

AMMONIATION OF LOW QUALITY ROUGHAGES USING UREA
TO IMPROVE THEIR NUTRITIVE VALUE FOR RUMINANT
FEEDING

By

John Njihia Njogah

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Department of ANIMAL SCIENCE

The University of British Columbia
Vancouver, Canada

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ABSTRACT

This study was carried out to examine the effect of urea treatment on different roughages with respect to their degradation in the rumen. The effect of urea treatment of barley straw on dry matter intake, digestibility and weight gain was also studied.

The first experiment involved urea treatment of wheat straw and orchardgrass hay. Three urea levels were used; 2, 4 and 6 g/100 g DM and the samples ensiled for 3, 6 and 9 weeks. Samples were incubated in the rumen for 1, 12, 24, 36, 48, 72 and 96 hours. Degradation constants were derived using the equation

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

where 'p' represents the amount degraded at time 't', 'a' represents the fraction which disappears rapidly, 'b' represents that fraction which will degrade in time and the rate of degradation of this fraction is represented by the 'c'. The fraction which is undegradable in the rumen can be derived as

$$100 - (a + b)$$

. Orchardgrass hay had significantly larger degradation constants than wheat straw ($P < 0.05$). Treatment increased the rapidly disappearing-'a' fraction of wheat straw but reduced the same fraction of orchardgrass hay ($P < 0.05$). The 'b' fraction of orchardgrass hay was increased significantly ($P < 0.05$) by urea treatment; in the case of wheat straw, the increase was not significant ($P > 0.05$). Crude protein content of

both materials was increased by treatment ($P < 0.05$). Rumen disappearance of CP did not show a consistent trend especially in the case of control samples and this was an indication of microbial contamination of the residue in the bags. In other words, part of dry matter that remained in the bags was contributed by microbial material. Such material also contributed to the nitrogen content of the residue thus masking some of the crude protein disappearance that may have occurred.

In the second experiment, some of the crop by-products that are fed to ruminants in Kenya, were treated. These included barley straw, maize stover, oat straw, rice straw, wheat straw and rhodesgrass hay which was considered to be of low quality. Water was added to raise the moisture content of the materials to 20%. Urea was applied at 6 g urea/100g DM and the materials were ensiled for 6 weeks. Samples of the ensiled materials and their controls were incubated in the rumen of a cannulated steer for 1, 12, 24, 36, 48 and 72 hours. Also incubated were untreated samples of napier grass, alfalfa hay and pyrethrum marc. Rhodesgrass hay had the largest degradation constants ('a', 'b' and 'a+b') before treatment and barley had the least; 13.59, 61.63, 75.22 and 7.16, 37.71, 44.87% respectively. Treatment had the largest impact on rice straw; the 'a', 'b' and 'a+b' increased from 10.24, 46.27 and 56.51% to 22.11, 70.79 and 92.91% respectively. In the case of rhodesgrass hay, treatment had no significant effect ($P > 0.05$). Urea treatment increased significantly ($P < 0.05$) the CP content of all materials. Analysis of data for CP disappearance indicated microbial contamination and this was more evident in the control samples where 24 and 72 hours incubation left the samples with more CP than the unincubated samples. Most of the CP in low quality roughages is bound to the lignin and is therefore undegradable. As degradation proceeds, the CP progressively becomes a larger fraction of the total dry matter which undergraded, thus there is a concentrating effect.

In the final experiment sixteen calves weighing from 86 to 176 kg were divided

into four weight categories. Animals within the same weight category were randomly allocated to four different diets; barley straw treated with 4 or 6 g urea/100g DM and ensiled for six weeks; or urea added to straw at feeding time to raise the crude protein to the levels in the ensiled straws. The straws were mixed with hay in 3:1 ratio (straw:hay) and offered *ad libitum*. In addition each calf received 1.5 kg of concentrate plus 20 g of a mineral mixture daily. A randomized complete block design was used and each group was on diet for four weeks after which the groups were randomly re-assigned to different diets. Treatment did not improve dry matter intake significantly ($P>0.05$). Acid insoluble ash (AIA) was used as an internal marker to calculate apparent digestibilities for DM, CP and ADF. Straw treated with 6 g urea/100g DM and ensiled had a higher dry matter digestibility ($P<0.05$) than the other straws; there was a 16.3% improvement over control. Ensiling reduced CP digestibility significantly ($P<0.05$). Acid detergent fibre digestibility was improved in the case of straw treated with 6 g urea/100g DM and ensiled compared to the control. There were no significant differences between the different diets in terms of average daily gain but there was a trend towards higher gain as the level of urea increased.

Urea as a source of ammonia for the treatment of low quality roughages has given encouraging results and is emerging as a viable treatment method. The method has some advantages over other chemicals in that it is safer to handle, cheaper, readily available and involves simple treatment procedures.

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Chapter 1

METHODS OF IMPROVING THE NUTRITIVE VALUE OF LOW QUALITY ROUGHAGES FOR RUMINANT FEEDING

1.1 Introduction

Worldwide, over 1.5 billion tons of crop residues are produced annually [1] and have been used for many purposes including feeding animals. The potential of these products as feed for ruminants is being increasingly appreciated. As the world population continues to increase, so does the demand for food. This means that the land allocated for forage production will continue to decrease and the amount of cereal grain fed to livestock is also likely to decrease in the future. Animals will therefore have to rely more on agricultural by-products such as straw. Feeding of such by-products is a good way of utilizing them. Alternative ways of disposal include burning which has lost its appeal due to increased concern about environmental pollution and the waste of potentially valuable feedstuffs.

1.2 Literature Review

Plant material can be separated into cell contents and cell walls. The cell contents are highly digestible by animals; however they constitute only a small proportion of the total dry matter in low quality roughages e. g. straw. Thus the major component is the cell wall fraction whose digestibility is variable depending on its components and the way they are associated with each other. These components include cellulose,

hemicellulose, lignin and minerals [2].

The extensive use of straws as ruminant feed is limited by their low nutritive value in terms of:

- Low crude protein in the dry matter (<50 g/kg DM).
- High lignin content and therefore low energy digestibility (35–50%).
- Low intake due to low rate and extent of degradation which result in low rumen turnover rates. In addition, such materials are bulky and this also reduces intake through gut fill.

Animal factors may also limit the extent of digestion of fibrous roughages. These include retention time in the rumen, rate of breakdown of large particles into small particles and the availability of other essential nutrients. The cell wall has physical and chemical factors which limit digestion. These include the cuticle, waxes, lignin and its bonding to other components, lignin–mineral and lignin–protein complexes. The chemicals which limit digestion include phenolic acids, acetyl groups, alkaloids and aromatic compounds [2].

The nutritional value of straw is affected by variety, location of production, physiological stage of the crops at which the grain is harvested and post harvest handling. Therefore there can be considerable variation [3]. Lesoing et al. [4] and Coombe et al. [5] showed that untreated wheat straw is of little nutritional value for growing calves. Oat straw might meet the energy needs for maintenance but is of little value for growing and lactating animals unless it is treated to improve its nutritional value [6]. To meet the energy requirements for growing and lactating ruminants, crop by-products need to be treated physically and/or chemically in addition to a requirement for supplementation in order to increase digestibility and intake [7]. Various treatments can

used to improve the digestibility of low quality crop by- products. They include the processes described in the following paragraphs.

1.2.1 Biological Treatments

Use can be made of certain organisms to degrade crop residues e. g. lignin-degrading fungi. This method is made difficult by the problem of finding microorganisms with metabolic pathways different from those of the rumen microbes. Such microorganisms should have a strong lignin metabolism and low degradation of cellulose and hemicellulose [8], otherwise they would be degrading the fractions which the rumen microbes are able to utilize. White rot fungi with selective lignin decomposition, can increase the in vitro digestibility of plant residue to 77 % [8]. Most of the work has been done on a laboratory scale and only a little is known about large scale processing.

1.3 Physical Processing

Most agricultural residues often give poor results when fed for production of milk, fibre or meat [9]. Physical processing is one of the methods used to improve the nutritive value of low quality roughages. Where roughage is the major or the only feedstuff available, maximization of microbial digestion and utilization of all ingested material should be the goal of the feeding programs. In this case extensive processing may be practical and justified. On the other hand, if both high quality feedstuffs and low quality roughages are available, elaborate processing may not be justified. Above about 65 % digestibility, bulk is not as important a factor in the control of feed intake as when one is dealing with materials with a digestibility of less than 65 %. Bulk then becomes a major but variable factor which can be modified by mechanical comminution to reduce the particle size [10]. This can be achieved by grinding, milling,

chopping and compacting. Mechanical comminution of coarse materials increases the intake by animals. This is partly due to an increase in the density of the feed (a reduction in bulk) and this increases the effective capacity of the animal. Another factor is a reduction in the chewing time required to reduce the particle size of the material to a size suitable for microbial digestion in the rumen or small enough to pass out of the rumen. Comminution also increases the surface area which is exposed to microbial attack and this increases the rate of rumen fermentation. Comminution is however accompanied by an increased rate of passage of the material through the digestive tract and thus nutrient digestion is not maximized [9]. This can however be more than compensated for by the increase in the DE intake [11]. Nitrogen usually becomes the factor limiting the effectiveness of ground, low quality material as most of them are low in nitrogen. One percent nitrogen appears to be adequate for cellulose digestion in the reticulo-rumen for materials up to 50 % DE; for higher levels the nitrogen requirement may be increased to 1.5 % [10]. It is unlikely that many of the commonly used milling methods actually increases the digestibility of cellulose or hemicellulose by rumen microorganisms [9]. Although grinding increases the surface area, the openings in the cell walls have been reported to be too small to allow surface access to the large protein molecules of carbohydrate digesting enzymes. Only extreme milling treatments which disrupt fibre structures of polymers at the molecular level are capable of increasing the digestibility of carbohydrate to any appreciable extent. Such milling procedures would include ball milling and vibratory ball milling [9]. Dehority and Johnson [12] demonstrated that wet ball milling increased the in vitro dry matter digestibility of mature forages. Rate and extent of digestion were increased by the milling procedure. This method however is of little practical significance due to the high processing costs involved and the high rate of passage of the fine particles produced by the method [13]. Pickard et al. [14] and White et al. [15]

did not get any differences in animal performance due to differences in particle size. Compaction of roughages through cubing or pelleting has several benefits such as increased density, reduced dust, improved handling, reduced segregation, wastage and increased intake. However the initial capital investments are high. Several workers have demonstrated that pelleting improves intake [11,16].

Treating material with steam at high pressure to increase their digestibility had considerable appeal due to its simplicity when energy costs were low. The emphasis has now shifted towards less expensive treatment processes [9]. Several crop residues have been steam treated with varying results [17,18,19]. Oji and Mowat [20] reported a large reduction in NDF and an increase in cell contents with steam treatment. Thermo-ammoniation and steam treatment increased ADF significantly. The increase in ADF may have been a result of formation of artefact lignin by non-enzymatic browning reaction [21].

1.3.1 Physico-Chemical Treatments

These includes different combinations of physical and chemical treatments e.g. sodium hydroxide and steaming or application of urea followed by pelleting. Another option is ammoniation at elevated temperatures; treatment of stover at ambient or elevated temperatures increased organic matter intake by 30 and 39 % respectively [22]. Increased intake with ammoniation may be a result of increased rate of passage of digesta. Oji et al. [22] reported that the mean retention time of particulate matter is significantly reduced with ammoniation.

1.3.2 Chemical Treatment

Chemical treatment is considered the most effective treatment method for agricultural by-products [23,24]. Many chemicals have been screened in laboratory experiments

for potential to enhance digestibility but the most commonly used in animal experimentation include sodium hydroxide, ammonium hydroxide, anhydrous ammonia, calcium hydroxide, potassium hydroxide and urea. Sodium hydroxide appears much more effective than either calcium hydroxide or ammonium hydroxide [25]. Modes of action of chemical treatment include solubilization of hemicellulose, increasing the extent of cellulose and hemicellulose digestion and increasing the rate of cellulose and hemicellulose digestion [25]. Generally lignin content is not reduced by chemical treatment [26]. The increase in the extent of digestion is probably due to breaking of bonds between lignin and hemicellulose or cellulose without actual removal of lignin [25].

1.3.3 Sodium Hydroxide

Sodium hydroxide is accepted as the most effective chemical for treatment of crop by-products and several methods of treatment have been developed. The Beckmann method [27] involved the soaking of straw in 1.5–2 % NaOH for three days. This is followed by washing of the straw to remove excess NaOH [3]. The method had the disadvantage of requiring a lot of water. Since the 1970's the use of the method has continued to decline due to tight restrictions on the pollution of streams being enforced. Several modifications to the Beckman method have been developed to overcome the above disadvantages [28] with the emphasis shifting towards dry-treatment methods. However wet treatment methods have given higher digestibility figures than dry methods [3,29].

Industrial dry treatment may turn straw into an energy rich feed for ruminants [30]. The production of alkali treated straw on an industrial scale was first started during world war one as an emergency measure due to scarcity of roughages and other feeds. Straw and wood were cooked under pressure for several hours, washed

and dried. The final product was an excellent feed but production was stopped after the war due to the high costs involved [30]. The in vivo organic matter digestibilities of treated material are reported to increase from 45–50 to 60–70 % and the energy value of the material is also increased [30].

On-farm methods have been developed for straw treatment [3] whereby straw can be treated manually or with the aid of a mixer. Average increase in digestibility of 13 percentage units has been reported using hand mixing and hand spraying [31].

Although sodium hydroxide is effective in treating crop residues, it has several drawbacks which include high cost of the chemical, concerns about human safety due to its corrosive nature, potential for contaminating soils and water as well as concern regarding possible mineral imbalances in animals consuming sodium treated material. There are different opinions as to whether Na^+ or OH^- or both represent any problems to the animals [28]. Heavier kidneys and lower blood-Ca and Mg have been related to Na load and it has been suggested that supplementation of K, Cl, and Mg may be helpful [28]. These factors do limit the use of NaOH as a chemical for improving the nutritive value of low quality roughages.

1.3.4 Ammonia

Treatment with ammonia has some advantages when compared to sodium hydroxide treatment [23,29]. First, the residual nitrogen can be used as a non-protein nitrogen source. Secondly, there are no mineral residues which could be detrimental to the animal or the soil to which the manure is added. Lawlor and O'Shea [32] treated straw with 3% (w/w) anhydrous NH_3 for 30 and 56 days. They reported a mean increase of 15 percentage units in dry matter digestibility in vivo irrespective of the treatment time. In vivo digestibility with wether lambs showed a mean increase in dry matter digestibility of 14.2 units. Crude protein content of the straw increased

from 3.1 to 7.6%. Ammoniation also had the effect of increasing the intake by more than 70%; 520 and 300 g for the treated and untreated straws respectively. Jewel and Campling [33] observed apparent increases in fibre (ADF and cellulose) which were accompanied by a fall in the hemicellulose content of ammoniated wheat straw. Ammoniation also resulted in small but significant increases in voluntary intake of straw, supporting earlier work by Mira and Kay [34]. Dryden and Kempton [35] found that ammonia treatment of barley straw improved its nutritive value. This was as a result of increased extent, but not the rate of organic matter and cellwall organic matter digestion. There was also an increased retention of a pool of readily available, water-soluble nitrogen in treated straw. Herrera-Saldana et al. [36] reported that treatment of wheat straw with NH_3 improved the digestibility of DM, OM, CP and ADF. The dry matter digestibilities of wheat straw treated with NH_3 or NH_4OH were higher than that obtained with the addition of urea to untreated straw. Addition of urea without ensiling doesn't allow the urea to breakdown into ammonia which is necessary for the breaking of some of the bonds between lignin and hemicellulose and thus increase digestibility [25]. The apparent digestibilities of DM and ADF were higher for NH_3 -treated wheat straw than for control.

1.3.5 Urea as Source of Ammonia

The use of urea as source of ammonia for treating agricultural by-products has given promising results and is emerging as a viable alternative [37,38,39,40]. Urea has certain advantages over anhydrous or aqueous ammonia; it is much safer to handle, cheaper and doesn't require elaborate equipment. Dias-Da-Silva and Sundstol [40] reported increased digestibility of urea-treated wheat straw and suggested that the improvement could be a result of slow release into the rumen of added nitrogen thus allowing a more intense microbial fermentation. Oji [22] observed a slower release

of bound nitrogen with NH_3 -treated straw. Ammonium hydroxide production that follows urea hydrolysis in the silo may cause cleavage of the alkali-labile linkages between lignin and structural carbohydrates [41,42] thus increasing fibre digestibility. Observed higher intake of ammoniated straws may be due to increased rate of breakdown of feed particles in the rumen which may intumescence, cause higher rates of passage of indigestible matter [22]. Williams and Innes [43] reported a significant increase in straw degradability as a result of urea treatment and that the effectiveness of the urea was related to the degree of its hydrolysis into ammonia.

Dias-Da-Silva et al. [44] successfully preserved maize stover without mould damage with urea treatment whereas there was extensive mould formation in the control. Highly digestible carbohydrates were also preserved from microbial degradation during the ensiling process. There was partial solubilization of the cell wall in urea treated maize stover and the rumen degradable nitrogen content of the material was markedly increased. Soya bean addition promoted a small increase in urea breakdown and even in those samples without an exogenous source of urease, there was extensive hydrolysis of the urea.

Aerobic storage of ryegrass hay with a dry matter of 56% without the addition of urea resulted in digestible organic matter losses of up to 25%. Treatment with urea reduced this loss to 3-10% for the hay treated with 60 and 30 g urea/kg dry matter respectively. Thus urea is a good preservative for moist material [45]. When the moisture level of straw is 300g/kg or more, then a concentration of urea of >40g/kg dry matter is required to achieve preservation [46].

Macdearmid et al. [46] observed higher dry matter disappearance from treated straw than from control after rumen incubation and this disappearance increased in proportion to the level of urea applied. The digestibility of dry matter, organic matter and acid detergent fibre was increased by treatment with 70 g urea or 40

g ammonia/kg dry matter while 20 g urea/kg dry matter yielded only a modest improvement. Straw intake was stimulated by 70 g urea or 40 g ammonia treatment. Fifty to seventy g urea/kg dry matter gave improvements in nitrogen content and digestibility comparable to those obtained from treatment with anhydrous ammonia [46].

Jayasuriya and Pearce [37] reported the optimum level of treatment to be 4% urea with an ensiling period of 3–4 weeks for the treatment of rice straw. Doubling of urea at ensiling from 4 to 8% had no significant effect on digestibility. Addition of soya bean meal to urea treated straw had the same effect as treating with urease. Addition of urease reduced the treatment time required to achieve a given level of digestibility and addition of soya bean meal also reduced the treatment time. Urea is a safe and relatively low cost method of preserving forage, increasing rumen degradable nitrogen as well as the digestibility of cell walls [45].

1.4 Other Treatments

These include mechanical separation of cereal straws into different parts and the treatment with ionizing radiation [47,48]. These methods have not been used extensively and it is doubtful whether they are economically justifiable.

1.5 Mode of Action of Alkali Treatment

Effects of chemical treatment include solubilization of hemicellulose and increasing the rate and extent of cellulose and hemicellulose digestion [25,23]. Lignin content is not reduced by chemical treatment [49,50,26]. Wilkinson and Gonzalez-Santillana [51] observed a decrease in NDF as the level of NaOH increased but ADF did not decrease with increasing level of NaOH and they concluded that the reduction in

NDF was produced by solubilization of hemicellulose in the neutral detergent. The major effect of alkali is an improvement in digestibility; the increase in the extent of digestion is thought to be as a result of breaking the bonds between lignin and hemicellulose or cellulose without removal of lignin [52]. If linkages between lignin and hemicellulose are broken this will make the latter and cellulose more accessible for hydrolyzing enzymes [53]. Lindberg et al. [54] indicated that xylans are partly translocated during NaOH treatment to a position in the straw cell walls where they are more available to ruminal digestion. Treatment also causes swelling of cellulose fibres within the cell wall matrix. When the cell wall is expanded and the surface is ruptured, the rumen microbes will have better access to the structural carbohydrates and consequently the digestibility is enhanced [55]. Spencer and Akin [56] reported that potassium hydroxide treatment of bermuda grass disrupted tissues and separated lignified thick-walled cells, which resulted in their being digested at a faster rate than the untreated tissues. Harbers et al. [57] using scanning electron microscopy observed that there was an inner cuticle enclosing parenchymal tissues and this cuticle was ruptured by ammonium hydroxide which also separated the parenchymal cells. The NH_4OH however, had no effect on vascular tissue, thick-walled sclerenchyma, outer cuticle or epidermal silica.

1.6 Utilization by the Animal

1.6.1 Digestibility

A reduction in particle size increases the feed intake and decreases the retention time of roughages in the rumen. To what extent the digestibility is improved by a treatment depends on the type of material, method of treatment and a number of process factors such as amount of chemical, length of treatment period, temperature,

moisture content and pressure [55]. When large amounts of concentrates are fed along with roughages the rumen pH drops and the activity of cellulolytic microorganisms is greatly reduced. Excess hydroxyl ions from chemical treatment may on the other hand, have a positive influence on the rumen environment.

1.6.2 Intake

The quantity of low quality roughage consumed will be restricted by the extent to which the animal can increase rumen load [58]. Rate of production of ruminants from low protein diets is restricted by the low intake of digestible nutrients. Non protein nitrogen and bypass protein will increase the nitrogen supply to the rumen microbes as well as amino acid supply to the animal and thus increase food intake. Intake of low quality diets is restricted by the physical size of the rumen and therefore animals may not be able to consume sufficient dry matter to meet energy requirements for maximum production. Metabolizable energy intake can be improved by increasing the digestibility of the basal diet or by supplementation with an energy form which does not suppress intake of the basal material [58]. Both physical and chemical treatment improve the voluntary intake of low quality roughages. Mechanical treatment such as chopping, grinding and pelleting improve intake simply because the rate of passage through the intestinal tract is increased. Jayasuriya and Owen [31] observed that the improvement in intake due to treatment seemed less for mixed rations than for all straw diets.

1.6.3 Supplementation

Production systems based on feeding ruminants on low quality diets often involve the use of supplements to alleviate nutritional deficiencies in the basal diet, to maintain or increase intake of the basal diet, to increase the efficiency of utilization of the

nutrients and to increase production. Cereal roughages generally contain $<0.5\%$ N which is insufficient to allow microbes to grow efficiently. Twenty to fifty mg NH_3/l is the critical level of ammonia in rumen fluid, below which microbial growth may be impaired or efficiency is reduced [59,60]. When the ammonia concentration falls below this level as may happen when animals are on low protein, low quality roughages; the rumen microbes may be N deficient and may respond to NPN supplements. Provision of readily soluble NPN supplements e.g. urea, increases the rumen NH_3 levels for only a short period post feeding and thus the N level may be below the critical level until the next feeding of supplement. Fluctuations in NH_3 concentration may decrease considerably the outflow of microbial protein from the rumen [61]. This is caused by a reduction in microbial activity which leads to reduced degradation as well as microbial proliferation.

Urea supplements sometimes increase the digestibility of cellulose and crude fibre of low protein rations. Urea is rapidly dissolved and hydrolyzed to ammonia by bacterial urease; the ammonia is utilized by the bacteria for the synthesis of amino acids required for their growth. Amino groups are also split from amino acids and intact protein and used by bacteria in the same manner. Protein synthesis within the rumen is closely associated with activity of the microbes in breaking down cellulose and other carbohydrate material and formation of organic acids as by-products of fermentation [62]. Efficiency of utilization of NPN for microbial protein synthesis in sheep can be increased by providing urea continuously in the rumen when compared with providing the same amount of urea in a single dose [64,65]. Orskov and Grubb [66] reported no increase in the digestibility of barley straw after NaOH treatment without urea supplementation. Supplementation with up to 18 g urea per kg gave no improvement in digestibility of untreated straw, but when added to NaOH-treated straw the organic matter digestibility increased from 42 to 62%. When cereal straw is

treated with 3% NH_3 the N content increases from about 0.5 to 1.0-1.5% of straw DM depending on the moisture content of the straw [55]. Lawlor and O'Shea [32] working with barley straw found that 58% of the anhydrous ammonia remained irreversibly bound to the straw. Oji and Mowat [20] as well as Solaiman et al. [67] reported no significant increase in acid detergent insoluble nitrogen. Therefore, it may be concluded that a considerable part of the N added during ammonia treatment is not bound and could be used as an ordinary NPN source for microbial protein synthesis in the rumen [35,68]. Kempton and Leng [69] reported that supplementation of low protein cellulosic diets with NPN and undegraded dietary protein had the effect of increasing food intake as well as an increase in the production and absorption of fermentation end products in lambs. Egan [70,71] found that supplementation with NPN and bypass protein did not increase digestibility of the basal diet. However, the rate of comminution of particles in the rumen and the rate of clearance of undigested feed residues from the rumen were increased, allowing food intake to be increased.

The efficiency of urea utilization and the amount of protein which can be replaced by urea depends on the amount of true protein in the diet. With a protein rich diet, efficiency of urea use is low; whereas efficiency is high with protein poor diets. Other factors include the type and amount of available carbohydrates as well as the level of essential mineral elements [62]. Campling et al. [72] fed cows straw (3% CP) and infused a solution of urea into the rumen. This led to an increase in digestibility of crude fibre and nitrogen free extracts. The mean retention of the ingesta in the rumen was reduced. They concluded that voluntary feed intake is related to the rate of disappearance of food from the rumen.

1.6.4 Carbohydrates

Urea is less well utilized when fed with hay or other forages alone than when starch or other cereal grains are included in the ration. McDonald [63] reported that the addition of starch to the rumen of sheep after feeding them with a diet containing casein (high rumen ammonia) effected a rapid decrease in ammonia level. He concluded that starch provided energy needed by the microbes to utilize the ammonia. Clark and Quin [73] showed that the digestibility of dry matter and cellulose by sheep was not changed by addition of urea-molasses; however the rate of digestion was increased, permitting greater feed intake. This led to decreased loss of body weight of animals on poor quality roughage.

1.6.5 Protein

Effects of protein supplements may be attributed to one or more of the following:

- Slow release of amino acids, ammonia, sulfur and energy in the rumen
- Increase in the proportion of nutrients absorbed as essential amino acids
- Supplementary energy including gluconeogenesis
- Stimulatory effect on intake.

Dietary protein supplements often have a greater effect than urea on intake and digestibility of low quality forages. This is because such supplements are useful sources of ammonia since they are degraded slowly as opposed to urea which is degraded rapidly [74]. Availability of energy in the rumen could be a limiting factor in animals on low quality roughage diets. Kellaway and Leibholz [75] reported that when starch or sucrose were sprayed onto hay, efficiency of bacterial protein synthesis was not

increased, although there were significant increases in dry matter intake, nitrogen flow to the abomasum and nitrogen balance. They concluded that the energy supplements increased total protein synthesis without changing the efficiency of synthesis. Rumen ammonia concentrations were lower with urea/energy supplements than with urea alone. This was an indication of more effective utilization of ammonia when energy is available [75]. Protein supplements can increase the supply of amino acids, ammonia and energy to the rumen and increase the proportion of nutrients absorbed as essential amino acids [75]. Supplements of NPN and protein are equally effective in stimulating forage intake when intake of rumen degradable nitrogen in the forage is low provided that the intake of NPN is not too infrequent. When intake of NPN is infrequent, protein supplements are likely to be more effective as a source of slowly released rumen degradable nitrogen.

1.7 Production Responses

Differences in digestibility to a great extent are reflected in the performance of animals, provided that their diets are balanced. The quality of cereal straw varies a great deal between species, varieties, growing season, region, weather conditions and handling [76]. Orskov et al. [77] reported that steers and heifers offered ammonia treated straw as the sole diet were able to grow 300-400 g/day. There are indications that supplementation with the deficient nutrient is the most feasible method of improving the utilization of low quality roughages. A number of experiments have shown that even small supplements to a diet based on untreated or urea/ammonia-treated straw may boost the growth rate of animals [55]. Sodium hydroxide treated straw must be supplemented primarily with protein or NPN to be able to show a satisfactory response [66]. Supplementation may also have an adverse effect on the utilization

of low quality roughages. Since cellulolysis is optimal at pH 6.2- 7.0 finely ground cereals or other readily available carbohydrates that lower rumen pH to 6 or below, reduce the rate of degradation and hence the utilization of roughages considerably [78]. Therefore in high concentrate diets, straw provides the fibre which is essential for production of butter fat in milk as well as for the maintenance of rumen function. If the straw is chopped or ground then the structural properties are lost and thus Kristensen [79] concluded that ground straw would be unsuitable in the diet of dairy cows.

1.8 Nylon Bag Technique

1.8.1 Introduction

A simple, accurate and rapid method of measuring forage digestibility would be of great value to both animal nutritionists and agronomists working with forage. Digestion trials have been the main method for the determination of the nutritive value of forages. Such trials are expensive, labor intensive, time consuming and require relatively large amounts of forage [80]. Thus alternative methods of determining dry matter digestibility are constantly being sought.

There has been considerable progress in the development of laboratory techniques for evaluation of feeds for ruminants. Such techniques have been developed with the aim of trying to mimic the in vivo processes [81,82]. The study of in vivo digestion of feeds using cannulated animals offers a simple and rapid alternative. Since it was first used by Quin et al. [83], the method has been adopted as a means of measuring dry matter, fibre and nitrogen digestion in the rumen [84].

One of the major advantages of the nylon bag technique is its simplicity; the only requirements being nylon bags, cannulated animals and facilities for drying the

samples. Nylon bag incubations have advantages since digestion is studied in an environment to which it is applicable, that is the *in vivo* environment. Studies of *in vivo* fermentations have the advantage of eliminating such problems as temperature control and removal of end products; problems which are encountered in *in vitro* fermentation studies [85]. The method measures the disappearance of feed constituents from bags after incubation in the rumen for varying periods of time. Thus the rate and extent of degradation can be determined. Knowledge of the rate at which degradation is occurring is important in that it provides information on factors affecting intake of roughages. The voluntary intake of roughages in ruminants is not only affected by the digestibility, but also by the rate at which digestion occurs since both factors affect the turn over rate [86,85]. The rate of protein degradation in the rumen is another important measurement since it influences protein supply to the host animal as well as the nitrogen available to the rumen microorganisms [81].

The nylon bag technique has some limitations; the kinetics of digestion of material placed in nylon bags will not duplicate exactly those of the same material taken in during the course of a normal meal and this is because materials in the bags are not able to circulate in the rumen as particles of normal ingesta are able to [87]. Food would normally leave the rumen once it has been broken down to suitable particle size but this is not possible due to confinement within the bag. What is actually measured is the breakdown of material to a size small enough to leave the bag and not necessarily a complete breakdown to simple chemical components. Thus the results should be treated with due caution, and in general be used as qualitative indicators [88]. The other limitation is microbial contamination of the residue that remains in the bags. This causes an underestimation of both dry matter and crude protein degradations.

1.8.2 Materials and Methodology

For consistent results certain factors have to be taken into account. These include material for the preparation of the bags, treatment and sample preparation, sample size relative to the bag size, position of the bags in the rumen, incubation time, replication, number of bags to be incubated, the diet of the animals and laboratory procedures after removal of the bags from the rumen e. g. washing of the bags [81,88, 89].

1.8.3 Applications of the Technique

The technique can be used to study degradation of protein supplements. When coupled with estimates of turnover rates of rumen digesta, the nylon bag technique offers the possibility of obtaining quantitative estimates of the true degradability within the rumen. Using the technique, one is able to obtain the degradation rate, the potential degradability and the effective degradability [88].

To study degradation of roughages and forages, the same general principles used for protein supplements can be applied to the study of the degradation of forages within the rumen. However the outflow rate of small particles cannot be directly applied [88,89]. This is because most protein supplements generally consist of particles which are sufficiently small to traverse the reticulo-omasal orifice without further reduction in particle size [88]. Thus the outflow of protein supplements is taken to be the same as that of small particles. Therefore the calculation of the effective degradation which takes into account the out flow rate of material from the rumen is possible with protein supplements. In the case of forages and roughages the picture is different since the material must be comminuted by rumination and mastication as well as degradation by the rumen microbes to particles sufficiently small to flow from

the rumen. Thus the outflow rate of small particles cannot be applied in the case of roughages [88].

The digestibility of a feedstuff is defined by the potential degradability of the material, the rate of degradation of this potentially degradable fraction and its residence time in the rumen. The outflow rate from the rumen will determine what the effective degradation will be. The effective degradation may not be the full potential degradation, although with cellulosic material, degradation is probably rapid relative to the possible outflow rate of the non-degradable fraction. This is because the non-degradable fraction has to be broken down physically to a size small enough to be able to leave the rumen. The retention time in the rumen of large particles is not easy to determine because accurate sampling of heterogeneous rumen contents is difficult [89]. Mansbridge [90] observed many difficulties in obtaining samples from the rumen of sheep and cattle fed on long diets while there were few problems in describing the dilution curve with ground diets where there is little or no distinction between liquid and solid phases in the rumen. Samples of hay or other fibrous material could be treated using the methods of Uden et al. [91], but since this treatment makes the material indigestible, it only measures the speed at which indigestible particles are broken down by physical means—chewing & rumination and cannot measure the combined effect of physical and microbial degradation.

To fully exploit the information provided by the description of degradation, it is important that the incubation times chosen are such that the sensitive part of the curve and the asymptote are adequately described. Orskov and McDonald [88] described degradation by the following exponential equation:

$$p = a + b(1 - e^{-ct}) \quad (1.1)$$

- 'p' is the degradation which has taken place during the time 't'

- 'a' is the intercept and represents the rapidly disappearing fraction
- 'b' is the fraction which will degrade with time
- 'c' is the degradation rate of the 'b' fraction
- 'a'+ 'b' represent asymptote and gives a measure of the potential digestibility of the feed.

Where the outflow rate of material from the rumen is known the effective degradation can be calculated using the following equation [88]

$$P = a + \frac{bc}{c + k} \quad (1.2)$$

- 'P' is effective degradation
- 'a', 'b' and 'c' are the same as in equation (1.1) above
- 'k' is the outflow rate of material from the rumen.

Chapter 2

TREATMENT OF WHEAT STRAW AND ORCHARDGRASS HAY WITH UREA

2.1 Objectives

The experiment was aimed at investigating the following:

- Effect of urea level on degradation of low quality roughages; 2, 4 or 6 g urea/100g DM were used.
- Effect of ensiling period on degradation; samples were ensiled for 3, 6 or 9 weeks.
- Effect of urea treatment on degradation of wheat straw and orchardgrass hay.

2.2 Materials and Methods

Wheat straw (*Triticum aestivum*) and orchardgrass hay (*Dactylis glomerata*) were coarsely chopped using a forage chopper. Duplicate samples of each material were taken for dry matter determination. The average dry matter was 89 and 86 % for the straw and hay respectively.

One hundred g (100 g) samples of each material were weighed. Two, four or six g feed grade urea (46% N) [46,37] was dissolved in enough water to raise the moisture content of the straw or hay to 20%. A small amount of the material was put in a plastic bag and some of the urea solution was added with thorough mixing. This was repeated until all the material and the urea

solution were in the plastic bag. Three g of soybean meal were added to each bag and thoroughly mixed after which the bags were sealed. Other workers have included the soybean at a higher level; treatment was reduced to 3 days by adding 8.5% soybean powder [92]. Dias-Da-Silva et al. [44] added soybean at 6 g/kg stover DM and reported only a slight increase in urea breakdown. Such levels of soybean inclusion may not be very practical. This procedure was repeated for all the different treatments. The plastic bags remained sealed for either 3, 6 or 9 weeks after which the bags were opened and the materials dried at 60°C for 24 hours. This was followed by grinding through a 1 mm screen using a C&N mill.

2.2.1 Laboratory analysis

Duplicate samples of the different treatments were analysed for dry matter using standard laboratory methods [93] after drying at 60°C for 24 hours. Crude protein was determined using the methods of Parkinson & Allen [94] and Wall & Gehrke [95]. The method of Waldern ([96]) was used for determination of acid detergent fibre.

2.2.2 Rumen Incubations

A rumen cannulated steer was used for the incubations and the procedures of Mehrez and Orskov [81] and Orskov [82] were followed. The steer was maintained on orchardgrass hay which was offered twice daily. The animal started receiving the hay diet ten days before the incubations were started. Five g dry matter samples were weighed in duplicate into pre-weighed nylon bags measuring 7x16 cm with a mean pore size of 40 μ . The bags were tied closed with 10

cm nylon strings and these strings were secured at three different points near the end of a 60 cm long nylon string. Each sample and its duplicate were tied at different points on the string and placed into the ventral sac of the rumen. The other end of the string was tied to the cannula plug. The bags remained in the rumen for 1, 12, 24, 36, 48, 72 or 96 hours. After removal from the rumen the bags were thoroughly washed under running water until the water squeezed from the bags was clear [88,84]. The washing time was approximately 1.5 minutes per bag. The bags were then dried at 60°C for 48 hrs. The bags were weighed and the dry matter of the material remaining in the bag was determined. Dry matter disappearance was calculated as the difference between the dry matter incubated and the dry matter remaining after incubation.

Duplicate samples of the residue remaining were analysed for CP and ADF using standard methods as mentioned above [94,95,96]. The loss of either ADF or CP was calculated by reference to the average composition of the original material and the initial weight of the samples.

2.3 Results and Discussion

2.3.1 Dry Matter Degradation

Dry matter disappearance (DMD) was calculated as the difference between the dry matter incubated and the dry matter after incubation as a percent of the dry matter incubated:

$$\%DMD = \frac{DM \text{ Incubated} - DM \text{ Remaining}}{DM \text{ Incubated}} * 100$$

This was done for all the rumen incubation periods and the data was fitted to the equation of Orskov and McDonald (1979) [88]:

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

A Eureka computer program which uses an iterative least squares procedure was used to calculate the degradation constants a,b and c. The asymptote was then calculated as (a+b). Analysis of variance was carried out on the constants using the general linear model of SAS [97] with material (hay or straw), urea (0, 2, 4 or 6 g urea/100g DM), and ensiling period (3, 6 or 9 weeks) as the main effects.

2.3.1.1 Rapidly Disappearing fraction-'a'

The orchardgrass hay had a significantly greater ($P < 0.05$) 'a' fraction than wheat straw (Table 2.1). The 'a' fraction is highly correlated to the soluble fraction and thus this fraction can be assumed to be highly degradable although solubility doesn't mean degradability in all cases. All the urea levels increased significantly ($P < 0.05$) the 'a' fraction of wheat straw compared to the control. However there were no significant differences between the three urea levels ($P > 0.05$). In the case of orchardgrass hay, urea treatment significantly decreased ($P < 0.05$) the 'a' fraction. This may have been as a result of the ammonia from urea reacting with some of the soluble carbohydrates to form insoluble complexes. If such complexes are degradable, their formation would be an advantage since they may be utilized more efficiently owing to their pattern of release compared to the soluble fraction which disappears rapidly. Ensiling time had no significant effect on the 'a' fraction ($P > 0.05$).

The 'a' fraction is the immediate source of nutrients to the rumen microorganisms post feeding. Thus if this fraction is substantial and provided the microbes are able to utilize it efficiently, then they will be adequately supplied until they can start degrading the 'b' fraction. Thus a boost in the 'a' fraction should be an advantage to the microbes provided that its utilization is efficient.

2.3.1.2 Insoluble degradable fraction 'b'

Orchardgrass hay had a significantly larger ($P < 0.05$) 'b' fraction than wheat straw (Table 2.1). This implies that the hay was more degradable than the straw and thus an animal would be able to degrade and get more nutrients from the hay than from the same quantity of straw.

Urea treatment had no significant effect ($P > 0.05$) on the 'b' fraction of wheat straw. However there was a trend towards an increase in 'b' fraction as the level of urea was increased. With orchardgrass hay, urea treatment increased significantly the 'b' fraction ($P < 0.05$) but there were no significant differences between the urea levels ($P > 0.05$) (Table 2.1). Thus urea treatment increased the potential degradability of the hay and the straw by increasing the 'b' fraction. However, the hay gave a better response when compared to the straw and this was probably due to the hay being less lignified and thus experiencing a larger swelling effect making it more degradable. An increase in the 'b' fraction should be of significance to the animal since with time this is the main source of nutrients to the microbes. Ensiling time had no significant effect on the 'b' fraction ($P > 0.05$) although there was a general trend towards an increase in the 'b' fraction as ensiling time was increased. Thus there was no added advantage to ensiling for longer than 3 weeks. This is an advantage since it means that

any treatment program will not be expensive with respect to time.

2.3.1.3 Rate of Degradation of the 'b' fraction-'c'

Orchardgrass hay had a significantly higher ($P < 0.05$) rate of degradation of than wheat straw. This implies that the hay would be more beneficial to an animal since more hay would be degraded per unit of time when compared to the straw. It also means that animals offered hay would consume more dry matter than those offered straw since a higher rate of degradation in the rumen would effect a higher turn over rate.

Urea treatment had no significant effect ($P > 0.05$) on the 'c' of either the hay or the straw. Probably the effect of ammoniation (swelling effect) was not large enough to cause a pronounced increase in the accessibility of the structural carbohydrates to rumen microbes.

2.3.1.4 Asymptote (a+b)

This represents the maximum potential degradability of a material. The hay had a significantly greater ($P < 0.05$) asymptote than the straw. Urea treatment had no significant effect ($P > 0.05$) on the asymptote of either of the materials. However the results indicate a trend towards an increase in the potentially degradable fraction as a result of urea treatment.

2.3.1.5 Dry Matter Disappearance

Material, urea and rumen incubation time were highly significant ($P < 0.05$). Orchardgrass hay had significantly higher ($P < 0.05$) dry matter disappearance

Table 2.1: Degradation Constants

Material	Urea g/100g DM	Degradation Constants			
		a \pm 1.2	b \pm 2.8	c \pm 0.01	a+b \pm 3.1
Wheat Straw	0	5.87 ^a	46.17 ^a	0.033 ^a	52.04 ^a
	2	8.12 ^{ab}	46.34 ^a	0.035 ^a	54.46 ^a
	4	10.59 ^{bc}	46.32 ^a	0.035 ^a	56.91 ^a
	6	9.74 ^b	47.54 ^{ab}	0.038 ^{ab}	57.28 ^a
Orchard Grass Hay	0	20.29 ^e	49.38 ^b	0.046 ^{ab}	69.66 ^{ab}
	2	12.38 ^{cd}	66.70 ^c	0.046 ^{ab}	79.08 ^b
	4	14.07 ^d	62.89 ^c	0.054 ^b	76.96 ^b
	6	13.34 ^d	68.40 ^c	0.045 ^{ab}	81.74 ^b

Values within the same column with different superscripts are significantly different at $p < 0.05$.

values than wheat straw and this means that the hay was more degradable than wheat straw. Dry matter disappearance increased significantly ($P < 0.05$) with urea treatment, however there were no significant differences ($P > 0.05$) between the urea levels (Table 2.2). Williams and Innes [43] reported that ammonia from urea hydrolysis increased the degradability of barley straw significantly. Jayasuriya and Pearce [37] observed that ensiling with urea increased the in vitro organic matter digestibility of rice straw significantly. An increase in the level of urea led to an increase in organic matter digestibility but the increase was small beyond 6 % level of application. Urea treatment effected a 10 % increase in in vitro organic matter digestibility. Ensiling of rice straw with urea significantly increased dry matter and organic matter digestibility in vivo. A level of urea higher than 4 % had no additional benefit in terms of increase in digestibility [37]. Macdearmid [46] observed higher dry matter disappearance from straw samples treated with urea than the untreated samples. They noted an increase in dry matter disappearance as the level of urea applied increased. Williams

[98] noted that treatment of barley straw with urea significantly increased the degradability of straw dry matter but the level of urea had no significant effect on straw digestibility. There was an increase in dry matter disappearance as the rumen incubation time increased. This is to be expected in view of the increased exposure time to rumen degradation (Figure 2.1 and 2.2). Wheat straw had no significant differences ($P>0.05$) between dry matter disappearance values for 36, 48 and 72 hours incubation. The largest dry matter disappearance occurred between 12 and 24 hours incubation. In the case of orchardgrass hay, there was also a general increase in the dry matter disappearance as the rumen incubation increased. However there were no significant differences between dry matter disappearance for 36, 48 and 72 hours incubation ($P>0.05$). The largest dry matter disappearance occurred between 12 and 24 hours incubation. This is in agreement with the findings of Hovell et al. [99]. The lack of significant differences between dry matter disappearance values for 36, 48 and 72 hours incubation could be a reflection of degradation tending towards the asymptote; thus a decreasing rate of degradation. This means that as incubation time increases beyond 36 hours, progressively less and less dry matter is lost per unit time since most of the degradable material has already disappeared by this time and the bulk of the material remaining is undegradable.

Ensiling time had no significant effect on the dry matter disappearance of either wheat straw or orchardgrass hay ($P>0.05$). This is in agreement with the results of Jayasuriya and Pearce [37]. The implication of the foregoing is that long ensiling periods have no beneficial effect on digestibility and 3 weeks seems to be enough time for urea to react with the straw [100,101].

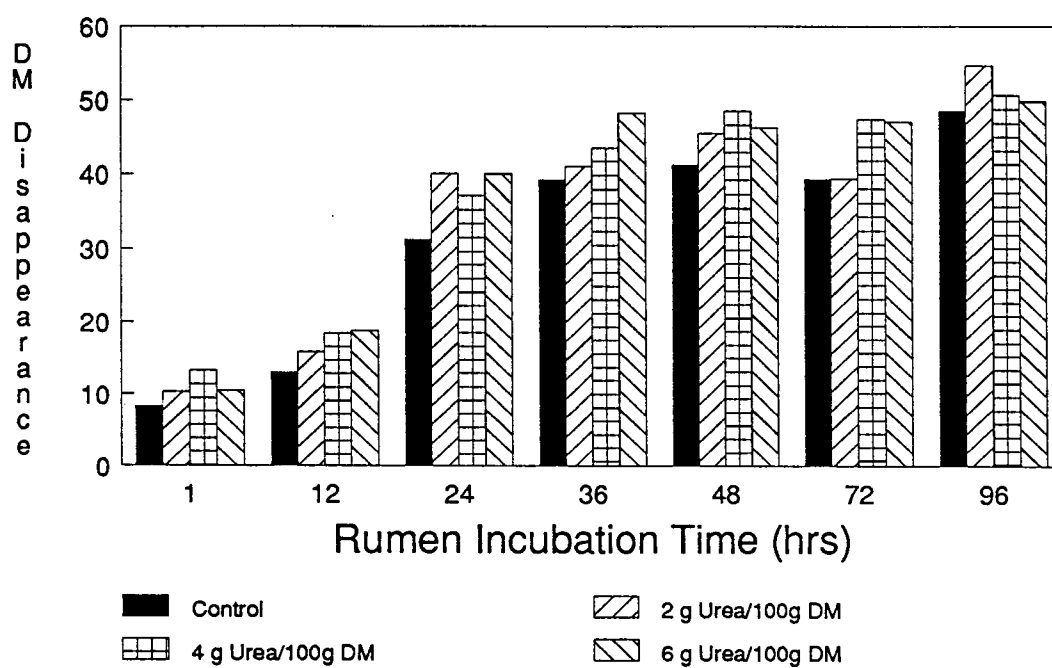


Figure 2.1: Dry matter disappearance from samples of wheat straw incubated in the rumen.

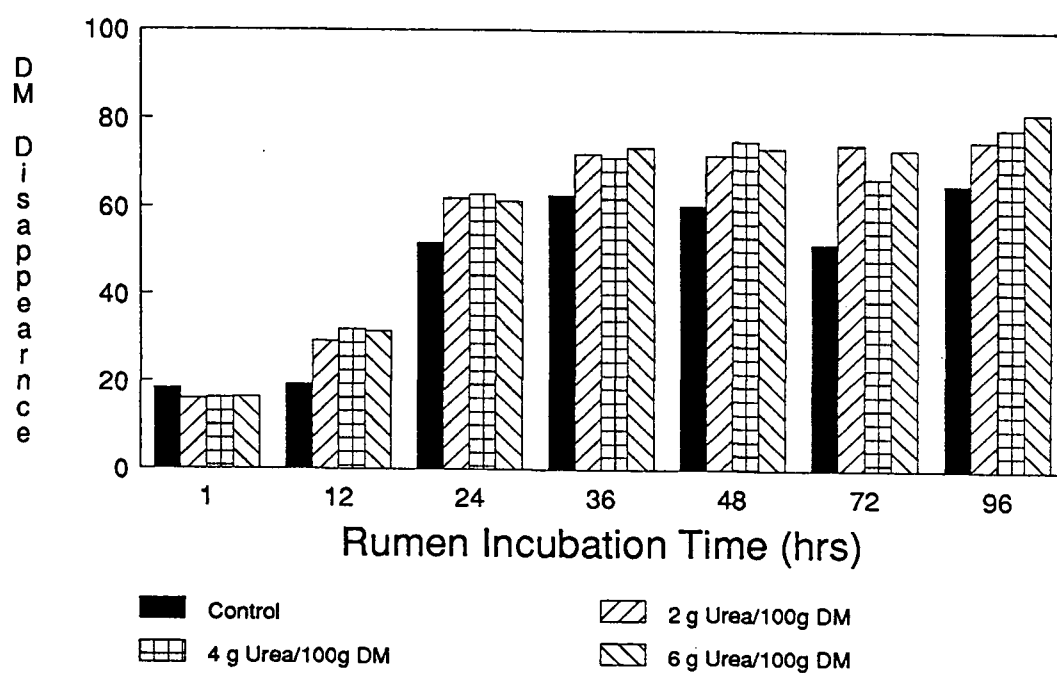


Figure 2.2: Dry matter disappearance from samples of orchardgrass hay incubated in the rumen.

Table 2.2: Percent Dry Matter Disappearance (DMD) after Rumen Incubation

MATERIAL	Incubation Time	Urea Level			
		Control DMD \pm 2.8	2g/100g DM DMD \pm 2.8	4g/100g DM DMD \pm 2.8	6g/100g DM DMD \pm 2.8
Wheat Straw	1 hour	8.28 ^a	10.31 ^a	13.21 ^a	10.45 ^a
	12 hours	12.94 ^a	15.80 ^a	18.38 ^a	18.76 ^a
	24 hours	31.23 ^a	40.06 ^b	37.13 ^{ab}	40.03 ^b
	36 hours	39.23 ^a	41.01 ^{ab}	43.56 ^{ab}	48.25 ^b
	48 hours	41.22 ^a	45.52 ^a	48.52 ^a	46.30 ^a
	72 hours	39.33 ^a	39.36 ^a	47.42 ^b	47.09 ^b
	96 hours	48.51 ^a	54.66 ^a	50.65 ^a	49.86 ^a
		DMD \pm 4.8	DMD \pm 2.8	DMD \pm 2.8	DMD \pm 2.8
Orchard Grass Hay	1 hour	18.52 ^a	15.97 ^a	16.27 ^a	16.50 ^a
	12 hours	19.33 ^a	29.21 ^{ab}	31.94 ^b	31.58 ^b
	24 hours	51.85 ^a	61.98 ^{ab}	62.95 ^b	61.31 ^{ab}
	36 hours	62.76 ^a	72.06 ^a	71.30 ^a	73.57 ^a
	48 hours	60.40 ^a	71.88 ^b	74.97 ^b	73.46 ^b
	72 hours	51.70 ^a	74.20 ^b	66.67 ^b	73.07 ^b
	96 hours	65.34 ^a	75.11 ^a	77.86 ^b	81.34 ^b

Values in the same row with different superscripts significantly different at $P < 0.05$.

Table 2.3: Percent Crude Protein Content after Ensiling with Different Urea Levels

Material	Urea(g/100g DM)	% CP \pm S.E.
Wheat	0	1.43 ^a \pm 0.64
	2	9.88 ^b \pm 0.64
Straw	4	14.53 ^c \pm 0.64
	6	19.21 ^d \pm 0.51
Orchard	0	13.77 ^e \pm 1.59
Grass	2	23.55 ^e \pm 0.64
	4	27.96 ^f \pm 0.64
Hay	6	32.44 ^g \pm 0.64

Values within the same column with different superscripts are significantly different at $P < 0.05$.

2.3.2 Crude Protein Content

Crude protein content data were analysed with the same model as that used for dry matter degradation. Orchardgrass had hay significantly more ($P < 0.05$) crude protein than wheat straw. Urea treatment increased significantly ($P < 0.05$) the crude protein of both wheat straw and orchardgrass hay. The crude protein content increased as the level of urea applied increased (Table 2.3). Thus urea acts as a source nitrogen besides acting on the cell wall. Ensiling time had no significant effect on the crude protein content of the materials. Jayasuriya and Perera [100] reported similar results for treated rice straw.

2.3.3 Crude Protein Disappearance

This was calculated as the difference in crude protein content of the samples before and after incubation expressed as percentage units. The data were analyzed using the same model as for dry matter disappearance. Crude protein

disappearance was increased by urea treatment, however there were no significant differences between the urea levels (Table 2.4). There was more crude protein disappearance from hay samples compared to straw samples.

Crude protein disappearance is expected to increase with increasing rumen incubation time. However this trend was absent in some cases and inconsistent in others. This was especially evident in the case of control wheat straw samples where there was a general increase in the CP content as the rumen incubation time increased (Figures 2.3 and 2.4). Crude protein increased from 1.43% for the unincubated samples to 5.64% for the 48 hr incubated samples. This can only be explained by microbial contamination of the incubated samples. In the case of the urea treated samples the crude protein content ranged from 9.88 to 20.42% (Table 2.4). In these samples there was a general decrease in CP content as rumen incubation time increased although the trend was not consistent in some cases.

In the case of orchardgrass hay the CP content of the unincubated samples ranged from 14.65% for control samples to 32.44% for the samples treated with 6 g urea/100 g DM. Crude protein content decreased as the rumen incubation time increased and the trend was much more consistent in this case (Figures 2.3 and 2.4). In spite of careful washing, the washed bag residue can still contain appreciable amounts of microbial material [102,103,104]. This can result in large errors when one is studying nitrogen degradation of feeds ([104]. Using ^{35}S incorporation into microbes, Mathers and Aitchison [104] were able to obtain the first quantitative estimate of microbial contamination of nylon bag residues. Their study showed a continuous increase in microbial nitrogen in the bag residue with increasing incubation time in the rumen. Varvikko

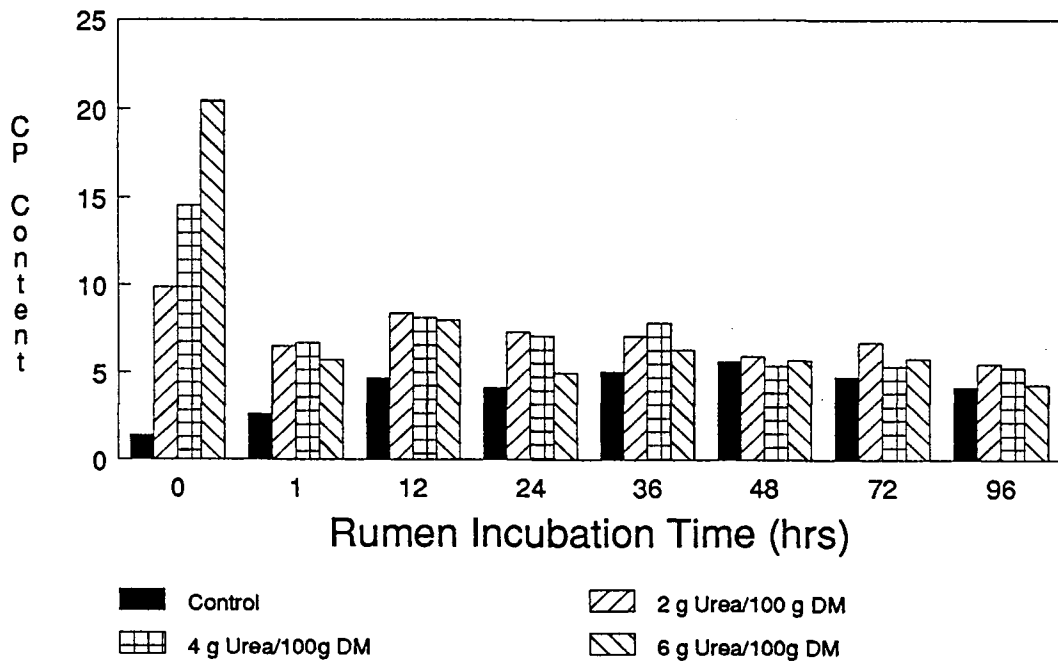


Figure 2.3: Crude protein content of wheat straw samples before and after incubation in the rumen.

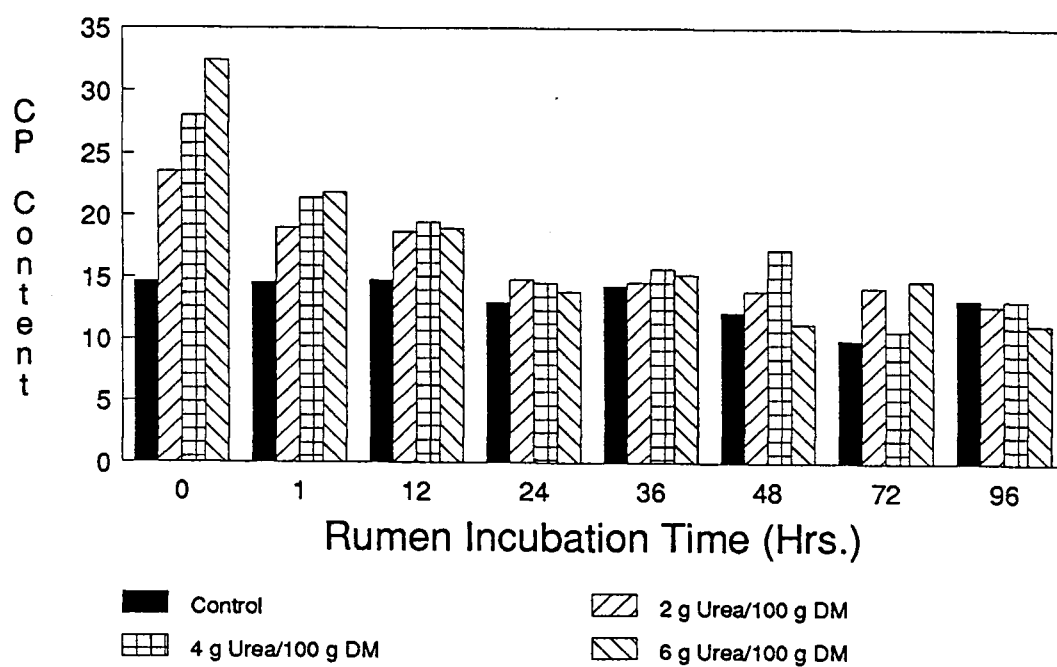


Figure 2.4: Crude protein content of orchardgrass hay samples before and after incubation in the rumen.

and Lindberg [105] estimated microbial contamination of nylon bag residues using ^{15}N -labelled feeds and observed that only small errors can be expected in estimates of nitrogen degradation due to microbial contamination in protein supplements and highly digestible forages. However in roughages with low nitrogen and slow degradability e.g. straw, errors can be high. Rooke et al. [106] measured microbial contamination of silage dry matter remaining in polyester bags and found that it ranged from 26 to 290 g N/kg residual N (2.6 and 29%) for 2 and 48 hours incubation respectively. As can be seen from figure 2.3 and 2.4, in some cases the CP content increased as the length of incubation increased and this is contrary to what is expected. This is a clear indication of microbial contamination. Degradation can also produce a concentrating effect on the CP in low quality roughages. This is because most of the nitrogen is lignin-bound and undegradable, so as degradation proceeds, the nitrogen becomes a bigger fraction of the dry matter which is undegraded. It is reasonable to assume that there was contamination even in the other cases but it was masked by the fact that the initial samples had a higher crude protein content and thus any microbial contamination would be a small fraction of the total CP. Thus the suitability of the *in sacco* technique to estimate nitrogen degradability of low protein feeds without correcting for microbial contamination is questionable.

2.3.4 Acid Detergent Fibre

The same model used to analyze dry matter degradation data was used for ADF values. Orchardgrass hay had significantly lower ADF values than wheat straw ($P > 0.05$) (Table 2.5). Increasing the ensiling period to 9 weeks increased the ADF significantly ($P < 0.05$) and ($P < 0.05$) for wheat straw and orchardgrass hay

Table 2.4: Crude Protein Disappearance (Percentage Units) After Rumen Incubation

Incubation Time	Urea g/100g DM	Wheat Straw Mean \pm 1.3	Orchardgrass Hay Mean \pm 1.3
1 Hour	0	-1.190 ^a	0.278 ^a
	2	3.354 ^b	4.575 ^{ab}
	4	7.825 ^c	6.563 ^b
	6	14.673 ^d	10.603 ^c
12 Hours	0	-3.569 ^a	0.062 ^a
	2	1.495 ^b	4.899 ^{ab}
	4	7.438 ^c	8.523 ^b
	6	12.415 ^d	13.503 ^c
24 Hours	0	-2.724 ^a	1.896 ^a
	2	2.575 ^b	8.730 ^b
	4	6.690 ^c	13.418 ^c
	6	15.419 ^d	18.597 ^d
36 Hours	0	-3.598 ^a	0.507 ^a
	2	2.803 ^b	8.934 ^b
	4	6.690 ^c	12.299 ^b
	6	14.107 ^d	17.249 ^c
48 Hours	0	-4.209 ^a	2.669 ^a
	2	3.933 ^b	9.645 ^b
	4	9.164 ^c	10.727 ^b
	6	14.677 ^d	21.233 ^c
72 Hours	0	-3.325 ^a	4.892 ^a
	2	3.153 ^b	9.358 ^a
	4	9.164 ^c	17.326 ^b
	6	14.618 ^d	17.705 ^b
96 Hours	0	-2.771 ^a	1.662 ^a
	2	4.362 ^b	10.851 ^b
	4	9.252 ^c	14.833 ^c
	6	16.083 ^d	21.267 ^d

Values in the same column within the same incubation time with different superscripts are significantly different at $P < 0.05$.

respectively (Table 2.5).

Urea treatment decreased the ADF content of wheat straw and as urea level increased, the ADF decreased. However there were no significant differences between the ADF values for 2 and 4 g urea levels ($P>0.05$); but increasing the urea level from 4 to 6 g/100g DM decreased significantly the ADF values ($P<0.05$). In the case of orchardgrass hay, urea treatment decreased the ADF values but there were no significant differences between the urea levels ($P>0.05$). High ADF values are generally associated with low digestibility and thus a reduction in ADF content would be expected to increase the digestibility of both wheat straw and the hay.

2.3.5 Acid Detergent Fibre Disappearance

Acid detergent fibre disappearance was the amount of ADF that was lost due to degradation of the incubated samples. It was calculated as the difference between the amount of ADF in the sample before incubation and the amount of ADF in the residue after incubation, expressed as a percentage of the amount of ADF before incubation.

Analysis of variance was done using the same model as for dry matter disappearance. In terms of ADF disappearance, orchardgrass hay and wheat straw were significantly different ($P<0.05$) and more ADF disappeared (*in situ*) from the hay compared to the straw. This is also a reflection of *in situ* DM disappearance; hay had significantly larger DM disappearance values than wheat straw. Acid detergent fibre disappearance will follow very closely dry matter disappearance as ADF is part of the DM that disappears due to degradation.

Disappearance of ADF increased as the rumen incubation time increased from

Table 2.5: Percent ADF Content of Forage After Ensiling With Different Levels of Urea

Material	Urea(g/100g DM)	LSMEAN \pm S.E.
Wheat	0	57.63 ^e \pm 0.41
	2	52.86 ^d \pm 0.41
Straw	4	51.89 ^d \pm 0.41
	6	48.92 ^c \pm 0.41
Orchard	0	36.78 ^b \pm 0.41
Grass	2	34.14 ^a \pm 0.41
	4	33.85 ^a \pm 0.41
Hay	6	34.59 ^{ab} \pm 0.41

Values within the same column with different superscripts are significantly different at $P < 0.05$.

1 hour to 96 hours (Table 2.6). However there were no significant differences between 36, 48 and 72 hours incubation values ($P > 0.05$). The greatest amount of ADF disappeared between 1 and 24 hours in the case of wheat straw. In the case of orchardgrass hay there was no significant differences between ADF loss values for 36, 48 and 72 hours. The greatest amount of ADF disappeared between 12 and 24 hours incubation. For both orchardgrass hay and wheat straw, very little ADF disappeared during the first hour of incubation and the ADF disappearance values were not significantly different from zero ($P > 0.05$). This is to be expected in view of the fact very little dry matter disappeared during the first hour of incubation. The bulk of what is lost during such a period is the 'a' fraction, while ADF is part of the 'b' fraction which requires more time before the microbes can start its degradation.

Table 2.6: Percent ADF Disappearance After Rumen Incubation (Means \pm 3.9)

MATERIAL	Incubation Time	Urea Level			
		Control	2g/100g DM	4g/100g DM	6g/100g DM
Wheat	1 hour	-1.62 ^a	-2.33 ^a	1.73 ^a	-6.52 ^a
	12 hours	7.37 ^a	15.46 ^a	20.72 ^b	7.55 ^a
	24 hours	19.47 ^a	28.99 ^a	24.57 ^a	22.08 ^a
	36 hours	31.13 ^a	23.71 ^a	28.69 ^a	31.74 ^b
Straw	48 hours	33.97 ^a	31.79 ^a	35.09 ^a	29.47 ^a
	72 hours	31.20 ^a	31.64 ^a	42.41 ^b	31.35 ^a
	96 hours	48.88 ^b	44.95 ^b	44.10 ^{ab}	33.39 ^a
Orchard	1 hour	-1.28 ^a	2.13 ^a	6.08 ^a	-0.89 ^a
	12 hours	8.91 ^a	8.68 ^a	12.23 ^a	10.44 ^a
	24 hours	42.50 ^a	46.40 ^a	44.61 ^a	35.03 ^a
	36 hours	45.23 ^a	58.67 ^a	57.28 ^a	59.34 ^a
Grass	48 hours	44.79 ^a	55.64 ^{ab}	61.37 ^b	56.73 ^{ab}
	72 hours	44.56 ^a	60.31 ^b	50.75 ^{ab}	61.12 ^b
	96 hours	49.79 ^a	61.84 ^{ab}	65.38 ^b	69.01 ^b

Values in the same row with different superscripts are significantly different at $P < 0.05$.

2.4 Summary and Conclusions

Orchardgrass hay was potentially more degradable than wheat straw and it gave a better response to treatment than the straw. The degradability of both materials was increased by urea treatment and there was a trend towards an increase in response as the level of urea increased. However the differences were not significant ($P > 0.05$).

Ensiling beyond three weeks was not beneficial in terms of improved degradability; a short treatment period is an advantage in any treatment program. Urea treatment increased the crude protein content of both materials and this is one of the advantages of using urea for ammoniation. Data on CP degradation indicated considerable microbial contamination of the incubated samples and goes to emphasize the need to correct for such contamination in order to obtain valid data for CP degradation, especially in materials that are low in crude protein. Due to microbial contamination of incubated samples, dry matter disappearance is underestimated.

Chapter 3

TREATMENT OF KENYAN SAMPLES USING UREA

3.1 Introduction

In Kenya, crop by-products are fed to ruminant animals especially in the dry season when there is a shortage of forage. Chemical treatment of some crop by-products using sodium hydroxide, ammonia and 'magadi' soda (a natural salt deposit of sodium sesquicarbonate) have shown that it is possible to improve their nutritive value and intake [107]. However routine treatment of such by-products is constrained by such factors as cost of the chemicals, handling problems and the need for capital investment. Urea would therefore be more convenient and cheaper to use especially by small scale farmers who cannot afford the more elaborate treatment methods.

For effective use of by-products knowledge of their nutritive value and their response to treatment is essential and there is a need to gather more data concerning the same. Samples of some of the main crop by-products and a few forages were therefore collected in Kenya during the summer of 1987. The materials were passed through a forage chopper and samples weighing about 300 g were taken.

3.1.1 Objectives

1. To determine the degradability of the roughages before treatment
2. To determine the response to urea treatment in terms of degradability

The materials consisted of the following:

- Oat straw *Avena sativa*, Rhodesgrass hay *Chloris gayana*
- Maize stover *Zea mays*, Wheat straw *Triticum aestivum*
- Rice straw *Oryza sativa*, Barley straw *Hordeum vulgare*
- Alfalfa *Medicago sativa*, Napier grass *Pennisetum purpureum*
- Pyrethrum Marc *Chrysanthemum cinerariaefolium*

Pyrethrum marc is a by-product of processing of pyrethrum flowers to extract pyrethrins and in Kenya it is used as a protein source for ruminant feeding.

3.2 Materials and Methods

Dry matter content of all the different materials was determined. The materials except alfalfa, pyrethrum marc and napier grass were treated with urea. One hundred g of each material and 6 g urea were weighed. The urea was dissolved in enough water to raise the moisture content of the material to 20%. Three g soybean meal were also weighed and included. Small amounts of each material were put in plastic bags and some of the urea solution was sprayed using a small hand sprayer. Some of the soybean meal was added followed by thorough mixing. This was repeated until all the appropriate material, soybean and urea solution were in the plastic bag; the bag was then sealed. The procedure was

repeated for the different materials. The bags remained sealed for six weeks before being opened for subsequent analysis. Samples of treated and untreated materials were ground through a 2 mm screen using a C&N mill.

3.2.1 Rumen Incubations

These were carried out as described in Chapter 2. Degradation was measured as the difference between the dry matter incubated and the dry matter remaining after incubation.

3.2.2 Laboratory Analysis

The incubated duplicate samples were composited before being analyzed for dry matter, crude protein and ADF using the same procedures described in Chapter 2. Unincubated samples were also analyzed for the same components.

3.3 Results and Discussion

The results for alfalfa, napier grass and pyrethrum marc are presented in appendix A.

3.3.1 Dry Matter Degradation

Similar procedures as those described in Chapter 2 were used to describe the degradation of the incubated samples. For the control samples, rhodesgrass hay had the largest 'a', 'b' and 'a+b' fractions; 13.59, 61.63 and 75.22% respectively. Barley straw had the smallest fractions; 7.16, 37.71 and 44.87% for 'a', 'b' and 'a+b' respectively. This ranking changed after urea treatment with rice

straw having the largest 'a' fraction (22.11%) and also the largest "a+b"(92.91). Wheat straw had the largest 'b' (74.71%) while barley straw had the smallest fractions; 60.65, 75.94% for 'b' and 'a+b' fractions respectively.

Treatment increased significantly ($P < 0.05$) the "a" fraction in barley, oat and rice straws. The largest increase occurred in rice straw; from 10.24 to 22.11% for control and treated samples respectively. In the case of maize stover, wheat straw and rhodesgrass hay, treatment had no significant effect ($P > 0.05$) on the 'a' fraction.

The 'b' fraction of all samples except rhodesgrass hay was increased significantly ($P < 0.05$) by urea treatment. Rice straw exhibited the largest increase from 46.27 to 70.79%, a 53% increase. Wheat straw was ranked second with an increase of 46.5%. Rhodesgrass hay had the smallest increase in 'b' fraction; 61.63 to 69.41%, an increase of 12.6%. In general, those materials with a small initial 'b' fraction exhibited the largest increase in the same after urea treatment, (Table 3.1).

Treatment had no significant effect on 'c' except in the case of maize stover. For rhodesgrass hay, treatment effect on the 'c' was close to being significant. So for materials which are relatively more degradable, it seems urea will have a moderate effect on the 'b' fraction but a bigger impact on the rate at which the fraction is degraded, (Table 3.1).

The asymptote (a+b) was increased significantly ($P < 0.05$) by urea treatment. The net increases were larger for those materials with low initial degradation constants than for those with high initial degradation constants. Rice straw showed the largest increase of 36.40 percentage units while rhodesgrass hay had the smallest increase of 11.91 percentage units, (Table 3.1).

Table 3.2 shows dry matter disappearance (DMD) values after various rumen incubation times. Analysis of the data showed that urea treatment increased the dry matter disappearance of all the materials. With each increase in incubation time, there was a resultant increase in dry matter disappearance, (Figures 3.1 and 3.2).

Urea treatment had the biggest impact on rice straw but no significant impact ($P>0.05$) on rhodesgrass hay. Materials with low initial DMD values were generally improved to a greater extent than those with higher initial DMD values. This is in agreement with the results of Tuah et al. [108]. Thus urea treatment was effective in improving the degradability of the various roughages and this is clearly demonstrated by both degradation constants and the dry matter disappearance values.

3.3.2 Acid Detergent Fibre Content

With the exception of barley straw, urea treatment had no significant effect ($P>0.05$) on the acid detergent fibre content of all the materials studied, (Table 3.3). Wilkinson and Gonzalez-Santillana [51] observed a decrease in neutral detergent fibre (NDF) as the level of NaOH increased but noted that acid detergent fibre (ADF) did not decrease with increasing level of sodium hydroxide. There was a wide range ADF content among the materials and there were significant differences ($P<0.05$). Rice straw had the highest ADF; 60.79% while rhodesgrass hay had the lowest; 41.77%, (Table 3.3).

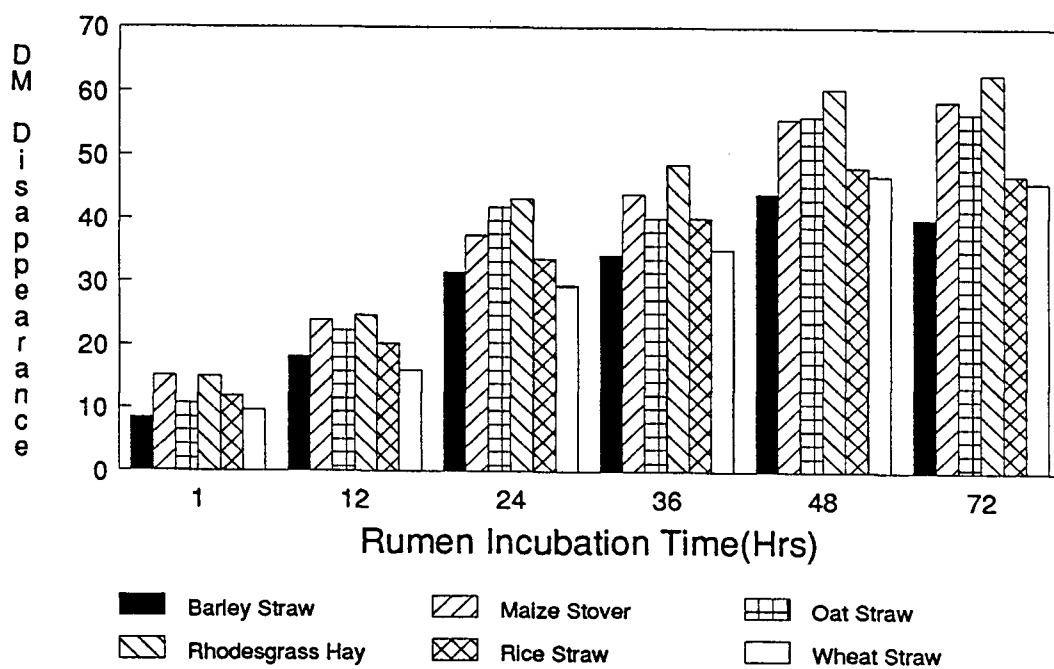


Figure 3.1: Percent dry matter disappearance from control samples of different materials, incubated in the rumen.

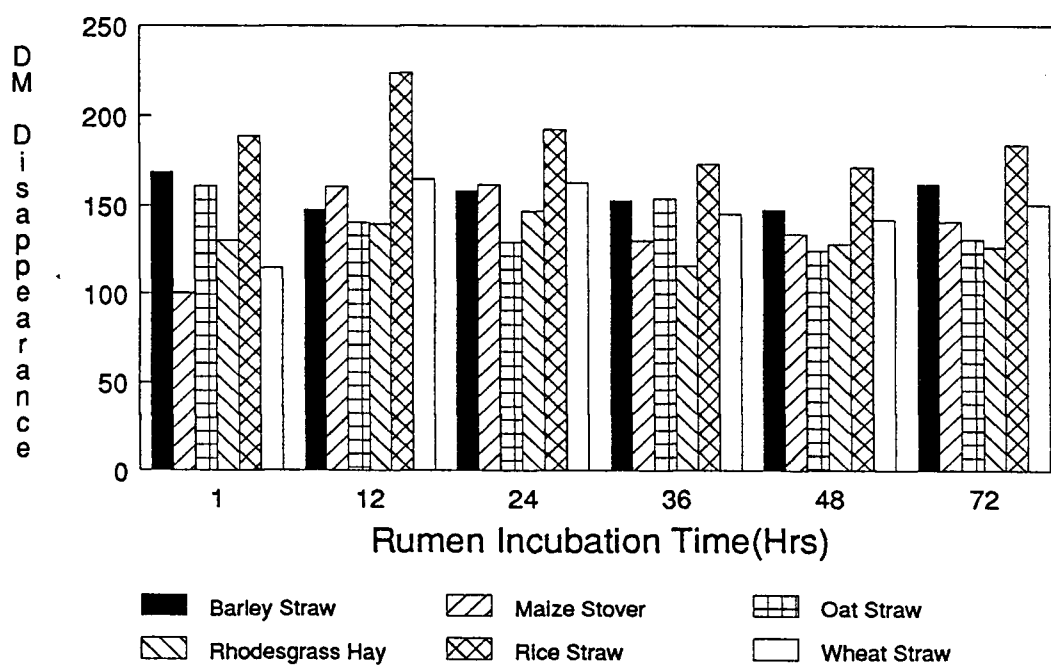


Figure 3.2: Dry matter disappearance from treated samples of different materials incubated in the rumen, expressed as a percent of the dry matter disappearance from control samples.

Table 3.1: Degradation Constants

MATERIAL	Degradation Constants			
	a \pm 1.6	b \pm 3.1	c \pm 0.01	a+b \pm 3.5
Barley Straw				
Control	7.16 ^a	37.71 ^a	0.039 ^a	44.87 ^a
Treated [§]	12.29 ^b	60.65 ^b	0.034 ^a	75.94 ^b
Maize Stover				
Control	13.55 ^a	58.28 ^a	0.023 ^a	71.83 ^a
Treated [§]	14.79 ^a	73.81 ^b	0.040 ^b	88.59 ^b
Oat Straw				
Control	9.06 ^a	54.98 ^a	0.033 ^a	64.02 ^a
Treated [§]	15.30 ^b	67.81 ^b	0.033 ^a	83.11 ^b
Rhodesgrass				
Control	13.59 ^a	61.63 ^a	0.026 ^a	75.22 ^a
Treated [§]	17.73 ^a	69.41 ^a	0.032 ^a	87.13 ^a
Rice Straw				
Control	10.24 ^a	46.27 ^a	0.031 ^a	56.51 ^a
Treated [§]	22.11 ^b	70.79 ^b	0.036 ^a	92.91 ^b
Wheat Straw				
Control	7.70 ^a	51.00 ^a	0.025 ^a	58.70 ^a
Treated [§]	10.21 ^a	74.71 ^b	0.025 ^a	84.92 ^b

§Treated with 6g Urea/100g DM and ensiled for six weeks.

Values within the same column within the same material with different superscripts are significantly different at P<0.05.

Table 3.2: Percent Dry Matter Disappearance after Rumen Incubation

MATERIAL	Rumen Incubation Time—Hours					
	1	12	24	36	48	72
Barley Straw						
Control	8.46	18.21	31.53	34.29	44.11	40.10
Treated	14.17	26.87	49.83	52.35	64.97	64.95
Maize Stover						
Control	15.09	23.87	37.36	43.97	55.64	58.60
Treated [§]	15.14	38.34	60.38	57.10	74.31	82.40
Oat Straw						
Control	10.68	22.28	41.88	40.12	56.04	56.63
Treated [§]	17.18	31.28	53.98	61.73	69.45	73.87
Rhodesgrass						
Control	15.00	24.63	43.17	48.59	60.40	62.70
Treated [§]	19.52	34.34	63.37	56.12	77.21	78.97
Rice Straw						
Control	11.89	20.21	33.74	40.15	48.20	46.98
Treated [§]	22.43	45.27	64.86	69.46	82.64	86.41
Wheat Straw						
Control	9.66	15.95	29.38	35.20	46.85	45.87
Treated [§]	11.00	26.28	47.80	51.03	66.48	68.96

[§]Treated with 6g Urea/100g DM and ensiled for six weeks.

3.3.3 Acid Detergent Fibre Disappearance

This was calculated as the difference between the amount of ADF in the sample before incubation and the amount of ADF after incubation as a percent of the amount of ADF before incubation.

The data were analyzed using the general linear model of SAS [97] with rumen incubation time and treatment as the main effects. There was an increase in ADF disappearance with increasing rumen incubation time, (Table 3.4). Very little ADF disappeared during the first hour of incubation. However the other incubation times caused significant ADF disappearance, (Table 3.4). There was no significant difference between ADF disappearance for 24 hours and 36 hours incubation nor was there any significant difference ($P>0.05$) between 48 and 72 hours ADF disappearance, (Table 3.4).

Urea treatment increased ADF disappearance significantly ($P<0.05$). There was a significant increase in ADF disappearance with increasing rumen incubation time and this increase was greater with urea treatment. This was a reflection of dry matter disappearance which increased as the rumen incubation time increased. Thus the pattern of ADF disappearance followed very closely that of dry matter disappearance, (Figures 3.3 and 3.4).

3.3.4 Crude Protein Content

Urea treatment increased significantly ($P<0.05$) the crude protein content of all the different materials, (Table 3.5). This is in agreement with the results of others [100,37,46,38]. For the untreated materials, rhodesgrass hay had the

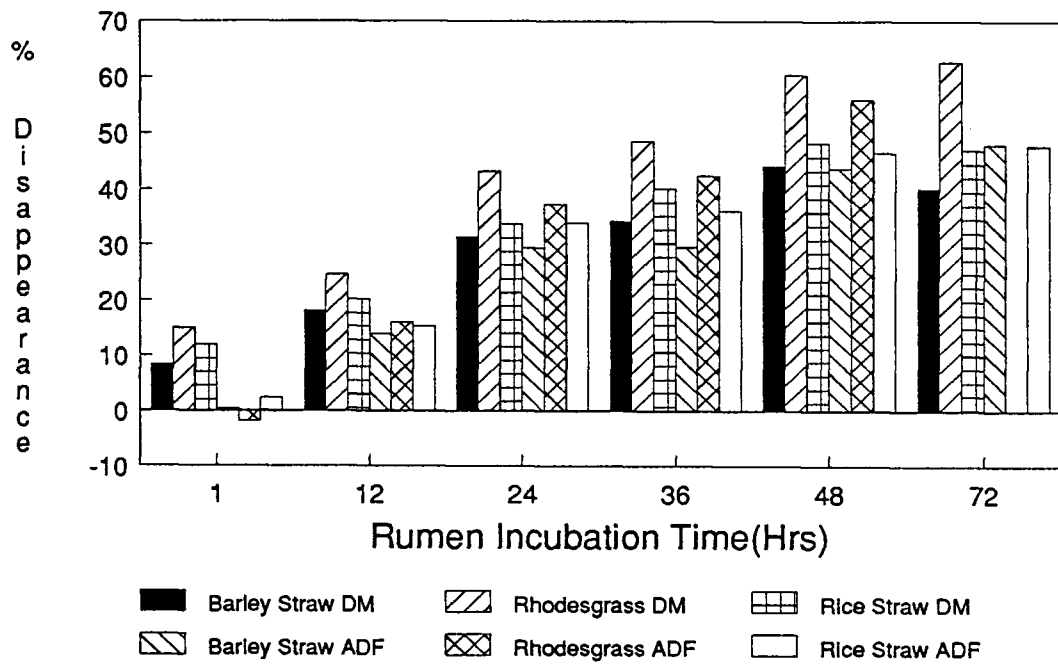


Figure 3.3: Dry matter and ADF disappearance from control samples incubated in the rumen.

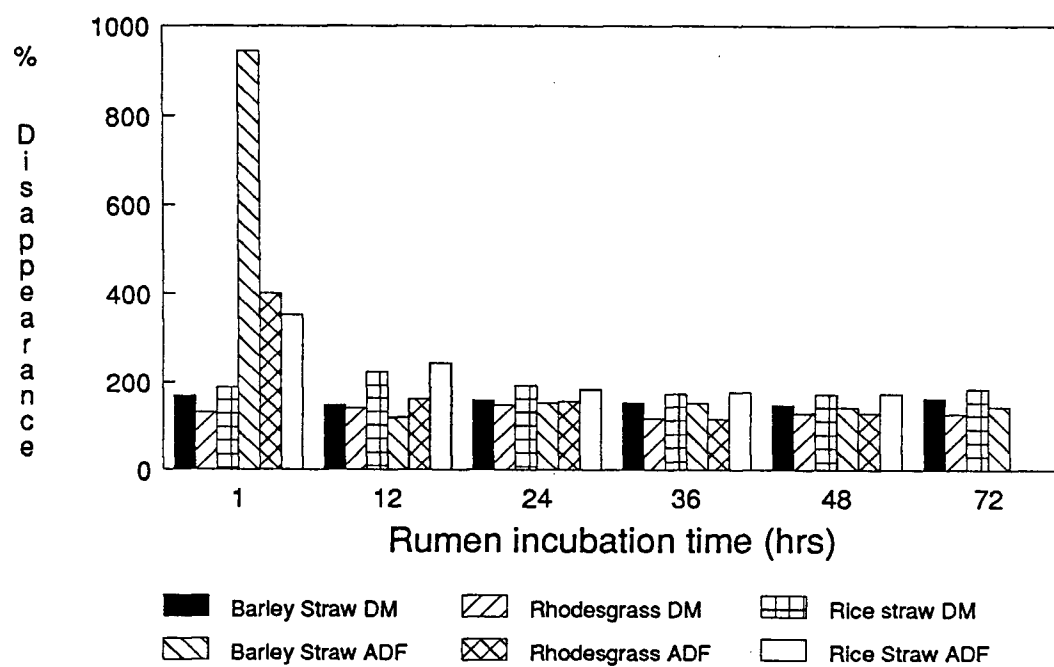


Figure 3.4: Dry matter and ADF disappearance from treated samples expressed as a percent of the disappearance from control samples.

Table 3.3: Percent ADF Content

MATERIAL	UNTREATED	TREATED [‡]
	MEAN ADF \pm 0.547	MEAN ADF \pm 0.547
Barley Straw	60.75 ^f	58.92 ^{ef}
Maize Stover	48.58 ^b	50.27 ^c
Oat Straw	57.70 ^{de}	57.05 ^d
Rhodesgrass	41.77 ^a	42.06 ^a
Rice Straw	60.79 ^f	59.80 ^{ef}
Wheat Straw	58.69 ^{def}	58.72 ^{def}

Values with different superscripts are significantly different at $P < 0.05$.

[‡]Treated with 6g urea/100g DM and ensiled for six weeks.

highest crude protein content; 11.82% followed by maize stover with a CP content of 6.56%. The crude protein content of barley straw was not significantly different ($P > 0.05$) from that of rice straw; 3.12 and 3.09% respectively. Wheat straw and oat straw had similar crude protein content; 2.51 and 2.32% respectively. Besides increasing the degradability of fibrous material, urea effectively elevated the nitrogen content of the treated material.

3.3.5 Crude Protein Disappearance after Rumen Incubations

Crude protein disappearance would be expected to increase as rumen incubation time increased due to degradation over a longer period of time. From Table 3.6 and from Figures 3.5 and 3.6, this trend is absent and this is more apparent in the control samples.

One hour incubation caused a decrease in crude protein content in all the materials except oat straw. This represents the soluble CP which is part of the

Table 3.4: Percent ADF Loss after Rumen Incubation

MATERIAL	Rumen Incubation Time-Hours					
	1	12	24	36	48	72
Barley Straw						
Control	0.36	13.98	29.44	29.62	43.69	47.98
Treated [§]	3.40	16.47	44.62	44.94	61.71	68.35
Maize Stover						
Control	3.26	15.65	36.29	36.47	50.11	60.34
Treated [§]	-2.05	26.74	53.35	47.45	69.51	—
Oat Straw						
Control	-0.07	16.93	32.93	41.86	52.89	58.61
Treated [§]	4.34	22.50	49.98	57.24	64.83	—
Rhodesgrass						
Control	-1.91	16.02	37.35	42.40	55.99	—
Treated [§]	5.73	25.76	58.08	48.39	72.25	75.20
Rice Straw						
Control	2.34	15.35	34.00	36.18	46.53	47.74
Treated [§]	8.24	37.52	62.34	64.00	80.23	—
Wheat Straw						
Control	2.75	11.15	28.38	31.23	44.52	52.74
Treated [§]	-0.68	17.22	44.49	46.81	65.56	71.317

[§]Treated with 6g Urea/100g DM and ensiled for six weeks.

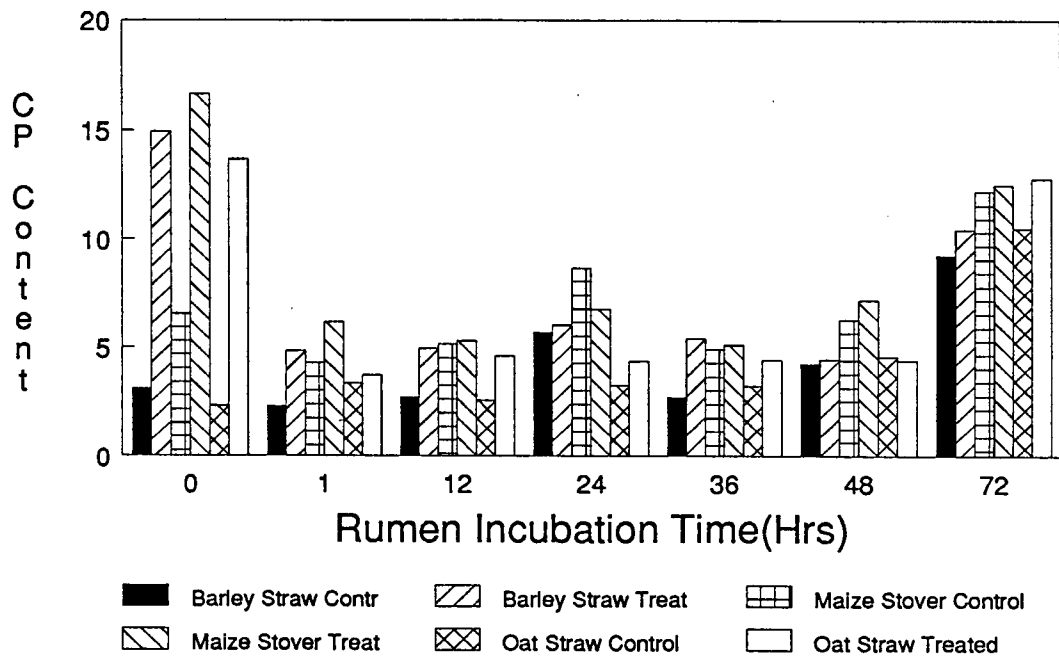


Figure 3.5: Percent CP content of samples before and after incubation in the rumen.

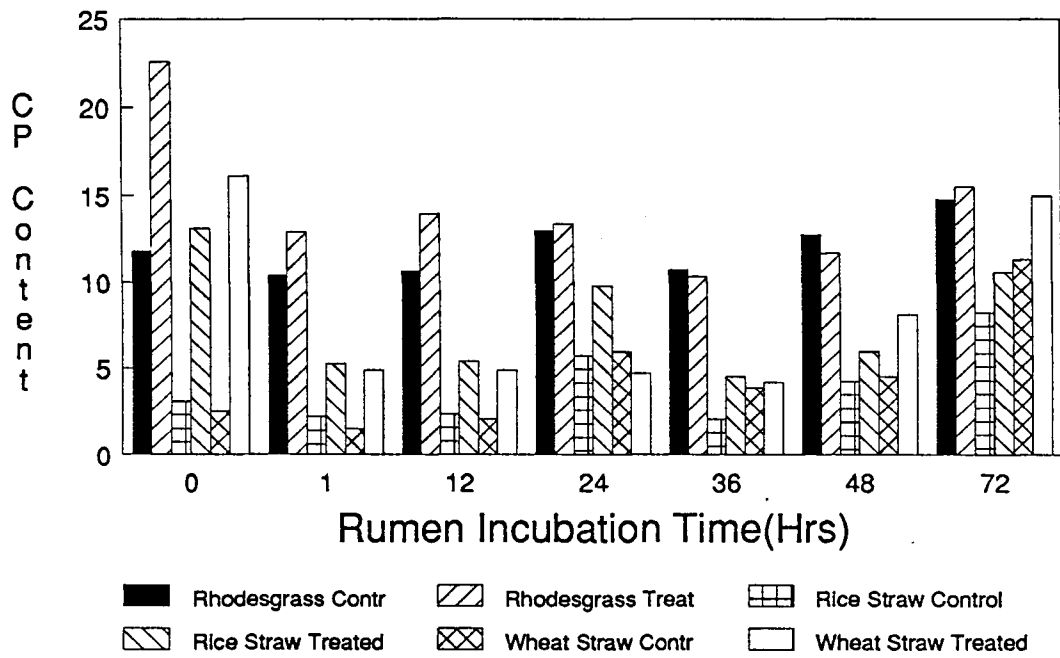


Figure 3.6: Percent CP content of samples before and after incubation in the rumen.

rapidly disappearing 'a' fraction. Longer incubation times resulted in a general increase in the CP content when compared to the CP content after one hour incubation. This can be explained in terms of microbial contamination of the incubated samples. This is apparent in the case of the control samples where 24 and 72 hours incubation resulted in the samples having more CP than the original material; thus the crude protein disappearance in such cases was negative. The untreated samples had considerably less crude protein when compared to the treated samples. Thus any microbial contamination made a bigger impact on the crude protein content of such samples than on the treated samples. For the latter, there was a general decrease in CP as rumen incubation time increased but the pattern was not consistent. In spite of careful washing, the washed bag residue can still contain appreciable amounts of bacterial material [54,102,104]. Using ^{35}S incorporation into microbes, Mathers [104] were able to quantify microbial contamination of nylon bag residues. They noted a continuous increase in microbial nitrogen in the residue with increasing rumen incubation time. Varvikko [105] estimated microbial contamination of the residue in the bags using ^{15}N -labelled feeds. They observed that only small errors can be expected in estimates of nitrogen degradation due to microbial contamination in proteins and highly digestible forages. However, in roughages with low nitrogen and slow degradability e.g. straw, errors can be high. Rooke [106] estimated microbial contamination of silage remaining in polyester bags and found that the degree of contamination increased with increasing rumen incubation time. This is in agreement with results of others [54,109,110,111]. It is reasonable to assume that there is also contamination in high protein samples incubated in the rumen. However the initial level of nitrogen in such samples and their degradability are high enough to mask an increase in nitrogen content

Table 3.5: Percent Crude Protein Content

MATERIAL	CONTROL	TREATED [†]	S.E.
	MEAN CP	MEAN CP	
Barley Straw	3.12 ^b	14.95 ^f	0.059
Maize Stover	6.56 ^c	16.62 ^g	0.059
Oat Straw	2.32 ^a	13.68 ^e	0.059
Rhodesgrass	11.82 ^d	22.57 ^h	0.059
Rice Straw	3.09 ^b	13.13 ^e	0.059
Wheat Straw	2.51 ^a	16.10 ^g	0.059

Values with different superscripts are significantly different at $P < 0.05$.

[†]Treated with 6g urea/100g DM and ensiled for six weeks

due to rumen microorganisms. All in all, the nylon bag technique is not suitable for studying degradation of nitrogen in fibrous feeds e.g. straw, without reliable estimates of microbial content of the residue in the bags [110].

3.4 Summary and Conclusions

Before treatment, rhodesgrass hay and maize stover had the highest potential degradabilities while barley straw had the lowest, . Urea treatment did not have the same effect on the different materials; rice straw showed the greatest response with an appreciable increase in its potential degradability. Rhodesgrass hay and maize stover exhibited the least response in terms of improvement in their potential degradabilities. Treatment had no significant effect ($P > 0.05$) on the rate of degradation ; 'c' except for maize stover.

Urea treatment was effective in improving the degradability of the various roughages and this is clearly demonstrated by both degradation constants and

Table 3.6: Crude Protein Disappearance after Rumen Incubation

MATERIAL	Rumen Incubation Time-Hours					
	1	12	24	36	48	72
Barley Straw						
Control	0.82	0.40	-2.59	0.41	-1.13	-6.12
Treated§	10.12	10.01	8.91	9.53	10.50	4.51
Maize Stover						
Control	2.27	1.42	-2.12	1.65	0.26	-5.64
Treated§	10.43	11.31	9.85	11.51	9.43	4.12
Oat Straw						
Control	-1.03	-0.25	-0.94	-0.91	-2.26	-8.18
Treated§	9.93	9.08	9.29	9.23	9.28	0.88
Rhodesgrass						
Control	1.41	1.60	-1.15	1.10	-0.92	-2.94
Treated§	9.66	8.60	9.19	12.25	10.91	7.08
Rice Straw						
Control	0.91	0.73	-2.63	1.03	-1.16	-5.15
Treated§	7.78	7.73	8.34	8.63	7.18	2.54
Wheat Straw						
Control	1.00	0.47	-3.43	-1.34	-2.00	-8.80
Treated§	11.22	11.22	11.40	11.90	7.98	1.14

§Treated with 6g urea/100g DM and ensiled for six weeks.

the dry matter disappearance values. Treatment generally had more effect on materials with low degradabilities. Besides improving degradability, urea treatment also increased the CP content of all the materials.

One the short comings of the nylon bag technique is microbial contamination of the residue in the bags. Therefore, to get a true picture of degradation, correction for this contamination is necessary.

Chapter 4

UTILIZATION OF UREA TREATED BARLEY STRAW BY YOUNG CALVES

4.1 Introduction

Different methods have been developed to improve the nutritive value of low quality roughages. To test the effectiveness of any treatment method, in vitro methods can be used. However the ultimate test is the feeding of such treated material to animals. Young animals are particularly suitable in view of the fact that they are growing rapidly and thus are a sensitive way of detecting any benefits accruing from such treatment.

4.2 Objective

This trial was carried out to evaluate the effect of ammoniation of barley straw using urea. Benefits of treatment were monitored in terms of animal performance through such measures as dry matter intake, digestibility and weight gain.

4.3 Materials and Methods

4.3.1 Preparation of the Straw

Core samples were taken to determine the dry matter of the straw so that to determine the amount of water to be added in order to raise the moisture content to 20%. The straw was then passed through a forage chopper and off-loaded into a mixer which had a weighing scale. Urea was added at 4 or 6 kg/100 kg DM; it was first dissolved in enough water to raise the moisture of the straw to 20%. Three kg soya bean meal per 100 kg DM was added followed by thorough mixing (20 minutes). The material was then put into plastic bags which were large enough to contain 150 kg of straw. The bags were sealed and the material allowed to ferment for at least six weeks before it was fed to the calves.

4.3.2 Animals

Sixteen Holstein calves were used; eleven bull calves and five heifer calves. At the start of the experiment their weight ranged from 86 to 176 kg. The calves were ranked according to weight, starting with the heaviest. The first four bull calves were randomly allocated to four different diets and this was repeated until all the eleven bull calves had been allocated. Then the first four heifer calves were randomly allocated to four different diets with the last one going to the group which was short of one calf. So any four calves which were in the same weight category were randomly allocated to the four different diets.

4.3.3 Experimental Design

A randomized complete block design was used with four groups of animals, four experimental diets and three experimental periods. The animal groups were allocated to the diets at random and after the end of each experimental period, the groups were randomly re-allocated to one of the remaining diets. Each experimental group consumed three of the four diets.

4.3.4 Diets

Before the calves were put on to the experimental diets, they were offered orchardgrass hay *ad libitum* and 1 kg concentrate per day for one week. The following week they were offered a combination of hay and untreated straw in a 2:1 ratio. During the third week, the ratio of the hay to straw was changed to 1:1 while concentrate was offered at 1 kg per day; this was an adjustment period. The calves were then put on experimental diets which were:

- Diet A–Barley Straw treated with 40g urea/kg straw DM ensiled for six weeks.
- Diet B–Barley straw supplemented with urea at time of feeding to raise the nitrogen content to the level of nitrogen in diet A.
- Diet C–Barley straw treated with 60 g urea/kg straw DM ensiled for six weeks.
- Diet D–Barley straw supplemented with urea at the time of feeding to raise the nitrogen content to the level of nitrogen in diet C.

Whenever a new silo was opened, its CP content was determined in order to determine the amount of urea to be added to the control diets. Before feeding

the straw was mixed with coarsely chopped orchardgrass hay in a 3:1 ratio (straw:hay). The straw-hay mixture was fed twice every day and it was offered at a level which ensured ad libitum intake. In addition, each calf consumed 1.5 kg of a calf concentrate mixture every day and which was offered in two portions; 1 kg in the morning and 0.5 kg in the afternoon. Twenty g of a mineral mixture was added to the concentrate every morning. The concentrate was offered in a different container from the hay-straw mixture. Straw-hay intake was calculated by weighing the weigh back every morning.

4.3.5 Samples

Every morning samples of the concentrate, the weighback and each of the forage mixtures were taken. The samples collected in each week were put together to make a weekly composite sample. During the last week (fourth week) of each experimental period, three fecal samples from each calf were collected. These were stored in a refrigerator and were later composited to make one sample per calf per experimental period.

4.3.6 Data Collected

Feed offered, the weighback and the intake were recorded every day. The animals were weighed at the beginning of each experimental week and this was done in the morning before the animals were fed.

4.3.7 Rumen Incubations

These were carried out following the methods described in chapter two.

Table 4.1: Chemical composition of the feeds offered to the calves.

Material	% CP	% ADF	% LIGNIN
Concentrate	19.49	7.99	2.88
Orchardgrass Hay	9.39	38.68	4.53
Barley Straw:			
4% urea ensiled	20.39		4.59
4% urea not ensiled	18.77		5.01
6% urea ensiled	19.31		4.35
6% urea not ensiled	18.86		4.63

4.3.8 Laboratory Analysis

The feed samples were dried at 60°C for 12 hours while the fecal samples were dried at 60°C for 60 hours with occasional turning to ensure uniformity in drying. The samples were then ground through a 1 mm screen using a C&N mill. Dry matter, crude protein and acid detergent fibre were determined using standard laboratory methods outlined previously [94,95,93,96]. Acid insoluble ash (AIA) of feed and fecal samples was determined using the method of Van Keulen and Young [112]. The AIA was used as an internal marker for the determination of apparent digestibilities of DM, ADF and CP.

The incubated duplicate samples were composited before being analysed for crude protein and acid detergent fibre [94,95,96].

4.4 Results and Discussion

4.4.1 Dry Matter Intake

The calves consumed 1.5 kg of concentrate (86.42% DM) every day; thus 1.3 kg DM. Since the calves were offered the same amount of concentrate, unless otherwise stated, dry matter intake refers only to the straw/hay combination. Analysis of variance was performed on dry matter intake values ($\text{g/kg } W^{0.75}/\text{day}$) using SAS. Period, urea level and ensiling were the main effects. Period had a significant effect ($P < 0.05$) on dry matter intake. Urea level and ensiling were not significant ($P > 0.05$), (Table 4.2).

Dry matter intake increased as the experiment progressed from period I to period III. This is to be expected since the calves were growing and intake increases with increase in body size. However the dry matter intake for period II and III were not significantly different ($P > 0.05$). Another possible explanation for increase in DM intake may be progressive adaptation of the rumen in favour of fibre digesting microbes as the experiment progressed. There was no significant difference ($P > 0.05$) between the DM intake for period II and III. The rumen may have adjusted fully by this time such that the only factor influencing intake would be the body size. Data from degradation constants indicated that there was an increase in the rate of degradation of the 'b' fraction as the level of urea increased to 6g/100g DM. This may have facilitated higher rumen turnover rates, leading to the increase in DM intake of the ensiled straw which had been treated with 6g urea/100g DM (Table 4.2). Other workers have reported an increase in dry matter intake as a result of straw treatment [113,20,32,40] while others did not observe any improvement in dry matter intake as a result of straw

Table 4.2: Mean Dry Matter Intake(DMI) g/kgW^{0.75}

Main Effect	DMI±5.39
Period I	75.874 ^a
II	92.020 ^b
III	100.73 ^b
Treatment	DMI±6.23
1. 4 g/100g DM [†]	87.86
2. 4 g/100g DM [§]	89.00
3. 6 g/100g DM [†]	91.79
4. 6 g/100g DM [§]	89.52

†Ensiled for six weeks; §not ensiled

Values within the same column with different superscripts are significantly different at P<0.05

treatment [101,46,40].

4.4.2 Apparent Dry Matter Digestibility

Analysis of variance was performed on the dry matter digestibility data using SAS [97]. Ensiling increased significantly (P<0.05) the apparent dry matter digestibility of the straw treated with 6g urea/100g DM, (Table 4.3). This is in agreement with the findings of other workers who have reported that ensiling of the treated material increases the dry matter digestibility as a result of the ammonia liberated from the urea reacting with the straw [40,38,101,46]. Such a reaction is thought to cause cleavage of alkali-labile linkages between structural carbohydrates and lignin [41,42]. The higher digestibility of ensiled urea-treated straw could also have resulted from a sustained release into the rumen of added nitrogen, thus allowing a more intense microbial fermentation. Oji and Mowat [20] as well as Dias-Da-Silva and Sundstol [40] reported a slower release of

Table 4.3: Apparent Dry Matter Digestibility (DMDIG)

Main Effect	DMDIG \pm 0.84
Period I	51.76 ^{ab}
II	50.01 ^a
III	53.08 ^b
Treatment	DMDIG \pm 0.96
1. 4 g/100g DM [†]	52.37 ^a
2. 4 g/100g DM [§]	50.91 ^a
3. 6 g/100g DM [†]	55.48 ^b
4. 6 g/100g DM [§]	47.70 ^c

†Ensiled for six weeks; §not ensiled

Values within the same column with different superscripts are significantly different at $P < 0.05$

bound nitrogen with ammonia treated straws. Such a pattern of release would ensure a fairly uniform rumen ammonia concentration when compared to direct feeding of urea which is highly soluble and thus hydrolyzed within a short time after feeding.

4.4.3 Apparent Crude Protein digestibility

Analysis of variance revealed that period and ensiling time had significant effects on CP digestibility ($P < 0.05$). Urea level was not significant ($P > 0.05$). Periods I and III had significantly higher crude protein digestibility than period two ($P < 0.05$); (Table 4.4). Ensiling reduced the crude protein digestibility significantly ($P < 0.05$). This is in agreement with the findings of Dias-Da-Silva and Sundstol [40] who reported lower apparent nitrogen digestibility for urea treated straw than for straw supplemented with urea at feeding time. This is to be expected since urea is highly soluble and digestible but when it reacts with

Table 4.4: Apparent Crude Protein Digestibility (CPDIG)

Main Effect	CPDIG \pm 0.075
Period I	61.96 ^a
II	55.97 ^b
III	64.76 ^c
Treatment	CPDIG
1. 4 g/100g DM [†]	56.39
2. 4 g/100g DM [§]	67.43
3. 6 g/100g DM [†]	54.01
4. 6 g/100g DM [§]	65.75

[†]Ensiled for six weeks; [§]not ensiled

Values within the same column with different superscripts are significantly different at $P < 0.05$

ensiled material, the nitrogen becomes bound to the material. This nitrogen is not as readily digestible as nitrogen from unreacted urea; thus the decrease in digestibility with ensiling.

4.4.4 Apparent ADF digestibility

Periods II and III had significantly higher apparent ADF digestibilities than period one ($P < 0.05$). This could have been due to rumen microbes becoming adjusted to digesting fibre as the experiment progressed; a shift in rumen microbial population in favour of fibre digesting microbes. Ensiling of the treated straw increased significantly ($P < 0.05$) the apparent ADF digestibility of the straw treated with 6g urea/100g DM compared to its control. For the samples treated with 4 g/100g DM there was no significant difference between the ADF digestibility of the ensiled straw and the control, (Table 4.5). Ensiling is essential in order to give the urea time to breakdown into ammonia which reacts

Table 4.5: Apparent Acid Detergent Fibre Digestibility (ADFDIG)

Main Effect	ADFDIG ± 0.786
Period I	52.32 ^a
II	56.72 ^b
III	54.96 ^b
Treatment	ADFDIG ± 0.99
1. 4 g/100g DM [†]	55.40 ^a
2. 4 g/100g DM [§]	55.44 ^a
3. 6 g/100g DM [†]	56.46 ^a
4. 6 g/100g DM [§]	51.38 ^b

†Ensiled for six weeks; §not ensiled

Values within the same column with different superscripts are significantly different at $P < 0.05$

with the cell walls via swelling effect and the release of phenolic acids, mainly coumaric and ferulic acids [114].

4.4.5 Average Daily Gain

Period had a significant ($P < 0.05$) effect on average daily gain; period I and III had significantly higher average daily gain than period II. There was a trend towards higher daily gains with increase in urea (from 4 to 6 g urea/100g DM) and also with ensiling, (Table 4.6). This can be explained as due to increased potential degradability as measured by 'a+b', improved degradation constants and increased dry matter digestibility due to treatment. Saadullah et al. [38] reported significant increase in daily gain with ensiled-urea treated rice straw over the straw supplemented with urea at feeding time. The straw used in their experiment was mixed with an equal amount of water before ensiling, thus it had a high moisture content and this could have enhanced the effect of urea on

Table 4.6: Average Daily Gain (ADG) kg/day

Main Effect	ADG Kg/day \pm 0.049
Period I	0.956 ^a
II	0.569 ^b
III	0.725 ^c
Treatment	ADG kg/day \pm 0.056
1. 4 g/100g DM [†]	0.705
2. 4 g/100g DM [§]	0.723
3. 6 g/100g DM [†]	0.807
4. 6 g/100g DM [§]	0.768

†Ensiled for six weeks; §not ensiled

Values within the same column with different superscripts are significantly different at $P < 0.05$

the straw.

4.4.6 Degradation Constants

The general linear model of SAS [97] was used to analyze the data for degradation constants with urea and time as the main effects.

4.4.6.1 Rapidly disappearing 'a' fraction

Ensiling time had no significant effect ($P > 0.05$) on the 'a' fraction. Treatment of straw with 4 g urea/100 g material increased the 'a' fraction but at the higher urea level (6 g/100 g material), the 'a' fraction was not significantly different ($P > 0.05$) from that of the control, (Table 4.7).

4.4.6.2 Slowly degradable 'b' fraction

Ensiling increased significantly ($P < 0.05$) the 'b' fraction from 68.62 to 72.84% for control and 6 weeks of ensiling respectively. Thus ammonia from urea reacted with the straw and increased degradability, (Table 4.7). An increase in the 'b' fraction is of benefit to the animals since they would degrade a bigger fraction of the dry matter consumed. Therefore, if other nutrients are not limiting and the rumen environment is optimal for degradation, this increased degradability should lead to improved animal productivity.

4.4.6.3 Rate of degradation of 'b' fraction - 'c'

Effect of urea treatment on rate of degradation was significant (Table 4.7). Degradation rate of the 'b' fraction increased from 12%/hr to 15%/hr for control and 6g urea/100g DM respectively. Thus treated material will have a higher turnover rate than control and therefore a higher dry matter intake as one of the factors affecting intake is gut fill. This is supported by data for dry matter intake, see table 4.2.

4.4.6.4 The Asymptote (a+b)

Ensiling increased the asymptote significantly ($P < 0.05$), (Table 4.7). This is of significance since the asymptote is a measure of the maximum potential degradability. So with the right rumen environment animals will degrade more of the treated straw than control.

Table 4.7: Degradation Constants

Main Effect	Degradation Constants			
Ensiling	a	b	c	a+b
control	15.81 ^a	68.62 ^a	0.014 ^a	84.43 ^a
6 weeks	16.50 ^a	72.84 ^b	0.014 ^a	89.34 ^b
Urea Level	a	b	c	a+b
0 g/100g DM	15.37 ^a	69.76 ^a	0.012 ^a	85.13 ^a
4 g/100g DM	17.27 ^b	70.93 ^a	0.013 ^a	88.20 ^b
6 g/100g DM	15.82 ^a	71.50 ^a	0.015 ^b	87.32 ^b

a=rapidly disappearing fraction, b= insoluble degradable fraction

c= rate at which the b fraction will be degraded per hr

a+b= maximum potential degradability.

Values within the same column with different superscripts are significantly different at $P < 0.05$.

4.5 Summary and Conclusions

Ensiling of treated material did not improve dry matter intake. This was unexpected since there was an improvement in the rate of degradation. This improvement would be expected to allow higher turn over rates which would in turn increase feed intake. Treatment improved the 'b' fraction, the rate of degradation of the 'b' fraction and also the asymptote. In addition, the apparent dry matter digestibility was also improved. These improvements would be expected to produce marked improvements in animal productivity. However data for average daily gain indicate small and insignificant improvements. Probably the improvements in the straw were not big enough to effect significant improvements in weight gain.

Response of material to treatment will vary depending on such factors as type of

material and optimization of treatment conditions. Moisture content influences the effectiveness of ammoniation through the use of urea. However one of the problems that may arise with high moisture material is mould damage. In the present trial, moisture content may have been inadequate for optimization of urea treatment. Another factor which influences animal response is retention time in the rumen. Oji et al. [22] has indicated that the mean retention time of particulate matter is significantly reduced with ammoniation. This will ultimately reduce degradation in the rumen and thus mask the beneficial effect of treatment. The amount of concentrate given can affect the rate of degradation of the straw. Preston [115] has indicated that it should not exceed 20% of the diet DM, otherwise straw degradation is depressed. Good quality forage should be included up to a maximum of 25% of the diet [115]. In the present study the hay and the concentrate constituted more than 45% of the total diet and this may have resulted in a substitution of the digestible energy of the basal diet. This may have masked the beneficial effects of treatment and thus the lack of any remarkable difference between the treated straw and the controls. The review of literature indicated considerable variation both in the way different materials respond to treatment as well as animal response to treated material.

Chapter 5

GENERAL SUMMARY

Different materials have different degradation constants and they respond differently to treatment. This is because the degree of lignification is different in different materials. Response to treatment is determined by various factors among them species, variety, post harvest handling, treatment method employed and the effectiveness of the treatment itself.

Urea treatment improves degradation through an increase in either the fraction which is soluble (a) or the fraction which is insoluble but degradable (b) or both. The least affected of the degradation constants is the 'c', which is the rate at which the 'b' is degraded. In the present study the only significant increase in 'c' was in the case of maize stover; from 2.3%/hr to 4.0%/hr for the treated and untreated stover respectively.

Analysis of dry matter disappearance (DMD) values indicated that there was no significant difference between DMD for 36, 48 and 72 hours incubation. Thus long rumen retention times do not result in greater benefits to the animal in terms of degradation. This is because intake is influenced by gut fill among other factors; thus low turnover rates lead to an accumulation in the rumen of material which is of very low degradability and this may reduce intake. A long rumen retention time tends to decrease the ratio of microbial protein to volatile fatty acids produced in the rumen and thus more energy substrates and less

microbial protein results from fermentation. Thus an increase in rumen outflow rate may increase the efficiency of conversion of feed to products such as meat, wool or milk [115].

Orchardgrass hay had larger degradation constants than wheat straw and it also gave a better response to treatment. Treatment had no significant effect ($P > 0.05$) on the degradation constants of the straw except for the 'a' fraction. Probably the straw was of such a low quality that treatment would have no effect at all.

Rice and wheat straws showed a remarkable response to treatment while oat and barley straws gave the least response to urea treatment. However since these materials had not necessarily been handled in the same way after harvest, a comparison of their response to treatment should be treated with caution. Thus to get a true picture, one would need to compare materials from crops which had been grown under the same conditions and the by-products should be handled the same way post harvest. Urea treatment can be recommended for the treatment of crop by-products in Kenya especially rice and wheat straws as well as maize stover. Emphasis should be placed on maize stover since it is the most abundant and is extensively used for feeding to ruminants especially by small scale farmers. Materials should be ensiled in air tight structures to avoid the loss of the ammonia generated from urea breakdown. The moisture content of the material is an important factor since it influences the degree of urea hydrolysis. Some workers have raised the moisture content of the straw to 60% [44]; others have mixed the straw to an equal weight of water [101]. It is difficult to say the exact moisture level necessary for effective treatment since one has to consider the need to have enough moisture for urea hydrolysis

while at the same time ensuring that the material is not too wet as to present handling problems. With high moisture material there is also the risk of mould damage; above 30% moisture level, a urea concentration of >40 g/kg is necessary to achieve preservation [46]. Another consideration is the need to supplement animals on ammoniated crop by-products with a good quality forage (10–20% of diet DM), a source of highly digestible by-pass nutrients e. g. cotton seed cake or by-products of cereal processing (10–20% of diet DM) and minerals [115]. The forage is thought to have an influence on microbial growth by supplying essential co-factors e. g. nicotinic acid. The by-pass nutrients are necessary as sources of glucogenic compounds and amino acids [115]. Whenever possible the straw should be removed from the field as soon as the grain is harvested otherwise, the longer the material is left in the field, the greater the loss of its nutritive value.

The response recorded during the calf growth trial were not what was expected. Animals were expected to do better on the straw which had been treated and ensiled than on the straw where the urea was added at feeding time. It is difficult to know the minimum improvement which is necessary to produce a significant response in animals. Animal response is influenced by such factors as intake, digestibility, rumen retention time, effectiveness of the treatment, other feeds which are offered together with the straw and the availability of other essential nutrients. To take full advantage of the effect of any treatment, it is important to optimize treatment conditions. However, this is not always possible since other factors have to be taken into account. In the case of urea for example, increasing the moisture content improves treatment effect. On the other hand, the higher the moisture content, the higher the risk of mould damage.

From the average daily gain data, one can conclude that the straw was of relatively good quality and thus treatment did not effect any remarkable improvement. Thus the straw where urea was added at feeding time and the one which was treated and ensiled produced similar response in the animals.

Treatment using urea has the added advantage of increasing the crude protein of the material and this is a source of non protein nitrogen for the rumen microbes. Data on nitrogen degradation have shown the need for correcting for microbial contamination of the incubated samples. Such a contamination also leads to an underestimation of dry matter degradation. This correction is especially critical when one is dealing with materials of low nitrogen content such as straw.

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Appendix A

Tables

Table A.1: Degradation Constants

MATERIAL	Degradation Constants			
	a \pm 1.6	b \pm 3.1	c \pm 0.01	a+b \pm 3.5
Barley Straw	7.16 ^a	37.71 ^a	0.039 ^b	44.87 ^a
Maize Stover	13.55 ^b	58.28 ^b	0.023 ^a	71.83 ^{cd}
Oat Straw	9.06 ^{ab}	54.98 ^b	0.033 ^{ab}	64.02 ^{bc}
Rhodesgrass	13.59 ^b	61.63 ^b	0.026 ^a	75.22 ^{cd}
Rice Straw	10.24 ^{ab}	46.27 ^a	0.031 ^a	56.51 ^b
Wheat Straw	7.70 ^a	51.00 ^b	0.025 ^a	58.70 ^b
Alfalfa Hay	15.21 ^{bc}	58.37 ^b	0.087 ^d	73.58 ^{cd}
Napier Grass	19.56 ^c	62.17 ^b	0.021 ^a	81.72 ^d
Pyrethrum Marc	14.97 ^{bc}	55.05 ^b	0.065 ^c	70.02 ^c

Values within the same column within the same material with different superscripts are significantly different at $P < 0.05$.

Table A.2: Percent ADF Content

MATERIAL	UNTREATED	TREATED [†]
	MEAN ADF \pm 0.547	MEAN ADF \pm 0.547
Barley Straw	60.75 ^f	58.92 ^{ef}
Maize Stover	48.58 ^b	50.27 ^c
Oat Straw	57.70 ^{de}	57.05 ^d
Rhodesgrass	41.77 ^a	42.06 ^a
Rice Straw	60.79 ^f	59.80 ^{ef}
Wheat Straw	58.69 ^{def}	58.72 ^{def}
Alfalfa Hay	35.47 ^a	
Napier Grass	49.04 ^c	
Pyrethrum Marc	41.97 ^b	

Values with different superscripts are significantly different at $P < 0.05$.

[†]Treated with 6g urea/100g DM and ensiled for six weeks.

Table A.3: Percent Dry Matter Disappearance after Rumen Incubation

MATERIAL	Rumen Incubation Time—Hours					
	1	12	24	36	48	72
Barley Straw						
Control	8.46	18.21	31.53	34.29	44.11	40.10
Treated [§]	14.17	26.87	49.83	52.35	64.97	64.95
Maize Stover						
Control	15.09	23.87	37.36	43.97	55.64	58.60
Treated [§]	15.14	38.34	60.38	57.10	74.31	82.40
Oat Straw						
Control	10.68	22.28	41.88	40.12	56.04	56.63
Treated [§]	17.18	31.28	53.98	61.73	69.45	73.87
Rhodesgrass						
Control	15.00	24.63	43.17	48.59	60.40	62.70
Treated [§]	19.52	34.34	63.37	56.12	77.21	78.97
Rice Straw						
Control	11.89	20.21	33.74	40.15	48.20	46.98
Treated [§]	22.43	45.27	64.86	69.46	82.64	86.41
Wheat Straw						
Control	9.66	15.95	29.38	35.20	46.85	45.87
Treated [§]	11.00	26.28	47.80	51.03	66.48	68.96
Alfalfa Hay	13.94	51.69	68.24	70.33	73.51	72.38
Napier Grass	21.42	29.48	44.44	51.67	62.47	65.71
Pyrethrum Marc	14.52	45.11	59.27	59.01	69.87	69.32

[§]Treated with 6g Urea/100g DM and ensiled for six weeks.

Table A.4: Percent ADF Loss after Rumen Incubation

MATERIAL	Rumen Incubation Time—Hours					
	1	12	24	36	48	72
Barley Straw						
Control	0.36	13.98	29.44	29.62	43.69	47.98
Treated [§]	3.40	16.47	44.62	44.94	61.71	68.35
Maize Stover						
Control	3.26	15.65	36.29	36.47	50.11	60.34
Treated [§]	-2.05	26.74	53.35	47.45	69.51	—
Oat Straw						
Control	-0.07	16.93	32.93	41.86	52.89	58.61
Treated [§]	4.34	22.50	49.98	57.24	64.83	—
Rhodesgrass						
Control	-1.91	16.02	37.35	42.40	55.99	—
Treated [§]	5.73	25.76	58.08	48.39	72.25	75.20
Rice Straw						
Control	2.34	15.35	34.00	36.18	46.53	47.74
Treated [§]	8.24	37.52	62.34	64.00	80.23	—
Wheat Straw						
Control	2.75	11.15	28.38	31.23	44.52	52.74
Treated [§]	-0.68	17.22	44.49	46.81	65.56	71.31
Alfalfa Hay	-1.43	31.06	50.29	48.83	54.61	55.09
Napier Grass	3.23	14.24	37.10	39.81	60.21	65.67
Pyrethrum Marc	-1.56	25.52	43.07	39.59	51.58	53.88

[§]Treated with 6g Urea/100g DM and ensiled for six weeks.

Table A.5: Crude Protein Disappearance after Rumen Incubation

MATERIAL	Rumen Incubation Time-Hours					
	1	12	24	36	48	72
Barley Straw						
Control	0.82	0.40	-2.59	0.41	-1.13	-6.12
Treated [§]	10.12	10.01	8.91	9.53	10.50	4.51
Maize Stover						
Control	2.27	1.42	-2.12	1.65	0.26	-5.64
Treated [§]	10.43	11.31	9.85	11.51	9.43	4.12
Oat Straw						
Control	-1.03	-0.25	-0.94	-0.91	-2.26	-8.18
Treated [§]	9.93	9.08	9.29	9.23	9.28	0.88
Rhodesgrass						
Control	1.41	1.60	-1.15	1.10	-0.92	-2.94
Treated [§]	9.66	8.60	9.19	12.25	10.91	7.08
Rice Straw						
Control	0.91	0.73	-2.63	1.03	-1.16	-5.15
Treated [§]	7.78	7.73	8.34	8.63	7.18	2.54
Wheat Straw						
Control	1.00	0.47	-3.43	-1.34	-2.00	-8.80
Treated [§]	11.22	11.22	11.40	11.90	7.98	1.14
Alfalfa Hay	1.49	0.79	7.39	10.13	10.81	8.67
Napier Grass	1.75	1.69	1.02	1.83	0.51	-2.34
Pyrethrum Marc	0.39	0.20	-0.86	1.26	0.81	-0.55

[§]Treated with 6g urea/100g DM and ensiled for six weeks.

Table A.6: Percent Crude Protein Content

MATERIAL	CONTROL	TREATED [‡]	S.E.
	MEAN CP	MEAN CP	
Barley Straw	3.12 ^b	14.95 ^g	0.17
Maize Stover	6.56 ^c	16.62 ^h	0.17
Oat Straw	2.32 ^a	13.68 ^f	0.17
Rhodesgrass	11.82 ^e	22.57 ^j	0.17
Rice Straw	3.09 ^b	13.13 ^f	0.17
Wheat Straw	2.51 ^a	16.10 ^h	0.17
Alfalfa Hay	20.86 ⁱ		0.17
Napier Grass	8.7 ^d		0.17
Pyrethrum Marc	13.55 ^f		0.17

Values with different superscripts are significantly different at $P < 0.05$.

[‡]Treated with 6g urea/100g DM and ensiled for six weeks