

THE NUTRITIONAL VALUE OF
AMMONIA TREATED ROUGHAGES AS
FEED FOR RUMINANTS

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IN THE
DEPARTMENT OF ANIMAL SCIENCE

BY
WICLIFF KHUZWAYO TEMBO

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THE NUTRITIONAL VALUE OF AMMONIA TREATED
ROUGHAGES AS FEED FOR RUMINANTS

BY

WICLIFF KHUZWAYO TEMBO

A thesis submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

MASTER OF SCIENCE

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ABSTRACT

Three trials were conducted to investigate the use of ammonia as a preservative of alfalfa hay and barley green feed harvested at high moisture (>20%) and its effect on the nutritional value of dry high protein and low protein roughages.

In the first part of trial 1, high moisture barley green feed (70% DM) and alfalfa hay (77% DM) were ammoniated by the conventional plastic cover method and temperature trends of only alfalfa hay treatments were monitored. A four wether 4x4 latin square design intake and digestion trial was carried out after assigning the four wethers to either treated straw or hay or their wet controls.

In the second trial, two separate experiments were conducted, involving four rams in each experiment in a 2, 2x2 switch back design. In the first experiment 2 rams of similar weight were randomly assigned to either good quality dry ammoniated alfalfa hay (NH) or its dry control (CH), to determine intake and digestibility. The second experiment was similar to the first except that low quality dry ammoniated barley straw (NB) or its dry control (CB) were used.

In the third trial, 10 lactating cows were randomly assigned to either high protein dry ammoniated hay or its dry control in a 5, 2x2 switch back design. Intake, DM digestibility, milk production and composition and physiological parameters were monitored. The barley straw and alfalfa hay used in the second and third trials were treated in an Fma-AN-STRA-VERTER oven.

Bale temperatures appeared to be higher for high moisture treated hay than wet untreated hay but showed a more rapid decline in temperature over time. Treated wet roughages did not show any apparent visible molding. Treatment with ammonia significantly lowered ($p < 0.05$) the acid detergent insoluble nitrogen (ADIN % of total N) of wet barley green feed but not alfalfa hay.

The nitrogen content of hay and green feed was significantly increased ($p < 0.05$) by 71% and 136% respectively upon ammoniation in the first trial.

The GE, ADIN (% of DM) and cellulose content of wet roughages was not affected ($p > 0.05$) by ammoniation. The ADF and lignin content of treated high moisture hay but not wet treated barley green feed, the hemi-cellulose content of wet barley green feed but not wet treated hay and the NDF content of both treated species of roughages were lowered ($p < 0.05$) upon ammoniation.

The DM and nitrogen intake of wet barley green feed and hay fed to rams in trial 1 and barley straw in trial 2 were increased ($p < 0.05$) by ammoniation. Ammoniation lowered ($p < 0.05$) the DM intake of long alfalfa hay when fed to cows with no effect ($p > 0.05$) when fed in the chopped form to rams.

The roughage DM, but not CP and GE digestibility of wet hay but not barley green feed was increased ($p < 0.05$) by ammoniation. Ammoniation had no effect ($p < 0.05$) on the DM, CP and GE digestibilities of hay or straw in the second and third trials. The ADF and cellulose digestibilities of wet barley green feed and also the NDF and hemi-cellulose digestibilities of both species of wet roughages were

significantly increased ($p < 0.05$) by ammoniation. In the second trial only the NDF and hemi-cellulose digestibilities of hay and straw respectively were increased ($p < 0.05$) by ammoniation.

Ammoniation significantly affected the ($p < 0.05$) IVDMD and nylon bag disappearance of DM, CP, ADF, NDF, cellulose and hemi-cellulose.

There was no abnormal behaviour exhibited by wethers consuming untreated or treated wet roughages in the first trial.

Milk production and composition was not affected by ammoniation in the third trial. Differences in ruminal pH, isobutyric and valeric concentrations, molar percent of acetic and isovaleric acid, and blood urea-N were significant ($p < 0.05$) at 2 hours post feeding.

Ammonia treatment of roughages offers potential to nutrient preservation of wet roughages and also to increasing the nutritional value of low protein high fiber roughages.

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Dedicated to dad and mom, my brothers, Muloyi,
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ABBREVIATIONS

ADIN	Acid-detergent insoluble nitrogen
ADF	Acid-detergent fiber
AIA	Acid-insoluble ash
Ammonia-N	Ammonia-nitrogen
BG	Blood glucose
BUN	Blood urea nitrogen
CP	Crude protein
DE	Digestible energy
DM	Dry matter
EE	Ether extract
GE	Gross energy
H-cell	Hemi-cellulose
IVDMD	In-vitro dry matter disappearance
NDF	Neutral detergent fiber
NH ₃ -N	Ammonia nitrogen
Molar %	Molar percent
P. Lignin	Permanganate lignin
VFA	Volatile fatty acids

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INTRODUCTION

Utilization of roughages for animal production has ranged from conserved forages as hay or silage to grazing of planted and native pastures and also crop residues. Conservation of forage for animals requires good management and is dependent on weather conditions (Hayhoe and Jackson, 1974). The main goals in conservation have been, production of high yields of dry matter per hectare, baling or ensiling the forage at proper moisture level that precludes or limits further respiration or spoilage, production of feed high in digestibility and reduction of all forms of quantity and nutrient losses. These goals are not easy to achieve.

Baling and storage of hay at a proper safe moisture level of 20% or less (Benham and Redman, 1980) requires curing the hay, and in absence of artificial drying, this is accomplished by leaving the cut crop in the field to dry. The time required is at least 3-5 good or fair consecutive drying days (Hayhoe and Jackson, 1974; Benham and Redman, 1980). It is however difficult to get consecutive good drying days. The probabilities of 2 and 3 consecutive good drying days at Nappan, N.S. were 0.41 and 0.29 respectively in June and 0.17 and 0.07 respectively for late August. The result of bad weather at harvest and baling time is losses in palatability and nutritional values of the hay. A few days of rain at the wrong time can reduce the value of the hay by 25 to 50% (Friesen, 1980) due to leaching of soluble carbohydrates, rotting, fermentation and delayed harvesting (Hayhoe, 1973). Even under good drying conditions, losses in nutritional values associated with harvesting operations can be as high as 39.8% (Klinner and Shepperson,

1975; Wilkinson, 1981).

One alternative available to farmers is to harvest and preserve the hay at higher moisture levels. This reduces nutrient losses resulting from leaf shatter in the field and weather damage. However, when hay is baled and stored at high moisture, the potential for nutrient loss during storage is greater due to high moisture levels in the hay. Fungal and bacterial activities in hay stored at high moisture increases leading to heating, which enhances losses of dry matter, sugars, fats, hemicellulose, starch and other components (Bechtel et al, 1945; Hodgson et al, 1946; Greenhill et al, 1961; Knapp et al, 1975), decreases digestibility of protein and nitrogen-free-extract (Bechtel et al, 1945; Van Soest, 1965; Miller et al, 1967; Thorlacius and Robertson, 1984) and reduces palatability (Mohanty et al, 1967). The use of anhydrous ammonia has shown promise as a preservative for high moisture hay (Knapp et al, 1975, Thorlacius and Robertson, 1984; Kersbergen and Barton, 1986; Henning et al, 1986).

Crop residues, unlike conserved roughages are inherently of low nutritional value since harvesting of grains is completed when the crop is nearly or fully mature. At this stage of maturity crop residues are characterized by low protein and phosphorus, marginal calcium, high fibre and lignin content and low energy. Consequently, digestibility is poor, passage rate slow and voluntary intake low (Anderson, 1978; Coombe, 1980). The result of low nutritional value and low intake is weight loss of mature animals and failure of young animals to achieve satisfactory growth rates. Hence, the need to improve their nutritional value. Chemical treatment of crop residues through ammoniation offers

potential to increase their nutritional value (Sundstol et al, 1978; Horton and Steacy, 1979; Sundstol and Coxworth, 1984).

This study was undertaken to investigate the nutritional value of high moisture hay that was preserved with ammonia and the effect of ammoniation on the nutritional value of low and good quality roughages.

LITERATURE REVIEW

DRY HAY MAKINGWeather and Weather Damage

Baling and storage of hay at proper moisture level requires curing the hay to safe moisture levels. In absence of artificial drying this is accomplished by leaving the cut crop in the field to dry. Drying is a process of water loss by evaporation and in the field, this is accomplished by solar radiation which provides the energy. The main factors affecting water loss are physiology and morphology of the plant and environmental conditions (Jones and Harris, 1980).

Jones and Harris (1980) have summarized the factors affecting water loss. The water content of a growing crop varies between 75-85% in the early stage of reproductive development of grass. To reduce the water content of such a hay crop (7t DM/ha) to 20% requires the removal of about 25 tonnes of water per hectare per day. Thus if this moisture loss per hectare was maintained for a mown crop, hay would be produced in a day, but in practice, at least 3 to 5 days are required. However, considerable limitations to water loss develop after mowing.

The factors limiting water loss can conveniently be divided into (a) those which restrict movement of water vapour from the plant tissues to the air and (b) those which restrict movement of water vapour from the air within the swaths to ambient air. Of concern here is the second limitation.

Green and Jagger (1978) studied the development of a macro-climate in a typical swath of 15-20 cm high, and 100-125 cm wide (quoted in

Jones and Harris 1980). They reported that in early stages of drying, the swath surface reflects 20% of the incident solar radiation and for the purpose of drying this is lost energy. The remainder is available for evaporation, but is rapidly attenuated at the surface of the swath. Radiation 2 cm below the surface was found to be half that at the surface, and at the base, it was only 20% (Green and Jagger, 1978).

This energy for evaporation of water within the swath is limited (Jones and Harris, 1980). The air flow in the middle of the swath was found to be as low as 0.2 m/s therefore limiting the supply of energy (Green and Jagger, 1978). Two reasons are given for low air flow within swaths: (a) wind speed decreases linearly with height and (b) the swath material acts as a windbreak (Jones and Harris, 1980). Despite the low air flow values, swath humidity increased by up to 15% relative humidity when a windbreak was placed alongside the swath (Green and Jagger, 1978) suggesting that airflow velocity may play a part in maintaining a gradient of humidity (Jones and Harris, 1980).

The humidity inside the swath depends on (a) the ambient humidity, (b) the water content of the crop, and (c) the airflow through the swath (Jones and Harris, 1980). During the early stages of drying, high water content of the crop and low ventilation lead to humidity values which rarely fall below 80% relative humidity during the day in the middle and base of a swath (Jones and Harris, 1980). Thus during the early stages of drying when the plant limitations remain low, the inside of a swath has a very unfavourable micro-climate for evaporation.

As drying proceeds, both the energy supply and the gradient of humidity within the swath increases, because the density of the crop is

reduced as water is lost (Jones and Harris, 1980). This therefore allows a greater penetration of solar radiation into the swath and a faster airflow through the swath. According to Jones and Harris (1980), this improved ventilation has three effects, (a) it increases the supply of sensible heat, (b) reduces the plant boundary resistance, and (c) reduces the humidity inside the swath, thus increasing the gradient of water vapour concentration between the site of evaporation and the ambient air.

Drying of hay to safer moisture levels is not only made difficult by the formation of a micro-climate formed due to swath formation but also by the ambient weather.

While most parts of the world have favourable climate for production of forage crops, it's unfortunate that the same conditions that favour production cause problems at harvesting. Rains, high humidity, dews, low temperatures and low wind speed result in slow drying rate of hay.

Generally, it's conceded that 3-5 good days are required to dry hay to a safe moisture level of 20% or less (Benham and Redman, 1980); several different criteria are used to define a good drying day. Hayhoe and Jackson (1974) looked at the probability of getting 2 or 3 consecutive good drying days for Nappan, N.S. They defined a good drying day as, one with less than 12.7 mm of rainfall on the previous day and the value of the drying index based on potential evaporation, was greater than or equal to 4.2 mm. They found that, the probabilities of 2 and 3 consecutive good drying days at Nappan, N.S. were 0.41 and 0.29 respectively between the 16th of June and the 30th of June, when

forages should be harvested. The probabilities for late August (for the second cut hay) were found to be 0.17 and 0.07 for 2 and 3 consecutive good drying days respectively. These probabilities are very low, therefore indicating the difficulty of making hay in an area like Eastern Canada.

The general result of bad weather conditions and micro-climate formulation in the swath is loss in quality and quantity of hay.

Good weather is a prerequisite to high quality hay and reduced losses of dry matter during hay making. Adverse weather during hay making and storage causes substantial losses in palatability and nutritional value of the crop (Hayhoe, 1973). A few days of rain at the wrong time can reduce the value of the hay by 25 to 50% (Friesen, 1980) due to leaching soluble carbohydrates, rotting, fermentation and delayed harvesting (Hayhoe, 1973). Hot dry winds can overdry the hay crop and cause high leaf losses (Friesen, 1980) and high humidity during wilting periods slows the drying rate and results in losses of crude protein, carotene and dry matter digestibility (Hart and Burton, 1967).

Hart and Burton (1967) studied bermuda grass hay during drying to less than 20% moisture. They reported dry matter losses of hay yielding 2 tonnes/ha to be 3.0% in dry weather and 9.6% when 5 cm of rain fell while losses of hay yielding 10 tonnes/ha were 4.6% and 11.1% respectively. Yield of cured hay was correlated with precipitation and yield at harvest ($R^2 = 0.99$). Carotene losses were over two-thirds of the original content on one brightest day and it was focused that 90% would be lost with such days in succession. Carotene loss was said to be entirely a function of total solar radiation received ($R^2 = 0.87$).

Carter (1960) has quoted loss values for carotene ranging from 90-95% for field-cured hay and 80-90% for barn-cured hay. Hart and Burton (1967) also noted that crude protein losses were small in good weather but increased rapidly in damp weather. Losses were closely correlated with the number of hours in which relative humidity equaled or exceeded 85% ($R^2 = 0.98$) but not rainfall amount. Therefore these losses were suspected to be due to microbial decomposition and respiratory losses of nitrogenous materials which are high under high humidity conditions. They concluded that leaching was not an important factor. However, leaching and high humidity were found to be a significant cause of digestible dry matter losses.

Losses reported by Hart and Burton (1967) may have been higher if some mechanical conditioning had been carried out. In previous work, Murdock and Bare (1960) observed higher losses in dry matter, crude protein and nitrogen-free-extract of tedded hay with rain than for hay which had just been turned. Their results (1963) were confirmed when they used sprinkler irrigation to simulate 1.14 cm of rain during curing of untouched and crimped hay.

Western European studies show similar trends in nutrient losses due to rains. Van Bockstaele et al (1980) noted that dry matter losses were much higher during wilting in rainy weather than when there was no rain. Losses and days to cure increased with more rainy days. Lingvall and Nilson (1980) reported a substantial decrease in organic matter, crude protein and crude fibre digestibility after rains although their nutrient losses were small. They also cited Danish researchers, Moller and Skovborg (1971) having observed dry matter losses of up to 12.5%

when artificial rain corresponding to 20 mm was applied for 24 hours. Most of these researchers noted that rain prolonged the time the hay took to cure and this led to increased losses due to biological activity.

Harvesting Losses Due to Delayed and Mechanical Harvesting

This includes losses due to the actual harvesting operation, occurring in the field and during baling. All these operations involve machinery use and therefore losses are machine related (Carter, 1960; Friesen, 1976 and 1980; Klinner and Shepperson, 1975). However, though machine related, successful storage of hay requires drying to 20% or less, moisture content.

Harvesting losses due to actual harvesting are in two forms, those related to postponing harvesting date due to bad weather (e.g. rains) and those due to actual mowing operation (Friesen, 1976).

It is now a well established fact that quality decreases with increasing maturity of plants. Kunelius and coworkers (1974) observed that, the crude protein content of Climax timothy grass decreased from 18.2% at vegetative stage to 9.1% at full flower and from 17.2% at boot stage to 7.1% at late flowering for Saratoga brome grass, and 18.0% at boot stage to 9.2% at late flowering for Frode orchard grass. This represented a daily decrease of 0.34, 0.37 and 0.33% in crude protein over a 27 day period, for Timothy, Brome and Orchard grass respectively. The in-vitro dry matter digestibility (IVDMD) also decreased with advancing maturity. Digestibility declined from 65.5 to 58.0% for Climax timothy, 74.1 to 66.2% for Saratoga brome grass and 67.6 to 56.5% for Frode orchard grass. High crude protein content did not coincide

with high dry matter yields.

Kilcher and Heinrichs (1974) noted similar results for Roamer alfalfa. They reported that crude protein decreased from 22.8% at an immature stage to 13.2% at late bloom. This represented a decrease of 42% over an 8 week period. Likewise percent in-vitro digestible energy declined at a relatively constant rate from 71.9 to 55.2%. In other experiments crude protein content declined from 30 to 7%, 25 to 9% and 23 to 13% for oats, brome grass and alfalfa hay crops respectively (Kilcher and Heinrichs, 1974). Decline in digestibility was from 65 to 50%, 73 to 46% and 72 to 55% for oats, brome grass and alfalfa hay respectively. The crude protein can be as low as 4.0, 4.1 and 3.2% for barley, oat and wheat straw after grain harvesting (NRC, 1985). Nicholson and McQueen (1984) showed decreases in dry matter intake and in-vitro digestibilities of energy and crude protein of five Timothy cultivars at four growth stages.

The decrease in crude protein content is accompanied by an increase in fibre components. The NDF, ADF and lignin content of alfalfa hay crop increases from 40 to 50%, 29 to 37% and 7 to 10% respectively from late vegetative stage to full bloom and from 55 to 70%, 29 to 40% and 3 to 7% for Timothy grass for the same stages of maturity as alfalfa (U.S.-Canadian Tables of feed composition, 1982).

A further source of field loss is from fragmentation of the plant caused by the action of machinery during cutting, swathing and baling.

Koegel and coworkers (1985) studied mechanical losses of alfalfa using different bale systems. Total mechanical losses varied between 6.4% and 27.1% with a seasonal mean of 15.7% at the 32.9% moisture

level. The bale chamber and mean total losses increased from 3.8% to 5.8% and 16.4% to 19.3% respectively when the moisture content decreased from 32.9% to 13.6%. Regression of total and chamber losses against bale moisture resulted in R^2 values of 32.2 and 42.5 respectively. However, these values increased to 49 and 53.3 respectively when the same losses were regressed on bale moisture content and conditioning method (fluted rolls vs. flail). When the same losses for the entire season were regressed on bale moisture content, the R^2 values were extremely low. They concluded that, this lack of correlation indicates the importance of other factors such as seasonal variation in leaf stem ratio and the disparity between mean moisture content as determined by coring the bale and the moisture content of the losses from the bale chamber.

Rotz and Sprott (1984) studied losses due to mowing and conditioning of alfalfa hay (from prebloom - full bloom, moisture content at 40%) by different machines. Weather conditions for the period (7/29/81 - 8/6/82) were as follows on average; temperature - 23.8°C (29°C maximum); relative humidity - 55.3% (90% maximum); rain - 0.05 cm (2 days); sunshine % possible 73.8% (maximum 99%); wind speed 11.5 km/hr (19 km/hr maximum). They reported shatter, respiration and leaching (no rain) losses ranging from 2.4 to 6.2%, 0.9 to 4.9% and 22.3 to 28.6% respectively. Total respiration, leaching and aerobic losses were found to be even higher in one test which was rained on. They concluded that the increase in the loss was due to splitting of stems thereby increasing leaching of internal stem material.

Whitney (1966) reported mechanical (total) (bale pickups and

chamber, chopper pickup and drift and rake and mower) losses ranging from 7 to 12% for a Timothy brome grass mixture with rake and mower losses contributing more to the total losses.

Other workers in their reviews have cited higher dry matter losses resulting from different operations. Raking at moisture levels less than 40% can result in losses of 10-25% for turning a windrow (Friesen, 1980) and baling losses of up to 15% when large balers are used (Friesen, 1976). Klinner and Shepperson (1975) and Wilkinson (1981) cited losses up to 39.8%. Zink (1936) reported that leaf shattering can start at moisture levels as high as 30%.

Storage Losses

Losses in stacks and bales have been studied in many areas. The magnitude of losses in both quality and quantity are dependent on the bale system used (Friesen, 1976 and 1980) which in turn determines the storage system (Lechtenberg et al, 1980; Verma and Nelson, 1983). Even the shape, density, small depressions or reduction in slope of stack is critical in determining the amount of rain penetration (Friesen, 1980).

Coates et al (1978) studied nutritional losses associated with "field dropped" and "hailed in" (stacked) storage of brome-alfalfa hay (11:3 ratio by weight) dried to about 25% moisture. Dry matter losses averaged 13.9% and 12.7% for "field dropped" and "hailed in" respectively after two months of storage. Crude protein and ash content tended to increase while organic matter digestibility decreased during storage. Increases in crude protein, ash and crude fibre and a decrease in nitrogen-free-extract had earlier been reported by Weeks et al (1975) during storage of mechanically stacked alfalfa hay. Dry matter losses

ranged from 4.2 to 9.9% and 8 to 15% for loose large stacks (6 m x 2.7 m x 3.7 m) and small loose stacks (4.3 m x 2.4 m x 2.7 m) respectively after almost a year of storage. The moisture content ranged from 18 to 49% and 22 to 54% respectively for the two bale systems of storage. Losses were higher for stacks baled at lower initial moisture content. Lechtenberg et al (1980) also reported that weathering reduced the in-vitro dry matter digestibility but increased the crude protein content of a grass and a mixed (alfalfa-grass) hay baled in large round bales stored on crushed rock or ground.

Increases in crude protein, crude fibre and ash are only apparent and reflect losses in non-protein constituents or fermentable carbohydrates (Hodgson et al, 1946; Weeks et al, 1975; Lechtenberg et al, 1980) and this reflects dry matter losses due to spoilage and metabolic activity (Coates et al, 1978).

Hodgson et al (1946) and Davies and Warboys (1978) studied losses in conventional rectangular bales. Hodgson and coworkers studied second cut alfalfa hay (with 20-25% ladino clover) baled at 43.6% and 19.2% for barn-cured and field-cured hay respectively. The gross losses for barn-cured forage were 20.8%, with field (from cut to storage) losses of 12.6% and a further reduction in dry matter during barn-curing of 9.3%. On the other hand gross losses of the field-cured hay were as high as 23.4%, of which 19.3% occurred in the field, with a further reduction in dry matter of 5.1% in the mow. They also reported a decrease in protein content decrease of 4.2% (from cut to mow) and an increase of 3.9% after storage for barn-cured hay. Field-cured hay protein content showed a 13.6% decrease (from cut to mow) and a 5.3% increase after

storage. Total carotene losses averaged 90.3% and 91.6% for barn-cured and field-cured hay respectively. When removed from storage, the barn-cured hay averaged 56% green colour and 50% leaves while field-cured hay averaged 54% green colour and 36% leaves.

Davies and Warboys (1978) looked at nutrient losses in storage of a mixed hay (perennial rye grass, timothy and white clover) baled at 33% and 25% moisture stored in a shed for 72 days. Dry matter and digestible organic matter losses averaged 12.6% and 20.8% respectively for hay at 33% baling moisture. Field-cured hay averaged 9.9% and 16.5% respectively for the same parameters. Though dry matter losses are lower compared to those obtained by Hodgson *et al* (1946) they however give an idea of the magnitude of losses. The differences in the result may be due to differences in baling moisture content. Davies and Warboys (1978) also noted a decrease in water soluble carbohydrates and an increase in crude protein during storage.

HIGH MOISTURE HAY

Nutrient Losses

The problem of nutrient losses during conventional hay making methods was long recognized by both farmers and researchers. The alternative to this method has been to bale the hay at high moisture content. When hay is baled at high moisture level, there is less nutrient losses due to reduced leaf shattering during baling and handling and less chance of weather damage due to reduced field drying time (Kersbergen and Barton, 1986). However, the potential for nutrient loss during storage is greater due to high moisture level and this problem has been long recognized (Bechtel *et al*, 1945; Miller, 1947;

Hodge, 1953; Gregory et al, 1963) and recently by (Knapp et al, 1975 and 1976, Sheaffer and Clark, 1975; Crawford et al, 1986; Kersbergen and Barton, 1986; Hlodversson and Kaspersson, 1986; Montgomery et al, 1986; Baxter et al, 1986).

Gregory and coworkers (1963) looked at microbial and biochemical changes during storage of high moisture and dry hays. They found that good hays (16% moisture content) contained a small diversity of microflora while hays baled at 40% moisture contained a large flora of thermophilic fungi and actinomycetes. Wet stacks developed a core of brown hay containing many spore forming bacteria and a few fungi surrounded by a layer of moldy hay. They also noted that proliferation and development of microbes was in succession as temperature increased. This succession of different microbes was accompanied by a general decrease in soluble carbohydrates, lipids, soluble nitrogen and an increase in volatile nitrogen. They attributed these changes to microbial degradation and plant enzyme activities. Nelson (1966) also noted mold under laboratory conditions in which alfalfa hay was baled at moisture content ranging from 27 to 48%.

Recent studies testify to these earlier findings. Visible mold was reported by Knapp et al (1975) in ladino-clover/tall fescue hay and alfalfa hay (Knapp et al, 1976) when baled at 32 to 33% moisture content. No molding was observed in hays baled at 14% moisture. Similar observations were made by Jafri and Bush (1979) and Weeks et al (1975) in alfalfa baled at 29 and 40% respectively. Severity of molding and browning increases with increased moisture content (Miller et al, 1967).

The effect of microbial activities is significant and direct on dry matter, soluble carbohydrates, ether extracts and to some extent structural carbohydrate losses due to fermentation (Miller, 1947). However Miller (1947) adds a parallel reaction involving degradation of proteins to amino-acids. He contends that, there's transformation from grass protein to bacteria protein. This is particularly true for silages in which protein content decreases.

Knapp et al (1975) observed dry matter losses of up to 15% of alfalfa hay baled at 32-33% moisture. They reported significant losses in total soluble carbohydrates and insoluble carbohydrates associated with molded hay. This loss was implicated in the 40-70% loss of digestible dry matter observed. Losses in dry matter and soluble carbohydrates have been reported by Gregory et al (1963), Nelson (1966), and Hlodversson and Kaspersson (1986).

However, Miller et al (1967) found little difference in water soluble carbohydrates and crude protein of alfalfa (25% red clover) and native hay stored in insulated boxes at moisture levels ranging from 26.2 to 58.5% and 19.2 to 50.8% respectively. These findings are at variance to those by Nelson (1966) who also used insulated boxes and showed a causal effect of moisture level on nutrient retention.

Increase in ash, crude fibre (Bechtel et al, 1945; Greenhill et al, 1961; Miller, 1967; Weeks et al, 1975), ADF, NDF and Cellulose (Jafri and Bush, 1979), lignin (Miller, 1967) and a decrease in nitrogen-free-extract and ether extract (Bechtel et al, 1945; Gregory et al, 1963; Miller et al, 1967; Weeks et al, 1975) have been reported in molded hays. These losses and apparent gains in nutrients during

storage result from destructive microbes which predominate in molded hay (Gregory et al, 1963; Hlodversson and Kaspersson, 1986).

Temperature changes during storage and their effects on the nutritional value of high moisture hay has received attention from many researchers. The main predisposing factor of self-heating (increased temperature) in such hays is high storage moisture. High moisture provides a conducive atmosphere for proliferation and growth of micro-organisms (Gregory et al, 1963; Weeks et al, 1975; Hlodversson and Kaspersson, 1986) resulting in generation of heat as a result of carbohydrate (Miller, 1947; Gregory et al, 1963; Nelson, 1966; Mohanty et al, 1967; Hlodversson and Kaspersson, 1986) and protein degradation (Miller, 1947) and also due to the normal proteolytic and oxidative reactions by plant enzymes (Gregory et al, 1963; Miller et al, 1967; Weeks et al, 1975).

Knapp et al (1975) observed that the temperature rose to 51°C and 22°C in 2-3 days for a grass-legume hay baled at 33% and 16% moisture respectively. Temperatures dropped to 12°C and 15-18°C after 3 and 6 days for wet and dry hay respectively. Kersbergen and Barton (1986) reported a maximum of 59°C and 33°C for a red-clover-timothy mixture baled at 30% and 15% moisture respectively. Generally, maximum temperatures reached are above 50°C for hay baled above 30% moisture (Miller et al, 1967; Crawford et al, 1986; Gregory et al, 1963) and at more than 40% moisture, temperatures reached are above 60°C (Gregory et al, 1963; Miller et al, 1967; Weeks et al, 1975). Nelson (1966) reported a maximum temperature of 78°C over six weeks for alfalfa baled at 48% stored in insulated boxes. Recently, Montgomery et al (1986)

have reported temperatures as high as 90°C, 54°C and 54°C in large round bales, baled at 23, 18 and 13%. These temperatures were reached in about five days. By 8-10 days the temperatures were between 20-30°C and 30-40°C for low and high moisture hay respectively. These temperatures are higher than those quoted earlier for similar moisture levels. These differences may be explained on the basis of bale density and size. The size of large round bales impedes dissipation of heat generated at the centre. Bale temperatures at the centre and surface were found to be 90°C and 63°C respectively. The effect of bale density on spontaneous heating was discussed by Nelson (1966). Similar observations were reported for large round and conventional bales at the same moisture level (24%) (Baxter et al, 1986).

Changes in nutrient composition discussed under molding also apply under increased temperatures. However, the largest and most direct effect of high temperatures is that shown in protein changes. While the general trend for protein is to decrease under ensiling conditions (McDonald, 1980; Bergen et al, 1974) this is not so with hay baled at high moisture level when compared to dry hay. Many workers have found no differences in crude protein or total nitrogen of hay baled at high and low moisture (Bechtel et al, 1945; Greenhill et al, 1961; Miller et al, 1967) and more recent work shows similar results (Knapp et al, 1975; Weeks et al, 1975; Jafri and Bush, 1979; Montgomery et al, 1986; Hlodversson and Kaspersson, 1986; Kersbergen and Barton, 1986). Other workers reported slightly higher crude protein for high moisture hay than dry hay (Grotheer et al, 1985 and Crawford et al, 1986). Johnson et al (1981) reported increased crude protein of high moisture hay after

storage. Such differences have been attributed to leaf loss in dry hays (Crawford et al, 1986), loss of non-fibrous and non-nitrogenous constituents (Weeks et al, 1975; Johnson et al, 1981) and also sampling variation (Montgomery et al, 1986).

The use of Acid-Detergent-Insoluble Nitrogen (ADIN) to assess protein damage due to increased temperature has been proposed and discussed (Van Soest, 1965; Goering and Van Soest, 1967).

Knapp et al (1975) reported that ADIN increased from 0.16% to 0.24% for alfalfa hay baled at 32% ($p < 0.05$). Jafri and Bush (1979) reported similar results for dry (19%) and high moisture (29%) alfalfa. ADIN values were 0.21% and 0.29% for dry and high moisture hays respectively. Recent results by Kersbergen and Barton (1986) showed ADIN values of 1.1% and 2.2%; and 0.9 and 2.5% for dry and wet red-clover-timothy mixture respectively in two separate trials. Similar results were observed by Hlodversson and Kaspersson (1986) under laboratory conditions.

Changes in ADIN are much higher for large round bales at high moisture. Montgomery et al (1986) noted that ADIN changed from 8.3% at baling time to 9.0% of the total N at feeding for bales at 13% moisture. While ADIN for bales at 23% moisture changed from 9.0% to 52.0% of the total N at feeding. The high moisture bales recorded a maximum temperature of 90°C.

Formation of ADIN has been attributed to binding of nitrogen to the fibre fraction (Van Soest, 1965; Goering and Van Soest, 1967) and also the Maillard reaction (Browning reaction) (Hodge, 1953; Miller, 1947; McQueen, 1982). Hodge (1953) has discussed in detail the Maillard

reaction and McQueen (1982) reported that browning reaction becomes significant at 50°C to 55°C in turn dissipating heat, and above 55°C the reaction becomes self-sustaining even after micro-organisms have been killed (70°C).

Intake, Digestibility and Performance on Heated and Molded Hays

Molded and heated hays have been shown to reduce palatability and therefore lower intakes. Reduced nutrient digestibilities, weight gains and milk production, have also been reported.

Bechtel et al (1945) reported that cows consuming brown and black hay consumed 26% and 50% less dry matter respectively than normal hay. Animals on brown and black hays lost considerable weight. Apparent digestibility coefficient of nutrients were much lower on heated hays than normal hay. The average coefficients for normal, brown and black hays, respectively were; DM-60, 41 and 27; crude fibre - 41, 36 and 14; and nitrogen-free-extracts - 72, 59 and 53. Protein was affected the most with average digestibility coefficients of 67, 16 and 3% for normal, brown and black hays respectively. These differences were attributed to excessive heating during storage.

Similar results have been observed by Mohanty et al (1967) and Miller et al (1967) in molded alfalfa hays. Mohanty et al (1967) noted that weight gains of steers receiving moldy (maximum temperature 50°C) hay supplemented with 1.82 and 0.91 kg of grain were 85% and 75% respectively of steers on non-moldy hay. Feed efficiency was higher for cured hay than molded hay averaging 14.54 and 17.44 for the two grain levels with well cured hay while steers receiving moldy hay averaged 16.29 and 22.71 kg/kg gain. Animals fed moldy hay also showed symptoms

of depraved appetite. This observation was earlier noted by Bechtel et al (1945).

Miller et al (1967) reported higher digestibility coefficients of dry matter, crude protein water-soluble-carbohydrates and gross energy ($p < 0.01$) for hay at low moisture than hay at high moisture (alfalfa and native hay). There were significant differences in the fibrous fraction as influenced by moisture content. Average weight gains of the calves and feed efficiency decreased significantly ($p < 0.01$) with increasing moisture. However daily feed consumption did not differ significantly. They explained these differences on the basis of differences in chemical composition and digestibility which was significantly affected by heating and molding. Rate of gain accompanied by improved feed conversion increased as the amount of fibrous material in the ration decreased and as the digestibility of energy and protein increased.

In-vitro dry matter disappearance (Knapp et al, 1975; Grotheer et al, 1985; Kersbergen and Barton, 1986) and in-vitro cell-wall disappearance (Knapp et al, 1975) have been shown to be significantly lower for wet hays compared to dry hays.

Recent results by Montgomery and coworkers (1986) show similar changes in apparent digestibilities. However they found no significant differences in dry matter intake between heated and molded hay and unheated-unmolded hay. There was a preference for heated hays. Similar observation was earlier reported by Weeks et al (1975) when heifers were restricted to a single forage. However the situation was reversed during cafeteria feeding. Caramalized hay has been shown to be more palatable than undamaged hay (Montgomery et al, 1986). Bechtel et al

(1945) and Weeks et al (1975) suggested the musty, moldy aroma of heated hay may be appealing to animals.

LOW QUALITY ROUGHAGES

The ability of ruminant animals to utilize high fibrous roughages has allowed man to convert these materials to animal protein which we can use. These materials include crop residues, low quality pasture, forestry by-products and other industrial by-products that are characterized by high fibre (Coombe, 1980; Hartley, 1981) but low in metabolizable energy (Greenhalgh, 1984). Generally these roughages constitute only a sub-maintenance ration for ruminant animals (Coombe, 1980).

A number of reasons have been put forward for the growing interest in low quality roughages particularly cereal straws. These include:

- (i) rising cost of grain formulated foodstuffs and therefore high cost of production (Anderson, 1978);
- (ii) a promising way of increasing animal production in parts of the world where grains are required for human food;
- (iii) an alternative source of feed for animals in times when man is not able to produce enough grains for himself and animals, e.g. in times of war, when production is disturbed;
- (iv) a better way of utilizing crop residues compared to the customary practice of burning them, which contributes to air pollution (Anderson, 1978);
- (v) an alternative way of utilizing crop residues in times of crop failure resulting from a drought;
- (vi) they are a potential feed for animals considering their

availability, abundance and the fact that ruminants are able to digest structural carbohydrates (Anderson, 1978; Coombe, 1980; Klopfenstein, 1978);

- (vii) high energy diet formulations of diets has created space for low energy roughages and not all feeding regimes require high energy diets (Greenhaugh, 1984), and;
- (viii) introduction of new methods of straw utilization, such as mechanical, physical and chemical treatments to improve the nutritive value (Greenhaugh, 1984).

Nutritional Characteristics, (Factors Limiting Utilization of Low Quality Roughages)

General

Chemical analyses show great variability in chemical composition and other nutritional characteristics depending on plant species, type of cereal or grain straw, variety, area of production and stage of harvest (Coxworth et al, 1981; Jackson, 1977; Van Soest, 1981). Whichever is the case, they are characterized by very low protein and phosphorous, marginal calcium, high fibre, lignin and silica content. Consequently, digestibility is poor, passage rate slow and voluntary intake is low (Anderson, 1978; Waiss et al, 1972; Jackson, 1977), thereby limiting energy intakes (Jackson, 1977). Typical composition data of low and high quality forages compiled from various sources are shown in Table 1 and 2.

Plant constituents related to utilization are discussed.

Crude Protein

The crude protein content of low quality forages is very low compared to the commonly used high quality roughages (Table 4). This difference is due to harvest date differences. Within the common crop plants, the residues of sorghum and corn (maize) are usually somewhat higher in crude protein than cereal straws, while legumes (Table 1) higher than the rest.

The importance of adequate nitrogen in promoting the digestion of roughages in the rumen has been indicated by Moir and Harris (1962), who reported high positive correlation between levels of nitrogen intake, number of rumen micro-organisms, and rates of digestion of cotton threads in the rumen. Coombe and Tribe (1963) found increased straw intake with urea supplementation which was associated with increased rate of cellulose digestion in the rumen, and reduced retention times of undigested particles in the rumen and whole alimentary tract of sheep. Maximum response in intake was at 8 g urea/head/day level. However increased apparent digestibility due to urea supplementation is not always true possibly because of lack of readily fermentable energy potentially available (Chesson and Orskov, 1984; Orskov and Grubb, 1978).

Cellulose

Considerable variation in cellulose content exists among the different plant residues. Cellulose is significantly higher in low quality roughages than high quality roughages (Table 2).

Cellulose is the most abundant carbohydrate in nature (Theander and Aman, 1984) making up to about 20-40% of the dry matter of higher

Table 1. Typical composition of non-fibrous constituents of roughages.

	Crude Protein	Nitrogen free extract	Calcium	Phosphorus	Ash
<u>RESIDUE</u> ¹					
Straw ²	1.9- 5.6	39.2-45.1	0.11-0.34	0.05-0.11	4.5-14.2
Rice straw	4.4- 5.6	32.5-42.4	0.24	0.09-0.16	16.7-17.5
Cornstalks	3.1- 8.1	40.6-47.8	0.30-0.41	0.08-0.15	5.6- 5.8
Sorghum Stalks	3.1- 7.5	47.9-48.1	0.34	0.13	8.5-12.8
Legume Crops	7.5-10.0	45.5			6.2
Pasture Legumes	7.5-11.9		0.9	0.03-0.10	
Grasses:					
Temperate	3.1- 5.6		0.27	0.04-0.06	2.7- 9.4
Tropical	0.25-5.6		0.20-0.40	0.04-0.07	
<u>NON-RESIDUE</u> ³					
Alfalfa hay					
Early bloom	18.0		1.41	0.22	
Mature	12.9		1.13	0.18	
Dehydrated	17.3		1.37	0.24	
Silage	17.8		1.50	0.28	
Brome grass hay					
Early vegetative	18.0		0.50	0.30	
Fresh mature	6.4		0.20	0.26	

¹From Coombe (1980).

²Wheat, oat and barley.

³From NRC, sheep nutrient requirements, 1985.

plants. Theander and Aman (1984) described the structure as a linear polymer composed of up to 10,000 β -1,4-linked glucopyranosyl units complicated by its three dimensional structure. In nature it occurs largely in a crystalline form, organized as fibrils, where the cellulose chain is tightly packed together in compact aggregates surrounded by a matrix of other cell-wall constituents such as lignin and silica (Van Soest, 1982). The glucans are held together by hydrogen bonds both between sugar units in the chain and between adjacent chains. Cellulose confirmation favours the formation of such bonds and explains the mechanical strength of cellulose as well as resistance to both biological degradation and acid hydrolysis (Theander and Aman, 1984; Van Soest, 1982).

Cellulose association with lignin is important because its nutritional availability varies from total indigestibility to complete digestibility (Van Soest, 1982). Cellulose is probably either esterified to lignin in a covalent bonding or physically associated (Van Soest, 1981 and 1982).

Hemi-Cellulose

Typical content of hemi-cellulose in roughages is shown in Table 2. Hemi-cellulose is higher in plant residues than conventional high quality roughages early in maturity but generally lower when compared to cellulose.

Structurally, hemi-cellulose has been described by Van Soest (1982) as a mixture of sugars mostly linked by β ,1-4 linkages to form xylan polysaccharides which are branched at some glucosidic linkages. The hemi-cellulose of leaves and stems of grasses and legumes seem to be

Table 2. Typical composition of fibrous components of roughages¹.

	Cell Wall	ADF	Crude Fibre	Cellulose	Hemi- Cellulose	Lignin	Silica
<u>RESIDUE</u>							
Straw	73-81	46-47	32.2-43.6	36.0-50.1	16-36	11.4-14.6	3-6
Rice straw	79		35.1-43.2	30.6	26	12.0	13
Cornstalks	66-92	40.0	38.8-46.1			5.5- 8.8	
Sorghum Stalks	74.0		29.1-34.5	31	30	11.0	3
Legume Crops	62.0		38.9	30	20		2
Pasture Legumes	69.0			38	19		1
Paddy Hulls	86.0	70.0		39	14	11	22
<u>NON-RESIDUE</u>							
Alfalfa hay							
Early bloom	47.0	35.0	23.0	24.0		8.0	
Mature	59.0	45.0	37.7	29.0		14.0	
Brome grass hay							
Early vegetative	54-69	33-41	24.0	27.0		3.0	
Fresh mature			38.0	35.0		9.0	

¹Compiled from Coombe (1980), Jackson (1977), Klopfenstein (1981), Preston (1983) and NRC, sheep nutrient requirements, 1985.

largely arabinoxylan with associated linkages to glucouronic acid and probably lignin. The xylan chain may be attached to lignin, through glucoside ester or directly to lignin. Linkages between xylose and arabinose are 1-3, while uronic acid may be 1-2, 1-3 or 1-4 linked.

Hemi-cellulose of plant seeds is also characterized by xyloglucans, mannoglucans and β -glucan gums, while galactose may form side chains in legumes.

The association of hemi-cellulose with lignin limits its digestion (Van Soest, 1981 and 1982; Hartley, 1981). There's evidence of direct bonding to phenolic constituents of lignin which includes ester linkages to xylose and possibly glucosidic linkages. Hemi-cellulose mostly occurs in lignified walls of forages and is insoluble in water.

To increase the digestibility of hemi-cellulose requires breaking of ester bonds (Van Soest, 1981 and 1982; Hartley, 1981; Jackson, 1977).

Lignin

Lignin is a non-carbohydrate polymer but has always been discussed together with structural carbohydrates because of its functional association with these constituents of plant cell-wall. Together with structural carbohydrates it offers protection of plants against destruction.

Although lignin content is lower when compared to cellulose and hemi-cellulose content its effect as protector of plants is profound. Like the other cell-wall constituents, lignin content increases with maturity (Table 2).

Structurally lignin is synthesized from phenyl-propane units which

have been identified as p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Theander and Aman, 1984; Van Soest, 1982). The synthesis of lignin is through a complex enzymatic dehydrogenation process, producing a polymer of high molecular weight. The compositions of lignin preparations varies according to the method of isolation, and upon oxidation with alkaline or nitro-benzene lignin yields a variety of products. Alcohols and acids of p-coumaryl and coniferyl yield p-hydroxy-benzaldehyde (p-hydroxyphenyl) and vanillin (guaiacyl), while sinapyl alcohol yields syringaldehyde (syringyl) (Van Soest, 1982; Theander and Aman, 1984) upon oxidation. In a lignin polymer the three precursors exist as the three corresponding oxidative products and their distribution in plants has been used to classify lignins (Theander and Aman, 1984). Softwood (gymnosperm) lignin is called a guaiacyl lignin because it is mainly a condensation product of coniferyl alcohol though small amounts of p-hydroxyphenyl and syringyl exists. Lignin in hardwoods (Angiosperm) belong to the guaiacyl-syringyl class and are thus copolymers of coniferyl and sinapyl alcohol. Substantial amounts of all the three aromatic residues are joined and thus lignin in grasses is called guaiacyl-syringyl-p-hydroxyphenyl. Recent work shows that another alcohol called p-ferulic alcohol is present in gramineae lignins (Hartley and Jones, 1976) and is known to be present only in grasses (Van Soest, 1982). Lignin polymer is largely a condensed structure containing primarily ether and C-C bonding in a three-dimensional structure (Van Soest, 1982).

Lignin association with structural carbohydrates has always been considered a negative factor by animal nutritionists because it limits

the extent of digestion (Van Soest, 1982). Present evidence shows linkage with hemi-cellulose in ester bonding. Linkage with cellulose is probable, but has not been demonstrated because of the difficulty in preparing soluble derivatives that can be characterized (Van Soest, 1982). Major bonding of lignin with carbohydrates is by ester linkages between lignin alcohols and carbohydrates (Van Soest, 1981 and 1982; Hartley and Jones, 1972).

A number of theories have been proposed to explain the reduced digestibility associated with increasing lignin content.

The lignin-carbohydrate linkages which are resistant to enzymatic degradation is one (Van Soest, 1982). Evidence for this theory is provided by the fact that ester linkages between lignin and carbohydrates are easily cleaved by alkali treatment (Van Soest, 1981 and 1982; Jackson, 1977).

Another theory is direct enzymatic inhibition by polyphenyl fractions of lignin possibly as competitive inhibitors. This has been demonstrated in wild and tropical legumes (Van Soest, 1982).

Bender et al (1970) proposed that the arrangement of lignin molecules is a major factor in reducing digestibility. Hardwood lignin molecules (guaiacyl and syringyl) prevent three dimensional cross linkage formation, while softwood lignin molecules allow cross linkage and therefore formation of three dimensional lignin polymers. The three dimensional arrangement could effectively block enzymatic degradation. This factor may also explain why legumes response to alkali treatment is limited (Forsberg, 1977).

Lignin is also chemically associated with nitrogen in grasses and

legumes but not in woody trees resulting in reduced availability of nitrogen (Van Soest, 1982; Theander and Aman, 1984; Hartley, 1981).

Silica

Unlike other structural components of plant cell-walls, silica is taken up by the plant roots from the soil as monosilicic acid, $\text{Si}(\text{OH})_4$, and transported to the shoots. When water is lost by transpiration, silica is deposited in cell-walls where it occurs in opaline form ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) (Jones *et al.*, 1963). Silica exists in both soluble and insoluble form (Hogan and Weston, 1971).

Silica is high in paddy rice straws and hulls compared to straws and hulls of other plant residues (Table 2).

There is evidence that the presence of silica in cell-walls limits the degradability of polysaccharides in the rumen (Van Soest and Jones, 1968) and in-vitro dry matter digestibility (Hartley, 1981). The effect of silica upon digestibility is greater in paddy rice straws and hulls than in other low quality cereal roughages (Jackson, 1977) and therefore of particular concern to nutritionists. Like lignin, silica is rendered soluble by sodium or potassium hydroxides and to a lesser extent by calcium and ammonium hydroxides because the corresponding salts are insoluble.

The mechanism by which silica limits digestibility is not entirely understood (Van Soest, 1981). Hogan and Weston (1971) suggested that, undissociated silicic acid polymerize to form polymeric silicic acid (Opaline polysilicic acid) which is deposited in the cell-walls which in turn acts as a barrier to microbiological degradation. The importance of silica in this respect does not seem to have received much research

attention compared to other structural components.

Van Soest (1981) indicated that silica reduces palatability and contributes to lower voluntary feed intake of fibrous material.

THE DETERGENT SYSTEM OF FIBRE ANALYSIS

This system was developed by Van Soest (1967) to replace the proximate analysis because of the inherent inadequacy of the proximate analysis to partition the fibre components. The method has therefore allowed us to partition fibre into its constituents and therefore a better understanding of exactly which fibre components are nutritionally important.

The Van Soest method partitions forage organic matter into (i) cell contents, which are soluble in a neutral-detergent. These are almost completely digestible and not lignified; (ii) cell-wall constituents which are insoluble in neutral-detergents. This component is further partitioned into (a) acid-detergent fibre (ADF), which is insoluble in acid detergent and includes cellulose, lignin, silica and lignified nitrogen and (b) neutral-detergent fibre (NDF), which is soluble in acid-detergent but insoluble in neutral-detergent. Neutral detergent fibre includes hemi-cellulose, cellulose, lignin, and fibre bound nitrogen. Lignified nitrogen known as Acid-detergent-Insoluble Nitrogen (ADIN), has been used as an indicator of heat damaged proteins (Goering and Van Soest, 1967).

Van Soest (1986) concluded from work by Mertens (1973) that lignin and ADF are better correlated with digestibility than intake, because the lignified matrix, in ADF is the most unavailable feed factor. On the other hand, cellulose, cell-wall and hemi-cellulose are better

correlated with intake than digestibility.

UTILIZATION OF LOW QUALITY ROUGHAGES

Utilization of low quality forages has ranged from grazing low quality pasture and crop residues to stall feeding of these materials after baling. Their limitation in animal production as a sole ration can take many forms, probably the most easily seen and widely reported being weight loss in mature animals and failure of young animals to achieve satisfactory growth rates. However, when physically or chemically treated to overcome their limitations to utilization they offer potential for increased animal production, particularly in mixed rations. They offer economic potential in replacing part of the concentrate because of the ever increasing concentrate prices.

Edye et al (1971) and Siebert (1971) reported weight losses in mature and young cattle grazing dry residues ranging from 0.2 to about 1.0 kg/day on Townsville stylo-spear grass and rangeland. Rush et al (1976) reported similar weight losses in cattle grazing tropical pastures. Weyreter and Englehardt (1984) observed a 20% decrease in weight of Blackhead sheep grazing straw while Heidschnucken and Merino breeds were able to maintain their weight. They attributed these breed differences to the ability by Heidschnucken and Blackhead sheep to adapt to cellulose rich roughage diets. Heidschnucken but not Merino Blackhead sheep increased their reticulo-rumen volume and prolonged the retention times of both particulate and liquid phases.

Other grazing studies show that gestating beef cows were able to maintain weight without protein supplementation and small gains have been realized with low level of supplementation. Ward (1978) quoted

work by Lamm (1976) and Shmitz (1976) in which gestating beef cows grazed cornstalks and sorghum respectively. They reported 0.08 kg daily weight increase with nitrogen supplementation, with soybean meal supporting better performance than urea or dehydrated poultry manure (Lamm, 1976). Shmitz (1976) reported a significant response in weight gains to soybean meal supplementation and the response was higher with mature cows than young cows. They attributed this age difference in performance to the bulkness of the roughage and less rumen capacity per unit of body weight of young cows. However, Mulholland et al (1976) found no response in weight gains to urea supplementation of cereal stubbles by grazing sheep even though the intake was deemed adequate. Mulholland et al (1976) concluded that the small amount of energy in the pasture and grain supplement ingested was adequate, hence limiting the response to urea supplementation.

Early and Anderson (1978) found Kentucky bluegrass straw fed ad-libitum with a 32% crude protein commercial liquid supplement was an adequate ration and supported normal reproductive performance as indicated by subsequent calf performance and conception rates. The five varieties of Kentucky bluegrass straw used ranged from 3.6 to 7.6% in crude protein, 47.9 to 56.6% acid-detergent-fibre and 24 to 44% in-vitro dry matter digestibility.

Supplementation with urea and grains has produced varied results as can be seen from above. In general the main effect of supplementation on live weight growth of sheep and cattle grazing low quality roughages has been to reduce the rate of weight loss, or to maintain weight gains when supplemented animals lose weight or promote weight gains when

control animals maintain weight. Even with heavy supplementation with barley-soybean meal concentrate, weight gains are not adequate for fattening (Durham and Hinman, 1979).

In recent years, research has concentrated on incorporation of straws in concentrate diets.

Weisenburger and Mathison (1976) investigated the incorporation of pelleted barley straw in a complete diet. Straw was incorporated at 40, 55 and 70% of the total ration and the diets were isonitrogenous. Digestible energy decreased while acid-detergent fibre increased with increasing straw levels. Daily gains decreased with increasing straw level averaging 0.82, 0.71 and 0.62 kg/day respectively. Corresponding feed conversions were 10.9, 12.0 and 14.1 kg/kg gain. The steers fed the least straw produced the heaviest and fattest carcasses indicating that these steers retained more energy and thus utilized the energy in the diet more efficiently than steers on higher straw levels, since approximately the same amount of digestible energy was consumed per kilogram of body weight. More animals on the low straw level graded A1 than those on higher straw levels. These results are important because they show the decreasing productivity with increasing straw levels. This is supported by evidence from a trial by Mathison (1976), in which beef cows were fed barley straw at 85, 95 and 100% of the diet. Beef cows fed barley straw at 95 and 100% showed body weight changes ranging from -17 to -14 kg in the 91 days of the trial, while those on 85% straw changes averaged from +8 to +34 kg. Daily straw intake was significantly lower for the 100% ration compared to the other rations. He also noted abomasal compaction problems of animals fed 95 and 100%

straw diets. He concluded that diets containing less than 15% supplemental concentrate cannot be recommended for wintering beef.

Intake studies with barley straw (Weinsenburger et al, 1976) show that cows fed pelleted straw ate more feed and gained more weight than those on ground or chopped straw. Coombe et al (1979) reported similar results for coarsely chopped versus pelleted wheat straw. Total feed consumption decreased as percentages of straw in the diet increased. However, grinding and pelleting decreased dry matter, energy and crude fibre digestibility. Campling and Freer (1966) and Coombe et al (1979) have reported lower dry matter digestibility on ground and pelleted oat straw and wheat straw respectively. Grinding reduced the particle size and increased rate of passage and therefore resulted in less time for reticulo-rumen fermentation. Other studies (Lamming et al, 1966; Pickard et al, 1969) show that dry matter intake was positively related to the proportion of straw at least up to the 50% level while dry matter digestibility and feed efficiency decreased. Level of roughage did not affect dry matter intake (Lesoing et al, 1981).

Carcass characteristics results reported by Weisenburger and Mathison (1976) are similar to those reported earlier by other researchers. Carcass weight and killing percentage were shown to decrease with increasing fibre level (Swan and Lamming, 1970) when Friesian steers were fed ground barley straw at 30, 50 and 70%; also supporting their earlier findings (Swan and Lamming, 1967) using pulverized barley straw at 10, 30 and 50% of the total diet. Pickard et al (1969) found no significant differences in carcass characteristics using pulverized barley straw at 15 and 30% of the diet. Swan and

Lamming (1967) determined that carcass lean muscle and growth rate increases up to 30% pulverized barley straw incorporation, in the diet thereafter the lean decreases with more straw incorporation. However, a previous study with milled barley straw (Lamming et al, 1966) showed no significant differences in carcass characteristics at 20 and 50% straw. The differences observed among trials likely can be attributed to differences in particle size, ration form, kind of basal diet and animal age. Additionally, in most studies varying levels of straw replaced cereal grains without keeping caloric intake equal. This means that at lower straw levels, energy intake and gain would be higher.

NUTRITIONAL VALUE OF AMMONIA TREATED ROUGHAGES

General

Various chemicals have been used to preserve high moisture hay and silages and improve the nutritional quality of low quality roughages. Treatment of low quality roughages with ammonia not only increases intake (Horton, 1978; Dias-da-silva and Sundstol, 1986) and digestibility (Horton and Steacy, 1979; Lawlor and O'Shea, 1979; Saenger et al, 1983), but also increases the nitrogen content (Horton and Steacy, 1979; Kernan et al, 1977; Horton, 1978; Saenger et al, 1983). Ammonia also has fungicidal properties (Lacey et al, 1981; Grother et al, 1985; Knapp et al, 1975) and bacteriostatic effects resulting from lowered pH (Kernan, personal communication 1986; Grotheer and Cross, 1986).

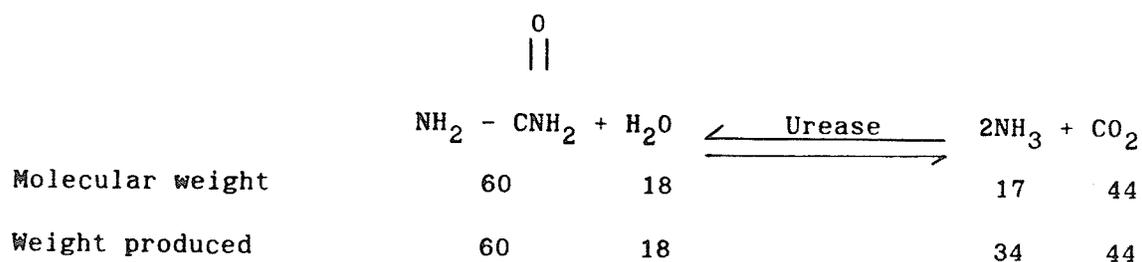
The two most common methods of roughage treatment are the oven and plastic cover methods. Excellent reviews are available describing the methods in detail (Kernan et al, 1977; Sundstol et al, 1978; Sundstol

and Coxworth, 1984).

Response to ammonia treatment is dependent on temperature, duration of treatment, moisture content, level of application of ammonia and type, initial quality and type of material (Sundstol et al, 1978; Sundstol and Coxworth, 1984) and also pressure (Chomyszyn and Ziotecka, 1972).

In most cases ammonia has been applied, either in aqueous or anhydrous form. Under normal pressure and temperature, ammonia is a colourless gas with a pungent odour. The gas is easily liquefied under pressure and dissolves readily in water. The boiling point at atmospheric pressure is 33.4°C and the freezing point is -77.7°C. Ammonia is normally available with a high degree of purity, >99.8% (weight basis). Anhydrous ammonia is packed in steel cylinders of varying sizes or in tank wagons and tankers.

Applying ammonia by using urea has received attention not only in developed countries but also in less developed countries because it is cheap and easy to handle. Urea is a crystalline solid produced technically from ammonia and carbon dioxide. Conversely, it decomposes completely at a temperature greater than 133°C in presence of urease enzyme yielding ammonia and carbon dioxide according to the equation below, (Sundstol and Coxworth, 1984).



Chemical Composition and Digestibility

High Moisture Roughages

Ammonia treatment has been shown to decrease dry matter losses and thereby reduce nutrient losses. Knapp et al (1975) reported that untreated alfalfa hay bales at 32% moisture lost 15.1% of its initial dry matter over a two month storage period compared to 9.9% loss with 1% anhydrous ammonia application. Jones and coworkers (1985) noted DM losses of up to 77.2% on untreated fescue hay in large round bales while treated bales with 2% ammonia had only 54.1% loss. Moisture averaged 18 to 20% at the time of baling. Thorlacius and Robertson (1984) and Rotz et al (1985) have also reported reduced dry matter loss of ammonia treated alfalfa hay.

The chemical composition of treated high moisture roughages shows that the effect of ammoniation is significant on levels of neutral-detergent fibre, hemi-cellulose and crude protein.

Grotheer and coworkers (1985) studied bermuda grass baled at 34% moisture and observed that hemi-cellulose and neutral-detergent fibre decreased from 38.5% to 27.3% and from 72.8% to 61.3% respectively after treatment with 3% ammonia, while crude protein increased from 16.8% to 26.9%. Decreases in hemi-cellulose have also been reported by Streeter and Horn (1984), and Buettner et al (1982) in wheat straw and tall-fescue hay respectively and Grotheer and Cross (1986) in coastal bermuda grass under laboratory conditions. Acid-detergent fibre did not show a clear trend due to treatment (Rotz et al, 1985). Knapp et al (1975) and Rotz et al (1985) reported only small increases in crude protein at 1% and 2.5% ammoniation levels respectively while Buettner et

al (1982) and Streeter and Horn (1984) noted increases comparable to that by Grotheer and coworkers (1985) at 3% ammoniation. Crude protein increases with rate of application though at a decreasing rate (Grotheer and Cross, 1986; Waagenpetersen and Thomsen, 1977) and the effect is higher with high moisture roughages than dry roughages (Grotheer et al, 1985; Grotheer and Cross, 1986; Waagenpetersen and Thomsen, 1977).

Grotheer and coworkers (1985) reported that 67.2% of the added N was retained in the treated high moisture hay. This compares to values of 60-80% reported by Knapp et al (1975) and 57% reported by Buettner et al (1982). Most of the nitrogen is retained as ammonium nitrogen. Of the added nitrogen 49.8%, 26.4% and 51.5% was retained as ammonium nitrogen as reported by Grotheer et al (1985), Buettner et al (1982) and Streeter and Horn (1984).

Acid-detergent-insoluble nitrogen increased significantly in both treated and untreated hay during storage (Knapp et al, 1975). However, when expressed as a percentage of the total nitrogen, the original samples were 7% ADIN compared to 6.7% ADIN in treated hay. ADIN was 9.1% of the total nitrogen in the untreated hay after storage. This suggests that only a small amount if any of the added nitrogen, became part of the indigestible ADIN fraction. Weiss et al (1982) had similar observations. Other workers, Streeter and Horn (1984) and Kersbergen and Barton (1986) reported higher ADIN for untreated than treated wheat straw and red-clover/timothy hay respectively. The effect of high temperatures has been discussed. Ammonia treatment reduced heating during storage of high moisture hay (Knapp et al, 1975; Lacey et al, 1981; Orskov et al, 1983).

Ammoniation increased ($p < 0.05$) apparent digestibility of nutrients both in-vitro and in-vivo (Grotheer et al. 1985) of coastal bermuda grass. Similar observations have been reported by Streeter and Horn (1984), Males and Gaskins (1982) and Buettner et al. (1982). Grotheer et al. (1985) reported a 21.4% increase in IVDMD of hay while Knapp et al. (1975) noted an 11% and 20% increase for treated alfalfa and clover-fescue respectively.

Low Quality Roughages

Treatment of low quality roughages with ammonia and other chemicals has been directed toward removing the digestibility-limiting factors outlined. There is enormous data with regard to chemical treatment and results are consistent in some areas but inconsistent in other areas. Differences in the nutritive values of ammoniated feeds seem to be caused primarily by differences in technology (Chomyszyn and Ziiolecka, 1972).

Most experiments conducted show that ammoniation either by aqueous or anhydrous ammonia, or urea or ammonium hydroxide results in elevated crude protein. Horton and Steacy (1979) reported improvement in crude protein content ranging from 50 to 276% after a 3% rate of application to different varieties of wheat, barley and oat straws. These findings are consistent with values quoted by Dupchak and Stewart (1984). They quoted improvements in crude protein content ranging from 72 to 125% when wheat chaff and straw, barley and oat straw were ammoniated with 3% ammonia. Similar improvements have been reported by Kernan et al. (1977), Horton (1978), Saenger et al. (1983), Herrera-Saldana et al. (1983); Dias-da-Silva and Sundstol (1986). Treatment with urea or

ammonium hydroxide give similar results (Herrera-Saldana et al, 1983; Dias-da-Silva and Sundstol, 1986). Crude protein content increases with increased dosage rate and duration of treatment (Waagenpetersen and Thomsen, 1977; Orskov et al, 1983) at least up to 5% dosage rate and four weeks of treatment time. Kernan et al (1977) showed no significant differences in crude protein content of roughages treated at 5% and 3% application rates. Lawlor and O'Shea (1978) showed no differences in crude protein content after 30 and 65 days of treatment. Initial quality and moisture level also influence the level of crude protein content after treatment. Relatively higher moisture levels gives better bonding in straws than dry straw and low quality roughages resulting in higher crude protein content than high moisture, high quality roughages (Waiss et al, 1972; Hartley and Jones, 1978; Sundstol et al, 1978).

Results of effect of ammoniation is unclear for cellulose and ADF while data for hemi-cellulose, lignin and NDF is quite consistent across experiments. Saenger et al (1983) reported that NDF and hemi-cellulose decreased from 74.8 to 69.6% and from 22.6 to 12.1% respectively upon ammoniation of wheat straw. However ADF slightly increased from 52.3 to 54.8% while Dias-da-Silva and Sundstol (1983) also reported a decrease in hemi-cellulose from 32.9 to 28.1 and 25.5% upon ammoniation with anhydrous ammonia and urea respectively. No differences in cellulose and lignin were shown between the treated and untreated wheat straw. Similar observations for ADF, cellulose and lignin (Garrett et al, 1979) and NDF (Waagenpetersen and Thomsen, 1977) have been reported. On the whole, where a decrease or increase in ADF and cellulose have

been reported, the change is small and statistically insignificant. Upon examination of data from sodium hydroxide treatment of crop residues similar inconsistencies in results are observed for ADF and cellulose and similar consistencies are noted for NDF and hemi-cellulose (Garrett et al., 1979; Ololade et al., 1970; Wignjosoestrol and Young, 1982; Van Eanaeme et al., 1981; Jackson, 1977). Two experiments which could elucidate these observations are those by Anderson and Ralston (1973) and Ololade and coworkers (1970). Ololade and coworkers (1970) treated alfalfa stems, barley straw and corn stover with increasing levels of sodium hydroxide (0, 2, 4 and 8% of the DM) at 23°C for 24 hours. ADF and cellulose content showed no significant differences upon treatment though 2 to 3 percentage points increases and in some other cases no increases were noted. Lignin content remained relatively constant while cell-wall constituents significantly decreased particularly for straw and stover. Results by Anderson and Ralston (1973) are at variance with respect to ADF and lignin. They treated rye grass with sodium hydroxide at 0, 0.5, 2, 4, 6 and 8% of the dry matter by soaking in the chemical for 24 hours. They reported a significant decrease in ADF and acid-detergent lignin (ADL). These differences may be due to the fact that Anderson and Ralston (1973) treated by soaking. It is highly likely that the solubilized cell-wall remained in the solution. The effect of washing upon fibre constituents after sodium hydroxide treatment has been pointed out (Jackson, 1977), and proved by Anderson and Ralston (1973) in a separate experiment.

Increase in the digestibility of nutrients is the most consistent result of ammonia treatment with the exception of the crude protein

increase. Saenger et al (1983) reported that IVDMD changed from 40.6 to 51.2% upon ammoniation of wheat straw representing a 26.1% percentage change. Lawlor and O'Shea (1979) and Eng (1984) reported and quoted respectively an increase in IVDMD of 34% and 23% respectively for wheat straw. In-vivo apparent dry matter digestibility of barley, oat and wheat straws increased by 2.2, 3.7 and 6.3 percentage units respectively (Horton and Steacy, 1979). The apparent dry matter digestibility of Neepawa wheat straw increased from 28.6 to 40.8% upon ammoniation, representing a 43 percentage change. These values changed from 51 and 57.1% respectively upon ammoniation when fed with a concentrate diet, representing a 12 percent change (Horton and Steacy, 1979). Lawlor and O'Shea (1979) reported similar percentage changes for treated barley straw when fed alone but the improvement in digestibility was reduced when barley grain or a concentrate was fed in combination with the treated straw. Improvement in apparent dry matter digestibility has also been reported by Horton (1978), and Orskov et al (1983) for treated barley straw, Horton (1978) for oat straw, Horton (1978), Herrera-Saldana et al (1983) and Dias-da-Silva and Sundstol (1986) for wheat straw although lower improvement than those obtained by Horton and Steacy (1979) and Lawlor and O'Shea (1979).

Apparent organic matter digestibility of Neepawa wheat straw improved by 50% upon ammoniation and this improvement was reduced to 12% when straw was fed with a concentrate (Horton and Steacy, 1979). Orskov et al (1983), Herrera-Saldana (1983), Dias-da-Silva and Sundstol (1986), and Lawlor and O'Shea (1979) have also reported improvements in apparent organic matter digestibility of treated barley and wheat straw

respectively, though values obtained were lower than those obtained by Horton and Steacy (1979).

Apparent digestibility of fibre components have been shown to increase upon treatment with ammonia. Al-Rabbat and Heaney (1978) reported a 45% increase in ADF apparent digestibility of ammoniated wheat straw fed at 64% of the diet. Herrera-Saldana et al (1983) and Orskov et al (1983) reported increases of up to 16% and 22% after treatment of wheat and barley straw respectively.

In-vitro NDF disappearance increased from 42.5 to 67.6% upon ammoniation of wheat straw (Saenger et al, 1983) while Mann and coworkers (1986) reported changes from 9.1 to 18.4%, 46.3 to 56.6% and -0.6 to 37.9% for wheat straw, wheat chaff and flax straw respectively.

Al-Rabbat and Heaney (1978) and Dias-da-Silva and Sundstol (1986) reported increased apparent digestibility of cellulose after treatment of wheat straw. They reported increases from 41 to 67% and 47.3 to 56.8% respectively. Garrett and coworkers (1979) also reported increased cellulose apparent digestibility of diets containing treated rice straw.

In-vitro hemi-cellulose disappearance increased from 12.1 to 100% after treatment of wheat straw (Saenger et al, 1983) while Dias-da-Silva and Sundstol (1986) got increases from 56.4 to 71.2% upon ammoniation of wheat straw incorporated into a mixed diet.

The importance of lignin digestion is not yet known and has not received much attention. Herrera-Saldana et al (1983) showed an increase in lignin digestion upon treatment of wheat straw while Dias-da-Silva and Sundstol (1986) showed negative digestibility.

Klopfestein (1978) suggested that the increase in extent of lignin digestion is probably due to breaking of bonds between lignin and hemi-cellulose or cellulose without actual removal of lignin.

Garrett and coworkers (1979) reported significant increase in gross energy digestibility. The digestibility increased from 50.2 to 57.2% and 59.2 upon treatment of rice straw with 7% and 4.7% ammonia respectively. Similar increases have been reported by Al-Rabbat and Heaney (1978), Horton (1978) and Horton and Steacy (1979) for diets containing treated wheat straw. Herrera-Saldana et al (1983) reported smaller increases. Most studies show significant improvement in crude protein apparent digestibility with increasing crude protein content. Dias-da-Silva and Sundstol (1986) reported an increase from 16.5 to 42% and 38.2% in apparent digestibility of nitrogen after treatment with anhydrous ammonia and urea, respectively. Horton (1978), Horton and Steacy (1979), Al-Rabbat and Heaney (1970) and Herrera-Saldana et al (1983) also showed increased apparent digestibility of crude protein. However, Garrett and coworkers (1979) and Oji et al (1977) reported lower nitrogen digestibility of sodium hydroxide and ammonia treated rice straw diets and sodium hydroxide treated corn-stover respectively. Recently Horton et al (1982) also reported lower crude protein digestibility with ammoniated wheat straw.

The exact mode of action of NH_3 on fibre fractions concentration and digestibility is not well understood. Basically the low digestibility of structural carbohydrates of cell-walls is a result of association of these fractions with lignin and response in concentration and digestibility upon chemical treatment is related to the lignin

content (Van Soest, 1967, 1982; and Feist et al, 1970). The hypothesis appears to be that NH_3 combines with moisture in the roughage to form a weak base of NH_4OH (Hodgkinson and Devlin, 1978). The NH_3 binds to the carbohydrates of the cell-wall to form R-CO-NH_2 (Buettner et al, 1982; and Barton II, 1986). Buettner and coworkers (1982) reported that ammoniation significantly reduced ester bond absorbance and increased amide bond absorbance. They concluded that the changes in infrared absorbance properties presumably results from breaking of ester bonds between lignin and structural carbohydrates resulting in greater fibre digestibility. All theories that have been discussed before have actually centred on cleavage of lignin/carbohydrate bonds. More evidence is also provided by chemical treatment with NaOH. Feist et al (1970) reported that lignin and intermolecular ester linkages between uronic acid groups of hemi-cellulose and cellulose are probably hydrolysed as a result of chemical treatment with NaOH and cleavage of ester bonds results in additional swelling in the water (accessibility). Swelling of cellulose resulting from NaOH treatment reducing the strength of intermolecular hydrogen bonds should be more easily penetrated by rumen fluid, accounting for the greater digestibility of cellulose. Cleavage of ester bonds and solubilization of lignin and silica (Feist et al, 1970; and Burney and Van Soest, 1984) and hemi-cellulose (Feist et al, 1970; Hartley, 1973; Saenger et al, 1983) by either NaOH or HN_3 treatment should provide rumen bacteria with greater substrate availability and therefore increased digestibility in DM and fibre fractions. Solubilization of these materials results in decreased hemi-cellulose, ADF and NDF content of treated roughages.

Other evidence of cleavage of lignin/carbohydrate bonds has been shown by the release of p-coumaric, ferulic, vanillic and other phenolic acids upon treatment of roughages with NaOH (Hartley, 1972, 1973, 1983). In summary, solubilization and cleavage of bonds increases the extent and rate of digestion of structural carbohydrates (Klopfenstein, 1978).

Intake

High Moisture Roughages

Voluntary dry matter intake results are varied across experiments. Weiss et al (1982) and Grotheer et al (1985) reported no significant differences in daily dry matter intake of treated alfalfa and coastal bermuda grass hay and untreated hays fed to lactating dairy cows and lambs, respectively. However, consumption tended to be higher on treated hays. Grotheer and coworkers (1985) reported ad libitum intake of 646 g/day and 583 g/day for treated and untreated bermuda grass, while Weiss et al (1982) reported intakes of 11.4 and 11.7 kg/day/head for treated and untreated alfalfa hay respectively. Atwal and Heslop (1984) also reported no significant differences in intakes of treated and untreated alfalfa hay baled at 25% moisture when fed to growing steers.

However, other studies show increased daily dry matter intake after ammoniation of high moisture roughages. Males and Gaskins (1982) noted that total dry matter intake increased from 5.7 kg/day/head on untreated dry wheat straw to 8.0 kg/day/head on wet ammoniated straw, while roughage intakes were 2.0 and 3.8 kg/day/head respectively. This represented a 90% increase in straw intake.

Similar observations were made by Streeter and Horn (1984) on wheat

straw and Buettner et al (1982) on tall fescue hay. However the increase in intake was lower than reported by Males and Gaskins (1982), Buettner et al (1982) reported a 32% and 51% increase in intake of ammoniated roughages over untreated roughage for sheep and heifers respectively, while Streeter and Horn (1984) noted that lambs fed ammoniated straw ate 22% more than those fed untreated straw. Recently, Jones et al (1985) also observed improvement in ad libitum intake of high moisture tall fescue after treatment with ammonia.

This discrepancy in ad libitum intake may be explained on the basis of nutrient content of the material used with respect to crude protein and fibre content, and whether the comparison is between untreated dry and treated high moisture hay. From the preceding discussion it appears that ammoniation of medium and high quality hay does not result in significant improvement in intake. This is supported by many studies on ammoniation of ensiled material (Bareeba et al, 1983; Hargreaves et al, 1984; Thorlacius and Robertson, 1984). Weiss et al (1982) and Grotheer et al (1985) used roughages whose crude protein and ADF content averaged 17% and below 40% respectively, in the untreated roughages while researchers who reported increased intake started with forages containing below 8.0% crude protein, and ADF content above 50%. Forage harvested at the proper stage of maturity has relatively high fibre digestibility and therefore ammoniation should not be expected to affect it as much, compared to straws (Horton and Steacy, 1979). Van Soest (1965) noted that on high quality forages where the fibre fraction is small, intake may not be limited by fibre mass. Thorlacius and Robertson (1984) suggested that since voluntary intake is related to

cell-wall digestibility and rate of passage, the potential of ammoniation to affect intake is more likely to be limited with higher quality forages except in instances where heating and spoilage would otherwise affect digestibility and perhaps acceptability.

Low Quality Roughages

Most experiments conducted so far indicate improvement in intake of low quality roughages upon ammoniation.

As a sole ration, ammonia treatment of barley straw has been shown to increase dry matter intake up to 50% over untreated barley straw (Orskov et al, 1983) when fed to Friesian heifers. Horton (1978) in an earlier study with barley, wheat and oat straw showed a 41% increase in voluntary intake upon ammoniation of these materials fed to Hereford steers. The voluntary intake increased from 39.1 to 46.5, 39.5 to 52.8 and 46.2 to 61.9 g/kgW^{.75} after treatment of wheat, barley and oat straw respectively. Recently, Dias-da-Silva and Sundstol (1986) have demonstrated increased intake of wheat straw when fed to male sheep after treatment with ammonia.

However, when treated roughages are fed in combination or in mixed diets, some studies have shown a slight depression in intake over the untreated. In some studies the increase in intake is lower compared to that obtained in experiments utilizing the roughages as a sole ration. In the experiment by Horton (1978) there were no significant differences in intake between untreated and treated wheat, barley and oat straw when they were supplemented with a concentrate. They attributed this observation to higher consumption of the concentrate (3.6 kg DM/day). These results are consistent with those obtained by Garrett et al (1979)

for rice straw, Al-Rabbat and Heaney (1978) for wheat straw and Rissanen et al (1981) for barley straw, but at variance with later findings by Horton and Steacy (1979) for the same straws. Light supplementation of treated wheat straw with corn showed significantly higher intake by yearling steers than untreated straw supplemented with soybean meal (Saenger et al, 1983). Dupchak and Stewart (1984) quoted results from Swift Current Research Station, which also showed higher intake of treated straw supplemented with 2.5 kg oats than untreated straw supplemented with 2.25 kg oats plus 1.4 kg alfalfa pellets.

The increase in intake has been attributed to increased dry matter and fibre fraction digestibility (Horton and Steacy, 1979; Orskov et al, 1983; Dias-da-Silva and Sundstol, 1986; Saenger et al, 1983) resulting in lower retention time. Nitrogen supplementation has also been shown to increase intake (Campling et al, 1962; Horton, 1978; Dias-da-Silva and Sundstol, 1986; Lee et al, 1985).

Al-Rabbat and Heaney (1978) suggested that the higher intake of untreated straw observed could be as a result of a compensatory feeding carry-over effect which was not anticipated with the experimental design. The untreated straw was fed in the trial immediately following the trial in which steamed aspen wood ration was fed. Heaney and Pidgen (1972) reported that, for grasses and legumes or grass-legume mixtures an adjustment or "pre-conditioning" period of 10 days was adequate to ensure reliable intake assays.

Performance

High-Moisture Roughages

Data on performance with respect to high moisture (ammoniated) hay is limited. However, the limited data suggests that, when incorporated into mixed diets, ammoniated high moisture hay or straw can produce results similar to good quality hay.

Males and Gaskin (1982) showed no significant differences in gain of male calves fed smooth-brome grass or wet ammoniated (reconstituted) wheat straw, though gains were slightly lower on stack ammoniated straw ($p < 0.05$). The roughages were fed at 56, 35, 48 and 42% of total diet for hay, chopped straw, wet NH_3 straw and stacked NH_3 straw respectively. They reported gains of up to 1.10, 1.04 and 0.91 kg/day/head for chopped smooth-brome grass hay, wet ammoniated straw and stacked ammoniated straw respectively. The feed/gain ratio did not differ significantly though animals on stacked ammoniated straw had a lower feed conversion efficiency. These findings were supported by results from heifers. Average daily gains were higher for heifers fed hay (0.98 kg/day) compared to wet ammoniated straw (0.84 kg/day) but values were not significantly different ($p > 0.05$). Heifers fed chopped untreated and stacked ammoniated straw had the lowest gains of 0.7 and 0.65 kg/day respectively. The feed/gain ratio was higher for stacked ammoniated straw and significantly different from the other treatments.

Davis (1979 and 1980) found a higher response in steer gain fed a diet containing only 25% high moisture ammoniated straw compared to the untreated straw.

However ammoniated high moisture roughages as a sole ration,

presently, does not seem to support gains as suggested by data from Jones and coworkers (1985). They reported negative weight gains for cows fed control and ammoniated high moisture alfalfa hay.

Data on milk production and related parameters shows that, alfalfa hay at 30 or 35% moisture could be treated with anhydrous ammonia and fed to lactating dairy cows with no detrimental effects (Weiss et al, 1982). They found no significant differences in actual milk production (kg/day), fat-corrected milk (FCM) and milk protein when lactating cows, 95 days postpartum were fed untreated (undamaged) and treated alfalfa hay. However, milk fat differences were significant at 0.10 level with ammoniated hay being slightly higher. In this study there was no soybean supplementation with the treated hay. Either the extra protein was not required or treatment with ammonia had a N sparing effect.

In another study, Hargreaves et al (1984) used ensiled ammoniated high moisture corn stalklage, they reported that dry matter intakes and milk yields were usually depressed when stalklage comprised 25% of the diet dry matter, but not at 10 or 20%. In all the trials, there was no significant differences in milk yields, milk fat, milk protein, feed efficiency and dry matter intake when ammonia treated stalklage was compared to untreated corn stalklage. The lower intakes of ammonia treated stalklages incorporated at 25% of the diet compared to corn silage and untreated stalklage explained the lower milk production observed. Song and Kennelly (1986a and 1986b) made similar observations with 1% ammonia treated barley silage.

Other studies with silages show that cows fed ammoniated silage as the main roughage produced more milk than those fed isonitrogenous

diets of control or urea silage (Huber et al, 1973), particularly at high intakes of non-protein nitrogen (NPN) (Huber et al, 1975). However, Bareeba et al (1983) found no differences ($p > 0.05$) in milk production and composition or dry matter intake when ammoniated corn silage was compared to urea treated corn silage.

These studies suggest that ammoniation as a method of preservation should allow dairymen to maintain hay quality under adverse conditions (Weiss et al, 1982).

Low Quality Roughages

Performance trials show great potential for utilization of ammoniated low quality roughages particularly in beef production for beef cows that require relatively low energy dense diets. Their value will vary according to availability of quality feeds and prices, time of year when to be used and therefore physiological stage of animals and production levels being aimed at.

Orskov and coworkers (1983) demonstrated that ammoniated barley straw could be used as a sole ration. They reported daily weight changes of -44.7 and 324 g/day of Friesian heifers fed untreated and treated straw, respectively. These results are at variance to those reported by Saenger et al (1983) with pregnant beef cows on corn supplemented diets. Cows receiving ammoniated wheat straw lost 7.2 kg while on untreated straw supplemented with soybean meal lost 40.7 kg in 90 days. These differences could be a result of differences in intake and physiological stage of the animals. Orskov and coworkers (1983) reported a 50% increase in intake upon ammoniated while Saenger et al

(1983) reported a 29% increase. Additionally, Saenger et al (1983) used pregnant cows and it may be argued the ration was not adequate to support weight gains or maintain weight as well as fetal growth. These results however do show potential for overwintering pregnant animals.

Horton et al (1982) compared shredded or pelleted untreated straw to the corresponding treated wheat straw fed to steers in a fattening program, with straw making up 40% of the total diet. Significantly higher gains with shredded ammoniated straw diets were obtained than the corresponding untreated diets (0.83 vs. 1.13 kg/day). Feed efficiency was not significantly different though steers fed untreated straw did eat more for a kilogram gain. They showed no significant differences in rib-eye area measurement but fat thickness and dressing percentage were significantly decreased with ammoniated straw being superior. Pelleted ammoniated straw was not superior over the corresponding untreated straw and gains were similar to ammoniated shredded straw. Higher gains and similar observations for carcass characteristics have been reported for ammoniated straws (Garrett et al, 1979; Mann et al, 1986; Al-Rabbat et al, 1978; and as quoted by Dupchak and Stewart, 1984).

Mann et al (1986) have shown that ammoniated low quality straw and chaff has a feeding value similar to medium quality hay both in terms of gains, carcass characteristics, calf birth weight, calving ease and vigour and therefore do offer an alternative to hay during the overwintering period of beef cows. Their results are similar to those quoted by Eng (1984) with respect to gains and carcass characteristics.

Zorrilla-Rios et al (1984) showed that increasing the crude protein of untreated wheat (4.2 to 11%) had a sparing effect of 0.585 kg of

soybean meal (50% CP DMB) for 280 kg steers gaining at a rate of 0.45 kg/day.

From the above data its quite apparent that ammoniated low quality roughages will increase animal performance and may support small gains if fed alone but better gains are realized in well formulated rations.

To date a limited amount of data is available on ammoniated low quality forages being fed to dairy cows. Rissanen et al (1981) reported no significant differences in intake, milk production and composition when Ayrshire dairy cows were fed untreated, or treated spring barley straw or hay together with 20 or 30 kg/day of grass silage plus concentrate. Kristensen (1984) quoted two experiments in which one of them the results agreed with those reported by Rissanen et al (1981) while another experiment showed that ammoniated barley significantly increased milk yields (21 vs. 19.7 kg/day) and fat content (4.12 vs. 3.89%). Mo (1978) as quoted by Kristensen (1984) showed that 3.5 kg high quality silage dry matter could be replaced by treated barley straw with no significant negative effect on milk production and fat. Recently Khalaf and coworkers (1986) reported higher gains in lactating dairy cows fed a diet in which 50% of the roughage source was treated wheat straw in place of untreated straw. However, there was no significant differences in milk production though milk fat percentage was increased by 0.6 unit by inclusion of untreated wheat or treated straw when compared to diets where alfalfa was the only roughage source. They concluded that wheat straw could be more useful than generally believed in diets for lactating cows of average milking ability.

It may also be suggested that treated straws offer great potential

for increasing milk production in developing countries by small milk producers who are generally not able to provide their animals with high quality roughages and concentrate.

TOXICITY OF AMMONIATED ROUGHAGES

Treating hay with anhydrous or aqueous ammonia produces many benefits. However, recent reports from the U.S. show that ammoniated hay may be toxic. A number of states in the U.S. have reported symptoms ranging from trembling, ear twitching and salivation to stampeding, convulsions and death in cows fed ammoniated brome grass, fescue, wheat bermuda grass and sorghum hay but not ammoniated low quality roughages (Eng, 1984; Anonymous, 1984; and Morgan and Edwards, 1986). Calves fed milk from cows eating such hays have shown similar symptoms. Similar symptoms have been produced in animals fed molasses-urea-protein blocks (Morgan and Edwards, 1986).

Recently, similar symptoms have been demonstrated under experimental conditions.

This problem is not new. The symptoms observed are similar to those observed in the early 50's with feeding ammoniated-cane-molasses (Tillman et al, 1957a; Tillman et al, 1957b). The condition was called "Stimulation". Today, the condition has been given different names such as Bovine Bonkers Syndrome or Ammoniated Feed Syndrome (Morgan and Edwards, 1986) and Crazy Cow Syndrome (Anonymous, 1984) or Crazy Calf Syndrome (Brown, 1984).

Tillman et al (1957a) proposed that the presence of imidazole type derivatives in ammoniated-cane-molasses was responsible. Citation by Nishie et al (1969) show that 10% imidazole and 20% pyrazine derivatives

in ammoniated molasses have been isolated. These compounds results from a reaction between reducing sugars with ammonia. This reaction supports the observation that only high quality hay produce problem hays upon ammoniation. High quality hays contain higher levels of soluble carbohydrates which can react with ammonia.

The convulsant and lethal effects of imidazole, 1-, 2-, and 4-methylimidazole and other related compounds have been demonstrated in mice, chicks and rabbits (Nishie et al, 1969; Nishie et al, 1970) under experimental conditions. Recently Ray and coworkers (1984) have isolated 2- and 4-methyl derivatives from problem rye, wheat, coastal hays and protein blocks, which caused toxicity on farms. Concentrations of the compounds ranged from 5 to 55 ppm. They however caution that evidence linking substituted imidazoles to toxicity in livestock is at this point presumptive.

Recent experimental work by Weiss and coworkers (1986) and Weiss (personal communication 1986) argues that, generally ammoniated forages contain less than 100 ppm of 4-methylimidazole which equates to less than 2 g/day consumption. After dilution in body fluids, it is in concentrations so low that it cannot be detected in blood and evidence from Ohio and Oklahoma shows that 4-methylimidazole is not transformed into milk. Work by Weiss and coworkers (1986) support the above observations. They have reported convulsions and death in sheep fed orchard grass hay treated with 4% ammonia. High levels of pyruvic and lactic acid were detected in blood. Similar observations were reported in calves fed milk from a cow fed ammoniated oat hay (5% of DM). However, when a potential 4-methylimidazole toxin was orally given to

sheep for five days, no toxic effects were produced. They noted from the literature that the neurological aberrations produced by ammoniated hay were similar to signs caused by certain alkaloids such as tryptamine, ergot and some penicillium. They therefore went ahead to test for alkaloids. When the crude alkaloid fractions isolated from toxic and control milk were injected subcutaneously into mice, no response occurred with control milk, but mice injected with toxic milk extract ran in circles, jumped into the walls of their cages and scratched violently. Signs developed in about three minutes. Weiss and coworkers have therefore suggested that the toxin is found in the alkaloid fraction and that it is a fluorescent compound which elutes at 8.1 minutes with the chromatographic system they used.

Certainly more work is needed to elucidate the position played by imidazoles and the newly proposed alkaloids in the etiology of this syndrome.

MATERIALS AND METHODS

PART I

Ammoniation Procedure

Ammonia-treated or untreated alfalfa hay (Medicago sativa var. Chimo) and barley green feed (Hordeum vulgare var. Bonanza) were obtained from two co-operators in Selkirk, Manitoba in 1984.

Alfalfa hay was harvested on September 4th, 1984 using a New Holland hay bind. Due to bad baling weather conditions, baling of high moisture hay took place on September 11th, 1984 using a New Holland round baler. Every second bale was left as a control (high moisture) or for ammoniation.

Moisture readings were taken from each bale using an electronic moisture tester (Del-mar moisture meter). An average moisture content of 23.2% was recorded.

Twenty-six bales of alfalfa hay at high moisture were ammoniated on September 14th, 1984. The bales weighed 697 kg (1,534 lbs.) on average at the time of ammoniation.

The procedure of ammoniation used, was as follows. The bales were stacked in a 3-2-1 configuration and covered with a 6 mil (35 um) black plastic. The plastic was weighted at the edge of the stack with sand to seal the plastic to the ground (approximately 2 feet of excess plastic) to prevent escape of ammonia.

The anhydrous ammonia was injected from a nurse tank at an estimated rate of 2% of dry matter forage weight. A steel pipe measuring 6.096 m long and 5.0 cm diameter joined to the nurse tank

hose-pipe through control valves was pushed into the stack to deliver the ammonia. The pipe had holes drilled at every 15.24 cm and the end was sealed by hammering to make a pointed end. After ammoniation the stack was left covered for the reaction to take place. The stack was opened in the first week of November 1984 making about seven weeks of treatment time.

Temperatures of the wet-treated and untreated hay were monitored using a potentiometer with thermocouple wires placed into the bales using a feed probe to place the wires. Some damage occurred to the thermocouples when placing in the hay bales.

Barley green feed (30% moisture) was obtained from River Bend farm in the Whitemouth area. The green feed was harvested on August 18th, 1984 and baled using a New Holland round baler. Forty bales with an average weight of 545 kg (1,200 lbs.) were ammoniated on August 20th, 1984. Ammonia was applied at a rate of 2% of the forage dry matter weight. The procedure of ammoniation was basically similar to that used for alfalfa hay. The barley green feed was harvested in the early milk stage.

Because of bad weather conditions for drying it was difficult to secure dry bales from this batch, therefore one dry bale of similar maturity was obtained from Wavey Creek farm, Clandiboye area, to act as a control. The bale weighed 637 kg (1,401 lbs.) with an average moisture content of approximately 21%.

The stack was opened on October 5th, 1984, resulting in a treatment time of about seven weeks. Removal of the covering plastic is necessary to release the excess ammonia before feeding.

The average ambient temperature for Selkirk was 20.9° C and 10.1° C for August and September respectively. The treatment time was therefore within the range of that recommended for low quality forages as suggested by Sundstol et al (1978) for such ambient temperatures.

Bales were visually inspected for any signs of molding characterized by whitish powder.

The forages were chopped using a Tub Grinder (Rotor Grind) and immediately packed into nylon and jute bags. The bags were brought to Animal Sciences Department (Ruminant Metabolism barn) where they were stored for feeding.

Intake and Digestion Trial

Four test forages were employed, ammonia-treated high moisture alfalfa hay (Medicago sativa var. Chimo), untreated-alfalfa hay, ammonia-treated high moisture barley green feed (Hordeum vulgare var. Bonanza) and untreated barley green feed in a 4 x 4 latin square design (Table 3) involving four periods and 4 mature wethers.

The wethers were shorn and weighed 39 kg on average at the start of the trial. They were also weighed on the last day of adjustment, intake and digestibility period. Weighing was always done in the morning before the feed was offered to reduce variations due to fill (Hughes and Harker, 1950).

They were housed in the basement of Animal Science building (University of Manitoba), in a single metabolism crate with 4 individual compartments and feeding troughs (Plate 1). They were randomly assigned to individual compartments and to one of the four forage test diets according to Snedecor & Cochran, (1980). The sheep on hay were

Table 3. Randomization of the 4 x 4 latin square design.

Animal I.D.	Period			
	1	2	3	4
1010	D ¹	A	C	B
0127	C	D	B	A
0131	B	C	A	D
0133	A	B	D	C

(Snedecor & Cochran, 1980).

¹Test forages; A - untreated-barley green feed; B - untreated-alfalfa hay; C - ammonia-treated alfalfa hay; and, D - ammonia-treated barley green feed.



PLATE 1: Metabolism crates for intake and digestibility trial for wethers.

supplemented with a vitamin-mineral premix at 3.3 gms/day (Table 4) but no grain, and those on straw received either urea-treated or untreated barley grain, plus 9.3 gms of vitamin-mineral premix (Table 4).

The urea was added to the barley grain at 2.5% of barley grain on an air dry basis as a 25% solution (W/W) according to Orskov et al (1974). The solution was sprinkled on the grain using a plastic container with a spout. This was thoroughly mixed using a commercial dough mixer (Hobart, The Hobart Manufacturing Co. Ltd., Toronto, Canada, Model B600).

Four periods were involved each consisting of a 10 day adjustment period to the diet, 7 days of intake, 3 days adjustment to 90% ad-libitum intake and 6 days of digestibility.

The animals were individually fed between 11:00 a.m. and 11:30 a.m., starting with grain, which was readily consumed. The animals on ammoniated barley green feed received 260 gms/day air dry of untreated grain and the animals on untreated barley green feed received 266 gms/day of urea-treated grain.

At the start of each period, the animals were given about 400 gms of forage. This amount was increased each day until they left some feed (about 5-10% weighback). This was done to determine maximum voluntary intake before the intake measurement. Maximum voluntary intake was reached within 5-6 days of the adjustment period leaving the last 4-5 days to confirm maximum intake. Water was available all the time through a nipple system.

After this initial 10 day adjustment period, hay and straw DM intake was measured for 7 days according to Heaney et al (1969) with a

Table 4. The composition of the vitamin-mineral supplement fed to wethers.

Item	Vitamin-Mineral Mix (Hay) gm/day	Vitamin-Mineral Mix (Barley) gm/day
Biophos	-	1.0
Limestone	-	5.0
Cobalt-iodized salt	2.5	2.5
DH premix ¹	0.8	0.8

¹DH premix - Dairy herd premix (amount per gm premix), vitamin A, D₃ and E, = 1750, 150 and 2 IU respectively; Se, Cu, Zn, Mn and Mg = 0.017, 2.2, 8.0 and 10.8 mg respectively.

few modifications. Approximately 5-10% more hay or green feed was fed than was consumed the previous day to assure free choice conditions throughout each day.

The feeding procedure during this period involved, removing the leftover feed, weighing it and taking a sample. Grain and forage samples were taken daily before feeding the appropriate amounts with the grain and vitamin-mineral mix being fed first. The feed and weigh-back samples were bagged in polyethylene plastic sample bags and stored at room temperature for further analysis.

Hay and straw digestibility was measured immediately after intake measurements according to the method described by Heaney et al (1969) as a basic procedure. The basic feeding procedure is as described for intake measurements except that the forage was given at 90% the Ad libitum intake (Forsberg, 1977; Heaney et al, 1969). Weigh-back samples if any were taken for DM analysis.

Fecal collection started 48 hrs. after the first day feeding of the 6 day digestibility period, and finished 48 hrs. after the 6th feeding day; that's assuming that the feed takes 48 hrs. to traverse through the entire gut. The feces were collected once/day between 11:00 a.m. and 12:00 p.m. in polyethylene plastic bags attached to the rumps of sheep using Bull Cement (Bull Cement, 3M Brand, 3M Company, 3M Centre, St. Paul, Minnesota) (Plate 1).

The fecal collection bags were made from a 6 mil (135 um) transparent polyethylene plastic measuring about 60 x 45 cm. The plastic was folded lengthwise and sealed using a commercial Heat Sealer (Audion Electro, type 535) making a diameter of approximately 20

cm. One end was fixed to the rumps leaving the other end open; only tied during fecal collection. Bags were fixed onto the sheep during the adjustment period to accustom the animals to the bags. During non-fecal collection times, bags were folded upwards to reduce tear and wear and also to prevent accumulation of any fecal material due to contact with the sides of the bags.

After collection, feces were weighed and bagged in polyethylene plastic sample bags and frozen for further analysis.

One animal went off feed immediately after the second period intake measurement. It was diagnosed as possible copper toxicity, but this was not the case. Pneumonia was suspected and was treated using an antibiotic (Ethalin, Rogar/STB Inc.). It was assumed to have recovered for the third period.

Daily feed and weigh-back samples were composited separately for intake and digestibility for each animal and period. Composite samples were resampled after thoroughly mixing.

Feces were also composited for each animal and period after thawing. Composites were thoroughly mixed using a commercial dough mixer (Hobart, Model B600) before a sample was taken. Both feed and fecal composite samples were dried in a forced air drier at 60° C for 48 hrs. to determine percentage dry-matter according to AOAC (1975). Dried samples were immediately ground through a 1 mm screen using a Wiley Mill (Standard Model No. 3). They were bagged in small polyethylene plastic sample bags and stored at room temperature for further analysis.

Feed and fecal samples were analysed for crude protein (Macro-Kjeldahl) (AOAC, 1975), ether extract (AOAC, 1984) and total ash.

Gross energy was determined with an "Adiabatic Parr Oxygen Bomb Calorimeter". All fiber components were determined according to Goering and Van-Soest (1970). A second dry matter was determined in-vacuo overnight at 95° C according to AOAC (1984). Calcium and phosphorus was determined by atomic absorption spectroscopy and photometric procedures, respectively.

Voluntary dry matter intake was expressed on a daily, metabolic weight and as a percent of body weight.

Percent digestibilities of dry matter, crude protein, ether-extract, gross-energy and fibre component were calculated using formula in Appendix 1a. Apparent digestibility coefficients for hay and straw DM, energy and crude protein were calculated after subtracting grain supplement contribution to total fecal output. Fecal contribution of grain supplement was calculated by considering a DM, energy, and crude protein digestibility of 91, 92 and 85% for barley, grain, rolled (Ref. No. 4 00 528 Canada) (N.A.S., 1982).

Fibre digestibility coefficients calculations ignored the contribution by grain. This was considered negligible since the animals received small amounts of grain.

Samples of each forage treatment were also sent to Texas Veterinary Medical Diagnostic Laboratory (Drawer, Texas) for analysis of 4-methylimidazoles and related compounds. Analysis of the chemicals was done by the gas chromatography/mass spectrometry (GC/MS) method (Reagor, 1986).

Analysis of variance was according to Cochran and Cox (1957).

Tukey's procedure was used to compare treatment means and missing data was completed by using the formula in Appendix 1b. The data was handled as a 4 x 4 Latin Square, using a SAS program (SAS User's Guide, 1982).

In-situ Studies

The disappearance of dry matter, crude protein and fibre components of the four treatment forages was determined using nylon bags according to Neathery (1969).

One mature Jersey steer fitted with a rumen cannula was used. The animal was fed a standard grass hay mixture (Appendix 10) during a 14 day preliminary period and a 1 day experimental period. The steer was offered about 3 kg of hay during the experimental period, which was below its maximum intake to allow for easy insertion and removal of bags from the rumen. A composite sample of the hay fed to the steer was taken from three bales for chemical analysis.

The sample of feeds used in this experiment are those collected during the sheep digestion trial.

The samples were ground in a Wiley mill through a 1 mm screen after being dried in a forced-air oven at 60° C for 48 hrs. A second dry matter was determined in-vacuo since the sample had been equilibrated with air. A 5 gram sample for each forage treatment was weighed and quantitatively transferred to bags (round bottom) measuring 13.3 x 8.2 cm on average. The bags were first washed, dried in a forced-air oven at 60° C for 24 hrs. and put in a dessicator before being weighed. There were two bags for each forage treatment at each stop time. Bags were securely tied with a wool string inserted inside the hem of the bags. Duplicate empty bags for each stop time were also included. Bags for

each stop time were connected to fish snap swivels on a series of rings. A small ring was permanently attached to the hem of each bag. The rings were then hooked to a metal weight using snaps. About a metre suspension nylon cord (inserted inside a polyethylene flexible tubing) was attached to the metal weight and the rings (Plate 2).

The metal together with bags were inserted in the ventral portion of the rumen approximately 15 minutes after the steer received its daily allotment of hay. The bags were partially wetted to aid digestion before insertion. Bags were withdrawn after 2, 6, 12 and 24 hrs. in the rumen. Immediately upon removal from the rumen, the bags were washed by hand and squeezed to remove adhering particles and rumen fluid which had impregnated the bags. Clear running wash water was used as an indicator to stop further washing. The bags were then dried in a forced-air oven at 60° C for 48 hrs. after which the bags were weighed and dry matter disappearance determined by weight differences.

The duplicate samples for each forage treatment at each stop time were mixed thoroughly and stored at room temperature for crude protein and fibre components analysis. The disappearance for these components were also determined by difference.

In-vitro Studies

The technique used here is an adaptation from that outlined by Pritchard et al (1963). The technique involves incubation of the test feed first with rumen liquor. The main aim in this technique is to try to simulate rumen digestion.

The forage samples used were those collected during the sheep digestibility trial. The samples were first dried in a forced-draft

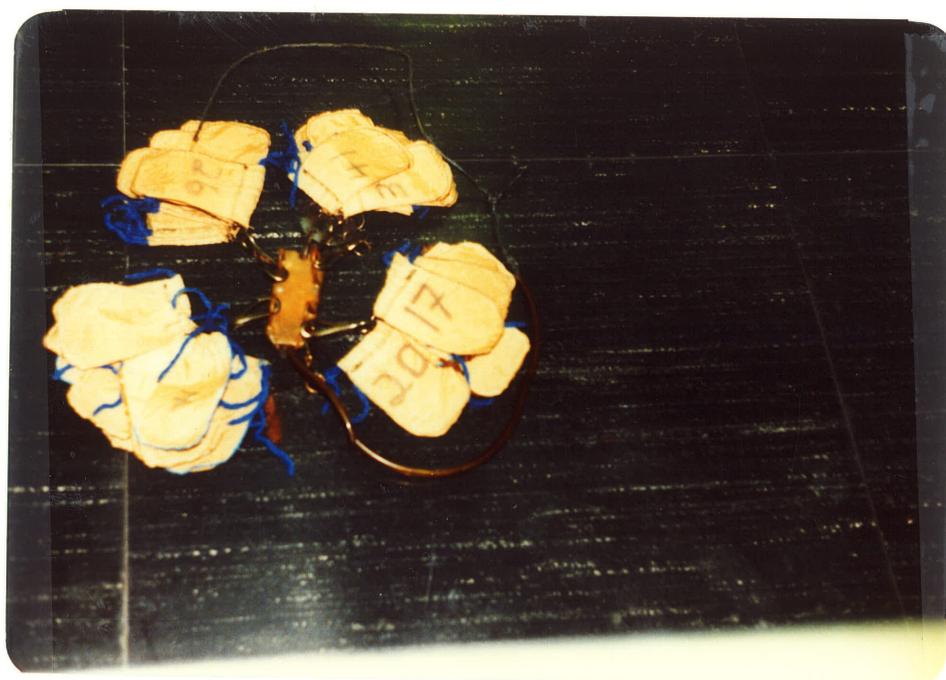


PLATE 2: Attachment of nylon bags to a metal weight ready for placement in the rumen.

oven at 60° C for 48 hrs. and then ground in a Wiley mill with a 1 mm screen.

The incubations were carried out in 50-milliliter centrifuge tubes equipped with a gas release valve as described by Tilley and Terry (1963). Test samples were weighed (0.25 grams DM) and quantitatively transferred into centrifuge tubes which had been previously washed, dried in a forced-draft oven at 60° C for 24 hrs. and then weighed. The tubes were left overnight in a water-bath maintained at 39° C. On the day of inoculation 12.5 milliliters of McDougall's buffer (pH 6.9) was added to each tube with the control barley green feed tubes receiving 1.0 milliliter of 0.1N ammonium chloride to bring up the nitrogen content. These additions were made a few hours before addition of rumen liquor to allow the temperature to equilibrate to 39° C.

Rumen liquor was taken from a single-fistulated steer (Jersey) fed a standard hay diet (Appendix 10). Grab samples of ingesta were taken out from the rumen and immediately strained by squeezing through three layers of warm cheese-cloth. The rumen fluid was collected in a previously warmed and CO₂ flushed insulated thermos flask, which was immediately closed after being filled with rumen fluid. The flask with the rumen liquor was immediately taken to the laboratory and put in a water-bath maintained at 39° C. Twelve and half milliliters of rumen liquor were immediately added to each tube using a syringe. Carbon dioxide was immediately flushed into each tube for a few seconds and then the tube was tightly closed with a gas release valve. After inoculating all tubes CO₂ was again flushed into each tube for about a second through the slit on the gas release valve.

Four replications for each forage treatment at 6, 12, and 24 hours stop time were incubated and only two replications were used at 48 hours. Another four replications for each stop time were included as a control. These contained the McDougall's buffer and rumen liquor in a 1:1 ration with no forage sample.

The buffer used in this study was prepared according to McDougall (1949) and therefore sometimes referred to as McDougall's solution. An attempt was made to equilibrate the buffer with CO₂ (for at least 20 minutes) immediately before use, to bring the pH to approximately 6.9. However a few drops of phosphoric acid were required to achieve this pH level (Johnson, 1961).

All tubes were hand shaken three times before being withdrawn from the water-bath.

Incubated forage samples were taken out of the water-bath after 6, 12, 24 and 48 hours of incubation. A random sample was immediately checked for pH and temperature. The gas release valve was immediately removed and the tubes were stored in a cooler at below 10° C after sitting in ice-cold water to arrest further fermentation. The tubes were left in the cooler overnight. Samples were washed 2-3 times followed by centrifuging after each washing at greater than 2000 rpm for 15-20 minutes. After decanting the last washing water, samples were dried in a forced-draft oven at 60° C for 48 hrs.

In-vitro dry matter digestibility was calculated by difference in dry matter lost and as a percent of total dry matter initially present.

The data were analysed as a factorial experiment according to Cochran and Cox (1957) for both the nylon bag and in-vitro studies.

Disappearance curves were also constructed (Appendices 11-17).

Regression equations were formulated by regressing the natural log of disappearance on the incubation time. The intercept and slope values were used to derive exponential equations to describe the nutrient disappearance over time. The exponential equations were derived as shown below:

In $Y = a + bx$

$$y = e^a \cdot e^{bx}$$

where $Y =$ Nutrient disappearance

$a =$ intercept

$b =$ slope

and $x =$ incubation time (t)

Pairwise comparison of treatment slopes for each nutrient were statistically tested by the t-test (Cochran and Cox, 1957). General linear model using a SAS program (SAS User's Guide) was used to analyse mean differences among means within each stop time and each roughage treatment.

PART II

Two experiments were carried out in the second part of the research project; i) a sheep intake and digestion trial, and ii) a dairy cow intake, digestion and performance trial.

Four dry forages were investigated in the sheep trial which included ammonia-treated alfalfa hay (NH), control alfalfa hay (CH) (89% DM), ammonia-treated barley straw (NB) and untreated barley straw (90% DM) (CB).

Only the alfalfa hays were used in the dairy cow experiments.

The alfalfa hay (Medicago sativa) was a 2nd cut hay and the barley straw (Hordeum vulgare var. Bonanza) were from the 1985 grain harvest. Both forages were obtained from the University farm (Glenlea Research Station).

Ammoniation Procedure

Ammoniation of the forages was done at Glenlea Research Station (University of Manitoba) by following the Fma method. This method of ammoniation involves the use of oven called the Fma-AN-STRA-VERTER manufactured by Fma maskinfabriker a/s Denmark. The Fma method works on the principle that the ammoniation process is a temperature dependent process. Therefore the oven has a heating element attached to the roof of the steel body. Other than the main steel body, the most important component of the oven is the El-Control Box. It is composed of a timer (watch), operation lamp, starter switch, switch for pilot current and a reset button.

The oven works on a 23 hour cycle broken into 3 main processes, viz: i) 15 hours of thermostatically controlled heating to 95° C, ii) 4

hours of reaction, and iii) 4 hours of ventilation. All these processes are controlled by the El-control Box.

A detailed description of the parts and their functions can be obtained from Fma maskinfabriker a/s brochure (4250 Fuglebjerg, Denmark).

The average weight of a bale was 18.3 kg (dry matter basis) and 16.2 kg for alfalfa hay and barley straw respectively. This was determined by weighing and sampling 6 bales of each forage before packing the bales in the oven. On average 922.59 kg (DM) of forage material was ammoniated daily over a four day period.

The anhydrous ammonia used was obtained from ENGRO (Granger and Till Inc., Winnipeg, Manitoba) and was transported under pressure in a nurse tank. The tank was weighed at the start and end of the 4 days to determine how much ammonia was used. The pressure of the nurse tank was read daily from the manometer before dosing.

Bales were manually inserted into the oven (Plate 3) and total weight on a wet basis was calculated. The gates of the oven were then tightly closed after removing any loose forages which could hinder tight closing against the seal. The hose from the nurse tank was then connected to a connecting socket attached to the hose from the oven (Plate 4).

The timer (watch) on the El-control Box was set at 23 hours, starter switch activated and operation lamp turned on. Dosing of ammonia is completed by slowly opening the fluid valve on the nurse tank and the 2 gas valves at the connection between the nurse tank and oven hoses. The ammonia was dosed for about 11.6 minutes on average in



PLATE 3: The Fma-AN-STRA-VERTER OVEN Packed with Alfalfa Hay and Barley Straw.



PLATE 4: Treatment period using an Fma-AN-STRA-VERTER OVEN.

order to achieve a 3% application rate as fed. In order to achieve this a dosing schedule was used to determine the dosing time for the particular quantity of forage material placed in the oven and particular pressure shown on the nurse tank.

The treated material was removed after 23 hours of treatment time at 95° C (180° F.).

The actual application rate by weight was calculated to be 3.35% (DM).

The bales were stored in a barn, ready for feeding.

Ram Intake and Digestion Trial

Two separate ram feeding experiments were conducted from May-July 1986. In the first experiment four rams of similar weight (average body weight 39 kg) were randomly assigned to either ammoniated alfalfa hay or its control in a 2, 2 x 2 switch back design involving 2 periods and two rams per treatment (Cochran & Cox, 1957).

The procedures used in the ram trials are basically the same as those used in the wether trials with minor modifications. The rams were offered chopped hay between 11:00 a.m. and 11:30 a.m. and then between 4:00 p.m. and 4:30 p.m. A 2 times/day feeding system was preferred to avoid excessive wastage. Almost half of what was planned to be offered that day was given in the morning and the rest in the afternoon.

Metal sheet trays were placed underneath each animal to collect spillage. The trays were weighed on the first and last day of the intake period. After thoroughly mixing of the spillage a sample was taken and dry matter immediately determined. Maximum ad-libitum intake was determined by subtracting the weigh-back from the amount of feed

offered and the amount of feed consumed was determined by subtracting the daily average spillage from it. This was definitely a labourious way of determining maximum ad-libitum intake. It was however justified because of feed spillage and also wetting of the spillage by water when the animals were drinking.

The next seven days after the intake measurement were used for total fecal collection (Plate 5).

Acid-Insoluble-Ash (AIA) was determined for alfalfa hay, feces and weigh-back according to Van Kenlen and Young (1977).

In the second ram experiment, four rams (average body weight of 41 kg) were randomly assigned to either ammoniated barley straw or its control in a 2, 2 x 2 switch back design involving 2 periods and two rams per treatment. Animals on ammoniated barley straw received 260 grams/day of rolled barley grain (Ref.: No. 4 00 528) (as fed) and those on untreated straw received 266 grams/day of urea-solution treated rolled barley grain. All animals were supplemented with 3.3 grams of a vitamin-mineral premix (Table 2). Weigh-back of grain if any was collected and dry matter determined on a composite sample. Grain was offered before the roughage.

Lactating Dairy Cows Intake, Digestion and Performance Trial

To determine the effect of ammoniation of alfalfa hay on intake, digestibility and performance, 10 lactating dairy Holstein cows (average body weight of 601.1 kg) were randomly assigned to either ammoniated alfalfa hay or its control in 5, 2 x 2 switch back design (Cochran & Cox, 1957). This is the same experimental hay that was used in the above digestion trial with rams. The cows were first grouped into high



PLATE 5: Fecal collection bags stuck to the rumps of rams.

and low milk production as reflected by days in milk and production level. They were then paired within each group on the basis of lactation number. Randomization of treatment assignment was carried out within each pair.

The animals were weighed on two consecutive days prior to initiation of the trial. Thereafter, they were weighed at the start and end of the second period. The mean value of the two weighings was taken as the weight of the animal.

The two experimental periods were 4 weeks duration. Long hay (unchopped) was offered to the animals ad-libitum for two weeks as the adjustment period. In the last two weeks of the 28 day period, intake was measured. The aim in the feeding program was a daily 1.0 kg weigh back to guarantee that hay was being offered ad-libitum. Feed was offered twice daily starting at 8:30 a.m. and 1:30 p.m. Weigh backs were taken once daily.

Fecal grab samples were taken in the last five days of each period in the morning and afternoon. To avoid any contamination from bedding and urine, fecal sampling was done directly from the rectum. The feeding system did not change during fecal collection. The samples were placed in polyethylene bags and frozen for further processing.

The high and low producing cows were given a 20% and 16% CP grain mixture (Table 5) respectively. This was given twice daily in a separate feeding trough from the hay. The aim here was to give the cows a quantity of grain in relation to the previous day hay intake to maintain a hay grain ratio of 45:55 and 55:45 for the high and low production groups respectively.

Table 5. Ingredients of experimental rations fed to cows.

	Grain Supplement	
	20% CP	16% CP
Ingredient (% as fed)		
Barley (rolled coarse)	61.40	90.10
Dairy pelleted supplement ¹	32.00	-
Pelleted dehydrated alfalfa	5.00	-
Tallow	1.60	-
Soybean meal (48%)	-	5.00
Urea	-	0.50
Biophos (17% Ca, 21% P)	-	0.20
Limestone	-	1.60
Dairy mineral mix ²	-	0.60
Dairy vitamin mix ³	-	0.30
Magox (baymag) (54% Mg)	-	0.40
Salt (cobalt-iodized)	-	0.40

¹See Appendix 32.

²See Appendix 34.

³Contained (IU/g): Vitamin A 500,000; Vitamin D₃ 500,000; Vitamin E 20,000 and Niacin

Water was available at all times.

Feed and total weigh back samples were taken in the last five days of each period. Total daily weigh back of hay and grain was put in separate bags individually for each animal for sampling. At the end of each period the feed samples and weigh back were composited and dry matter determined in a forced air oven at 60° C for 48 hours. The hay and weigh back samples were then ground through 1 mm screen using a Wiley mill (Standard Model No. 3). They were stored in sample bags at room temperature.

Daily milk production was measured in the last two weeks of each period and milk samples were taken for three consecutive days of the last week of each period. The samples were sent to Manitoba Agriculture Complex (University of Manitoba) on the same day for fat, protein and lactose analysis by the Near Infrared method (Standard methods for examinations of dairy products, 1985).

Rumen samples were taken in the last week of each period on a single day. The samples were taken through a stomach tube attached to a "strainer bullet" and pH was immediately determined using a portable "Photovolt Model 120A" pH meter. Samples were placed in plastic sample bottles and stored in a freezer for further processing and analysis. Rumen samples were taken just before the a.m. feeding and two hours post feeding.

Blood samples were taken on the same days as rumen sampling. Samples were taken from the tail (Ventral Coccygeal vein) with "Vacutainers" before the a.m. feeding and two hours after. The samples were chilled overnight and centrifuged the following day using a

swinging-bucket "International Model U Centrifuge". Centrifuging was done at 3,000 rpm for 20 minutes (Forsberg, 1977) and about 15 ml of blood plasma was recovered. This was decanted into sample vials which were immediately capped with a screwable cap and frozen for blood urea analysis.

Feed (hay and grain), weigh back and fecal samples were analysed for dry matter, crude protein, energy, acid-detergent fibre, neutral detergent fibre, hemi-cellulose and acid-insoluble ash. Acid insoluble ash was analysed according to Van Keulen and Young (1977) and all other nutrients were analysed according to methods described in the wether intake and digestion trial. No chemical analysis were carried out on grain weigh back samples because there was no apparent selection when eating, or contamination from the forage material. There was seldom any grain weigh back left. Prior to analysis a.m. and p.m. fecal grab samples were composited and analysed for dry matter. After grinding a five day composite sample was made for each animal.

Total diet nutrient digestibilities were computed by the indicator method using acid insoluble ash. Voluntary feed intake of hay and total diet was computed for the last 2 weeks in each period on a daily, metabolic size and percentage of body weight basis.

About 15 ml of thawed rumen fluid sample was centrifuged using a swinging-bucket "International Model U Centrifuge" at 2300 rpm for 20 minutes (Forsberg, 1977). Five milliliters of the top layer was frozen in sample vials after adding 1 ml of 25% metaphosphoric acid to coagulate the proteins. On the day of volatile fatty acids (VFA) analysis the samples were thawed overnight and then centrifuged for 10

minutes at 2000-2500 rpm. Two milliliters of the supernatant was decanted into clean dry vials and capped for gas chromatograph analysis. Acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acid were determined with a "Burrell Single Flame" gas chromatograph packed with a 20% NPGS + 2% H₃PO₄ on a firebrick 60/80 column. The rest of the rumen samples after centrifuging were mixed again with the original sample and stored in a cooler after adding 1 ml of concentrated sulphuric acid to prevent rumen ammonia loss. Rumen ammonia nitrogen was determined by the method of known addition with low-selective electrode (Orion Research Manual, Ammonia Electrode Model 95-10).

Blood urea nitrogen and ammonia were determined using a "Technicon Flame Photometer IV" auto-analyzer according to Marsh et al (1965) and Bolleter et al (1961) respectively.

All data was handled as a 5, 2 x 2 Latin Square according to Cochran and Cox (1957), using a SAS program (SAS User's Guide, 1982).

RESULTS

PART 1: NUTRITIONAL VALUE OF AMMONIATED HIGH MOISTURE ALFALFA HAY AND BARLEY GREEN FEED

Composition and Temperature

Bale temperature trends for wet ammonia treated and wet untreated alfalfa hay are shown in Figure 1 and Appendix 2. Generally the temperature tended to be lower for the untreated hay than the treated hay. The maximum temperature for the treated hay was 49.0°C compared to 41.0°C for the untreated control hay. Within 4 days, the temperature for treated hay reached the maximum and then declined over the next 2 days to the mid 40's and then cooled to 19.4°C over a two week period. The corresponding untreated hay heated to 32.7°C in one day and then dropped to 13.0°C in two days. The temperature then rose to a maximum of 41.0°C in two days and thereafter dropped to 15.7°C to the next two weeks with variations in between. Temperature changes were not monitored for the barley green straw.

Visual observation of both alfalfa hay and barley green feed, showed no apparent visible molding of treated roughages while the untreated roughages did show signs of molding characterized by white powdery spores of fungus. Although, the untreated hay showed signs of molding, it still retained a light greenish color, while the treated hay tended to have a dull color after treatment.

Chemical composition of ammoniated and untreated roughages is shown in Table 6. Crude protein content of hay and the green feed was increased as a result of treatment with ammonia, and differences between the treated roughages and the corresponding untreated control were

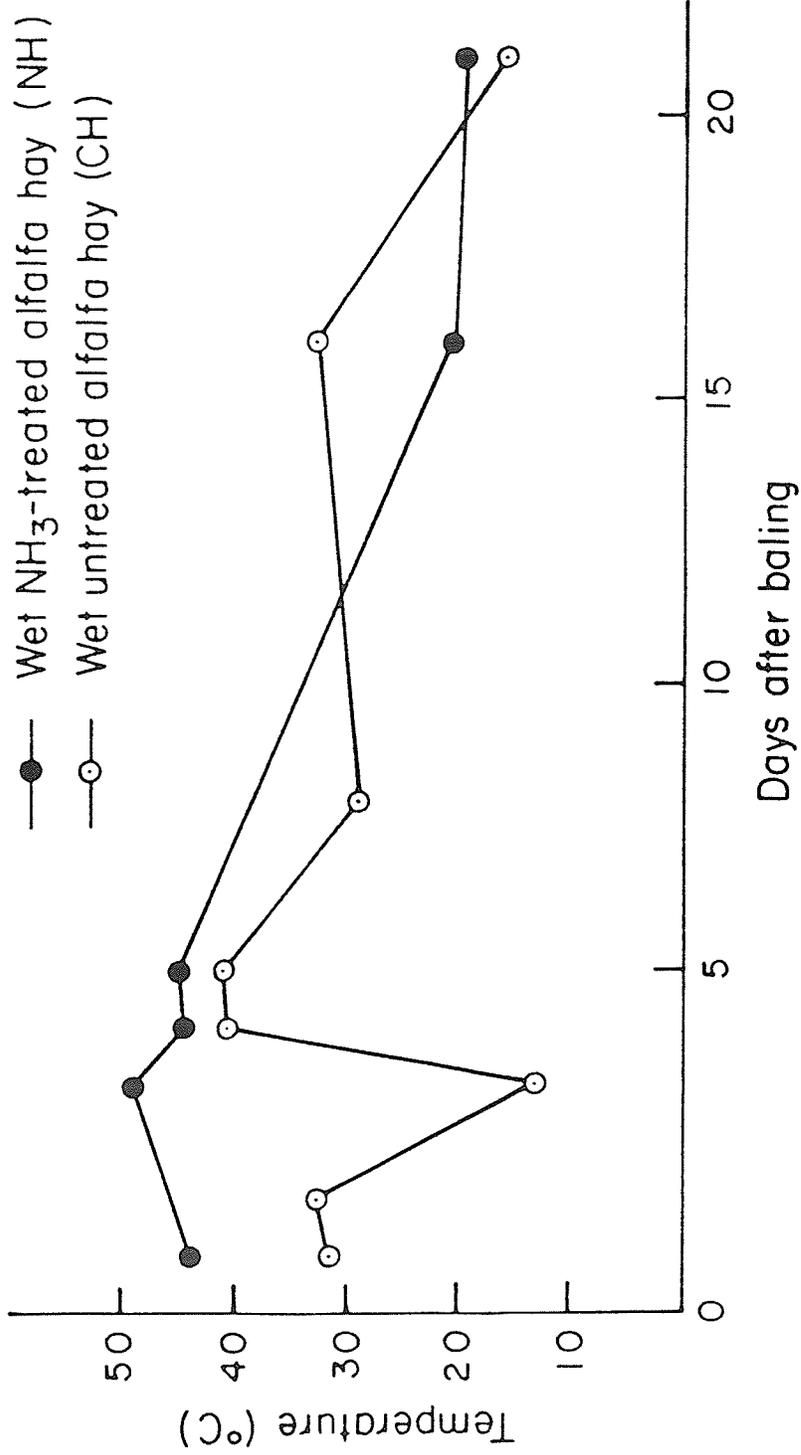


Figure 1: Temperature trends in wet (77% DM) ammonia-treated and wet untreated alfalfa hay.

Table 6. The effect of ammoniation on the chemical composition of barley green feed and alfalfa hay and the proximate analysis of grain supplements fed to wethers (% DM).

Item	Test Roughages 1,2				S.E.	Grain Supplements	
	CB	NB ⁴	CH	NH ⁴		Barley Grain	Barley + 2.5% Urea
DM (1 st)	93.5	92.8	93.7	92.3		89.8	91.00
CP	6.9 ^c	16.3 ^b	14.3 ^b	24.5 ^a	0.43	12.7	18.5
EE	1.9	2.0	1.7	2.1	0.13	2.7	3.4
Ash	6.4 ^b	7.5 ^a	7.1 ^{ab}	7.5 ^a	0.16	3.56	3.8
GE Kcal/gm	4.79 ^b	4.45 ^b	4.50 ^a	4.62 ^a	0.01	4.30	4.33
ADF	38.4 ^{ab}	42.8 ^{ab}	44.4 ^a	36.4 ^b	1.80		
NDF	66.3 ^a	61.8 ^{bc}	57.8 ^b	49.2 ^c	1.21		
ADIN	0.5	0.6	0.5	0.8	0.06		
ADIN (% Total N)	46.0 ^a	28.0 ^b	23.3 ^b	20.5 ^b	2.80		
P. Lignin ³	6.9 ^c	6.5 ^c	12.3 ^a	9.5 ^b	0.42		
Cellulose	29.4 ^{ab}	33.5 ^a	31.9 ^{ab}	26.4 ^b	1.06		
Hemi-cellulose	28.0 ^a	19.0 ^b	13.5 ^c	12.8 ^c	0.43		
Ca	0.24	0.41	1.37	1.56			
P	0.23	0.29	0.15	0.16			

¹Test roughages:- CB - control barley green feed (untreated).

NB:- Ammoniated barley green feed (treated)

CH:- Control alfalfa hay (untreated)

NH:- Ammoniated alfalfa hay (treated)

²Means with different superscripts within the row are significantly different (p<0.05).

³P. Lignin:- Permanganate lignin.

⁴Ammonia retention after treatment: 99.5% (hay)
And 91.7% (green feed) calculated by using $[(24.5\%CP - 14.3\%CP)/6.25] / [2.0\%NH_3 \times 0.82\%N]$ and $[(16.3\%CP - 6.9\%CP)/6.25] [2.0\%NH_3 \times 0.82\%N]$ formula for hay and green feed respectively.

significantly different ($p < 0.05$). Alfalfa hay crude protein increased from 14.3% to 24.5% (Table 6, Appendices 3 and 19) after treatment representing a 71 percentage increase, while barley green feed crude protein increased from 6.9% to 16.3% after treatment representing a 136 percentage increase ($p < 0.05$) (Table 6, Appendices 3 and 19). Based upon ammonia treatment rate, 91.7% and 99.5% of the ammonia-nitrogen ($\text{NH}_3\text{-N}$) added during treatment was retained as $\text{NH}_3\text{-N}$, in the barley green feed and alfalfa hay respectively.

Ammoniated hay and straw had higher acid detergent insoluble nitrogen (ADIN) than their corresponding untreated roughages, although differences among the treatments did not reach statistical significance ($p > 0.05$) (Table 6, Appendices 4 and 19). However, when ADIN was expressed as a percent of total nitrogen, untreated barley green feed had a significantly higher ($p < 0.05$) ADIN than the treated barley green feed (Table 6, Appendices 4 and 19).

There were no significant differences in ether-extract (EE) content among treatment means ($p > 0.05$) (Table 6, Appendices 3 and 19).

Ash content of treated roughages was higher compared to their corresponding untreated roughages, but the only difference that was significant ($p < 0.05$) was that between untreated green feed and the treated roughages (Table 6, Appendices 3 and 19).

There was no significant difference ($p > 0.05$) in gross energy (GE) content between untreated and treated roughages of hay or green feed (Table 6, Appendices 3 and 19).

The acid detergent fibre (ADF) increased by 11.5% and decreased by 18% after treatment of straw and hay respectively. However, the only

significant difference ($p < 0.05$) in ADF content was that between treated and untreated hay (Table 6, Appendices 4 and 19).

On the other hand neutral detergent fibre (NDF) content decrease ($p < 0.05$) from 66.3% to 61.8% and from 57.8% to 49.2% after treatment of the green feed and hay respectively (Table 6, Appendices 4 and 19). This represented a 7.3% and 15% decline in NDF for the green feed and hay respectively.

Cellulose content was not significantly affected ($p > 0.05$) by ammoniation. The content tended to increase from 29% to 33.5% for green feed and declined from 32% to 26.4% for hay after treatment. Treated green feed had significantly higher cellulose content ($p < 0.05$) than treated hay but similar to untreated hay (Table 6, Appendices 4 and 19).

Hemi-cellulose content calculated as a difference between NDF and ADF was significantly decreased ($p < 0.05$) after treatment of barley green feed (Table 6, Appendices 4 and 19) but not hay. The content declined by 32.2% and 5.2% after treatment of green feed and hay respectively.

Lignin content was higher ($p < 0.05$) for untreated hay compared to treated hay (Table 6, Appendices 4 and 19). The lignin content of the hay was higher ($p < 0.05$) than that of the green feed.

Voluntary Intake of Roughages by Wethers

Generally, the grain supplement offered before the green feed was readily consumed by wethers. There were no visible signs by the wethers to indicate that the treated hay was in anyway unpalatable. Animals appeared to be more selective in eating untreated roughages leaving stemy parts unconsumed.

Daily dry matter intake was significantly increased ($p < 0.05$) by ammonia treatment (Table 7, Appendices 5 and 20). There was no significant difference ($p > 0.05$) in daily dry matter intake between treated barley green feed and untreated hay though hay intake was somewhat higher than for treated green feed. When daily dry matter intake was expressed on unit metabolic weight basis similar results were noted in intake (Table 7, Appendices 5 and 20). However, when daily dry matter intake was expressed as a percent of body weight, the significant difference in intake between treated and untreated hay disappeared (Table 7, Appendices 5 and 20).

Wether Digestion Trial

The apparent in-vivo digestibility of dry matter, crude protein, gross energy and restricted intake of the test roughages is shown in Table 8 (Appendices 6, 7 and 20). Although intake was at 90% of the maximum ad-libitum intake animals offered untreated roughages still tended to be selective in consuming the roughages.

There was no significant difference ($p < 0.05$) in restricted total dry matter intake among treatment means (Table 8, Appendices 6 and 20). Restricted roughage dry matter intake was only significant ($p < 0.05$) between treated hay and untreated green feed means. Restricted total crude protein was statistically significant ($p < 0.05$) only when treated hay was compared to any one of the other treatments with treated hay being higher (Table 8). Ammoniation significantly increased ($p < 0.05$) the crude protein intake of treated hay but not treated barley green feed (Table 8).

Table 7. Daily voluntary intake of dry matter of treated and untreated green feed and hay fed to wethers in trial 1.¹

Treatment ³	Dry Matter Intake ²		
	g/head/day	g/BW ^{0.75} kg	%BW
CB	841.0 ^c	43.7 ^c	1.64 ^c
NB	1229.0 ^b	68.0 ^b	2.60 ^b
CH	1308.0 ^b	76.9 ^b	3.00 ^{ab}
NH	1687.0 ^a	93.3 ^a	3.57 ^a
S.E.	70.0	3.30	0.13

¹All values are averaged over four periods and four wethers.

²Means with different superscripts within the same column are significantly different ($p < 0.05$).

³See Table 6.

Table 8. Dry matter and crude protein intake during digestion trial and the effect of ammonia treatment on apparent digestibility of barley green feed and alfalfa hay by wethers in trial 1.¹

Item	Test Roughages ^{2,3}				S.E.
	CB	NB	CH	NH	
Intake, g/head/day					
Total DM	1302.0	1607.0	1436.0	1820.0	95.7
Roughage DM	1060.0 ^b	1374.0 ^{ab}	1436.0 ^{ab}	1820.0 ^a	95.7
Total CP	117.0 ^b	253.0 ^b	207.0 ^b	448.0 ^a	27.5
Roughage CP	72.0 ^b	223.0 ^b	207.0 ^b	448.0 ^a	27.5
Digestibility %					
Total DM	62.8 ^{ab}	68.4 ^a	56.3 ^b	64.0 ^a	1.30
Roughage DM	56.2	64.3	56.3	64.0	1.64
Total CP	62.5	64.6	70.0	73.2	2.18
Roughage CP	44.8 ^b	61.7 ^{ab}	70.0 ^a	73.1 ^a	3.89
Roughage GE	55.3	62.7	57.0	63.3	1.74
Roughage Digestible Energy (kcal/gm)	2.43 ^b	2.80 ^{ab}	2.61 ^{ab}	2.93 ^a	0.08

¹All values are averaged over four periods and four wethers.

²See Table 6.

³Means with different superscripts within the same row are significantly different ($p < 0.05$).

Total apparent dry matter digestibility was higher when animals were fed treated roughage rather than when fed untreated roughage (Table 8, Appendices 7 and 20). However the only difference which was significant was that between untreated hay and any of the treated roughages. Roughage apparent dry matter digestibilities suggests that treated roughages had higher digestibility than their corresponding untreated roughages. Although the latin square ANOVA showed significant differences ($p < 0.05$) among means, Tukey's procedure could not detect which treatment means were actually significantly different (Table 8). Ammoniation increased roughage digestibility by 14.5% and 13.6% for straw and hay respectively.

There were no significant differences ($p < 0.05$) in apparent digestibility of total crude protein among treatments (Table 8). However, within roughage species comparison shows that treated roughages had a higher total apparent crude protein digestibility than untreated roughages and within treatment comparison shows that hay treatments had higher total crude protein digestibility than their corresponding green feed treatments. Roughage crude protein digestibility was only significant ($p < 0.05$) between treated hay and untreated green feed. Although not statistically significant ($p > 0.05$) ammoniation increased crude protein digestibility by 37.7% and 4.3% for green feed and hay, respectively.

Ammoniation did not significantly ($p > 0.05$) affect the apparent gross energy digestibility of either green feed or hay (Table 8). However, the gross energy digestibility tended to be increased by 13.5% and 11% after treatment of green feed and hay respectively. When gross

energy digestibility was expressed as digestible energy per gram of roughage, treated hay had a significantly higher ($p < 0.05$) digestible energy than untreated green feed (Table 8). Although, there were no apparent significant ($p > 0.05$) differences between any other pair-wise comparisons, treated roughages had higher digestible energy than their corresponding untreated roughages.

The digestibility of ADF was significantly ($p < 0.05$) higher when treated green feed was fed rather than untreated green feed (Table 9, Appendices 8 and 20). This was also true when treated hay was fed, rather than when untreated hay was fed but the improvement in digestibility did not reach statistical significance ($p > 0.05$). Percent improvement in digestibility was 37.6% and 15.5% as a result of treatment of green feed and hay respectively.

Neutral detergent fibre was more digestible ($p < 0.05$) when either treated green feed or hay was fed (Table 9, Appendices 8 and 20) rather than when either untreated green feed or hay was fed. Percent improvement in digestibility resulting from ammoniation was 34.8% and 25% for green feed and hay respectively.

The apparent digestibility of hemi-cellulose was significantly improved ($p < 0.05$) by 44% and 52% for green feed and hay respectively as a result of ammoniation (Table 9, Appendices 8 and 20).

Ammoniation significantly increased ($p < 0.05$) the apparent digestibility of cellulose of green feed but not hay (Table 9, Appendices 8 and 20) ($p < 0.05$). Percent improvement in digestibility of cellulose was 32.7% and 13.8% for green feed and hay respectively.

There were no significant differences among treatment means

Table 9. Effect of ammoniation treatment on apparent fibre digestibility of barley green feed and alfalfa hay by wethers in trial 1.¹

Treatment ²	Mean									
	Fibre Digestibility Coefficients % ³									
	ADF	S.E.	NDF	S.E.	CELL	S.E.	H-CELL	S.E.	P.LIGNIN	S.E.
CB	43.3 ^c	1.30	50.0 ^c	1.20	52.3 ^b	1.71	60.0 ^b	2.47	14.0 ^a	3.67
NB	59.6 ^a	1.30	67.7 ^a	1.20	69.4 ^a	2.21	86.0 ^a	3.20	21.0 ^a	4.73
CH	46.5 ^{bc}	1.30	47.8 ^c	1.20	52.7 ^b	1.71	50.4 ^b	2.47	32.7 ^a	3.67
NH	53.7 ^{ab}	1.30	59.7 ^b	1.20	60.0 ^{ab}	1.71	76.7 ^a	2.47	36.5 ^a	3.67

¹All values are averaged over four periods and four wethers.

²Treatments, see Table 6.

³Means with different superscripts within the same column are significantly different (p<0.05).

($p < 0.05$) (Table 9, Appendices 8 and 20) in the digestibility of permanganate lignin.

In-vitro and In-situ Studies

Treatment with ammonia increased ($p > 0.05$) the IVDM of hay at 24 hours and the green feed at 12 hours (Table 10, Appendices 11, 21 and 23). Generally, IVDM increased ($p < 0.05$) over time within each treatment as expected (Table 10, Appendices 11, 21 and 23).

Ammoniation increased ($p < 0.05$) the DM, CP, ADF, NDF, cellulose and hemi-cellulose disappearance from the nylon bags for both hay and the green feed at 2 and 24 hours time (Tables 11 to 16, Appendices 12 to 17, 21 and 22). This was also true for CP disappearance of the green feed at 6 hours. At 12 hours time, ammoniation significantly increased ($p < 0.05$) the disappearance of all nutrients for the green feed and DM, CP and hemi-cellulose for hay (Tables 11 to 16, Appendices 12 to 17, 21 and 22). Generally, the disappearance of DM, CP, ADF, NDF cellulose and hemi-cellulose increased with time within each roughage treatment as would be expected (Tables 11 to 16, Appendices 12 to 17, 21 and 23).

Exponential equations derived and their treatment plots for each nutrient are shown in Figures 2 through 8. The coefficients of t (incubation time) in the exponential equations (Figures 2 to 8) are slope (b) values derived from regression equations shown in Appendix 18. Slope differences were not significant ($p > 0.05$) due to treatment.

Dry matter disappearance from the nylon bag for each treatment measured at 6, 12 and 24 hours of incubation time was always higher than that measured by the in-vitro method (Table 17). At 24 hours, in-vivo dry matter digestibility for untreated roughages and treated hay was in

Table 10. Effect of ammoniation on the in-vitro DMD disappearance of alfalfa and barley roughages incubated at different times (% DM).

Incubation time ² , hr.	Treatments ¹				S.E.
	CB	NB	CH	NH	
6	10.4 ^y	18.3 ^x	30.3	30.2 ^x	6.5
12	23.0 ^{bx}	23.5 ^{bx}	24.7 ^b	42.0 ^{awx}	4.0
24	27.4 ^{cx}	41.7 ^{bw}	43.8 ^{ab}	53.5 ^{aw}	2.6
48	48.0 ^w	57.6 ^w	47.3	52.8 ^w	2.0
S.E.	1.6	3.8	7.0	3.5	

¹abcd means in the same row are significantly different ($p < 0.05$).

²wxyz means in the same column are significantly different ($p < 0.05$).

Table 11. Effect of ammoniation on the DM disappearance of alfalfa and barley roughages from nylon bags incubated at different times (% DM).

Incubation time ² , hr.	Treatments ¹				S.E.
	CB	NB	CH	NH	
2	26.3 ^{bz}	37.6 ^{ay}	25.8 ^{bz}	37.9 ^{ax}	0.7
6	29.9 ^{bx}	41.2 ^{aby}	38.6 ^{aby}	45.7 ^{ax}	2.2
12	40.4 ^{dx}	49.3 ^{cx}	53.8 ^{bx}	64.4 ^{aw}	0.8
24	54.3 ^{cw}	66.6 ^{bw}	60.9 ^{bw}	72.6 ^{aw}	1.0
S.E.	0.5	1.2	0.7	2.2	

¹abcd means in the same row are significantly different (p<0.05).

²wxyz means in the same column are significantly different (p<0.05).

Table 12. Effect of ammoniation on the CP disappearance of alfalfa and barley roughages from nylon bags incubated at different times (%).

Incubation time ² , hr.	Treatments ¹				S.E.
	CB	NB	CH	NH	
2	58.9 ^{CZ}	74.7 ^{az}	48.9 ^{dz}	62.0 ^{by}	0.4
6	62.9 ^{CY}	78.7 ^{ay}	68.4 ^{cby}	71.0 ^{bx}	1.1
12	74.0 ^{CX}	84.5 ^{bx}	84.5 ^{bx}	90.0 ^{aw}	0.3
24	81.4 ^{CW}	90.3 ^{bw}	88.9 ^{bw}	92.5 ^{aw}	0.3
S.E.	0.3	0.4	0.4	1.1	

¹abcd means in the same row are significantly different ($p < 0.05$).

²wxyz means in the same column are significantly different ($p < 0.05$).

Table 13. Effect of ammoniation on the ADF disappearance of alfalfa and barley roughages incubated from nylon bags incubated at different times (% DM).

Incubation time ² , hr.	Treatments ¹				S.E.
	CB	NB	CH	NH	
2	-3.95 ^{CV}	12.69 ^{AV}	-3.76 ^{CZ}	6.19 ^{BX}	1.0
6	-4.18 ^V	14.84 ^{XY}	11.08 ^Y	15.91 ^X	3.5
12	9.66 ^{BX}	24.41 ^{AX}	26.12 ^{AX}	31.16 ^{AW}	1.4
24	29.66 ^{BW}	49.56 ^{AW}	36.05 ^{BW}	47.02 ^{AW}	1.6
S.E.	0.8	1.8	1.0	3.6	

¹abcd means in the same row are significantly different (p<0.05).

²wxyz means in the same column are significantly different (p<0.05).

Table 14. Effect of ammoniation on the NDF disappearance of alfalfa and barley roughages from nylon bags incubated at different times (% DM).

Incubation time ² , hr.	Treatments ¹				S.E.
	CB	NB	CH	NH	
2	11.13 ^{bz}	24.16 ^{ay}	9.99 ^{by}	13.95 ^{bx}	0.9
6	15.75 ^y	27.34 ^y	19.35 ^x	22.52 ^x	3.1
12	28.37 ^{cx}	36.72 ^{bx}	48.88 ^{aw}	44.00 ^{aw}	1.1
24	45.00 ^{bw}	58.90 ^{aw}	45.25 ^{bw}	56.10 ^{aw}	1.3
S.E.	0.6	1.4	0.9	3.2	

¹abcd means in the same row are significantly different (p<0.05).

²wxyz means in the same column are significantly different (p<0.05).

Table 15. Effect of ammoniation on the cellulose disappearance of alfalfa and barley roughages from nylon bags incubated at different times (% DM).

Incubation time ² , hr.	Treatments ¹				S.E.
	CB	NB	CH	NH	
2	3.96 ^{by}	16.17 ^{ay}	5.01 ^{bz}	14.24 ^{ax}	0.9
6	8.20 ^v	21.84 ^v	16.28 ^v	22.42 ^x	3.1
12	18.39 ^{cx}	33.46 ^{bx}	38.58 ^{abx}	44.67 ^{aw}	1.1
24	40.77 ^{cw}	58.92 ^{abw}	53.02 ^{bw}	61.45 ^{aw}	1.3
S.E.	0.8	1.5	0.9	3.1	

¹abcd means in the same row are significantly different (p<0.05).

²wxyz means in the same column are significantly different (p<0.05).

Table 16. Effect of ammoniation on the hemi-cellulose disappearance of alfalfa and barley roughages from nylon bags incubated at different times (% DM).

Incubation time ² , hr.	Treatments ¹				S.E.
	CB	NB	CH	NH	
2	26.34 ^{CZ}	42.39 ^{ay}	34.14 ^{by}	28.75 ^{CY}	0.7
6	34.69 ^Y	47.22 ^Y	36.07 ^Y	47.18 ^X	2.6
12	47.29 ^{CX}	56.41 ^{bx}	55.01 ^b	68.22 ^{aw}	0.7
24	60.32 ^{bw}	73.73 ^{aw}	63.99 ^{bw}	73.46 ^{aw}	0.8
S.E.	0.5	1.0	0.6	2.6	

¹abcd means in the same row are significantly different (p<0.05).

²wxyz means in the same column are significantly different (p<0.05).

Table 17. Dry matter digestibility measured by different techniques at different incubation times compared to in-vivo digestibility¹.

Technique	Incubation time and delay, hr.				
	2	6	12	24	48
CB					
In-vivo ²	-	-	-	51.0	56.2
In-situ	26.3	30.0	40.4	54.3	-
In-vitro	-	12.0	23.0	28.9	48.0
NB					
In-vivo	-	-	-	62.7	64.3
In-situ	37.6	41.2	49.3	53.8	-
In-vitro	-	18.3	23.5	38.6	57.6
CH					
In-vivo	-	-	-	55.0	56.3
In-situ	25.8	38.6	53.8	60.9	-
In-vitro	-	17.9	24.7	46.7	47.3
NH					
In-vivo	-	-	-	63.9	64.0
In-situ	37.9	45.7	64.4	72.6	-
In-vitro	-	24.2	37.1	55.0	52.8

¹See Appendices 11 to 17.

²Calculated on the basis of 24 or 48 hour delay between feed intake and fecal collection.

general lower than nylon bag dry matter disappearance but higher than in-vitro dry matter disappearance. In-vitro dry matter disappearance was lower for all treatments at 48 hours incubation when compared to in-vivo dry matter digestibility. The in-situ values at 24 hours where all three methods can be compared appear to be much closer to the in-vivo values than do the in-vitro values. In general calculating in-vivo digestibility by delaying fecal collection by 24 or 48 hours from intake data resulted in similar digestion values for DM.

Toxicity of Ammoniated Roughages

There was no abnormal behavior shown by any of the sheep consuming ammoniated roughages throughout the experimental period.

Chemical analysis showed that neither 2-methylimidazole or 4-methylimidazole was detected in any of the roughages.

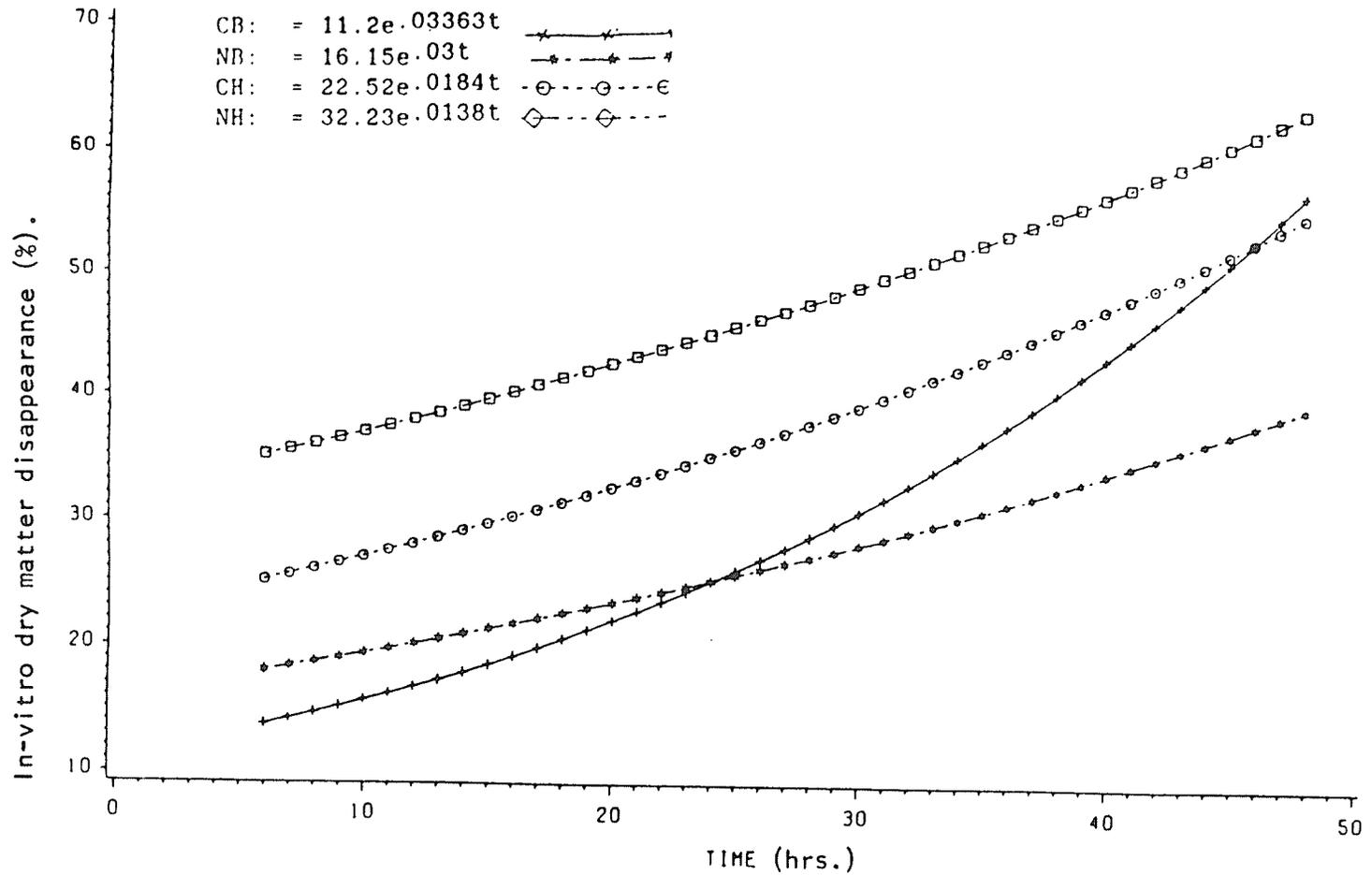


Figure 2. Relationship between in vitro dry matter disappearance (IVDMD) of alfalfa hay and barley green feed treatments and incubation time.

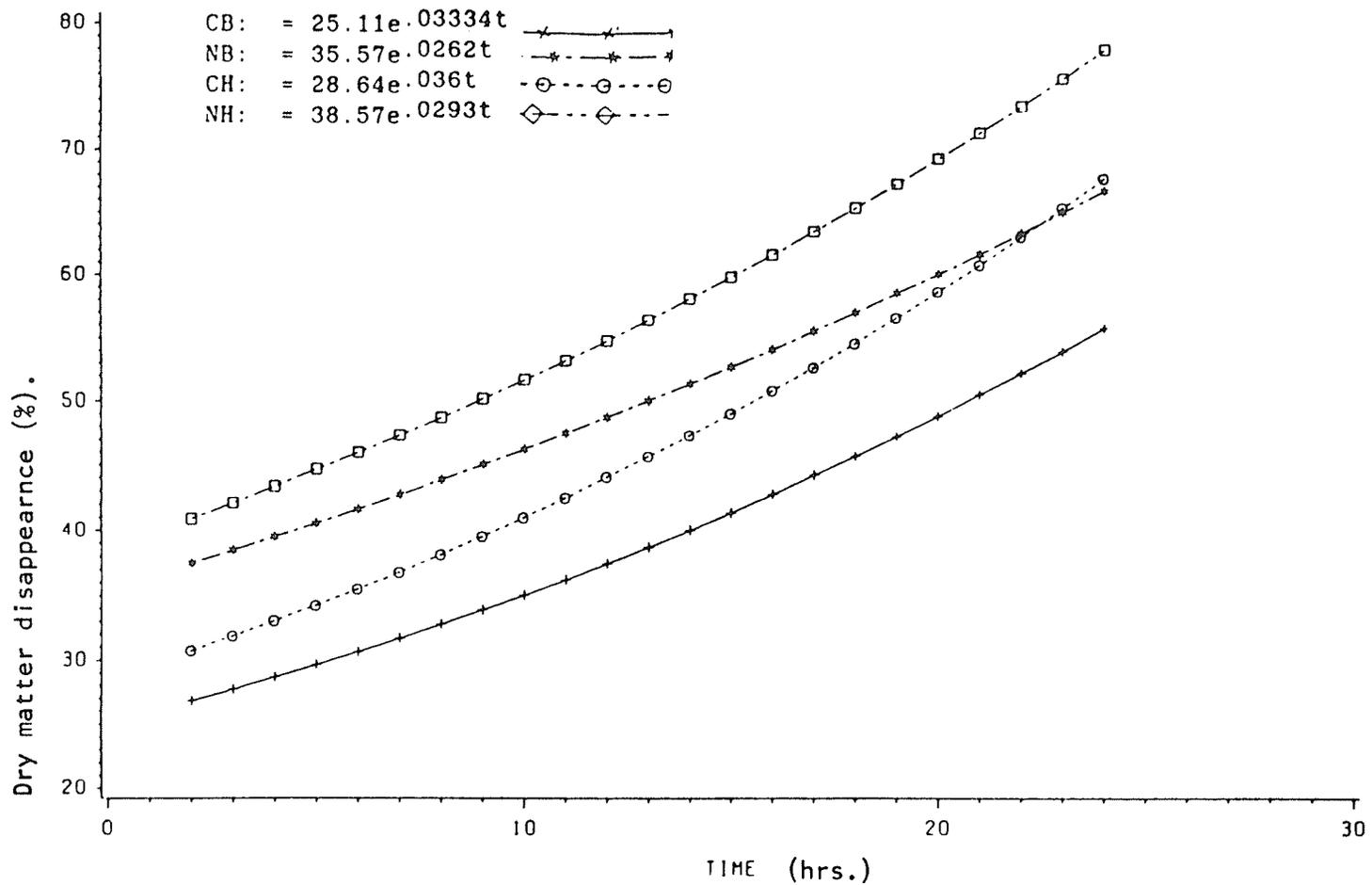


Figure 3. Relationship between dry matter disappearance of alfalfa hay and barley green feed treatments from the nylon bag and incubation time.

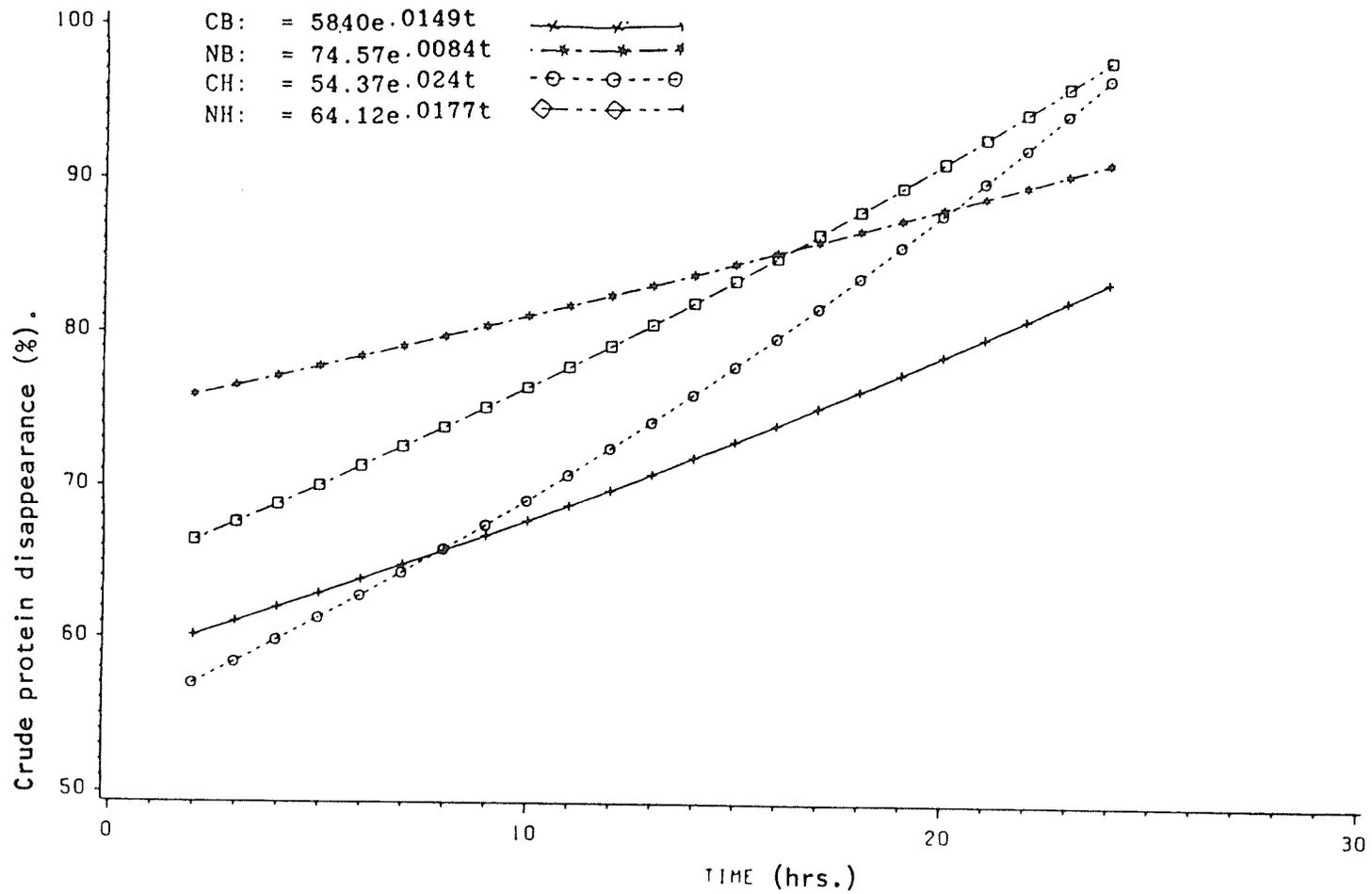


Figure 4. Relationship between crude protein disappearance of alfalfa hay and barley green feed treatments from the nylon bag and incubation time.

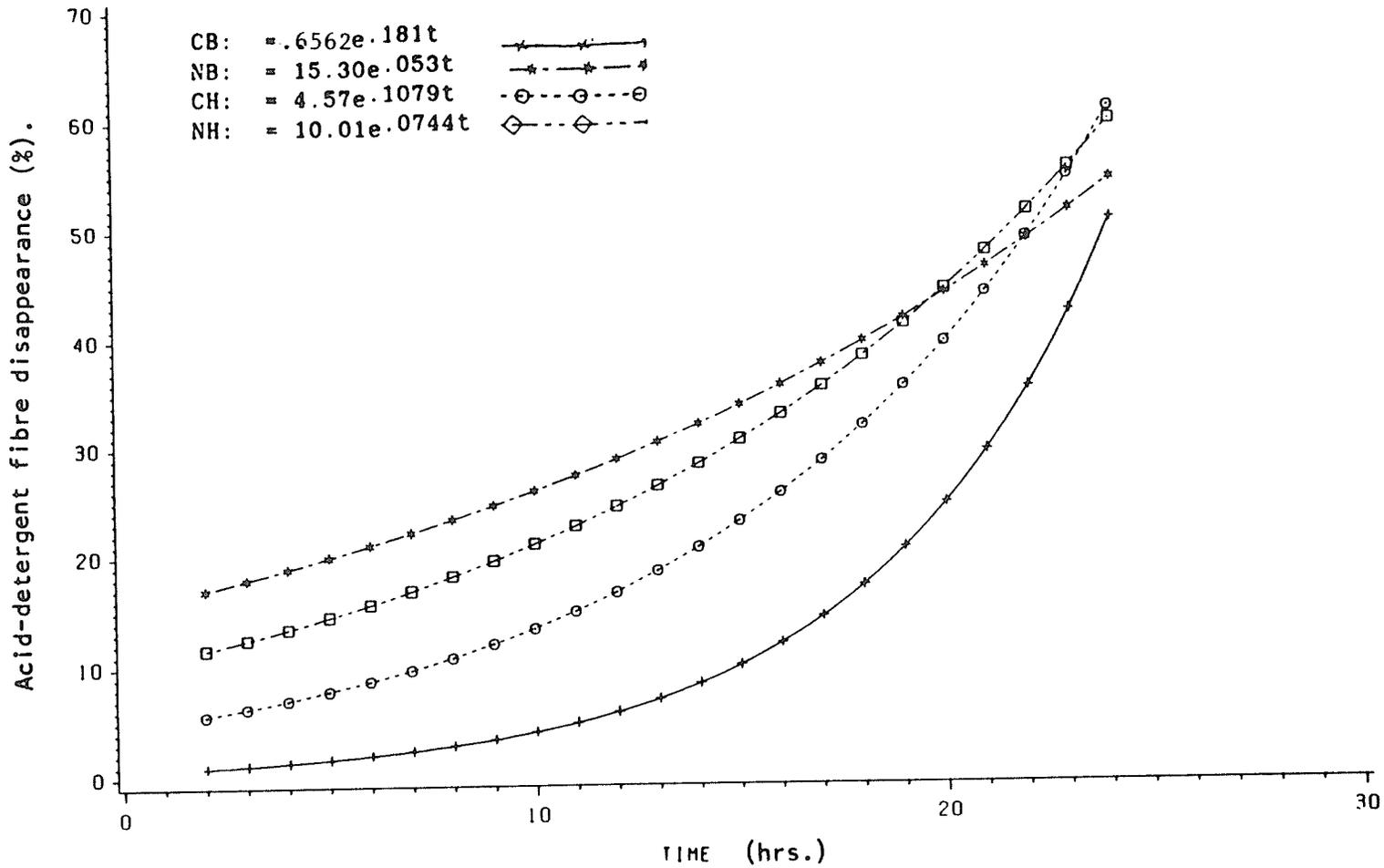


Figure 5. Relationship between acid-detergent fiber disappearance of alfalfa hay and barley green feed from the nylon bag and incubation time.

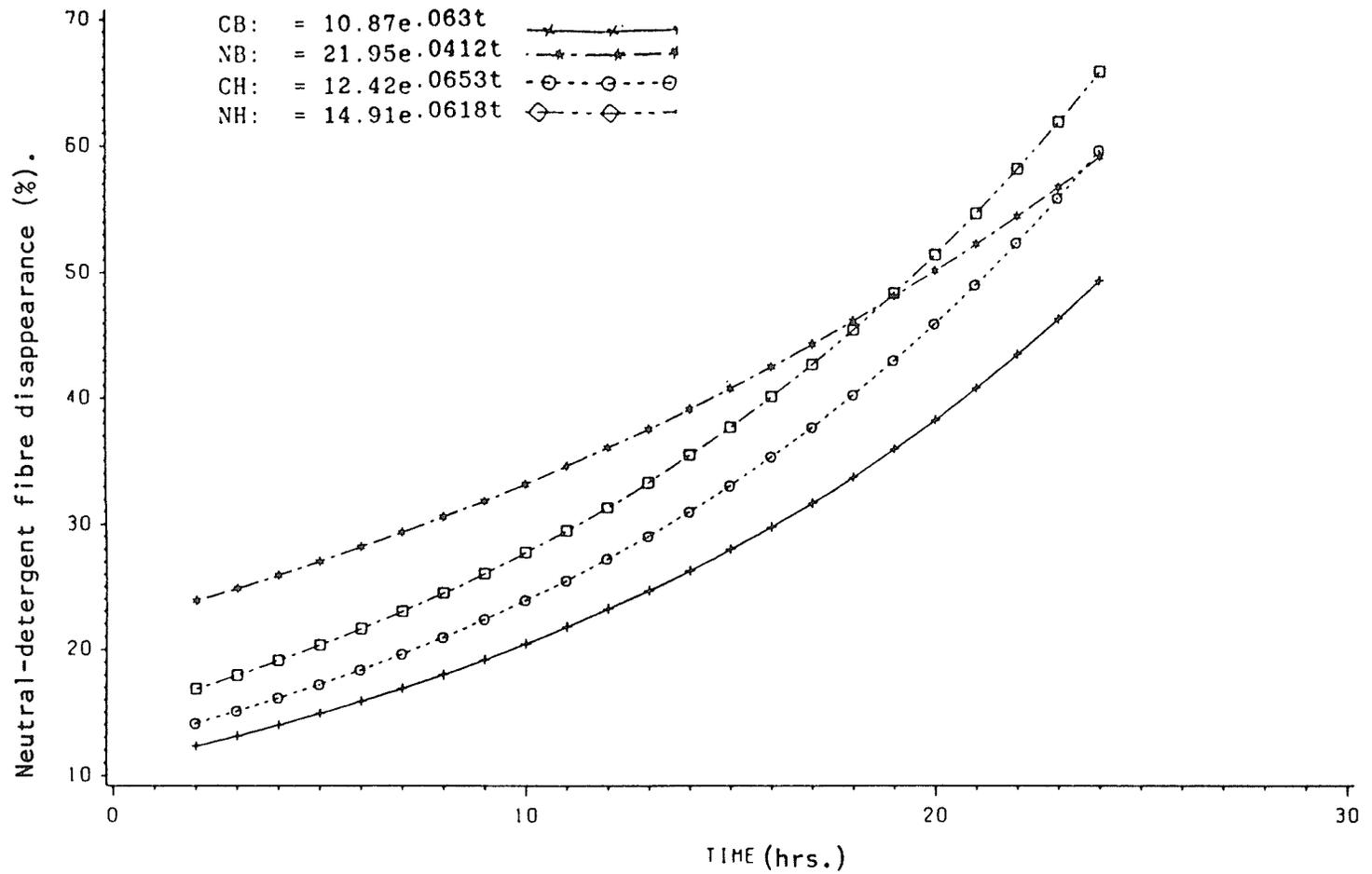


Figure 6. Relationship between neutral-detergent fiber disappearance of alfalfa hay and barley green feed treatments from the nylon bag and incubation time.

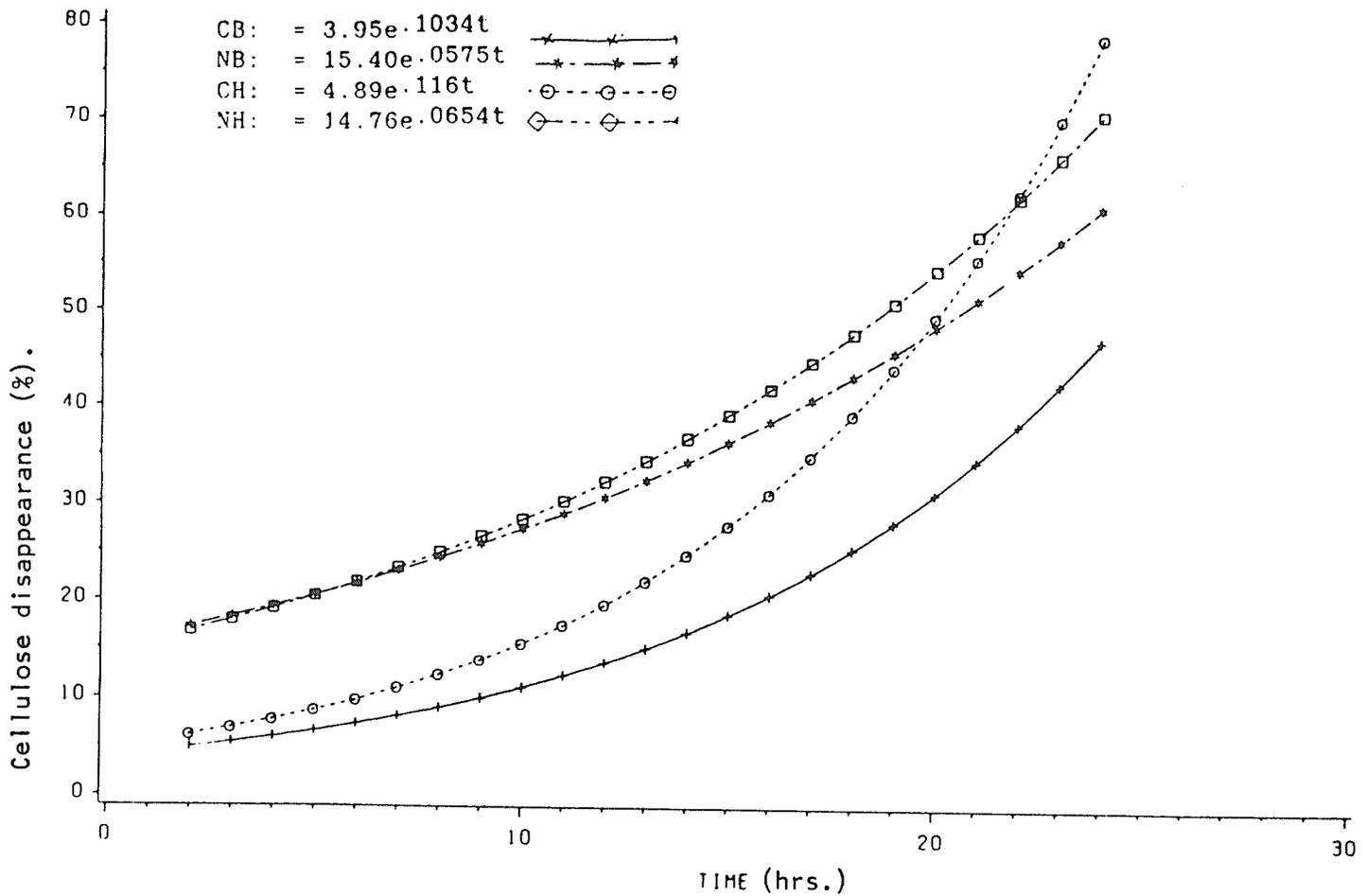


Figure 7. Relationship between cellulose disappearance of alfalfa hay and barley green feed treatments from the nylon bag and incubation time.

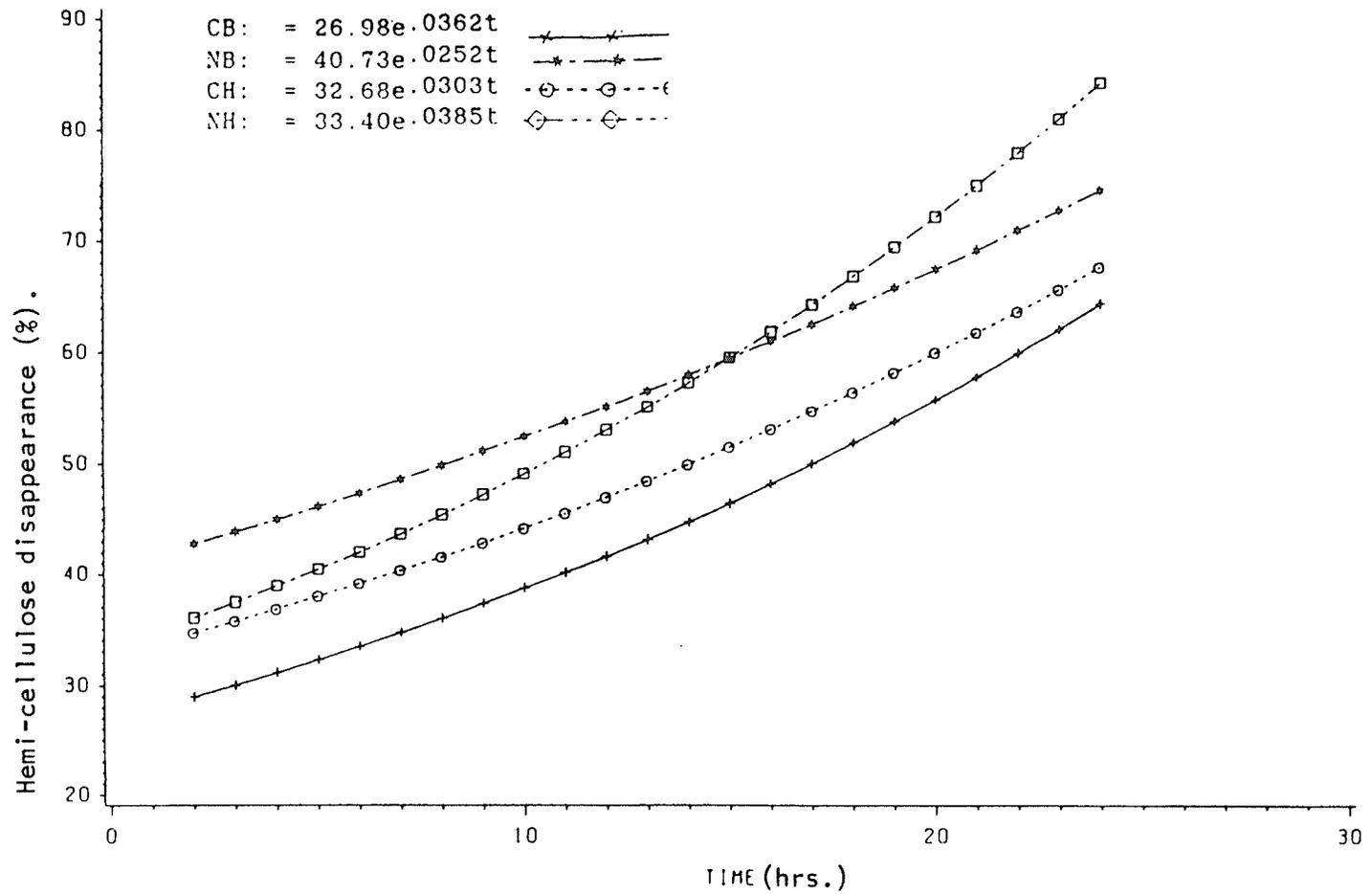


Figure 8. Relationship between hemi-cellulose disappearance of alfalfa hay and barley green feed treatments from the nylon bag and incubation time.

PART II: NUTRITIONAL VALUE OF DRY AMMONIATED ALFALFA HAY AND BARLEY STRAW

Chemical Composition

Effect of ammoniation of dry high protein alfalfa hay is shown in Table 18 (Appendix 24). Ammoniation increased the crude protein content from 15.9% to 24.3%. This represented a 52.7 percentage improvement in crude protein. Of the added ammonia-N, 54.5% was retained as ammonia-N at the time of feeding. Treated hay (NH) appeared to have higher ADF, NDF and hemi-cellulose content than untreated hay (CH) (Table 18).

Results from the second lamb (ram) experiment in which dry low protein barley straw was fed are shown in Table 19 (Appendix 24). Treatment of straw with ammonia increased the crude protein from 5.5% to 9.8%. This represented a 78.8 percentage improvement in crude protein content. Of the added ammonia-N 28.0% was retained as ammonia-N (Table 19). Treated barley straw appeared to have higher ADF content but lower NDF and hemi-cellulose content. Chemical analysis of grain supplements fed to rams offered either treated or untreated barley straw are indicated in Table 20.

Voluntary Intake and Digestibility of Roughages by Lambs

The ad-libitum intake of alfalfa hay is shown in Table 21 and Appendices 25 and 28. Ammoniation did not significantly affect the ad-libitum intake of alfalfa hay ($p > 0.05$). The above observation did not change when the daily dry matter intake was expressed on a metabolic weight basis or a percent of body weight.

Treatment of barley straw with ammonia significantly increased ($p < 0.05$) the consumption of straw by rams (Table 22, Appendices 25 and

Table 18. Effect of ammoniation of alfalfa hay on chemical composition.

Treatment ¹	Nutrients ²							CP Percent Improvement	NH ₃ -N Retained
	CP	ADF	NDF	H-CELL	Ca	P	GE ³		
	% DM basis								
CH	15.9	34.6	47.3	12.7	1.34	0.30	4.36		
NH	24.3	37.2	51.0	14.0	1.20	0.33	4.52	52.7	54.5 ⁴

¹Treatments: CH - Dry control alfalfa hay, NH - Dry ammonia treated alfalfa hay.

²Based upon average of samples taken in periods I and II.

³K cal/gm (DM basis).

⁴Calculated as follows: $[(24.29\% \text{ CP} - 15.91\% \text{ CP}) / 6.25] / [3.0\% \text{ NH}_3 \times 0.82\% \text{ N}]$.

Table 19. Effect of ammoniation of barley straw on chemical composition.

Treatment ¹	Nutrients ²							CP Percent Improvement	NH ₃ -N Retained
	CP	ADF	NDF	H-CELL	Ca	P	GE ³		
% DM basis									
CB	5.5	53.6	80.0	24.0	0.22	0.10	4.36		
NB	9.8	56.0	77.6	21.6	0.21	0.10	4.36	78.8	28.0 ⁴

¹Treatments: CB - Dry control barley straw, NB - Dry ammonia treated straw.

²Based upon average of samples taken in periods I and II.

³K cal/gm (DM basis).

⁴Calculated as follows: $[(9.76\% \text{ CP} - 5.46\% \text{ CP}) / 6.25] / [3.0\% \text{ NH}_3 \times 0.82\% \text{ N}]$.

Table 20. Nutrient analysis and amount of grain supplements fed to lambs given either treated or untreated barley straw.

Stem	Rolled barley grain alone	Rolled barley grain & 2.5% urea
1st DM %	91.0	86.5
DM Intake g/head/day	236.3	230.3
CP %	13.0	17.9
GE cal/gm	4360.6	4355.7
Ca %	0.90	0.90
P %	0.35	0.50

Table 21. Effect of ammoniation of alfalfa hay on voluntary intake by rams.¹

Treatment ²	Dry matter intake			N-intake	
	g/head/day	g/BW ^{.75} kg	% BW	g/head/day	g/BW ^{.75} kg
CH	1629.0	92.2	3.56	37.7	2.13
NH	1681.6	93.0	3.54	56.0	3.10
S.E.	91.7	4.85	0.18	3.67	0.18

¹All values are averaged over two rams in two periods.

²See Table 18.

Table 22. Effect of ammoniation of barley straw on voluntary intake by rams.¹

Treatment ²	Dry matter intake ³			N-intake ³	
	g/head/day	g/BW ^{.75} kg	% BW	g/head/day	g/BW ^{.75} kg
CB	652.4 ^a	38.79 ^a	1.51 ^a	4.24 ^a	0.25 ^a
NB	838.8 ^b	49.14 ^b	1.91 ^b	10.69 ^b	0.63 ^b
S.E.	2.80	0.41	0.02	0.01	0.004

¹All values are averaged over two rams in two periods.

²See Table 19.

³Means with different superscripts within the same column are significantly different ($p < 0.05$).

30). The daily dry matter consumption was improved by 28.6% as a result of ammoniation. The result was similar when the daily dry matter intake was expressed on a metabolic weight basis or as a percent of body weight. The daily nitrogen intake was improved by 152% as a result of ammoniation and the difference in intake was significant ($p < 0.05$) both on a daily nitrogen intake and metabolic weight basis.

The effect of ammoniation of alfalfa hay on in-vivo apparent digestibility of various nutrients is shown in Table 23 and Appendices 26 and 29. Ammonia treatment of the hay did not significantly affect ($p < 0.05$) the apparent digestibilities of dry matter, crude protein and gross energy. Dry matter digestibility tended to be higher for untreated hay than treated hay when the digestibility was determined by the Acid-Insoluble-Ash (AIA) indicator method but the difference was not statistically significant ($p > 0.05$) (Table 23, Appendices 26 and 29). The dry matter digestion coefficients determined by AIA indicator method were somewhat different than their corresponding in-vivo dry matter digestion coefficients.

The apparent digestibility of NDF improved ($p < 0.05$) and ADF and hemi-cellulose tended to improve (Table 23, Appendices 26 and 29) as a result of ammoniation. The percent improvement was 17.4%, 13.6% and 24.6% for ADF, NDF and hemi-cellulose, respectively.

The total dry matter and gross energy digestibilities tended to be higher when treated straw with grain was fed rather than when untreated straw with grain was fed (Table 24, Appendices 27 and 31). The percent improvement in digestibility was 12.8% and 12.9% for total dry matter, and gross energy, respectively, although the percent improvements did

Table 23. Effect of ammoniation of alfalfa hay on apparent in-vivo digestibility by rams.¹

Treatment ²	DM intake g/head/day	Apparent digestibility ³						AIA ^{3,4,5} DM
		Total Collection						
		DM	CP	GE	ADF	NDF	H-CELL	
CH	1536.0	63.4	73.3	61.7	47.7	50.7 ^a	58.7	67.0
NH	1718.0	62.5	70.5	61.7	54.0	59.5 ^b	73.0	59.0
S.E.	17.0	0.28	1.10	0.41	0.64	0.21	1.37	4.66

¹All values are averaged over two rams in two periods.

²See Table 18.

³Means with different superscripts within the same column are significantly different ($p < 0.05$).

⁴AIA - digestibility by Acid-Insoluble-Ash procedure.

⁵Average AIA content were 1.02% and 0.38% for treated and untreated hay respectively.

Table 24. Effect of ammoniation of barley straw on apparent in-vivo digestibility by rams.¹

Treatment ²	DM ³ intake g/head/day	Total ³			Straw			Fibre ⁴		
		DM	CP	GE	DM	CP	GE	ADF	NDF	H-CELL
CB ⁵	620.0	48.6	51.2	47.4	36.0	21.7	35.0	32.6	37.5	40.2 ^a
NB	793.7	54.8	52.5	53.5	43.8	37.0	42.0	41.7	50.6	73.2 ^b
S.E.	11.0	1.08	3.48	1.52	1.11	1.50	1.08	2.22	1.74	0.75

¹All values are averaged over two rams in two periods.

²See Table 19.

³Straw plus grain supplement.

⁴Contribution of grain was considered negligible.

⁵Means with different superscripts within the same column are significantly different ($p < 0.05$).

not reach statistical significance ($p > 0.05$). The calculated dry matter, crude protein and gross energy digestibilities for straw treatments alone (separate from grain) tended to be higher for treated straw than untreated straw (Table 24) but not statistically ($p > 0.05$) different. Percent improvement in digestibility was 21.8%, 70.3% and 20.3% for straw dry matter, crude protein and gross energy respectively. As expected the percent improvement in digestibilities of straw calculated by assuming a digestibility value for barley grain supplement were higher than digestibilities calculated on a total ration basis.

Treatment of straw with ammonia also tended to increase ($p > 0.05$) the apparent digestibilities of ADF and NDF (Table 24, Appendices 27 and 31). Hemi-cellulose digestibility was increased ($p < 0.05$) by ammonia treatment. Percent improvement were 28%, 35% and 82% in digestibilities of ADF, NDF and hemi-cellulose respectively.

Voluntary Intake, Digestibility and Performance of Cows on Ammoniated Hay

The chemical analysis of the hay and grain supplement fed to the cows during the experimental period are shown in Table 25 (Appendix 32). Cows consumed 14% more ($p < 0.05$) of the untreated long hay (Table 26) than they did long ammonia treated hay (Appendices 35 and 42). These observations did not change when the daily dry matter intake of long hay was expressed on a metabolic weight basis or as a percent of body weight (Table 26). The total dry matter intake was also higher ($p < 0.05$) when cows were offered untreated long hay than the treated long hay. Grain was fed on a ratio basis relative to the amount of hay consumed. Differences in dry matter intake expressed on metabolic

Table 25. Proximate analysis of alfalfa hay and grain supplement fed to lactating dairy cows (% DM).

Item	TEST ROUGHAGES ¹		GRAIN SUPPLEMENTS ²	
	CH	NH	Low Protein	High Protein
DM	94.0	94.0	94.5	94.2
CP	17.3	26.6	16.0	20.0
GE K cal/gm	4.0	4.2	4.5	4.3
ADF	31.5	33.2	7.0	10.8
NDF	42.0	47.4		
HEMI-CELL	10.6	14.2		
Ca	1.34	1.20	0.78	-
P	0.30	0.33	0.58	-

¹Test roughages: CH - Dry control alfalfa hay (untreated),
NH - Dry ammoniated alfalfa hay (treated).

²Grain supplements: Low protein (16%) and High protein (20%).

Table 26. Effect of ammoniation of alfalfa hay on voluntary intake by lactating dairy cows.

Treatment ²	Mean dry matter intake ³						
	Hay			Grain	Total		
	Kg/head/day	g/BW ^{.75} kg	% BW	Kg/head/day	Kg/head/day	g/BW ^{.75} kg	% BW
CH	9.4 ^a	21.2 ^a	1.59 ^a	10.5 ^a	19.8 ^a	45.0 ^a	3.64 ^a
NH	8.3 ^b	18.4 ^b	1.39 ^b	9.8 ^b	18.0 ^b	40.5 ^b	3.04 ^b
S.E.	0.32	0.49	0.03	0.15	0.20	0.54	0.04

¹All values are averaged over five cows in two periods.

²See Table 25.

³Means with different superscripts within the same column are significantly different (p<0.05).

weight basis and as a percent of body weight differed significantly ($p < 0.05$), with untreated long hay being higher than treated long hay. Cows offered the treated hay snorted while consuming the hay.

The daily intake of hay, grain and the total dry matter intake during the five days of fecal collection period are shown in Table 27, and Appendices 36 and 43. The cows consumed more ($p > 0.05$) of the untreated hay than they did treated hay and thus this was also true for grain and total dry matter consumption. The apparent dry matter digestibility determined by using Acid-Insoluble-Ash (AIA) as an indicator, tended to be higher for cows consuming long untreated hay than those consuming treated hay; however the difference was not statistically significant ($p > 0.05$) (Table 27, Appendices 36 and 43).

Actual milk, 4% FCM, butterfat and protein yields and also butterfat, protein and lactose content were not significantly affected ($p > 0.05$) by ammoniation (Table 28, Appendices 37 and 44). One of the cows (No. 80-32) Lorna) on untreated hay suffered from mastitis during the second period thereby reducing the level for a period of time. It was decided that square one animals be removed from statistical analysis.

Ruminal pH was the same ($p > 0.05$) for cows consuming treated hay and those consuming untreated hay before the feed was offered. The pH was higher ($p < 0.05$) at 2 hours post-feeding for cows consuming ammoniated hay (Table 29, Appendices 38 and 45).

Treatment of alfalfa hay with ammonia did not ($p > 0.05$) affect ruminal ammonia-N level (Table 29, Appendices 38 and 45).

Total VFA level and the acetate: propionate ratios were not

Table 27. Dry matter intake during fecal collection and the effect of ammoniation on dry matter digestibility of treated and untreated alfalfa hay fed to lactating dairy cows.¹

Treatment ²	DM intake, kg/head/day			DM digestion coefficient % ^{3,4}
	Hay	Grain	Total	
CH	7.0	10.0	17.0	77.0
NH	5.8	9.7	15.5	73.4
S.E.	0.40	0.25	0.83	2.70

¹All values are averaged over five cows in two periods.

²See Table 25.

³Determined by Acid-Insoluble-Ash (AIA) as an indicator.

⁴AIA contents, CH = 0.38%, NH = 1.02%, 20% CP grain supplement = 0.033% and 16% CP grain supplement = 0.08%.

Table 28. Effect of ammoniation of alfalfa hay on lactating dairy cows milk production and composition.¹

Items ²	Treatments ²		S.E.
	CH	NH	
Milk yield (kg/day)	24.8	24.4	0.28
4% FCM ³ (kg/day)	22.2	21.8	0.29
Butterfat (%)	3.35	3.35	0.08
Butterfat yield (kg/day)	0.82	0.80	0.02
Protein content (%)	3.00	2.95	0.12
Protein yield (kg/day)	0.74	0.71	0.01
Lactose (%)	4.79	4.80	0.10

¹All values are averaged over five cows in two periods.

²See Table 25.

³FCM - Fat corrected milk.

Table 29. Effect of ammoniation of alfalfa hay on lactating dairy cows ruminal pH and ammonia nitrogen.¹

Items ³	Sampling time ²	Treatments ⁴		
		CH	NH	S.E.
pH	0	7.0	7.0	0.08
	2	6.8 ^b	7.0 ^a	0.03
Ammonia N (mg/l)	0	100.5	110.0	15.9
	2	221.0	235.4	18.5

¹All values are averaged over five cows in two periods.

²Zero hours before and two hours after feeding.

³Means with different superscripts within the same row are significantly different ($p < 0.05$).

⁴See Table 25.

($p > 0.05$) affected by treatment (Table 30, Appendices 39 and 45) at 0 or 2 hours post feeding.

Treatment differences in VFA concentration were significant ($p < 0.05$) only for isobutyric and valeric acids at 2 hours post feeding (Table 30, Appendices 39 and 45). Both acids were lowered as a result of ammoniation. When VFA concentration were expressed on a molar % basis, ammoniation treatment resulted in higher ($p < 0.05$) acetic and lower ($p < 0.05$) isobutyric, valeric and isovaleric acid at 2 hours post feeding (Table 31, Appendices 40 and 46).

Ammoniation of alfalfa hay appeared to elevate blood urea nitrogen (BUN) of cows both before and 2 hours after the feed was offered (Table 32, Appendices 41 and 45). The differences in the levels reached statistical significance ($p < 0.05$) only at 2 hours post feeding.

Blood glucose levels and blood glucose to blood urea-N ratios were not different ($p > 0.05$) between treatments (Table 32, Appendices 41 and 45).

Table 30. Effect of ammoniation of alfalfa hay on lactating dairy cows volatile fatty acids (VFA) concentration and molar ratios.¹

Items ²	Sampling time ³	Treatments ⁴		
		CH	NH	S.E.
Total VFA (m moles/100ml)	0	6.00	6.68	0.53
	2	6.58	6.24	0.22
Acetic	0	3.80	4.13	0.32
	2	3.88	3.97	0.18
Propionic	0	1.24	1.42	0.13
	2	1.40	1.22	0.10
Butyric	0	0.70	0.85	0.10
	2	0.89	0.80	0.05
Isobutyric	0	0.07	0.08	0.01
	2	0.08 ^a	0.07 ^b	0.004
Valeric	0	0.10	0.10	0.01
	2	0.10 ^a	0.08 ^b	0.004
Isovaleric	0	0.10	0.11	0.01
	2	0.13	0.10	0.01
Acetate: propionate ratio	0	3.44	3.20	0.21
	2	3.04	3.50	0.12

¹All values are averaged over five cows in two periods.

²Means with different superscripts within the same row are significantly different ($p < 0.05$).

³Zero hours before and two hours after feeding.

⁴See Table 25.

Table 31. Effect of ammoniation of alfalfa hay on lactating dairy cows volatile fatty acids (VFA) molar percent (%).¹

Items ²	Sampling time ³	Treatments ⁴		
		CH	NH	S.E.
Acetic	0	64.7	62.5	1.3
	2	60.6 ^b	64.8 ^a	0.7
Propionic	0	19.9	20.7	0.9
	2	21.2	19.3	1.0
Butyric	0	11.4	12.6	0.5
	2	13.3	12.0	0.7
Isobutyric	0	0.1	0.1	0.01
	2	0.08 ^a	0.07 ^b	0.004
Valeric	0	1.3	1.3	0.1
	2	0.5 ^a	1.2 ^b	0.004
Isovaleric	0	1.7	1.6	0.04
	2	2.0 ^a	1.6 ^b	0.1

¹All values are averaged over five cows in two periods.

²Means with different superscripts within the same row are significantly different ($p < 0.05$).

³Zero hours before and two hours after feeding.

⁴See Table 25.

Table 32. Effect of ammoniation of alfalfa hay on lactating dairy cows blood urea N and blood glucose levels.¹

Items ²	Sampling time ³	Treatments ⁴		
		CH	NH	S.E.
Blood urea	0	16.4	18.4	0.5
Nitrogen (mg/100 ml)	2	19.0 ^b	21.4 ^a	0.5
Blood glucose (mg/100ml)	0	65.2	61.7	1.8
	2	72.0	71.5	1.85
Blood glucose:	0	0.25	0.30	0.01
Blood urea nitrogen ratio	2	0.27	0.30	0.01

¹All values are averaged over five cows in two periods.

²Zero hours before and two hours after feeding.

³Means with different superscripts within the same row are significantly different ($p < 0.05$).

⁴See Table 25.

DISCUSSION

NUTRITIONAL VALUE

Chemical Composition

The data presented in this study suggests that treatment of high moisture alfalfa hay with ammonia (23.2% moisture) was effective in preserving the nutritional quality. However the data must be interpreted with caution because (i) bale temperatures for untreated hay were in most cases lower than those of the treated hay, (ii) some damage occurred with thermocouple wires which may account for the lack of consistent readings and (iii) the best design of such an experiment would be one involving dry untreated, wet-untreated and wet-treated hay. Quantitation of fungal and bacterial spores and pictures of open bales would also enhance the interpretation of such data.

Bale temperature for wet-treated hay were higher (with a maximum of 49°C) than those of wet-untreated hay in the first 7 days (Figure 1). Untreated hay showed somewhat sustained high temperatures. The short, early rise in temperature of NH₃-treated hay has been attributed to the heat of hydration of NH₃ gas reacting with the moisture of the hay (Knapp et al., 1975; Thorlacius and Robertson, 1984). The changes in temperature reported here are at variance to those of Knapp et al. (1975), Thorlacius and Robertson (1984), Henning et al. (1986) and Kersbergen and Barton (1986) who reported lower bale temperatures for wet-treated roughages than wet-untreated roughages.

Lack of visible molding in NH₃-treated hay is indicative of the fungicidal effect of ammonia (Thomas, 1978; Lacey et al., 1981) resulting

in declining bale temperatures. Knapp et al (1975), Thorlacius and Robertson (1984) and Weiss et al (1982) reported similar results. Other researchers have reported reduction in actual mould and bacterial counts as a result of ammoniation (Grotheer et al, 1985; Henning et al, 1986; Woolford and Tetlow, 1984).

The lower ADIN (as a percent of total N) of the treated green feed versus untreated green feed suggests that, only a small portion of the ammonia-N became part of the ADF-N fraction (Table 6). Increase in ADIN results from the non-enzymic browning (Maillard) reaction which form indigestible carbon to nitrogen bonds between proteins and sugars (Van Soest, 1965). Increased ADIN for treated roughages (Table 6) but not in the ratio of ADIN to total nitrogen following ammoniation has also been reported by other researchers (Knapp et al, 1975; Weiss et al, 1982; Moore et al, 1985; Thorlacius and Robertson, 1984). Van Soest (1965) suggested that, forages with 20% or more of the total nitrogen as ADIN would be considered heat damaged. Although no temperature readings were taken on barley green feed treatments, the lower ADIN to total nitrogen of the treated barley green feed (Table 6) compared to the untreated green feed strongly suggests that NH_3 may have helped resist self heating in high moisture barley green feed.

Increase in the crude protein content of high moisture barley green feed and alfalfa hay is a result of the added NH_3 which contains 82% N (Table 6). The increase in CP content is also supported by findings in the 2nd and 3rd experiments (Ram experiments) (Table 18 and 19) using dry high protein alfalfa hay and dry low protein barley straw.

However, percentage improvement in CP content and NH_3 -N retained was

greater for high moisture roughages in the first trial (Table 6 and Tables 18 and 19) despite the NH_3 dosage rate having been lower than that used in the other two experiments. However the method of ammonia application was different. Ammoniation of high moisture low protein barley green feed resulted in CP content similar to the untreated alfalfa (good quality) (Table 6) while dry ammoniation of low protein barley straw resulted in CP content similar to a medium quality alfalfa hay (Table 18 and 19). Percentage improvement in CP content was higher for high moisture low protein barley green feed than high moisture high protein alfalfa hay although high moisture high protein hay retained more of the added $\text{NH}_3\text{-N}$. These data suggest that improvement in CP is dependent on moisture, quality of initial material and retention of the added $\text{NH}_3\text{-N}$ is different for legumes and cereal straw. Moore and coworkers (1985) have clearly demonstrated the importance of moisture in retention of NH_3 . Percentage improvement in CP content of high moisture alfalfa hay are similar to those reported by Thorlacius and Robertson (1984) for high moisture brome-grass hay and Grotheer et al (1985) for bermuda grass hay. These workers had 63% and 60% improvement in CP content upon ammoniation. Knapp et al (1975) and Weiss et al (1982) reported lower increases ranging from 17% to 35% for treated high moisture alfalfa, clover-fescue hay and fescue hay. These differences could be accounted by the fact that Knapp and coworkers (1975) used high protein hays (>10% CP) and a lower dosage rate (1%) while Weiss et al (1982) used high quality alfalfa hay (18.82% CP). Data reported and quoted by Horton (1978) and Dupchack and Stewart (1984) respectively indicate that the percent improvement in CP content was higher for

cereal straws with a lower CP content than those straws with a higher CP content. Waagenpertersen and Thomsen (1977), Orskov et al (1983) and Moore et al (1985) showed that increases in CP content is partly dependent on dosage rate. The high percent improvement in CP content observed for high moisture barley green feed is not unusual considering that this material had a low protein content. Males and Gaskins (1982) reported a 172% improvement of reconstituted high moisture low protein wheat straw while Streeter and Horn (1984) reported a percentage improvement close to 500% for reconstituted high moisture low protein content wheat straw; however, it must be noted that these workers used a higher application rate of NH_3 .

Percentage improvement in CP content reported for dry ammoniated alfalfa hay and barley straw (Tables 18 and 19) are similar to those of Horton and Steacy (1979) for dry ammoniated barley straw and those quoted by Dupchak and Stewart (1984). Lawlor and O'Shea (1979), Kernan et al (1979) and Mann et al (1986, unpublished data) realized a percentage improvement greater than 100% as a result of dry ammoniation of various cereal straws.

Ammoniation of high moisture roughages (Table 6) produced conflicting results with respect to ADF content. Although not significantly different, ADF content of high moisture barley green feed slightly increased while that for high moisture alfalfa hay decreased upon ammoniation. The ADF content also slightly increased upon ammoniation of dry alfalfa hay (Table 18) and barley straw (Table 19). If lignin and silica are dissolved during chemical treatment as shown by Feist et al (1970) then the ADF content should decrease. Weiss et al

(1982) and Buettner et al (1982) reported no significant differences in ADF content of treated and untreated high moisture alfalfa hay and dry medium quality tall fescue respectively. Other researchers have reported slight increases in ADF content upon ammoniation of high moisture brome grass (Thorlacius and Robertson, 1984) and dry low protein wheat straw (Mann et al 1986, unpublished data). The increase in ADF content could also be due to the Maillard reaction which produces a component insoluble in the acid detergent media as suggested by Van Soest (1965) and Kernan et al (1980). This explanation seems logical particularly if heat damage of protein occurred which is supported by data on ammoniation of high moisture barley green feed but not alfalfa hay (Table 6).

Ammoniation decreased the NDF content of high moisture barley green feed and alfalfa hay by 11% and 15% respectively (Table 6). These values are similar to 12% and 18% reported by Thorlacius and Robertson (1984) for high moisture brome grass hay and alfalfa hay. Paterson and coworkers (1981) and Grotheer et al (1985) reported percentage decreases in the same range for early and late harvest stalkages and reconstituted high moisture coastal bermuda grass, respectively. A percentage decrease as low as 4% has been reported by Weiss et al (1982) for high moisture alfalfa hay. Such differences in percentage may be attributed to duration of treatment, temperature and application rate. The other researchers used a higher application rate and longer treatment compared to Weiss et al (1982). Moore and coworkers (1985) reported that the decrease in NDF content was dependent on application rate as well as duration of treatment. Grotheer et al (1985) noted the

decrease in NDF content was higher for high protein materials than low protein roughages. The data in this study seem to support this suggestion. The difference in response in NDF content after ammoniation of low and high protein material may be related to lignification of these materials. It is reasonable to suggest that longer treatment time would be required for dry low quality roughages to maximize breaking of bonds between lignin and carbohydrates in order to realize a higher decrease in NDF content. Dry short term ammoniation appeared to have little effect on the NDF content of alfalfa hay and barley straw (Tables 18 and 19). The percentage decrease in NDF content of the barley straw observed here was much lower than that for high moisture barley green feed in trial 1 even though the NDF level was higher in the later barley straw. The difference could be attributed to differences in ammoniation procedures and moisture content since a higher application rate was used in case of dry ammoniation. Solubilization of cell-wall constituents was probably responsible for the decreased NDF content.

Previous work by some researchers showed that ammoniation did not affect cellulose of high moisture roughages (Weiss et al, 1982; Grotheer et al, 1985) and also of dry roughages (Buettner et al, 1982; Grotheer et al, 1985). In this study the differences were not significant but the cellulose content tended to be higher for high moisture barley green feed while that for high moisture alfalfa hay decreased upon ammoniation (Table 6). Moore and coworkers (1985) also reported an apparent increase in cellulose content of reconstituted treated high moisture orchard grass hay and attributed the increase to microbial respiration which occurred during reconstitution of the hay.

Hemi-cellulose content decreased ($p < 0.05$) upon ammoniation of the high moisture low protein barley green feed (32% decrease). There was a non significant (> 0.05) decrease for high moisture high protein alfalfa (7% decrease) (Table 6). Data on dry ammoniation showed no change in hemi-cellulose of high protein alfalfa hay while that for low protein barley straw decreased by 10% (Table 18 and 19). The percentage decrease for high moisture barley green feed is similar to the 29% decrease reported by Grotheer et al (1985) for high moisture coastal bermuda grass while that for high moisture alfalfa hay is lower than the 14% decrease reported by Weiss et al (1982) for high moisture alfalfa hay. A decrease of 52% was reported for reconstituted high moisture low protein wheat straw by Streeter and Horn (1984). A combination of high moisture (65%), high application rate and treatment time may have contributed to the enhanced decrease in hemi-cellulose content. Moore et al (1985) have demonstrated the effect of varying treatment time, dosage rate and moisture content, on hemi-cellulose content. Solubilization of some hemi-cellulose was probably responsible for the decreased hemi-cellulose content upon ammoniation of roughages.

Klopfenstein (1978) noted that lignin content was not generally reduced by chemical treatment. In this study permanganate lignin content of high moisture barley green feed was not affected by ammoniation while that for high moisture alfalfa hay was significantly decreased (Table 6). Weiss et al (1982) and Moore et al (1985) reported no differences in lignin content of high moisture treated and untreated roughages, while Streeter and Horn (1984) and Buettner et al (1982) reported a slight increase and decrease respectively.

Intake and Digestibility

Wethers and rams readily consumed high moisture ammoniated and dry ammoniated roughages. Aeration and chopping of these forages would enhance volatilization of the free NH_3 , which has a pungent smell. Lactating dairy cows did not readily consume the treated unchopped alfalfa hay. Animals tended to be snorting probably due to the pungent smell of ammonia. This might be due to the fact that the hay was direct fed without chopping. Bales were taken from the stack and fed; thus aeration may have been limited.

Streeter and Horn (1984) noted that airing high moisture ammoniated wheat straw before feeding increased ($p < 0.05$) straw DM intake from 1.60% to 2.00% of body weight and they attributed the depressed intake in direct fed straws as a result of high NH_3 -N levels.

Ammoniation in a stack significantly increased ($p < 0.05$) the daily DM intake of high moisture alfalfa hay and barley green feed by 29% and 46% respectively when fed to wethers (Table 7). In trial two there was no change in daily DM intake ($p > 0.05$) by sheep for dry ammoniated alfalfa hay (3% improvement) (Table 21). This difference in the results may in part be explained by the fact that the high moisture alfalfa hay was more mature than the dry alfalfa hay as indicated by their ADF contents in Tables 6 and 18. Intake was improved ($p < 0.05$) for dry ammoniated barley straw by 32% (Table 22). However, in the lactating dairy cows experiment, both total and roughage dry matter were significantly ($p < 0.05$) lower for cows consuming treated alfalfa hay than those consuming untreated hay (Table 26). The lactating dairy cows results are at variance with those of Weiss et al (1982), Rissanen et al

(1981) and also Thorlacius and Robertson (1984) who found no significant differences in dry matter intake of untreated and treated roughages, but in agreement with the third experiment of Rissanen et al (1981). Whether the lower intake of treated alfalfa hay by lactating cows is actually true, is subject to argument. The lowered intake may be due to the pungent smell of NH_3 . Streeter and Horn (1984) have show increased intake after aeration of high moisture ammoniated wheat straw while Rounds et al (1976) found lowered consumption of 50% DM corn cobs treated with 4% NH_4OH compared to 4.0% NaOH. In any case there is very little improvement in intake expected from ammoniation of high protein roughage, it would however been interesting to find out the result had the forage been fed in a chopped or pelleted form. Weiss et al (1982) and Thorlacius and Robertson (1984) used high quality alfalfa hay (CP around 20's) in their studies. Since voluntary intake is related to cell-wall content, digestibility and rate of passage, the potential of ammonia treatment of roughages to affect intake is more likely to be limited with higher quality forages except in instances of where heating and spoilage would otherwise severely affect digestibility and perhaps acceptability (Thorlacius and Robertson, 1984). The percentage increase in ad-libitum intake by lambs observed for high moisture alfalfa hay in the present experiment is similar to the 22% increase reported by Streeter and Horn (1984) and Horton (1978) for high moisture wheat straw and dry cereal straws respectively. Buettner et al (1982) reported 32% and 51% increase in intake of tall fescue fed to lambs and cattle respectively. This is similar to the 46% increase observed for high moisture barley straw. The results obtained in these studies indicate

that improvement in ad-libitum intake is higher for barley roughages than alfalfa hay. Other workers have also reported significant increases in DM intake following ammoniation of dry crop residues such as wheat straw (Males and Gaskins, 1982; Horton and Steacy, 1979; Saenger et al, 1982; Dias-da-Silva and Sundstol, 1986), barley straw (Horton, 1978; Orskov et al, 1983), oat straw (Horton, 1978) and coastal bermuda grass hay (Grotheer et al, 1985). In addition to improved DM intake, nitrogen intake is significantly improved by ammoniation of low protein roughages (Tables 8, 21 and 22). The nitrogen intake was improved by 152% (6.5 gm) as a result of ammoniation of dry low protein barley straw compared to 46% (18.3 gm) observed for dry high protein alfalfa hay.

Increased intake resulting from ammoniation may be due to (i) improved palatability of treated roughages resulting from caramelization of carbohydrates and nitrogen (Bechtel et al, 1945; and Forsberg, 1977) and also the wetness (moisture) of treated roughages may contribute to acceptability and (ii) increased digestibility of fibre fractions (Buettner et al, 1982; Saenger et al, 1983; Streeter and Horn, 1984) and therefore DM. Saenger et al (1982) noted that the dry matter intake of low protein roughage particularly, is limited by digestibility, rumen turnover rate, and rumen fill. Intake may also be limited by insufficient crude protein intake, but this does not seem to be the case in our studies as shown by the fact that both untreated high moisture low protein and dry untreated barley straw was supplemented with urea treated barley grain to provide an adequate level of N. Campling et al (1962) and Horton (1978) demonstrated that intake and digestibility can be improved by N supplementation, while in another study, Streeter and

Horn (1984) attributed greater response in intake and digestibility of treated straw over urea supplemented untreated straw to improved treated straw digestibility rather than the protein sparing effect of the added N due to ammoniation.

Although not significant except for high moisture high protein alfalfa hay, ammoniation increased the apparent in-vivo DM, CP and GE digestibilities for high moisture roughages and dry treated roughages when fed to wethers and rams (Tables 8, 23 and 24). Although limited data is available on high moisture ammoniated roughages, the results observed in the first digestion trial are similar to those reported for high moisture high quality brome grass (Thorlacius and Robertson, 1984), high moisture high protein coastal bermuda grass (Grotheer et al., 1985) and wet wheat straw (Males and Gaskins, 1982) but higher than those for reconstituted high moisture low protein wheat straw (Streeter and Horn, 1984). However Thorlacius and Robertson's (1984) data showed significant difference in DM digestibility of unsupplemented untreated and treated hay which is in agreement with the results for high moisture high protein alfalfa hay but at variance with that for high moisture low quality barley green feed which was supplemented with barley grain. It is possible that supplementing the treated high moisture barley green feed may have depressed the DM digestibility as shown by the fact that percentage improvement in digestibility resulting from supplementation was higher for untreated green feed than treated green feed (11% versus 6%). Mean separation by Tukey's procedure on roughage DM digestibility could not detect which treatment means were actually significantly different, although the original latin square ANOVA indicated

significant differences among means. Non-significant differences of total CP, roughage CP, GE digestibility is not an invariable finding. Garrett et al (1979) reported similar findings although at variance to reports by other researchers who have shown significant differences in these parameters (Thorlacius and Robertson, 1984; Dias-da-Silva and Sundstol, 1986; Horton and Steacy, 1979). Large standard errors may also have prevented differences from reaching statistical (Table 8) significance of these parameters.

Though data are limited on dry ammoniation of high protein hay the results for dry ammoniated hay are in agreement with those reported by Grotheer et al (1985) for coastal bermuda grass hay. The greater increased digestibility reported for high moisture roughages compared to dry ammoniation may be due to differences in moisture content and/or duration of treatment. Sundstol et al (1978) noted that improvement is somewhat more pronounced for untreated materials with a relatively low digestibility than for more digestible materials and this has been demonstrated by Waiss et al (1972).

Generally, ammoniation increased significantly ($p < 0.05$) or non-significantly ($p > 0.05$) the apparent digestibility of fibre fractions of both high moisture and dry ammoniated roughages (Tables 9, 23 and 24). Apparent digestibilities and percentage increase of digestibilities of fibre fractions were higher for treated high moisture low protein barley green feed compared to treated high moisture high protein alfalfa hay. Although dry ammoniation indicated higher fibre fractions digestibilities for alfalfa hay than dry treated low protein barley straw (Tables 23 and 24), percentage improvement in

digestibilities were higher. In addition to the above observation, apparent digestibilities and percentage improvements were higher for roughages ammoniated at high moisture than at low moisture (dry). As earlier noted, improvement of digestibility as a result of ammoniation is more pronounced for roughages with relatively low digestibility (Sundstol et al, 1978; Waiss et al, 1972) and also moisture dependent (Sundstol et al, 1978; Moore et al, 1985). Although fibre fractions digestibilities for high moisture and dry roughages markedly increased, large standard errors may have prevented differences from reaching statistical significance in case of fractions that did not show significant differences (Tables 9, 23 and 24). Increased fibre fractions digestibilities have also been reported for wet ammoniated coastal bermuda grass (Grotheer et al, 1985) wet ammoniated alfalfa hay and brome grass hay (Thorlaciuss and Robertson, 1984), wet reconstituted wheat straw (Streeter and Horn, 1984), dry ammoniated wheat straw (Streeter and Horn, 1984; Dias-da-Silva and Sundstol, 1986; Herrera-Saldana et al, 1983) and dry treated tall fescue (Buettner et al, 1982).

In-vitro and In-Situ Disappearance

Increased digestibility of nutrients observed in-vivo upon ammoniation of high moisture roughages was also demonstrated using the in-vitro and in-situ methods. Basically the CP and fibre fractions disappearance in-situ paralleled the DMD, as shown by the similarity in curves (Appendices 11 to 17). No chemical analysis was done on in-vitro samples because of the small sample used in incubation.

Dry matter digestion coefficients calculated for a 24 hour

incubation time showed that, while nylon bag DMD for treatments were higher than those obtained by both in-vivo and in-vitro techniques, the values were more similar to the in-vivo digestion coefficients, than the in-vitro values. The in-vitro system may not have been as active as it should have been. However, Tilley and Terry (1963) noted that, the failure to predict exactly in-vivo digestibility from in-vitro results reflects not only the inherent analytical errors in the two methods, but also the fact that in-vivo digestibility is not a constant characteristic of a herbage. In-vivo digestibility varies according to whether sheep or cattle are used in the trial, age and health status of animals, the level of feed intake and the manner in which the feed is prepared. Lawlor and O'Shea (1979) reported that DMD in-vivo values agreed quite well with those obtained in-vitro for treated and untreated cereal straw. In-situ values would be expected to be similar to in-vivo in that there is very little modification in this procedure in simulating in-vivo digestibility.

Although limited data is available on high moisture treated studies involving all three techniques, increases in DM and fibre digestibilities have been reported in high moisture treated alfalfa, clover-fescue hay (Knapp et al, 1975), fescue (Jones et al, 1985), reconstituted high moisture treated orchard grass hay (Moore et al, 1985) and dry ammoniated low quality wheat straw (Saenger et al, 1983).

The higher ($p > 0.05$) nutrient disappearances per unit time (Appendix 8) observed for untreated roughages relative to the treated roughages is unexplainable considering the fact that treated roughages had higher potentially digestible nutrients as shown by the higher

nutrient digestibilities.

Toxicity of Ammoniated Roughages

Lack of any visible symptoms of toxicity or abnormal behaviour of lambs suggests, either lack of toxic materials or low levels of toxic materials in the treated high moisture roughages. Chemical analysis of 4-methylimidazole and related compounds showed that these compounds were not present in treated high moisture roughages, however; oven drying before chemical analysis may have resulted in volatilization of the compounds (Dr. Reagor, personal communication, 1986).

LACTATION AND PHYSIOLOGICAL STUDIES

The data from lactation studies showed no significant difference in actual milk and FCM yield and composition (Table 28) despite the fact that cows fed untreated hay had a significantly higher total DM intake than those consuming treated hay. This may be due to the fact that all test animals satisfied both their protein and energy requirements at the level of milk production found in this trial. In spite of one cow, consuming untreated hay, having mastitis in the 2nd period of the trial, the average milk production was not markedly affected. Observations in this study support previous findings by other researchers (Weiss et al, 1982; Rissanen, 1981; Khalaf et al, 1986).

The alfalfa hay used in this study was of good quality with a crude protein similar to mid bloom hay (NRC, 1985) and well preserved. The beneficial effect of ammoniation may be in a situation where adverse drying conditions are threatening field curing of a good quality hay crop (Weiss et al, 1982). Khalaf et al (1986) suggested that ammoniated

wheat straw could be more useful than generally believed in diets of lactating cows of average milking ability. Further work in this area is required.

The increased ($p < 0.05$) ruminal pH at 2 hours postfeeding and a non-significant ($p > 0.05$) increase in ruminal ammonia-N (Table 29) due to feeding of ammoniated hay is an indication of the higher ammonia-N found in the treated roughages. Dissolution of the larger quantity of NH_3 in the ruminal fluid and subsequent ionization to NH_4^+ and OH^- could have increased the pH and ruminal NH_4^+ -N (Streeter and Horn, 1984). The results (Table 29) obtained in this study are similar to those reported by Streeter and Horn (1984) and Males and Gaskins (1982) for wet treated wheat straw but lower than those reported by Horton (1978) for treated barley, wheat and oat straw. Horton (1978) sampling time was more than three hours after the feed was offered compared to 2 hours post feeding used in our study. The longer time may have resulted in more dissolution of NH_3 .

Differences in VFA productions between cows fed untreated and treated hay were significant ($p < 0.05$) for isobutyric and valeric acid measured 2 hours post feeding (Table 30). On a molar percent basis, ammoniation significantly increased ($p < 0.05$) acetic acid production but decreased ($p < 0.05$) the isobutyric, valeric and isovaleric acid production at 2 hours post feeding (Table 31).

The data seem to suggest that ammoniation pushed the fermentation to one favouring production of acetic acid (Table 30 and 31) which would be supported by the increased fibre digestion. This was not reflected in the butterfat content, as both treatments resulted in acetate:

propionate ratios of at least 3 to 1. These results are supported by reports by Streeter and Horn (1984) and Males and Gaskins (1982).

Higher blood urea-N (BUN) of cows fed treated hay (Table 32) may have been due to the elevated ruminal ammonia-N and may be an indication of low nitrogen utilization due to limited energy supply (Tagari et al, 1964), or excess ruminal ammonia-N due to the daily nitrogen intake (4160 gm/day) being higher than that recommended by NRC (1978) at this level of milk production of the cows in this study. Normal blood urea when a supplement was fed, shows that the increased N in ammoniated straw may be utilized effectively provided that dietary energy is not limiting (Dror et al, 1969). The data reported in this study shows that both BUN and blood glucose levels (Table 32) were in the normal range of 6-27 mg/100ml and 40-70 mg/100ml (Swenson, 1984) respectively. Blood glucose measurement is also an indirect measure of energy release, although little glucose is absorbed from the gastro-intestinal tract of ruminants. Since these two parameters were not excessively elevated and the difference in intake didn't influence milk yield one may speculate that energy supply was not limiting.

SUMMARY AND CONCLUSIONS

Three experiments were carried out to investigate the use of ammonia as a preservative of hay and barley green feed harvested at high moisture (>20%) and its effect on the nutritional value of dry high protein and low protein roughages.

Although it wasn't possible to run statistical analysis, bale temperatures were higher for high moisture treated alfalfa hay than wet untreated hay but showed a more rapid decline in temperature over time. Ammonia treated alfalfa hay and treated barley green feed did not show any apparent visible molding while the corresponding untreated roughages did. ADIN as a percentage of the total nitrogen was significantly lower ($p < 0.05$) for ammonia wet treated barley green feed than the untreated green feed. There was no significant differences ($p > 0.05$) in the ADIN (% of total N) content of untreated and treated hay though the ADIN content tended to be lower for treated hay.

Ammoniation of high moisture roughages significantly increased ($p < 0.05$) the nitrogen content of the roughages in the first trial. Although no statistical analysis was not carried out, ammoniation markedly increased the nitrogen content of the dry treated roughages in the second trial.

Ammoniation did not affect ($p > 0.05$) the GE, ADIN (% of DM) and cellulose content of high moisture roughages. The ADF and lignin content of treated high moisture hay but not treated barley green feed, the hemi-cellulose content of wet treated barley green feed but not treated hay and the NDF content of both treated species of roughages were significantly lower ($p < 0.05$) than the untreated roughages.

Although not statistically significant ($p>0.05$), the ADF content of treated barley green feed and hemi-cellulose content of treated hay tended to be higher and lower respectively than the corresponding untreated roughages.

The voluntary DM and nitrogen intake of barley green feed and hay fed to lambs in trial 1 and barley straw in trial 2 was significantly increased ($p<0.05$) by ammoniation. Ammoniation of dry hay significantly lowered ($p<0.05$) the dry matter intake of alfalfa hay when fed to lactating dairy cows with no effect ($p>0.05$) when fed to rams. The pungent smell of ammonia was suspected as the probable cause of the lowered intake by cows. The hay was chopped before feeding to the rams but not for the cows.

The roughage DM digestibility of both species of wet roughages was significantly increased ($p<0.05$) by ammoniation in trial one. Although statistically not significant ($p>0.05$), the total DM digestibility of barley green feed, the total crude protein, roughage crude protein, roughage GE digestibilities and DE of the treated hay and straw tended to be higher than the corresponding untreated roughages. The DM, CP and GE digestibilities of roughages in the second and third trials were not significantly affected ($p>0.05$) by ammoniation. Dry matter and GE digestibilities tended to be higher for treated barley straw than untreated barley straw.

The ADF and cellulose digestibility of wet barley green feed and also the NDF and hemi-cellulose digestibility of both species of wet treated roughages were significantly increased ($p<0.05$) by ammoniation. Though not statistically significant ($p>0.05$), the ADF, and cellulose

digestibilities of wet treated alfalfa hay and the lignin digestibility of both species of treated roughages tended to be higher than the corresponding untreated roughages. In the second trial, only the NDF and hemi-cellulose digestibilities of alfalfa hay and barley straw respectively were significantly increased ($p < 0.05$) by ammoniation. Though, not statistically significant ($p > 0.05$), the ADF digestibility of both treated roughage species, the hemi-cellulose and NDF of the dry treated alfalfa hay and barley straw respectively, tended to be higher than the corresponding untreated roughages. Large standard errors may have prevented differences reaching statistical significance.

Ammoniation significantly increased ($p < 0.05$) the IVDMD and the nylon bag disappearances of DM, CP, ADF, NDF, cellulose and hemi-cellulose of wet treated roughages. The disappearances also significantly increased ($p < 0.05$) with increasing incubation time. The nylon bag DMD was more similar to the in-vivo DM digestibility than the IVDMD. Correlations among the three methods were not calculated due to the limited data points. Ammoniation did not significantly affect the DM, CP, ADF, NDF, and hemi-cellulose disappearance per unit time.

No 4-methylimidazoles or any of the related compounds were detected in either of the wet roughages. Lambs on these roughages did not show any signs of abnormal behaviour which could be associated with symptoms of toxicity of ammoniated roughages.

There were no significant differences ($p > 0.05$) in the milk production and composition of cows fed either dry treated or untreated hay. Differences in the ruminal pH, isobutyric, valeric acid and blood urea nitrogen concentrations were significant ($p < 0.05$) at 2 hours post

feeding. Acetic and isovaleric acids were different ($p < 0.05$) at 2 hours post feeding when expressed on a molar percent basis. The fermentation appeared to be pushed towards more acetate production than propionic acid with treatment of the forage.

It should be noted here that, the original plan in trial 2 and 3 was to use high moisture hay. However, the material obtained at the end of 1985 heated up excessively and spoiled, therefore could not be used.

In conclusion, the results indicate that ammonia treatment;

1. can effectively reduce or minimize the deterioration of the nutritional value of high moisture hay,
2. increases the crude protein of roughages,
3. increases the voluntary DM intake of wet treated roughages,
4. decreases the NDF and hemi-cellulose content but not EE, GE and cellulose content,
5. increases the nutrient digestibilities of barley green feed or barley straw.

In the last four conclusions the benefits from ammoniation are greater for low quality roughages than good quality roughages.

6. The alleged toxic compounds in ammoniated high moisture roughages were not found,
7. no benefits were obtained in milk parameters resulting from ammoniation of dry good quality alfalfa hay.

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Appendix 1. Reference formula for calculation of digestibility, and missing values.

$$(a) \quad \% \text{ digestibility} \quad = \frac{(\text{intake} - \text{fecal output})}{(\text{intake})} \times 100$$

$$(b) \quad \text{Missing value (y)} \quad = \frac{rC + t(R + T) - 2G}{(t - 1)(r - 2)}$$

where:

C, R and T = totals of the column, row and treatment with missing value

G = grand total

t = no. of treatments

r = replications

Appendix 2. Temperature ($^{\circ}\text{C}$) trends in wet NH_3 -treated and wet untreated alfalfa hay.

Treatment	September							October
	14	15	17	18	19	22	30	4
Wet ammoniated alfalfa hay	44.0	-	49.0	44.5	45.0	-	20.9	19.4
Wet-untreated alfalfa hay	31.5	32.7	13.0	40.6	41.0	28.7	32.9	15.7

Appendix 3. Nutrient analysis of roughages fed to individual wethers from period (week) 1 to 4¹ in trial 1.

Period/ Feed Type	Animal No.	Nutrients % (DM basis)					GE k cal/gm	
		CP	EE	Ash	Ca	P		
PI	CB	0133	7.73	1.69	6.11	0.28	0.25	4.35
	NB	1010	16.57	2.62	6.95	-	-	4.44
	CH	0131	13.46	1.68	6.82	1.28	0.14	4.58
	NH	0127	24.54	1.86	7.73	1.52	0.16	4.65
PII	CB	1010	6.34	1.69	5.90	0.21	0.24	4.40
	NB	0127	16.50	1.75	7.88	0.38	0.31	4.45
	CH	0133	13.83	1.62	6.91	-	0.14	4.62
	NH	0131	24.02	2.11	7.46	1.56	0.16	4.62
PIII	CB	0131	7.28	2.34	6.39	0.21	0.23	4.42
	NB	0133	15.79	1.82	7.86	0.41	0.29	4.46
	CH	0127	14.00	1.58	7.06	1.36	0.14	4.60
	NH	1010	25.27	2.31	7.95	1.58	0.16	4.61
PIV	CB	0127	6.12	1.86	7.11	0.27	0.19	4.39
	NB	0131	16.12	1.97	7.53	0.43	0.28	4.44
	CH	1010	15.87	1.92	7.46	1.46	0.16	4.59
	NH	0133	24.30	1.97	7.65	1.56	0.16	4.60

¹Roughage samples collected during digestion trial.

Appendix 4. Fiber fractions analysis of roughages fed to individual wethers from period (week) 1 to 4¹ in trial 1.

Period/ Feed Type	Animal No.	Fiber constituents % (DM basis)						ADIN % of total N	
		ADF	NDF	Hemi- cellulose	Cellulose	Lignin	ADIN		
PI	CB	0133	35.94	64.27	28.63	27.10	6.33	0.49	40
	NB	1010	40.26	59.47	19.21	31.12	6.64	0.67	25
	CH	0131	44.98	57.61	12.63	32.13	11.90	0.57	27
	NH	0127	37.03	49.26	12.23	26.75	9.36	0.87	22
PII	CB	1010	35.81	62.59	26.98	26.77	7.14	0.49	49
	NB	0127	41.27	61.21	19.94	30.90	7.53	0.54	20
	CH	0133	43.10	56.95	13.85	30.61	12.09	0.53	22
	NH	0131	37.03	49.18	12.15	26.45	10.01	0.71	18
PIII	CB	0131	37.98	66.25	28.27	29.86	5.77	0.63	54
	NB	0133	46.63	66.27	19.64	37.29	6.43	0.67	26
	CH	0127	45.62	59.27	13.65	31.67	13.34	0.38	17
	NH	1010	35.71	48.93	13.22	24.48	10.70	0.73	18
PIV	CB	0127	43.75	72.07	28.32	33.75	8.44	0.40	41
	NB	0131	42.92	60.07	17.15	34.48	5.36	0.55	21
	CH	1010	43.68	57.37	13.69	32.00	11.61	0.68	27
	NH	0133	36.00	49.44	13.44	27.95	8.24	0.92	24

¹Roughage samples collected during digestion trial.

Appendix 5. Individual wether data on voluntary D.M. intake of test roughages in trial 1.

Period/ Feed Type	Animal No.	DM %	Total roughage DM intake g/head	Mean daily DM intake g/head/day	DM intake percent of body wt.	DM intake g/kg W.75	
PI	CB	0133	92.66	5,994.44	856.35	2.13	53.52
	NB	1010	93.73	8,225.44	1,175.49	3.00	74.87
	CH	0131	93.01	5,837.02	833.86	2.18	54.15
	NH	0127	95.85	11,600.92	1,657.27	4.34	107.61
PII	CB	1010	92.22	8,303.40	1,186.20	2.19	59.61
	NB	0127	90.58	10,620.10	1,517.16	3.24	84.76
	CH	0133	93.32	9,155.19	1,307.88	3.09	78.79
	NH	0131	91.99	8,724.10	1,246.30	2.91	74.63
PIII	CB	0131	94.57	3,370.39	481.48	0.82	22.60
	NB	0133	91.01	9,645.79	1,377.97	2.62	70.67
	CH	0127	92.24	9,956.77	1,422.40	3.30	84.67
	NH	1010	88.25	13,610.37	1,944.34	3.72	100.22
PIV	CB	0127	93.39	5,869.47	838.50	1.40	39.00
	NB	0131	91.59	5,921.34	845.91	1.53	41.67
	CH	1010	91.98	11,671.16	1,667.31	3.41	90.12
	NH	0133	90.56	13,287.69	1,898.24	3.30	90.82

Appendix 6. Individual wether data on total and roughage DM consumption from period 1 to 4 of trial 1.

Period/ Feed Type	Animal No.	1st DM %	2nd DM %	Mean daily roughage DM consumed g/head/day	Total mean daily DM consumed g/head/day	Roughage CP daily intake g/head/day	Total CP daily intake g/head/day	
PI	CB	0133	94.18	92.45	859.55	1,101.53	66.44	111.18
	NB	1010	94.06	92.21	1,230.14	1,463.72	203.83	233.59
	CH	0131	94.24	93.80	855.86		115.20	
	NH	0127	94.45	93.17	1,630.99		400.24	
PII	CB	1010	93.87	93.60	1,197.00	1,438.98	75.89	120.63
	NB	0127	92.67	92.49	1,675.47	1,909.05	276.45	306.21
	CH*	0133	94.34	92.99	1,466.50		202.82	
	NH	0131	93.33	92.88	1,276.75		306.68	
PIII	CB	0131	92.13	93.63	1,194.77	1,436.75	86.98	131.72
	NB	0133	92.92	92.55	1,666.52	1,900.10	263.14	292.90
	CH	0127	93.44	93.33	1,656.08		231.85	
	NH	1010	91.75	93.54	2,477.09		625.96	
PIV	CB	0127	93.91	94.08	989.50	1,231.48	60.56	105.30
	NB	0131	91.52	93.88	922.52	1,156.10	148.71	178.47
	CH	1010	92.69	93.70	1,763.89		279.93	
	NH	0133	89.77	94.04	1,893.40		460.10	

*Pseudo values (Cochran and Cox, 1957).

Appendix 7. Individual wether data on the apparent in vivo nutrient digestibility of test roughages from period 1 to 4 of trial.¹

Period/ Feed Type	Animal No.	Roughage DM	Total DM	Roughage CP	Total CP	G.E.	DE K cal/gm	Roughage DM (24 hrs.)	
PI	CB	0133	57.96	65.22	48.18	65.02	56.13	2.44	57.20
	NB	1010	66.96	70.79	62.26	65.16	64.76	2.89	66.47
	CH	0131	56.16		70.27		55.97	2.56	57.98
	NH	0127	63.63		73.66		62.93	2.93	65.22
PII	CB	1010	56.36	62.19	37.98	57.28	55.31	2.43	53.89
	NB	0127	65.06	68.23	63.22	65.34	64.15	2.85	64.81
	CH*	0133	57.26		68.16		57.92	2.63	
	NH	0131	64.28		74.01		63.74	2.94	63.80
PIII	CB	0131	64.01	68.55	64.14	72.94	63.86	2.82	50.95
	NB	0133	64.58	67.83	61.43	63.83	63.50	2.83	61.91
	CH	0127	57.59		70.13		58.42	2.69	56.70
	NH	1010	65.21		74.25		64.91	2.99	63.76
PIV	CB	0127	46.34	55.11	29.05	54.96	45.94	2.02	45.74
	NB	0131	60.76	66.87	59.78	63.99	58.76	2.61	57.40
	CH	1010	53.97		71.78		55.93	2.57	50.45
	NH	0133	62.73		70.61		61.76	2.84	62.74

*Pseudo values (Cochran and Cox, 1957).

¹(DM basis).

Appendix 8. Individual wether data on the apparent in vivo fiber fractions digestibility from period 1 to 4 of trial 1¹.

Period/ Feed Type	Animal No.	ADF	NDF	Hemi- cellulose	Cellulose	Lignin	
PI	CB	0133	44.15	51.63	61.52	51.86	16.34
	NB	1010	61.32	65.41	74.00	69.44	43.53
	CH	0131	45.79	45.46	44.29	49.05	40.03
	NH	0127	54.20	58.68	72.26	56.94	49.23
PII	CB	1010	40.57	48.41	58.91	48.64	17.37
	NB	0127	60.82	70.50	90.54	69.81	34.96
	CH*	0133	46.97	50.64	57.36	55.06	29.94
	NH	0131	54.45	59.68	75.60	61.51	34.59
PIII	CB	0131	51.54	56.97	64.26	58.81	18.15
	NB	0133	63.00	71.73	92.45	73.94	15.97
	CH	0127	51.90	50.70	46.70	55.36*	44.20*
	NH	1010	54.99	61.30	78.37	59.25	43.23
PIV	CB	0127	36.87	43.92	54.81	49.70	4.95
	NB	0131	53.42	63.24	87.82	64.44	-10.08
	CH	1010	41.49	44.25	53.06	51.50	16.63
	NH	0133	51.08	59.09	80.54	62.09	18.73

*Pseudo values (Cochran and Cox, 1957).

¹% (DM basis).

Appendix 9. Nutrient analysis of roughages used in the in-vitro and nylon bag studies.¹

Feed Type ²	1st DM	2nd DM	CP	ADF	NDF	Cellulose	Hemi-Cellulose
CB	94.05	96.18	6.89	38.25	76.15	32.84	37.90
NB	93.72	94.51	15.97	43.59	71.01	37.87	27.42
CH	93.10	96.40	14.66	44.07	65.88	34.68	21.81
NH	93.75	94.65	23.91	36.95	56.35	31.55	19.40

¹% (DM basis).

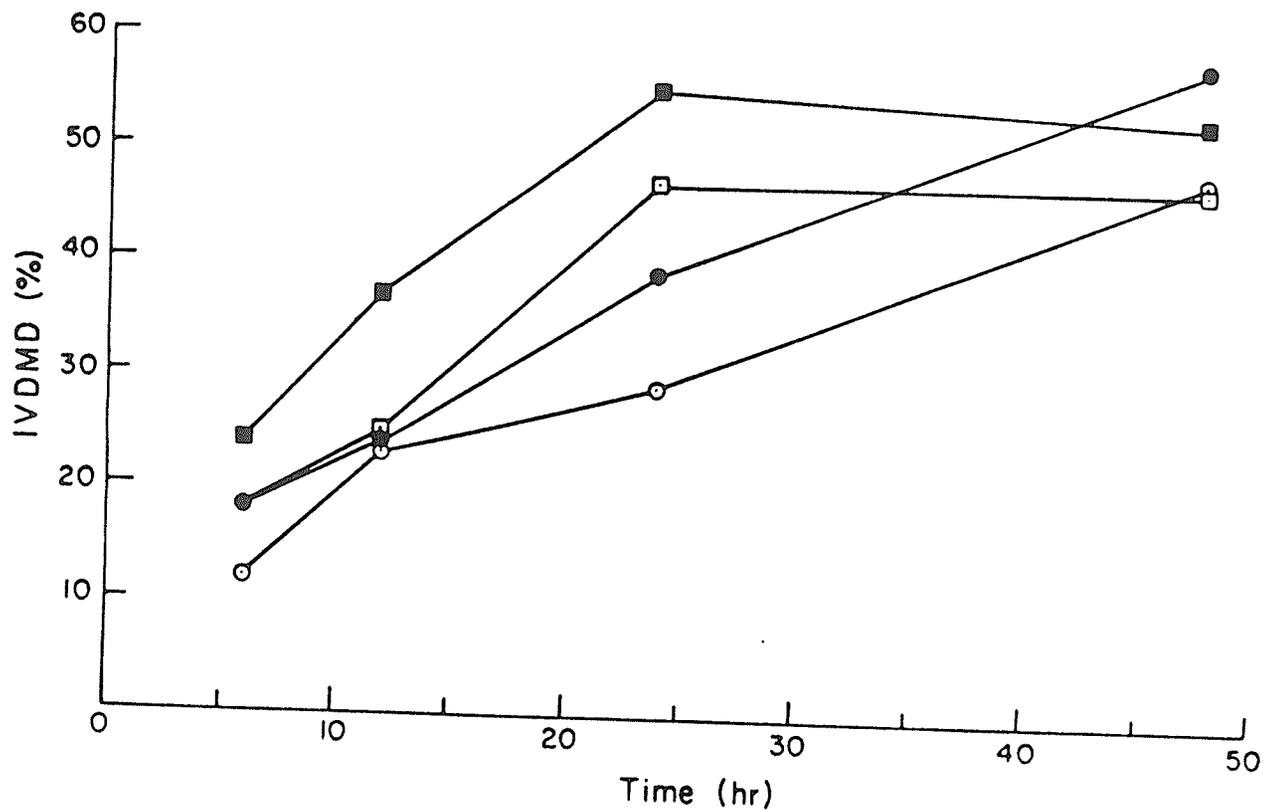
²See Table 6.

Appendix 10. Chemical analysis of grass hay fed to the steer for insitu studies and providing rumen inoculum for in-vitro studies.¹

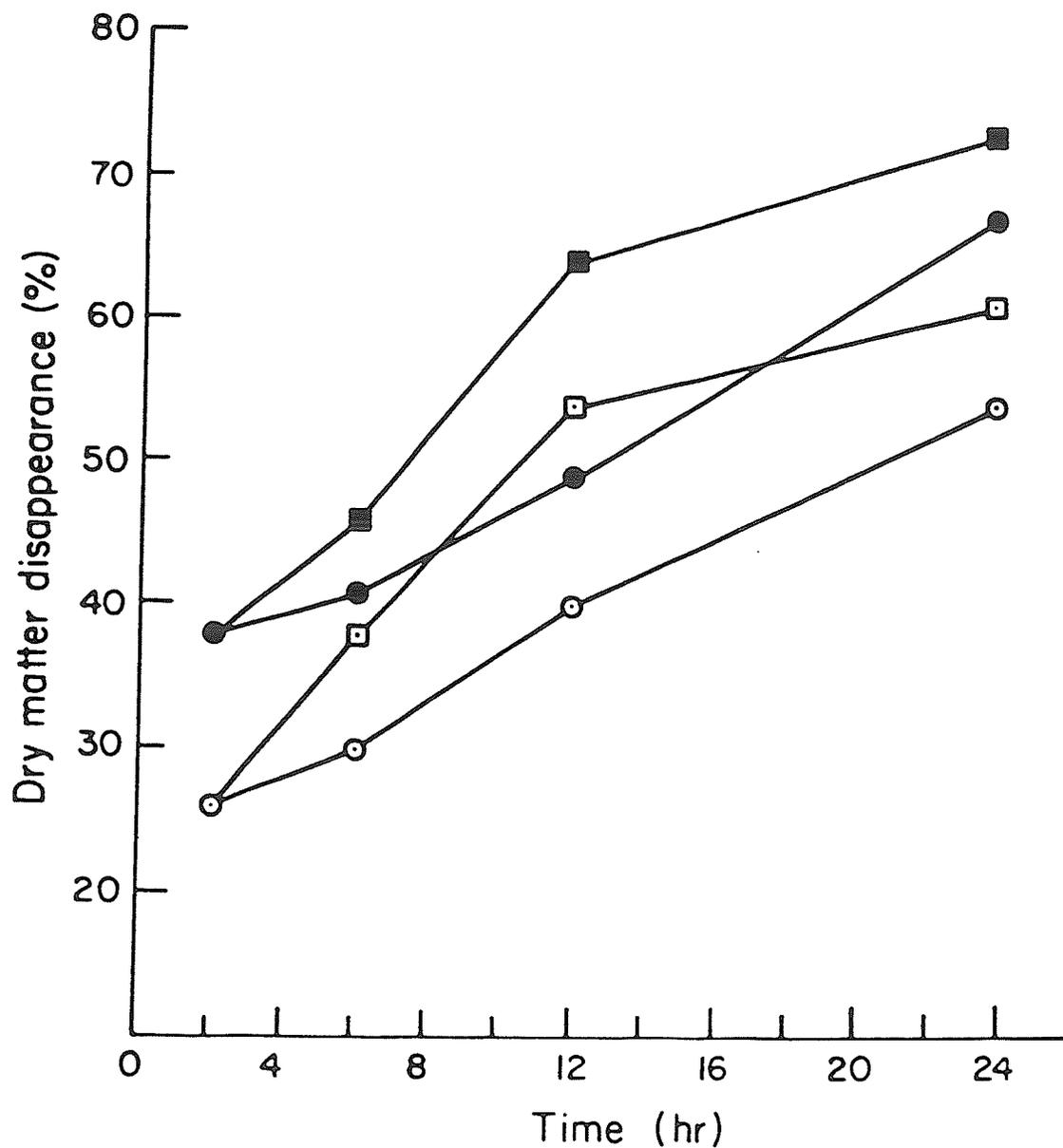
1st DM	2nd DM	CP	ADF	NDF	G.E. ²	Hemi-Cellulose	Cellulose	Ash
88.71	97.09	11.01	38.58	66.71	3.63	28.13	29.41	4.79

¹% (DM basis).

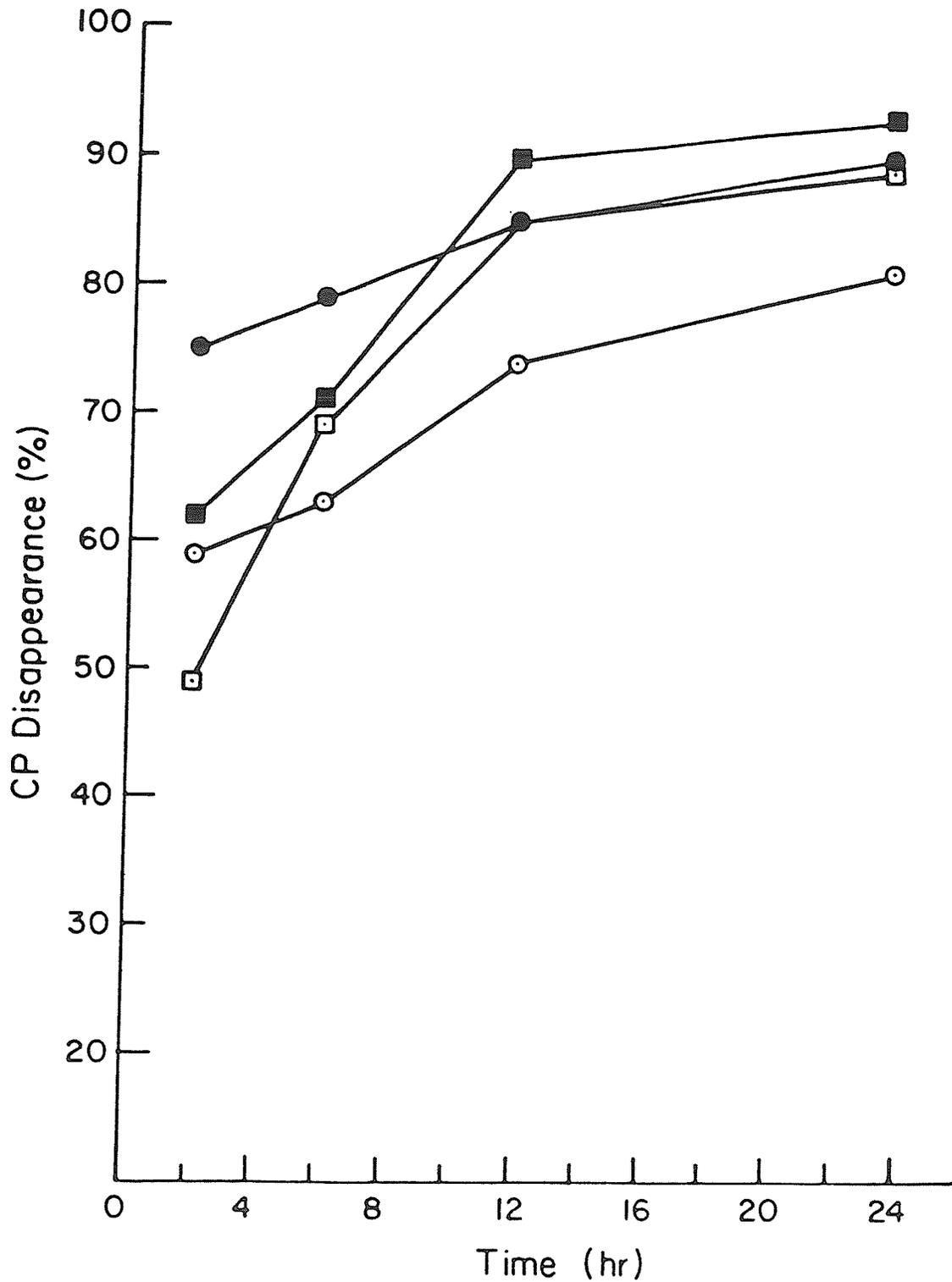
²G.E. in K cal/gm (DM basis).



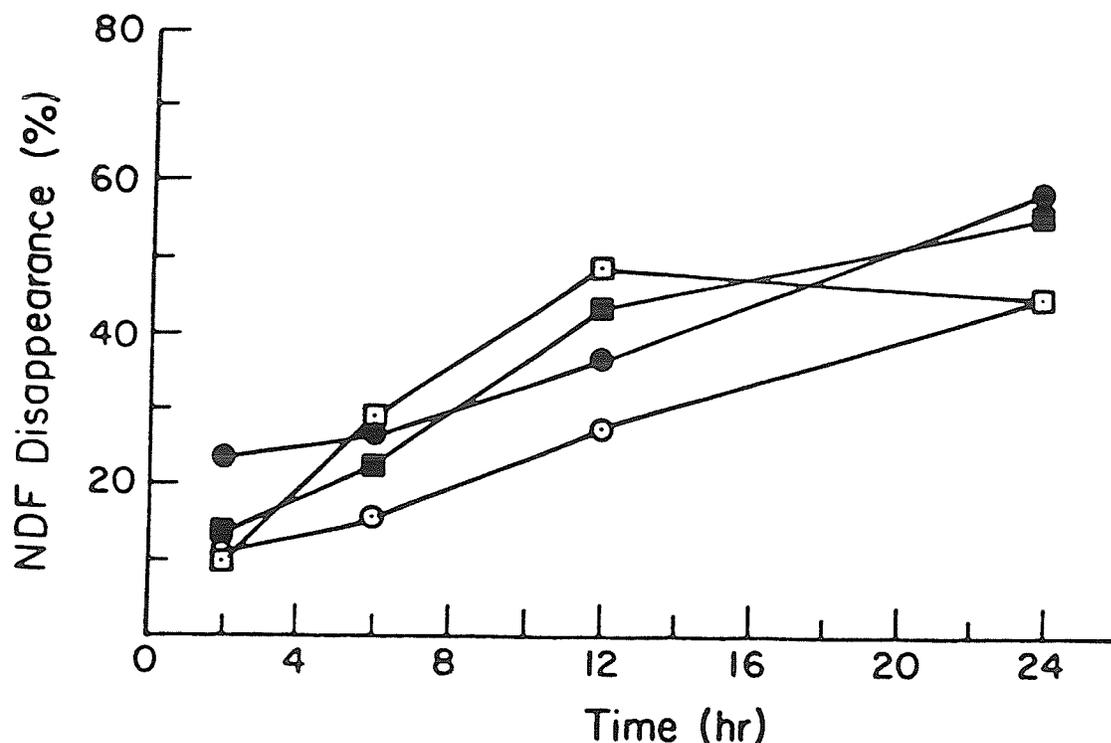
Appendix figure 11: In vitro dry matter disappearance (IVDMD) of untreated barley green feed (○—○), untreated alfalfa hay (□—□), ammonia-treated barley green feed (●—●) and ammonia-treated alfalfa hay (■—■) incubated at different periods of time.



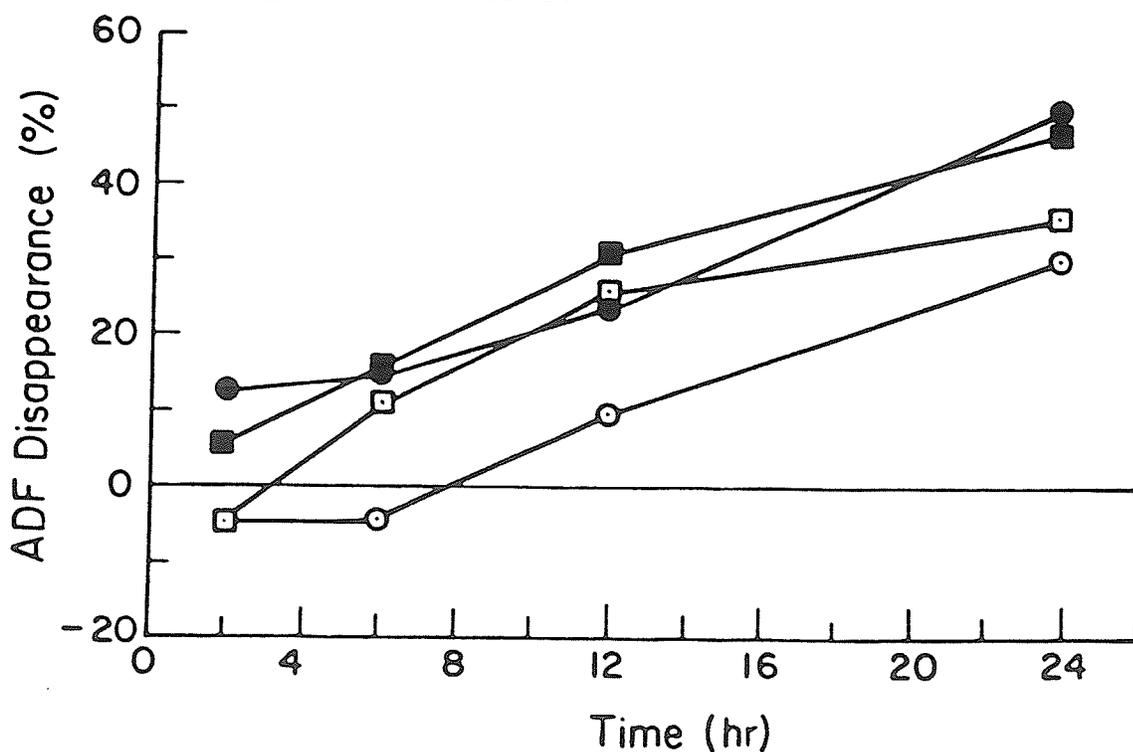
Appendix figure 12: Dry matter disappearance (DMD) of untreated barley green feed (○—○), untreated alfalfa hay (□—□), ammonia-treated barley green feed (●—●) and ammonia-treated alfalfa hay (■—■) incubated in situ at different periods of time.



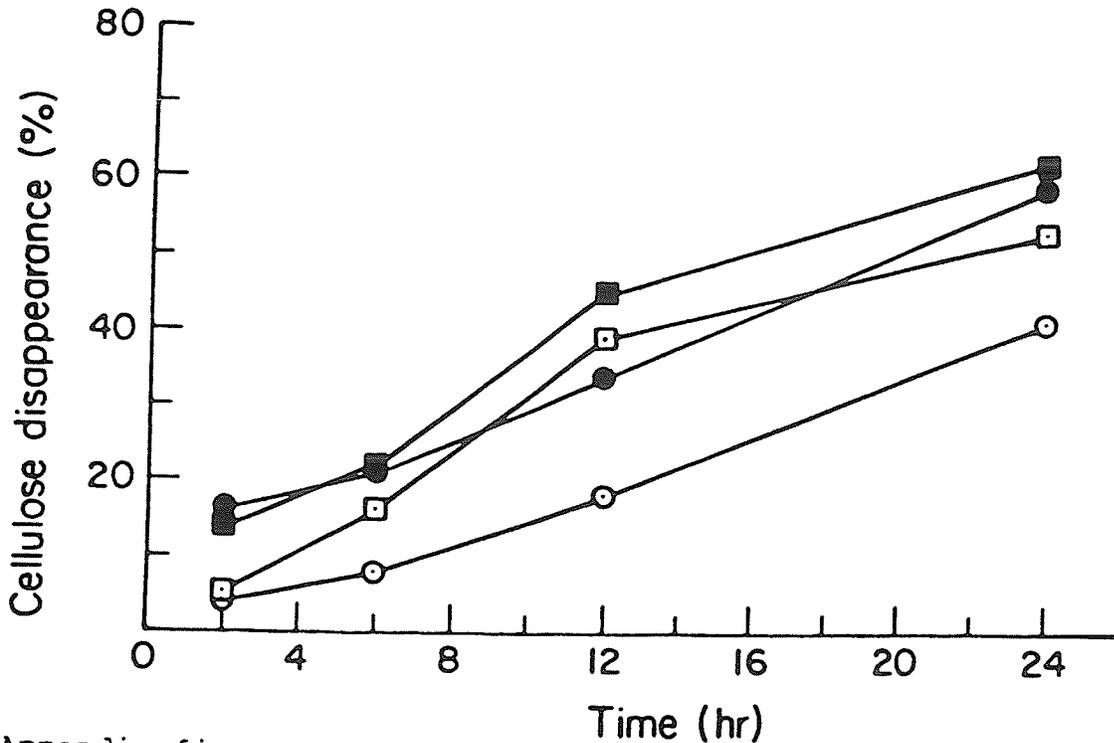
Appendix figure 13: Crude protein disappearance of untreated barley green feed (○—○), untreated alfalfa hay, (□—□), ammonia-treated barley green feed (●—●) and ammonia-treated alfalfa hay (■—■) incubated in situ at different periods of time.



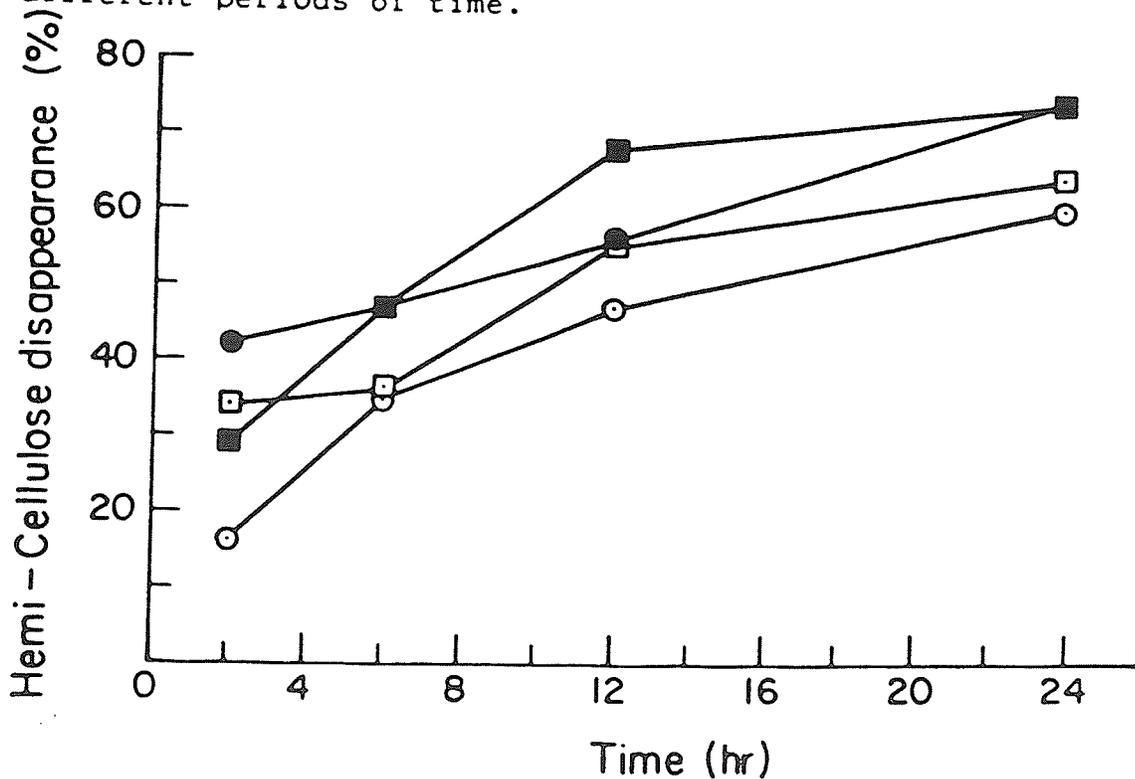
Appendix figure 14: Neutral-detergent fibre disappearance (NDFD) of untreated barley green feed (o—o), untreated alfalfa hay (□—□), ammonia-treated barley green feed (●—●) ammonia-treated alfalfa hay (■—■) incubated in situ at different periods of time.



Appendix figure 15: Acid-detergent fibre disappearance (ADFD) of untreated barley green feed (o—o), untreated alfalfa hay (□—□), ammonia-treated barley green feed (●—●) and ammonia-treated alfalfa hay (■—■) incubated in situ at different periods of time.



Appendix figure 16: Cellulose disappearance of untreated barley green feed (○—○), untreated alfalfa hay (□—□), ammonia-treated barley green feed (●—●) and ammonia-treated alfalfa hay (■—■) incubated in situ at different periods of time.



Appendix figure 17: Hemi-cellulose disappearance of untreated barley green feed (○—○), untreated alfalfa hay (□—□), ammonia-treated barley green feed (●—●) and ammonia-treated alfalfa hay (■—■) incubated in situ at different periods of time.

Appendix 18. Regression equations derived for each nutrient disappearance.

Nutrient	Feed Type	In $Y = a + bx$	R^2	Pooled EMS	S.E.	Slopes $p < 0.05$
IVDMD	CB	$2.42 + 0.03363X$	0.69 (0.66) ¹	0.10	0.009	a
	NB	$2.78 + 0.0300X$	0.69 (0.66)			
	CH	$3.11 + 0.0184X$	0.34 (0.30)			
	NH	$3.47 + 0.0138X$	0.44 (0.39)			
Nylon bag disappearances						
DMD	CB	$3.22 + 0.0333X$	0.97 (0.97)	0.01	0.007	
	NB	$3.57 + 0.0262$	0.99 (0.99)			
	CH	$3.35 + 0.0359X$	0.79 (0.76)			
	NH	$3.65 + 0.0293X$	0.84 (0.81)			
CP	CB	$4.10 + 0.0149X$	0.93 (0.91)	0.008	0.005	a
	NB	$4.31 + 0.0084X$	0.95 (0.94)			
	CH	$4.00 + 0.024X$	0.72 (0.68)			
	NH	$4.16 + 0.0177X$	0.77 (0.74)			
ADF	CB	$-1.52 + 0.181X$	0.78 (0.75)	0.42	0.04	
	NB	$2.73 + 0.0528X$	0.99 (0.97)			
	CH	$1.52 + 0.1079X$	0.61 (0.54)			
	NH	$2.30 + 0.0744X$	0.76 (0.72)			
NDF	CB	$2.39 + 0.063X$	0.94 (0.93)	0.08	0.02	
	NB	$3.09 + 0.0412X$	0.99 (0.98)			
	CH	$2.52 + 0.0653X$	0.67 (0.62)			
	NH	$2.70 + 0.0618X$	0.81 (0.78)			
Cellulose	CB	$1.37 + 0.1034X$	0.93 (0.92)	0.19	0.03	
	NB	$2.73 + 0.0575X$	0.98 (0.98)			
	CH	$1.59 + 0.1159X$	0.67 (0.62)			
	NH	$2.69 + 0.0654X$	0.84 (0.81)			
Hemi-cellulose	CB	$3.30 + 0.0361X$	0.92 (0.91)	0.02	0.008	
	NB	$3.71 + 0.0252X$	0.99 (0.99)			
	CH	$3.50 + 0.0303X$	0.88 (0.85)			
	NH	$3.51 + 0.0385$	0.72 (0.68)			

¹Adjusted R^2

Appendix 19. Analysis of variance of the 4 x 4 Latin square design of test roughages fed to wethers in trial 1 for nutrient analysis.

Source of Variation	Periods (weeks)	Animals (wethers)	Treatment (roughages)	Error
Degrees of freedom	3	3	3	6
Items	(mean squares)			
CP	0.17	0.52	210.83**	0.73
EE	0.04	0.14	0.11	0.07
GE	0.0004	0.0002	0.05**	0.0006
ADF	5.98	6.32	54.58**	6.72
NDF	7.77	8.15	210.68*	5.90
ADIN	0.006	0.009	0.07	0.02
ADIN (% Total N)	27.56	70.40	526.06**	36.38
Perm. lignin	0.57	1.83	28.37**	0.85
Cellulose	8.41	4.41	35.24*	5.43
Hemi-cellulose	0.28	1.30	198.30**	0.87
Ash	0.24	0.14	1.42**	0.10

*Significant differences at $p < 0.05$.

**Highly significant differences at $p < 0.01$.

Appendix 20. Analysis of variance of the 4 x 4 Latin square design of test roughages fed to wethers in trial 1 for voluntary intake and digestibility.

Source of Variation	Periods (weeks)	Animals (wethers)	Treatment (roughages)	Error
Degrees of freedom	3	3	3	6
Items (mean squares)				
Roughage DM intake (Ad-Libitum):				
daily, g/head/day	32,588.15	320,859.90**	481,210.27**	19,351.62
g/kg W.75	62.19	920.30**	1,716.79**	43.59
% body weight	0.22	1.33**	2.65**	0.07
Digestion trial intake:				
Total DM	246,790.73*	261,472.67*	200,170.30	43,942.40
Roughage DM	246,790.73*	261,472.67*	388,624.43*	43,942.40
Total CP	8,441.61	11,932.61	78,112.44**	3,616.95
Roughage CP	8,440.50	11,932.73	97,014.85**	3,616.77
Digestion coefficients %:				
Total DM	20.17	5.83	101.34**	8.03
Roughage DM	35.09	23.05	84.26*	12.92
Total CP	20.02	13.94	95.76	22.78
Roughage CP	67.79	45.15	655.28*	72.58
Roughage DE K cal/gm	0.07	0.01	0.19*	0.03
GE	34.72	5.91	65.33	14.50
ADF	62.61	2.64	214.83**	8.04
NDF	40.71*	8.17	335.45**	5.26
Cellulose	21.95	9.64	257.62**	14.61
Hemi-cellulose	51.17	42.30	1,045.37**	24.49
Perm. lignin	662.61*	177.10	422.91*	67.26

*Significant differences at $p < 0.05$.

**Highly significant differences at $p < 0.01$.

Appendix 21. Analysis of variance of the 4 x 4 factorial experiment of test roughages used in the in-vitro and in-situ studies.

Source of Variation	Treatment (roughage)	Time (hour)	Treatment x time	Error
Degrees of freedom	3	3	9	16(40) ¹
Items	(mean squares)			
Invitro DMD	649.30**	2,018.16**	114.64	79.18
Nylon bag disappearances:				
DM	424.00**	1,585.57**	25.61**	3.50
CP	266.89**	1,216.88**	59.55**	0.84
ADF	82.45**	2,263.36**	34.17**	8.76
NDF	204.21**	2,242.17**	64.15**	6.67
Cellulose	492.20**	3,053.74**	37.64**	6.84
Hemi-cellulose	299.05**	1,953.91**	45.75**	4.30

¹Error mean square for invitro studies in parenthesis.

**p < 0.01

Appendix 22. Analysis of variance of in-vitro and nylon bag disappearances by time of incubation (hr.)

Source of variation	2	6	12	24	48
Degrees of freedom	3	3	3	3	3
Items	(mean square) ¹				
In-vitro DMD		377.82 (169.58)	337.42* (64.80)	463.98* (26.91)	7.93 (45.84)
Nylon bag disappearances					
DM	1.10 (91.03)	87.15* (9.63)	199.50* (1.24)	123.14* (2.07)	
CP	224.61 (0.45)	86.46* (2.62)	88.35* (0.12)	46.13* (0.18)	
ADF	117.78 (2.03)	121.86 (24.18)	170.76* (3.86)	174.56* (4.97)	
NDF	83.30 (1.57)	48.39 (19.01)	159.68* (2.83)	105.30* (3.28)	
Cellulose	93.45 (1.82)	89.56 (19.71)	252.30* (2.64)	169.82* (3.18)	
Hemi-cellulose	101.24 (0.88)	93.82 (14.00)	149.47* (0.96)	91.76* (1.35)	

¹Figures in parenthesis - Error mean squares

*p < 0.05

Appendix 23. Analysis of variance of in-vitro and nylon bag disappearances by treatment (roughages)

Source of variation	CB	NB	CH	NH
Degrees of freedom	3	3	3	3
Items	(mean square) ¹			
In-vitro DMD	646.18* (10.36)	910.55* (56.92)	375.57 (199.99)	429.77* (49.44)
Nylon bag disappearances				
DM	314.56* (0.60)	333.67* (2.87)	493.68* (0.99)	520.50* (9.54)
CP	212.62* (0.15)	92.80* (0.29)	656.04* (0.40)	434.07* (2.52)
ADF	493.05* (1.30)	571.74* (6.40)	558.52* (1.97)	742.55* (25.38)
NDF	459.52* (0.80)	491.64* (4.17)	733.20* (1.58)	750.26* (20.15)
Cellulose	543.77* (1.38)	712.77* (4.60)	990.07* (1.67)	920.04* (19.71)
Hemi-cellulose	441.61* (0.53)	381.62* (1.96)	424.87* (0.79)	843.06* (13.92)

¹Figures in parenthesis - Error mean squares

*p < 0.05

Appendix 24. Nutrient analysis of test roughages fed to rams in experiment 1 and 2 of trial 2 (% DM basis).

Period/ Square	Feed Type	1st DM	2nd DM	CP	GE K cal/gm	ADF	NDF	Hemi- cellulose		
1	1	NH	92.63	94.19	25.95	4.57	36.35	53.13	16.78	
	2	NH	92.47	94.19	25.95	4.57	36.35	53.13	16.78	
	1	CH	91.78	94.62	16.18	4.39	33.88	45.51	11.63	
	2	CH	91.23	94.62	16.18	4.39	33.88	45.51	11.63	
	1	NB	92.85	93.97	10.11	4.41	55.70	76.96	21.26	
	2	NB	92.39	93.97	10.11	4.41	55.70	76.96	21.26	
	1	CB	92.67	94.06	4.66	4.43	55.54	82.86	22.40	
	2	CB	92.69	94.06	4.66	4.43	55.54	82.86	22.40	
	2	1	CH	89.14	93.94	15.64	4.32	35.28	49.10	13.82
		2	CH	89.14	93.94	15.64	4.32	35.28	49.10	13.82
		1	NH	88.28	93.64	22.63	4.47	37.98	48.93	10.95
		2	NH	88.28	93.64	22.63	4.47	37.97	48.93	10.95
1		CB	88.81	92.74	6.26	4.29	51.68	77.11	25.43	
2		CB	88.81	92.74	6.26	4.29	51.68	77.11	25.43	
1		NB	89.44	93.61	9.41	4.31	56.47	78.31	21.84	
2		NB	89.44	93.61	9.41	4.31	56.47	78.31	21.84	

Appendix 25. Individual ram data on voluntary roughage DM and CP intake in experiments 1 and 2 of trial 2.

Period/ Square	Feed Type	Animal I.D.	DM %	Roughage			N intake ad-libitum			
				DM intake (ad-libitum)			g/head/day	g/kg W.75		
				g/head/day	g/kg W.75	% B.wt.				
1	1	NH	250	87.76	1,719.45	97.31	3.79	64.73	3.66	
	2	NH	218	87.76	1,691.39	97.32	3.76	55.38	3.19	
	1	CH	249	88.36	1,733.84	105.79	4.16	40.33	2.46	
	2	CH	194	88.36	1,583.29	90.16	3.47	35.80	2.04	
	1	NB	181	88.74	946.20	55.92	2.18	10.96	0.65	
	2	NB	162	88.74	786.33	46.09	1.79	9.86	0.58	
	1	CB	158	90.03	643.16	38.86	1.52	4.40	0.27	
	2	CB	148	90.03	589.06	35.44	1.39	4.68	0.28	
	2	1	CH	250	88.65	1,681.83	90.76	3.43	42.76	2.31
		2	CH	218	88.86	1,517.55	82.07	3.10	31.71	1.71
		1	NH	249	89.97	1,541.78	86.96	3.34	44.71	2.52
		2	NH	194	89.66	1,773.67	90.08	3.34	59.25	3.01
1		CB	181	90.18	681.89	40.16	1.56	3.47	0.20	
2		CB	162	89.85	695.39	40.69	1.58	4.42	0.26	
1		NB	158	90.33	789.57	44.97	1.75	9.78	0.58	
2		NB	148	88.64	862.92	49.59	1.91	12.14	0.70	

Appendix 26. Individual ram data on roughage DM consumption and apparent in-vivo nutrient digestibility in experiment 1 (trial 2).

Period/ Square	Feed Type	Animal I.D.	Mean daily DM consumed g/head/day	Digestion coefficients, %							
				DM	DM (AIA)*	CP	GE	ADF	NDF	Hemi- cellulose	
1	1	NH	250	2,013.23	69.52	62.66	76.34	69.24	64.57	72.03	88.13
	2	NH	218	1,598.71	59.87	54.31	69.41	59.41	53.24	62.03	81.23
	1	CH	249	1,707.77	64.99	69.89	74.16	63.79	49.54	51.95	58.98
	2	CH	194	1,567.91	60.41	68.31	71.62	58.88	40.90	44.21	53.79
2	1	CH	250	1,495.09	65.74	60.50	73.51	63.52	52.82	55.44	62.10
	2	CH	218	1,373.41	62.59	69.69	73.82	60.42	47.53	51.02	59.89
	1	NH	249	1,505.61	60.10	64.97	68.96	59.22	48.83	52.52	65.67
	2	NH	194	1,754.72	60.43	54.56	67.45	58.72	49.83	51.56	57.43

*AIA - Acid-Insoluble Ash

Appendix 27. Individual ram data on roughage consumption and apparent in-vivo nutrient digestibility in experiment 2 (trial 2).

Period/ Square	Feed Type	Animal I.D.	Digestibility coefficients										
			Total			Straw							
			DM	CP	GE	DM	CP	GE					
1	1	NB	181	56.19	52.89	54.51	47.45	42.30	45.38				
	2	NB	162	51.76	50.79	50.83	39.39	36.70	38.07				
	1	CB	158	51.25	56.44	50.55	36.22	15.79	35.15				
	2	CB	148	52.24	63.33	51.62	33.98	24.87	32.95				
2	1	CB	181	43.85	33.22	40.63	41.24	24.64	39.76				
	2	CB	162	47.00	51.95	46.74	32.40	21.51	31.52				
	1	NB	158	54.30	49.05	53.22	42.55	32.88	40.80				
	2	NB	148	56.76	57.09	55.46	45.80	35.95	43.76				
				Mean daily roughage DM consumed g/head/day				ADF		NDF		Hemi-cellulose	
1	1	NB	181	940.58			45.32	54.15		77.17			
	2	NB	162	749.02			38.41	44.94		62.10			
	1	CB	158	609.09			35.35	38.89		32.28			
	2	CB	148	488.59			31.35	34.65		26.78			
2	1	CB	181	689.19			37.94	44.52		57.75			
	2	CB	162	693.83			25.90	31.93		44.21			
	1	NB	158	737.93			39.98	50.53		76.79			
	2	NB	148	747.05			43.78	52.78		77.04			

Appendix 28. Analysis of variance of the 2, 2 x 2 switch back design of test hays fed to rams in experiment 1 of trial 2 for voluntary DM and N intake.

Source of variation	Squares	Period (square)	Animal (square)	Treatments (roughages)	Error
Degrees of freedom	1	2	2	1	1
Items	(mean squares)				
Mean daily roughage					
DM intake:					
g/head/day	1,540.40	6,628.88	4,712.86	5,501.48	33,624.73
g/kg W.75	56.13	109.89	2.83	1.04	94.20
% body weight	0.14	0.25	0.01	0.001	0.12
Mean daily roughage nitrogen intake:					
daily, g/head/day	13.50	38.69	70.92	674.73	53.92
g/kg W.75	0.13	0.24	0.13	1.86	0.14

Appendix 29. Analysis of variance of the 2, 2 x 2 switch back design of test hays fed to rams in experiment 1 of trial 2 for DM consumption and nutrient digestibility.

Source of variation	Squares	Period (square)	Animal (square)	Treatments	Error
Degrees of freedom	1	2	2	1	
<hr/>					
Items	(mean squares)				
<hr/>					
Mean daily DM consumed g/head/day	22,785.79	65,039.20	26,230.86	66,264.38	1,155.12
Digestion					
coefficients					
DM (in-vivo)	36.34	10.33	13.26	1.81	0.32
CP	14.23	8.07	7.82	14.99	4.82
GE	42.04	13.33	12.50	.00007	0.67
DM (AIA)	15.54	6.60	17.27	127.12	86.92
ADF	73.57	20.70	57.82	82.43	1.62
NDF	66.82*	33.75	103.45*	157.71*	0.18
Hemi-cellulose	63.51	85.92	193.54	416.16	7.49

*Significant differences at $p < 0.05$

Appendix 30. Analysis of variance of the 2, 2 x 2 switch back design of test straws fed to rams in experiment 2 of trial 2 for voluntary DM and N intake.

Source of variation	Squares	Periods (square)	Animals (square)	Treatments (roughages)	Error
Degrees of freedom	1	2	2	1	1
Items	(mean squares)				
Mean daily roughage					
DM intake:					
daily, g/head/day	1,179.04	6,916.77*	6,458.95*	69,474.00*	31.68
g/kg W.75	8.20	21.21	19.14	214.45*	0.67
% body weight	0.01	0.03	0.03	0.31*	0.002
Mean daily roughage					
nitrogen intake:					
daily, g/head/day	0.76**	1.07**	0.81**	83.00**	0.0001
g/kg W.75	0.002	0.004	0.002	0.28**	0.0001

*Significant differences at $p < 0.05$

**Highly significant at $p < 0.01$

Appendix 31. Analysis of variance of the 2, 2 x 2 switch back design of test straw fed to rams in experiment 2 of trial 2 for total and roughage nutrient digestibility.

Source of variation	Squares	Periods (square)	Animal (square)	Treatments (roughages)	Error
Degrees of freedom	1	2	2	1	1
<hr/>					
Items	(mean squares)				
<hr/>					
Mean daily DM consumed: g/head/day	11,122.86	7,042.15	15,360.44	60,183.68	554.11
Digestion coefficients total:					
DM	0.59	10.80	16.90	76.08	4.67
CP	124.50	94.76	86.02	2.98	48.41
GE	4.12	15.72	20.61	74.91	9.29
Straw:					
DM	31.56	2.92	20.28	122.85	4.91
CP	1.46	2.15	42.59	465.43	8.99
GE	27.34	2.27	16.87	102.46	4.64
ADF	47.78	0.97	21.43	166.99	19.63
NDF	70.75	3.78	24.63	343.35	12.18
Hemi-cellulose	143.31	209.67	84.30	2,180.64*	2.23

*Significant at $p < 0.05$

Appendix 32. Nutrient analysis of hays fed to lactating dairy cows in period 1 and 2 of trial 3 (% DM).

Period	Feed Type	1st DM	2nd DM	CP	GE K cal/gm	ADF	NDF	Hemi- Cellulose
1	CH	89.38	92.63	18.02	4.42	32.64	43.44	10.80
	NH	89.04	92.21	27.58	4.47	32.47	47.07	14.60
2	CH	89.43	95.21	16.48	4.39	30.25	40.62	13.72
	NH	89.04	95.52	25.65	4.38	33.91	47.63	10.37

Appendix 33. Dairy-pelleted supplement.

	% as fed
<hr/>	
Ingredients:	
Distillers corn grain (W.S.)	26.00
Canola meal	48.80
Soybean meal (48%)	10.00
Limestone	5.00
Biophos	1.20
Urea	1.20
Dairy mineral mix ¹	2.40
Dairy vitamin mix	1.20
Salt (cobalt-iodized)	1.00
Magox	0.60
Tallow	2.60

¹See Appendix 34.

Appendix 34. Dairy mineral mix.

		% as fed
Ingredients:		
Copper sulfate	(25% Cu)	0.011
Selenium	(200 mg/kg)	0.280
Zinc oxide	(72% Zn)	0.014
Manganous oxide	(60% Mn)	0.014
Magnesium oxide	(54% Mg)	15.380
Potassium chloride	(50.5% K)	1.000
Salt (cobalt-iodized)	(22% Na, 35% Cl)	43.311
Potassium sulfate	(41% K, 17% S)	40.000

Appendix 35. Individual cow data on voluntary DM intake of roughages fed in periods 1 and 2 of trial 3.

Period/ Square	Feed Type	Animal I.D.	Hay			Grain		Total			
			kg/head/day	g/kg W.75	% B. wt.	kg/head/day	kg/head/day	g/kg W.75	% B. wt.		
1	1	CH	80.45	10.06	21.14	1.59	14.49	24.55	51.59	3.87	
	1	NH	80.32	8.62	13.96	1.05	12.15	20.77	33.63	2.52	
	2	CH	81.30	8.83	16.92	1.27	11.53	20.36	39.00	2.93	
	2	NH	83.36	7.85	20.59	1.54	10.76	18.61	48.82	3.66	
	3	NH	83.04	7.70	18.22	1.37	11.26	18.96	44.86	3.36	
	3	CH	83.42	9.30	24.45	1.83	12.47	21.77	57.22	4.29	
	4	NH	76.16	12.41	27.42	2.06	10.24	22.65	50.04	3.75	
	4	CH	83.28	9.08	22.38	1.68	7.96	17.04	42.00	3.15	
	5	NH	83.33	9.77	20.02	1.63	9.37	19.14	42.72	3.20	
	5	CH	83.35	9.93	23.08	1.73	9.04	18.97	44.08	3.31	
	2	1	NH	80.45	6.36	13.69	1.03	9.24	15.60	33.58	2.52
		1	CH	80.32	5.28	8.75	0.66	6.64	11.92	19.74	1.48
		2	NH	81.30	6.68	12.98	0.97	9.59	16.27	31.62	2.37
		2	CH	83.36	7.77	20.26	1.52	10.70	18.47	48.17	3.61
		3	CH	83.04	8.67	20.59	1.54	11.65	20.32	48.25	3.62
3		NH	83.42	7.57	20.01	1.50	10.77	18.34	48.47	3.64	
4		CH	76.16	12.96	28.15	2.11	10.22	23.18	50.36	3.78	
4		NH	83.28	7.36	17.84	1.34	6.83	14.19	34.40	2.58	
5		CH	83.33	11.78	26.08	1.96	9.93	21.71	48.06	3.60	
5		NH	83.35	8.14	18.88	1.42	8.08	16.22	37.63	2.82	

Appendix 36. Individual cow data on DM consumption and digestibility (AIA)¹ in periods 1 and 2 of trial 3.

Period/ Square	Feed Type	Animal I.D.	1st DM	2nd DM	DM intake kg/head/day			DMD %		
					Hay	Grain	Total			
1	1	CH	80.45	79.43	94.11	9.36	14.29	23.65	79.51	
	1	NH	80.32	86.33	94.11	8.29	12.20	20.49	71.74	
	2	CH	81.30	89.61	95.11	6.25	11.23	17.48	51.59	
	2	NH	83.36	87.86	93.53	4.68	9.79	14.47	87.80	
	3	NH	83.04	88.01	94.06	6.39	10.94	17.33	51.96	
	3	CH	83.42	90.08	95.79	6.67	12.09	18.76	82.28	
	4	NH	76.16	60.48		12.36	9.89	22.25	76.04	
	4	CH	83.28	88.41	95.07	7.66	7.64	15.30	85.82	
	5	NH	83.33	85.49	93.86	7.82	8.06	15.88	77.99	
	5	CH	83.35	88.30	94.92	7.32	9.24	16.56	76.10	
	2	1	NH	80.45	77.09	95.02	3.74	9.86	13.60	90.40
		1	CH	80.32	87.70	96.76	1.64	3.29	4.93	78.26
		2	NH	81.30	85.01	94.67	2.69	9.61	12.30	39.17
		2	CH	83.36	85.66	96.15	5.28	11.04	16.32	86.66
		3	CH	83.04	90.01	96.22	6.34	11.27	17.61	72.07
3		NH	83.42	87.58	94.44	5.01	11.55	16.56	90.88	
4		CH	76.16	83.87	94.96	11.39	8.89	20.28	87.00	
4		NH	83.28	82.74	95.01	4.65	6.96	11.61	70.20	
5		CH	83.33	87.24	96.80	8.65	8.91	17.56	69.42	
5		NH	83.35	86.63	94.61	2.52	8.13	10.65	81.56	

¹Acid-insoluble ash

Appendix 37. Individual cow data on milk yield and composition in period 1 and 2 of trial 3.

Period/ Square	Feed Type	Animal I.D.	Yield, kg/ head/day				Milk composition %				
			Actual Milk	4% FCM	CP	BF	CP	BF	Lactose		
1	1	CH	81.30	25.34	20.64	0.77	0.70	3.04	2.76	4.77	
	1	NH	83.36	25.60	22.24	0.77	0.80	2.99	3.12	4.53	
	2	NH	83.04	27.61	25.44	0.78	0.96	2.82	3.49	4.77	
	2	CH	83.42	31.23	25.39	0.82	0.86	2.65	2.75	5.11	
	3	NH	76.16	26.09	21.54	0.76	0.74	2.92	2.82	4.69	
	3	CH	83.28	21.90	19.56	0.61	0.72	2.79	3.29	4.86	
	4	NH	83.33	19.40	20.51	0.71	0.85	3.65	4.38	4.78	
	4	CH	83.35	21.25	20.50	0.65	0.80	3.08	3.75		
	2	1	NH	81.30	26.58	22.78	0.74	0.81	2.77	3.07	4.92
		1	CH	83.36	25.57	23.58	0.72	0.89	2.81	3.48	4.55
		2	CH	83.04	26.92	26.82	0.80	1.07	2.96	3.99	4.68
		2	NH	83.42	28.54	23.72	0.73	0.82	2.56	2.88	5.01
3		CH	76.16	25.10	21.44	0.75	0.76	2.98	3.01	4.71	
3		NH	83.28	20.67	18.32	0.57	0.67	2.78	3.25	4.93	
4		CH	83.33	20.76	20.00	0.76	0.78	3.67	3.75	4.94	
4		NH	83.35	20.54	19.92	0.64	0.78	3.11	3.81	4.80	

Appendix 38. Individual cow data on ruminal ph and ammonia-N in period 1 and 2 of trial 3.

Period/ Square	Feed Type	Animal I.D.	ph		Ammonia-N mg/l			
			T-0	T-2	T-0	T-2		
1	1	CH	80.45	7.19	6.93	92.43	211.70	
	1	NH	80.32	6.77	6.86	229.19	309.50	
	2	CH	81.30	7.26	6.65	69.36	189.20	
	2	NH	83.36	6.69	6.35	179.76	392.93	
	3	NH	83.04	6.49	6.63	62.47	240.78	
	3	CH	83.42	6.55	5.90	147.10	246.08	
	4	NH	76.16	6.78	7.35	83.29	202.42	
	4	CH	83.28	7.72	6.87	62.57	118.54	
	5	NH	83.33	7.30	6.85	110.25	198.45	
	5	CH	83.35	6.78	6.79	107.26	158.76	
	2	1	NH	80.45	7.19	7.14	125.02	280.53
		1	CH	80.32	7.14	6.71	105.92	531.92
		2	NH	81.30	7.59	7.67	55.69	84.39
		2	CH	83.36	6.99	7.33	91.95	145.66
		3	CH	83.04	7.16	6.79	78.19	248.47
3		NH	83.42	7.51	6.57	61.39	275.60	
4		CH	76.16	6.87	7.06	72.24	148.51	
4		NH	83.28	7.43	6.92	59.93	184.21	
5		CH	83.33	7.01	6.93	178.50	211.34	
5		NH	83.35	7.37	7.45	132.95	185.64	

Appendix 39. Individual cow data on molar concentration and ratio of volatile fatty acids (VFA) in periods 1 and 2 of trial 3 (mmoles/100 ml).

Period/ Square	Feed Type	Animal I.D.	Total VFA		Acetic		Propionic			
			T-0	T-2	T-0	T-2	T-0	T-2		
1	1	CH	80.45	4.89	5.42	3.36	3.24	0.82	1.28	
	1	NH	80.32	8.36	5.27	5.17	3.24	1.38	1.07	
	2	CH	81.30	4.62	7.08	3.23	4.28	0.75	1.58	
	2	NH	83.36	7.06	9.74	4.05	5.69	1.95	2.47	
	3	NH	83.04	6.36	8.00	3.68	5.08	1.40	1.65	
	3	CH	83.42	7.89	8.94	4.22	4.54	2.46	2.85	
	4	NH	76.16	5.60	4.31	3.72	2.98	1.05	0.78	
	4	CH	83.28	2.24	2.61	1.36	1.72	0.50	0.46	
	5	NH	83.33	4.02	6.56	2.83	4.54	0.61	1.10	
	5	CH	83.35	7.00	6.06	4.05	3.96	1.30	0.92	
	2	1	NH	80.45	6.05	6.24	3.71	4.12	1.39	0.98
		1	CH	80.32	5.68	7.85	3.72	4.70	1.00	1.10
		2	NH	81.30	7.25	2.52	4.39	1.55	1.57	0.51
		2	CH	83.36	8.97	5.33	5.58	3.21	2.08	1.17
		3	CH	83.04	7.86	7.37	4.70	4.20	1.81	1.70
3		NH	83.42	9.62	8.77	5.53	5.28	2.62	1.78	
4		CH	76.16	4.50	6.60	3.34	4.22	0.69	1.49	
4		NH	83.28	6.46	6.00	4.27	4.11	1.31	1.07	
5		CH	83.33	6.30	7.50	4.69	4.70	0.97	1.48	
5		NH	83.35	6.02	4.97	3.98	3.13	0.92	0.77	

... continued

Appendix 39 (cont'd)

Period/ Square	Feed Type	Animal I.D.	Butyric		Isobutyric		Valeric			
			T-0	T-2	T-0	T-2	T-0	T-2		
1	1	CH	80.45	0.48	0.67	0.089	0.059	0.053	0.078	
	1	NH	80.32	1.34	0.73	0.140	0.062	0.140	0.078	
	2	CH	81.30	0.43	0.93	0.077	0.083	0.054	0.110	
	2	NH	83.36	0.76	1.14	0.089	0.110	0.100	0.140	
	3	NH	83.04	1.06	1.04	0.054	0.051	0.094	0.099	
	3	CH	83.42	0.83	1.18	0.102	0.086	0.150	0.160	
	4	NH	76.16	0.62	0.40	0.069	0.045	0.049	0.041	
	4	CH	83.28	0.25	0.24	0.041	0.040	0.031	0.045	
	5	NH	83.33	0.43	0.67	0.055	0.078	0.035	0.075	
	5	CH	83.35	1.25	0.89	0.110	0.074	0.130	0.090	
	2	1	NH	80.45	0.71	0.89	0.065	0.063	0.082	0.065
		1	CH	80.32	0.73	1.63	0.058	0.110	0.075	0.110
		2	NH	81.30	0.96	0.33	0.100	0.041	0.110	0.032
		2	CH	83.36	0.94	0.71	0.100	0.066	0.120	0.065
		3	CH	83.04	1.12	1.13	0.057	0.088	0.110	0.110
3		NH	83.42	1.12	1.43	0.079	0.064	0.140	0.110	
4		CH	76.16	0.35	0.60	0.036	0.092	0.036	0.090	
4		NH	83.28	0.61	0.57	0.079	0.072	0.087	0.071	
5		CH	83.33	0.61	0.91	0.069	0.130	0.065	0.110	
5		NH	83.35	0.87	0.83	0.074	0.068	0.076	0.064	

... continued

Appendix 39 (cont'd)

Period/ Square	Feed Type	Animal I.D.	Isovaleric		A:P ratio ¹			
			T-0	T-2	T-0	T-2		
1	1	CH	80.45	0.092	0.090	4.10	2.53	
	1	NH	80.32	0.190	0.098	3.75	3.02	
	2	CH	81.30	0.080	0.093	4.31	2.71	
	2	NH	83.36	0.110	0.190	2.08	2.30	
	3	NH	83.04	0.073	0.081	2.63	3.08	
	3	CH	83.42	0.130	0.120	1.72	1.59	
	4	NH	76.16	0.094	0.062	3.54	3.82	
	4	CH	83.28	0.057	0.100	2.72	3.74	
	5	NH	83.33	0.062	0.100	4.64	4.13	
	5	CH	83.35	0.160	0.130	3.12	4.30	
	2	1	NH	80.45	0.088	0.120	2.67	4.20
		1	CH	80.32	0.110	0.200	3.72	4.27
		2	NH	81.30	0.120	0.052	2.80	3.04
		2	CH	83.36	0.150	0.110	2.68	2.74
		3	CH	83.04	0.063	0.140	2.60	2.47
3		NH	83.42	0.130	0.110	2.11	2.97	
4		CH	76.16	0.051	0.110	4.84	2.83	
4		NH	83.28	0.100	0.110	3.26	3.84	
5		CH	83.33	0.094	0.170	4.63	3.18	
5		NH	83.35	0.100	0.110	4.33	4.06	

¹Acetic:Propionic acid ratio

Appendix 40. Individual cow data on molar percent of volatile fatty acids in periods 1 and 2 of trial 3.

Period/ Square	Feed Type	Animal I.D.	Acetic		Propionic		Isobutyric		
			T-0	T-2	T-0	T-2	T-0	T-2	
1	1	CH	80.45	68.71	59.78	16.77	23.62	1.82	1.09
1	1	NH	80.32	61.84	61.30	16.51	20.30	1.67	1.18
2	1	NH	80.45	61.32	66.03	22.98	15.71	1.07	1.01
2	1	CH	80.32	65.50	59.87	17.61	14.01	1.02	1.40
1	2	CH	81.30	69.91	60.45	16.23	22.32	1.67	1.20
1	2	NH	83.36	57.37	58.42	27.62	25.36	1.26	1.13
2	2	NH	81.30	60.55	68.89	21.66	22.67	1.38	1.82
2	2	CH	83.36	62.21	60.23	23.19	21.95	1.11	1.24
1	3	NH	83.04	57.86	63.50	22.01	20.63	0.85	0.64
1	3	CH	83.42	53.50	50.78	31.18	31.88	1.30	0.96
2	3	CH	83.04	59.80	56.99	23.03	23.07	0.73	1.20
2	3	NH	83.42	57.48	60.21	27.23	20.30	0.82	0.73
1	4	NH	76.16	66.43	69.14	18.75	18.10	1.23	1.04
1	4	CH	83.28	60.71	65.90	22.32	17.62	1.83	1.53
2	4	CH	76.16	74.22	63.94	15.33	22.58	0.80	1.40
2	4	NH	83.28	66.10	68.50	20.28	17.83	1.22	1.20
1	5	NH	83.33	70.40	69.21	15.17	16.77	1.40	1.20
1	5	CH	83.35	57.86	65.35	18.57	15.18	1.57	1.22
2	5	CH	83.33	74.44	62.67	15.40	19.73	1.10	1.73
2	5	NH	83.35	66.11	62.98	15.28	15.50	1.23	1.37

... continued

Appendix 40 (cont'd)

Period/ Square	Feed Type	Animal I.D.	Butyric		Isovaleric		Valeric		
			T-0	T-2	T-0	T-2	T-0	T-2	
1	1	CH	80.45	9.82	12.36	1.88	1.66	1.08	1.44
1	1	NH	80.32	16.03	13.85	2.27	1.86	1.67	1.48
2	1	NH	80.45	11.74	14.26	1.45	1.92	1.36	1.04
2	1	CH	80.32	12.85	20.76	1.94	2.55	1.32	1.40
1	2	CH	81.30	9.31	13.14	1.73	1.31	1.17	1.55
1	2	NH	83.36	10.76	11.70	1.56	1.95	1.42	1.44
2	2	NH	81.30	13.24	5.98	1.66	0.94	1.52	0.58
2	2	CH	83.36	10.48	13.32	1.67	2.06	1.34	1.22
1	3	NH	83.04	16.67	13.00	1.15	1.01	1.48	1.24
1	3	CH	83.42	10.52	13.20	1.65	1.34	1.90	1.80
2	3	CH	83.04	14.25	15.33	0.80	1.90	1.40	1.50
2	3	NH	83.42	11.64	16.31	1.35	1.25	1.46	1.25
1	4	NH	76.16	11.07	9.28	1.68	1.44	0.88	0.95
1	4	CH	83.28	11.16	9.20	2.54	3.83	1.38	1.72
2	4	CH	76.16	7.78	9.09	1.13	1.67	0.80	1.36
2	4	NH	83.28	9.44	9.50	1.55	1.83	1.35	1.18
1	5	NH	83.33	10.70	10.21	1.54	1.52	0.87	1.14
1	5	CH	83.35	17.86	14.70	2.30	2.15	1.86	1.50
2	5	CH	83.33	9.68	12.13	1.50	2.30	1.03	1.47
2	5	NH	83.35	14.45	16.70	1.66	2.21	1.26	1.30

Appendix 41. Individual cow data on blood glucose and urea-N concentration and ratio in period 1 and 2 of trial 3 (mg/100 ml).

Period/ Square	Feed Type	Animal I.D.	Blood Glucose		BUN		BG:BUN ratio ¹			
			T-0	T-2	T-0	T-2	T-0	T-2		
1	1	CH	80.45	66.70	68.30	18.60	22.80	0.28	0.33	
	1	NH	80.32	64.70	72.10	22.35	23.60	0.35	0.33	
	2	CH	81.30	62.80	67.50	16.55	20.10	0.26	0.30	
	2	NH	83.36	64.20	73.60	18.95	24.00	0.30	0.33	
	3	NH	83.04	56.20	73.50	18.00	21.80	0.32	0.30	
	3	CH	83.42	67.90	74.70	19.65	20.15	0.29	0.27	
	4	NH	76.16	62.10	72.30	17.60	21.30	0.28	0.29	
	4	CH	83.28	65.40	71.70	16.65	21.45	0.25	0.30	
	5	NH	83.33	70.10	78.30	15.95	19.45	0.23	0.25	
	5	CH	83.35	69.50	82.70	14.05	16.80	0.20	0.20	
	2	1	NH	80.45	53.80	64.20	16.90	19.75	0.31	0.31
		1	CH	80.32	78.20	80.40	12.35	13.60	0.16	0.17
		2	NH	81.30	56.80	68.10	18.45	19.10	0.32	0.28
		2	CH	83.36	55.60	62.30	18.55	19.75	0.33	0.32
		3	CH	83.04	54.80	68.60	17.10	21.55	0.31	0.31
3		NH	83.42	65.60	72.00	19.45	22.35	0.30	0.31	
4		CH	76.16	64.20	68.90	15.20	17.55	0.24	0.25	
4		NH	83.28	62.40	71.30	16.50	19.35	0.26	0.27	
5		CH	83.33	66.80	74.20	14.90	17.55	0.22	0.24	
5		NH	83.35	61.00	70.00	19.75	23.00	0.32	0.33	

¹Blood glucose: blood urea-N ratio

Appendix 42. Analysis of variance of 5, 2 x 2 switch back design of test hays fed to cows in trial 3 for voluntary roughage DM intake.

Source of Variation	Squares	Periods (square)	Animal (square)	Treatment (roughages)	Error
Degrees of Freedom	4	5	5	1	4
Items	(mean squares)				
Mean daily DM intake					
Hay					
kg/head/day	6.72**	2.83*	4.92*	6.27*	0.38
g/kg W.75	56.92**	10.04*	27.57*	39.73	2.41
% body weight	0.33**	0.06*	0.16**	0.20**	0.01
Grain, kg/head/day	5.28**	6.15**	3.08*	2.01*	0.22
Total:					
kg/head/day	1.73	17.22**	15.09*	15.38*	1.05
g/kg W.75	117.11**	58.25**	126.16**	91.16**	2.92
% body weight	0.66**	0.33**	0.71**	91.16**	0.016

*Significant differences at $p < 0.05$

**Highly significant differences at $p < 0.01$

Appendix 43. Analysis of variance of the 5, 2 x 2 switch back design of test hays fed to cows in trial 3 for DM consumption and digestibility.

Source of Variation	Squares	Periods (square)	Animal (square)	Treatment (roughages)	Error
Degrees of Freedom	4	5	5	1	4
Items	(mean squares)				
Mean daily DM consumed, kg/head/day:					
Hay	10.17	9.69	9.35	7.70	1.63
Grain	7.62*	5.04*	2.34	0.95	0.64
Total	5.68	36.03	21.20	8.86	6.94
Dry matter digestibility, % (AIA)	116.58	66.07	496.50*	61.15	72.89

*Significant differences at $p < 0.05$

Appendix 44. Analysis of variance of the 4, 2 x 2 switch back design of test hays fed to cows in trial 3 for milk yield and composition.

Source of Variation	Squares	Periods (square)	Animal (square)	Treatment (roughages)	Error
Degrees of Freedom	3	4	4	1	3
Items	(mean squares)				
Yield, kg/head/day					
Actual milk	47.24**	1.14*	6.56**	0.58	0.64
4% FCM	23.43	0.95	2.61**	0.75	0.70
Crude protein	0.01*	0.001	0.01*	0.002	0.001
Butterfat	0.03*	0.003	0.01	0.001	0.002
Composition, %					
Crude protein	0.31**	0.01	0.11**	0.01	0.002
Butterfat	0.61*	0.07	0.30	0.0001	0.06
Lactose	0.03*	0.01	0.06**	0.001	0.002

*Significant differences at $p < 0.05$

**Highly significant differences at $p < 0.01$

Appendix 45. Analysis of variance of the 5, 2 x 2 switch back design of test hays fed to cows in trial 3 for ruminal and blood parameters.

Source of Variation		Squares	Periods (square)	Animal (square)	Treatments	Error
Degrees of Freedom		4	5	5	1	4
Items		(mean squares)				
ph	T-0	0.04	0.17	0.20	0.01	0.07
	T-2	0.23**	0.26**	0.11*	0.17*	0.01
Ammonia-N	T-0	3,443.66	1,623.50	2,167.58	447.74	2,514.6
	T-2	18,073.05	10,594.57	9,990.37	1,040.69	3,418.35
Total VFA's	T-0	5.89	2.24	2.35	2.35	2.80
	T-2	5.90*	6.25*	2.60	0.28	0.47
Acetic acid	T-0	1.08	1.17	0.58	0.47	1.01
	T-2	1.26	2.32*	0.83	0.04	0.32
Propionic	T-0	0.99	0.34	0.10	0.17	0.17
	T-2	0.71*	0.27	0.43	0.17	0.06
Butyric	T-0	0.17	0.04	0.11	0.11	.07
	T-2	0.30*	0.14	0.01	0.04	.03
Iso-butyric	T-0	0.001	0.001	0.001	0.0002	0.005
	T-2	0.0003	0.001	0.001	0.002*	0.0002

*Significant differences at $p < 0.05$

**Highly significant differences at $p < 0.01$

... continued

Appendix 45 (cont'd)

Source of Variation		Squares	Periods (square)	Animal (square)	Treatments (roughages)	Error
Degrees of Freedom		4	5	5	1	4
Items		(mean squares)				
Valeric acid	T-0	0.003	0.0005	0.001	0.0004	0.001
	T-2	0.002*	0.002*	0.001	0.002*	0.0002
Isovaleric acid	T-0	0.001	0.001	0.002	0.0003	0.001
	T-2	0.001	0.002	0.002	0.003	0.001
Acetic:propionic acid ratio	T-0	2.00	0.40	0.85	0.35	0.45
	T-2	1.43*	0.60	0.19	0.84	0.15
Blood urea-N (BUN)	T-0	4.23	8.42	1.59	20.60	2.68
	T-2	3.02	12.72	3.08*	25.10	2.10
Blood glucose (BG)	T-0	35.70	18.36	52.56	61.25	32.04
	T-2	36.66	24.33	21.23	0.76	23.76
BG:BUN ratio	T-0	0.003	0.002	0.001	0.01	0.002
	T-2	0.002	0.001	0.003	0.005	0.001

*Significant differences at $p < 0.05$

Appendix 46. Analysis of variance of the 5, 2 x 2 switch back design of test roughages fed to cows in trial for volatile fatty acids molar %.

Source of Variation		Squares	Periods (square)	Animal (square)	Treatments (roughages)	Error
Degrees of Freedom		4	5	5	1	4
Items		(mean squares)				
Acetic acid	T-0	66.90	19.73	39.89	22.85	16.25
	T-2	47.66	11.14	12.03	89.06*	4.92
Propionic acid	T-0	56.68	5.11	23.04	3.09	9.35
	T-2	38.60	16.37	8.18	17.69	10.50
Isobutyric acid	T-0	0.0007	0.0007	0.0005	0.0002	0.0007
	T-2	0.0003	0.0009	0.0005	0.002	0.0002
Butyric acid	T-0	9.02	3.06	13.87	7.25	2.22
	T-2	25.01	7.67	9.10	7.72	4.50
Isovaleric acid	T-0	0.24	0.19	0.22	0.08	0.02
	T-2	0.48	0.27	0.54	1.15*	0.14
Valeric	T-0	0.11	0.03	0.16	0.0001	0.08
	T-2	0.03	0.09	0.05	0.56*	0.02

*Significant differences at $p < 0.05$