performance of these quality models was almost similar. These results suggest that there is no benefit to be gained by using quality models which include CP and ACP. Even though a strong relationship exists between these quality models, the included chemical components (CP, ACP) that distinguish them showed a significant ($P<0.0001$) but relatively poor relationship with RFV ($r^2 = 0.35$ and $r^2 = 0.41$ respectively).

Significant ($P<0.0001$) but lower ($r^2 = \pm 0.50$) correlations were found between LQI and RFV, TFI or ATFI respectively. In contrast with the other models LQI includes IVOMD and MP. The poorer relationship probably indicates that, because of these variables, LQI is a more accurate measure of the quality of lucerne hay.

### 3.4 Correlations between essential amino acids (EAA)

Linear regressions and prediction statistics for essential amino acids (EAA) are presented in Table 4.

**Table 4 Simple linear regression equations and the coefficients of determination of essential amino acids**

| Independent variate ($X_1$) | Dependent variate (Y) | $r^2$ | n | Regression equation $Y = b_0 + b_1X_1$ |
|---|---|---|---|---|
| Isoleucine | Valine | 0.52 | 118 | $Y = 1.67 + 1.05X_1$ |
| Leucine | Valine | 0.76 | 117 | $Y = 0.23 + 0.80X_1$ |
| Phenylalanine | Valine | 0.67 | 118 | $Y = 2.36 + 0.83X_1$ |
| Leucine | Isoleucine | 0.83 | 117 | $Y = 0.41 + 0.56X_1$ |
| Phenylalanine | Isoleucine | 0.60 | 117 | $Y = 1.63 + 0.53X_1$ |
| Valine | Lysine | 0.54 | 118 | $Y = 1.28 + 0.59X_1$ |
| Isoleucine | Lysine | 0.81 | 118 | $Y = 0.56 + 1.10X_1$ |
| Phenylalanine | Lysine | 0.65 | 118 | $Y = 2.01 + 0.70X_1$ |
| Phenylalanine | Arginine | 0.65 | 118 | $Y = 1.69 + 0.52X_1$ |

Of the nine EAA measured in the present study, methionine, histidine and threonine were not significantly ($P>0.05$) predicted from other EAA. The highest ($P<0.0001$) correlation occurred between isoleucine and leucine and isoleucine and lysine. According to Schwab *et al.* (1992), Rulquin & Deluby (1994), Erasmus (2000) and NRC (2001), methionine, lysine and histidine are the three most limiting EAA for milk synthesis. From Table 4 it is evident that only lysine would be significantly ($P<0.0001$) predicted from other EAA. Phenylalanine showed a significant ($P<0.0001$) relationship with several EAA (valine, isoleucine, lysine and arginine) (Table 4).

Linear correlations between EAA in lucerne hay are of academic interest as the standard procedure for EAA determinations include nine of the ten EAA. However, near infrared reflectance spectroscopy (NIRS) could provide a solution to the problem of individual EAA determinations and needs further investigation.

### 3.5 Multiple regressions

IVOMD and ERPD are important components of LQI. Stepwise regression was performed on the data for possible combinations of chemical analysis that may predict IVOMD, ERPD and LQI accurately. Significant ($P<0.0001$) regression equations are indicated in Table 5.

The partial contribution of fat, ADF, CP, NFC and CF to the prediction of IVOMD in multiple regression equations was relatively small. Therefore the NDF content of lucerne hay alone can be used to predict IVOMD. From the coefficient of determination, it seems that a more reliable function for prediction of IVOMD was found with 108 compared to 208 lucerne hay samples. Thus the effect of prediction using different sample sizes was inconsistent. Regressions calculated from smaller sample sizes should therefore be interpreted with care.

When the components of LQI were evaluated as factors in a multiple regression equation it seems that the ADF, ERPD and CP content of lucerne hay played a major role in predicting LQI. In accordance with the small CV of IVOMD calculated in Chapter 1, the partial contribution to the coefficient of determination was negligible. However, the small number of samples used in this multiple regression analysis make it difficult to arrive at final conclusions. This also applies for the prediction equation of ERPD.

**Table 5** **Multiple regression equations for predicting in vitro organic matter digestibility, crude protein degradability and lucerne quality index and the coefficients of determination ($r^2$) between dependent and independent variates**

| Independent variate (X) | Dependent variate (Y) | $r^2$ | n | Regression equation $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4$ |
|---|---|---|---|---|
| Neutral detergent fibre ($X_1$) | IVOMD[1)] | 0.54 | 208 | $Y = 81.33 - 0.37X_1$ |
| Fat ($X_2$) | | 0.57 | 208 | $Y = 81.73 - 0.34X_1 + 1.32X_2$ |
| Acid detergent fibre ($X_3$) | | 0.58 | 208 | $Y = 82.25 - 0.25X_1 + 1.32X_2 - 0.12X_3$ |
| Crude protein ($X_4$) | | 0.59 | 208 | $Y = 77.95 - 0.24X_1 + 1.31X_2 - 0.10X_3 + 0.15X_4$ |
| Neutral detergent fibre ($X_1$) | IVOMD[1)] | 0.75 | 108 | $Y = 89.42 - 0.44X_1$ |
| Non-fibre carbohydrates($X_2$) | | 0.77 | 108 | $Y = 82.46 - 0.35X_1 + 0.13X_2$ |
| Crude fibre ($X_3$) | | 0.78 | 108 | $Y = 78.34 - 0.15X_1 + 0.22X_2 - 0.12X_3$ |
| Acid detergent fibre ($X_1$) | LQI[2)] | 0.5 | 30 | $Y = 126.59 - 0.62X_1$ |
| ERPD[3)] ($X_2$) | | 0.79 | 30 | $Y = 173.96 - 1.06X_1 - 56.59X_2$ |
| Crude protein ($X_3$) | | 0.94 | 30 | $Y = 133.18 - 0.79X_1 - 71.85X_2 + 2.00X_3$ |
| IVOMD[1)] ($X_4$) | | 0.97 | 30 | $Y = 36.56 - 0.19X_1 - 81.87X_2 + 2.00X_3 + 1.15X_4$ |
| ADF-N[4)] ($X_5$) | | 0.98 | 30 | $Y = 50.59 - 0.19X_1 - 76.98X_2 + 2.14X_3 + 0.92X_4 - 17.89X_5$ |
| IVOMD[1)] ($X_1$) | ERPD[3)] | 0.56 | 30 | $Y = -0.43 + 0.01X_1$ |
| ADF-N[4)] ($X_2$) | | 0.65 | 30 | $Y = -0.89 + 0.02X_1 + 0.65X_2$ |

[1)] *In vitro* organic matter digestibility

[2)] Lucerne quality index

[3)] Effective ruminal protein degradabilty

[4)] Acid detergent fibre-nitrogen

## 4. CONCLUSIONS

From the results of the present study it seems that compared to ADF, NDF is a better predictor of IVOMD in lucerne hay. This contrasts with reports by several researchers (Van Soest, 1964; Rohweder *et al.*, 1976a; Hvelplund *et al.*, 1997; Taylor, 1997) and points to differences among forages. In fact NDF showed a strong correlation with RFV, TFI and ATFI respectively. Accordingly a high relationship occurred between these various models.

These high coefficients of determinations suggest that NDF analysis is a good indicator of lucerne hay quality. A moderate ($P<0.0001$) correlation was however observed between LQI and RFV, TFI and ATFI respectively. Accordingly ADF and NDF showed a moderate ($P<0.0001$) relationship with LQI. These results possibly indicate that LQI is a better evaluation system (model) of lucerne hay quality.

Calculation of LQI requires the determination of IVOMD (ME), NDF, CP, fat, ERPD fractions (a, b and c) and ADF-N. IVOMD, ERPD fractions (a, b and c) and ADF-N are probably the most problematic to determine in the laboratory. However, according to the results of the present study, NDF could be used to predict fairly accurately the IVOMD and ERPD but not the fractions a, b and c of lucerne hay. Thus the calculation of DUP, and accordingly of MP from these predictions would be inaccurate. ACP could be accurately predicted from CP. The low ADF-N values of lucerne hay seem to indicate a negligible contribution. Another possibility is to predict LQI directly from ADF ($r^2 = 0.50$) or ADF, ERDP and CP ($r^2 = 0.94$) in a multiple regression. However, the conclusion on predicting LQI is based on a relatively small quantity ($n = 30$) of lucerne hay samples. A further study is needed to confirm these results with a larger quantity of lucerne hay samples. The usage of near infrared reflectance spectroscopy (NIRS) for the rapid nutritional evaluation of lucerne hay (LQI) also needs urgent investigation.

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## GENERAL CONCLUSIONS

Lucerne hay is one of the most important high quality hay crops in South Africa. The results of the present study clearly indicated the variation in energy and protein composition as well as the utilisation of nutrients in lucerne hay. The highest CV was observed for the effective degradation parameters a, b and c. On the other hand IVOMD showed a low CV. Nevertheless a CV of approximately 15% was detected for both ADF and NDF. Accordingly DMI, and thus energy intake, varied.

The results of the present study did not support the findings in literature that lucerne hay CP has a fairly high RDP fraction. These differences could be attributed to the larger particle size of samples used in the present study. The most representative particle size of lucerne hay in the rumen of dairy cows after mastication needs further investigation to improve the accuracy of *in situ* studies. According to the results of the present study, lucerne hay is a potential source of RUP in dairy cow diets. Therefore the EAA contribution of lucerne hay, though small in some instances, especially as regards methionine, could contribute to the amino acid requirements of dairy cow diets.

The variation that occurred in the nutritive value of lucerne hay (Chapter 1) emphasized the need for an accurate quality evaluation system (model) for lucerne hay. Lucerne hay is mostly used in dairy cow diets and such an evaluation system should, in the first place, as in the current study, be directed at dairy cows. Such an evaluation system would probably also be applicable for other ruminants like beef, cattle and sheep.

Lucerne hay is also utilised for poultry, horse and especially ostrich nutrition in South Africa. Since the alimentary tracts of these species differ from ruminants, an alternative evaluation system should probably be developed for them.

From the results of the present study it seems that existing quality evaluation models (RFV, TFI and ATFI) for lucerne hay consumed by dairy cattle do not differ in accuracy. In fact NDF alone seems to be a good predictor of the outcome of these models. These models were however a moderate predictor of LQI. The same applied for ADF and NDF. These

results suggested that crude protein quality (MP) should be an integral part of an evaluation system for lucerne hay consumed by ruminants. Therefore LQI, which considers energy intake potential and MP, seems to be the most appropriate and accurate quality evaluation model for lucerne hay. However, the LQI however overlooked EAA composition. Therefore further investigation on models for lucerne quality evaluation should be directed at the inclusion of EAA composition.

The successful implementation of a quality evaluation system (model) for lucerne hay in practice should inter alia satisfy the following requirements:

a) It should be simple to carry out
b) It should be able to be implemented in a relatively short time
c) It must be accurate
d) Visual (subjective) judging must be part of the system. Chemical analysis does not identify mould and foreign materials that could be present in lucerne hay.
e) Objective measurement should be mainly put into practice.
f) It should be acceptable for all participants.

At this stage the determination of ERPD fractions (a, b and c) seems to be the most problematic for the implementation of LQI in practice. According to the results of the present study, it seems that LQI could be predicted directly from ADF ($r^2 = 0.50$) or from ADF, ERDP and CP ($r^2 = 0.94$) in a multiple regression. However, these regressions are based on a relatively small quantity ($n = 30$) of lucerne hay samples. Further research is needed to confirm these results with a larger quantity of lucerne hay samples.

The usage of near infrared reflectance spectroscopy (NIRS) for the rapid determination of IVOMD (ME), NDF, CP, fat, ERPD fractions (a, b and c), ADF-N and eventually LQI should enjoy further investigation. This will largely determines whether it is possible to fulfil the above-mentioned requirements. In practice it means obtaining and milling a representative sample of a cutting of lucerne hay. This sample could be sent to a laboratory with a NIRS. The needed determinations can then be completed in 5 seconds and the results sent back by means of fax or e-mail.

The LQI evaluation model developed and identified in the present study is based on the AFRC (1992)-protein requirement system. This evaluation system should be compared in a further study with the most recent systems of Cornell net carbohydrate and protein system (Russell *et al.*, 1992; Sniffen *et al.*, 1992) and NRC (2001).

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## ABSTRACT

A study was conducted to develop and identify an evaluation system (model) to predict lucerne hay quality. Two hundred and ten lucerne hay samples for chemical analyses and *in vitro* digestibility determinations were obtained from different cuttings during two seasons (100 samples for 1998/1999 and 110 for 1999/2000), at different times in a season and from different lucerne producing areas (sites) in the Northern Cape, South Africa. One hundred and eighteen of these samples were used for essential amino acid analysis. Thirty of the 210 samples and an additional 42 lucerne hay samples were obtained during one season (1999/2000) to estimate protein degradation (*in sacco*). Twelve Dorper lambs fitted with a rumen cannula were used in the degradation study.

The variation in nutritive value of South African lucerne hay was evaluated as an initial study. The highest moisture content recorded (9.35%) was safely below the critical moisture level of 15% for effective storage. The coefficient of variation (CV = ±15%) of crude fibre (CF), acid detergent fibre (ADF) and neutral detergent fibre (NDF) emphasized the need for analysis to ensure accurate diet formulation. This also applied for ash (CV = 22.11%), crude protein (CP) (CV = 11.6%), non-fibre carbohydrate (NFC) (CV = 34.0), effective ruminal protein degradability (ERPD) (CV = 18.9%) and essential amino acids (EAA) (CV = 14.5% - 27.1%). The effective protein degradation parameters a, b and c showed the highest CV (106.8% - 306.4%). The mean *in vitro* organic matter digestibility (IVOMD) value (67.6%) seems to be representative (CV = 5.4%) of the IVOMD for the lucerne hay population used.

Lower ERPD values (average 47.7%) than usually reported, were observed. The influence of acid detergent fibre nitrogen (ADF-N) on the rumen undegradable protein (RUP) of lucerne hay was negligible. Heat damage occurred to a small percentage (3.5%) of the samples. Lucerne hay had a high lysine (4.55g/100g CP) and low methionine (0.44g/100g CP) content. Methionine, lysine and isoleucine were, in a descending order, the least detectable in lucerne hay for milk synthesis.

A second study was conducted to include protein quality, according to the United Kingdom metabolizable protein system (MP), into the total forage index (TFI) system to determine the quality of lucerne hay more accurately. This model (lucerne quality index) was compared to the chemical analysis and existing models for the determination of lucerne hay quality. The following models were used to estimate lucerne hay quality:

a) Relative feed value (RFV) = % digestible dry matter (DDM) × dry matter intake as a % of body weight (DMI) × 0.775

b) Total forage index (TFI) = RFV + % CP × 6

c) Adjusted total forage index (ATFI) = RFV + adjusted crude protein (ACP) × 6.

d) Lucerne quality index (LQI) = IVOMD × DMI + MP × 6, expressed as an index.

NDF ($r^2$ = 0.57) compared to ADF ($r^2$ = 0.48) was a more accurate estimator of IVOMD. The NDF content of lucerne hay also played an important ($P<0.0001$) role in regression equations to predict the quality models RFV ($r^2$ = 0.86), TFI ($r^2$ = 0.82) and ATFI ($r^2$ = 0.84) respectively as well as IVOMD ($r^2$ = 0.57) and ERPD ($r^2$ = 0.50). A high relationship occurred between these various models ($r^2>0.90$). A moderate correlation ($P<0.0001$) was however observed between LQI and respectively ADF ($r^2$ = 0.50), NDF ($r^2$ = 0.50), IVOMD ($r^2$ = 0.56), CP ($r^2$ = 0.50), RFV ($r^2$ = 0.51), TFI ($r^2$ = 0.56) or ATFI ($r^2$ = 0.55). No significant ($P>0.05$) predictor for the ERPD fractions (a, b and c) were observed. LQI could be predicted from ADF ($r^2$ = 0.50) or ADF, ERPD and CP ($r^2$ = 0.94) in a multiple regression equation.

The results of the present study clearly indicated that a large variation occur in the energy and protein composition as well as the utilization of nutrients in South African lucerne hay. This emphasizes the need for a rapid and accurate quality evaluation system for lucerne hay in practice. LQI seems to be a better quality evaluation system than to those currently available. The relationships with LQI in the present study is based on a relatively small number (n = 30) of lucerne hay samples. Further research with a larger number of lucerne hay samples is needed to confirm these results. The usage of near infrared reflectance spectroscopy (NIRS) for the rapid nutritional evaluation of lucerne hay (LQI) also needs urgent investigation.

## OPSOMMING

Navorsing is onderneem om 'n evalueringstelsel (model) te ontwikkel en identifiseer vir die bepaling van lusernkwaliteit. Tweehonderd en tien lusernhooimonsters vir chemiese ontledings en *in vitro* verteerbaarheidsbepalings is ingesamel vanaf verskillende snysels gedurende twee seisoene (100 monsters gedurende 1998/1999 en 110 gedurende 1999/2000), verskillende tye binne 'n seisoen en vanaf verskeie lusernproduserende gebiede in die Noord-Kaap provinsie van Suid-Afrika. Eenhonderd en agtien van hierdie monsters is vir essensiële aminosuurontledings gebruik. Dertig van hierdie 210 monsters en 'n verdere 42 lusernhooimonsters is verkry gedurende een seisoen (1999/2000) om proteïendegradering (*in sacco*) te beraam. Twaalf Dorperlammers toegerus met rumenfistels is vir die studie gebruik.

Die variasie in voedingswaarde van Suid-Afrikaanse lusernhooi is in 'n eerste studie geëvalueer. Die hoogste voginhoud gemeet (9.35%) was veilig onder die kritiese vogvlak van 15% vir effektiewe opberging. Die koëffisient van variasie (CV = ±15%) vir ruvesel (CF), suurbestande vesel (ADF) en neutraalbestande vesel (NDF) het die noodsaaklikheid van ontledings vir akkurate dieetformulering beklemtoon. Dit het ook gegeld vir as (CV = 22.11%), ruprotein (CP) (CV = 11.6%), nie-veselagtige koolhidrate (NFC) (CV = 34.0%), effektiewe rumendegradeerbaarheid van protein (ERPD) (CV = 18.9%) en esensiële aminosure (EAA) (CV = 14.5% - 27.1%). Die effektiewe. proteïendegradeerbaarheids parameters a, b en c het die hoogste CV (106.8% - 306.4%) getoon. Die gemiddelde *in vitro* organiese materiaal verteerbaarheid (IVOMD) waarde (67.6%) blyk verteenwoordigend te wees (CV = 5.4%) van die lusernhooi populasie wat gebruik is.

Laer ERPD waardes (gemiddeld 47.7%) wat algemeen gerapporteer word, is verkry. Die invloed van suurbestandeveselstikstof (ADF-N) op die rumenondegradeerbareprotein (RUP) van lusernhooi was onbeduidend. Hittebeskadiging is by 'n klein persentasie (3.5%) van die lusernhooimonsters waargeneem. Lusernhooi het 'n hoë lisien (4.55g/100g CP) en 'n lae metionieninhoud bevat. Metionien, lisien en isoluesien, was in 'n dalende volgorde, die mees beperkte EAA vir melksintese.

'n Tweede studie is uitgevoer om proteinkwaliteit, ooreenkomstig die metaboliseerbare proteinstelsel (MP) van die Verenigde Koninkryk, in te sluit by die totale ruvoerindeksstelsel (TFI) om die kwaliteit van lusernhooi meer noukeurig te bepaal. Hierdie model (lusernkwaliteit-indeks) is vergelyk met chemiese ontledings en bestaande modelle om die gehalte van lusernhooi te bepaal. Die volgende modelle is gebruik om die kwaliteit van lusern te bereken:

a) Relatiewe voerwaarde (RFV) = % verteerbare droë materiaal (DDM) × droëmateriaalinname as % van liggaamsgewig (DMI) x 0.775

b) Totale ruvoerindeks (TFI) = RFV + % CP × 6

c) Aangepaste totale ruvoerindeks (ATFI) = RFV x aangepaste ruprotein (ACP) × 6

d) Lusernkwaliteitsindeks (LQI) = IVOMD × DMI + MP × 6, as 'n indeks uitgedruk.

NDF ($r^2$ = 0.57) in vergelyking met ADF ($r^2$ = 0.48) was 'n meer akkurate beramer van IVOMD. Die NDF inhoud van lusernhooi het ook 'n belangrike rol ($P<0.0001$) gespeel in regressievergelykings om onderskeidelik die kwaliteitmodelle RFV ($r^2$ = 0.86) en TFI ($r^2$ = 0.84) asook IVOMD (r = 0.57) en ERDP (r = 0.50) te voorspel. 'n Hoë verwantskap is tussen hierdie verskillende modelle ($r^2 > 0.90$) gevind. 'n Matige korrelasie ($P<0.0001$) is egter waargeneem tussen LQI en onderskeidelik ADF ($r^2$ = 0.50), NDF ($r^2$ = 0.50), IVOMD ($r^2$ = 0.56), CP ($r^2$ = 0.56), RFV ($r^2$ = 0.51), TFI ($r^2$ = 0.56) of ATFI ($r^2$ = 0.55). Geen betekenisvolle ($P>0.05$) beramer vir die ERPD fraksies (a, b en c) is waargeneem nie. LQI kon voorspel word vanaf ADF ($r^2$ = 0.50) of ADF, ERPD en CP ($r^2$ = 0.94) in 'n meervoudige regressievergelyking.

Die resultate van die huidige studie het duidelik getoon dat daar 'n groot variasie voorkom in die energie- en proteïensamestelling, asook benutting van voedingstowwe, in Suid-Afrikaanse lusernhooi. Dit beklemtoon die noodsaaklikheid van 'n vinnige en akkurate evalueringstelsel vir lusernhooi in die praktyk. Dit wil voorkom asof LQI 'n beter evalueringstelsel van lusernkwaliteit is as ander stelsels wat tans gebruik word. Die verwantskap met LQI in die huidige studie is gebaseer op betreklik min (n = 30) lusernhooimonsters. Verdere navorsing met meer lusernhooimonsters is nodig om bogenoemde resultate te staaf. Die gebruik van infrarooispektroskopie (NIRS) vir vinnige bepaling van die voedingswaarde van lusernhooi verg ook dringende aandag.