# EFFECT OF UREA-AMMONIATION OF DIETARY ROUGHAGE AND CONCENTRATE RATIO ON RUMINAL MICROBIAL ACTIVITY IN JERSEY COWS

by

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## SUMMARY

The effect of untreated roughages on digestibility and rumen fill of the gut was reviewed as physical mechanism influencing the regulation of roughage intake. The review of literature also focused on identifying factors that affect the way in which urea-ammoniation alters the roughage intake, digestibility and performance of ruminant animals. Trials were carried out with fistulated cows to address to what extent concentrate proportion and urea-ammoniation affected microbial colonization and degradation of roughage diets in the rumen. One interest of this study was to develop a model that would help to predict the benefit associated with urea-treatment of roughages.

The first trial (Chapter 3) investigated the effect of urea-ammoniation of roughage and concentrate proportion of the diet on degradation of roughages, and the benefit associated with the treatment of roughages. Four rumen-fistulated Jersey cows were fed on a basal diet composed of either urea treated (3 kg of urea per 100 kg of straw) or untreated *Eragrostis curvula* hay. These basal diets were supplemented with concentrate composed of maize meal (78%) and cotton seed cake (22%). The concentrate contributed 0, 25, 50 and 75% of the total ration and hay the rest. The experiment consisted of 6 periods. Each period lasted 19 days, comprising 12 days of adaptation to the experimental diet followed by 6 days degradability measurements and 1-day rumen fluid collection. During each period the 4 Jersey cows were randomly allocated to 4 of the 8 dietary treatments, ensuring that each diet was fed to 3 animals during the entire experimental period.

The experimental roughages used in this trial were wheat (*Triticum sativum*) straw, barley (*Hordeum Vulgare*) straw, coastcross ($k_{11}$) (*Cynodon hybrid*) hay, veld hay (natural grass), oat (*Avena sativa*) straw, oat (*Avena sativa*) hay, maize (*Zea mays*) stover, kikuyu (*Pennisetum clandestinum*) grass, weeping love grass (*Eragrostsis curvula*) and Italian rye (*Lolium multiflorum*) grass. Each roughage (sample) was subdivided into two equal portions, one of which was then treated with urea. The urea solution was prepared by dissolving 30 g of urea in 0.4 liter of water. The solution was fully distributed over 1 kg of roughage. Treated roughages were sealed tightly and stored at room temperature for 5 weeks in plastic bags. Immediately after opening, the

different roughages, including the untreated ones, were sun dried, chopped fine by hand and ground through a 2-mm screen in a laboratory mill. About 3 g of each sample was weighed into labeled nylon bags. The bags were tied to a stainless steel disc with 10 evenly spaced small holes drilled through the periphery of the disc serving as anchor points. The bags were incubated (in duplicate per time interval) in the rumen for 120, 96, 72, 48, 24, 12, 6 and 3 h, sequentially. The treated samples were incubated in animals fed treated hay, while untreated samples were incubated in animals given untreated hay. Immediately after removal from the rumen, the bags, including the 0 hour ones, which had not been incubated but soaked in warm water for 1 hour, were washed in 6 cycles (each lasting 4 minutes) in a semi-automatic washing machine. The washed bags were then dried in a forced draught oven at 60 $^{0}$C for 48 hours, cooled in a desicator and weighed.

The pH of the rumen fluid ranged between 6.5 and 6.8 for all diets. Rumen ammonia concentration was higher ($P<0.002$) when the basal diet consisted of urea treated hay. Increasing the concentrate proportion in the diet had the desired effect of increasing rumen ammonia concentration without severely affecting pH. Urea-ammoniation increased ($P<0.0001$) the slowly degradable fraction (B), potential degradability (PD), effective degradability (ED) of dry matter and neutral detergent fiber (NDF), decreased ($P>0.05$) lag time (LT) but had no effect on the rate of degradation (c) of dry matter. Concentrate proportions affected ($P<0.05$) the slowly degradable fraction, potential degradability, lag time and effective degradability but had no effect ($P>0.05$) on the rate of degradation of dry matter (DM). Maximum and minimum values of the slowly degradable fraction, potential degradability and effective degradability of DM and NDF were obtained at the 25 and 75% concentrate levels, respectively. Within urea-ammoniation, roughage type affected ($P<0.001$) the B-fraction, PD and ED of DM and NDF degradation. Rate of degradation of DM of untreated roughages varied from 0.022 $h^{-1}$ in wheat straw to 0.087 $h^{-1}$ in rye grass, while for urea treated roughages it varied from 0.022 $h^{-1}$ in oat straw to 0.082 $h^{-1}$ in rye grass. Rye grass degraded almost three to four times faster than urea treated oat or untreated wheat straw. Urea-ammoniation was less effective in increasing DM and cell wall degradation rates (c) of rye grass compared to wheat straw. The results showed that low quality roughages such as wheat straw benefited relatively the most from urea-ammoniation.

The effect of urea-ammoniation and dietary manipulation on microbial colonization (Chapter 4) of fiber particles in the rumen of animals was also investigated in two experiments. In Experiment 1, the cows were fed on rations comprising either urea-ammoniated or untreated *Eragrostis curvula* hay supplemented with concentrate at hay to concentrate ratio of 100:0, 75:25, 50:50, 25:75, resulting in eight different rumen environments. The experiment consisted of two periods. Each period lasted 12 days of adaptation to the experimental diet followed by one-day incubation of urea-ammoniated and untreated barley straw. Experiment 2 consisted of two urea-ammoniated (7.5 kg of urea per 100 kg of hay) hay levels (20 and 40% of the total ration) and concentrate levels (60 and 80%). Fistulated Jersey cows were adapted for 12 days after assigning to the dietary treatment. Feed was given at the rate of 9.0 kg $day^{-1}$ per animal portioned into equal meals of 4.50 kg each and offered at 08:00 and 16:00 every day. About 3 g of urea-ammoniated or untreated barley (*Hordeum vulgare*) straw, ground through a 2-mm screen, was weighed into a labelled nylon bag and incubated for 3, 6 or 12 h in the rumen of the fistulated cows. Microbes adhering to incubated fiber particles were examined under the Environmental Scanning Electron Microscopy (ESEM) and analysed on the image analyser. Depending on morphology, the microbes were divided into three groups: bacilli (rod), cocci (round) and others (spiral, fimbrea and cluster; not specifically defined or undefined microbes).

Urea-ammoniation of dietary roughage decreased ($P<0.001$) bacilli counts and total bacteria count but had no effect on count of the undefined group of microbes on fiber particles in the rumen of cows (Experiment 1). Concentrate proportions had no effect ($P>0.05$) on bacilli, cocci and total bacterial count on fiber particles. However, the results from electron micrograph observations revealed that the total bacterial count tended to decrease as the concentrate level increased in the diet of cows. Bacilli, cocci, undefined group of microbes and total count of microbes increased ($P<0.05$) as length of incubation increased. In Experiment 2, incubated feed, concentrate proportion and time of incubation had no effect ($P>0.05$) on bacilli, others (undefined group of microbes) and total count of fiber-adhering microbes in the rumen of cows. However, increasing concentrate in the diet of cows tended to decrease ($P<0.07$) the count of fiber-adhering cocci. The total count of microbes on fiber particles was higher in animals fed 80% concentrate as compared to 60% concentrate.

The benefit derived from urea treatment in terms of B-fraction, effective degradability and potential degradability of DM and fiber of roughages increased with increasing the NDF content. Therefore, the important conclusions drawn from the results of the present study is that urea-ammoniation of roughages should be done strategically and that high quality roughages may give little return per unit of cost of ammoniation. This means that the benefit associated with urea-ammoniation would be justified for poor quality roughages only.

**DECLARATION**

The experimental work described in this dissertation was carried out in the Discipline of Animal Science and Poultry Science, School of Agricultural Sciences and Agribusiness, Faculty of Science and Agriculture, University of Natal, Pietermaritzburg, under the supervision of Dr. I. V. Nsahlai.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any other University. Where use has been made of the work of others, it has been duly acknowledged in the text.

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Signed.................. Date..04-12-2003

I, Dr. Nsahlai, I. V., Chairperson of the Supervisory committee, approve the release of this thesis for examination.

Signed.................. Date.......................

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CHAPTER 1

# GENERAL INTRODUCTION

## 1.1 Background

The problems of ruminant feeding have received considerable attention in the tropics and sub-tropics. Most of the research has focused on treating roughage in the late dry season when the quality and quantity of food supply from natural pastures become limiting. Moreover, ruminant animals have evolved the ability to utilize and digest fibrous material. In contrast to the situation in the tropics and sub-tropics, in many developed countries, foods that are suitable for human consumption are very often used for feeding both monogastric and ruminant animals. It has been suggested that ruminants should be fed, as much as possible, roughage based diets and other feeds that are not directly used by humans (Ørskov, 1998). Thus, straw is becoming an important and staple feed for ruminants in most parts of the developing world. This is because of an increase in animal population density and failure to modify traditional grazing practices, especially in the arid tropics and subtropics, which have caused serious deterioration of natural vegetation cover. In many parts of the world today straw makes up 60 to 90 percent of the bovine diet (Verma and Jackson, 1984).

Even though straw is the most abundant of all agricultural residues and has a great potential as a feed-stuff for ruminants, it appears that livestock production based on these straw is rather low. Verma and Jackson (1984) reported that nearly half of the world's bovine population is reared and maintained on diets composed of 50 % or more straw. Thus, these animals are the world's least productive in terms of annual output per animal. The reasons for this low level of production are the low digestibility and intake of the straw based diets. Coxworth *et al.* (1977) reported that the voluntary intake and digestibility of straw are limited by its high lignin content, the manner in which this indigestible material is bound to the digestible cellulose and hemicellulose, and its low nitrogen concentration. Kamstra *et al.* (1958) and Van Soest (1967) reported that poor digestibility is related to the extent of lignification of the cell wall components of the low quality roughages. The degree of fill in the reticulo-rumen has also been suggested as the dominant factor limiting voluntary intake of poor quality roughage diets because they have relatively long rumen retention times (Grovum and Williams, 1979).

However, decreasing the retention time by increasing the rate of passage tends to decrease the extent of fiber digestion in the rumen (Van Soest, 1982).

Chemical up-grading of straws by means of ammoniation with gaseous or liquid ammonia has received considerable attention in many countries (Sundstøl and Coxworth, 1984). An alternative method of ammoniation, using urea as the source of ammonia has been reported by several research workers (Saadullah *et al.*, 1981; Hadjipanayiotou, 1982a; Cloete and Kritzinger, 1984; Dias-Da-Silva and Sundstøl, 1986). According to Davis *et al.* (1983), of all the alkali's tested, ammonia is the most preferred because it provides both the alkaline effect and a source of nitrogen. However, alkali treatments are generally expensive and the chemicals are not readily available in many parts of developing countries. Consequently, urea has been studied as a source of ammonia for treating roughages (Hadjipanayiotou, 1982a; Cloete *et al.*, 1983; Khanal *et al.*, 1999). Many of the factors influencing the effectiveness of straw treatment with urea (Cloete and Kritzinger, 1984, 1985), like type and level of chemical reaction, period, ambient temperature and amount of water (moisture level), are closely related to the economics of straw treatment (Hadjipanayiotou, 1989). With the increase in human and animal populations, and a consequent reduction in the cropping and grazing land, it is possible that this strategy will be attractive to many farmers in the future. However, unless adequate feed resources are made available, grazing animals will continue to be undernourished with consequent low productivity.

Urea-ammoniation of straws generally results in increased digestibility and intake (Cloete and Kritzinger, 1984; Djajanegara and Doyle, 1989b; Flachowsky, *et al.*, 1996). Although urea-ammoniation of straws increases digestibility and intake, there is a view that urea-ammoniated diets such as barley, oat and wheat straw and oat hay may not be adequate for production functions like growth, pregnancy and lactation (Brand *et al.*, 1991). Therefore, addition of concentrate to urea-ammoniated diets could give better results in the production function of animals. This is due to the fact that concentrate based diets are more digestible (Ørskov and Ryle, 1990) and therefore more volatile fatty acid, (VFA) are produced per unit weight than from forage. Evidence from the literature suggests that the inclusion of protein and energy at low levels may improve fiber digestibility (Williams, 1983/84). High levels of soybean meal and maize gluten meal were, however, found to suppress the digestibility of cellulose and hemicellulose in wheat straw diets (Streeter *et al.*, 1983). The apparent digestibility of acid detergent fiber (ADF) of low quality roughages was also adversely affected by high levels of barley (Lamb and Eadie, 1979) and molasses (Williams, 1983/84).

## OBJECTIVES OF THE STUDY

The main objectives of the study were:

I. To develop a deterministic model for predicting the benefit associated with urea-treatment of roughages; and

II. To find out how dietary manipulation and urea ammoniation affect colonization and degradation of dietary roughage.

CHAPTER 2

# LITERATURE REVIEW

## 2.1 Roughages

Under most tropical conditions, where roughage is the basal diet of ruminants, the availability of nutrients to the animal is the main factor limiting productivity. The type and quality of roughages found in a given area will depend on local ecological and climatic conditions. Thus, success in improving livestock productivity in each region, demands basic knowledge of the nutritive values of available feed resources.

## 2.2 Chemical composition and nutritive value of roughages

The chemical composition of roughages (in DM) is variable; for instance, the crude protein content may range from as little as 30 g $kg^{-1}$ in mature herbage plants to over 300 g $kg^{-1}$ in young heavily fertilized grasses (McDonald *et al.*, 1995). Fiber forms the bulk of most tropical roughages and is considered as the sum of cellulose, hemicellulose (xylans, mannans, glucomannans, arabino-galactans) and pectin and all are broadly related inversely to the crude protein content. Crude fiber may vary from 200 to as much as 450 g $kg^{-1}$ in mature plant materials. In straws the digestible cell contents constitute usually less than 250 g $kg^{-1}$ of the total dry matter (FAO, 1982) and therefore, it is plausible to make a minor contribution to the evaluation of feeds depending on their nutritive value and nutritional importance. Generally, cellulose contents fall within the ranges 200 – 300 g $kg^{-1}$ DM and hemicellulose within the range of 100 – 300 g $kg^{-1}$ DM (McDonald *et al.*, 1995). These polysaccharide components increase with the maturity of the plant. The lignin concentration increases in the same manner and adversely affects the digestibility of nutrients, except soluble carbohydrates (Akin and Benner, 1988).

A large number of roughages have been documented as useful livestock feed during the dry season. The main features of these roughages are low contents of soluble dry matter, crude protein and mineral content (Khanal *et al.*, 1999). A summary of the chemical composition of various alkali treated and untreated roughages is presented in Table 2.1. From the Table it is seen that the nitrogen (N) content of untreated roughages varies from a low of 4 g $kg^{-1}$ for barley straw to a high of 12 g $kg^{-1}$ for rye grass, while for the treated roughages it varies from a low of 8 g $kg^{-1}$ for the barley straw to a high of 25 g

**Table 2.1** The chemical composition (g DM kg$^{-1}$) of various alkali treated (g DM kg$^{-1}$) and untreated roughages.

| Animal | Feed | Alkali | T/U | Level | N | NDF | ADF | Lignin | CF | HC | CELL | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sheep | BS | $NH_3$ | 0 | 30 | 4.2 | 830 | 520 | 85 | 451 | 310 | - | Castrillo *et al.*, 1995 |
| | | | 1 | | 17.0 | 778 | 493 | 78 | 439 | 285 | - | |
| Cattle | BS | Urea | 0 | 40 | 7.8 | 731 | 451 | 41 | - | 280 | - | Hadjipanayiotou and |
| | | | 1 | | 18.0 | 729 | 457 | 47 | - | 272 | - | Economides, 1997 |
| Cattle | WS | Urea | 0 | 50 | 5.4 | 800 | 540 | 120 | 440 | 260 | - | Zhang, 1995 |
| | | | 1 | | 15.8 | 700 | 560 | 100 | 430 | 210 | - | |
| Cattle | WS | $NH_3$ | 0 | 35 | 7.4 | 813 | 386 | - | - | 426 | - | Zorilla-Rios *et al.*, |
| | | | 1 | | 14.9 | 807 | 377 | - | - | 430 | - | 1984 |
| Ewes | BS | Urea | 0 | 35 | 7.2 | 776 | 498 | 51 | - | 278 | - | Orr *et al.*, 1985 |
| | | | 1 | | 18.6 | 714 | 501 | 57 | - | 213 | - | |
| Ewes | RGH | Urea | 0 | 35 | 12.0 | 733 | 450 | 58 | - | 283 | - | Orr *et al.*, 1985 |
| | | | 1 | | 25.0 | 698 | 465 | 59 | - | 233 | - | |
| Lamb | TFH | $NH_3$ | 0 | 30 | 7.2 | 763 | 456 | 81 | - | 307 | 365 | Buettner *et al.*, 1982 |
| | | | 1 | | 16.7 | 698 | 443 | 67 | - | 255 | 367 | |
| Cattle | WS | $NH_3$ | 0 | 30 | 8.2 | 806 | 550 | - | - | 256 | 417 | Jewell and Campling, |
| | | | 1 | | 16.8 | 789 | 562 | - | - | 277 | 426 | 1986 |
| Sheep | WS | $NH_3$ | 0 | 30 | 4.3 | - | - | 59.8 | - | 329 | 413 | Dias-Da-Silva and |
| | | | 1 | | 14.0 | - | - | 59.5 | - | 281 | 419 | Sundstøl,1986 |
| Goats | Rice | urea | 0 | 40 | 7.5 | 781 | 538 | 87 | - | 243 | 351 | Tuen *et al.*, 1991 |
| | | | 1 | | 17.4 | 790 | 556 | 92 | - | 234 | 364 | |
| Lambs | BS | $NH_3$ | 0 | 40 | 4.0 | 812 | 534 | - | - | 278 | - | Abidin and Kempton |
| | | | 1 | | 8.0 | 739 | 515 | - | - | 224 | - | 1981 |
| Steers | BS | $NH_3$ | 0 | 35 | - | 684 | 437 | 79 | - | 247 | 373 | Horton, 1981 |
| | | | 1 | | - | 656 | 421 | 74 | - | 235 | 371 | |
| Steers | OS | $NH_3$ | 0 | 35 | - | 755 | 486 | 97 | - | 269 | 428 | Horton, 1981 |
| | | | 1 | | - | 720 | 470 | 83 | - | 250 | 423 | |
| Sheep | WS | $NH_3$ | 0 | 75 | 4.1 | 702 | 368 | - | 352 | 334 | - | Cloete *et al.*, 1983 |
| | | | 1 | | 16.1 | 694 | 393 | - | 351 | 301 | - | |
| Sheep | WS | urea | 0 | 50 | 5.0 | 829 | 486 | - | - | 343 | - | Cloete and |
| | | | 1 | | 15.5 | 822 | 505 | - | - | 317 | - | Kritzinger, 1984 |
| Sheep | WS | $NH_3$ | 0 | 35 | 7.7 | 770 | - | - | - | - | - | Alibes *et al.*, 1983/8 |
| | | | 1 | | 11.7 | 741 | - | - | - | - | - | |
| Sheep | MS | $NH_3$ | 0 | 35 | 5.8 | 769 | - | - | - | - | - | Alibes *et al.*, 1983/8 |
| | | | 1 | | 14.1 | 730 | - | - | - | - | - | |
| Sheep | WS | $NH_3$ | 0 | 35 | 4.8 | 806 | - | - | - | - | - | Alibes *et al.*, 1983/8 |
| | | | 1 | | 7.0 | 805 | - | - | - | - | - | |
| Sheep | MS | $NH_3$ | 0 | 35 | 8.6 | 785 | - | - | - | - | - | Alibes *et al.*, 1983/8 |
| | | | 1 | | 15.2 | 732 | - | - | - | - | - | |

T, alkali treated (1); U, untreated (0); BS, barley straw; WS, wheat straw; OS, oat straw; RGH, ryegrass hay; TFH, tall fescue hay; MS, maize stover; N, nitrogen; NDF, neutral detergent fiber; ADF, acid detergent fiber; CF, crude fiber; HC, hemicellulose; CELL, cellulose.

$kg^{-1}$ for ryegrass. In a review by Butterworth (1967) the mean crude protein percentage for good quality hay was 7.7% (N content 12.3 g $kg^{-1}$). Low protein in roughages is generally considered as one of the major constraints to optimum digestion. The range of neutral detergent fiber (NDF) content of 70-81% is reduced, compared to 73-83% for the untreated roughages. The high variability in chemical constituents could be attributed to the stage of maturity of the plant, plant part, harvesting regime, season, location and type of the roughage plant.

The beneficial effects of feeding urea treated roughages to ruminants include increased metabolizable energy intake, increased animal performance and feed efficiency, increased availability of nutrients and improved rumen function (Pirie and Greenhalgh, 1978; Mgheni *et al.*, 1993). Habib *et al.* (1998) improved the nitrogen content of wheat straw from 4.12 to 9.83% through ammoniation and reported that this improvement in nitrogen content (9.83%) is close to that found normally in the non-legume green fodders. The authors then concluded that the added nitrogen in straw is one of the main advantages of ammonia treatment, which per se could increase digestibility.

Table 2.2 highlights the dry matter (DM) intake, average daily gain and digestibility coefficient of various alkali treated and untreated roughages as reported by different researchers. It is seen that in all cases where the basal diet was treated with alkali, there was an improvement in voluntary dry matter intake, dry matter digestibility and live weight gain irrespective of the nature or type of the basal feed. Feeding of non-supplemented or non-treated roughages such as maize stover, rice, barley and wheat straw resulted in live weight losses in sheep and cattle while others like alkali-treated and good pasture hay elicited modest levels of gains. It is therefore, possible that in order to meet requirements for a given level of productivity, the type of supplement or treatment is dependent on the quality of the basal roughage.

### 2.3 Limitations of straw and crop residues

The most important factor influencing the production response of an animal is the total quantity of nutrients absorbed (Poppi *et al.*, 2000). Thus, intake and digestibility are key parameters in any feed evaluation system, and of this intake is the most important, as it accounts for most differences between feed types. The prime physical factor in a plant

**Table 2.2** Dry matter intake (g DM $kg^{-1}$ $W^{0.75}day^{-1}$), live body weight gain (g $day^{-1}$) and digestibility coefficient (%) of various alkali treated (g DM $kg^{-1}$) and untreated roughages as reported by different workers.

| Source | Feed | Alkali | T/U | Level | DMI | DMD | OMD | LWG | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Sheep | | | | | | | | | |
| | Barely straw | $NH_3$ | 0 | 35 | 28.8 | 49.0 | 51.0 | - | Silva *et al.*, 1989 |
| | | | 1 | | 51.7 | 57.0 | 60.0 | - | |
| | Wheat straw | Urea | 0 | 55 | 49.5 | - | 57.3 | - | Brand *et al.*, 1991 |
| | | | 1 | | 62.7 | - | 61.8 | - | |
| | Barley straw | $NH_3$ | 0 | 40 | 56.5 | - | 49.0 | 93 | Abidin and Kempton, |
| | | | 1 | | 59.0 | - | 57.0 | 108 | 1981 |
| | Wheat straw | $NH_3$ | 0 | 30 | 58.9 | 42.1 | - | - | Dias-Da-Silva and |
| | | | 1 | | 74.9 | 49.4 | - | - | Sundstøl, 1986 |
| | Wheat straw | $NH_3$ | 0 | 50 | 27.4 | 44.2 | 47.1 | - | Cloete and Kritzinger, |
| | | | 1 | | 40.2 | 51.9 | 54.1 | - | 1984 |
| | Barley straw | Urea | 0 | 75 | 55.3 | - | 52.7 | - | Cloete *et al.*, 1983 |
| | | | 1 | | 67.4 | - | 57.5 | - | |
| | Wheat straw | $NH_3$ | 0 | 35 | 24 | - | 55.4 | | Fahmy and Ørskov, |
| | | | 1 | | 41.5 | - | 62.8 | | 1984 |
| | Tall fescue | $NH_3$ | 0 | 30 | 34 | 40.0 | - | - | Buettner *et al.*, 1982 |
| | | | 1 | | 46 | 57.0 | - | - | |
| | Wheat straw | $NH_3$ | 0 | 35 | 29.7 | 56.0 | 45.8 | - | Alibes *et al.*, 1983/84 |
| | | | 1 | | 36.5 | 57.8 | 50.6 | - | |
| | Maize stover | $NH_3$ | 0 | 35 | 29.2 | 61.1 | 54.2 | - | Alibes *et al.*, 1983/84 |
| | | | 1 | | 38.7 | 65.9 | 64.3 | - | |
| | Wheat straw | $NH_3$ | 0 | 35 | 33.5 | 49.4 | 42.6 | | Alibes *et al.*, 1983/84 |
| | | | 1 | | 40.0 | 55.0 | 50.8 | | |
| | Maize stover | $NH_3$ | 0 | 35 | 33.2 | 60.5 | 53.2 | | Alibes *et al.*, 1983/84 |
| | | | 1 | | 40.9 | 65.2 | 61.0 | | |
| Goat | Rice straw | Urea | 0 | 20 | 49.5 | 45.8 | 52.7 | - | Tuen *et al.*, 1991 |
| | | | 1 | | 54.3 | 50.1 | 57.8 | - | |
| Cattle | | | | | | | | | |
| | Barley straw | $NH_3$ | 0 | 35 | 34.1 | 49.9 | 47.0 | -447 | Ørskov *et al.*, 1983 |
| | | | 1 | | 51.8 | 58.8 | 56.9 | 324 | |
| | Barley straw | $NH_3$ | 0 | 30 | 56.9 | - | 40.3 | 130 | Jewell and Campling, |
| | | | 1 | | 69.5 | - | 49.6 | 360 | 1986 |
| | Wheat straw | $NH_3$ | 0 | 30 | 62.4 | - | 42.5 | 210 | Jewell and Campling, |
| | | | 1 | | 78.1 | - | 54.6 | 370 | 1986 |
| | Rice straw | Urea | 0 | 40 | 64.6 | 45.9 | - | - | Khanal *et al.*, 1999 |
| | | | 1 | | 73.8 | 54.2 | - | - | |
| | Barley straw | $NH_3$ | 0 | 35 | 24 | - | 55.4 | - | Mira *et al.*, 1981 |
| | | | 1 | | 41.5 | - | 62.8 | - | |

T, Alkali treated (1); U, untreated (0); DMI, dry matter intake; DMD, dry matter digestibility; OMD, organic matter digestibility; LWG, live weight gain.

which influences voluntary intake is the rate at which it is broken down to particles small enough to leave the rumen (Minson, 1982a). Crampton (1957) suggested that the most significant effect of lignification is on the rate of forage digestion rather than its possible relation to the proportion of dry matter ultimately digested. Plant maturation is accompanied by an increase in the proportion of fiber and a reduction in the protein and non-structural carbohydrates of the cell content (Donefar *et al.*, 1963; Van Soest, 1965).

In most tropical roughages, the quality of feed at the beginning of the rainy season is high but because of high temperatures, rapid physiological maturation takes place leading to early lignification with the protein and phosphorus contents falling to very low levels while the fiber content increases (Becker and Lohrmann, 1992; McDonald *et al.*, 1995; Nyamangara and Ndlovu, 1995). Lignification confers resistance to roughage fiber, thus decreasing mechanical and microbial degradation in the rumen, which could explain the long retention time of tropical roughages in the rumen. Long retention time facilitates rumen fill and consequently decreases feed intake (Thorton and Minson, 1973; Aitchison *et al.*, 1986).

Most tropical grass species belong to the $C_4$ category of plants in which carbon dioxide is first fixed in a reaction involving a 4-carbon compound, oxalate (Egan, 1986), while temperate species belong to the $C_3$ category of plants in which a 3-carbon compound, phosphoglycerate, acts as an important intermediate in the photosynthetic fixation of carbon dioxide (Wilson, 1993). The low protein and sulphur contents usually found in tropical grasses are inherent characteristics of $C_4$ plant metabolism (Egan, 1986) that is associated with survival under conditions of low soil fertility. In tropical grasses, starches are the main storage carbohydrates, but these are replaced by fructans in temperate ones.

The plant cell wall has been shown to be the primary restrictive determinant of forage intake (Van Soest, 1994). Tropical and subtropical forages are more stemmy and have more cell wall than the temperate forage species (Meissner, 1997). This results in low digestibility, slow rate of fermentation and particle size reduction, which slow down the passage rate of residue from the rumen, increase rumen fill and thereby reduce intake (Minson, 1982a). In South Africa, cell wall constituents that have been shown to be correlated with intake include NDF (Meissner *et al.*, 1991b), ADF (Cilliers and Van der

Merwe, 1993) and acid detergent lignin (ADL) (Pietersen *et al.*, 1993). Van Soest (1965) reported that the intake was limited above NDF concentrations of 550-600 g $kg^{-1}$ DM but not below. Similar evidence was presented by Meissner *et al.* (1991b). Non-cell wall constituents that limit the intake and digestibility of tropical and subtropical forages include phenolic compounds (ferulic, deferulic, P-coumaric acids and vanillin).

This limitation could be overcome by physical or alkali treatment or by improving the activity of the rumen microbiota. Treatment with alkali (e.g. ammonia and/or urea) hydrolyses lignin-hemicellulose linkages, thus opening up the structure for bacterial attachment (Sundstøl and Owen, 1984), and hence increasing the availability of roughage energy.

### 2.4 Ways of improving intake and digestibility of roughages

Studies on factors influencing the quality of feeds have clearly indicated that various factors substantially change nutrient concentration and availability to the animal. Among the major factors identified, genetic make up of the plant, its environment and management practices are the major ones (Norton, 1982; Wilson, 1982). Thus, no absolute figures of nutritional characteristics of a feed could be established across regions and genotypes. Many techniques are available for improving the nutritive value of roughages. Methods currently employed to enhance digestibility and intake of the basal roughage diet range from physical through chemical treatment to supplementation.

#### 2.4.1 Physical or mechanical treatment

Physical or mechanical treatments, such as chopping, grinding, pelleting and steaming have long been used to improve the nutritive value of low quality roughages, including straw (Minson, 1963; Walker, 1984). All the above treatments cause physical disruption of cells and have limited effect on digestibility, but often improve roughage intake. Improved digestibility is partly a result of enlarged surface area caused by grinding and thus improving the possibility for the attachment of rumen microbes. Improved intake is achieved through a faster rate of passage through the rumen, which in turn might cause a decrease in digestibility. Besides, it is probable that species, maturity of the animal, origin of the plant material, and the conditions under which the material is fed also affect utilization of the feed, irrespective of the particle size (Harris and Crampton, 1972). This method facilitates maximal use of roughage by creating more favorable condition for the host animal to eat more feed.

### 2.4.2 Chemical treatment

Several alkali compounds (NaOH, $Ca(OH)_2$, KOH) have been tested, but sodium hydroxide has been the most successful in improving nutritive value of roughages (Church, 1984). The use of sodium hydroxide (NaOH) treatment to increase digestibility of straws has been known for more than a century. The earliest method, developed by Kellner and Kohler (1900; cited by Homb, 1984) involved the pressure-cooking of straws in dilute solutions of sodium hydroxide, followed by washing with clean water to remove the alkali. Clearly this was an expensive method because of the severe processing and problems of environmental pollution. This method was later modified by Beckman (1921), who replaced pressure-cooking with simple soaking. In the Beckman method, rye straw is treated in 1.5% NaOH solution for three days and thereafter rinsed with water. This method of treatment increased the organic matter digestibility (OMD) of rye straw from 46 to 71%, which was lower than the 88% achieved by Kellner and Kohler (1900; cited by Wilson and Pigden, 1964). Straw treated by the Beckman method turned out to be more expensive than other feeds (Jackson, 1977).

Although NaOH is effective in improving the digestibility of low quality roughage it has some drawbacks. The alkali solution is dangerous to handle. Besides being a potential pollutant in case of storage leakage, the large quantities of sodium (Na) being imported to the farm and excreted in urine and faeces are far above what is required for plant growth. In most countries NaOH is expensive and not available. Due to this concern it was necessary to look for alternatives that were cheap and effective in improving the nutritive value of straw and safe for the environment.

Treatment with ammonia (Sundstøl and Coxworth, 1984) and urea (Jewell and Campling, 1986; Flachowsky *et al.*, 1996) has resulted in increased forage digestibility, voluntary intake and animal performance. Accordingly, Djibrillou *et al.* (1998) reported that urea and/or ammonia is preferred over other treatments as it has an added advantage of increasing the N content of the straw. As a result of several advantages over sodium hydroxide treatment, like ease of application, nitrogen addition and absence of undesirable residues, ammoniation is also a popular chemical method of upgrading crop residues (Sundstøl, 1983/84). However, limited availability and increased regulation on transportation may limit the use of anhydrous ammonia in certain regions of the tropics.

Urea is widely available and has been used as a source of ammoniation to improve the feeding value of various grasses and crop residues (Sundstøl and Coxworth, 1984). Urea treatment is relatively easy to apply and its ability to swell cellulosic fibers is as effective as that of NaOH (Khanal *et al.*, 1999). In addition to the upgrading effect of urea treatment the added nitrogen from ammonia also enhances microbial activity in the rumen, resulting to increased synthesis of microbial protein. The following sections attempt to review research done so far on effect of urea and/or ammonia treatment on various roughages to improve the intake and digestibility.

## 2.5 Effect of urea and/or ammonia treatment on voluntary intake of roughages

The quality of any roughage depends on the voluntary intake of that roughage and on the extent to which its dry matter (DM) can supply dietary energy, protein, minerals and vitamins when eaten by the animal. Many factors influence the intake of roughages among which are feed characteristics, animal species, physiological state and management practices. Most straws contain about 70-80% cell wall constituents, which represent an energy source for ruminants. Voluntary feed intake (VFI) is the amount of food eaten by an animal during a given period of time when an excess of the food is available. Food intake is important in defining food conversion efficiency (FCE). Efficient food conversion, however, will be achieved only if an animal is able to obtain from the food a substantial margin of nutrients above maintenance requirements. In many animal production systems, maximum intake may not be sufficient to ensure maximum production, or may be critical to the system.

Treatment of roughages with either urea or ammonia is an effort to increase intake (Castrillo *et al.*, 1995; Flachowsky *et al.*, 1996) through alkaline hydrolysis of ligno-cellulose bonds (Sundstøl and Owen, 1984) and to increase nitrogen concentration in the roughage. This would allow an even release of ammonia in the rumen, creating favourable conditions for intense microbial fermentation. Voluntary feed intake has been found to increase when treated roughage is made available to ruminants (Jewell and Campling, 1986; Silva *et al.*, 1989; Brand *et al.*, 1991). Aitchison *et al.* (1988) offered urea treated and urea supplemented straw (i.e. straw sprayed with urea before feeding) to mature sheep and found a 21% increase in dry matter (DM) intake for animals fed urea treated straw. Increased roughage intake due to urea treatment has been reported (Joy *et al.*, 1992; Fahmy and Klopfenstein, 1994; Brown and Adjei, 1995; Schiere and de Wit, 1995). Similarly, Fahmy and Ørskov (1984) reported that the DM intake of ammonia treated barley straw was 73% higher than for the untreated straw and the intake of digestible organic matter was improved by 98%. A linear increase in intake of cereal straws has also been observed with urea treatment up to 7% (Macdearmid *et al.*, 1988) and 8% (Jayasuriya and Perera, 1982) of the roughage DM. The digestible organic matter intake of rice straw was also increased by 0.42 and 0.27 kg $day^{-1}$ due to urea and ammonia treatment, respectively compared with untreated straws.

In an experiment, Manyuchi *et al.* (1992) reported that treatment of straw with ammonia or supplementing straw with 200 or 400g of ammonia treated straw resulted in an 80, 56 and 59% increase in intake, respectively. The report by Silva *et al.* (1989) showed an increase of DM intake from 414 to 729 g/day in sheep and from 4.75 to 6.09 kg $day^{-1}$ in cattle due to ammoniation. Mira *et al.* (1983) observed that steers offered urea treated straw consumed 1.36 $\pm$ 0.236 kg $day^{-1}$ more than those offered untreated straw. Hadjipanayiotou *et al.* (1997) reported in on experiment with higher values of voluntary intake of urea treated straw relative to untreated straw. Superiority of urea treatment as opposed to urea supplementation has also been reported for voluntary intake. Khanal *et al.* (1999) reported an increase of 17.4% in DM intake after animals were fed urea treated wheat straw. Experimental evidence (Cloete and Kritzinger, 1984) indicates that the voluntary intake of ammoniated wheat straw by sheep was increased by 8.1% and 46.7% over that of urea supplemented and non-supplemented straw, respectively.

The beneficial effect of ammonia and/or urea treatment in ruminant diets has been associated mainly with the increase in N for better utilization of roughages. Significant improvement in rumen environment (Silva and Ørskov, 1988) and higher live weight gain (Castrillo *et al.*, 1995; Flachowsky *et al.*, 1996) were found after urea-treated barley straw diets were fed to ruminants. Hadjipanayiotou *et al.* (1997) identified a

12.4% improvement in weight gain of crossbred heifers fed urea treated barley straw relative to urea-supplemented diet.

Figures 2.1, 2.2 and 2.3 summarize changes in DMI from various experiments on urea and/or ammonia treated roughages. The mean increase in DMI owing to urea-ammoniation of roughages is about 33.8% (s.d. 22.18). Figure 2.1 indicates that the change in DMI is inversely related to the corresponding DMI of the untreated roughage

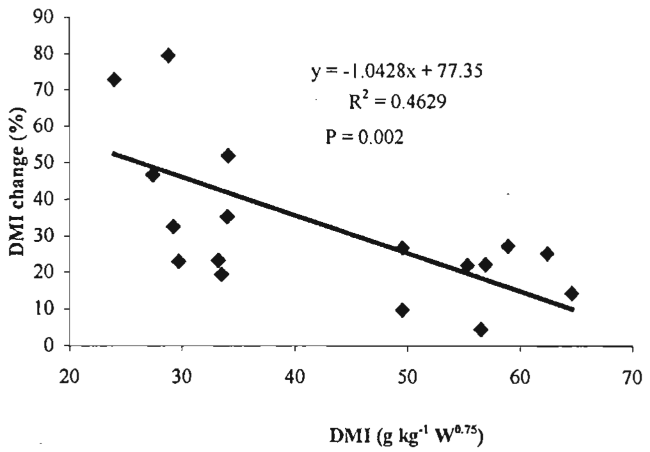



**Figure 2.1** Plot of percentage change in DMI after treating roughages with urea and/or ammonia against the corresponding DMI of untreated roughages (source of data: Table 2.2).

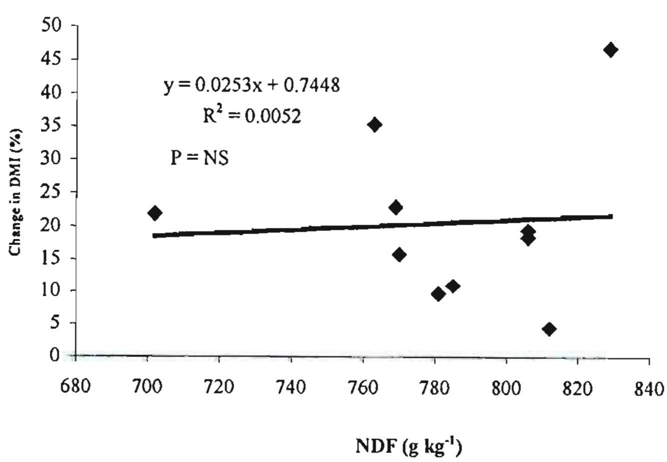



**Figure 2.2** Plot of percentage change in DMI after treating roughages with urea and/or ammonia against the corresponding NDF content of untreated roughages (source of data: Abidin and Kempton, 1981; Buettner *et al.*, 1982; Cloete *et al.*, 1983; Alibes *et al.*, 1983/84; Cloete and Kritzinger, 1984; Jewell and Campling, 1986; Tuen *et al.*, 1991).
NS = Non significant

but had no relationship with the change in NDF due to ammoniation. It is thus of no benefit to treat roughages that elicit high intakes in the untreated form. The lack of a positive relationship between the change in DMI and either NDF content or change in NDF (%) could be the result of the varied nature of experiments from which these data were derived (Figure 2.2). It is also possible that this poor relationship could be linked to different factors influencing the effectiveness of urea-ammoniation, like adequate moisture, physical form of the forage (i.e., chopped or long), and uniform mixing of the urea solution with the forage to ensure uniform distribution of ammonia (Cloete and Kritzinger, 1985; Ibrahim *et al.*, 1986).

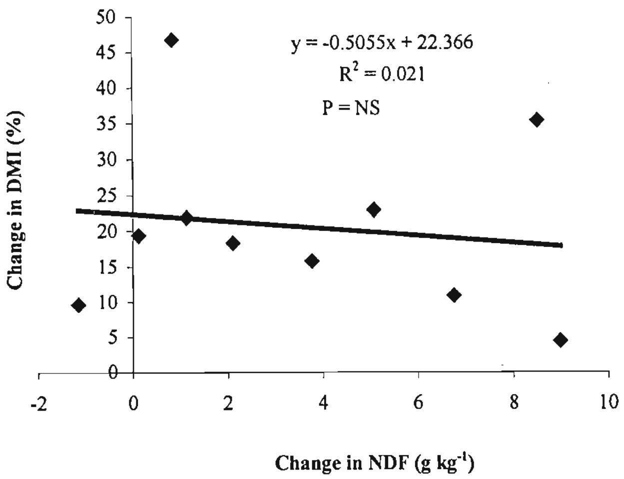



**Figure 2.3** Plot of percentage change in DMI after treating roughages with urea and/or ammonia against the corresponding change in NDF content of roughages (source of data: Abidin and Kempton, 1981; Buettner *et al.*, 1982; Cloete *et al.*, 1983; Alibes *et al.*, 1983/84; Cloete and Kritzinger, 1984; Jewell and Campling, 1986; Tuen *et al.*, 1991).

## 2.6 Effect of urea and/or ammonia treatment on the digestibility of roughages

Work reported in the literature indicate that treatment of cereal straws with urea/ammonia results in changes in the chemical composition of the straw (Abidin and Kempton, 1981; Cloete and Kritzinger, 1984; Seed *et al.*, 1985; Dias-Da-Silva and Sundstøl, 1986; Flachowsky *et al.*, 1996), which improve digestibility.

Several authors have reported the effect of chemical treatment on the digestibility of roughages. Schneider and Flachowsky (1990) reported an increase in the rumen dry matter degradability of wheat straw from 36.7 to 74.4% after ammonia treatment. The apparent digestibility of wheat straw was also increased from 43.9 to 52.7% due to ammonia treatment and from 46.5 to 53.0% due to urea-ammoniation (Flachowsky *et al.*, 1996). Similarly, the apparent digestibility of rice and wheat straw was improved by 18.1 and 13.3% units respectively, due to urea treatment (Khanal *et al.*, 1999). A mean improvement in organic matter digestibility of 13.0 $\pm$ 5.5% has been found following urea treatment (Dolberg *et al.*, 1981). The report of Dryden and Leng (1988) showed that the disappearance of total nitrogen and digestibility of dry matter from ammoniated barley straw was significantly greater than that of untreated or supplemented straw. Fahmy and Ørskov (1984) also reported an improved DM degradability of barley straw due to ammonia and sodium hydroxide (NaOH) treatments. The rumen dry matter loss of rice straw was significantly higher after treatment with urea and ammonia as compared with untreated straw (Macdearmid *et al.*, 1988). Using barley, oat, and wheat straw, Horton (1981) reported that ammoniation increased the mean digestibility value of NDF, ADF and cellulose by 5.8, 4.7 and 5.8%, respectively. There is no doubt therefore that ammoniation of the basal roughage diet increases intake (Nelson *et al.*, 1984; Zorrila-Rios *et al.*, 1984; Nelson *et al.*, 1985) through increased digestibility (Males and Gaskins, 1982; Mira *et al.*, 1983; Zorrila-Rios *et al.*, 1984).

For an appreciable microbial digestion of plant material to occur in the rumen, a close physical association is essential between the plant tissue and the microbes responsible for the digestion (Cheng *et al.*, 1983/84; Orpin, 1983). It is known that enzymatic activity is likely to be proportional to the mass of cellulolytic microorganisms. Cheng *et al.* (1990) have shown, using cotton thread as cellulosic substrate, that the rate of cellulose digestion is correlated with the mass of attached colonizing microbes. It has always been assumed that colonization of fiber entering the rumen is from the free floating pool of bacteria in the rumen. Krebs *et al.* (1989) suggested that colonization of bacteria occurs from fiber to fiber without passing through the free-floating pool. Leng (1990) thus extrapolated from this theory that the beneficial effects of incorporating high quality roughage in an otherwise low digestibility forage diet could be that it provides a highly colonized fiber source to "seed" bacteria onto the less digestible fiber. Treating roughages with urea contributes fermentable energy to the rumen in the form of available cellulose and hemicellulose, which stimulate fiber digestion (Silva and Ørskov, 1988). According to Bauchop (1981) it is possible that offering such material in the daily ration may induce a greater degree of colonization of straw by rumen bacteria and by rumen fungi, responsible for the breakdown of fiber. Other factors may be involved. For instance, Ørskov and Dolberg (1984) stated that if animals fed untreated straws or poor quality roughages are offered substrates that increase the fermentation rate of cellulose, the rumen environment would become similar to that of animals receiving ammonia treated straws.

According to Akin (1989) it is likely that any change in the degradation of the basal diet as a result of an increase in microbial activity may depend on the number of available sites for microbial attachment. The cuticle layer and extent of lignification of some roughage are barriers to microbial colonization, so that an increase in rumen microbial population may not be reflected in an increase in rate of degradation. Chenost and Kayouli (1997) published data showing that the degradation of the cell walls requires the microbes to attach themselves to the feed particles so that the fibrolytic enzymes can penetrate inside the fibrous structures. Hence, there is a need for the microflora, which secrete these enzymes, to have sufficiently wide access paths through the lignocellulose complex. Unfortunately, low quality roughages show a high proportion of lignified walls, encrusted with lignin in a very complex manner, especially in the case of grasses. Thus, lignin impedes the microbial colonization of the fibers and, consequently, the action of the cellulolytic enzymes.

Figures 2.4, 2.5 and 2.6 summarize changes in DMD and OMD from various

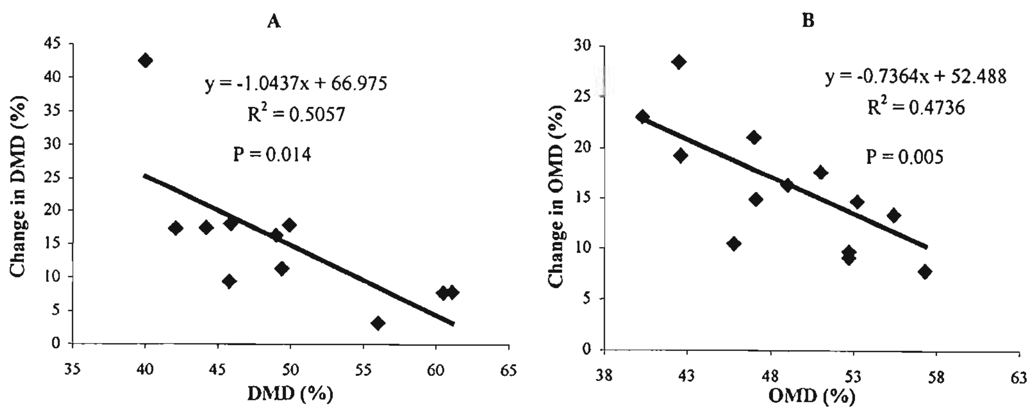



**Figure 2.4** Plots of percentage changes in DMD (A) and OMD (B) after treating roughages with urea and/or ammonia against the corresponding contents of untreated roughages (source of data: Table 2.2).

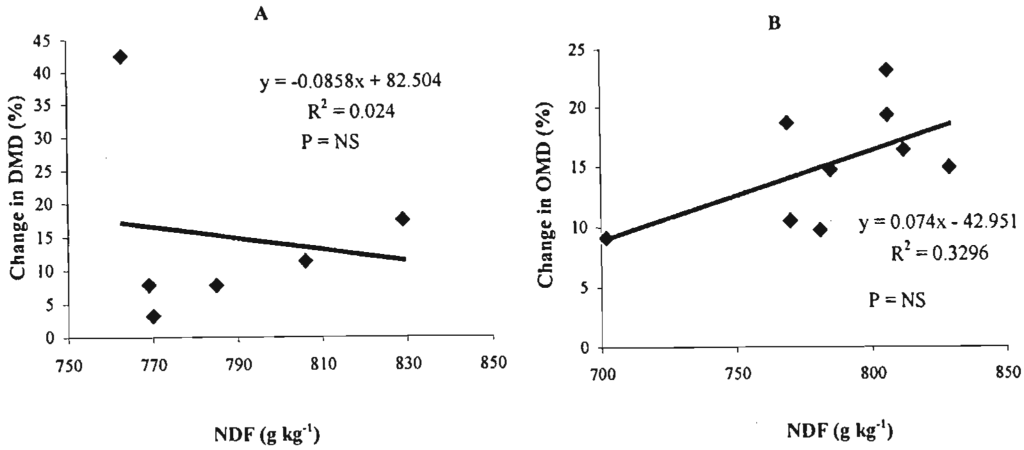



**Figure 2.5** Plots of percentage changes in DMD (A) and OMD (B) after treating roughages with urea and/or ammonia against NDF contents of untreated roughages (source of data: Abidin and Kempton, 1981; Buettner *et al.*, 1982; Alibes *et al.*, 1983/84; Cloete *et al.*, 1983; Cloete and Kritzinger, 1984; Jewell and Campling, 1986; Tuen *et al.*, 1991).

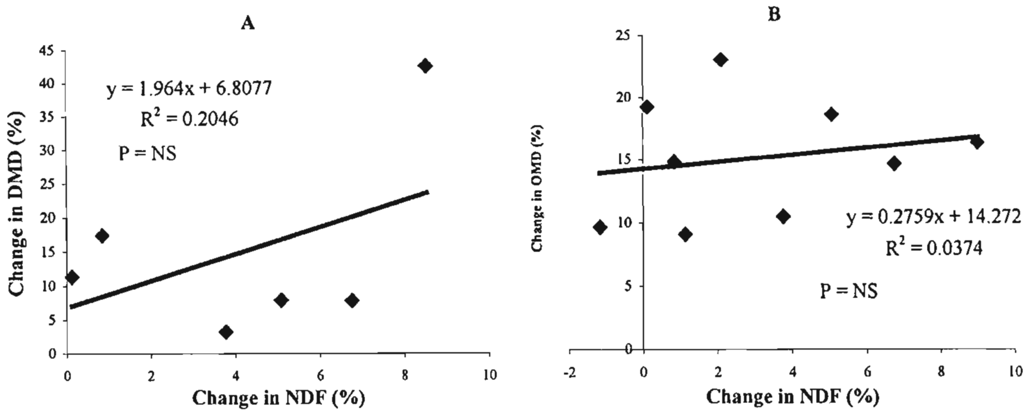



**Figure 2.6** Plots of percentage changes in DMD (A) and OMD (B) after treating roughages with urea and/or ammonia against the corresponding change in NDF contents of roughages (source of data: Abidin and Kempton, 1981; Buettner *et al.*, 1982; Alibes *et al.*, 1983/84; Cloete *et al.*, 1983; Cloete and Kritzinger, 1984; Jewell and Campling, 1986; Tuen *et al.*, 1991).

experiments on urea and/or ammonia treated roughages. The mean increase in DMD and OMD owing to urea or ammonia treatment was 15.4% (s.d. 10.36) and 15.9% (s.d. 5.69), respectively. Figures 2.4A and B suggest that digestibility responses to treatment are high for feeds with intrinsic low digestibility, but then decreases as the digestibility of the untreated feed increases. As expected the change in OMD owing to urea-ammoniation increased as their corresponding NDF contents of roughages increased. These summaries are in agreement with previous postulates that the change in DMD and OMD of roughages could be due the initial quality of the untreated roughages (Habib *et al.*, 1998).

## 2.7 Changes in cell wall components due to urea and/or ammonia treatment

This section reviews changes in the cell wall components (CWC) of roughages

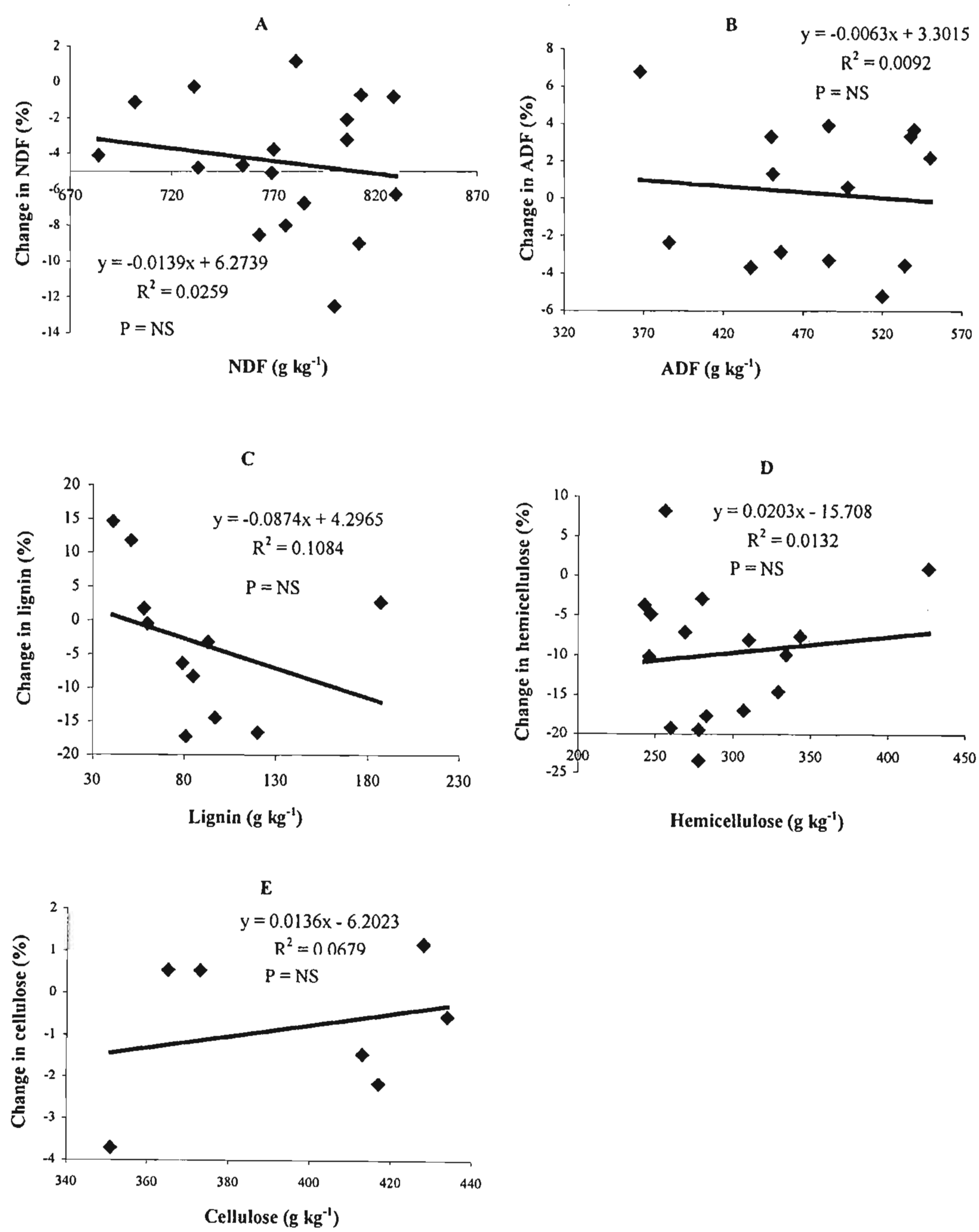



**Figure 2.7** Plot of percentage changes in NDF (A), ADF (B), lignin (C), hemicellulose (D) and cellulose (E) after treating roughages with urea and/or ammonia against the corresponding contents of untreated roughages (source of data: Table 2.1).

following urea and/or ammonia treatment. Where the change is referred to in terms of a percentage change in NDF, ADF, lignin, hemicellulose and cellulose it signifies a response over the untreated roughages. Summarized changes in the CWC of urea and/or ammonia treated roughages are presented in Figure 2.7. It can be seen that the change in NDF is negatively correlated to the NDF content of the untreated roughages. This implies that the effectiveness of urea-ammonaition on roughage quality increases with roughage NDF content. Most reports in the literature indicate that ADF and hemicellulose increase while NDF decreases after treating with chemicals (Van Soest *et al.*, 1983/84; Cloete and Kritzinger, 1984; Habib *et al.*, 1998).

Oji and Mowat (1979) reported a slight, but significant decrease in the NDF and a non-significant increase in ADF when maize stover was treated with 3% ammonia. Similarly, Abidin and Kempton (1981) reported a reduction of 20% in the hemicellulose content of barley straw after ammoniating with anhydrous ammonia. However, results reported by Brown and Adjei (1995) showed no difference in NDF content due to urea-ammoniation of guinea grass hay.

In a series of experiments with maize stover, barley and wheat straw, Joy *et al.* (1992) found that urea-ammoniation reduced NDF concentration when NDF concentration of untreated straw was 86%, but not when it was 76%. Similar results, reported by Kiangi *et al.* (1981), Williams *et al.* (1984b), and Fahmy and Klopfenstein (1994) for a range of crop residues (70 to 75% NDF), suggest that in some cases urea-treatment may not effect any change in chemical composition. The mean values of NDF (g $kg^{-1}$), ADF (g $kg^{-1}$), hemicellulose (g $kg^{-1}$), cellulose (g $kg^{-1}$) and lignin (g $kg^{-1}$) contents of urea-ammoniated and untreated roughages were 739.8 (s.d. 45.26) & 774.4 (s.d. 41.55), 479.9 (s.d. 58.99) & 478.6 9 (s.d. 57.04), 267.8 (s.d. 55.15) & 296.2 (s.d. 47.71), 401.4 (s.d. 32.60) & 397.3 (s.d. 33.42) and 73.3 (s.d. 16.88) & 77.4 (s.d. 23.06), respectively.

### 2.8 Nutrient utilization

The efficiency with which absorbed nutrients are converted to animal products (live weight, milk, etc.) is dependent on precisely meeting the animal's requirements for the individual nutrients required for the particular function (Preston and Leng, 1987). Roughage is produced in large amounts in many countries. However, because of its relatively poor nutritive value and low digestibility only a small proportion is included in animal diets, and roughages remain as under-utilized sources of nutrients for ruminants.

It has been reported that ammoniation increases the utilization of roughages. However, the protein:energy ratio (P/E) is an important factor that is associated with the efficiency of feed utilization (Devendra, 1995). Since anaerobic fermentation in the rumen is associated with the synthesis of microbial cells, which supply protein to the host animal, the efficiency of microbial growth therefore influences the P/E ratio. Poor microbial growth due to inadequate dietary nitrogen, for example, will result in a low P/E ratio and, conversely, adequate supply of nitrogen and good rumen function enable a good balance in the nutrients available to the animal (Leng, 1990). The crude protein (CP)/total digestible nutrient (TDN) ratio in urea treated straw varies between 0.14 and 0.22 which is generally higher than the requirements for maintenance (0.15) and growth of older animals (0.13), but lower than the requirements for growth of young animals (0.27) and for milk production (0.27) (NRC, 1976). The CP/TDN ratio of untreated straw varies between 0.09 and 0.11, i.e. too low for all functions, even for maintenance.

The required CP/TDN ratio for microbial growth in the rumen is between 0.14 and 0.21, depending on the rumen degradability of CP, the rumen degradability of the organic matter, the recycling of N and the percentage of degradable protein which can be captured effectively in the rumen (Rohr *et al.*, 1979; Durand, 1989). There are indications that energy utilization in the rumen is low for untreated straw, i.e. the efficiency of microbial nitrogen production is low, causing a relative protein shortage in the small intestine (Zorrila-Rios *et al.*, 1984; Doyle and Panday, 1990).

The patterns of feeding of urea supplements or alkali treated feeds are important for optimising total nutrient supply. The availability of nitrogen, sulphur and other minerals are important for maximal rate of fermentation and microbial growth will depend on the energy substrates being utilized. Where fibrous crop residues are fed and where the energy is derived from slowly fermented hemicellulose and cellulose, nitrogen and sulphur will be needed continuously over a 24-hour feeding cycle (Dixon, 1986). This author reviewed the literature on the effect of different methods of administering urea and the use of slow release non-protein nitrogen compounds. In all the experiments reviewed, the differences in intake of organic matter in animals receiving no urea supplement as compared to those receiving urea every day or every second day was much greater than the differences among the other methods of urea administration. Dixon (1986) concluded that the decision to provide a urea supplement was far more important than the method of urea administration. Another way of maximizing utilization of roughages, in addition to chemical treatment, is proper supplementation. The effect of supplementation with forage legume and concentrate on roughages is discussed in the subsequent section.

## 2.9 Supplementation

### 2.9.1 Supplementation of roughages with forage legume

Early works on the use of forage supplements were mainly concerned with the need to improve the nitrogen content of diets based on poor quality roughages in order to overcome a deficiency of nitrogenous substrates for the rumen microorganisms. Recent evidence indicated that other changes occur with supplementation resulting to enhanced intake and digestibility of the diet (Topps, 1995). These changes seem to be related to the level of supplementation, the quality of the basal diet and the quality of the forage supplement.

The beneficial effect of forage legumes in ruminant diets has been associated with the need for better utilization of low quality feeds. Significant improvement in rumen environment and higher weight gain (Tolera and Sundstøl, 2000) was reported in response to *Desmodium intortum* supplementation of maize stover diet. In a study, McMeniman *et al.* (1988) reported increased degradation of straw due to supplementation of rice straw with each of the forage legumes: mung bean, cowpea, pigeonpea, peanut and Lucerne. Ndlovu and Buchanan-Smith (1985) also found that Lucerne increased the rate of degradation of barley straw, brome grass and maize cobs. Olayiwale *et al.* (1986) observed a 41% improvement in weight gains of crossbred heifers in response to *Trifolium* hay supplementation. Furthermore, Mosi and Butterworth (1985) supplemented four different cereal straws (maize stover, oat hay, teff (*Eragrostis*) straw and wheat straw) with *Trifolium tembense* hay and found that the forage legume increased intake and digestibility of the roughages although the extent of substitution of the basal diet tended to be greater with the good quality straws (oat and maize). The substitution rate depends on the quality of both the basal roughage and the supplement, and it is generally known to be lower in poor quality roughages than the better quality roughages (Nsahlai *et al.*, 1996; Chenost and Kayouli, 1997; Tolera and Sundstøl, 2000). This is probably due to a better response of poor quality roughages than good quality roughages to supplementation.

### 2.9.2 Supplementation of roughages with concentrate

Most of the beef and dairy cattle enterprises use high concentrate levels in diets to achieve higher performance. However, the main constraints with the use of high levels of concentrate in diets, especially in dairy cattle, is the formation of acid in the rumen, resulting to increased proportion of propionice acid (Brake and Hutcheson, 1996). The higher level of propionic acid production can be attributed to a reduction in fiber digestion. Many studies (Harvey *et al.*, 1968; Foster and Wood, 1970; Brent, 1976) have shown that the incidence of acidosis increases as diet roughage level decreases. Conversely, replacing concentrate with high levels (>20%) of roughage depresses growth rate, which Bartle *et al.* (1994) and Zinn *et al.* (1994) ascribed to a reduction in digestibility of the diets.

When ruminants consume too much starch (primarily grain) within a short period of time or when there is an abrupt change of feeding from a high roughage diet to high concentrate diet without adequate adaptation, it results in rapid production and absorption of acids from the rumen. In a study with beef and dairy cattle Jensen and Mackey (1965) reported that acidosis also occurs during the first 3 weeks of the fattening period, during which beef and dairy cattle are still adapting to concentrate diets. This could be due to the effect of concentrate on the rumen pH. The optimum pH at which cellulolytic bacteria can multiply and digest fibers range from 6.2 to 7.0 (Ørskov and Ryle, 1990). This range is maintained on roughage-based diets, but not with more intensive concentrate feeding. For instance, Cronje (1992) noted that *Eragrostis curvula* hay degradation decreased when the rumen pH falls from 6.2 to 5.8 for high and low roughage diets, respectively. The decrease in fiber digestion could be due to the fact that cellulolytic bacteria are known to be sensitive to changes in pH. Moreover, growth of cellulolytic bacteria is reported to be inhibited once pH falls below 6.2 (Ørskov, 1982; Mould and Ørskov, 1984). Similarly, Brand *et al.* (1991) observed that dry matter digestibility of wheat straw was depressed at the highest inclusion of maize meal (20%) when the pH decreased from 6.31 to 6.26 eight hours after feeding. It is also possible that the rumen pH could vary with the level of concentrate component of the ration, duration of feeding, type and species of animal. The following section will consider the effect of concentrate on urea-ammoniated diets.

### 2.9.3 Supplementation of urea-ammoniated roughages with concentrate

Several studies have reported the effect of concentrate on the utilization of urea-ammoniated roughages (Buettner *et al.*, 1982; Silva *et* al., 1989; Tuen *et al.*, 1991; Mgheni *et al.*, 1993). However, information regarding the effect associated with inclusion of high concentrate on urea-ammoniated diets is limited. The effect of ammoniated roughage on performance of animals is dependent on the concentrate level of the diets fed to animals (Garrett *et al.*, 1979; Horton, 1979). Seed *et al.* (1985) showed that ammoniation of maize residues increased lamb performance at a high concentrate level (60%). Zemicael (2003) fed 60% concentrate and 40% urea-ammoniated diets to steers for a period of two months and found no negative effect of increased concentrate proportion on the incidence of liver abscesses. Flachowsky *et al.* (1993) fed 5 different rations varying in straw to concentrate ratio to rumen fistulated male sheep. It was observed that the decrease in DM degradability was much higher for untreated (from 50.8 to 45.9%) than for ammoniated wheat straw (from 55.2 to 53.3%), when the concentrate increased from 25 to 50% of the ration. Brake and Hutcheson (1996) reported that under optimal rumen fermentation conditions, the acetate: propionate ratio should be greater than 2.2 to 1. Wise *et al.* (1961) obtained an acetate: propionate ratio of 2 to 1 after 1% urea was added to a concentrate diet. The authors then concluded that it is possible that the ammonia released from urea and converted to the cation form ($NH_4^+$) may have been present in sufficient quantities to increase the buffering capacity of the rumen and to aid in neutralization of the rapidly produced VFAs.

### 2.10 Performance of animals fed treated straws

Chemically treated roughages have been reported to increase milk yield when fed to lactating ruminants. Urea-treated roughages are enriched with nitrogen, have high digestibilities, higher rate of volatile fatty acid (VFA) production, elicit short rumen retention time, resulting in high overall intake, thus enhancing the performance of ruminants, as compared to untreated roughages (Wanapat *et al.*, 2000). Consequently, live weight gain and food efficiency were significantly increased when ammonia treated straw was offered (Mira *et al.*, 1983). Orr *et al.* (1985) fed pregnant ewes spring barley straw or hay which was either untreated or treated with anhydrous ammonia, from day 105 of pregnancy until lambing. Ewes offered treated forage gained slightly more weight during pregnancy (138 vs. 104 g $day^{-1}$) than ewes fed untreated forage. In another study Silva *et al.* (1989) fed sheep and cattle with fish meal and unmolassed sugar beet pulp either separately or in combination, as supplement to ammonia treated and untreated barley straw for 24 weeks. All animals offered untreated straw as the sole feed lost more body weight than those animals offered ammoniated straw. The study conducted by Djibrillou *et al.* (1998) showed that all animals offered untreated rice straw lost significantly more weight than the animals fed urea treated rice straw. Similar results have been reported in other research (Tuen *et al.*, 1991; Mgheni *et al.*, 1993; Flachowsky *et al.*, 1996).

The experiment conducted by Khanal *et al.* (1999) reported that feeding urea treated rice and wheat straw to lactating buffaloes increased milk yield by 1.15 and 1.12 kg $day^{-1}$. Wanapat *et al.* (2000) conducted a study in Thailand in which dairy cattle were fed urea treated rice straw (UTRS), UTRS and whole sugar cane (WSC) at 75:25% DM, UTRS and WSC at 25:75% DM and WSC. It was found that a combination of UTRS and WSC at 75:25 ratio (DM) enhanced DM intake, milk yield, milk fat and protein percentages as compared to WSC alone. Djibrillou *et al.* (1998) fed lactating goats untreated straw, urea treated straw and untreated straw plus cotton seed cake during the first eight weeks of lactation and found that milk yield was improved from $2.73 \pm 0.24$ to $3.11 \pm 0.37$ kg $week^{-1}$ due to urea-ammoniation. These reports suggest that urea-ammoniation can improve the feeding value of tropical roughages.

### 2.11 Conclusions

Constraints that limit the productivity of ruminants in the tropics were highlighted and prospects of using urea-ammoniation to improve the nutritive value of roughage were reviewed. Urea-ammoniation of roughages tended to decrease NDF content. Urea-ammoniation increased the DMI, DMD and OMD of roughages by 33.8% (s.d. 22.18), 15.4% (s.d. 10.36) and 15.9% (s.d. 5.69), respectively. From this review, however, it is clear that roughages do not respond uniformly to urea-ammoniation. It appears that the ammonia released from urea-ammoniated roughage could act as a buffer to reduce drastic changes in ruminal pH that usually accompany the consumption of high concentrate diets, thus dampening the negative effect of high concentrate diet on roughage digestion and utilization.

CHAPTER 3

# EFFECT OF UREA TREATMENT AND CONCENTRATE PROPORTIONS ON DRY MATTER DEGRADATION OF DIFFERENT ROUGHAGES IN THE RUMEN OF JERSEY COWS

## 3.1 Abstract

A study was conducted to determine the interaction of roughage quality and urea-ammoniation on the ruminal degradation properties of low quality roughage diets. Four-rumen fistulated Jersey cows were fed on a basal diet composed of either urea-treated or untreated *Eragrostis curvula* hay. These basal diets were supplemented with concentrate composed of maize meal (78%) and cotton seed cake (22%). The concentrates contributed 0, 25, 50 and 75% of the total ration and hay the rest. The experiment consisted of 6 periods. Each period lasted 19 days, comprising 12 days of adaptation to the experimental diet followed by 6 days degradability measurements and 1-day collection of rumen fluid. During each period the 4 Jersey cows were randomly allocated to 4 of the 8 dietary treatments, ensuring that each diet was fed to 3 animals during the entire experimental period.

The pH of the rumen fluid ranged between 6.5 and 6.8 for all diets. Rumen ammonia concentration was higher ($P<0.002$) when the basal diet consisted of urea-treated hay. Increasing the concentrate proportion in the diet had the desired effect of increasing rumen ammonia concentration without severely affecting pH. Urea-ammoniation increased ($P<0.0001$) the slowly degradable fraction (B), potential degradability (PD), effective degradability (ED) of dry matter and neutral detergent fiber (NDF), decreased ($P>0.05$) lag time (LT) but had no effect on the rate of degradation (c) of dry matter. Concentrate proportions affected ($P<0.05$) the slowly degradable fraction, potential degradability, lag time and effective degradability but had no effect ($P>0.05$) on the rate of degradation of dry matter (DM). Maximum and minimum of the slowly degradable fraction, potential degradability and effective degradability of DM and NDF were obtained at the 25 and 75% concentrate levels, respectively for both urea-treated and untreated diets. Within urea-ammoniation, roughage type increased ($P<0.001$) the B-fraction, PD and ED of DM and NDF degradation. Rate of degradation of DM of untreated roughages varied from 0.022 $h^{-1}$ in wheat straw to 0.087 $h^{-1}$ in ryegrass, while for urea-treated roughages it varied from 0.022 $h^{-1}$ in oat straw to 0.082 $h^{-1}$ in ryegrass. Ryegrass degraded almost three to four times faster than urea-treated oat or untreated wheat straw. Urea-ammoniation was less effective in increasing DM and cell wall degradation rates (c) of ryegrass compared to wheat straw. The observations show that low quality roughages such as wheat straw benefited relatively the most from urea-ammoniation.

**Keywords: Urea-ammoniation, rumen ammonia, dry matter, fiber fractions, degradability.**

## 3.2 Introduction

Over the last decade there has been a lot of research conducted to determine the benefit of using low quality roughages in livestock feeding in the tropics, particularly during the winter season when green pasture resources are not available. These roughages contain 70-80% cell wall components, which represent potential protein and energy for ruminants. Availability of this energy and protein to the animals is generally limited by the low voluntary intake, the chemical association between lignin and cell wall carbohydrates, and physical limitation of the cell wall components to microbial fermentation. Despite the limiting features roughages have enormous potential and can be used as sole feed, if their feeding value is improved.

Considerable efforts have been made worldwide to find ways of modifying roughages to improve degradability and upgrade their nutritive value to feed ruminants. Some of the methods for improving the availability of energy in roughages for ruminants involve treatment with ammonia (Sundstøl and Coxworth, 1984), NaOH (Dixon and Parra, 1984; Escobar *et al.*, 1984) and urea (Hadjipanayiotou, 1982a; Cloete and Kritzinger, 1984; Dias-Da-Silva and Sundstøl, 1986). Urea is one of the major chemical agents used to improve the nutritive value of cereal straws and other fibrous by-products (Rai *et al.*, 1989; Goto *et al.*, 1991; Tuen *et al.*, 1991; Mgheni *et al.*, 1993). Treatment with urea can break the ester bonds between lignin, hemicellulose and cellulose, and physically cause structural fiber to swell. It has been suggested (Mason *et al.*, 1988; Manyuchi *et al.*, 1992) that urea-ammoniation affects cell wall composition and improves rumen degradability.

*Eragrostis curvula* is a popular hay grass, produced in vast quantities in Natal, with a mean crude protein (CP) contents of less than 6 g $kg^{-1}$ DM (Galloway, 1980), which does not meet the minimum dietary CP concentration required for milk producing dairy cows (Flachowsky *et al.*, 1993). A large proportion of the potential hay crop is lost each year because difficulties are experienced with harvesting the hay at the correct time. As a result a large proportion of the *E. curvula* hay produced is of little feed value. If urea has the same effect on *E. curvula* hay as on cereal straws then urea-ammoniation of such low quality hay could increase its potential as a ruminant feedstuff. Concentrate supplementation for such low quality roughages also increase the rumen fermentation due to the supply of readily fermentable energy. However, it has been reported that high concentrate levels in the diet of animals reduce rumen fermentation and rumination time (Ørskov and Ryle, 1990), consequently saliva production is low. It is not known, therefore, to what extent the changes in the rumen environment induced by feeding urea treated roughages and concentrate supplements could affect fiber degradation.

The main objectives of the study were: (i) to determine how dietary manipulation and urea-ammoniation affect degradation of roughage diets; and (ii) to develop a model that would help to predict the benefit associated with the treatment of roughages.

### 3.3 Materials and methods

#### 3.3.1 Study area

The experiment was conducted at the University of Natal's Ukulinga Research Farm outside Pietermaritzburg, in the subtropical hinterland of KwaZulu-Natal Province, South Africa. It lies at $30^0$ 24'S, $29^\circ$ 24'E and approximately 700 m above sea level. The mean annual rainfall for the study site is 735 mm, falling mostly in summer, between October and April, and the maximum and minimum mean annual temperatures are 25.7 and 8.9 °C, respectively (Camp, 1999). Light to moderate frost occurs occasionally in winter.

#### 3.3.2 Animals

Four adult Jersey cows (average body weight of 425.5 ± SD 58.4 kg), fitted with a permanent rumen cannulae of 120 mm internal diameter were used in this study. They were kept in individual feedlot pens under a roofed shed with floor beddings. Cows were provided with clean water and a mineral block supplement at all times throughout the experiment.

#### 3.3.3 Diet and feeding

All rumen fistulated Jersey cows were fed on a basal diet composed of either urea-treated or untreated *Eragrostis curvula* hay. These basal diets were supplemented with a concentrate mixture composed of maize meal (78%) and cottonseed oilcake meal (22%). The concentrate was formulated to contain 15 % crude protein (CP). Concentrate contributed 0, 25, 50 or 75% of the total ration and treated or untreated *E.curvula* hay the rest. Thus 8 different dietary treatments were formed.

### 3.3.4 Experimental design

The experiment comprised six periods. Each period lasted 19 days, comprising 12 days of adaptation to the experimental diet followed by 6-days of degradability measurements and 1-day of rumen fluid collection. During each period the 4 Jersey cows were randomly assigned to 4 of the 8 dietary treatments. It was however ensured that each diet was fed to 3 animals during the entire experiment (Table 3.1).

Before the initiation of the experiment, cows were allowed free access to diets to determine *ad libitum* intake. Cows were then subsequently fed eighty percent (80%) of the *ad libitum* intake to ensure complete consumption of feed so as to adhere to the roughage: concentrate ratio. Diets were fed twice a day, during the morning (08:00) and afternoon (16:00).

**Table 3.1** Experimental design of feeding program (regime) in different periods

| Periods | Treated: Concentrate Ratio (T: C)** | | | | Untreated: Concentrate Ratio (UT: C)** | | | |
|---|---|---|---|---|---|---|---|---|
| | 100: 0 | 75: 25 | 50: 50 | 25: 75 | 100: 0 | 75: 25 | 50: 50 | 25: 75 |
| $P_1$* | 1**** | | | 4 | 3 | 2 | | |
| $P_2$ | | 1 | 4 | | | | 3 | 2 |
| $P_3$ | 2 | 3 | 1 | | | | 4 | |
| $P_4$ | | | 2 | 1 | | 4 | | 3 |
| $P_5$ | 3 | 2 | | | 1 | | | 4 |
| $P_6$ | | | | 3 | 4 | 1 | 2 | |

Where:
* $P_1$, $P_2$, $P_3$, ... $P_6$ = period.
**T: C = Urea-ammoniated hay: Concentrate ratio; UT: C = Untreated: Concentrate ratio.
**** 1 – 4 = Animal number (ID).

### 3.3.5 Preparation of the urea ammoniated hay

Urea solution was prepared in a plastic container by adding 3 kg urea into 40 litre of water whilst stirring until the urea was all dissolved. The entire 40-litre solution was then sprayed onto 100 kg of hay (on a dry basis). Before the solution was made, a total of 240 bales of *Eragrostis curvula* hay were collected from Ukulinga Research Farm. One hundred and twenty of the 240 bales were ammoniated with urea and divided into four layer groups. The bottom layer, comprising 30 bales, was placed on the concrete floor. Using a 15-litre capacity plastic watering can fitted with a spray nozzle, the prepared urea solution was distributed evenly over the top surface of each layer of hay. The same procedures were applied until the fourth layer. The urea treated hay was covered with large plastic sheets and tightly sealed with tape at the four corners and round the edges with soils to exclude air. Immediately after sealing tightly, weights were strategically put on the top of the plastic sheet to hold it down. After 5 weeks the treated hay was opened and aerated before feeding to the experimental animals. This treatment resulted in moisture content of approximately 35%, as calculated according to Chenost and Kayouli (1997). Representative samples of hay were taken for chemical analysis prior to and after treatment.

### 3.3.6 Preparation of roughages for rumen degradation

Samples (2 kg) of ten different roughages were chosen for incubation. They were collected from different parts of South Africa, to study the degradability of dry matter. The experimental roughages were wheat (*Triticum sativum*) straw, barley (*Hordeum vulgare*) straw, coastcross ($k_{11}$) (*Cynodon* hybrid) hay, veld hay (natural grass), oat (*Avena sativa*) straw, oat (*Avena sativa*) hay, maize (*Zea mays*) stover, Kikuyu (*Pennisetum clandestinum*) grass, weeping love grass (*Eragrostis curvula*) hay and italian rye grass (*Lolium multiflorum*).

The experimental barley, wheat and oat straws examined in this study originated from the Western Cape Province and oat hay from the Free State Province. The rest of the roughages used in this experiment were collected from Ukulinga Research Farm. Italian ryegrass was collected (cut) fresh and then sun-dried before use. Each sample was sub-divided into two equal portions. Each portion was then treated with or without urea. The solution was wholly distributed over 1 kg of roughage. Addition of the urea solution would increase the moisture content of the roughages to 35%. Treated roughages were sealed tightly and stored at room temperature for 5 weeks in plastic bags. Immediately after opening, the different roughages, including the untreated ones, were sun dried, chopped fine by hand and ground through a 2-mm screen in a laboratory mill.

### 3.3.7 Nylon bag incubation procedure

About 3 g of each ground forage sample was weighed into labelled nylon bags (ANKOM Co, Fairport, New York, USA; internal dimensions: 5cm x 9cm; pore size 50μm). The bags were tied to a stainless steel disc with 10 evenly spaced small holes drilled through the periphery of the disc serving as anchor points. The bags were incubated (in duplicate per time interval) in the rumen for 120, 96, 72, 48, 24, 12, 6 and 3 h sequentially. The treated samples were incubated in animals fed treated hay, while untreated samples were incubated in animals given untreated hay. Immediately after removal from the rumen, the bags, including the zero hour ones, which had not been incubated but soaked in warm water for one hour, were washed in 6 cycles (each lasting 4 minutes) in a semi-automatic washing machine. The washed bags were then dried in a forced draught oven at 60 °C for 48 hours, cooled in a desicator and weighed.

### 3.3.8 Determination of neutral detergent fiber content of incubated roughages

Bags containing residues for each incubated time from animals on the same diet were pooled and analysed for their concentration of neutral detergent fiber (NDF). The neutral detergent solution (NDS) was prepared using the method described by Van Soest *et al.* (1991). During the determination process, 0.1 g of each pooled sample was weighed into a labelled nylon bag and then 50 such bags were placed into a 21-litre vat to which 8-litres of NDS was added. Using an electric heater, the mixture was quickly brought to boiling point. At this point, 2 ml of heat-stable alpha amylase (Sigma A-3306) was added and the mixture was allowed to boil for one hour, and then poured into a bag made out of silk cloth and rinsed with tap water. These bags were subsequently washed in a washing machine as described above. The final residue was dried at 60 °C in a forced draught oven for 48 h. To validate our method, six feed samples were analysed in duplicate using both the method described by Van Soest *et al.* (1991) and the one described above. The mean value for the method described by Van Soest *et al.* (1991) was 81.3% (± SD 4.2), while the one for the nylon bag method was 78.1% (± SD

8.3). The disparity in this method of extraction compared to the standard Van Soest's (1991) method was 4.1 percent.

### 3.3.9 Rumen ammonia concentration and pH

After each incubation period, animals were maintained on the same diets and rumen fluid was collected from the rumen of each animal for determination of ammonia concentration and rumen pH during the following times after morning feeding: 2, 4, 6, 8, 10, 12 and 24 hours. Immediately after collection, the rumen fluid was strained through a double layer of cheesecloth. The rumen pH was measured using a Crison portable pH meter model 507 (Crison Instruments, SA. 08328 Alella, Barcelona, Spain). Samples of about 100 ml were put in 250 ml containers to which 5 drops of concentrated sulfuric acid were added and stored in a freezer maintained at –20 °C until they were needed for ammonia analysis.

### 3.3.10 Chemical analysis of basal diet and concentrate

Dry matter (DM), organic matter (OM) and ash were analysed using the procedures described by the Association of Official Analytical Chemists (AOAC, 1990). Nitrogen (N) or crude protein (6.25*N) content in feeds was determined using an automatic protein determinator (LECO FP2000, LECO, Pretoria, South Africa). Rumen ammonia concentration ($NH_3$-N) was measured using an auto analyser with no sample preparation. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents in feeds were analysed using the method described by Van Soest *et al.* (1991) while hemicellulose was estimated as the difference between the NDF and ADF (Van Soest *et al.*, 1991).

### 3.3.11 Calculations and statistical analysis

The degradation of DM and detergent fiber fractions were estimated by fitting the non-linear model proposed by McDonald (1981) and modified by Dhanoa (1988) to the degradation data of each component: variables were determined using the secant method (SAS, 1987).

$$\mathbf{P = A + B\,[1\text{-}exp^{-c\,(T\text{-}LT)}]} \qquad \mathbf{(3.1)}$$

Where P is the disappearance of DM or fiber fraction at time T, A = the part of dry matter which is water soluble (washing losses) and is considered being immediately degradable at time zero, B = degradable part of the insoluble fraction, c = rate of disappearance of degradable fraction "B", T is time of exposure and LT = the lag time. The PD was calculated as A + B. A passage rate (k) of $0.03h^{-1}$ was assumed in order to calculate the effective degradability (ED) of DM (Bonsi *et al.*, 1994; Nsahlai *et al.*, 1998a):

$$\mathbf{ED = A + B \times c/(k + c)} \qquad \mathbf{(3.2)}$$

The model used for statistical analysis of DM degradation was:

$$\mathbf{Y_{ijklm} = \mu + A_i + P_j + U_k + C\,(U)_{kl} + F\,(U)_{km} + \varepsilon_{ijklm}} \qquad \mathbf{(3.3)}$$

Where

Y = individual observation;

μ = Overall mean;

A = Animal effect;

P = Period effect;

U = Effect of urea-ammoniation;

C (U) = Effect of concentrate within urea-ammoniation;

F (U) = Effect of roughage within urea-ammoniation; and

ε = Random variation (assumed independent, identical and normally distributed).

A similar statistical model was applied for the degradation of detergent fiber fractions with a notable difference that animal and period were dropped from the model. The data on DM and NDF degradation parameters of roughages incubated are presented in appendix Table 2 and 3, respectively.

The statistical model for the analysis of rumen ammonia concentration and rumen pH was:

$$\mathbf{Y_{ijklm} = \mu + T_i + TA_{ij} + TP_{ik} + TC_{il} + TU_{im} + TC\,(U)_{ilm} + \varepsilon_{ijklm}} \qquad \mathbf{(3.4)}$$

Where

Y = individual observation;

$\mu$ = Overall mean;

T = Effect of time;

A = Animal effect;

P = Period effect;

C = Effect of concentrate;

U = Effect of urea-ammoniation;

C (U) = Effect of concentrate within urea; and

$\varepsilon$ = Random variation (assumed independent, identical and normally distributed).

Time was introduced in the model as a repeated measure.

Association effects between chemical composition and the degradation characteristics of dry matter and neutral detergent fiber of the roughages were detected using correlation and stepwise multiple regression analysis. Two additional variables were included in the stepwise multiple regression procedure to determine the benefit of treating roughages with urea. One was NDF x $T_{dummy}$ and the other was nitrogen times $T_{dummy}$, where $T_{dummy}$ equals 0 and 1 for the untreated and treated roughages, respectively. The NDF x $T_{dummy}$ will be referred to as the benefit axis. The equation used was of the form below:

$$\mathbf{Y = a\ (s.e) + b_1\ (s.e)\ x_1 + b_2\ (s.e)\ x_2 + \ldots + b_n\ (s.e)\ x_n,} \qquad \mathbf{(3.5)}$$

where "a" is constant, Y is the dependent variable, s.e. the standard error, $x_1$, $x_2$,…,$x_n$ are the explanatory variables and $b_1$, $b_2$, …, $b_n$ are the regression coefficient.

## 3.4 Results

### 3.4.1 Chemical composition of the feeds

The chemical composition of the urea treated and untreated roughages are given in Table 3.2. Urea-ammoniation resulted in marked increase in nitrogen (N) content. There was a wide variation in the chemical composition of the treated and untreated roughage samples. Most of the roughages (Table 3.2) were characterised as high fibrous feedstuffs, with a high NDF value but low crude protein (CP) content. NDF and hemicellulose contents of most roughage were decreased while ADF was increased due to urea ammoniation. Among the untreated roughages, rye grass had the highest crude protein content, followed by coastcross hay, oat hay, maize stover and the lowest was barley straw in a descending order. The rest of the untreated roughages fell in between the two extremes.

### 3.4.2 Ammonia ($NH_3$) concentration and pH in the rumen

The effects of urea-ammoniation of dietary roughage and varying concentrate proportions on rumen ammonia concentration and rumen pH of cows are presented in Figure 3.1, while the least square means are given in Table 3.3. Urea-ammoniation ($P<0.01$) and concentrate proportions ($P<0.001$) increased ammonia concentration in the rumen of Jersey cows but concentrate proportions within urea-ammoniation had no effect ($P>0.05$). The lowest and highest rumen ammonia concentrations were observed at 2 and 4 hrs, respectively, after feeding for animals fed urea treated hay, while for those on the untreated hay the lowest and highest were recorded at 24 and 4 h after morning feeding. The profile of rumen ammonia concentration of the untreated diet was maintained at a lower level than for urea-treated diet throughout the period of measurement.

The effect of urea-ammoniation and concentrate proportions on rumen pH levels was not significant ($P>0.05$; Table 3.3). There was, however, a tendency for increased ($P<0.098$) rumen pH on animals fed urea-treated diets. Concentrate proportion within urea-ammoniation had no effect on rumen pH of the cows.

**Table 3.2** The chemical composition (g DM kg$^{-1}$) of feed ingredients and incubated sample of feeds.

| Ingredients | Urea | DM | CP | Ash | NDF | ADF | HEM |
|---|---|---|---|---|---|---|---|
| Feed ingredients | | | | | | | |
| *E. curvula* hay | 1 | 937 | 88.3 | 56.1 | 754 | 431 | 234 |
| *E. curvula* hay | 0 | 938 | 64.2 | 59.2 | 752 | 420 | 332 |
| Maize Meal | | 897 | 80.4 | 11.1 | 85 | 24 | 61 |
| Cotton seed cake | | 895 | 363.5 | 56.7 | 287 | 234 | 544 |
| Incubated roughages | | | | | | | |
| Barley straw | 1 | 938 | 79.4 | 52.7 | 739 | 523 | 217 |
| *E. curvula* hay | 1 | 923 | 10.1 | 59.2 | 772 | 432 | 340 |
| Coastcross hay | 1 | 924 | 153.2 | 69.8 | 758 | 419 | 339 |
| Kikuyu hay | 1 | 909 | 117.6 | 78.9 | 659 | 381 | 279 |
| Maize stover | 1 | 912 | 124.3 | 46.9 | 720 | 434 | 287 |
| Oat hay | 1 | 887 | 177.5 | 105.4 | 479 | 318 | 162 |
| Oat straw | 1 | 938 | 80.4 | 68.5 | 724 | 516 | 208 |
| Rye grass | 1 | 913 | 348.6 | 139.4 | 495 | 247 | 248 |
| Veld hay | 1 | 924 | 106.0 | 60.6 | 721 | 492 | 230 |
| Wheat straw | 1 | 932 | 76.0 | 48.3 | 727 | 518 | 209 |
| Barley straw | 0 | 930 | 37.0 | 54.5 | 795 | 541 | 254 |
| *E. curvula* hay | 0 | 936 | 71.2 | 58.1 | 765 | 426 | 339 |
| Coastcross hay | 0 | 951 | 118.3 | 70.2 | 767 | 398 | 369 |
| Kikuyu hay | 0 | 930 | 75.7 | 77.2 | 692 | 365 | 327 |
| Maize stover | 0 | 929 | 85.9 | 46.3 | 720 | 448 | 272 |
| Oat hay | 0 | 905 | 97.0 | 107.9 | 487 | 302 | 185 |
| Oat straw | 0 | 937 | 43.4 | 69.2 | 769 | 531 | 239 |
| Rye grass | 0 | 924 | 302.7 | 136.2 | 438 | 233 | 206 |
| Veld hay | 0 | 945 | 64.7 | 79.3 | 698 | 454 | 245 |
| Wheat straw | 0 | 935 | 45.4 | 56.9 | 752 | 521 | 230 |

1, Urea-ammoniated; 0, Untreated; *E. curvula* hay, *Eragrostis curvula* hay, HEM, hemicellulose (HEM= NDF-ADF)

### 3.4.3 Degradation of dry matter (DM)

**Effect of roughage type**

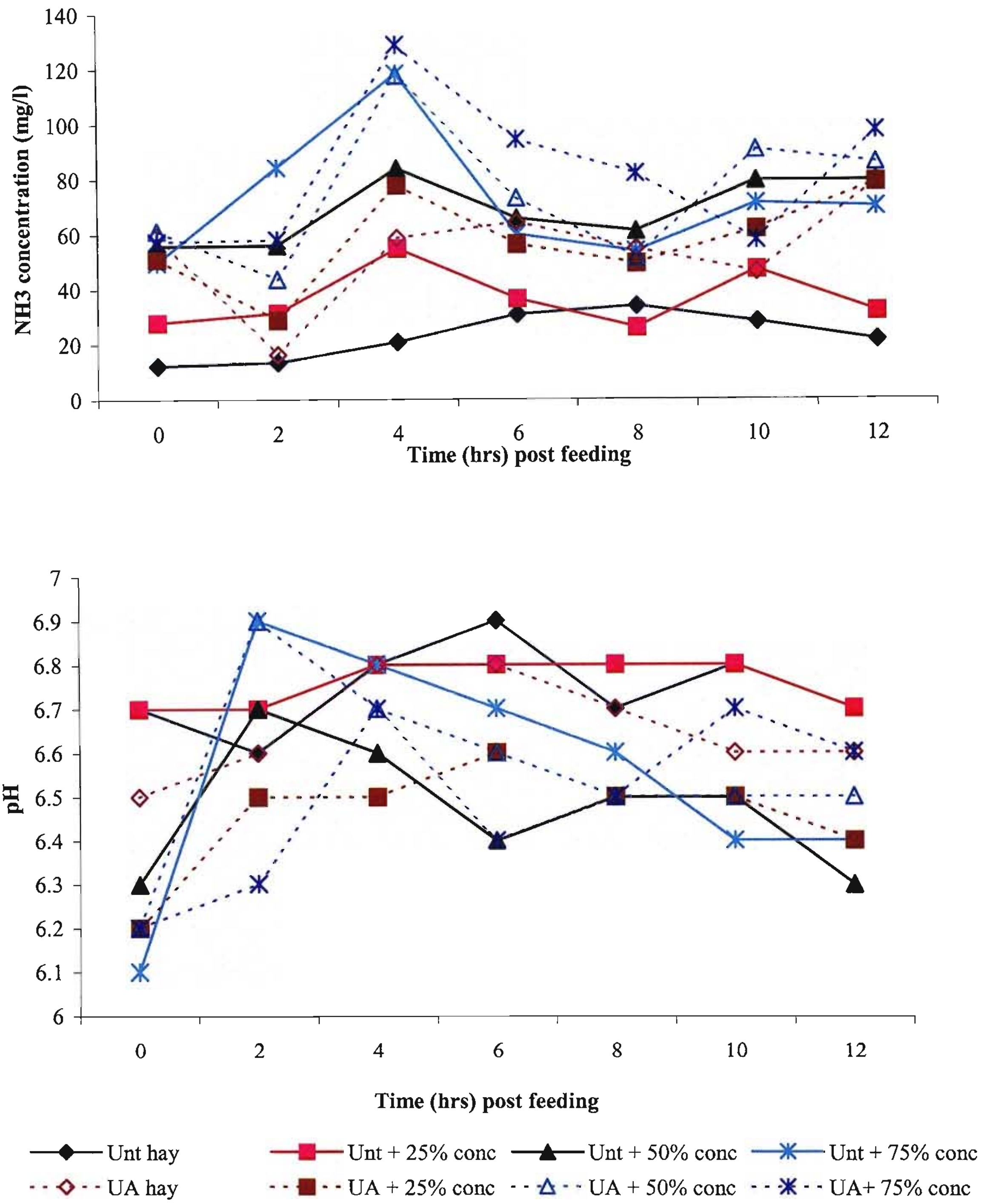
The effect of roughage type within urea-ammoniation on DM degradability of incubated roughages (treated and untreated) is given in Table 3.4. Within urea-ammoniation, roughage type affected ($P<0.0001$) all the DM degradation parameters.

The slowly degradable fraction of untreated roughages varied from 442 g $kg^{-1}$ of wheat straw to 650 g $kg^{-1}$ of veld hay with a mean value of 527 g $kg^{-1}$, while for urea-treated roughages it varied from 483 g $kg^{-1}$ of oat hay to 742 g $kg^{-1}$ of veld hay with a mean value of 599 g $kg^{-1}$. The rate of degradation of DM of untreated roughages varied from 0.022 $h^{-1}$ for wheat straw to 0.087 $h^{-1}$ for ryegrass, while for urea-treated roughages it varied from 0.022 $h^{-1}$ for oat straw to 0.082 $h^{-1}$ for ryegrass. Ryegrass degraded almost three to four times faster than urea-treated oat straw. The potential degradability ranged from 625 g $kg^{-1}$ of untreated wheat straw to 950 g $kg^{-1}$ of ryegrass, and from 733 g $kg^{-1}$ of treated wheat straw to 964 g $kg^{-1}$ of treated ryegrass. The effective degradability was lowest in untreated wheat straw (370 g $kg^{-1}$) and highest in ryegrass (799 g $kg^{-1}$), while in treated roughages wheat straw had the lowest (434 g $kg^{-1}$) and ryegrass the highest (797 g $kg^{-1}$) effective degradability. For the untreated feed the lag time ranged from 0.6 h in *E. curvula* hay to 3.2 h in veld hay, and from 0.3 h in treated oat straw to 3.0 h in treated veld hay.

**Table 3.3** Least square means of the main effects of urea-ammoniation of dietary roughage and variation in dietary concentrate proportion on the rumen ammonia concentration and rumen pH of Jersey cows.

| Parameters | Urea | | SED | P value | Concentrate (%) | | | | SED | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | | | 0 | 25 | 50 | 75 | | |
| $NH_3$ $mgl^{-1}$ | 49.9 | 66.9 | 2.54 | 0.002 | 38.2 | 46.8 | 71.5 | 77.0 | 3.59 | 0.000 |
| pH | 6.6 | 6.5 | 0.03 | 0.098 | 6.7 | 6.6 | 6.5 | 6.5 | 0.05 | 0.169 |

0, untreated; 1, urea-ammoniated.




Unt, untreated; UA, Urea-ammoniated; Conc, concentrate.

**Figure 3.1** Effect of urea-ammoniation and concentrate proportions on variations in ammonia concentration (top) and rumen pH (bottom) in the rumen of Jersey cows fed either urea treated or untreated hay with or without concentrate.

**Table 3.4** Effect of roughage type within urea-ammoniation on DM degradability of treated and untreated roughages incubated in nylon bags in cows fed either treated or untreated hay with or without concentrate.

| Feed type | Urea | Degradation parameters | | | | |
|---|---|---|---|---|---|---|
| | | B ($g\ kg^{-1}$) | c ($h^{-1}$) | PD ($g\ kg^{-1}$) | ED ($g\ kg^{-1}$) | LT (h) |
| Barley straw | 1 | 603 | 0.026 | 812 | 479 | 2.2 |
| *E. curvula* hay | 1 | 728 | 0.031 | 853 | 467 | 1.9 |
| Kikuyu hay | 1 | 551 | 0.043 | 755 | 517 | 0.9 |
| Coastcross hay | 1 | 622 | 0.032 | 808 | 498 | 2.7 |
| Maize stover | 1 | 593 | 0.032 | 799 | 504 | 1.5 |
| Oat hay | 1 | 483 | 0.050 | 912 | 723 | 0.6 |
| Oat straw | 1 | 578 | 0.022 | 788 | 439 | 0.3 |
| Rye grass | 1 | 572 | 0.082 | 964 | 797 | 1.8 |
| Veld hay | 1 | 742 | 0.027 | 882 | 476 | 3.0 |
| Wheat straw | 1 | 517 | 0.023 | 733 | 434 | 1.6 |
| Barley straw | 0 | 559 | 0.027 | 719 | 423 | 2.2 |
| *E. curvula* hay | 0 | 629 | 0.025 | 787 | 442 | 0.6 |
| Kikuyu hay | 0 | 447 | 0.032 | 665 | 448 | 3.2 |
| Coastcross hay | 0 | 578 | 0.029 | 730 | 435 | 1.5 |
| Maize stover | 0 | 485 | 0.026 | 711 | 448 | 0.7 |
| Oat hay | 0 | 455 | 0.066 | 881 | 731 | 1.4 |
| Oat straw | 0 | 464 | 0.029 | 635 | 397 | 2.1 |
| Rye grass | 0 | 565 | 0.087 | 950 | 799 | 1.5 |
| Veld hay | 0 | 650 | 0.026 | 808 | 453 | 3.2 |
| Wheat straw | 0 | 442 | 0.022 | 625 | 370 | 1.9 |
| SED | | 46.3 | 0.0106 | 46.3 | 26.9 | 1.15 |
| Effect of | | | | | | |
| Feed type (urea) | | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

1, Urea-ammoniated; 0, Untreated; c, rate of degradation of slowly degradable fraction; B, slowly degradable fraction; h, hour; PD, potential degradability; ED, effective degradability and LT, lag time.

**Table 3.5** The effect of urea-ammoniation and concentrate proportions on DM degradability of treated and untreated roughages incubated in the rumen of Jersey cows.

| Concentrate (%) | Urea | Degradation parameters | | | | |
|---|---|---|---|---|---|---|
| | | B (g $kg^{-1}$) | c ($h^{-1}$) | PD (g $kg^{-1}$) | ED (g $kg^{-1}$) | LT (h) |
| 0 | 1 | 596 | 0.036 | 827 | 534 | 1.4 |
| 25 | 1 | 614 | 0.040 | 846 | 548 | 1.6 |
| 50 | 1 | 592 | 0.038 | 823 | 541 | 2.3 |
| 75 | 1 | 594 | 0.033 | 826 | 511 | 1.3 |
| 0 | 0 | 529 | 0.039 | 753 | 507 | 2.3 |
| 25 | 0 | 553 | 0.039 | 776 | 511 | 1.8 |
| 50 | 0 | 525 | 0.036 | 749 | 488 | 1.9 |
| 75 | 0 | 503 | 0.034 | 727 | 473 | 1.4 |
| Main effect of urea | | | | | | |
| | 1 | 599 | 0.037 | 831 | 533 | 1.6 |
| | 0 | 527 | 0.037 | 751 | 495 | 1.8 |
| SED (conc level) | | 32.8 | 0.0075 | 32.8 | 19.0 | 0.81 |
| SED (urea) | | 16.4 | 0.0038 | 16.4 | 9.5 | 0.41 |
| Effect of | | | | | | |
| Conc (urea) | | 0.0353 | 0.2589 | 0.0353 | 0.0001 | 0.0256 |
| Urea | | 0.0001 | 0.9706 | 0.0001 | 0.0001 | 0.2804 |

1, Urea-ammoniated; 0, Untreated; c, rate of degradation of slowly degradable fraction; B, slowly degradable fraction; h, hour; PD, potential degradability; ED, effective degradability and LT, lag time.

**Effect of urea-ammoniation**

The main effect of urea and concentrate proportions within urea-ammoniation on dry matter (DM) degradability of treated and untreated roughages is presented in Table 3.5; while the effect of urea-ammoniation and concentrate levels on B-fraction, potential and effective degradability of DM degradation of urea-ammoniated and untreated roughages is presented in Figure 3.2. Urea-ammoniation increased ($P<0.0001$) the slowly degradable fraction, potential degradability, and effective degradability, decreased

(P>0.05) the lag time that preceded the onset of degradation of DM but had no effect on rate of degradation (c) of DM.

**Effect of concentrate proportion**

Concentrate proportion affected ($P<0.05$) the slowly degradable fraction, potential degradability, lag time and effective degradability but had no effect ($P>0.05$) on the rate of degradation of DM. For both urea treated and untreated roughages, the slowly degradable fraction, potential degradability and effective degradability of DM increased to their maximum at 25% concentrate levels. Beyond this level, they decreased with increasing concentrate level reaching a minimum at 75% concentrate level (Table 3.5). The pattern of decrease of each of these variates was gentle for treated but rapid for untreated ones.

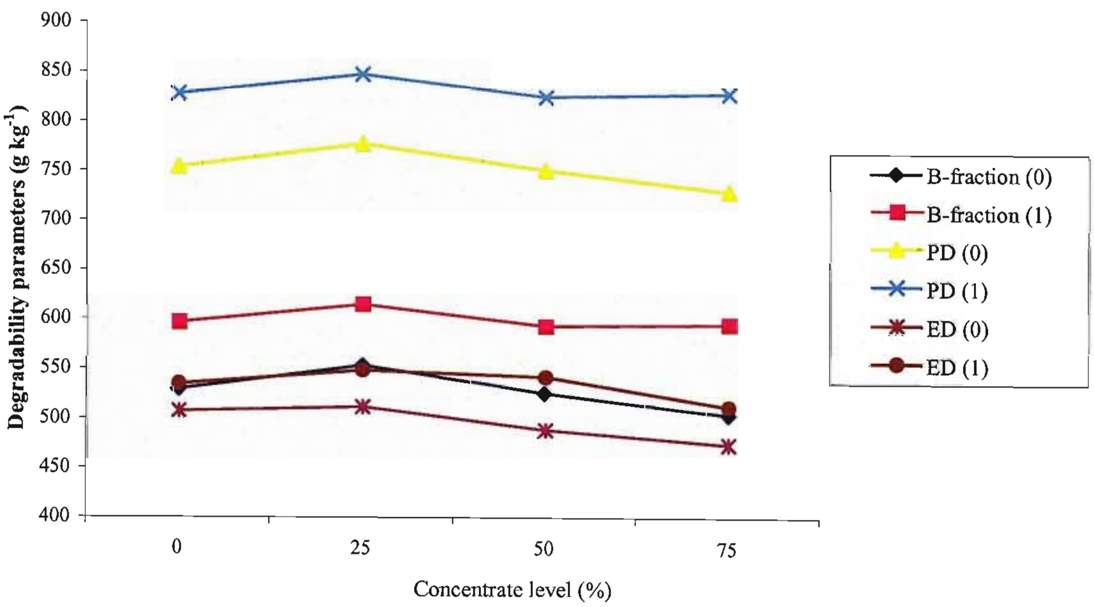



**Figure 3.2** Effect of urea-ammoniation and concentrate proportions on B-fraction, potential and effective degradability of DM degradation of urea-ammoniated (1) and untreated (0) roughages incubated in the rumen of Jersey cows.

**Table 3.6** Effects of roughage type within urea-ammoniation on degradability of fiber fraction of treated and untreated roughages.

| Feed type | Urea | Degradation parameters | | | | |
|---|---|---|---|---|---|---|
| | | B (g kg$^{-1}$) | c (h$^{-1}$) | PD (g kg$^{-1}$) | ED (g kg$^{-1}$) | LT (h) |
| Barley straw | 1 | 455 | 0.020 | 843 | 567 | 9.8 |
| *E. curvula* ha | 1 | 570 | 0.027 | 837 | 534 | 6.8 |
| Kikuyu hay | 1 | 433 | 0.050 | 757 | 582 | 4.3 |
| Coastcross hay | 1 | 603 | 0.020 | 911 | 532 | 5.7 |
| Maize stover | 1 | 533 | 0.049 | 795 | 583 | 5.0 |
| Oat hay | 1 | 306 | 0.048 | 921 | 802 | 7.7 |
| Oat straw | 1 | 709 | 0.009 | 1032 | 462 | 13.6 |
| Rye grass | 1 | 451 | 0.010 | 962 | 847 | 3.2 |
| Veld hay | 1 | 712 | 0.016 | 995 | 519 | 7.8 |
| Wheat straw | 1 | 540 | 0.033 | 708 | 446 | 0.1 |
| Barley straw | 0 | 552 | 0.024 | 820 | 500 | 0.9 |
| *E. curvula* hay | 0 | 502 | 0.310 | 799 | 547 | 5.1 |
| Kikuyu hay | 0 | 386 | 0.040 | 701 | 534 | 2.8 |
| Coastcross hay | 0 | 528 | 0.025 | 797 | 508 | -1.7 |
| Maize stover | 0 | 466 | 0.024 | 792 | 533 | 1.7 |
| Oat hay | 0 | 373 | 0.063 | 897 | 773 | 0.5 |
| Oat straw | 0 | 394 | 0.025 | 657 | 434 | 7.2 |
| Rye grass | 0 | 442 | 0.085 | 945 | 828 | 4.5 |
| Veld hay | 0 | 528 | 0.042 | 808 | 545 | 12.7 |
| Wheat straw | 0 | 457 | 0.050 | 726 | 468 | 3.5 |
| SED | | 85.9 | 0.0175 | 85.9 | 22.7 | 4.72 |
| Effect of | | | | | | |
| Feed type (urea) | | 0.0001 | 0.0001 | 0.0003 | 0.0001 | 0.0484 |

1, urea-ammoniated; 0, untreated; c, rate of degradation of slowly degradable fraction; B, slowly degradable fraction; h, hour; PD, potential degradability; ED, effective degradability and LT, lag time.

**Table 3.7** The main effect of urea-ammoniation and concentrate within urea on fiber degradation of treated and untreated roughages.

| Concentrate (%) | Urea | Degradation parameters | | | | |
|---|---|---|---|---|---|---|
| | | B (g $kg^{-1}$) | C ($h^{-1}$) | PD (g $kg^{-1}$) | ED (g $kg^{-1}$) | LT (h) |
| 0 | 1 | 463 | 0.034 | 808 | 579 | 8.9 |
| 25 | 1 | 594 | 0.043 | 938 | 600 | 8.2 |
| 50 | 1 | 552 | 0.037 | 897 | 594 | 2.8 |
| 75 | 1 | 516 | 0.035 | 861 | 576 | 3.7 |
| 0 | 0 | 452 | 0.041 | 783 | 571 | 7.8 |
| 25 | 0 | 517 | 0.036 | 848 | 576 | 0.03 |
| 50 | 0 | 470 | 0.045 | 802 | 568 | 4.0 |
| 75 | 0 | 412 | 0.041 | 743 | 553 | 5.8 |
| Main effects of urea | | | | | | |
| | 1 | 531 | 0.037 | 876 | 587 | 6.4 |
| | 0 | 463 | 0.041 | 794 | 567 | 3.7 |
| SED con (urea) | | 60.8 | 0.0124 | 60.8 | 16.1 | 3.34 |
| SED urea | | 30.4 | 0.0062 | 30.4 | 8.03 | 1.67 |
| Effects of | | | | | | |
| Conc (urea) | | 0.0535 | 0.9276 | 0.0535 | 0.1989 | 0.0234 |
| Urea | | 0.0052 | 0.4345 | 0.001 | 0.002 | 0.0434 |

1, urea-ammoniated; 0, untreated; c, rate of degradation of slowly degradable fraction; B, slowly degradable fraction; h, hour; PD, potentially degradable; ED, effective degradability and LT, lag time; conc, concentrate.

### 3.4.4 Degradation of fiber (or NDF)

**Effect of roughage**

The effects of roughage type within urea-ammoniation on degradability of fiber fraction of treated and untreated roughages are given in Table 3.6. Within urea-ammoniation, roughage type had no effect on the lag time (P>0.05) but affected the rest of the parameters. For treated roughages, the insoluble but slowly degradable fiber fraction varied from 305.7 g $kg^{-1}$ oat hay to 712.4 g $kg^{-1}$ of veld hay, while for untreated roughages it varied from 373.45 g $kg^{-1}$ of oat hay to 551.5 g $kg^{-1}$ of barley straw. The rate of fiber degradation of urea treated roughages was slowest in oat straw (0.009 $h^{-1}$) and fastest in kikuyu hay (0.05 $h^{-1}$), whereas for the untreated roughages barley straw had the slowest (0.024 $h^{-1}$) and rye grass the fastest (0.085 $h^{-1}$) degradation rate.

The potential degradability of fiber of urea treated roughages ranged from 707.5 g $kg^{-1}$ of wheat straw to 1031.8 g $kg^{-1}$ of oat straw, while that of untreated roughages ranged from 657.25 g $kg^{-1}$ of oat straw to 944.8 g $kg^{-1}$ of rye grass. The effective degradability varied from 445.8 g $kg^{-1}$ of urea treated wheat straw to 847.3 g $kg^{-1}$ of rye grass, while for the untreated roughages it ranged from 434.4 g $kg^{-1}$ of oat straw to 827.8 g $kg^{-1}$ of rye grass. The lag time preceding the onset of fiber degradation in urea treated roughages was shortest in wheat straw (0.1 h) and longest in oat straw (13.6 h), whereas in the untreated roughages coastcross hay had the shortest (-1.7 h) and veld hay the longest (12.7 h).

**Effect of urea-ammoniation**

The main effects of urea and concentrate proportion within urea-ammoniation on fiber degradation are given in Table 3.7. Urea-ammoniation increased ($P<0.05$) the slowly degradable fraction, potential degradability, effective degradability and lag time, but had no effect ($P>0.05$) on the rate of degradation of fiber.

**Effect of concentrate proportions within urea treatment**

The effect of urea-ammoniation and concentrate levels within urea treatment on B-fraction, potential and effective degradability of fiber degradation of urea-ammoniated and untreated roughages is presented in Figure 3.3. Concentrate proportion within urea-ammoniation had an effect ($P=0.0535$) on the degradable part of the insoluble fraction, potential degradability and the time that preceded the onset of degradation of the fiber fraction. The slowly degradable fraction, potential degradability and effective degradability of fiber tended to increase up to their maximum values at 25% concentrate before declining to minimum values at 75% concentrate on both urea-treated and untreated diets (Table 3.7).

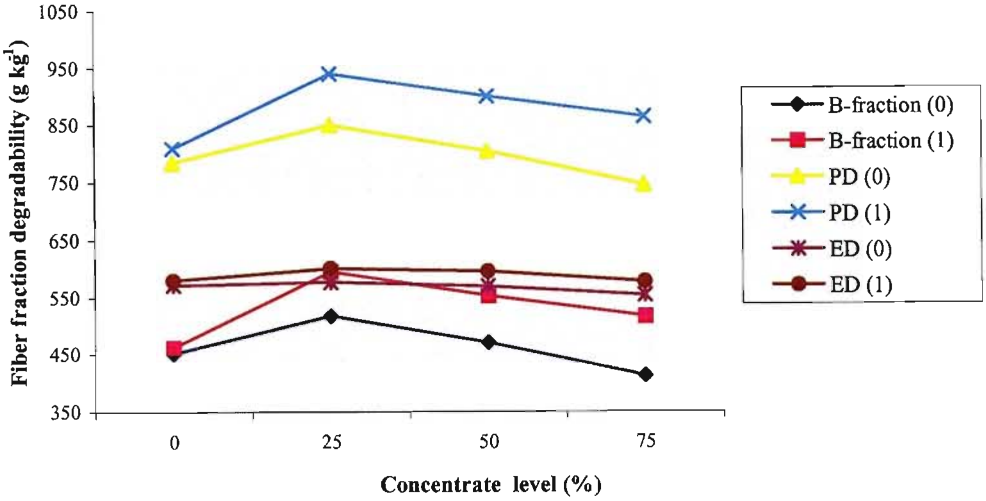



**Figure 3.3** Effect of urea-ammoniation and concentrate levels within urea on B-fraction, potential and effective degradability of fiber degradation of urea-ammoniated (1) and untreated (0) roughages.

### 3.4.5 Relationships among variables

**Correlation of chemical constituents with degradation of roughages**

**DM**: Correlations were established between chemical constituents and the potential, effective and rate of degradation of dry matter of roughage. The rate of degradation, potential and effective DM degradability were highly correlated ($P<0.0001$) with the concentration of CP, ash, ADF and NDF (Table 3.8). The potential, effective and rate of degradation of dry matter increased with increasing CP and ash contents but decreased in the direction of increasing concentration of either NDF or ADF.

**NDF**: The rate of degradation and effective degradability of NDF were highly correlated ($P<0.001$) with the CP, ash, ADF and NDF in the roughages (Table 3.8). The rate of degradation and effective degradation of NDF increased with increasing CP and ash contents but decreased in the direction of increasing concentration of either NDF or ADF.

**Table 3.8** Correlation between the chemical composition of roughages and the rate, potential and effective DM and NDF degradation (N = 40).

| | C ($h^{-1}$) | | PD ($g\ kg^{-1}$) | | ED ($g\ kg^{-1}$) | |
|---|---|---|---|---|---|---|
| **DM ($g\ kg^{-1}$)** | 1 | 0 | 1 | 0 | 1 | 0 |
| Crude protein | 0.88** | 0.83** | 0.67** | 0.72** | 0.81** | 0.80** |
| Ash | 0.85** | 0.93** | 0.75** | 0.78** | 0.89** | 0.91** |
| NDF | -0.86** | -0.94** | -0.74** | -0.80** | -0.95** | -0.97** |
| ADF | -0.86** | -0.84** | -0.67** | -0.77** | -0.88** | -0.87** |
| | | | | | | |
| **NDF ($g\ kg^{-1}$)** | | | | | | |
| Crude protein | 0.73** | 0.52** | $0.19^{NS}$ | 0.54** | 0.75** | 0.77** |
| Ash | 0.60** | 0.61** | 0.31* | 0.51** | 0.83** | 0.87** |
| NDF | -0.69** | -0.63** | $-0.19^{NS}$ | -0.57** | -0.92** | -0.94** |
| ADF | -0.69** | -0.52** | $-0.12^{NS}$ | -0.53** | -0.86** | -0.88** |

1, urea treated; 0, untreated; NDF, neutral detergent fiber; ADF, acid detergent fiber, c, rate of degradation of slowly degradable fraction; h, hour; PD, potential degradability; ED, effective degradability

** =(P<0.001), * = (P<0.05), NS = (P>0.05).

**Regression between chemical composition and DM and NDF degradation properties**

The best fitting equations using stepwise multiple regressions are given in Table 3.9. The rate of degradation, the potential and effective DM degradability decreased (P<0.0001) whereas the B-fraction of DM increased (P<0.0007) as the NDF content of the roughages increase. The rate, B-fraction, potential degradability and effective degradability of DM increased with increasing crude protein (P<0.01) and of the benefit axis (P<0.01).

The rate of degradation, potential and effective degradability of the fiber fraction decreased (P<0.0014) but the B-fraction of NDF increased (P<0.0008) with increasing NDF content. The rate of degradation, the B-fraction, effective degradability and potential degradability of the fiber fraction increased (P<0.05) with increasing crude protein and benefit axis of the roughages.

**Table 3.9** The regression relationships between chemical composition and ruminal DM and NDF degradation characteristics of roughage.

| | Rate of degradation ($h^{-1}$) | B-fraction ($g\ kg^{-1}$) | PD ($g\ kg^{-1}$) | ED ($g\ kg^{-1}$) |
|---|---|---|---|---|
| **DM ($g\ kg^{-1}$)** | | | | |
| Intercept | 0.096 (0.0084) | 205.7 (88.73) | 1058.5 (67.40) | 1129.9 (42.67) |
| NDF | $-9.7\ 10^{-5}$ ($1.03 \times 10^{-5}$) | 0.387 (0.1097) | -0.49 (0.083) | -0.948 (0.0524) |
| CP | $10.4 \times 10^{-4}$ ($1.64 \times 10^{-5}$) | 0.576 (0.1744) | 0.30 (0.132) | 0.281 (0.0832) |
| NDF*$T_{dummy}$ | - | 0.109 (0.0235) | 0.12 (0.018) | 0.061 (0.0112) |
| CP*$T_{dummy}$ | - | - | - | - |
| Concentrate proportion | $-4.4 \times 10^{-5}$ ($2.76 \times 10^{-5}$) [a] | - | - | -0.297 (0.1400) |
| Root MSE | 0.0069 | 73.3 | 55.7 | 35.0 |
| Adj-$R^2$ | 0.873 | 0.307 | 0.679 | 0.929 |
| **NDF($g\ kg^{-1}$)** | | | | |
| Intercept | 0.101 (0.0257) | -44.12 (151.619) | 1096.4 (84.34) | 1230.8 (36.01) |
| NDF | $-10.5 \times 10^{-5}$ ($3.16 \times 10^{-5}$) | 0.65 (0.188) | -0.44 (0.122) | -0.96 (0.049) |
| CP | $11.4\ 10^{-5}$ ($5.05 \times 10^{-5}$) | 0.58 (0.298) | - | - |
| NDF* $T_{dummy}$ | - | 0.11 (0.040) | 0.12 (0.041) | - |
| CP* $T_{dummy}$ | - | - | - | 0.17 (0.082) |
| Root MSE | 0.2125 | 125.3 | 127.0 | 47.4 |
| Adj-$R^2$ | 0.455 | 0.207 | 0.177 | 0.863 |

PD, potential degradability; ED, effective degradability; NDF, neutral detergent fiber; CP, crude protein; Tdummy is a dummy variable which = 1 for urea treated or 0 for untreated roughages.
All the independent variables were significant at the ($P < 0.05$) except for concentrate proportion in the rate of degradation of DM.
[a]non significant.
The values in parenthesis indicate standard error.

### 3.5 Discussion

### 3.5.1 Chemical composition

The nitrogen content of urea-treated diet was higher than that of untreated diets due to urea-ammoniation, as was reported previously (Cloete and Kritzinger, 1984; Brand *et al.*, 1991). Urea-ammoniation generally caused a reduction in the NDF and hemicellulose content of the low quality roughages. It was the reverse for the high quality roughages especially rye grass and oat hay. Similar results regarding neutral detergent fiber and hemicellulose content for low quality roughages have been reported before (Cloete and Kritzinger, 1984) due to solubilization of hemicellulose (Van Soest *et al.*, 1983/84; Mason *et al.*, 1988). Urea-ammoniation may have removed some linkages within hemicelluloses and thus enhanced their solubility in detergent solution.

### 3.5.2 Rumen environment

Rumen ammonia concentration steadily increased with increased concentrate proportion in the diet. The highest dry matter and neutral detergent fiber degradation was observed when the ruminal ammonia concentrations were close to a value of 50 mg/l recommended by Satter and Slyter (1974). Ammonia is the preferred source of nitrogen for a large proportion of the rumen microbes (Bryant and Robinson, 1963). The results of the present study indicate that the urea-treated hay, compared to the untreated diets, caused an increase in the DM degradation of the roughage. This might be attributed to the fact that urea-treated hay provided more available fermentable energy and nitrogen to the rumen microbes than the untreated diets (Van Soest *et al.*, 1983/84; Goto *et al.*, 1991; Tuen *et al.*, 1991). Although the concentrate level had no effect ($P>0.05$) on the ruminal pH of cows, it tended to be lower in cows receiving 50 and 75% concentrate than in cows receiving 25% concentrate. This might have reduced the degradability parameters in the rumen of cows.

### 3.5.3 Effect of roughage type on DM degradability

The difference observed between the degradable part of the insoluble fraction, potential degradability and effective degradability of the dry matter and neutral detergent fiber of urea treated and untreated roughage might be related to the quality of the roughage before urea-ammoniation. Roughages such as barley, wheat and oat straw, coastcross hay, *E. curvula* hay and maize stover, unlike ryegrass and oat hay, consist of cell walls that have undergone secondary thickening (Adebowale and Nakashima, 1992; Jung and Allen, 1995; Wilson and Martens, 1995) consequently they are very slowly degraded. This may explain why the washing losses of ryegrass and oat hay were almost two times higher than for the rest of the roughages. The high potential degradability values for ryegrass followed by oat hay resulted partly from very high washing losses of these roughages. This could be linked to their low NDF concentrations since the potential degradability of the roughages was found to be negatively correlated ($r = -0.797$, $P<0.0001$) to NDF. This observation confirms a report that DM digestibility was positively correlated to crude protein content and negatively correlated to crude fiber, NDF and ADF (Minson, 1982b). This may also explain why wheat, oat and barley straws had low effective DM degradabilities. The low potential degradability of wheat, oat and barley straws, veld, Kikuyu and *E. curvula* hay are closely linked to their low values of washing losses. The high effective degradability of dry matter for the rye grass and oat hay before urea-ammoniation could be linked to their low neutral detergent fiber concentrations.

Mature plants (straws) like barley, wheat and oat straw, *E. curvula* hay, coastcross hay and maize stover are more lignified than the young grasses (rye grass and oat hay). The deposition of lignin polymer commences with the initiation of secondary wall thickening (Terashima *et al.*, 1993). In addition to deposition of lignin in these plants during secondary wall thickening, there is apparently an incorporation of some of the arabinoxylan ferulate esters of the primary wall into cross-linkages of the xylans to lignin (Iiyama *et al.*, 1990) and p-coumaric acid (Lam *et al.*, 1992). The phenolic nature of lignin developed in the secondary wall thickening may act as an enzyme inhibitor and interfere with the digestion of other cell wall components (Van Soest, 1994). Thus, the high slowly degradable fraction, potential degradability and effective degradability values of the neutral detergent fiber observed in this experiment, in rye grass and oat hay may be attributed to the low levels of soluble phenolic compounds as compared to wheat, barley and oat straws. These compounds are present in most mature grasses (Hartley *et al.*, 1985) and were reported to be linked to lignin, consequently limiting the degradability of cell walls (Theander, 1985). Variation in lignin content has been reported to have a greater effect on neutral detergent fiber degradability of grasses than the variation in structural polysaccharides (Buxton *et al.*, 1987). Lignin equally depresses the degradability of hemicellulose (Wedig *et al.*, 1986; Albrecht *et al.*, 1987; Fort and Elliott, 1987; Hatfield and Weimer, 1995). Non-lignified tissues may also be poorly degraded due to binding with low molecular weight phenolic compounds (Vadiveloo and Fadel, 1992).

### 3.5.4 Effect of urea-ammoniation on the degradation of DM and fiber fractions

Significantly higher rumen ammonia concentration may partly be responsible for a better rumen environment for roughage degradation in the animals fed urea-treated diets. Urea-ammoniation increases the slowly degradable fraction, potential degradability and effective degradability except the rate of degradation of dry matter and neutral detergent fiber fraction. This improvement in slowly degradable fraction, potential degradability and effective degradability of DM and NDF degradation of urea treated roughages could in part be attributed to increased availability of carbohydrates in the dietary fiber for microbes. According to Akin (1989), it is likely that any change in the degradation of the basal diet as a result of an increase in microbial activity may depend on the number of available sites for microbial attachment.

Treatment with urea increases the B-fraction. The mathematical procedure used to derive degradation parameters shows that an estimate of 'B' is negatively correlated with the rate of degradation. Consequently, an increase in the B-fraction following urea-ammoniation might reduce the possible effect of treatment on the rate of degradation. Urea-ammoniation is thought to weaken the crystalline structure of cellulose and hemicellulose in feeds, rendering them more permeable to cellulolytic microorganisms (Van Soest *et al.*, 1983/84; Mason *et al.*, 1988). In addition, urea-ammoniation might reduce the effect of lignin on the degradability of fiber constituents through the hydrolysis of the ether bonds that link lignin to hemicellulose as well as reduce the strength of the cuticular wall. This could facilitate the capacity to penetrate and digest the lumina of cells by rumen microbes.

The effect of urea-ammoniation might have been more pronounced on degradability of low quality roughages, such as wheat straw, relative to good quality roughage like rye grass because urea-ammoniation works best for low quality roughages. It has been observed (Goto *et al.*, 1991) that the extent of improvement in degradability following ammonia treatment of Golden Promise (variety of wheat straw which had a dry matter degradation of 41% before ammonia treatment and 53.5% after treatment) was about four times higher than the corresponding value in Doublet (a variety of wheat straw which had a dry matter degradation of 57% before ammonia treatment and 59% after treatment), indicating that the effect of ammoniation was more pronounced for materials of lower inherent degradability. Tuah *et al.* (1986) also reported that the poorest quality straw benefited the most from urea-ammoniation.

### 3.5.5 Effect of concentrate proportions on the degradation of DM and fiber fractions

Although the highest rumen ammonia concentration was recorded in animals fed urea-treated *E. curvula* hay supplemented with 75% concentrate, its failure to improve the dry matter and neutral detergent fiber degradability of the incubated roughages may relate to the fact that higher concentrate proportions in a diet may lower the cellulolytic activities and thus decrease fiber degradability. Galyean and Goetsch (1993) proposed that the inclusion rates of concentrate in a diet for dairy and beef cattle should be less than 40 and 20% of the total ration, respectively. In this experiment, however, addition of concentrate up to 25% to urea treated and untreated hay has resulted in positive associative effects on dry matter and neutral detergent fiber degradation. Similar results were reported in another study (Nianogo *et al.*, 1993) after goats and sheep were offered urea treated sorghum straw with low (less than 25% of DM) concentrate supplementation. Beyond the 25% concentrate level, there was a negative associative effect on dry matter and neutral detergent fiber degradation. The pattern of negative effect was less pronounced in the urea-ammoniated diets. This might be due to the fact that urea is alkaline, which can neutralize the acidity caused by adding higher amount of concentrate in the diets of cows. Flachowsky *et al.* (1993) also reported that dry matter degradability of ammonia treated and untreated straw decreased when dietary straw was replaced by concentrate and that the extent of the depression was higher for the untreated straw.

### 3.5.6 Quantitative benefits of treating roughages with urea.

The chemical composition accounted for a high proportion of the variation in the rate and effective degradability of DM. Among all the independent terms, the benefit axis contributed highly ($P<0.05$) to explain the variation associated with the slowly degradable fraction, potential degradability and effective degradability of dry matter degradation of roughages (Table 3.9). The benefit associated with urea-ammoniation of roughages in terms of effective degradability ($Y_{ED}$) and slowly degradable fraction ($Y_B$) is given in Figure 3.4.

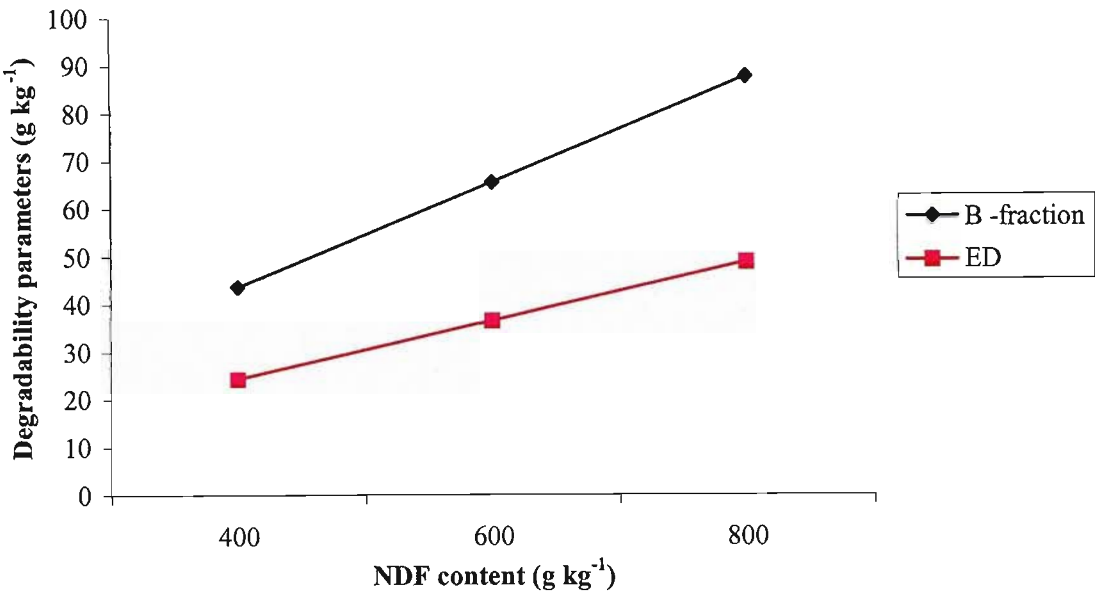



**Figure 3.4** Benefits associated with treating roughages.

This graph demonstrates that the greater the NDF content of the roughages, the greater the effect of urea-ammoniation on degradability parameters. The present findings agree with Goto *et al.* (1991) and Habib *et al.* (1998) who reported that the effect of ammonia was more pronounced for plant materials with an initially low digestibility. Quantifying this benefit of urea-ammoniation is highly desirable as a decision support tool.

### 3.6 Conclusions

The results of this study have shown that urea-ammoniation increased CP content, decreased NDF and hemicellulose contents of roughages. It also improved the disappearance of DM and NDF of roughages. The benefit associated with treatments of roughages has also shows that roughages with low quality such as barley, wheat and oat straw benefited relatively the most from urea-ammoniation. Addition of 25% concentrates in either urea-ammoniated or untreated *E. curvula* hay diets increase the

the DM and NDF degradation. Increasing concentrate level beyond 25% of the diet was associated with decreased *in sacco* DM and NDF degradation. It was observed that urea-ammoniation tended to reduce the negative effect of feeding high concentrate compared to untreated diets. Therefore, this study suggests that there is much to gain by treating low quality roughages.

CHAPTER 4

# EFFECT OF UREA-AMMONIATION AND CONCENTRATE PROPORTIONS ON MICROBIAL COLONIZATION OF FIBER PARTICLES

## 4.1 Abstract

Two experiments were carried out with rumen-fistulated Jersey cows to measure the effect of urea-ammoniated roughages and concentrate proportions on microbial colonization of fiber particles. In Experiment 1, the cows were fed on rations comprising either urea-ammoniated or untreated *Eragrostis curvula* hay supplemented with concentrate at hay to concentrate ratio of 100:0, 75:25, 50:50, 25:75 resulting to eight different rumen environments. The experiment consisted of two periods. Each period comprised of 12 days of adaptation to the experimental diet followed by one-day incubation of urea-ammoniated or untreated barley straw. Experiment 2 consisted of two urea-ammoniated hay levels (20 and 40% of the total ration) and concentrate levels (60 and 80%). The animals were adapted for 12 days after assigning to a dietary treatment. Feed was given at the rate of 9.0 kg $day^{-1}$ per animal portioned into equal meals of 4.50 kg each and offered at 08:00 and 16:00 every day. Microbes adhering to fiber particles were examined under the Environmental Scanning Electron Microscope (ESEM) and analysed with an image analyser. Depending on morphology the microbes were divided into three groups: bacilli (rod), cocci (spherical) and others (spiral, fimbrea and cluster; not specifically defined or undefined microbes).

About 3 g of urea-ammoniated or untreated barley (*Hordeum vulgare*) straw, ground through a 2-mm screen, was weighed into a labelled nylon bag and incubated for 3, 6 and 12 h in the rumen of fistulated Jersey cows. During Experiment 1, urea-ammoniation of dietary roughage decreased ($P<0.001$) bacilli counts and total bacteria count but had no effect on count of the undefined group of microbes on fiber particles in the rumen of cows. Concentrate proportions had no effect ($P>0.05$) on bacilli, cocci and total bacterial count on fiber particles. However, the visual observation of electron micrographs revealed that the total bacterial count tended to decrease as the concentrate level increased in the diet of cows. Bacilli, cocci, undefined group of microbes and total count of microbes increased ($P<0.05$) as length of incubation increased. During Experiment 2, incubated feed, concentrate proportion and time of incubation had no effect ($P>0.05$) on bacilli, others (undefined group of microbes) and total count of fiber-adhering microbes in the rumen of cows. However, increasing concentrate in the diet of cows tended to decrease ($P<0.07$) the count of fiber-adhering coccal microbes. The total count of microbes on fiber particles was higher in animals fed 80% concentrate as compared to 60% concentrate.

**Keywords: microbial colonization, roughage, urea-ammoniation, electron microscopy, concentrate level.**

## 4.2 Introduction

Ruminants have an advantage over monogastric animals in that the rumen or fore stomach is well equipped with a wide range of symbiotic organisms, which under favourable conditions can break down indigestible roughage. In addition, the rumen ecosystem of ruminants contains a diversity of microbial types that contribute a myriad of carbohydrases responsible for plant cell wall degradation and utilization (Akin, 1993; Goto *et al.*, 1993). Thus, the microbes require a receptive environment for desirable fermentation patterns. Microbial population and fermentation patterns vary with changing rumen environment. A continual supply of substrate, and salivary buffering salts and the removal of fermentation end-products and residues will result in a relatively stable rumen environment, thus promoting high microbial populations and increased fiber digestion. If the basal diet has low nitrogen content as to constrain rumen microbial activity, the addition of a urea supplement or treatment with urea will increase the nitrogen content of the total diet which is likely to increase microbial colonization of fiber particles in the rumen. Such positive effects are well known and were originally thought to be the main effect of urea-ammoniation on roughage-based diets (Tuen *et al.*, 1991; Habib *et al.*, 1998). In a study by Silva and Ørskov (1988), three diets (urea-ammoniated, and untreated barley straw and hay) were used to examine degradation characteristics and microbial colonization. The three diets differed markedly in crude protein content (23 to 62 g $kg^{-1}$) and in acid detergent fiber (710 to 780 g $kg^{-1}$). Microbial colonization was higher in untreated barley straw, which was incubated in the rumen of animals given ammonia treated straw compared with those given untreated straw. The authors then concluded that it would be of interest to create a favourable rumen environment for plant cell wall degrading microorganisms. Thus, it is necessary to treat chemically to upgrade the roughage for microbial attack. Part of this difference was probably due to overcoming a deficiency of nitrogen in the diets.

Strategies for the utilization of roughages using urea-ammoniation should aim at establishing an efficient rumen environment in order to maximize microbial activity on fiber. An efficient rumen ecosystem requires available nitrogen, energy and mineral to support microbial growth. Ørskov and Ryle (1990) reported that a group of microorganisms might be particularly important for the degradation of the plant structural materials, which predominate in coarse roughage, although lignin does not appear to be susceptible to attack by rumen microorganisms. Cellulolytic and amylolytic bacteria both require ammonia ($NH_3$) and branched chain fatty acids as growth factors. Dietary urea can provide $NH_3$ and so promote the utilization of fibrous roughage if the rumen pH does not fall below about 6.0 (Ørskov and Ryle, 1990). Microbial colonization efficiency is also associated with the availability of carbohydrates contained in the fiber particles.

The present experiment was designed to examine how dietary manipulation and urea-ammoniation affect colonization of fiber particles.

## 4.3 Materials and methods

### Experiment 1

### 4.3.1 Animals, diets, feeding management and design

Eight experimental test diets were given to four-rumen fistulated Jersey cows in two periods. These test diets were prepared in the same way as described in section 3.3. Each animal was assigned to a dietary treatment. Each period lasted 12 days of adaptation to the experimental diets followed by 1-day incubation of urea-ammoniated and untreated barley straw samples to study microbial colonization of fiber (sample preparation and urea-ammoniation were described in Chapter 3). Animals and feeding management used in this experiment were described in section 3.3.

### Experiment 2

### 4.3.2 Animals and diet

Four cows were used as in experiment 1. The cows were fed two diets. The diets comprised 20% hay and 80% concentrate or 40% hay and 60% concentrate, respectively. Dietary hay was treated with 7.5% urea. The method of treatment was described in section 3.3. These diets were deemed to create two different rumen environments. The animals were adapted for 12 days after assigning to a dietary treatment before starting incubation. Feed (9.0 kg $day^{-1}$ per animal) was divided into two equal portions and fed to cows twice a day at 08:00 and 16:00. Dietary ingredient composition and chemical analysis are given in Table 4.1.

**Table 4.1** Ingredient composition (g kg$^{-1}$, as feed basis) and chemical analysis (g kg$^{-1}$, DM basis) of the total ration[1].

| | Rations | |
|---|---|---|
| Ingredients | Diet 1 | Diet 2 |
| Maize meal | 630.0 | 430.0 |
| Cane molasses | 50.0 | 50.0 |
| Cotton oil cake meal | 85.4 | 85.4 |
| Lime stone | 18.3 | 17.5 |
| Urea | 5.0 | 5.0 |
| Salt | 2.5 | 2.5 |
| Vitamin & min. premix | 2.0 | 2.0 |
| Phosphoric acid | 6.3 | 6.9 |
| Rumensin 20% (mg kg$^{-1}$) | 20.0 | 20.0 |
| Sulphur (mg kg$^{-1}$) | 358.0 | 486.0 |
| Urea treated veld hay | 200 | 400 |
| Chemical analysis | | |
| Dry matter | 877.0 | 876.0 |
| Crude protein | 142.0 | 166.0 |
| Crude fiber | 52.0 | 57.2 |
| Ash | 56.8 | 73.6 |
| Calcium | 10.9 | 16.5 |
| Phosphorus | 4.9 | 6.4 |
| | Urea treated | Untreated |
| Dry matter | 913.3 | 944.0 |
| Crude protein | 131.0 | 40.1 |
| Ash | 83.9 | 77.5 |
| Calcium | 3.5 | 2.4 |
| Phosphorus | 0.5 | 0.4 |
| Crude fiber | 423.7 | 422.6 |
| Neutral detergent fiber | 706.9 | 795.3 |
| Acid detergent fiber | 583.3 | 527.6 |
| Hemicellulose | 123.6 | 267.7 |

[1] Diet 1: 20% urea treated veld hay, 80% concentrate mixture.
Diet 2: 40% urea treated veld hay, 60% concentrate mixture.

### 4.3.3 Sample incubation procedure

Barley (*Hordeum vulgare*) straw was chosen as incubation material in this study. The straw was ground through a 2-mm screen in a hammer mill and then divided into two portions; one for urea-ammoniation at the rate of 30 g urea $kg^{-1}$ (dry basis), the rest left untreated.

About 3 g of urea-treated and untreated barley straw samples were weighed into nylon bags (internal bag dimension: 5 cm x 9 cm; pore size 50 μm; ANKOM Co, Fairport, New York, USA). The bags were incubated in the rumen of fistulated Jersey cows for 12, 6 and 3 h. The treated and untreated samples of barley were incubated in all the animals irrespective of their diet. Immediately after removal from the rumen, the bags were opened and a few particles transferred into a fixative solution containing 3% glutaraldehyde in 0.05 M cacodylate buffer (pH 6.99).

### 4.3.4 Critical point drying

In preparing the particles for critical point drying (CPD), the collected particles were washed twice with 0.05 M cacodylate buffer at pH 6.99 for 30 minutes each and then dehydrated in ethanol of increasingly concentration 30, 50, 70, 80 and 90 % for ten minutes minimum per solution. Finally, samples were dehydrated 3 times with 100 % ethanol solution. Then the particles were transferred to the CPD baskets under 100 % ethanol, i.e. the baskets were placed in a petridish containing 100 % alcohol. The CPD apparatus was pre-cooled before the baskets were placed in the chamber.

### 4.3.5 Environmental Scanning Electron Micrograph (ESEM)

All the samples were coated with gold (40%) and palladium (60%) for pure images and observed with a Philips ESEM XL 30 microscope under high vacuum at 15 kV. From each sample, five images were taken (8000 x magnification and 15-16 working distance) randomly and analysed under the image analyser with touch counting. The microbes were divided into three groups depending on their shape: Bacilli (rod), cocci (spherical) and others (spiral, fimbrea and cluster; not specifically defined).

### 4.3.6 Statistical analysis

Data obtained from ESEM were analysed using the General Linear Model (GLM) procedure of SAS (1987) statistical package.

The model used for statistical analysis of bacterial colonization in experiment 1 was:

$$Y_{ijkl} = \mu + C_i + U_j + C(U)_{ij} + F_k + U(F)_{jk} + C((U)F)_{ijk} + T_l \qquad (4.1)$$

Where

Y = Individual observations;

μ = Overall mean;

C = Effect of concentrate proportion;

U = Effect of urea-ammoniation;

C (U) = Effect of concentrate proportion within urea-ammoniation;

F = Effect of incubated roughage;

U (F) = Effect of incubated roughage within urea-ammoniation;

C ((U) F) = Effect of concentrate proportion and effect of incubated roughage within urea-ammoniation; and

T = Effect of time.

Contrasts between urea-treated and untreated, among incubation times and among concentrate proportions were obtained by applying the probability of difference (PDIFF) option of the LSmeans statement available in the GLM (SAS, 1987).

The statistical model for the analysis of bacterial colonization in experiment 2 was:

$$Y_{ijk} = \mu + C_i + U_j + C(U)_{ij} + T_l \qquad (4.2)$$

Where

Y = Individual observations;

μ = Overall mean;

C = Effect of concentrate proportion;

U = Effect of urea-ammoniation;

C (U) = Effect of concentrate proportion within urea-ammoniation; and

T = Effect of time.

Contrasts were done as described in experiment 1.

## 4.4 Results

### 4.4.1 Experiment one

**Effect of dietary roughage on colonization:** The effect of urea-ammoniation on microbial colonization of treated and untreated roughages is shown in Table 4.2, while the main effect is given in Table 4.3. Cows fed on urea-treated roughage had decreased ($P<0.001$) bacilli count and total bacteria count but similar counts of other colonizers of fiber relative to cows fed on untreated roughage. Figures 4.1 and 4.2 show the electron micrographs of urea-treated and untreated barley straw after it had been incubated for 3, 6 and 12 hrs in animals fed either treated or untreated hay, respectively. It was observed that the treated fiber particles were highly degraded as compared to the untreated. The ESEM revealed that rumen microbial colonization of treated barley straw incubated in animals fed untreated hay was slightly higher after each incubation time when compared with untreated barley straw incubated in animals fed untreated hay.

**Effect of incubated roughage:** Incubated roughage had no effect ($P>0.05$) on microbial colonization of fiber particles in the rumen of Jersey cows. However, the results from ESEM showed that there was a higher number of microbes on urea-treated barley straw.

**Effect of concentrate proportions:** The effect of concentrate proportion on microbial colonization of treated and untreated roughages is shown in Table 4.2, while the main effect is given in Table 4.3. Concentrate proportions had no effect ($P>0.05$) on bacilli, cocci and total count of microbes on fiber particles in the rumen of Jersey cows. However, concentrate proportion significantly ($P<0.05$) affected the count of unidentified group of microbes on fiber particles whose numbers decreased with increasing concentrate proportion. Figure 4.3 shows the electron micrographs of urea-treated and untreated barley straw after it had been incubated for 6 hrs in animals consuming 0, 25, 50, and 75% concentrate proportion in their diet. The results from ESEM observations revealed that the total bacterial count tended to decrease as the concentrate proportions increased in the diet of cows (Table 4.3).

Bacilli, cocci, unidentified group of microbes and total count of microbes increased ($P<0.05$) as length of incubation increased (Table 4.3).

**Table 4.2** Effect of urea-ammoniation, concentrate proportions and incubated feed on microbial colonization of Jersey cows fed either urea-treated or untreated hay supplemented with concentrate proportions.

| Concentrate proportions (%) | Urea-ammoniation of dietary roughage | Incubated feed | Type of bacteria (number/1700μm$^2$) | | | |
|---|---|---|---|---|---|---|
| | | | Bacilli | Cocci | Others | Total |
| 0 | 0 | 0 | 598 | 178 | 34 | 810 |
| 0 | 0 | 1 | 658 | 231 | 16 | 905 |
| 0 | 1 | 0 | 250 | 84 | 12 | 346 |
| 0 | 1 | 1 | 255 | 93 | 11 | 359 |
| 25 | 0 | 0 | 556 | 263 | 118 | 937 |
| 25 | 0 | 1 | 258 | 131 | 47 | 436 |
| 25 | 1 | 0 | 308 | 110 | 18 | 435 |
| 25 | 1 | 1 | 363 | 102 | 26 | 491 |
| 50 | 0 | 0 | 404 | 176 | 57 | 638 |
| 50 | 0 | 1 | 336 | 103 | 33 | 472 |
| 50 | 1 | 0 | 412 | 173 | 33 | 617 |
| 50 | 1 | 1 | 316 | 168 | 55 | 539 |
| 75 | 0 | 0 | 325 | 117 | 10 | 452 |
| 75 | 0 | 1 | 636 | 129 | 15 | 779 |
| 75 | 1 | 0 | 228 | 117 | 35 | 380 |
| 75 | 1 | 1 | 389 | 189 | 75 | 654 |
| SED | | | 128.8 | 56.5 | 21.5 | 178.6 |
| Effect of | | | | | | |
| Concentrate (urea-ammoniation (incubated feed)) | | | 0.0613 | 0.2881 | 0.2275 | 0.0572 |

0, untreated hay; 1, urea-ammoniated hay

**Table 4.3** Main effect of urea-ammoniation, concentrate proportions and time of incubation on microbial count on fiber particles.

| Main effect | | Type of bacteria (number/1700μm$^2$) | | | |
|---|---|---|---|---|---|
| | | Bacilli | Cocci | Others | Total |
| Treatment of dietary roughage | 0 | 471 | 166 | 41 | 679 |
| | 1 | 315 | 130 | 33 | 478 |
| Concentrate | 0 | 440 | 147 | 18 | 605 |
| | 25 | 371 | 152 | 52 | 575 |
| | 50 | 367 | 155 | 45 | 566 |
| | 75 | 394 | 138 | 34 | 566 |
| Incubated feed | 0 | 385 | 152 | 39 | 577 |
| | 1 | 401 | 143 | 35 | 579 |
| Time | 3 | 316 | 122 | 29 | 467 |
| | 6 | 384 | 151 | 31 | 566 |
| | 12 | 479 | 171 | 51 | 701 |
| SED of urea | | 45.5 | 19.9 | 7.6 | 63.2 |
| SED of concentrate | | 64.4 | 28.2 | 10.8 | 89.3 |
| SED of time | | 55.8 | 24.5 | 9.3 | 77.3 |
| Effect of | | | | | |
| Treatment of dietary roughage | | 0.0018 | 0.0784 | 0.2864 | 0.0034 |
| Concentrate | | 0.6561 | 0.9414 | 0.0205 | 0.9682 |
| Incubated feed | | 07264 | 0.6538 | 0.5439 | 0.9702 |
| Time | | 0.0231 | 0.1493 | 0.0443 | 0.0180 |

0, untreated hay; 1, urea-ammoniated hay; 0-75 concentrate proportion in the diet; 3, 6 and 12, time of incubation

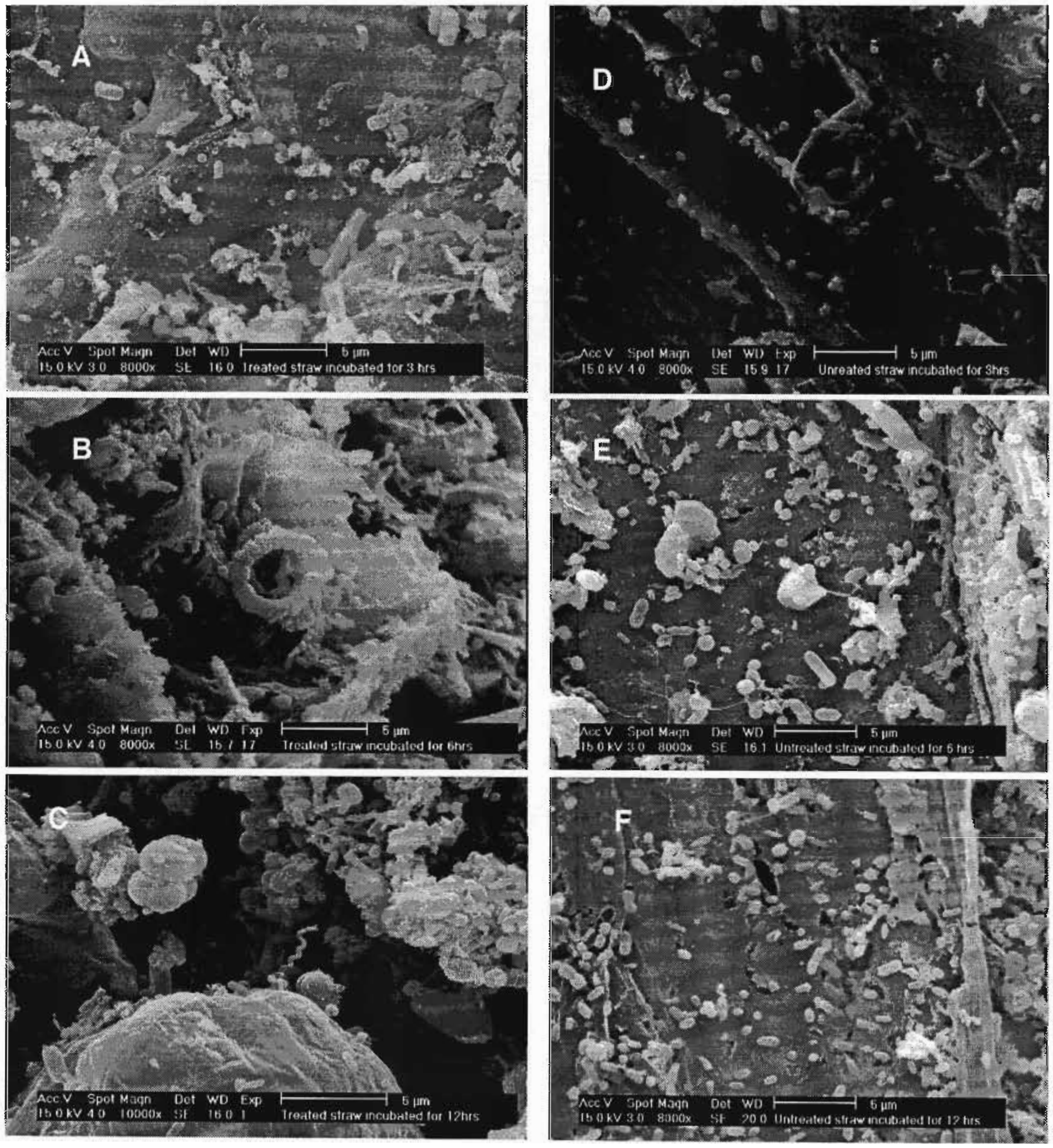



**Figure 4.1** Treated (A, B, C) and untreated (D, E, F) barley straw incubated for 3 (A, D), 6 (B, E) and 12 (C, F) hrs in animals consuming urea ammoniated hay.

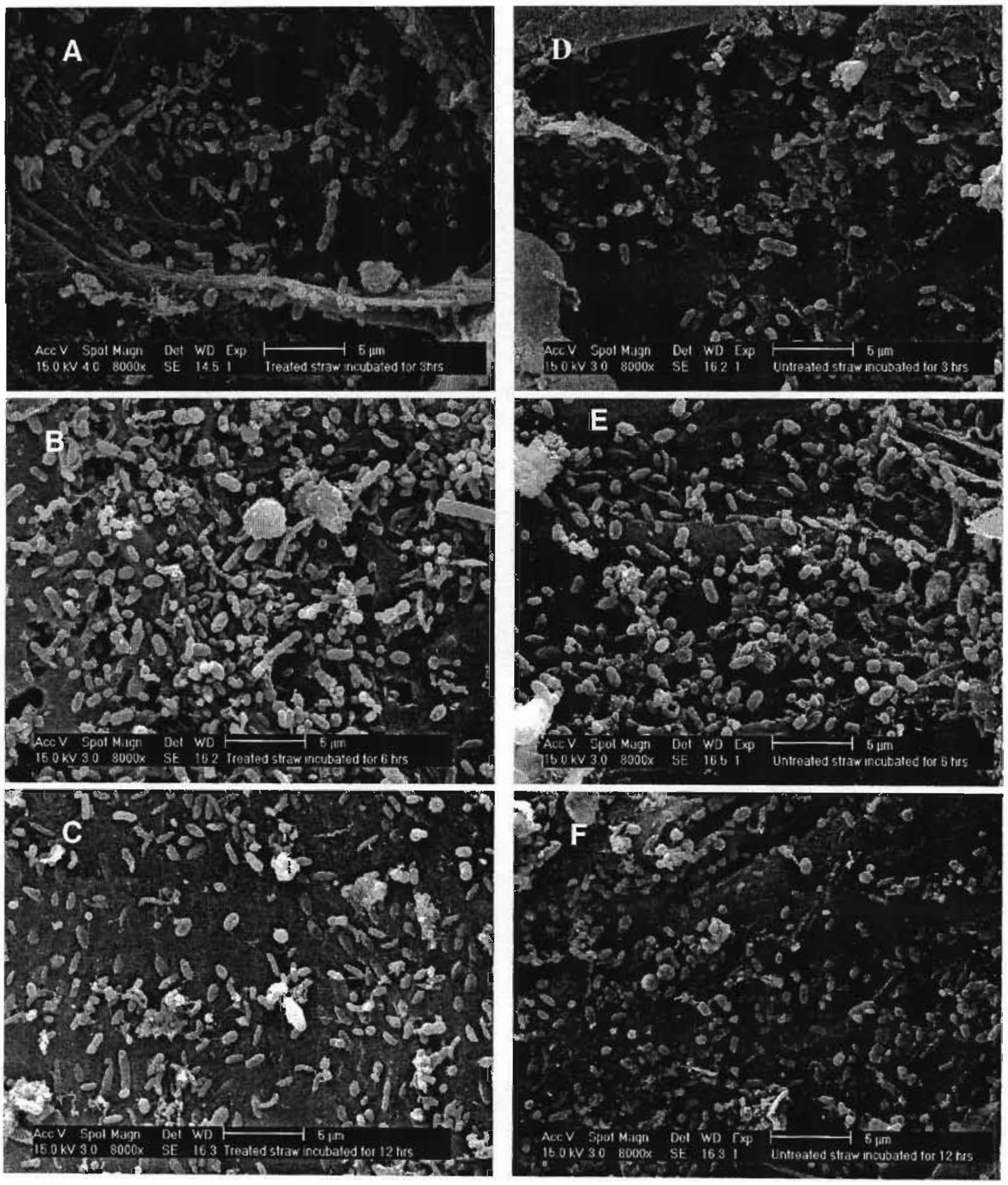



**Figure 4.2** Treated (A, B, C) and untreated (D, E, F) barley straw incubated for 3 (A, D), 6 (B, E) and 12 (C, F) hrs in animals consuming untreated hay.

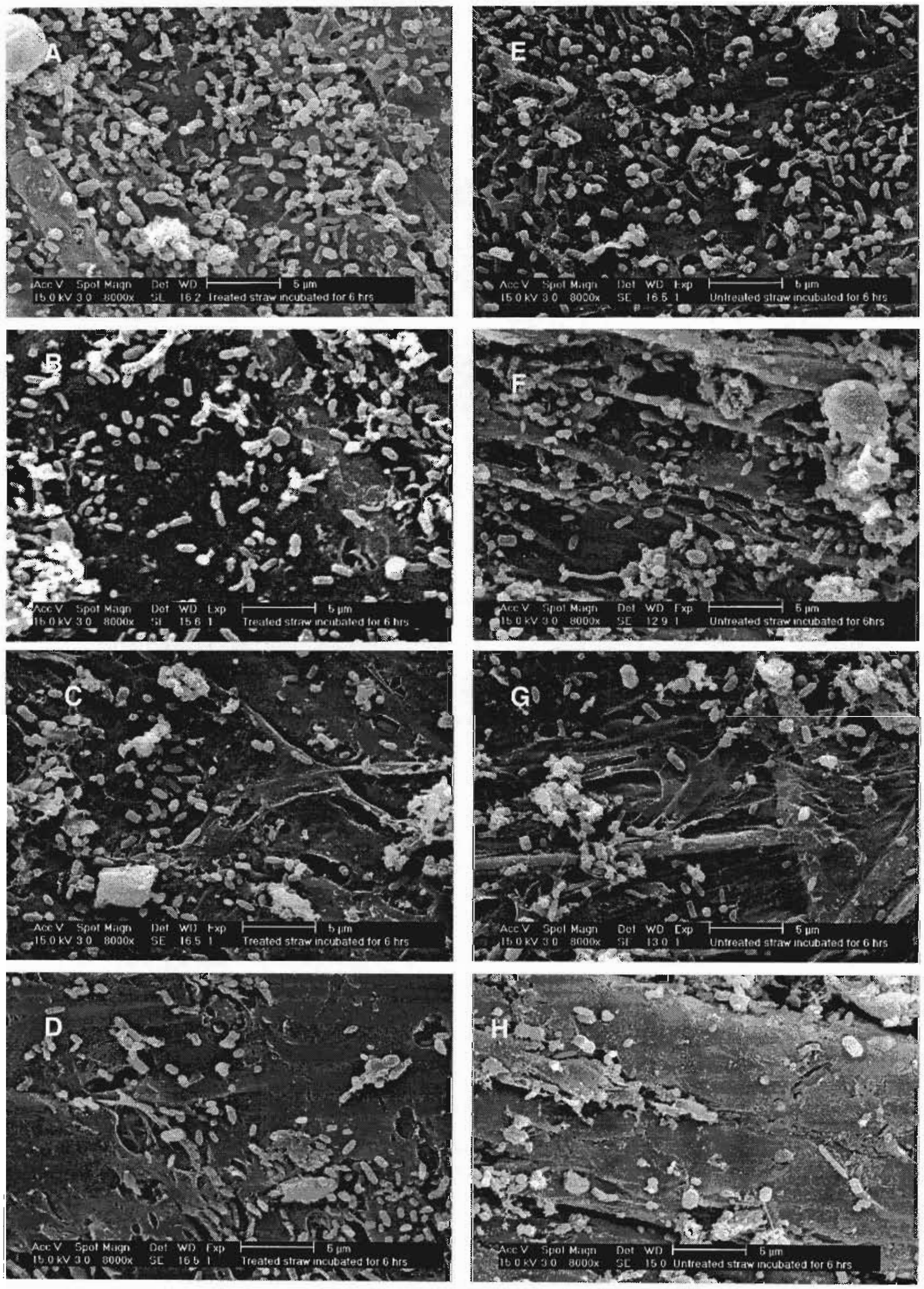



**Figure 4.3** Treated (A, B, C, D) and untreated (E, F, G, H) barley straw incubated for 6 hrs in animals consuming 0 (A, E), 25 (B, F), 50 (C, G) and 75% (D, H) concentrate proportion in their diet.

### 4.4.2 Experiment two

In the second experiment the main effects of incubated feed, concentrate proportion and incubation time on microbial colonization of fiber particles are presented in Table 4.4, while the electron micrographs are shown in Figures 4.4 and 4.5. Incubated feed, concentrate proportion and time of incubation had no effect (P>0.05) on microbial colonization of fiber particles in the rumen of cows (Table 4.4). Urea-ammoniated barley straw had lower total microbial populations on fiber particles than the untreated

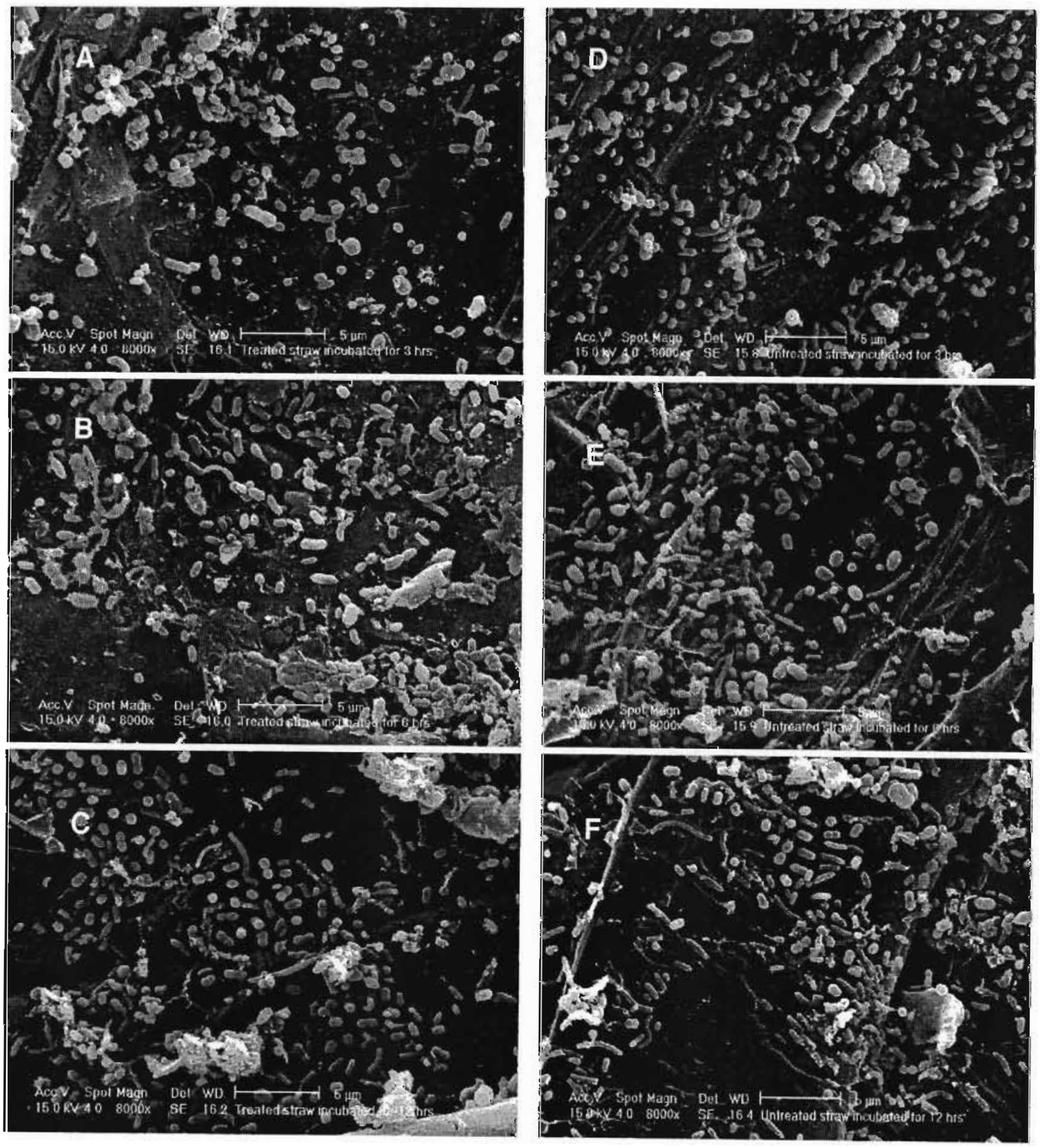



**Figure 4.4** Treated (A, B, C) and untreated (D, E, F) barley straw incubated for 3 (A, D), 6 (B, E) and 12 (C, F) hrs in animals consuming urea-ammoniated veld hay and 60% concentrate.

**Table 4.4** The main effect of incubated feed, concentrate proportion and time of incubation on microbial colonization of urea-ammoniated and untreated barley straw incubated in the rumen of Jersey cows.

| Concentrate (%) | Incubated feed | Type of bacteria (number/1700μm$^2$) | | | |
|---|---|---|---|---|---|
| | | Bacilli | Cocci | Others | Total |
| 60 | 0 | 433 | 211 | 31 | 676 |
| 60 | 1 | 343 | 182 | 29 | 555 |
| 80 | 0 | 519 | 145 | 30 | 694 |
| 80 | 1 | 497 | 159 | 25 | 681 |
| Main effect | | | | | |
| Incubated feed | 0 | 476 | 178 | 31 | 685 |
| | 1 | 421 | 171 | 27 | 618 |
| Concentrate | 60 | 389 | 197 | 30 | 615 |
| | 80 | 509 | 152 | 27 | 688 |
| Time | 3 | 370 | 160 | 20 | 550 |
| | 6 | 502 | 214 | 32 | 748 |
| | 12 | 473 | 149 | 34 | 656 |
| SED of concentrate * incubated feed | | 115.9 | 33.1 | 9.4 | 127.4 |
| SED of incubated feed | | 81.9 | 23.4 | 6.7 | 90.1 |
| SED of concentrate | | 81.9 | 23.4 | 6.7 | 90.1 |
| SED of time | | 100.3 | 28.6 | 8.2 | 110.3 |
| Effect of | | | | | |
| Concentrate * incubated feed | | 0.68 | 0.37 | 0.85 | 0.55 |
| Incubated feed | | 0.5 | 0.76 | 0.5 | 0.4 |
| Concentrate | | 0.16 | 0.07 | 0.6 | 0.4 |
| Time | | 0.4 | 0.07 | 0.2 | 0.2 |

0, untreated barley straw; 1, urea-treated barley straw; 60 and 80, concentrate proportion in the diet; 3, 6 and 12, time of incubation straw when incubated in both environments. The total microbial count was numerically higher in animals fed 80% concentrate than those on 60% concentrate (Table 4.4).

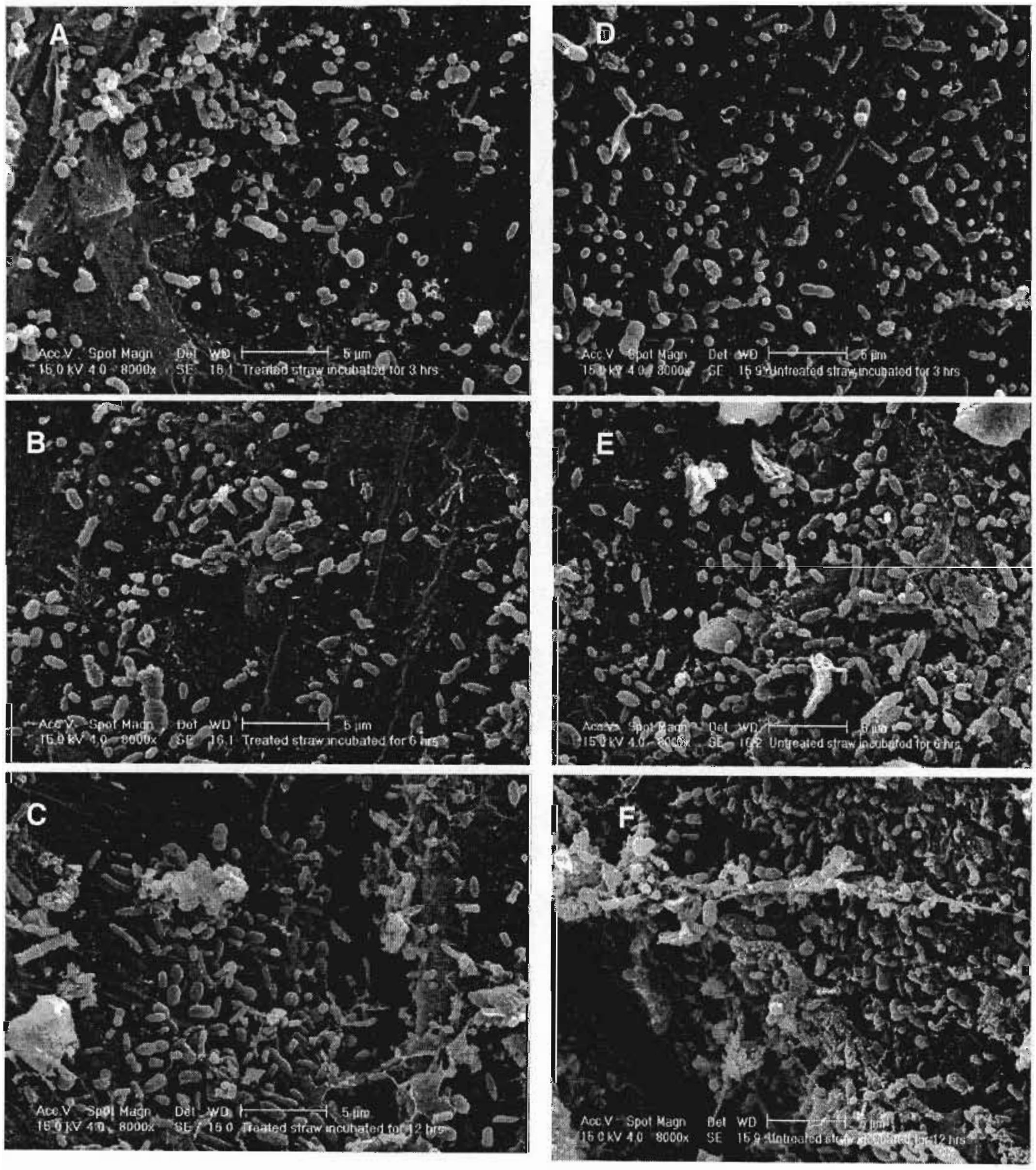



**Figure 4.5** Treated (A, B, C) and untreated (D, E, F) barley straw incubated for 3 (A, D), 6 (B, E) and 12 (C, F) hrs in animals consuming urea-ammoniated veld hay and 80% concentrate.

## 4.5 Discussion

### 4.5.1 Effect of incubated roughage

Spencer and Akin (1980) and Goto *et al.* (1993) reported that rumen microorganisms colonize the more easily digested tissues (mesophyll) and the more slowly degraded tissues (parenchyma bundle sheath) faster in treated blades than in untreated blades. Similarly, Spencer and Akin (1980) reported that treated lignified cell walls were attacked by rumen bacteria and the intercellular layers were easily degraded, whereas similar untreated tissues were resistant to microbial attack. In this experiment, a large number of microbes was seen adhering to the fiber when urea-treated roughages were incubated in the rumen of cows, as was reported previously (Cheng *et al.*, 1983/84). Comparable observations were reported by Latham *et al.* (1979) who found that sodium hydroxide treatment of straw increased microbial colonization. Microbial colonization does not show the same distribution in the same plant material at the same incubation time. This could be related to the fact that forages vary in the degree to which they are colonized by microbes (Akin *et al.*, 1973). Secondly, the rumen ecosystem differs in the quantity and type of bacteria depending on the type of feed that the animal consumes. Fiber particles are differentially colonized in the rumen, in that degraded parenchyma cell walls are heavily colonized and even rapidly degraded, whereas the thick parts of the vascular and sclerenchyma tissue of the same plant are sparsely colonized and no degraded pitting is seen. One possible factor, which may restrict colonization and the spread of bacteria over the outer surfaces of the plant fiber, is the presence of an intact cuticle (Akin and Burdick, 1975; Akin, 1979). Breaking down of this barrier using urea-ammoniation could have improved microbial colonization of fiber particles in the rumen of cows.

### 4.5.2 Effect of dietary roughage

Microbial growth and digestion can be limited by the supply of readily fermentable substrates (Demeyer, 1981), and such limitations are more likely for untreated than for urea treated diets. Silva and Ørskov (1988) demonstrated an increase in microbial density in the rumen of animals given ammoniated straw, possibly due to a more abundant supply of digestible cellulose/hemicellulose. Microbial colonization of fiber particles depends on the extent to which the rumen environment allows an adherent cellulolytic microbial population to develop (Silva *et al.*, 1987). In addition, Cheng and

Hungate (1976) have shown that cellulolytic counts increased from 2.9 x $10^9$ colonies $ml^{-1}$ of rumen content to 5.5 x $10^9$ colonies $ml^{-1}$ when alkali treated alfalfa fiber was included in the medium. This could be a result of the alkali treatment, which partially cleaves the bonds between lignin and hemicellulose (Fernandez Carmona and Greenhalgh, 1972) thus making it available to bacterial colonization. Results found from treating dietary roughage with urea in the present study however failed to demonstrate increased bacterial count owing to urea-ammoniation of dietary roughage. Generally, it is well known that ammoniated dietary roughage contains more soluble carbohydrate than untreated diets and this was true for the ammoniated dietary roughage used in this trial but it is not possible to explain the low number of bacterial count when ammoniated dietary roughage was fed.

### 4.5.3 Effect of concentrate proportions

Although concentrate proportion had no effect on total microbial count, the results from the ESEM examination showed that the total microbial count decreases as concentrate proportion increases in the diet. There is some evidence that a high level of concentrate in the diet causes a decrease in microbial colonization. High concentrate feeding reduces the amount of saliva production, which serves as a buffer for the microbes (Ørskov and Ryle, 1990), and lowered cellulolytic activities (Chimwano *et al.*, 1976; Brink and Steele, 1985; Miller and Muntifering, 1985). This may explain why fewer bacteria were seen adhering to the barley straw, which were incubated in animals given 75% concentrate in their diet (Experiment 1). Further evidence was given by Flachowsky and Schneider (1992) who observed that decreased degradation of dry matter of wheat and barley straws was accompanied by decreased bacterial colonization when the amount of concentrate in the diet was increased from 20 to 80%. However, it is unclear why in Experiment 2, total microbial count was somewhat greater when the amount of concentrate shifted from 60 to 80%.

### 4.5.4 Effect of length of incubation

Examination of incubated samples using ESEM revealed that total population of microbes that colonized fiber particles increased with increasing length of incubation, as was reported previously (Spencer and Akin, 1980; McAllister *et al.*, 1990; Ho *et al.*, 1996). As more bacteria colonize and penetrate the cells, degradation of the cell walls occurs with increasing length of incubation (Spencer and Akin, 1980; Goto *et al.*, 1993). The report of McAllister *et al.* (1990) showed a limited number of bacteria, after incubating for 2h in the rumen of Holstein steers, colonized the endosperm of barley, wheat, sorghum and maize grains but after 8h of incubation the grains were colonized by a variety of bacteria.

### 4.6 Conclusions

In this study concentrate supplementation up to 25% to untreated basal diet of *Eragrostis curvula* hay brought about improvements in the rumen environment and the highest microbial colonization was recorded under this environment. Concentrate proportion had no effect on total count of microbes on fiber particles. However, visual observation of the Environmental Scanning Electron micrographs revealed that the total bacterial count tended to decrease as the concentrate proportions increased in the diet. Urea-ammoniation of incubated roughage increased the total bacterial count on fiber particles. However, under the conditions of this study urea-ammoniation of dietary roughage did not increase microbial colonization of fiber particles.

# CHAPTER 5

# GENERAL DISCUSSION AND CONCLUSIONS

## 5.1 Introduction

The problems encountered in feeding ruminant livestock in the tropics and sub-tropics were highlighted at the beginning of this work. To reiterate, the key problems are seasonal variation in the availability and quality of pastures and low intake and digestibility due to the fibrous nature of roughage from pastures and crop residues. More than 340 million tonnes of fibrous crop residues are produced in Africa per year (Kossila, 1984), the great majority of which are from cereals. The utilization of these low quality roughages (which Leng (1990) defined as roughages which are less than 55% digestible, having less than 8% crude protein and low soluble sugars) as sole feed by ruminants is limited because they are high in ligno-cellulose compounds and low in nitrogen and thus do not meet the maintenance requirements of ruminants, let alone those for production (Wilman *et al.*, 1999).

On the other hand, many authors have reported on the use of urea-ammoniation (Cloete *et al.*, 1983; Seed *et al.*, 1985; Schiere and Nell, 1993; Khanal *et al.*, 1999), to increase animal production, in terms of live weight gain, growth and milk production, owing to the improved nutritive value and intake of straw after treatment, but the information on the interaction of concentrate proportion and urea-treated roughage diets is rather scanty. Experiments were therefore designed to: (i) determine the effect of concentrate proportion and urea treatment on the degradation of roughage diets and microbial colonization; and (ii) determine the benefits associated with treatment of roughages. In this work, a model was proposed that would help to predict the benefit associated with the treatment of roughages with urea, which would help as a decision support tool to decide on whether or not to treat a roughage.

## 5.2 Effects of urea treatment, concentrate proportion and their interaction

The roughages differed widely in the effective degradability of dry matter in the rumen for the urea-treated (434 to 797 g $kg^{-1}$ DM) and untreated (370 to 799 g $kg^{-1}$ DM) roughages. Differences in solubility and potential degradability among the roughages within the treated and untreated groups were primarily attributed to their NDF concentration. Correlation analysis showed that NDF ($r = -0.97$) and ADF ($r = -0.87$) were negatively associated with DM degradation. This observation is in accordance with previous reports (Goto *et al.*, 1991; Habib *et al.*, 1998) on the negative effects of NDF and ADF on forage digestion.

The data obtained from the degradability study indicated that urea-ammoniation improved the insoluble but slowly degradable fraction, potential degradability and effective degradability of DM and NDF degradation. The improvement in the insoluble but slowly degradable fraction, potential degradability and effective degradability of DM and NDF degradation of urea-ammoniated roughages could in part be attributed to the availability of energy in the diet for the microbes due to the chemical changes in the roughage arising from the effect of ammoniation (Tuen *et al.*, 1991; Mgheni *et al.*, 1993). This improvement in degradability parameters following ammoniation could be as a result of an increase in microbial colonization due to the availability of carbohydrates for microbes. Horton (1981) has also reported that the improvement in digestibility following ammoniation was largely the result of increased disappearance of cellulose and hemicellulose.

The benefit associated with urea-treatment of the roughage was positively associated with the NDF content; which for the B-fraction, potential degradability and effective degradability of DM degradation can be computed as: 0.109 x NDF, 0.12 x NDF and 0.061 x NDF, respectively, where NDF is in g $kg^{-1}$. This agrees with Habib *et al.* (1998) who observed a negative linear correlation ($r = -0.79$) between the quality of untreated straw and increase in dry matter digestibility following ammoniation. These authors suggested that the response to ammoniation was maximum in poor quality straw. Chesson and Murison (1989) suggested that the extent or nature of the bonding between lignin and hemicellulose could be a key factor affecting degradation. They postulated that straws with a high degradability (high quality) would have fewer alkali-labile lignin-carbohydrate linkages than straws of low degradability. Thus, in the more highly degradable straws limited substitution of hemicelluose might be expected to promote cell wall degradability but to limit the response shown to ammonia treatment whereas in more poorly degraded straws a more extensive substitution would act to restrict degradation but would allow a greater response to ammonia treatment.

In the present study, the maximum improvement in the degradation of DM and NDF of urea-treated and untreated roughages was realized at the 25% concentrate. Further increase in the concentrate level negatively affected DM and NDF degradation; the negative effect being milder for the urea-treated than for the untreated roughages. This might be an indication that urea has a positive effect on high concentrate diets, due to the fact that urea is an alkali. Seed *et al.* (1985) fed untreated and ammoniated maize residues at five concentrate levels (0, 20, 40, 60 and 80%) to sheep and found that concentrate level (at 0 and 20%) in the diet had a significant effect on feed intake, average daily gain, and digestibility when animals were fed ammoniated maize residue.

Microbial colonization is an essential process for efficient utilization of crop residues by ruminants. The sequential colonization of fiber particles begins with the substrate specific colonization of the selected regions of the fiber by rumen bacteria (McAllister *et al.*, 1990). There is some evidence that increasing concentrate level in a diet cause in a reduction of microbial activity in the rumen. This result was confirmed from the electron micrograph that the total bacterial count tended to decrease as the concentrate levels increased in the diet.

### 5.3 Conclusions

The results of this work have confirmed other reports that urea-ammoniation improved the B-fraction, effective degradability and potential degradability of DM and fiber in roughages. The benefit associated with urea treatment of roughages increased with increasing roughage NDF content. Furthermore, urea-treated roughage diets tended to reduce the negative effect associated with feeding high concentrate diets. Electron micrographs revealed that microbial colonization decreased with increasing concentrate proportion in the diet. Therefore, the approach of using ammoniated roughage diets can be one method of reducing the nutritional disorders that result from feeding high concentrate diets.

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**Appendix Table 1**
DM degradation parameters of urea treated and untreated roughages incubated in the rumen of Jersey cows

| Phase | Animal | Urea | Conc | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 0 | BS | 209 | 607 | 0.023 | 817 | 473 | -2.03 |
| 1 | 1 | 1 | 0 | ETH | 125 | 643 | 0.070 | 768 | 575 | 1.94 |
| 1 | 1 | 1 | 0 | K | 204 | 562 | 0.037 | 767 | 516 | -0.25 |
| 1 | 1 | 1 | 0 | $K_{11}$ | 186 | 585 | 0.040 | 771 | 522 | 1.93 |
| 1 | 1 | 1 | 0 | MS | 206 | 556 | 0.054 | 762 | 564 | 0.89 |
| 1 | 1 | 1 | 0 | OH | 429 | 461 | 0.050 | 890 | 716 | -0.52 |
| 1 | 1 | 1 | 0 | OS | 209 | 572 | 0.024 | 782 | 466 | 0.55 |
| 1 | 1 | 1 | 0 | RG | 392 | 573 | 0.050 | 965 | 750 | -2.55 |
| 1 | 1 | 1 | 0 | VH | 140 | 694 | 0.040 | 835 | 536 | 3.37 |
| 1 | 1 | 1 | 0 | WS | 216 | 510 | 0.031 | 726 | 475 | 3.31 |
| 1 | 2 | 0 | 25 | BS | 161 | 518 | 0.036 | 678 | 443 | 2.32 |
| 1 | 2 | 0 | 25 | ETH | 158 | 741 | 0.020 | 899 | 451 | 1.36 |
| 1 | 2 | 0 | 25 | K | 218 | 501 | 0.027 | 719 | 455 | 3.39 |
| 1 | 2 | 0 | 25 | $K_{11}$ | 152 | 625 | 0.023 | 777 | 422 | 0.66 |
| 1 | 2 | 0 | 25 | MS | 227 | 540 | 0.020 | 767 | 445 | 1.42 |
| 1 | 2 | 0 | 25 | OH | 426 | 469 | 0.042 | 895 | 699 | -0.12 |
| 1 | 2 | 0 | 25 | OS | 171 | 528 | 0.019 | 699 | 376 | 3.44 |
| 1 | 2 | 0 | 25 | RG | 385 | 566 | 0.090 | 952 | 810 | 0.34 |
| 1 | 2 | 0 | 25 | VH | 158 | 639 | 0.028 | 796 | 464 | 2.79 |
| 1 | 2 | 0 | 25 | WS | 184 | 465 | 0.020 | 648 | 368 | 2.74 |
| 1 | 3 | 0 | 0 | BS | 161 | 526 | 0.029 | 686 | 420 | 2.89 |
| 1 | 3 | 0 | 0 | ETH | 158 | 645 | 0.021 | 803 | 423 | 0.56 |
| 1 | 3 | 0 | 0 | K | 218 | 429 | 0.035 | 647 | 449 | 4.38 |
| 1 | 3 | 0 | 0 | $K_{11}$ | 152 | 550 | 0.033 | 702 | 439 | 2.59 |
| 1 | 3 | 0 | 0 | MS | 227 | 450 | 0.028 | 677 | 445 | 1.19 |
| 1 | 3 | 0 | 0 | OH | 426 | 467 | 0.050 | 893 | 719 | 0.75 |
| 1 | 3 | 0 | 0 | OS | 171 | 409 | 0.032 | 580 | 382 | 4.38 |
| 1 | 3 | 0 | 0 | RG | 385 | 569 | 0.094 | 954 | 817 | 2.51 |
| 1 | 3 | 0 | 0 | VH | 158 | 616 | 0.035 | 774 | 491 | 5.19 |

**Appendix Table 1 (continued)**
DM degradation parameters of urea treated and untreated roughages incubated in the rumen of Jersey cows

| Phase | Animal | Urea | Conc | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 0 | 0 | WS | 184 | 420 | 0.020 | 604 | 350 | 0.11 |
| 1 | 4 | 1 | 75 | BS | 209 | 637 | 0.017 | 846 | 443 | 2.30 |
| 1 | 4 | 1 | 75 | ETH | 125 | 845 | 0.015 | 970 | 412 | 1.64 |
| 1 | 4 | 1 | 75 | K | 204 | 506 | 0.046 | 710 | 511 | 1.39 |
| 1 | 4 | 1 | 75 | $K_{11}$ | 186 | 598 | 0.028 | 785 | 477 | 3.12 |
| 1 | 4 | 1 | 75 | MS | 206 | 635 | 0.023 | 841 | 481 | 0.77 |
| 1 | 4 | 1 | 75 | OH | 429 | 472 | 0.035 | 900 | 684 | -0.51 |
| 1 | 4 | 1 | 75 | OS | 209 | 831 | 0.008 | 1040 | 376 | -3.13 |
| 1 | 4 | 1 | 75 | RG | 392 | 564 | 0.050 | 956 | 745 | -0.27 |
| 1 | 4 | 1 | 75 | VH | 140 | 838 | 0.015 | 978 | 420 | 2.69 |
| 1 | 4 | 1 | 75 | WS | 216 | 533 | 0.018 | 749 | 413 | 4.67 |
| 2 | 1 | 1 | 25 | BS | 209 | 614 | 0.033 | 823 | 528 | 3.54 |
| 2 | 1 | 1 | 25 | ETH | 125 | 766 | 0.029 | 891 | 500 | 1.10 |
| 2 | 1 | 1 | 25 | K | 204 | 580 | 0.041 | 784 | 538 | 0.34 |
| 2 | 1 | 1 | 25 | $K_{11}$ | 186 | 637 | 0.033 | 823 | 522 | 1.82 |
| 2 | 1 | 1 | 25 | MS | 206 | 627 | 0.036 | 833 | 546 | 2.44 |
| 2 | 1 | 1 | 25 | OH | 429 | 498 | 0.053 | 927 | 748 | 2.40 |
| 2 | 1 | 1 | 25 | OS | 209 | 558 | 0.027 | 767 | 475 | 0.28 |
| 2 | 1 | 1 | 25 | RG | 392 | 576 | 0.053 | 968 | 760 | 1.83 |
| 2 | 1 | 1 | 25 | VH | 140 | 779 | 0.028 | 919 | 516 | 3.04 |
| 2 | 1 | 1 | 25 | WS | 216 | 541 | 0.028 | 757 | 476 | 1.44 |
| 2 | 2 | 0 | 75 | BS | 161 | 498 | 0.018 | 659 | 346 | -0.34 |
| 2 | 2 | 0 | 75 | ETH | 158 | 518 | 0.022 | 676 | 375 | -0.91 |
| 2 | 2 | 0 | 75 | K | 218 | 395 | 0.027 | 612 | 405 | 3.49 |
| 2 | 2 | 0 | 75 | $K_{11}$ | 152 | 415 | 0.036 | 567 | 379 | -0.30 |
| 2 | 2 | 0 | 75 | MS | 227 | 378 | 0.022 | 605 | 388 | -0.64 |
| 2 | 2 | 0 | 75 | OH | 426 | 454 | 0.039 | 879 | 683 | 1.74 |
| 2 | 2 | 0 | 75 | OS | 171 | 331 | 0.034 | 502 | 348 | 2.34 |

**Appendix Table 1 (continued)**
DM degradation parameters of urea treated and untreated roughages incubated in the rumen of Jersey cows

| Phase | Animal | Urea | Conc | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 2 | 0 | 75 | RG | 385 | 513 | 0.083 | 899 | 762 | 2.35 |
| 2 | 2 | 0 | 75 | VH | 158 | 522 | 0.025 | 680 | 395 | 5.99 |
| 2 | 2 | 0 | 75 | WS | 184 | 321 | 0.025 | 505 | 331 | 0.94 |
| 2 | 3 | 0 | 50 | BS | 161 | 513 | 0.040 | 674 | 453 | 1.75 |
| 2 | 3 | 0 | 50 | ETH | 158 | 659 | 0.019 | 817 | 415 | -3.53 |
| 2 | 3 | 0 | 50 | K | 218 | 439 | 0.028 | 656 | 430 | 1.26 |
| 2 | 3 | 0 | 50 | $K_{11}$ | 152 | 582 | 0.029 | 735 | 438 | 0.81 |
| 2 | 3 | 0 | 50 | MS | 227 | 513 | 0.020 | 740 | 434 | 0.15 |
| 2 | 3 | 0 | 50 | OH | 426 | 457 | 0.033 | 883 | 666 | -1.88 |
| 2 | 3 | 0 | 50 | OS | 171 | 433 | 0.035 | 604 | 403 | 4.22 |
| 2 | 3 | 0 | 50 | RG | 385 | 590 | 0.046 | 976 | 744 | 0.03 |
| 2 | 3 | 0 | 50 | VH | 158 | 504 | 0.043 | 662 | 456 | 3.52 |
| 2 | 3 | 0 | 50 | WS | 184 | 390 | 0.029 | 573 | 376 | 0.45 |
| 2 | 4 | 1 | 50 | BS | 209 | 629 | 0.029 | 838 | 518 | 1.23 |
| 2 | 4 | 1 | 50 | ETH | 125 | 756 | 0.032 | 881 | 514 | 1.36 |
| 2 | 4 | 1 | 50 | K | 204 | 599 | 0.046 | 803 | 568 | 0.35 |
| 2 | 4 | 1 | 50 | $K_{11}$ | 186 | 656 | 0.039 | 843 | 556 | 2.80 |
| 2 | 4 | 1 | 50 | MS | 206 | 634 | 0.034 | 839 | 543 | 1.27 |
| 2 | 4 | 1 | 50 | OH | 429 | 491 | 0.085 | 920 | 792 | 1.28 |
| 2 | 4 | 1 | 50 | OS | 209 | 590 | 0.028 | 799 | 495 | 0.42 |
| 2 | 4 | 1 | 50 | RG | 392 | 580 | 0.124 | 972 | 859 | 2.27 |
| 2 | 4 | 1 | 50 | VH | 140 | 777 | 0.031 | 917 | 532 | 2.84 |
| 2 | 4 | 1 | 50 | WS | 216 | 579 | 0.022 | 795 | 463 | 0.94 |
| 3 | 1 | 1 | 50 | BS | 209 | 595 | 0.031 | 804 | 510 | 3.77 |
| 3 | 1 | 1 | 50 | ETH | 125 | 709 | 0.028 | 834 | 470 | 2.82 |
| 3 | 1 | 1 | 50 | K | 204 | 550 | 0.040 | 755 | 519 | 2.17 |
| 3 | 1 | 1 | 50 | $K_{11}$ | 186 | 618 | 0.035 | 805 | 519 | 4.71 |
| 3 | 1 | 1 | 50 | MS | 206 | 588 | 0.035 | 793 | 522 | 2.18 |

**Appendix Table 1 (continued)**
DM degradation parameters of urea treated and untreated roughages incubated in the rumen of Jersey cows

| Phase | Animal | Urea | Conc | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 1 | 1 | 50 | OH | 429 | 473 | 0.060 | 902 | 744 | -0.33 |
| 3 | 1 | 1 | 50 | OS | 209 | 503 | 0.029 | 712 | 455 | 2.35 |
| 3 | 1 | 1 | 50 | RG | 392 | 572 | 0.069 | 964 | 792 | 2.62 |
| 3 | 1 | 1 | 50 | VH | 140 | 743 | 0.027 | 883 | 493 | 4.07 |
| 3 | 1 | 1 | 50 | WS | 216 | 600 | 0.013 | 816 | 394 | -0.46 |
| 3 | 2 | 1 | 0 | BS | 209 | 580 | 0.020 | 789 | 439 | 2.56 |
| 3 | 2 | 1 | 0 | ETH | 125 | 757 | 0.015 | 882 | 381 | 0.50 |
| 3 | 2 | 1 | 0 | K | 204 | 594 | 0.022 | 798 | 453 | -0.92 |
| 3 | 2 | 1 | 0 | $K_{11}$ | 186 | 564 | 0.031 | 750 | 475 | 4.58 |
| 3 | 2 | 1 | 0 | MS | 206 | 566 | 0.021 | 772 | 440 | 1.85 |
| 3 | 2 | 1 | 0 | OH | 429 | 440 | 0.046 | 869 | 694 | 2.24 |
| 3 | 2 | 1 | 0 | OS | 209 | 646 | 0.012 | 855 | 392 | 2.82 |
| 3 | 2 | 1 | 0 | RG | 392 | 577 | 0.061 | 969 | 780 | 3.61 |
| 3 | 2 | 1 | 0 | VH | 140 | 687 | 0.021 | 828 | 419 | 4.49 |
| 3 | 2 | 1 | 0 | WS | 216 | 360 | 0.030 | 576 | 396 | 3.81 |
| 3 | 3 | 1 | 25 | BS | 209 | 588 | 0.028 | 798 | 494 | 2.67 |
| 3 | 3 | 1 | 25 | ETH | 125 | 654 | 0.044 | 779 | 512 | 3.81 |
| 3 | 3 | 1 | 25 | K | 204 | 531 | 0.055 | 735 | 548 | 1.22 |
| 3 | 3 | 1 | 25 | $K_{11}$ | 186 | 528 | 0.047 | 714 | 508 | 2.96 |
| 3 | 3 | 1 | 25 | MS | 206 | 526 | 0.040 | 732 | 507 | 1.12 |
| 3 | 3 | 1 | 25 | OH | 429 | 466 | 0.060 | 895 | 740 | 1.28 |
| 3 | 3 | 1 | 25 | OS | 209 | 512 | 0.026 | 721 | 448 | -0.55 |
| 3 | 3 | 1 | 25 | RG | 392 | 561 | 0.153 | 954 | 862 | 2.62 |
| 3 | 3 | 1 | 25 | VH | 140 | 694 | 0.040 | 835 | 536 | 3.78 |
| 3 | 3 | 1 | 25 | WS | 216 | 575 | 0.015 | 791 | 406 | -0.84 |
| 3 | 4 | 0 | 50 | BS | 161 | 592 | 0.025 | 752 | 429 | 3.66 |
| 3 | 4 | 0 | 50 | ETH | 158 | 533 | 0.037 | 691 | 451 | 1.94 |
| 3 | 4 | 0 | 50 | K | 218 | 436 | 0.040 | 654 | 466 | 4.41 |
| 3 | 4 | 0 | 50 | $K_{11}$ | 429 | 473 | 0.060 | 902 | 744 | -0.33 |

**Appendix Table 1 (continued)**
DM degradation parameters of urea treated and untreated roughages incubated in the rumen of Jersey cows

| Phase | Animal | Urea | Conc | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 4 | 0 | 50 | MS | 227 | 348 | 0.053 | 575 | 449 | 3.60 |
| 3 | 4 | 0 | 50 | OH | 426 | 454 | 0.087 | 880 | 764 | 2.70 |
| 3 | 4 | 0 | 50 | OS | 171 | 527 | 0.022 | 698 | 393 | 0.90 |
| 3 | 4 | 0 | 50 | RG | 385 | 567 | 0.110 | 952 | 831 | 2.23 |
| 3 | 4 | 0 | 50 | VH | 158 | 692 | 0.023 | 850 | 460 | 4.31 |
| 3 | 4 | 0 | 50 | WS | 184 | 480 | 0.021 | 664 | 379 | 4.74 |
| 4 | 1 | 1 | 75 | BS | 209 | 744 | 0.011 | 953 | 412 | -1.43 |
| 4 | 1 | 1 | 75 | ETH | 125 | 717 | 0.018 | 842 | 392 | 1.65 |
| 4 | 1 | 1 | 75 | K | 204 | 527 | 0.034 | 732 | 483 | 2.01 |
| 4 | 1 | 1 | 75 | $K_{11}$ | 186 | 638 | 0.027 | 825 | 490 | 1.45 |
| 4 | 1 | 1 | 75 | MS | 206 | 553 | 0.022 | 759 | 442 | 2.34 |
| 4 | 1 | 1 | 75 | OH | 429 | 512 | 0.029 | 940 | 682 | -0.94 |
| 4 | 1 | 1 | 75 | OS | 209 | 404 | 0.025 | 613 | 392 | 0.58 |
| 4 | 1 | 1 | 75 | RG | 392 | 552 | 0.101 | 944 | 818 | 3.04 |
| 4 | 1 | 1 | 75 | VH | 140 | 547 | 0.028 | 687 | 402 | 3.58 |
| 4 | 1 | 1 | 75 | WS | 216 | 432 | 0.024 | 648 | 408 | 2.55 |
| 4 | 2 | 1 | 50 | BS | 209 | 466 | 0.026 | 675 | 427 | 4.45 |
| 4 | 2 | 1 | 50 | ETH | 125 | 631 | 0.030 | 756 | 442 | 4.63 |
| 4 | 2 | 1 | 50 | K | 204 | 436 | 0.053 | 640 | 482 | 2.17 |
| 4 | 2 | 1 | 50 | $K_{11}$ | 186 | 640 | 0.020 | 827 | 442 | 3.46 |
| 4 | 2 | 1 | 50 | MS | 206 | 505 | 0.031 | 711 | 463 | 3.24 |
| 4 | 2 | 1 | 50 | OH | 429 | 493 | 0.030 | 922 | 675 | 1.91 |
| 4 | 2 | 1 | 50 | OS | 209 | 528 | 0.019 | 737 | 417 | 0.28 |
| 4 | 2 | 1 | 50 | RG | 392 | 569 | 0.043 | 961 | 728 | 1.23 |
| 4 | 2 | 1 | 50 | VH | 140 | 728 | 0.020 | 869 | 428 | 5.22 |
| 4 | 2 | 1 | 50 | WS | 216 | 411 | 0.028 | 627 | 413 | 3.40 |
| 4 | 3 | 0 | 75 | BS | 161 | 596 | 0.019 | 756 | 391 | 1.45 |
| 4 | 3 | 0 | 75 | ETH | 158 | 562 | 0.031 | 720 | 445 | 1.67 |

**Appendix Table 1 (continued)**
DM degradation parameters of urea treated and untreated roughages incubated in the rumen of Jersey cows

| Phase | Animal | Urea | Conc | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 3 | 0 | 75 | K | 218 | 458 | 0.026 | 675 | 431 | 2.90 |
| 4 | 3 | 0 | 75 | $K_{11}$ | 152 | 586 | 0.026 | 738 | 425 | 2.94 |
| 4 | 3 | 0 | 75 | MS | 227 | 512 | 0.022 | 738 | 441 | 1.81 |
| 4 | 3 | 0 | 75 | OH | 426 | 453 | 0.062 | 879 | 731 | 1.27 |
| 4 | 3 | 0 | 75 | OS | 171 | 441 | 0.029 | 612 | 390 | 0.65 |
| 4 | 3 | 0 | 75 | RG | 385 | 573 | 0.075 | 959 | 795 | 0.99 |
| 4 | 3 | 0 | 75 | VH | 158 | 657 | 0.022 | 814 | 438 | 1.21 |
| 4 | 3 | 0 | 75 | WS | 184 | 456 | 0.020 | 640 | 365 | 1.29 |
| 4 | 4 | 0 | 25 | BS | 161 | 594 | 0.026 | 754 | 434 | 0.16 |
| 4 | 4 | 0 | 25 | ETH | 158 | 650 | 0.028 | 808 | 472 | 0.87 |
| 4 | 4 | 0 | 25 | K | 218 | 448 | 0.041 | 665 | 475 | 3.93 |
| 4 | 4 | 0 | 25 | $K_{11}$ | 152 | 589 | 0.038 | 741 | 480 | 1.83 |
| 4 | 4 | 0 | 25 | MS | 227 | 505 | 0.025 | 732 | 456 | 0.16 |
| 4 | 4 | 0 | 25 | OH | 426 | 457 | 0.086 | 882 | 764 | 2.31 |
| 4 | 4 | 0 | 25 | OS | 171 | 440 | 0.038 | 611 | 418 | 1.90 |
| 4 | 4 | 0 | 25 | RG | 385 | 565 | 0.113 | 951 | 832 | 1.95 |
| 4 | 4 | 0 | 25 | VH | 158 | 706 | 0.022 | 864 | 457 | 1.58 |
| 4 | 4 | 0 | 25 | WS | 184 | 515 | 0.019 | 699 | 386 | 0.75 |
| 5 | 1 | 0 | 0 | BS | 161 | 578 | 0.032 | 739 | 458 | 3.36 |
| 5 | 1 | 0 | 0 | ETH | 158 | 654 | 0.027 | 812 | 469 | 1.65 |
| 5 | 1 | 0 | 0 | K | 218 | 471 | 0.032 | 688 | 459 | 3.21 |
| 5 | 1 | 0 | 0 | $K_{11}$ | 152 | 613 | 0.028 | 765 | 448 | 0.66 |
| 5 | 1 | 0 | 0 | MS | 227 | 547 | 0.025 | 773 | 475 | 0.37 |
| 5 | 1 | 0 | 0 | OH | 426 | 455 | 0.082 | 881 | 759 | 1.92 |
| 5 | 1 | 0 | 0 | OS | 171 | 524 | 0.028 | 695 | 424 | 2.21 |
| 5 | 1 | 0 | 0 | RG | 385 | 576 | 0.085 | 962 | 811 | 1.59 |
| 5 | 1 | 0 | 0 | VH | 158 | 677 | 0.028 | 834 | 484 | 3.53 |
| 5 | 1 | 0 | 0 | WS | 184 | 450 | 0.029 | 633 | 404 | 4.59 |

**Appendix Table 1 (continued)**
DM degradation parameters of urea treated and untreated roughages incubated in the rumen of Jersey cows

| Phase | Animal | Urea | Conc | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 2 | 1 | 25 | BS | 209 | 648 | 0.020 | 857 | 471 | 2.70 |
| 5 | 2 | 1 | 25 | ETH | 125 | 815 | 0.016 | 940 | 410 | 0.83 |
| 5 | 2 | 1 | 25 | $K_{11}$ | 186 | 736 | 0.014 | 923 | 423 | 0.53 |
| 5 | 2 | 1 | 25 | MS | 206 | 655 | 0.020 | 861 | 466 | 0.61 |
| 5 | 2 | 1 | 25 | OH | 429 | 481 | 0.044 | 909 | 715 | 0.54 |
| 5 | 2 | 1 | 25 | OS | 209 | 671 | 0.014 | 880 | 422 | -0.22 |
| 5 | 2 | 1 | 25 | RG | 392 | 564 | 0.083 | 956 | 806 | 1.91 |
| 5 | 2 | 1 | 25 | VH | 140 | 793 | 0.021 | 933 | 465 | 3.15 |
| 5 | 2 | 1 | 25 | WS | 216 | 539 | 0.022 | 755 | 447 | 0.42 |
| 5 | 3 | 1 | 0 | BS | 209 | 608 | 0.033 | 818 | 526 | 3.96 |
| 5 | 3 | 1 | 0 | ETH | 125 | 732 | 0.030 | 857 | 489 | 2.02 |
| 5 | 3 | 1 | 0 | $K_{11}$ | 186 | 625 | 0.028 | 812 | 487 | 3.23 |
| 5 | 3 | 1 | 0 | MS | 206 | 607 | 0.037 | 813 | 541 | 1.36 |
| 5 | 3 | 1 | 0 | OH | 429 | 495 | 0.036 | 924 | 698 | -1.09 |
| 5 | 3 | 1 | 0 | OS | 209 | 549 | 0.027 | 759 | 472 | 1.01 |
| 5 | 3 | 1 | 0 | RG | 392 | 572 | 0.080 | 964 | 808 | 2.54 |
| 5 | 3 | 1 | 0 | VH | 140 | 859 | 0.015 | 1000 | 427 | -2.26 |
| 5 | 3 | 1 | 0 | WS | 216 | 542 | 0.022 | 758 | 448 | 0.31 |
| 5 | 4 | 0 | 75 | BS | 161 | 580 | 0.023 | 741 | 414 | 1.10 |
| 5 | 4 | 0 | 75 | ETH | 158 | 653 | 0.024 | 811 | 450 | 0.40 |
| 5 | 4 | 0 | 75 | K | 218 | 464 | 0.032 | 682 | 458 | 3.46 |
| 5 | 4 | 0 | 75 | $K_{11}$ | 152 | 607 | 0.027 | 759 | 440 | -0.01 |
| 5 | 4 | 0 | 75 | MS | 227 | 544 | 0.020 | 771 | 444 | -1.73 |
| 5 | 4 | 0 | 75 | OH | 426 | 459 | 0.086 | 885 | 766 | 2.72 |
| 5 | 4 | 0 | 75 | OS | 171 | 501 | 0.028 | 672 | 412 | 0.68 |
| 5 | 4 | 0 | 75 | RG | 385 | 574 | 0.104 | 959 | 831 | 2.32 |
| 5 | 4 | 0 | 75 | VH | 158 | 715 | 0.021 | 872 | 455 | 0.57 |
| 5 | 4 | 0 | 75 | WS | 184 | 510 | 0.017 | 693 | 368 | 0.83 |

**Appendix Table 1 (continued)**

DM degradation parameters of urea treated and untreated roughages incubated in the rumen of Jersey cow

| Phase | Animal | Urea | Conc | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 1 | 0 | 25 | BS | 161 | 565 | 0.036 | 725 | 467 | 3.14 |
| 6 | 1 | 0 | 25 | ETH | 158 | 645 | 0.029 | 803 | 475 | 1.41 |
| 6 | 1 | 0 | 25 | K | 218 | 457 | 0.032 | 675 | 455 | 1.70 |
| 6 | 1 | 0 | 25 | $K_{11}$ | 152 | 594 | 0.038 | 746 | 485 | 2.88 |
| 6 | 1 | 0 | 25 | MS | 227 | 528 | 0.027 | 755 | 479 | 0.93 |
| 6 | 1 | 0 | 25 | OH | 426 | 461 | 0.097 | 887 | 778 | 2.09 |
| 6 | 1 | 0 | 25 | OS | 171 | 510 | 0.029 | 681 | 421 | 1.42 |
| 6 | 1 | 0 | 25 | RG | 385 | 585 | 0.052 | 970 | 757 | 0.32 |
| 6 | 1 | 0 | 25 | VH | 158 | 687 | 0.027 | 845 | 483 | 3.22 |
| 6 | 1 | 0 | 25 | WS | 184 | 470 | 0.024 | 653 | 394 | 2.48 |
| 6 | 2 | 0 | 50 | BS | 161 | 623 | 0.018 | 784 | 396 | 3.19 |
| 6 | 2 | 0 | 50 | ETH | 158 | 692 | 0.019 | 850 | 428 | 0.87 |
| 6 | 2 | 0 | 50 | K | 218 | 460 | 0.034 | 677 | 461 | 2.05 |
| 6 | 2 | 0 | 50 | $K_{11}$ | 152 | 638 | 0.022 | 790 | 420 | 0.19 |
| 6 | 2 | 0 | 50 | MS | 227 | 489 | 0.028 | 716 | 463 | 0.71 |
| 6 | 2 | 0 | 50 | OH | 426 | 454 | 0.064 | 880 | 734 | 2.44 |
| 6 | 2 | 0 | 50 | OS | 171 | 468 | 0.029 | 639 | 402 | 2.37 |
| 6 | 2 | 0 | 50 | VH | 158 | 742 | 0.016 | 899 | 420 | 2.97 |
| 6 | 2 | 0 | 50 | WS | 184 | 471 | 0.018 | 654 | 361 | 2.15 |
| 6 | 3 | 1 | 75 | BS | 209 | 481 | 0.038 | 690 | 477 | 3.02 |
| 6 | 3 | 1 | 75 | ETH | 125 | 670 | 0.035 | 795 | 487 | 1.02 |
| 6 | 3 | 1 | 75 | $K_{11}$ | 186 | 599 | 0.040 | 786 | 528 | 2.07 |
| 6 | 3 | 1 | 75 | MS | 206 | 628 | 0.027 | 834 | 503 | -0.20 |
| 6 | 3 | 1 | 75 | OH | 429 | 483 | 0.070 | 912 | 767 | 1.22 |
| 6 | 3 | 1 | 75 | OS | 209 | 542 | 0.022 | 751 | 438 | -1.02 |
| 6 | 3 | 1 | 75 | RG | 392 | 567 | 0.104 | 959 | 832 | 2.39 |
| 6 | 3 | 1 | 75 | VH | 140 | 724 | 0.032 | 865 | 514 | 2.04 |

**Appendix Table 1 (continued)**
DM degradation parameters of urea treated and untreated roughages incubated in the rumen of Jersey cows

| Phase | Animal | Urea | Conc | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 3 | 1 | 75 | WS | 216 | 551 | 0.020 | 767 | 439 | -0.66 |
| 6 | 4 | 0 | 0 | BS | 161 | 562 | 0.032 | 723 | 449 | 3.29 |
| 6 | 4 | 0 | 0 | ETH | 158 | 636 | 0.031 | 794 | 478 | 1.29 |
| 6 | 4 | 0 | 0 | K | 218 | 451 | 0.035 | 669 | 460 | 3.84 |
| 6 | 4 | 0 | 0 | $K_{11}$ | 152 | 588 | 0.031 | 741 | 454 | 1.95 |
| 6 | 4 | 0 | 0 | MS | 227 | 499 | 0.032 | 726 | 483 | 0.46 |
| 6 | 4 | 0 | 0 | OH | 426 | 460 | 0.067 | 886 | 743 | 1.17 |
| 6 | 4 | 0 | 0 | OS | 171 | 499 | 0.030 | 670 | 420 | 1.03 |
| 6 | 4 | 0 | 0 | RG | 385 | 570 | 0.110 | 955 | 833 | 1.97 |
| 6 | 4 | 0 | 0 | VH | 158 | 688 | 0.023 | 846 | 455 | 3.44 |
| 6 | 4 | 0 | 0 | WS | 184 | 392 | 0.032 | 576 | 387 | 1.84 |

Urea = either treated (1) or untreated (0) roughage.
Conc = concentrate proportion;
Ftype = type of feed incubat
Where:
BS = barley straw;
ETH = *Eragrostis curvula* hay;
K = kikuyu hay;
$K_{11}$ = coastcross hay;
MS = maize stover;
OH = oat hay;
OS = oat straw;
RG = rye grass;
VH = veld hay;
WS =wheat straw.

A = dry matter loss;
B = slowly degradable fraction;
c = rate of disappearance of degradable fraction "B";
PD = potential degradability;
ED = effective degradability; and
LT = the lag time.

**Appendix Table 2**
NDF degradation parameters of urea treated and untreated roughages

| Htype | Concentrate | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | BS | 268 | 507 | 0.031 | 776 | 528 | 9.01 |
| 0 | 0 | ETH | 298 | 489 | 0.040 | 786 | 577 | 8.71 |
| 0 | 0 | K | 315 | 385 | 0.037 | 700 | 529 | 6.23 |
| 0 | 0 | $K_{11}$ | 269 | 543 | 0.023 | 811 | 505 | 2.37 |
| 0 | 0 | MS | 326 | 458 | 0.022 | 784 | 520 | 5.23 |
| 0 | 0 | OH | 523 | 354 | 0.075 | 877 | 776 | 5.35 |
| 0 | 0 | OS | 263 | 316 | 0.025 | 580 | 406 | 28.80 |
| 0 | 0 | RG | 503 | 443 | 0.097 | 947 | 842 | 5.07 |
| 0 | 0 | VH | 280 | 551 | 0.041 | 831 | 597 | 6.01 |
| 0 | 0 | WS | 269 | 476 | 0.015 | 745 | 430 | 1.70 |
| 0 | 25 | BS | 268 | 686 | 0.013 | 954 | 470 | -5.70 |
| 0 | 25 | ETH | 298 | 592 | 0.023 | 889 | 553 | -0.19 |
| 0 | 25 | K | 315 | 424 | 0.047 | 739 | 574 | 3.20 |
| 0 | 25 | $K_{11}$ | 269 | 550 | 0.028 | 819 | 536 | -6.67 |
| 0 | 25 | MS | 326 | 490 | 0.031 | 816 | 574 | 0.20 |
| 0 | 25 | OH | 523 | 389 | 0.045 | 912 | 756 | -6.91 |
| 0 | 25 | OS | 263 | 373 | 0.036 | 636 | 466 | 3.57 |
| 0 | 25 | RG | 503 | 447 | 0.103 | 950 | 849 | 5.04 |
| 0 | 25 | VH | 280 | 572 | 0.026 | 852 | 548 | 15.78 |
| 0 | 25 | WS | 269 | 645 | 0.010 | 913 | 430 | -8.04 |
| 0 | 50 | BS | 268 | 549 | 0.023 | 817 | 505 | 0.26 |
| 0 | 50 | ETH | 298 | 472 | 0.030 | 769 | 534 | 1.76 |
| 0 | 50 | K | 315 | 369 | 0.036 | 684 | 515 | 2.64 |
| 0 | 50 | $K_{11}$ | 269 | 556 | 0.021 | 825 | 495 | -4.05 |
| 0 | 50 | MS | 326 | 424 | 0.029 | 750 | 533 | 4.24 |

**Appendix Table 2 (continued)**
NDF degradation parameters of urea treated and untreated roughages

| Htype | Concentrate | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|
| 0 | 50 | OH | 523 | 373 | 0.073 | 897 | 788 | 4.84 |
| 0 | 50 | OS | 263 | 514 | 0.014 | 777 | 430 | -5.76 |
| 0 | 50 | RG | 503 | 458 | 0.074 | 962 | 830 | 1.36 |
| 0 | 50 | VH | 280 | 644 | 0.016 | 924 | 501 | 17.03 |
| 0 | 50 | WS | 269 | 343 | 0.139 | 611 | 550 | 8.20 |
| 0 | 75 | BS | 268 | 464 | 0.029 | 732 | 498 | -0.15 |
| 0 | 75 | ETH | 298 | 455 | 0.029 | 752 | 523 | 10.23 |
| 0 | 75 | K | 315 | 365 | 0.038 | 680 | 518 | -1.04 |
| 0 | 75 | $K_{11}$ | 269 | 465 | 0.029 | 734 | 498 | 1.54 |
| 0 | 75 | MS | 326 | 493 | 0.017 | 819 | 504 | -2.82 |
| 0 | 75 | OH | 523 | 377 | 0.058 | 900 | 771 | -1.32 |
| 0 | 75 | OS | 263 | 373 | 0.026 | 636 | 436 | 2.39 |
| 0 | 75 | RG | 503 | 418 | 0.066 | 921 | 791 | 6.74 |
| 0 | 75 | VH | 280 | 344 | 0.086 | 624 | 535 | 12.09 |
| 0 | 75 | WS | 269 | 364 | 0.034 | 633 | 462 | 12.29 |
| 1 | 0 | BS | 388 | 456 | 0.019 | 844 | 565 | 3.04 |
| 1 | 0 | ETH | 267 | 521 | 0.034 | 788 | 544 | 9.52 |
| 1 | 0 | K | 324 | 438 | 0.030 | 762 | 544 | 7.06 |
| 1 | 0 | $K_{11}$ | 307 | 466 | 0.025 | 773 | 520 | 1.50 |
| 1 | 0 | MS | 262 | 499 | 0.068 | 761 | 609 | 3.92 |
| 1 | 0 | OH | 616 | 281 | 0.040 | 897 | 777 | 10.55 |
| 1 | 0 | OS | 322 | 432 | 0.016 | 754 | 470 | 11.37 |
| 1 | 0 | RG | 511 | 455 | 0.054 | 966 | 803 | 0.10 |
| 1 | 0 | VH | 282 | 579 | 0.019 | 861 | 510 | 18.66 |
| 1 | 0 | WS | 167 | 505 | 0.038 | 672 | 449 | -7.60 |

**Appendix Table 2 (continued)**
NDF degradation parameters of urea treated and untreated roughages

| Htype | Concentrate | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|
| 1 | 25 | BS | 388 | 439 | 0.027 | 826 | 594 | 11.01 |
| 1 | 25 | ETH | 267 | 582 | 0.024 | 849 | 528 | 5.89 |
| 1 | 25 | K | 324 | 448 | 0.069 | 772 | 637 | 7.37 |
| 1 | 25 | $K_{11}$ | 307 | 585 | 0.017 | 892 | 521 | 12.03 |
| 1 | 25 | MS | 262 | 532 | 0.042 | 794 | 573 | 7.23 |
| 1 | 25 | OH | 616 | 301 | 0.048 | 917 | 801 | 9.42 |
| 1 | 25 | OS | 322 | 1204 | 0.004 | 1526 | 468 | 17.58 |
| 1 | 25 | RG | 511 | 439 | 0.152 | 950 | 878 | 5.45 |
| 1 | 25 | VH | 282 | 792 | 0.016 | 1074 | 553 | 12.18 |
| 1 | 25 | WS | 167 | 615 | 0.026 | 783 | 451 | 0.64 |
| 1 | 50 | BS | 388 | 481 | 0.018 | 869 | 565 | 12.21 |
| 1 | 50 | ETH | 267 | 571 | 0.028 | 838 | 545 | 8.52 |
| 1 | 50 | K | 324 | 391 | 0.075 | 715 | 603 | 7.84 |
| 1 | 50 | $K_{11}$ | 307 | 867 | 0.011 | 1175 | 540 | 7.10 |
| 1 | 50 | MS | 262 | 511 | 0.054 | 772 | 591 | 7.15 |
| 1 | 50 | OH | 616 | 304 | 0.044 | 919 | 795 | 6.18 |
| 1 | 50 | OS | 322 | 775 | 0.008 | 1097 | 478 | 13.51 |
| 1 | 50 | RG | 511 | 459 | 0.072 | 970 | 835 | 4.73 |
| 1 | 50 | VH | 282 | 662 | 0.019 | 944 | 541 | 11.21 |
| 1 | 50 | WS | 167 | 500 | 0.038 | 667 | 447 | 3.20 |
| 1 | 75 | BS | 388 | 443 | 0.016 | 831 | 543 | 12.87 |
| 1 | 75 | ETH | 267 | 607 | 0.021 | 874 | 519 | 3.22 |
| 1 | 75 | K | 324 | 456 | 0.028 | 780 | 545 | -5.00 |
| 1 | 75 | $K_{11}$ | 307 | 495 | 0.028 | 802 | 545 | 2.26 |
| 1 | 75 | MS | 262 | 591 | 0.030 | 853 | 558 | 1.68 |

**Appendix Table 2 (continued)**
NDF degradation parameters of urea treated and untreated roughages

| Htype | Concentrate | Ftype | A | B | c | PD | ED | LT |
|---|---|---|---|---|---|---|---|---|
| 1 | 75 | OH | 616 | 337 | 0.055 | 952 | 834 | 4.66 |
| 1 | 75 | OS | 322 | 426 | 0.010 | 749 | 432 | 11.85 |
| 1 | 75 | RG | 511 | 452 | 0.119 | 963 | 873 | 2.51 |
| 1 | 75 | VH | 282 | 817 | 0.009 | 1099 | 472 | -10.69 |
| 1 | 75 | WS | 167 | 540 | 0.030 | 708 | 436 | 4.30 |

Htype = either urea treated (1) or untreated (0) roughage;
Ftype = type of feed incubated;
Where:
BS = barley straw;
ETH = *Eragrostis curvula* hay;
K = kikuyu hay;.
$K_{11}$ = coastcross hay;
MS = maize stover;
OH = oat hay;
OS = oat straw;
RG = rye grass;
VH = veld hay;
WS =wheat straw.

A = neutral detergent fiber loss;
B = slowly degradable fibre fraction;
c = rate of disappearance of fiber fraction;
LT = lag time;
PD = potential degradability of fiber fraction; and
ED = effective degradability of fiber fraction.