STUDIES OF THE EFFECTS OF "MIMOSA BARK EXTRACT" CONTAINING CONDENSED TANNINS ON MILK PRODUCTION BY GRAZING DAIRY COWS AND ON RUMINAL PROTEIN METABOLISM IN SHEEP

A thesis presented in partial fulfilment of the requirements
for the degree of
Master of Agricultural Science in Animal Science
at
Massey University, Palmerston North, New Zealand

M A S H U D I

1996

ABSTRACT

Mashudi, 1996. Studies of the effects of "Mimosa bark extract" containing condensed tannins on milk production by grazing dairy cows and on ruminal protein metabolism in sheep. M.Agr.Sc. Thesis, Massey University, Palmerston North, New Zealand.

Tannins, particularly condensed tannins (CT), either added to the diet or occuring naturally in the forage are advantageous because they protect dietary protein from degradation in the rumen. The aim of this study was to measure the effect of Mimosa bark extract which contained approximately 70% CT on grazing dairy cow performance and on ruminal protein metabolism in sheep.

Two experiments were carried out over the spring season (September and October 1994). In experiment I, effects of CT in Mimosa bark extract upon blood urea concentration, milk yield and milk composition, liveweight and condition score of grazing dairy cows were evaluated. Thirty Friesian cows were allocated at random to 3 treatments :(1) Control : no Mimosa bark extract (no CT); (2) Low CT : 50 g/cow daily of Mimosa bark extract (2.4 g CT/ kg DM eaten); (3) high CT : 100 g/cow daily of Mimosa bark extract (4.8 g CT/kg DM eaten). Mimosa bark extract was given twice daily as a suspension by oral drenching during each milking. In experiment II, effects of CT in Mimosa bark extract on ruminal protein metabolism in sheep were evaluated. Six mature Romney sheep fitted with permanent ruminal cannulae were randomly assigned into 2 treatments in a cross-over design. The two treatments were (1) Control : no Mimosa bark extract (no CT); (2) High CT : 6.66 g/sheep daily of Mimosa bark extract (4.8 g CT/kg DM eaten). Mimosa bark extract was given twice daily as a suspension by oral drenching just after feeding. Dry matter intake, rates of DM disappearance by the *in sacco* method, rumen ammonia and blood urea concentration and apparent digestibility of dry matter and and nitrogen were measured.

In experiment I, liveweight and condition score as well as milk yield and composition, were not influenced by CT. Lactose concentrations were higher in the low CT group

than in the high CT group in all weeks of the experiment. Cows drenched with high CT had a lower ($P<0.05$) blood urea concentration than cows in the control group, and, in week I they were lower ($P<0.05$) than cows in the low CT group. In experiment II, rumen metabolism parameters, including dry matter intake, *in sacco* DM disappearance parameters (A, B, C and A+B) and apparent digestibility of DM and N were not influenced by Mimosa bark extract. However sheep drenched with high CT had lower rumen ammonia and blood urea concentrations ($P<0.05$) than the control in the whole period.

These results indicate that Mimosa bark extract had no significant effect on milk production. However it did consistently and significantly reduce blood urea concentration in both cows (high CT group) and sheep and it reduced rumen ammonia concentration in sheep. This indicates that the CT did have some biological effect in the rumen namely, a reduced protein degradation in the rumen.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to **Dr. I.M. Brookes** and **Associate Professor C.W. Holmes** for their supervision, understanding and kind encouragements throughout my study and especially for their guidance, constructive criticism, patience in interpreting the results and correction of my English throughout the research component of the thesis.

My sincere thanks also go to **Martin** and **Louie**, the staff of the Dairy Cattle Research Unit and **Jeff**, technician of the Animal Science Department, to **Wang Yuxi and Yu feng**, Phd. students for their friendly encouregement and all technical help. The help of **Roel Mulder**, a Dutch student who was involved in the experimental work is also gratefully acknowledged. The advice on condensed tannins and guidance provided by **Professor T.N. Barry** and constructive criticism and guidance by **Dr. G.F. Wilson** are also gratefully acknowledged. Appreciative thanks are also due to **Dr. P.C.H. Morel** and **Dr. Nicholas**, for their advice on statistical analysis.

Thanks also to all the Animal Science Department staff and postgraduate students; **Barbara, Kusmartono, Aderina, Cesar, Hamsun, Chandana, Annor, Min, Francis, Nigel and others**, who have given me help and support and made my life easier and more enjoyable during my study.

Thanks to the New Zealand Official Development Assistance (NZODA) Programme for providing the scholarship which enabled me to undertake this study.

Finally I would like to express my appreciation and gratitude to my parents, **Samud Mahmud and Almarhumah Salmah**, also to my elder sister, **Badriyah** and her husband, **Rupiono** who looked after me since childhood and educated me to the present level with a lot of self denial, and also great thank to my wife; **Chotmaniyah** and my son; **Vahrus Nuril Albi** for their patience, understanding, support and persistent encouragement throughout my study.

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CHAPTER 1
INTRODUCTION

Sheep and dairy cattle farming systems of New Zealand are based on the grazing of fresh temperate forage plants. Using the United States and British feeding standards for calculated protein requirements of dairy cows, the diet of the New Zealand cow grazing pasture, has an excess of rumen degradable protein (Penno and Carruthers 1995). New Zealand fresh forages contain high quantities of crude protein (20-25% CP), about 70-80% of which is degraded in the rumen (Waghorn and Barry, 1987; Penno and Carruthers, 1995) with only 30% escaping to the small intestine for absorption (Waghorn and Barry, 1987).

It is possible that increasing the dietary concentration of rumen undegradable protein could increase ruminant production by increasing the quantity of amino acids that is made available for absorption in the small intestine. Amino acids, especially essential amino acids, are key nutrients which are required by the mammary gland in adequate quantities if milk production and efficiency of feed utilization are to be maximized.

However quantity of amino acids is not the sole factor limiting milk production. The ratio of protein and energy has a significant effect on utilisation of both protein and energy in the rumen (Clark and Davis, 1980) and therefore their ratio also must be considered in establishing requirements for these nutrients.

Attempts that have been made to control the rate and extent of microbial degradation of dietary protein, function primarily to improve the efficiency of utilisation of dietary protein by the ruminants. Tannins, particularly Condensed Tannin (CTs) either added to the diet or occurring naturally in the forage are advantageous because they protect dietary protein from degradation in the rumen. Interest in CTs first developed in New Zealand as a means of reducing forage protein solubility and rumen degradation, and hence improving the efficiency of dietary N utilisation in ruminants fed solely on high quality fresh forage (Barry, 1989).

The objective of this study was to measure the effects of Mimosa bark extract which contain approximately 70% CTs on milk yield and milk composition, liveweight and condition score and blood urea concentration by grazing dairy cows and on dry matter intake, dry matter and nitrogen digestibility, rumen ammonia and blood urea concentration and rumen dry matter disappearance in sheep.

CHAPTER 2
REVIEW OF LITERATURE

2.1. Protein digestion in ruminants

Ruminants depend on the supply of amino acids derived from dietary protein which escapes ruminal degradation, and the microbial protein which is formed as the result of rumen fermentation. A scheme to estimate the flow of available amino acids to the host animal which depends on the properties of the feed and some assumptions has been proposed (Wallace, 1994).

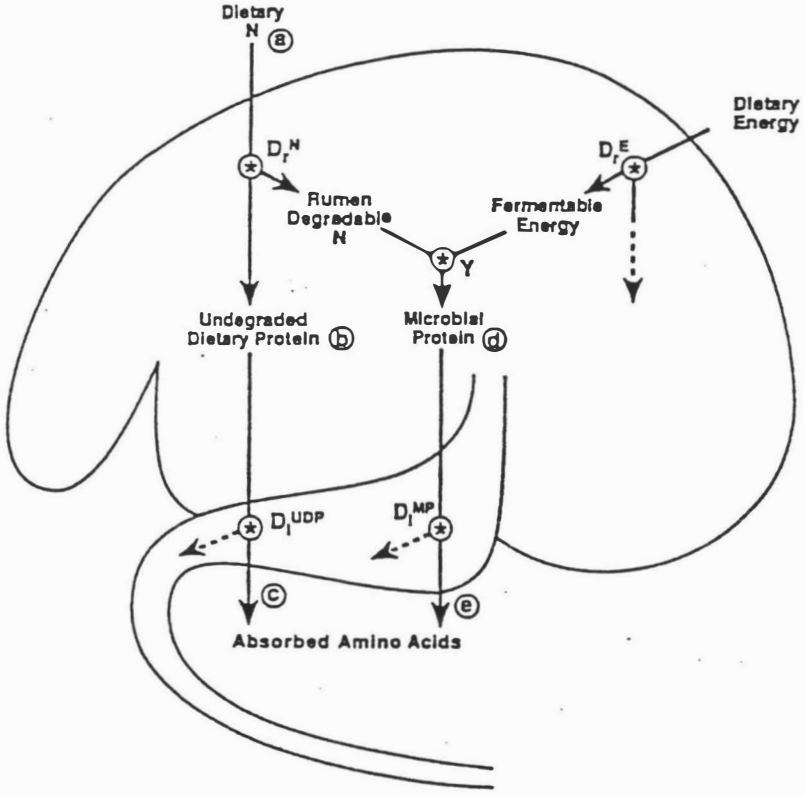


Figure 2.1. Factors influencing amino acid availability in ruminants (Adapted from Wallace, 1994)

Protein and energy degradabilities (DrN and DrE) vary with the rumen outflow rate which also depends on the level of intake (Figure 2.1). They are not constants, but

variables, and a kinetic approach to degradability is necessary for their evaluation. Microbial yield (Y) is affected by outflow rate, as well as the extent of protein recycling within the rumen. Y is also a variable. Any equation relating the content of amino acid in the feed to its availability in the intestine, therefore must to take into account the variability of DrN, DrE, DiUDP (intestinal digestibilities of undegraded dietary protein) and DiMP (microbial protein) as well as Y and the changes in amino acid composition (a to e) at each stage during the digestion process.

Nitrogen metabolism is a complex of N pathways. Those involving the absorption of ammonia and its conversion to urea for either excretion (urine) or recycling, represent a net cost to the animal of the energy required to convert ammonia to urea (Waghorn and Barry, 1987) (see Figure 2.2).

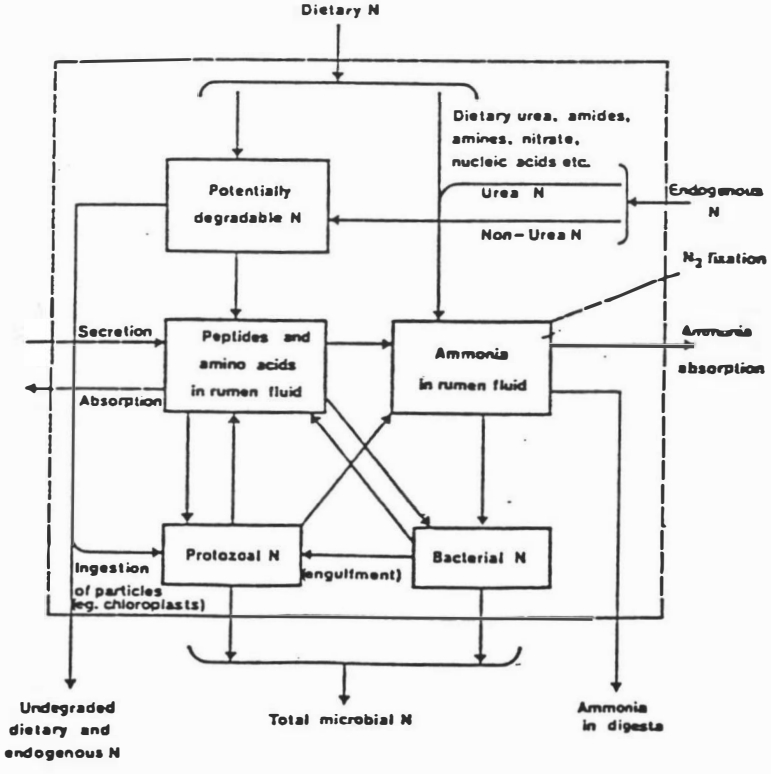


Figure 2.2. A model of the metabolism of nitrogen in the rumen (Adapted from Leng and Nolan,1984)

2.1.1. Protein in forages

The protein ingested by ruminants is largely in the forms of plant protein. True protein makes up about 60-80 of the total plant N, with soluble NPN and a small amount of lignified N making up most of the remainder (Van Soest, 1994).

Plant proteins can be conveniently divided into two major groups, the leaf proteins and the seed proteins, both of which constitute major sources of N in animal diets (Oram and William, 1976 and Van Soest, 1994). The former represent the actively metabolizing matter of the living plant; the latter are reserves.

Most seed proteins contain some fractions include albumins, globulins, prolamines and glutelins. Dicotyledonous sources, especially legumes, tend to contain potentially more soluble globulins and albumins, but these are sensitive to heat denaturation, which will render them water-insoluble. Seed proteins are compounds stored for the infant plant, which will be capable of synthesizing all its organic essentials. Unfavourable amino acid ratios and poor solubility as well as antiproteolytic factors in seeds make them less desirable to animal predators (Van Soest, 1994).

Leaf proteins are higher in quality than seed proteins, although they are diluted with variable amounts of NPN (Van Soest, 1994). Table 2.1 shows the distribution of protein in the leaves typical of temperate forages containing 20-30% of crude protein.

Approximately half of the leaf protein is soluble and located in chloroplast, mitochondria and cytoplasm; up to 50% of the total soluble leaf protein is made up of the enzyme ribulose diphosphate carboxylase or Fraction 1 protein (Reid, 1994). The insoluble protein is associated with membranes.

Table 2.1. Distribution of protein in leaves of temperate forages (Adapted from Brady, 1976)

Intracelluler location		% Total protein
Chloroplast :	- membrane	30 - 35
	- soluble	25 - 35
Mitochondria :	- membrane	3 - 4
	- soluble	2 - 3
Cytoplasm :	- membrane	5 - 15
	- soluble	15 - 20
Nucleus		1 - 2
Cell wall		1 - 2

The amino acids composition of forage proteins is reasonably constant. Analysis of extracted leaf protein showed that lysine was present in relatively high concentrations. Approximately 10 to 35% of the N in forages is present in non-protein compounds, mainly as free amino acid, amines, ureides, nucleotides, peptides, chlorophyl and amino acids in bound non-protein form, with small amount of alkaloids and inorganic N (Reid, 1994).

2.1.2. Protein digestion (degradation) in the ruminant

N consumed by animals originates from crude protein and NPN contained in the diet. NPN entering the rumen can be used directly by microorganisms to synthesise protein in their bodies. However, the amount of crude protein that can be digested in the rumen varies between diets and depends on the degree of their degradability or solubility.

With regard to difference between degradability and solubility, generally more dietary protein passes to the lower gut when solubility of dietary protein is decreased. Preston and Leng (1987) postulated that for practical purposes, the solubility of protein in buffer

solution indicates the degradability of the protein in the rumen. However, low solubility of protein does not guarantee that ruminal degradation will be low. For example, solubility of protein in cotton seed meal is low, but degradability of this protein is high (Clark and Davis, 1980).

Protein degradation in the rumen is the process whereby a dietary protein compound is fermented by proteolytic microorganisms (mostly bacteria and protozoa). In this case bacteria and protozoa produce proteolytic enzymes such as proteases, peptidases and deaminases to ferment protein into amino acids, peptides, and finally into ammonia. About 20-100% of the protein in many diets based on high protein forage, protein meal and grain may be soluble (Preston and Leng, 1987). Variations in the degradation value of fresh forages have been widely studied. For instance, Walker *et al* (1975) working with White clover and perennial Ryegrass found that 50% of the N consumed was degraded in the rumen. Fresh forages contain as much as half of their true protein in a water soluble form most of which is degraded in the rumen. Fresh forages also contain larger cell wall bound protein fractions, and contain moderate levels of tannins, which may promote more efficient nitrogen use (Van Soest, 1994). By-pass protein or undegraded protein may be beneficial, since it will be utilized efficiently in the postruminal digestion as long as it digestible and contains essential amino acids.

According to Robinson *et al.* (1991) protein available for maintenance, growth, and lactation is derived from digestible protein that escapes the forestomachs. Under almost all circumstances, net microbial protein yield is assumed to be insufficient to meet the total metabolizable protein requirements of lactating cows. Thus, some additional dietary protein that escapes degradation in the rumen is necessary to allow dairy cattle to express their full production potential.

In dairy cattle, diets providing at least the calculated requirement for undegradable intake protein (UIP) were associated with highest yields of true protein in milk. Diets grossly excessive or deficient in UIP produced intermediate or lowest yields of true protein in milk (Roseler *et al.*, 1993).

To achieve the greatest flow of amino acids to the small intestine, dietary protein must escape ruminal degradation without decreasing the efficiency of synthesis of microbial protein. The ratio between degradable and non-degradable dietary protein in the rumen is a critical factor affecting the quantity and pattern of amino acid flowing to the small intestine. This ratio must be considered if feed efficiency and milk production are to be maximized (Clark and Davis, 1980).

2.1.2.1. Rumen ammonia

Ammonia (NH_3) is produced in the rumen by degradation of the crude protein entering the rumen in forage and saliva. According to Chalupa (1984), sources of entry into the ammonia pool include components soluble in the rumen liquid phase, peptides and amino acid arising from fermentative hydrolysis of peptide linkages, influx into the rumen via saliva, protozoal excretion of ammonia, and turn over of microbial protein and endogenous protein. Ammonia is lost irreversibly from rumen fluid by incorporation into microbial cells that pass out the rumen, by absorption through the rumen wall, and in fluid passing out of the rumen.

According to Hoover (1986) cited by Minson (1990) maximum microbial growth rate and organic matter digestibility are achieved when the concentration of rumen ammonia is 10 to 60 mg N/l. In other studies, higher requirements of about 70 - 76 mg N/l of rumen fluid have been reported. However, Roffler *et al.* (1976) have reported that concentration of rumen ammonia of 50 mg N/l rumen fluid in steers was sufficient to support maximum growth rates of rumen microbes. They observed that once ammonia began to accumulate at this level, there was a linear relationship between urea intake and ruminal ammonia concentration, and there was no increase in plasma amino acids concentration.

Early work, (Bryant and Robinson, 1962) indicated that about 90% of bacterial species isolated from the rumen could utilize NH_3 as the main source of N for growth. However, further studies have demonstrated a potential for free amino acid and peptides

to become incorporated into microbial protein without passing through the rumen ammonia pool (Mackie and White, 1990).

According to Satter and Roffler (1976), the amount of metabolizable protein (MP) available per unit of crude protein intake is higher when ruminal ammonia is utilized totally for microbial protein synthesis than when NH_3 is in excess (Figure 2.3). Below the point of NH_3 accumulation, all sources of N are approximately equal in providing MP. However, above this point, additional NPN contributes nothing to the amount of MP. Additional true protein in the diet adds to the amount of available protein for absorption to the extent that it escapes degradation in the rumen and is digested in the lower tract.

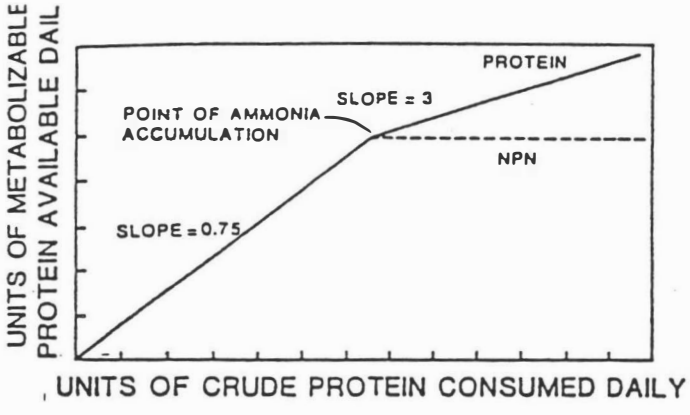


Figure 2.3. Schematic relationship between MP, crude protein, and NPN (Adapted from Satter and Roffler,1976).

Since ammonia N is the end product of dietary protein degraded in the rumen, its concentration in the rumen is often used as an indicator of the extent of protein degradation occurred. A high ammonia concentration is indicative of high levels of degradable protein in the diet. Conversely, a low level of rumen ammonia is found when the quantity of degradable crude protein is low, either because the crude protein has a low degradability coefficient or because the total crude protein concentration is low (Minson, 1990). An experiment conducted by Doyle and McLaren (1988) using sheep

given green-Persian and mature subterranean clover, recorded rumen ammonia concentrations of 113 and 13 mg NH_3 N/l, respectively. This was because the level of degradability of green-persian clover protein was greater than that of mature subterranean clover.

2.1.2.2. Blood urea

As a consequence of the extensive turn over of N-containing compounds in the digestive tract of ruminants, significant ruminal protein degradation can be measured by the concentration of urea in the blood. Blood urea concentration has been suggested to be a realistic predictor of both nitrogen utilisation (Egan and Kellaway, 1971) and nitrogen intake (Nolan et al., 1970). Blood urea N reflects the ratio of dietary crude protein to ruminally fermentable organic matter (Oltner *et al.*, 1985) and post ruminal protein metabolism (Roseler, 1993). Urea N concentration in blood or milk provides a useful tool for assessing protein metabolism in lactating cows (Buttler *et al.*, 1995). Blood urea is also used as an indicator of milk urea concentration due to the free diffusion of urea across the mammary tissues (Gustafsson and Palmquist, 1993).

Preston *et al.* (1965) concluded from their experiment on growing-finishing lambs that increasing protein concentration in the ration markedly increased ($P<0.005$) blood urea concentration. In terms of protein adequacy, a blood urea concentration in excess of 100 mg/l would indicate adequate protein intake.

Blood samples are usually easier to collect than samples of rumen fluid and the use of blood urea to predict ammonia level in the rumen has been suggested (Minson, 1990). The concentrations of blood urea and rumen ammonia are related but the correlation is low, possibly because the concentration of blood urea is also affected by the level of energy in the diet (Topp and Thomson, 1984).

2.1.3. Measurement of protein disappearance by *in sacco* methods

The *in sacco* method measures the disappearance of feed components from a polyester bag containing the test diet after incubation, for a variable period of time, in the rumen of an animal fitted with a rumen cannula. The quantity of dry matter (DM) or of one of its constituents which disappears from the bag is considered to have been degraded in the rumen (Michalet-Doneau and Ould-Bah, 1992).

The artificial fibre bag technique provides a powerful tool for the initial evaluation of feedstuffs and for improving the understanding of the processes of degradation which occur within the rumen (Perdock, 1990). Comparative studies have shown that there is a close relationship between *in sacco* degradability and *in vivo* degradability of DM in all feeds and of N in concentrates.

In sacco techniques have the advantage of giving a very rapid estimate of the rate and extent of the degradation of the feedstuff in the functioning rumen, without necessitating any procedure more complicated than simple weighing. Moreover, this method is inexpensive and reasonably reproducible (Orskov *et al.*, 1980). ARC (1984) proposed that protein requirements of ruminants should be given in terms of rumen degradable (RDP) and undegradable protein (UDP). Results indicated that evaluation of feeds for their RDP and UDP value by *in sacco* have been extensively published. However, it must be pointed out that the technique has three important limitations (Orskov *et al*, 1980). Firstly, the samples are confined within the bag, and are not exposed to any breakdown due to chewing and rumination. Secondly, feed would normally be able to leave the rumen once it had been broken down to a specific particle size. Thirdly, this technique measures the break down of material to a size small enough to leave the bag, and not necessarily the rate of degradation to simple chemical compounds.

Several factors can affect the *in sacco* measurement of degradability. The most important factors are; sample preparation, nylon bag (pore size etc.), sample size, position in the rumen, number of bags per animal, diet of animal, duration of the incubation period, and animal species (Orskov *et al.*, 1980).

The nylon bag technique has been used to determine the value of the percentage DM or N disappearance (Y) at each of a series of incubation times (t) (Orskov and McDonald, 1979). The relationship between Y and t has been found to be exponential and can be described by equations of the form :

$$Y = A + B (1 - e^{-ct})$$

where A, B, and c are constants particular to each protein and type of diet. The A value may be interpreted as a measure of the rapidly-soluble DM and N-fraction. The B value represents that fraction which will degrade in time t, while c represents the rate at which the B fraction degrades. The potential degradability of the samples is given by A + B. It follows that 100 - (A+B) represents the fraction which will appear to be undegradable in the rumen. A small value for c and a large value for B implies a DM or protein source which degrades relatively slowly, but which will be highly degraded if the incubation period is of sufficient duration.

2.1.4. Outflow of dietary and microbial nitrogenous from the rumen

The N-containing compounds entering the small intestine of ruminants comprise dietary proteins which escape degradation in the rumen, microbial proteins produced during fermentation in the rumen and endogenous proteins. The amino acids content of these fractions determines the amounts of amino acids supplied to the intestine and their subsequent digestion and the amount of amino acids absorbed for productive purposes (Hvelplund, 1987).

Preston and Leng (1987) stated that the amount of dietary N leaving the rumen is determined principally by the total N in the diet and the rate of fermentation and residence time in the rumen. The amount of microbial protein synthesised in the rumen has been estimated in various ways with data indicating that 40-80% of ingested protein may be converted to microbial protein (Church, 1979).

According to McRae (1976) proteins that are less extensively degraded may have direct effects on increasing the total supply of amino acids in digesta passing into the small intestine. In ruminants consuming large quantities of fresh forages in which the protein is highly soluble, the extent of microbial degradation of these amino acids into ammonia is likely to greatly exceed the capacity of the microbes to utilise this ammonia for microbial protein synthesis. In this situation a large amount of excess ammonia will be absorbed from the rumen and excreted as urea in the urine.

Beever and Siddon (1986) observed that the amount of Non ammonia-Nitrogen (NAN) entering the small intestine of sheep fed White clover was higher (44 g/kg DM intake) than those fed Ryegrass (30 g/kg DM intake). This is an indication that legumes can be used more efficiently as source of protein, because they contain a smaller proportions of degradable protein than grass.

Waghorn and Barry (1987) stated that plant and microbial amino acids reaching the small intestine are extensively digested (about 90% digestibility) but endogenous secretions and microbial synthesis in the hind gut add amino acid N to the faeces, so apparent amino acid digestion is about 70%. Of the amino acids absorbed, 40-60% is utilised by the small intestine itself and only a small proportion of apparently digested amino acids enter the portal blood, especially with diets low in crude protein.

2.1.5. Absorption of amino acids from the intestine

It is now commonly accepted that ruminants as well as non ruminants should be supplied at the tissue level with sufficient essential amino acids. The inefficient use of amino acids by dairy cows for milk production must reflect inadequate delivery of nutrients for peripheral tissue metabolism or poor retention of synthesized protein in the mammary gland.

Protein digestion is initiated by pepsin in the abomasum and is continued in the intestines by pancreatic proteases. Free amino acids are absorbed from the lumen of the

small intestine. Essential amino acids are apparently absorbed more rapidly than non essential amino acids. Amino acids availability from the small intestine depends not only on the quality and composition of mixed digesta but also on the true absorption coefficient for individual amino acids in the microbial, endogenous and by-pass protein fraction (Nolan, 1981).

Microbial proteins make up the main part of intestinal amino acids. As a result, they have smoothing effects on the variation in the amino acid profiles of the whole duodenal protein. Therefore it has been generally assumed that the duodenal amino acid profiles vary little; thus reflecting the composition of microbial amino acids (Rulquin and Verite, 1993).

However, the similarity between duodenal and microbial amino acid profiles is expected to be closer when microbial protein flow is relatively high. This can be proven from experiments where the composition of intestinal proteins from sheep fed only with NPN was close to the composition of bacterial proteins. Moreover, as the proportion of undegraded feed protein in the diet increases, the duodenal amino acid composition differs increasingly from the microbial amino acid composition. Thus, the composition of absorbable amino acids is also related to the amount and composition of undegraded dietary proteins. This could be of importance in high yielding dairy cows because, owing to energy limitation of microbial protein synthesis, the proportion of undegraded protein increases as the production level increases (Rulquin and Verite, 1993).

Amino acids absorbed from the small intestine consist of essential amino acids (EAA) and non essential amino acids (NEAA). If the ratio of EAA and NEAA is too low , it will limit animal production and performance. The spectrum of amino acids in microbial protein as compared to ruminant tissue suggest that, if microbial protein is not increased by by-pass protein, then methionine, lysine, histidine, threonine and arginine are most likely to limit growth (Chalupa, 1976). When a limiting amino acid is identified, it may be possible to alter the composition of absorbed amino acids or to increase the total supply by dietary supplementation.

2.1.6. Protein protection

The dietary protein can be degraded by attack of microorganisms in the rumen. It is therefore possible to protect them using several procedures such as heat treatment, chemical modification, inhibition of proteolytic activity, and identification of naturally protected protein.

Because of the increase in interest in the protection of protein from rumen degradation, a great deal of effort has been directed towards identifying chemicals or plant secondary compounds which could cause a reduction in the rumen degradation of protein. This can theoretically be achieved by limiting the hydrolysis of peptides, and also by limiting the subsequent deamination of the amino acids. For instance, monensin appears to reduce ammonia concentration, either due to selective inhibition of deamination or to a decrease in rumen proteolytic activity (Broderick *et al.*, 1991). Feed treated with formaldehyde to increase the amount of protein escaping ruminal degradation and therefore the amount reaching the duodenum of ruminants, has increased wool growth (Ferguson, 1975) and weight gains in young ruminants (Tamminga, 1979) compared to untreated proteins. However, in other studies, for example, Broderick and Lane (1978) and Crooker *et al.*(1983), formaldehyde treatment of protein has failed to produce significant improvements of milk yield and milk composition and body weight change in the dairy cow.

Plant secondary compounds such as tannins, either added to the diet or occurring naturally in the forage are often found to be relatively resistant to degradation in the rumen. Tannins appear to cause cross-linkages between protein and other molecules. Using tannin as a deliberate method of protecting protein aims to increase or optimize amino acid supply in the lower digestibility tract and hence ruminant production.

According to Broderick *et al.*(1991) the main requirements for the successful use of tannins are: a) the tannins-protein complexes should be stable in the rumen but not interfere with the digestion of the protein in the lower gut b) metabolism of rumen microorganisms should not be adversely affected and c) that tannins should be harmless

to the animals. If the concentration of tannin in the diet becomes too high, microbial enzyme activities may be depressed (due to the over protection of protein).

According Crooker *et al* (1983) failure to increase productivity through protection of dietary proteins from ruminal degradation could result because : 1) factors other than absorption of essential amino acids are limiting productivity, 2) protected protein is of low biological value, 3) protein is protected inadequately, 4) protein is overprotected, and 5) protected protein is naturally resistant to microbial degradation 6) microbial protein production in the rumen is decreased.

Another factor that can influence the animal production response to protection of dietary protein is the effect of treatment upon the rumen microbial population. If the method of protection decreases microbial protein production in the rumen and thereby decreases the amount of microbial protein reaching the duodenum, then the benefit of the increases in dietary protein that escapes ruminal fermentation will be decreased by the reduced contribution of microbial protein to absorbable amino acids (Crooker *et al.*, 1983).

2.2. Tannins

II.2.1. Chemical structure

Bate Smith and Swain (1962), cited by Jansman (1993) defined tannins as naturally occurring water soluble polyphenolic compounds with a Molecular Weight (MW) of between 500 to 3000 units capable of precipitating alkaloids as well as gelatin and other proteins from aqueous solutions. Tannins are not well defined chemically but rather they are a group of substances with some common properties. Polyphenols, referred to as tannins, have a considerable number of phenolic groups. They are capable of forming effective cross-links with other molecules. According to White (1957) phenolic compounds with a low MW (<500) do not form stable crosslinks with other molecules. On the other hand, compounds with a much higher MW (>3000) do not show tanning properties because they appear to be too large to penetrate into the collagen fibrils in hides.

Horvarth (1981) cited by Reid (1994) defined tannin as any phenolic compound of sufficiently high MW containing sufficient phenolic hydroxyls and other suitable groups to form effectively strong complexes with protein and other macromolecules under the particular environmental conditions being studied.

Tannins have been classified by Kummar and D'Mello (1995) into two groups based on structure and reactivity toward hydrolytic reagents namely the hydrolysable tannins (HTs) and the condensed tannins (CTs). Pathways of tannin synthesis shown in Figure 2.4.

According to Kumar and D'Mello (1995), the HTs consist of a carbohydrate moiety in which hydroxyl groups are esterified to gallic acid or m-digallic acid (gallotannins) or hexahydroxydiphenic acid. These types of tannins are readily hydrolysed by acids, bases or by enzymes, eg. *Penicillium* tannase. Tannic acid is a well known example of this group and contains 8-10 mol of gallic acid/mol glucose.

In contrast the CTs are the most widespread and typical of the plant tannins and consist of an oligomer of the flavan-3-ols (the catechins) and related flavanol residues which typically produce anthocyanidins (eg. cyanidin and pelargonidin) on acid degradation. Thus CTs are also referred to as proanthocyanidins (Mangan, 1988). CTs have no carbohydrate core and are usually derived by condensation of flavonoid precursors (McLeod, 1974), which occur as a range of polymers, an example being procyanidin (Mangan, 1988).

In this work only CT will be discussed. CTs have advantages over HTs because the relationship between pH and protein binding is more favourable, and because CTs are more stable and less toxic than HTs.

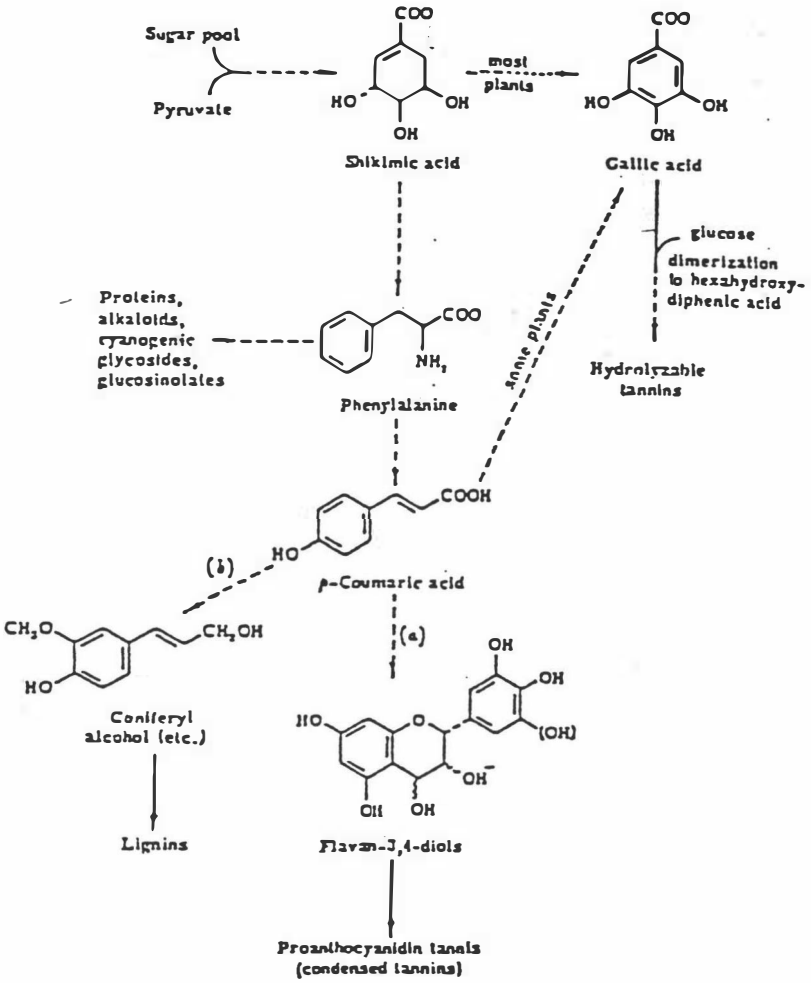


Figure 2.4. Biosynthetic origins of lignins and condensed and hydrolysable tannins in plants (Swain, 1979 cited by Barry, 1989)

2.2.2. Occurrence in plants

Tannins are most widely distributed in forage plants. The nature, content and location of tannins in plants varies considerably among species. Tannins are most commonly found in dicotyledons, particularly in leguminosae. Some legume herbages such as Lespedeza (*Lespedeza Creneata L*), Sainfoin (*Onobrychis viciaefolia Scop*), and Sweet

clover (*Melilotus officinalis L*), are known to contain considerable amounts of hydrolysable and/or condensed tannin (Salunkhe et al, 1990, cited by Jansman, 1993).

CTs have generally been found in the leaves of tree and shrubs, but occur in leaves and stems of only a number of specialized forage plants (Barry, 1989). These include legumes such as sainfoin (MW 17,000-28,000), lotus species (MW 6000-7000) and sulla and grasses such as yorkshire fog. Generally CTs have not been found in the leaves and stems of other grasses and legumes commonly used as temperate forage plants in agricultural systems. They occur in the flower petals of White clover and Red clover. According to Ohara (1994) CTs are present at very high concentrations in the bark of timber species such as conifers, eucalypts and leguminous hardwoods. CTs which have been identified in some of the common forage species are listed in Table 2.2.

Table 2.2. Condensed tannin found in some forages species (Adapted by Kumar and D'Mello, 1995)

Pasture/browse legume	Predominant tannin
Acacia aneura	CT
Acacia cyanophylla	CT
Acacia nilotica (pods)	CT
Acacia sieberiana (pods)	HT
Leucaena leucocephala	CT
Lotus corniculatus	CT
Lespedeza cuneata	CT

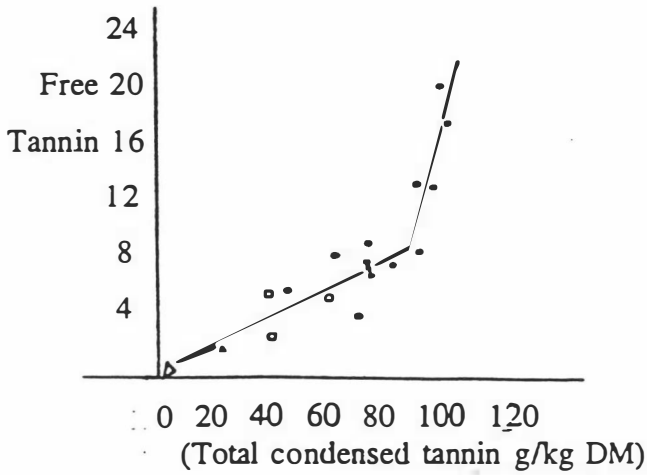
The exact function of tannins in the plant is not clear. McLeod (1974) stated that tannins act as a type of redox buffer in the cell. In contrast, White (1957) stated that true tannins are rarely found in actively metabolising tissues and considered their role to be the protection of vulnerable parts of the plant from microbial attack. Swain (1979) agreed that CTs in plants act as a defence mechanism against attack by pathogenic

bacteria and fungi, relying on the complexing of proteins, polysacharides and nucleic acid by CT. Swain further stated that in the ecological significance of plants, production of CTs acted as a defence mechanism against attack by insects and herbivores, and suggested 20 g CT/kg DM as the minimum plant concentration to deter feeding by both species, due to the astringent taste and properties of CTs.

The concentration of CTs in forages are affected by plant genotype, soil fertility and season. *Lotus pedunculatus* (CV Grassland Maku) has higher CTs concentration than *Lotus corniculatus* (Barry, 1989). Low soil fertility resulted in a marked elevation in CT concentration; with the increase being much greater for *Lotus pedunculatus* than *Lotus corniculatus* (except for cv empire) (Barry, 1989). With regard to seasonal effects, the Sericea species have highest CTs concentration in mid summer (Cope *et al.*, 1971). Seasonal changes may reflect the influences of light intensity and temperature on the occurrence of CTs in the plants. High tannin content may be related to high light intensity, high temperature and severe drought (McLeod, 1974). CTs and lignin are both produced in plants from the shikimic acid biochemical pathway (see Figure 2.4) and share many common intermediates (Barry, 1989).

Barry and Forss (1983) defined free CTs as the proportion of total CTs which exceeded the capacity of the macerated plant tissue to bind it, and Barry (1989) showed that free CT concentration was related to the total plant concentration by the relationship illustrated in Figure 2.5. The ability of macerated plant tissue to bind CTs was saturated at a total concentration of 90 g/kg DM.

Barry and Manley (1984) concluded that free CTs were most likely to have been responsible for depression of voluntary intake. This could be due to their reaction with microbial enzymes in the rumen, the tannin content of the plant exceeding the capacity of the plant protein to bind it, or to reaction with enzymes secreted into the small intestine following the absorption of amino acid. The authors added that free CT could also react with protein of the gut wall.



Lotus pedunculatus (cv. Grasslands Maku) grown under o (high) and • (low) soil fertility conditions; *Lotus corniculatus* cultivars △ (Winnar) ▲ (El Boyero) and ▲ (Granger) grown under low soil fertility conditions.

Figure 2.5. Free condensed tannin concentration as a function of total condensed tannin in macerates of fresh legumes (Adapted from Barry, 1989)

Barry and Manley (1984) investigated the interrelationships between total CT (TCT), free CT (FCT) of lotus on the theory that the reacted CT-protein complex (TCT-FCT) was beneficial in allowing dietary true protein to reach the duodenum undegraded, but that FCT inhibits rumen fermentation of hemicellulose and other desirable fermentation functions.

Linear equations for different populations of *Lotus pedunculatus* and *Lotus corniculatus* are given as follows :

FCT = 0.15+0.099 TCT r=0.856 (TCT up to 90 g/kg DM)

FCT = -61.3+0.73 TCT r=0.797 (TCT above 90 g/kg DM)

Tannins include various chemical entities and there is a diversity of analytical methods, some of which have been used without adequate consideration of their limitations. It must be pointed out that some analytical methods specifically measure CTs, and not such entities as total phenolics or total tannins. Currently the two most common methods used for measuring CT are the Vanillin-HCl procedure (Broadhurst and Jones, 1978) and the Butanol-HCl procedure (Bate - Smith, 1973) as cited by Barry (1989).

2.2.3. Reaction with plant constituents

Tannins can react and form complexes not only with proteins but also with other plant constituents such as carbohydrates and minerals. There are three types of reactions that tannins may undergo, namely, hydrogen bonding which is reversible and dependent on pH, when the pH is less than 8.0; ionic interaction which depends on pH and is reversible and which may weaken or reinforce the hydrogen bonding depending on the conditions; and oxidative coupling which operates under aerobic conditions (McLeod, 1974). Hydrogen bonding has been proposed as the most likely mechanism for forming the linkages and which satisfactorily explains the reversibility and substrate non specificity (McLeod, 1974).

Tannin has been found to have a greater affinity for protein than carbohydrate, which has been attributed to the strong hydrogen bond affinity of the carbonyl oxygen of the peptide group. The large numbers of phenolic groups in the tannin molecule provide many points of attachment with favourable steric opportunities for linkage by hydrogen-bonding with the peptides of adjacent protein chains to form protein-tannin complexes (White, 1957).

According to Kumar and D'Mello (1995), the process of tannin-protein complex formation is normally reversible, and both proteins and tannins can, in principle, be recovered unchanged from the complex. However, if the protein and tannins are brought into contact under some conditions (eg. alkaline, O_2) then polyphenol may become oxidized and the guinones then form covalent linkages with nucleophillic amino acid side chains such as lysine or cysteine of protein, making the association irreversible.

Many factors can affect tannin-protein complexes including the characteristics of the tannins (MW, structural heterogeneity), the protein source (degree of glycosylation, amino acid composition and MW) and reaction conditions (pH, temperature, reaction time, relative concentrations of the reactants) (Hagerman and Butler, 1989 cited by Jansman, 1993). The fixation of tannin by hydrogen binding has been shown to be independent of pH in the range of 2 to 8, but at higher pH the fixation of tannin

declines sharply due to the breaking of hydrogen bonds by the formation of phenolate ions (McLeod, 1974).

Jones and Mangan (1977) stated that the CT-protein complex is stable and insoluble in the pH range 3.5 - 7.0, but that it is unstable and releases protein at pH<3.0 and at pH >8.0. The pH dependence of tannin-protein interactions may be especially important in consideration of the role of tannins in digestibility of nutrients, because the various regions of the gastrointestinal tract have different pH values.

The reaction of tannins with protein depends also on the spatial configuration of the molecules and availability of the reactive phenolic groups. Such spatial relationships contribute to the degree to which different tannins react with protein (Mangan, 1988). White (1957) suggested that the size of the tannin molecule is an important factor affecting its ability to cross-link with proteins. They should be small enough to penetrate into the conformational structure of the molecule but should also posses sufficient reactive groups to form effective cross link with protein molecules. Tannins bind to proteins by the interaction of their reactive hydroxyl groups with the carbonyl groups of proteins. Hydrogen bonds and hydrophobic interactions appear to be the principal linkages involved (Jansman, 1993)

Increase in reactivity (or bonding) also implies a corresponding increase in spatial compatibility between pairs of active sites on the adjacent tannin and protein surfaces. This suggests that the MW as well as the molecular configuration and the number of reactive groups determine the reactivity of the tannin (McLeod, 1974). Astringency, a measure of protein precipitated per unit weight of CT, was less for the high MW CT (17,000-28,000) isolated from sainfoin than for the low MW CT (6,000-7,000) isolated from lotus (Jones, 1976). It seems reasonable to suggest that lotus CT are more effective in causing increased duodenal NAN flow than sainfoin CT. It is apparent that any definition of the ideal concentration of CT in the diet of a high producing ruminant animal will depend upon its MW. With lotus CT, a desired concentration would appear to be 20-40 g/kg DM, while a slightly higher concentration would be expected to be most effective for sainfoin CT (Barry, 1989).

2.2.4. Enzyme and microbial inhibition

Since tannins are able to form complexes with protein, it is not surprising that they also bind to enzymes. Griffiths (1979) reported that activities of trypsin, chymotrypsin and α-amylase *in vitro* assays were reduced after addition of tannin-containing extracts from hulls of coloured flowering varieties of Faba bean. The resistance to enzyme attack of substrate complexed with tannin was illustrated by Feeny (1969) who found that casein was 83% hydrolysed when incubated with mammalian trypsin for 3 hours at pH 7.6 and at 26°C, but that casein complexed with oak-leaf tannin was only 1% hydrolysed. Fahey and Jung (1989) stated that the extent of inhibition of digestive enzymes may depend on several factors such as the amount of dietary protein available, the formation of tannin-protein complexes prior to ingestion, the relative amounts of different enzymes present, the order in which they are encountered and differences in affinities of enzymes for tannins. In addition, species and age of the animal concerned may influence the magnitude of the effect of tannins on the activity of digestive enzymes.

Most organisms, even microorganisms, are sensitive in some degree to the inhibitory effects of tannin, although some can tolerate high concentrations of tannin and even degrade and utilize the carbon of tannin for growth (McLeod, 1974). The degree of inhibition depends on the type of tannin and on the microorganism. The main site of action of tannins on sensitive microorganisms is the cell wall because tannins have been found to produce pleomorphism in microorganisms which increase in size without dividing. Reaction of tannins with cell wall can impair permeability (McLeod, 1974).

There is concern that if tannin concentration in the diet becomes too high, microbial enzyme activities including cellulase and intestinal digestion may be depressed.

2.3. Effect of CT on protein and amino acid digestion

Kinetic studies on the rate of degradation of soluble proteins in the rumen show characteristics of a pseudo-1st-order reaction, each soluble protein having its own rate of degradation (Mangan, 1972). The most abundant protein in nature is fraction 1 (F1) leaf protein and this protein predominates in the dietary intake of herbivores consuming fresh forage. F1 protein degrades rapidly in the rumen unless protected. Figure 2.6 describes the effect of CT on protein protection in the rumen.

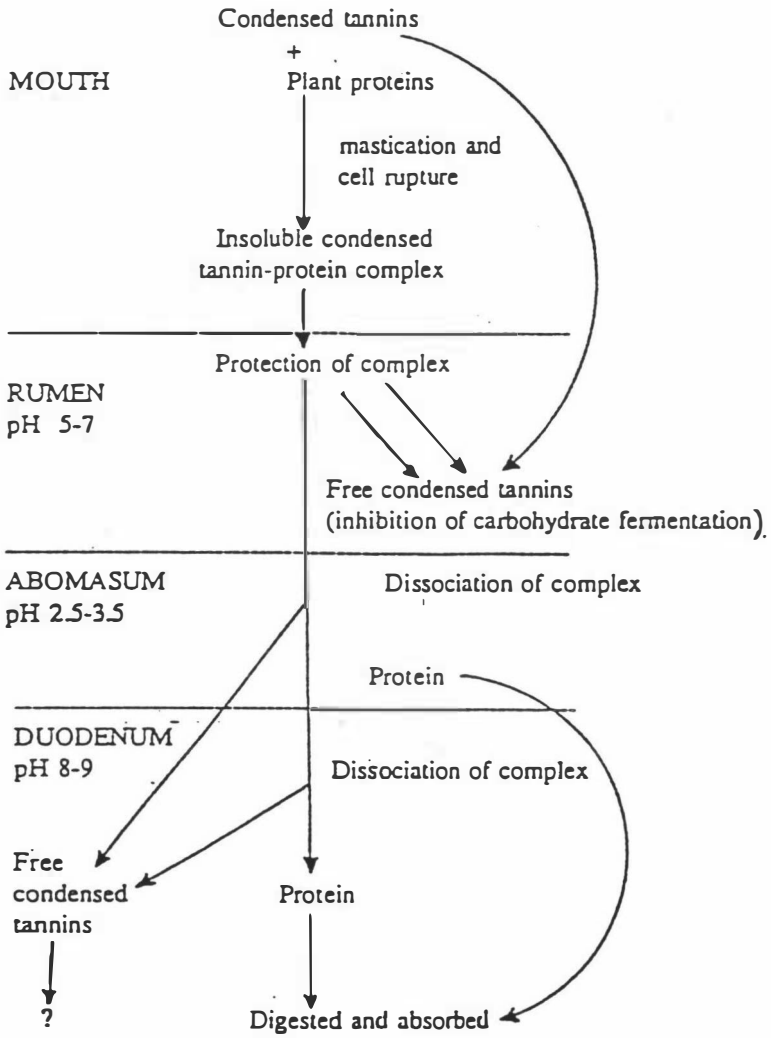


Figure 2.6. Condensed tannins and protein protection in the rumen (after Mangan, 1988). From D'Mello (1992).

Figure 2.6 shows the F1 leaf protein-CT complex is stable in the pH range 4-7 but readily dissociates at pH on either side of this range. Consequently, it has been proposed that the F1 leaf protein-CTs complex escapes degradation in the rumen where pH ranges from 5-7, but dissociates on exposure to gastric (pH 2.5 - 3.5) and pancreatic (ph 8-9) secretions. The clear implication is that CTs protect F1 leaf proteins in the rumen and therefore increase the supply of high quality protein entering the duodenum (Kumar and D'Mello, 1995).

Because CTs bond to proteins more strongly than they do to carbohydrate, it is natural that ruminant nutritionists should be interested in CT principally as a means of reducing rumen degradation of soluble leaf proteins. The presence of CTs uniformly distributed throughout leaf tissue may have potential for protecting forage proteins from rumen degradation, while also allowing release of the protein in the abomasum and small intestine, and hence may increase both duodenal NAN flow and the absorption of amino acid from the small intestine (Barry, 1989).

Ruminants consuming CT containing herbage have more NAN flux to the small intestine than those consuming non CT containing herbage (see Figure 2.7) (Waghorn and Barry, 1987).

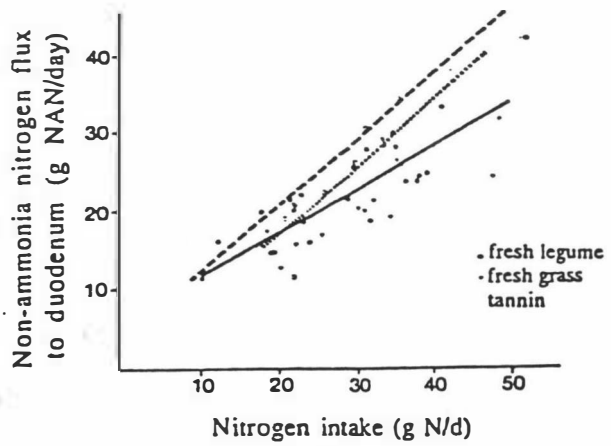


Figure 2.7. Relationship between N intake and NAN passing the duodenum of sheep offered (a) fresh grasses and legumes (-), and dry feeds (--) and tannin containing legumes (...) (source: as cited by Waghorn and Barry, 1987)

2.3.1. The effect of CT on N metabolism in the rumen

As discussed previously, rumen proteolysis is often used as an indicator of the extent of protein degradation in the rumen. The effect of CT on the rumen proteolysis can be seen from studies of Waghorn et al (1994) on effects of CT in *Lotus pedunculatus* (5.5% CT in DM) on its nutritive value for sheep (see Table 2.3). In this experiment sheep were given *Lotus pedunculatus* as the control compared with those given CT inactivated by infusion of PEG (polyethylene glycol). PEG can forms a soluble complex with CT (Jones and Mangan, 1977) and this principle can be used to either prevent protein reacting with CT or to displace protein from a pre-formed CT protein complex (Barry, 1989).

The higher flux of feed NAN to the intestine of the tannin sheep (without affecting microbial NAN flow) (19.2 vs 14.3) and lower rumen ammonia pools (0.98 vs 1.15 g) correspond with a lower rumen degradation of feed protein, compared to the PEG (polyethylene glycol) sheep. They argued that this may be due to the CT-protein binding which is established in the rumen and this can result in a precipitation of soluble proteins. Subsequently, due to the increased flux of feed NAN more feed protein was available for absorption from small intestine, and therefore the CT appeared to overcome one of the major constraints to improved ruminant production from high quality forages, namely the wastage of plant protein through microbial degradation in the rumen. However, the beneficial effects of CT in reducing rumen degradation of feed protein were negated in part by a reduction in the total amount of microbial N reaching small intestine and in the fractional absorption of amino acid from the small intestine. Fractional absorption of EAA was 0.66 in tannin sheep and 0.79 in PEG sheep; corresponding values for NEAA were 0.59 and 0.73. The amino acid concentrations in blood were similar for both groups, but tannin sheep had lower plasma urea concentrations, a more rapid plasma urea turnover rate and a higher IRL (Irreversible Loss) than those receiving PEG. It is clear that with *Lotus pedunculatus* the potential gains from reduced rumen degradation of feed protein are partly negated by low apparent absorption of the extra amino acid from the intestine.

Table 2.3. Nitrogen dynamics (based primarily on 15N enrichment) in sheep fed *Lotus pedunculatus* with and without an intraruminal infusion of polyethylene glycol (PEG).(Adapted from Waghorn *et al*, 1994).

	Tannin group Mean ± SE (n=8)	PEG group Mean ± SE (n=6)
N intake (g/d)	43.6 ± 1.57	47.5 ± 0.9
Rumen ammonia (mg/kg)	283 ± 17	507 ± 23
Rumen pools (g)		
ammonia-N	0.98 ± 0.012	1.15 ± 0.146
Non-microbial NAN	12.5 ± 1.40	8.6 ± 0.86
microbial N	8.4 ± 0.86	6.8 ± 0.72
total NAN	20.9 ± 2.22	15.4 ± 1.54
Abomasal fluxes (g/d)		
ammonia-N	2.9 ± 0.25	4.0 ± 0.23
Non-microbial NAN	19.2 ± 0.99	14.3 ± 1.30
microbial N	14.8 ± 1.03	17.0 ± 0.84
total NAN	34.0 ± 1.91	31.3 ± 0.78
Proportional distribution of NAN		
rumen NAN in microbes	0.40 ± 0.010	0.44 ± 0.012
abomasal NAN in microbes	0.44 ± 0.010	0.54 ± 0.034
plasma urea N from rumen NH3-N	0.47 ± 0.043	0.58 ± 0.066
Rumen microbial N turnover (per d)	1.84 ± 0.201	2.63 ± 0.273
Sources of abomasal microbial N (g/d)		
rumen ammonia-N	6.6 ± 0.70	6.8 ± 0.40
rumen non ammonia-N	8.2 ± 0.79	10.2 ± 0.67
Ammonia-N absorbed from rumen (minimum g/d)	9.6 ± 1.10	14.8 ± 1.28
Urea N from rumen ammonia (g/d)*	24.9 ± 4.45	18.7 ± 3.0

*From urea irreversible loss and the proportion of plasma urea from rumen ammonia.
NAN, non-ammonia nitrogen

It has been observed that due to their reactivity with proteins, increasing dietary reactive CT concentration in the range 0 - 110 g/kg DM linearly increases the duodenal flow of non ammonia nitrogen (NAN) per unit of total N consumed (D) in sheep fed fresh lotus diets (Barry and Blaney, 1987). The equations for primary (1) and secondary (2) growth plant material are as follows :

$$D = 0.79 + 0.0052\ CT \qquad r = 0.99 \qquad (1)$$

$$D = 0.54 + 0.0047\ CT \qquad r = 0.92 \qquad (2)$$

Barry and Blaney (1987) concluded that dietary CT can prevent the large loss of N across the rumen which occurs in sheep and cattle fed fresh diets of Ryegrass and White clover. CT reduces the extent to which proteins are degraded in the rumen so that a higher proportion of forage protein passes to the intestine in the presence of CT than is normally the case. Regression analysis of data summarised from several trials showed the slope of the line relating NAN-N flux to the intestine relative to nitrogen intake was significantly greater for forages containing CT (0.85) than for forages without CT (0.57) (Waghorn *et al.*, 1987). It was also observed that increased passage can result (but not always) in improved amino acid absorption.

2.3.2. The effect of CT on the post ruminal metabolism of N

F1 leaf protein has a high nutritional value and the passage of F1-tannin complexes through the rumen which could be digested readily by the gastric and pancreatic secretions in the small intestine could be of benefit to the animal (Van Soest, 1994).

By growing *Lotus pedunculatus* under conditions of high and low soil fertility, Barry and Manley (1984), were able to induce low (46 g/kg DM) and high (106 /kg DM) concentrations of CT in the herbage. In both the low and high tannin Lotus there were gains of 1.8 and 10.5 g total N/d across the rumen, compared with predicted losses of 3.7 and 2.1 g total N/d for non-tannin species at the same N intakes. The post rumen digestion of NAN was only slightly less, 71 and 67% respectively, than published values for white clover (76%), and NAN apparently digested in duodenum of 0.71 and 0.91 g/g

total N feed intake respectively. This indicates clearly the advantage of the higher tannin content in Lotus. Similarly, Barry et al (1986) reported that post rumen digestibility of NAN in the intestines was greater for sheep fed on low PEG lotus (45 g CT) than for sheep fed in control (14 g CT) and high PEG diets (95 g CT/kg DM). It was concluded that a recommended concentration of CT in *Lotus corniculatus* to improve the efficiency of N digestion is 30-40 g/kg DM.

Concerning post ruminal amino acid digestion, Waghorn and Barry (1987) concluded that tannin containing plants have higher ratios of EAA:NEAA (1.14:1 in *Lotus corniculatus*, 1.10;1 in sainfoin) and very high ratios (1.40:1) were found in abomasal digesta of sheep on high intakes of *Lotus corniculatus* containing 2.2 % CT. This implies that the potential of quantitative absorption of EAA were much higher with CT containing than CT free forages.

Wang *et al.* (1995a) conducted an experiment on sheep held in metabolism cages and given *Lotus corniculatus* containing 27 g extractable CT and 8 g bound CT per kg DM compared with inactive CT (by PEG infusion). They reported that CT in *Lotus corniculatus* significantly reduced the apparent digestibility of nitrogen. As discussed previously, tannins have been reported to inhibit digestive enzyme activities and to increase endogenous N excretion in single stomached animals. The rapidly increasing pH along the small intestine may enable CT-protein complexes to be reformed to protect protein against being hydrolysed by endogenous enzymes or the dissociated CT may bind to gut epithelium and affect amino acid absorption. This phenomenon could reduce the apparent N digestibility in the post-ruminal digestive tract and hence reducing apparent N digestibility (Wang *et al,* 1995a). They suggested that research in this area should be conducted to quantify the effects of low dietary CT levels on post ruminal protein digestion, in particular on endogenous N excretion.

2.3.3. The effect of CT on absorption of amino acids.

McNabb *et al.* (1993) carried out an experiment on the effect of CT in *Lotus pedunculatus* (5.5% CT in DM) on the digestion and metabolism of methionine, cystine and inorganic sulphur in sheep. The results indicate that apparent absorption of methionine from the small intestine was 27% higher in the CT sheep than inactive CT sheep (PEG group) sheep, but both groups had a similar apparent absorption of cystine. They argued that differences in amino acid absorption depend on the relative flows through the duodenum of undegraded protein, microbial protein and endogenous protein, together with their amino acid composition and the apparent absorption of each amino acid. Subsequently they suggested that CT affects the apparent absorption of individual amino acids from the small intestine to different extents. It is therefore essential to understand the mechanism by which CT affects the apparent absorption of amino acid from the small intestine in order to maximize any potential nutritional benefits from including CT in ruminant diets.

McNabb *et al.* (1993) also reported that CT had no effect on plasma methionine IRL, but markedly increased the IRL of cystine (39.8 vs 22.4 umol/min). They concluded that CT reduced the proteolysis of forage protein in the rumen resulting in increased net absorption of methionine and increased utilisation of cystine for body synthesis reactions in sheep with a high capacity wool growth.

Waghorn *et al.* (1987) conducted an experiment on sheep given low CT concentration in *Lotus corniculatus* (as control) compared with inactive CT (achieved by giving PEG to sheep through infusion). The presence of tannin in the control diet increased net apparent absorption of threonine (57%), valine (89%), isoleucine (94%), leucine (30%), tyrosine (41%), phenylalanine (93%), histidine (96%) and lysine (59%) and reduced NEAA absorption by 10% compared with PEG sheep.

According to Waghorn *et al.* (1994) the mechanisms by which CT may affect amino acids absorption include: direct effects on endogenous enzymes by binding with the enzyme and reducing activity, binding with digesta proteins and reducing the ability of

endogenous proteolytic enzymes to cleave off peptides and amino acids, and association with the intestinal mucosa, thus reducing transport and absorption of peptides and amino acids.

In contrast, Waghorn and Shelton (1992) reported that apparent absorption of EAA from the intestine was only 66% (78 g/d) in tannin sheep compared with 78% (83 g/d) in PEG sheep. They suggested that a possible partial inhibition of EAA hydrolysis prior to absorption in sheep where *Lotus pedunculatus* CT had bonded to forage protein. A similar effect was evident with NEAA. It must be remembered that any reduction in apparent absorption has major implications for productivity, because the intestine utilises a considerable quantity of amino acid during absorption, the remainder being available for protein synthesis by other tissues (Tagari and Bergman, 1978).

2.3.4. The effect of CT on production by ruminants

It is well-known that ruminants rarely approach their genetic potential for meat, milk or wool production. The reason normally advanced for this is that the forages which form an important source of nutrients for ruminants vary widely in feeding value. Feeding value which reflects the productivity of the animal is defined as animal response to the total forage consumed and it is a function of intake and nutritive value (Ulyatt, 1973).

FV = Animal Production = VFI * NV

Where :

FV = Feeding Value
VFI = Voluntary Feed Intake
NV = Nutritive Value

In assessing the feeding value of forages, VFI is the main parameter. The failure of animals to attain a high VFI is usually followed by the a decrease in their level of production (Ulyatt, 1973).

Kumar and D'Mello (1995) stated that high levels of tannin may depress the feed intake in two ways. Firstly, they may reduce the rate of digestion of DM in the rumen, react with the outer cellular layer of the gut, and thus diminish the permeability of the gut wall, all of which give signals of physical distension - an important feed back signal in the ruminant for controlling feed intake. Secondly plant tissue may precipitate salivary proteins, causing an unpalatable astringent taste in the mouth.

Barry (1989) also observed, that because of their role in evolution, it would be expected that forages containing high concentration of CT would not be consumed by ruminants in large amounts. For example, Burns et al (1972) as cited by Barry (1989) found that VFI by dairy heifers was lower for the subtropical legume *Lespedenz cuneata* containing 60-120 CT g CT/kg DM than for lucerne (non CT containing).

Barry (1989) reported that high concentrations of CT in *Lotus pedunculatus* depressed ME intake, due to depression in the VFI. Barry and Duncan (1984) reported that high concentrations of CT (50-100 g extractable/kg DM) have depressed VFI. However, the CT containing herbage in a specific range of concentrations have potential benefits. Concentrations of dietary CT below 63 g/kg do not depress DM intake by herbivores. For the lotus species, levels of extractable CT in the range 20-40 g/kg DM are thought to be beneficial for animal production (Barry, 1989).

It was seen in the previous section that low CT levels do not have a significant adverse effect on either VFI or digestibility, but do significantly increase N retention. Therefore the high feeding value of low CT herbage, such as sainfoin or lotus would appear to be due not to the changes in apparent digestibility, but rather to the effect of improving N utilisation and retention which would be expected to improve animal production.

Wang *et al.* (1994) reported that liveweight and wool growth were similar for sheep grazing lucerne (with and without PEG supplementation) and for sheep grazing lotus with PEG supplementation. However, in sheep grazing lotus, the actions of CT increased wool production compared with the PEG group (12.1 vs 10.9 g/d) and slightly increased liveweight gain (203 vs 188 g/d). They argued that CT in *Lotus corniculatus* increased

the amount of cystine available for body synthetic reactions and hence increase wool growth. In a similar experiment, Wang *et al.* (1994) reported that compared to lambs grazing lucerne, lambs grazing lotus had slightly lower VFI, and higher liveweight gain, carcass weight gain, carcass dressing-out percent and wool growth. They concluded that the action of CT in lotus increased wool growth and improved the efficiency of feed utilisation. Lambs grazing lotus had higher rates of carcass gain, higher carcass dressing out percentage and better efficiency of feed utilisation than lambs grazing lucerne.

In addition Wang *et al.* (1995b) reported that the action of CT in *Lotus corniculatus* significantly increased milk production after weeks 5 (Figure 2.8) in ewes rearing twin lambs and increased the secretion rates of milk protein in weeks 8 and 9 and lactose in weeks 6 to 9. However control and PEG ewes produced similar amount of fat in all weeks of the experimental period (Figure 2.9).

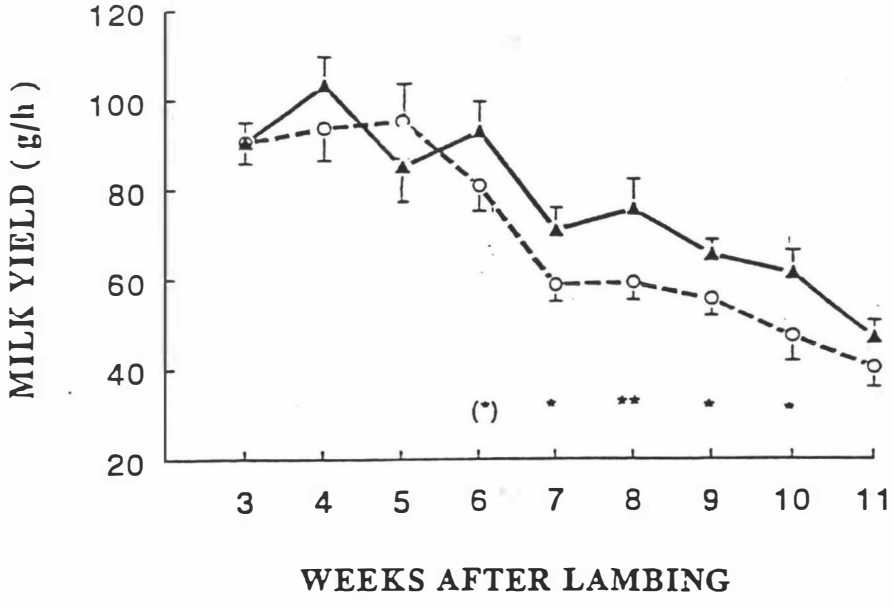


Figure 2.8. Milk yield (g/h) of twin bearing lactating ewes grazing *Lotus corniculatus*. ▲—▲ = control; O—O = PEG ewes (Adapted from Wang *et al.*, 1994).

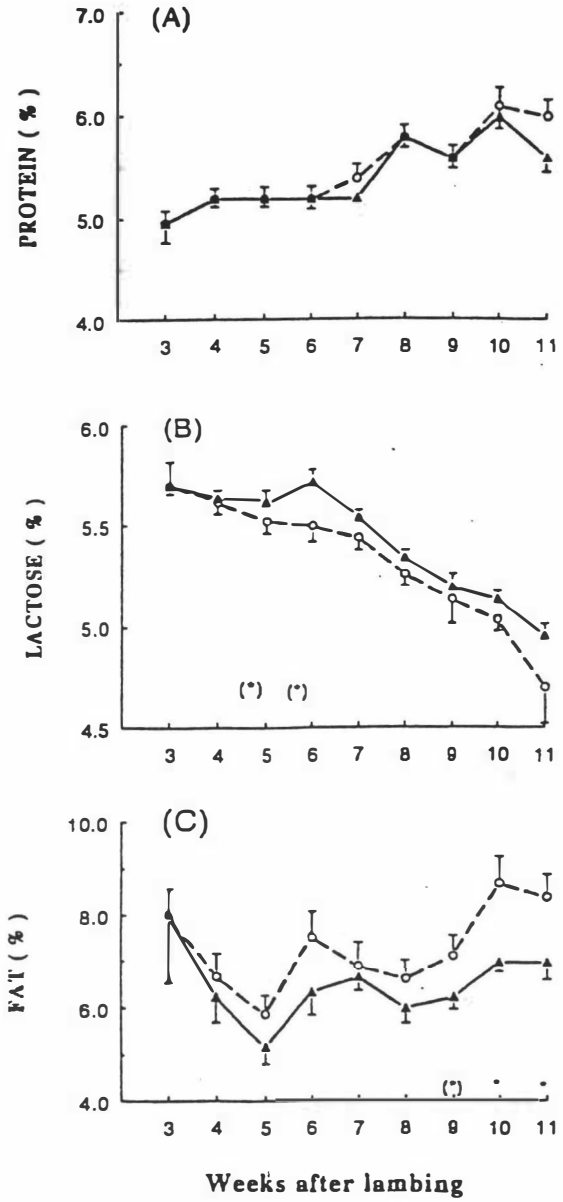


Figure 2.9. Milk protein (A), lactose (B) and fat (C) yield (g/h) of twin bearing lactating ewes grazing *Lotus corniculatus*. ▲—▲ = control; O--O = PEG ewes (Adapted from Wang *et al.*, 1994).

They argued that the effect of CT in increasing milk volume and milk protein yield is thus probably due to its effect in increasing EAA absorption from the small intestine. West *et al.* (1993) carried out an experiment on the effect of dietary peanut skins containing a tannin on cow performance. Increased percentage of peanut skins in the diet increased DMI and milk and FCM yields quadratically and increased both milk and FCM per DMI linearly. Milk fat percentage improved quadratically as peanut skins content of the diet increased.

There is no information on the effect of CT on the performance of grazing dairy cows. However rumen undegradable protein (UDP) supplementation using fish meal slurry drenching twice daily to provide 150 and 300 g UDP has been reported to have no effect on daily milk, fat, protein or lactose yield, or on liveweight of cows grazing temperate spring Ryegrass and White clover pastures in early lactation (Penno and Carruthers, 1995).

It is clear from the foregoing review that the action of CT in the forage (at relatively low levels) can reduce protein degradation in the rumen and provide extra amino acids for absorption, and hence increase ruminant production.

However evidence from long term studies, suggests that the effects of tannin on ruminants production can be variable. Price and Buttler (1980) have suggested that the beneficial protective, and deleterious effects of tannin are nearly equal, the balance favouring one or the other depending upon conditions.

II.4. Scope and objectives of the present study

New Zealand pastures typically contain high quantities of crude protein, most of which is extensively broken down in the rumen with considerable loss of NH_4. Reducing this loss and increasing the proportion of plant protein escaping ruminal degradation, and thus increasing the absorption of amino acids in the small intestine, is one of the principal objectives of ruminant nutrition research in New Zealand. Little information is available about the effects of CTs on milk production of grazing dairy cows. Therefore a series of two experiments related to this subject was conducted.

In the first experiment, effects of CT in Mimosa bark extract upon milk yield and milk composition, live weight, condition score and blood urea concentration of grazing cows were evaluated. In the second experiment, effects of CT in Mimosa extract on ruminal protein metabolism in sheep were also evaluated in terms of pasture intake, DM and N digestibility, rumen ammonia, blood urea and rates of DM disappearance by *in sacco*.

CHAPTER 3
MATERIALS AND METHODS

Experiment 1 : Effects of Mimosa bark extract on milk production by grazing dairy cows.

3.1. Experimental design

3.1.1. Location

The experiment was carried out in September 1994 at the Dairy Cattle Research Unit, Massey University. The farm area was 45 ha divided into 60 paddocks each of approximately 0.75 ha.

The soil type is Tokomaru silt loam, a soil which consists of a 15-30 cm layer of heavy silt above a mottled clay loam. All paddocks have been drained and are topdressed annually with 300 kg potassic superphosphate and 100 kg urea per ha.

3.1.2. Animals and treatment

Thirty Friesian cows (3-9 years old, range of average of milk yield 19.6 - 33.8 kg/cow/day and with the calving date spread from 24/7/1994 to 19/8/1994) were used in the experiment. These cows were assigned to block of 3 according to milk yields and allocated at random to treatments from within blocks.

The Mimosa bark extract was administered at 3 different rates (three treatments) as follows

- Control : 0g/d/cow of Mimosa bark extract to 10 cows
- Low dose : 50g/d/cow of Mimosa bark extract to 10 cows
- High dose : 100g/d/cow of Mimosa bark extract to 10 cows

The low and high doses corresponded to approximately 0.24 and 0.48 g condensed tannin per 100 g DM pasture eaten.

During the experiment these cows were grazed, milked and managed normally with the main herd and the Mimosa extract was given twice daily as a suspension by oral drenching (see detail in section 3.1.4).

Experimental periods;

Preliminary period : (one week; commencing : 5/9/1994)

Milk yield and composition were measured on two days during this week. Blood samples were also taken on one day. These data were used as covariates in the analysis of the effects of treatment on milk production and blood urea concentration measured during the subsequent experimental period.

Experimental period : (three weeks; commencing : 12/9/1994)

Three groups of ten cows (control, low and high dose rate treatments) were managed as part of the main herd. Herbage intake, milk yield, milk fat, milk protein, milk lactose, blood urea, liveweight, and body condition score were measured during this period. Weekly treatment means for daily yields of milk, milk fat, milk protein, milk lactose and the concentration of blood urea were adjusted by covariance analysis using the values measured for each cow during the preliminary period as covariates.

3.1.3. Pasture

The pastures at the Dairy Cattle Research Unit, Massey University consisted principally of a mixture of Ryegrass varieties (*Lolium perenne*) and White clover (*Trifolium repens*) with a small proportion of Cocksfoot (*Dactylis glomerata*), Phalaris (*Phalaris tuberosa*) and Prairie grass (*Bromus catharticus*).

3.1.4. Mimosa bark extract (Condensed tannin source)

The experiment used Mimosa bark extract (powder) as source of condensed tannin in the experiment. This Mimosa was produced by Wattle Bark Industry, South Africa. The full specification presented in Table 3.1.

Table 3.1. Specification of Mimosa bark extract as source of condensed tannin

	SLTC Shake Method (%)	Filter Bell Method (%)
Tannins	67.5	71.5
Non-Tannins	24.8	20.8
Insolubles	0.4	0.4
Water	7.3	7.3

The liquid which was given to the cows by oral drench was made up as follows :
4.2 kg Mimosa bark powder was dissolved in water and made up into 15 litre mixture. Therefore 90 ml mixture = 25 g Mimosa bark powder.

The condensed tannin of Mimosa bark powder was also determined in the Animal Science Nutrition Laboratory Massey University using the Butanol-HCl method (Porter *et al.*, 1986) modified by Terril *et al.* (1992).

3.2. Measurements

3.2.1. Feed measurement

Samples of herbage (plucked to simulate grazing by the cows) offered on each paddock were collected. These samples were then combined weekly into samples I, II and III. The sample from each week was then mixed thoroughly to produce three sub-samples which were stored and freeze dried. The freeze dried sub-samples were ground to pass through a 1 mm sieve and analysed for :

1. Dry matter
2. Nitrogen concentration (Kjeldahl method)
3. *In vitro* digestibility (Roughan and Holland, 1977)
4. Ash concentration (500^0C/24 hrs)

3.2.2. Animal measurements

3.2.2.1. Pasture intake

The average amount of herbage eaten by each cow was estimated as the difference between the pre-grazing herbage mass and the residual herbage mass, multiplied by the area allocated daily and divided by the number of cows grazing during that time.

It was assumed that the pasture dry matter (DM) which disappeared during grazing had been consumed by the cows. No correction was made for growth of pasture during the grazing period, or for material that may have disappeared through other means such as by trampling or decomposition.

Herbage mass before and after grazing were measured by Ellinbank Plate Meter (Earle and Mc Gowan, 1979).

3.2.2.2. Milk production and its constituents

Daily milk yields were recorded as the yield of milk at the evening milking plus the yield at the next morning's milking for each cow.

The milk samples from the two milkings were combined and tested for milk composition (fat, protein and lactose) using Milkoscan 140 A/B (Foss electric, Denmark). Milk yield and composition were measured for each cow on two consecutive days per week.

Milk, milk fat, milk protein and milk lactose (yields and percentage) were calculated for individual cows and the figures averaged during the experimental period to give mean values for daily yields per cow. These data were subject to analysis of covariance using average yields measured during the pre experimental week as covariates.

3.2.2.3. Liveweight and body condition score.

Cows were weighed at the start (the end of preliminary period) and end (third week) of the experimental period immediately after the morning's milking. The liveweight change was defined as the difference in liveweight between the start and the end of the experimental period.

Body condition score for each cow was estimated at the same time as the liveweight. The scoring system used was that reported by Scott *et al.* (1980), with range of 1 - 10. The body condition score change was defined as the difference in body condition score between the start and the end of the experimental period.

Changes in liveweight and body condition score during the experimental period were subject to analysis of variance to test the difference due to treatment.

3.2.2.4. Blood urea

Samples of blood were taken by venipuncture from the the tail of each cow using EDTA vacutainers (Nipro Industries, Japan) during morning's milking. These samples were immediately placed in crushed ice, until the blood plasma could be separated by centrifugation at 3000 rpm for 20 minutes. Plasma was then stored at -20°C for analysis for urea concentration. The plasma samples were determined for plasma urea using Cobas Fara II Autoanalyser belonging to the Department of Animal Science.
The basis of the test is as follows :

$$Urea + 2H_2O \text{ -----urease-------> } NH^{+4} + HCO^{-3}$$

$$NH^{+4} + 2\text{-oxoglutarate} + NADH \text{ <---GIDH---> L-glutamate } + NAD^{+} + H_2O$$

In the presence of urease, glutamate dehydrogenase and NADH, urea reacts to form L-glutamate. The decrease in NADH concentration is proportional to the urea concentration and is determined by monitoring the decrease in absorbance at 340 nm.

3.3. Statistical analysis

All data were analysed using the Statistic Analysis System (SAS) computing package (SAS Institute, 1985).

Data for change in liveweight and body condition were analysed using analysis of variance (Steel and Torrie, 1986) using the following model .

$$y_{ij} = \mu + \alpha_i + e_{ij}$$

Where

y_{ij} = the observation on the j^{th} individual exposed to the i^{th} treatment.
i=1,2,3 ; j=1,2....10.

μ = the unknown population mean.

α_i = the effect of the i^{th} treatment

e_{ij} = the random error associated with the j^{th} individual exposed to the i^{th} treatment. It is assumed that e_{ij} normally distributed with mean 0 and variance σ_e^2

Data for milk yield, milk composition and blood urea were analysed using weekly repeated measures method. These data were subjected to analysis of covariance using the means obtained for each cow during the preliminary period as covariates (Finn, 1974). This method of analysis removed the between cow variation in milk production, milk composition and blood urea during the treatment period that was associated with between cow variation existing before the treatments were applied.

The model is as follows :

$$y_{ijk} = \mu + \alpha_i + w_j + \alpha w_{ij} + \beta x_{ijk} + e_{ijk}$$

Where;

y_{ijk} = the observation on the k^{th} individual exposed to the i^{th} treatment, in the j^{th} week. i=1,2,3 ; j=1,2,3 k=1,2,...10

μ = the overall mean

α_i = the effect of i^{th} treatment

w_j = the effect of the j^{th} week

X_{ijk} = the initial observation on the k_{th} individual in the j^{th} week exposed to the i^{th} treatment.

β = partial regression coefficient associated with x_{ijk}

αw_{ij} = the interaction between the i^{th} treatment and the j^{th} week

e_{ijk} = the random residual effects associated with the k^{th} individual exposed to the i^{th} treatment in the j^{th} week. It is assumed that the e_{ijk} normally distributed with mean 0 and variance σ_e^2 2

Experiment 2 : Effects of Mimosa bark extract on aspects of rumen metabolism in sheep.

3.4. Experimental design

3.4.1. Location

The experiment was conducted in September and October 1994 at the Animal Physiology Unit, Massey University.

3.4.2. Animal and treatments

Six mature Romney sheep (55.9 to 79.5 kg liveweight) fitted with permanent ruminal cannulae were used in the experiment. They were held in the metabolism crates. There was no evidence of lameness or other adverse effects from confinement in crates and all sheep remained in good health throughout the trial. A 7 day period of confinement in the crates allowed animals to adjust indoor conditions before the experimental treatment measurements began.

The six sheep were randomly assigned into 2 treatments in a Cross-over design.
The two treatments were as follows :

1. Control : No Mimosa bark extract to 3 sheep (these sheep received a daily drench of pure water).
2. High dose level : 6.66 g/d/sheep of Mimosa bark extract to 3 sheep. This level of Mimosa bark extract corresponds to the high level used in experiment I, at 0.48 % total dry matter intake.

Mimosa bark extract was given twice daily as a suspension (see section 3.1.4) by oral drenching just after feeding.

The experiment was divided into period I (19 - 28 September 1994) and period II (29 September to 12 October 1994) and based on a Cross-over design. The three sheep drenched Mimosa bark extract in period I were drenched water in period II, whilst those drenched water in period I were drenched Mimosa bark extract in period II. Each period was divided into a preliminary period (5 and 9 days in period I and II respectively) and experimental period (5 days).

3.4.3. Feed

The sheep were fed on freshly cut grass consisting principally of perennial Ryegrass and White clover, similar to the pasture fed in experiment I (grazing dairy cows). The pasture was offered as two equal meals at 08.00 and 15.30 h at maintenance level (approximately 1 kg DM/day). During the experiment water were always available.

3.5. Measurements

3.5.1. Feed

Samples of pasture offered and refused were collected from individual sheep and weighed, dry matter was determined (in triplicate for each sample). About 200 g samples of pasture offered were taken daily, pooled and kept at -20^{0}C during the experiment. Prior to laboratory analysis, sub-samples of pooled pasture offered were freeze-dried and ground to pass 1 mm mesh diameter sieve (Wiley mill, USA). These samples were then subjected to analysis for :

1. Dry matter
2. Nitrogen concentration (Kjeldahl method)
3. *In vitro* digestibility (Roughan and Holland, 1977)
4. Ash concentration (500°C/24h)

3.5.2. Pasture intake

Daily pasture intake was recorded throughout the experiment for each sheep. The weight of fresh feed offered and of feed residues from the previous day were recorded daily for each sheep. Representative samples of the feed offered and the refusal were taken daily and dry matter determined in triplicate. About 100 g of sample were oven dried overnight at 80°C. The difference between the feed DM offered and the residue DM remaining the following day was the daily DM intake of the animal. The data of pasture intake analysed in this experiment were taken between days 13 to 17 (period I) and days 27 to 31 (period II).

3.5.3. Digestibility of pasture

The digestibility measurement was carried out between days 13 to 17 (period I) and days 27 to 31 (period II). During the 5 day digestibility trial (the final 5 days of each period), the weights of oven dried feed offered, feed refusals and faecal outputs were recorded. The faeces from each animal were bulked for each of two 5-day periods and kept at -20^{0}C. At the end of each period, the faeces were allowed to thaw and were mixed thoroughly. Two 100 g subsamples were taken for DM determination in a forced draught oven at 80°C until constant weight had been attained (approximately 72 hours).

The subsamples of faecal output per animal were bulked over the 5-day period and stored at -20^{0}C, and subsequently freeze-dried, ground and stored for Nitrogen analysis.

$$\text{Digestibility} = \frac{\text{DM or N pasture intake for 5 days} - \text{DM or N in faeces for 5 days}}{\text{DM or N pasture intake for 5 days}} * 100\%$$

3.5.4. Rumen ammonia

After adaptation to the treatments (5 and 9 days in period I and II respectively) rumen liquor was collected on day 19 and 20 (period I) and 29 and 30 (period II) from each sheep.

Rumen liquor samples were taken through the rumen fistula using a rumen sampler (a syringe of 50 ml) inserted through the fistula. The samples were taken on day 3 and 4 (two consecutive days) during the digestibility trial at 0 (just before feeding), 2, 4, and 6 hours after the feeding in the morning (at 08.30 am). The samples of rumen liquor (20 ml each) were added to 5 ml of deproteinising reagent (1M sulphuric acid, saturated with magnesium sulphate), centrifuged at 3000 rpm for 20 minutes, and the supernatant was stored at -20^{0}C until rumen ammonia analysis by the Kjeldahl method (Kjeltec Auto 1030 Analyser).

Rumen ammonia was determined by distilling samples which had been made weakly alkaline with sodium tetraborate (pH 9.5) into boric acid (10 g/l) and titrated against 0.02 M-HCl using mixed bromocresol green/methylred indicator (Kjeltec Auto 1030. Analyser, Sweden)

3.5.5. Blood urea

Blood samples for each sheep were collected at the same time as the rumen liquor, to determine the urea concentration in the blood. Samples of blood were taken by venipuncture from the external jugular vein of each sheep using EDTA vacutainers (Nipro Industries, Japan). These samples were immediately placed in crushed ice, until the blood plasma could be separated by centrifugation at 3000 rpm for 20 minutes. Plasma was then stored at -20^{0}C prior to analysis for urea concentration.

3.5.6. Measurement of rates of disappearance (*in sacco*)

In this experiment, the progressive breakdown of dietary DM of pasture was measured using the polyester bag method of Mehrez and Orskov (1977). Small samples of 'minced' (fresh) pasture, contained in small porous polyester bags, were inserted into rumen. These were removed at 1, 2, 4, 8 and 24 hours after feeding, on two consecutive sampling days.

30 polyester bags (Estal-mono, 7*14 cm, 47 μm pore size, Swiss Screens, Sydney, Australia) each containing a marble (approximately 5 g) to ensure that the bags would not float in the rumen, plus aproximately 6.5 g of the 'minced' pasture, were lowered into the rumen of each sheep. Bags were removed from the rumen after 1, 2, 4, 8 and 24 hours incubation and were rinsed with tap water until the rinse fluid was clear. Bags with their contents were then dried in a forced-draught oven at 60^0C for 48 h, cooled in a dessicator and weighed. Loss in dry weight was reported as DM breakdown in the rumen.

Dry matter disappearence in the rumen due to simple solubilization was estimated by rinsing with tap water until the rinse fluid was clear. This represented initial solubility.

Dry matter disappearance and disappearance rate in the rumen was calculated using the following equation (Orskov and McDonald, 1979).

$$Y = A + B\,(1 - e^{-ct})$$

Where;

Y = Percent DM disappearance at time (t) in hours spent in the rumen

A = Instantly soluble fraction

B = Proportional disapperance in time t

C = Disappearance rate of the 'B' fraction

The constants A, B, C, and (A+B) for each animal were calculated by using NLIN (non-linear regression) procedures (SAS, 1985).

3.6. Statistical analysis

The data were analysed using the General Linear Model (GLM) procedure (SAS, 1985), for a cross-over design. Effects analysed for dry matter intake and apparent digestibility of dry matter and nitrogen were treatments, periods and interaction between treatment and period. The effects analysed for rumen ammonia and blood urea concentration were treatments, time and interaction between treatment and time. Generalized Linear Model (GLM) procedures with repeated measurements through time was used to test the differences between the effects mentioned above.

In the *in sacco* experiment, the significance of differences between means for A, B, A+B, and C was also established using the GLM procedures with repeated measurements through time with the factors examined being treatments, periods, and interaction between treatments and period.

CHAPTER 4
RESULTS

Effects on milk production by grazing cows (Experiment 1)

4.1. Chemical analysis of the pastures and Mimosa bark extract

The chemical analysis of the pastures in each week, and of the Mimosa bark extract used in the experiment are presented in Table 4.1.

Table 4.1.a. Data for the chemical analysis of the pastures (percentage of dry matter; N = nitrogen; DMD = dry matter digestibility; OMD = organic matter digestibility; DOMD = organic matter digestibility of dry matter).

			In vitro		
	Ash (%)	N (%)	DMD (%)	OMD (%)	DOMD (%)
Pastures					
Week I	11.54	3.62	82.50	75.69	85.98
Week II	9.95	2.84	77.02	75.44	84.41
Week III	13.20	3.94	81.80	74.67	86.30

Table 4.1.b. Data for the chemical analysis of the Mimosa bark extract, DM = dry matter; CT = condensed tannin

DM (%)	Extractable tannins (%)	Protein -bound (%)	Fibre-bound (%)	Total CT (%)
88.47	50.69	ND	ND	50.69

ND = Not detected

4.2. Apparent dry matter eaten

The mean values for the amounts of dry matter herbage apparently eaten by the group of cows, measured by the Plate Meter method in each week, are presented in Table 4.2. as kg DM per cow.

Table 4.2. Mean values (±sem) for the amounts of dry matter herbage apparently eaten by each cow in each week.

Period	Dry matter eaten (kg DM/cow/day)
Week I	17.0 (±3.7)
Week II	20.2 (±4.9)
Week III	16.1 (±4.9)

4.3. Blood urea concentration

Mean values for blood urea concentration for the three treatments are given in Table 4.3. Cows in the high CT treatment had significantly ($P<0.05$) lower blood urea concentrations than the control groups. In weeks II and III, values for the higher level were lower than for the control group, and in week I values for the higher level were lower than for the lower level.

Table 4.3. Mean values (adjusted by covariance* ± sem) for concentration of blood urea (uM/ml) for the three treatments; control (no CT) and the low and high levels of Mimosa bark (low and high CT).

Mean values for	Control	Low CT[1]	High CT[2]	Level of significance for difference between treatment
Initial	10.47 (±0.39)	10.47 (±0.41)	9.95 (±0.39)	NS
Week I	10.64^{ab}(±0.28)	11.20^{b} (±0.30)	10.13^{a}(±0.28)	*
Week II	11.36^{b} (±0.38)	11.24^{ab}(±0.40)	10.17^{a}(±0.38)	*
Week III	10.25^{b} (±0.22)	10.32^{b} (±0.23)	9.49^{a}(±0.22)	*

** = Significant (P<0.01)
* = Significant (P<0.05)
NS = Not Significant
a, b = Values in the same line but with different superscript letters (a or b) are significantly different from each other (P<0.05)
* = Values for the initial week are unadjusted means
1 = Corresponded to 50 g/c/d of Mimosa bark (= 0.24% CT daily DM eaten)
2 = Corresponded to 100 g/c/d of Mimosa bark (= 0.48% CT daily DM eaten)

4.4. Yields of milk, milk fat, milk protein and milk lactose

Mean values for yields of milk, milk fat, milk protein and milk lactose for the three treatments are presented in Table 4.4. and described in Figures 4.1 and 4.2. There were no significant (P>0.05) differences between treatment groups in any of the yields. There were also no significant (P>0.05) differences between weeks for the individual treatments.

Table 4.4. Mean values (adjusted by covariance[+]) (± sem) for yields of milk, milk fat, milk protein and milk lactose (kg/cow/day) for the three treatments; control (no CT) and the low and high levels of Mimosa bark (low CT and high CT).

Mean values for yields	Control	Low CT[1]	High CT[2]	Level of significance for difference between treatment
Milk				
Initial	27.65[a](±0.82)	26.31[ab](±0.82)	25.12[b](±0.82)	*
Week I	26.24 (±0.58)	25.76 (±0.57)	26.43 (±0.58)	NS
Week II	24.82 (±0.58)	25.62 (±0.57)	25.11 (±0.58)	NS
Week III	25.10 (±0.64)	24.67 (±0.62)	25.81 (±0.63)	NS
Fat				
Initial	1.19[a](±0.04)	1.15[ab](±0.04)	1.04[b](±0.04)	*
Week I	1.08 (±0.03)	1.09 (±0.03)	1.10 (±0.03)	NS
Week II	1.09 (±0.05)	1.13 (±0.05)	1.05 (±0.05)	NS
Week III	1.15 (±0.05)	1.11 (±0.05)	1.12 (±0.05)	NS
Protein				
Initial	0.91 (±0.03)	0.90 (±0.03)	0.84 (±0.03)	NS
Week I	0.86 (±0.02)	0.86 (±0.02)	0.87 (±0.02)	NS
Week II	0.81 (±0.02)	0.85 (±0.02)	0.83 (±0.02)	NS
Week III	0.84 (±0.02)	0.84 (±0.02)	0.88 (±0.02)	NS
Lactose				
Initial	1.38[a](±0.04)	1.35[ab](±0.04)	1.24[b](±0.04)	*
Week I	1.30 (±0.03)	1.28 (±0.03)	1.30 (±0.03)	NS
Week II	1.24 (±0.03)	1.28 (±0.03)	1.23 (±0.03)	NS
Week III	1.24 (±0.03)	1.23 (±0.03)	1.27 (±0.03)	NS

*, NS, [+], a or b, [1] and [2] refer to the foot of Table 4.3.

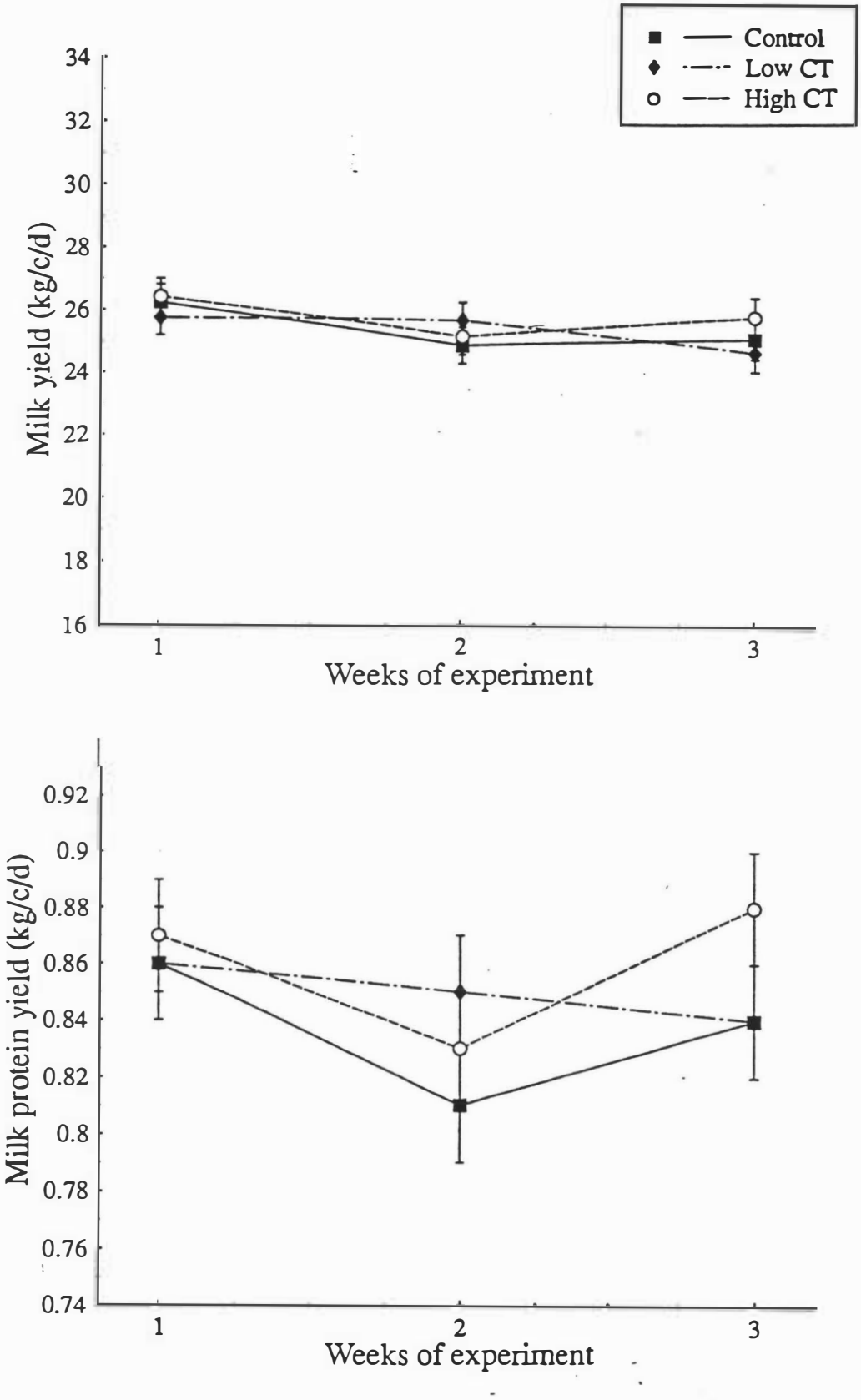


Figure 4.1. Milk (A) and protein (B) yield (kg/cow/day) of lactating dairy cows grazing Ryegrass and White clover pastures.

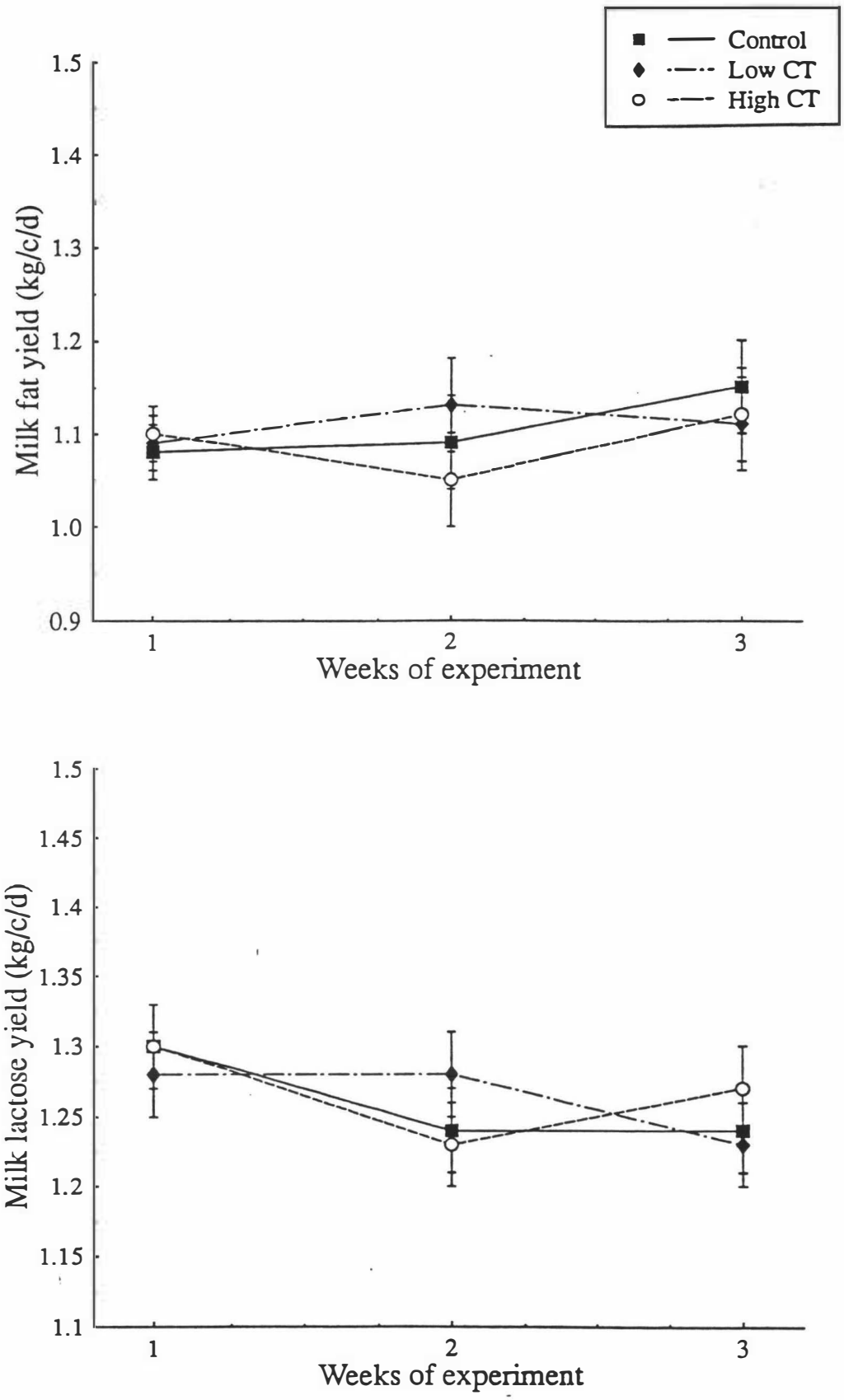


Figure 4.2. Fat (C) and lactose (D) yield (kg/cow/day) of lactating dairy cows grazing Ryegrass and White clover pastures.

4.5. Milk composition

Mean values for the concentrations of milk fat, milk protein, and milk lactose for the three treatments are presented in Table 4.5. There were few significant differences between treatment groups although protein concentrations were lower in the control group than in the low CT groups in week I, and lactose concentrations were higher in the low CT group than in the high CT group in weeks I, II and III.

Table 4.5. Mean values (adjusted by covariance^{+} ± sem) for concentrations of milk fat, milk protein and milk lactose (%) for the three treatments; control (no CT) and the low and high levels of Mimosa bark (low CT and high CT).

Mean values for	Control	Low CT[1]	High CT[2]	Level of significance for difference between treatment
Fat (%)				
Initial	4.32 (±0.14)	4.40 (±0.14)	4.15 (±0.14)	NS
Week I	4.11 (±0.09)	4.27 (±0.09)	4.20 (±0.09)	NS
Week II	4.28 (±0.13)	4.46 (±0.13)	4.25 (±0.13)	NS
Week III	4.49 (±0.12)	4.50 (±0.12)	4.42 (±0.12)	NS
Protein (%)				
Initial	3.30^{a}(±0.04)	3.43^{b}(±0.04)	3.36^{ab}(±0.04)	*
Week I	3.28^{a}(±0.02)	3.34^{b}(±0.02)	3.33^{ab}(±0.02)	*
Week II	3.27 (±0.03)	3.32 (±0.03)	3.30 (±0.02)	NS
Week III	3.37 (±0.02)	3.40 (±0.03)	3.41 (±0.02)	NS
Lactose (%)				
Initial	5.00^{ab}(±0.04)	5.11^{a}(±0.04)	4.94^{b} (±0.04)	*
Week I	4.95^{ab}(±0.03)	5.01^{a}(±0.03)	4.91^{b} (±0.03)	*
Week II	4.97^{ab}(±0.02)	5.01^{a}(±0.03)	4.91^{b} (±0.03)	*
Week III	4.93^{ab}(±0.03)	5.01^{a}(±0.03)	4.91^{b} (±0.03)	*

*, $^{+}$, NS, a or b, [1] and [2] refer to the foot of Table 4.3.

4.6. Liveweight and body condition score

Mean values for the initial and final liveweight and condition score, and for changes in liveweight and condition score are presented in Table 4.6. There were no significant (P>0.05) differences between treatment groups in the initial liveweight and in liveweight change. Control groups had a higher (P<0.05) final liveweight than the low CT groups but similar to the high CT groups; the values for the low CT and high CT groups were not significantly different (P>0.05). Initial, final and change of condition score over the experiment were not affected by the CT treatments.

Table.4.6. Mean values (± sem) for initial weight, final weight, liveweight change (kg/period) initial condition score, final condition score and condition score change (unit/period) for the three treatments; control (no CT) and the low and high levels of Mimosa bark (low CT and high CT).

Mean values for	Control	Low CT[1]	High CT[2]	Level of significance for difference between treatment
Liveweight (kg)				
Initial	464 (±11)	448 (±11)	472 (±11)	NS
Final	458[a](±10)	431[b](±10)	458[ab](±10)	*
Change	-6 (±4)	-17 (±4)	-14 (±4)	NS
Condition score (units)				
Initial	4.15 (±0.10)	4.24 (±0.10)	4.26 (±0.10)	NS
Final	4.24 (±0.08)	4.23 (±0.08)	4.37 (±0.08)	NS
Change	0.09 (±0.07)	-0.01 (±0.07)	0.11 (±0.07)	NS

*, NS, a or b, [1] and [2] refer to the foot of table 4.3.

Effects on rumen digestion in sheep (Experiment 2)

4.7. Chemical analysis of the pastures fed to the sheep

The chemical analysis of the pastures used in the experiment are presented in Table 4.7.

Table 4.7. Data for the chemical analysis of the pastures fed to the sheep (percentage of dry matter; DM = dry matter; N = nitrogen; DMD = dry matter digestibility; OMD = organic matter digestibility; DOMD = digestible organic matter digestibility).

				In vitro		
	%DM*	%N	Ash	%DMD	%OMD	%DOMD
Period I	17.82	4.07	15.10	71.99	75.59	67.56
Period II	16.10	4.65	12.10	71.92	77.97	68.83

* = DM as fed to sheep

4.8. Dry matter intake

The mean values for the amount of pasture dry matter eaten by each sheep are presented in Table 4.8. There were no significant ($P>0.05$) differences in dry matter intake between control and high CT sheep in both periods I and II or in the whole period. The interaction between treatment and period was not significant. However, dry matter intake of the control group was slightly higher than that of the high CT sheep in both periods.

Table 4.8. Mean values (± sem) for dry matter intake (kg DM/day/sheep) for the two treatments; control (no CT) and the high level of Mimosa bark (high CT).

Dry matter intake	Control (No Mimosa bark)	High[1] Mimosa bark	Level of significance for difference between treatment
Period I	1.16 (±0.10)	1.02 (±0.10)	NS
Period II	0.76 (±0.10)	0.72 (±0.10)	NS
Whole period	0.96 (±0.05)	0.87 (±0.05)	NS

NS refers to the foot of table 4.3
[1] = Corresponded to 6.66 g Mimosa bark/sheep daily (= 0.48% CT daily DM eaten)

4.9. Dry matter disappearance

Mean values for percentage DM disappearance from nylon bag in the rumen at various times of incubation of pasture and mean values for *in sacco* disappearance parameters (constants) are presented in Table 4.9. The actual percentage DM disappearance from the nylon bag at various times of incubation of pastures are illustrated in Figure 4.3. Increases in DM disappearance with time were found for both groups up to 24 hours of incubation and there were no significant ($P>0.05$) differences between the two groups.

Table 4.9 also shows that there were no significant ($P>0.05$) differences in the instantly soluble fraction (A), proportional disappearance at time t (B), rate of disappearance (C) and potential DM disappearance (A+B) between control and high CT sheep. The interaction of treatment and period was also not significant.

Mean values for rate of disappearance of the high CT sheep was slightly slower, but, surprisingly, proportional disappearance (B) of the high CT sheep was slightly higher than in the control groups.

Table 4.9. Mean values (± sem) for *in sacco* dry matter disappearance (%) from the rumen of fistulated sheep for the two treatments; control (no CT) and the high level of Mimosa bark (high CT).

Time (hour) After feeding	Control	High CT[1]	Level of significance for difference between treatment
0[2]	32 (±2)	32 (±2)	NS
1	34 (±1)	34 (±1)	NS
2	42 (±2)	38 (±2)	NS
4	45 (±1)	47 (±1)	NS
8	60 (±2)	56 (±2)	NS
24	77 (±2)	76 (±2)	NS
Constants			
A (%)	31 (±1)	31 (±1)	NS
B (%)	51 (±5)	60 (±5)	NS
C (%/h)	0.1 (±0.01)	0.09 (±0.01)	NS
A+B (%)	83 (±5)	91 (±5)	NS

NS refers to the foot of table 4.3

[1] refer to the foot of table 4.8

[2] = DM disappearance in the bag due to simple solubilization was estimated by rinsing with tap water until the rinse fluid was clear.

Sheep were drenched with Mimosa bark just after feeding (morning:8.30 am and afternoon: 03.30 pm).

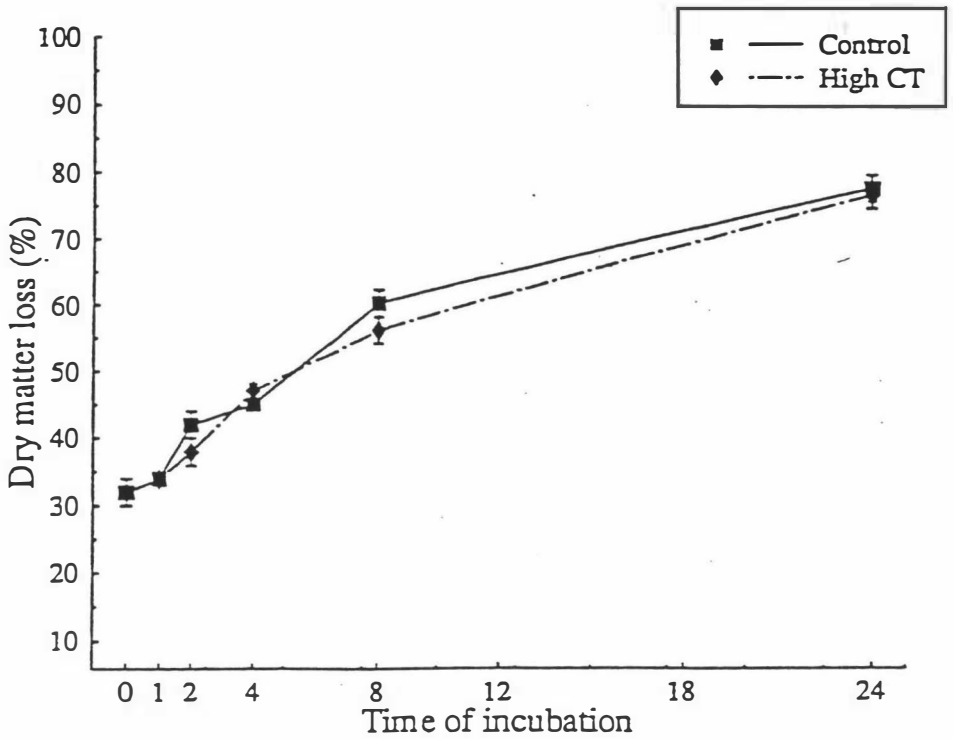


Figure 4.3. The actual percentage DM disappearance from nylon bag at various times of incubation of pastures.

4.10. Rumen ammonia concentration

Mean values for rumen ammonia concentration of sheep are given in Table 4.10 and described in Figure 4.4. At all times the values for the treatment group were lower than for the control group, but the differences were significant only at 2 hours after feeding (Table 4.10). There were significant ($P<0.05$) differences between the treatment and control groups in the whole period as analysed by the repeated measures method.

Rumen ammonia concentration reached its first peak in both groups at 2 hours after feeding. There were significant (P<0.05) differences between times of measurement in rumen ammonia concentration. In hour 2 and 4 the values of rumen ammonia concentration were higher (P<0.05) than in hour 0 and 6, but the values in hours 0 and 6 were not significantly different (P>0.05).

Table 4.10. Mean values (±sem) for rumen ammonia concentration (μg/ml) for the two treatments; control (no CT) and the high level of Mimosa bark (high CT).

Time (hour) After morning feed	Control	High CT[1]	Level of significance for difference between treatment	Mean values at each time of measurement
0 (before feeds)	179 (±10)	162 (±10)	NS	171[a](±23)
2	340 (±23)	232 (±23)	*	286[b](±23)
4	314 (±37)	229 (±37)	NS	271[b](±23)
6	219 (±17)	171 (±17)	NS	195[a](±23)
Whole period			*	

a and b means within a column with unlike superscripts differ (P<0.05)
* and NS refer to the foot of table 4.3
[1] refers to the foot of table 4.8
Sheep were drenched with Mimosa bark just after feeding (morning:8.30 am and afternoon: 03.30 pm).

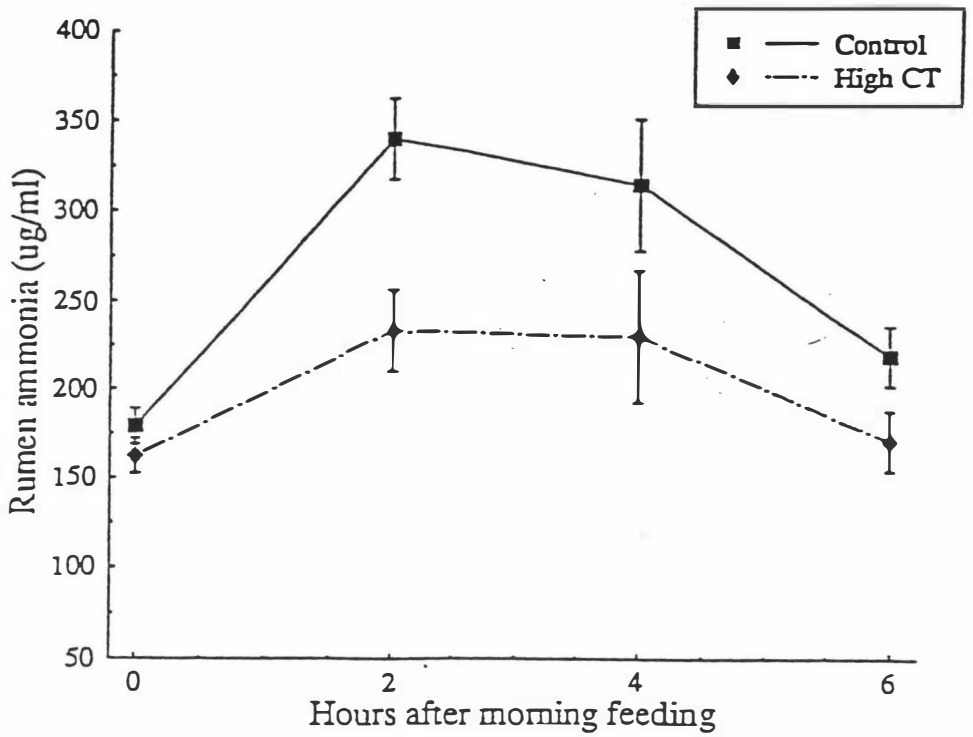


Figure 4.4. The variations in rumen ammonia concentration between treatments at various times of measurement.

4.11. Blood urea concentration

Mean values for blood urea concentration of sheep are presented in Table 4.11 and described in Figure 4.5. There were no significant ($P>0.05$) differences between the treatment and control groups at 0 and 2 hours after feeding but the differences were significant ($P<0.05$) at 4 and 6 hours after feeding. However there were significant differences ($P<0.05$) between the control and treatment in the whole period as analysed by the repeated measures method. Mean blood urea concentrations were generally lower for the treatment group. The interaction between treatment and time was not significant ($P>0.05$). There were no significant ($P>0.05$) differences between the times of measurement in blood urea concentration.

Table 4.11. Mean values (± sem) for blood urea concentration (μM/ml) for the two treatments; control (no CT) and the high level of Mimosa bark (high CT).

Time (hour) After morning feed	Control	High CT[1]	Level of significanc for difference between treatment	Mean values at each time of measurement
0 (before feed)	7.41 (±0.21)	7.13 (±0.21)	NS	7.27[a](±0.17)
2	7.86 (±0.24)	7.29 (±0.24)	NS	7.58[a](±0.17)
4	7.98 (±0.15)	7.33 (±0.15)	*	7.66[a](±0.17)
6	7.86 (±0.18)	7.18 (±0.18)	*	7.52[a](±0.17)
Whole period			*	

a = refers to the foot of table 4.9
* and NS refer to the foot of table 4.3
[1] refers to the foot of table 4.8
Sheep were drenched with Mimosa bark just after feeding (morning:8.30 am and afternoon: 03.30 pm).

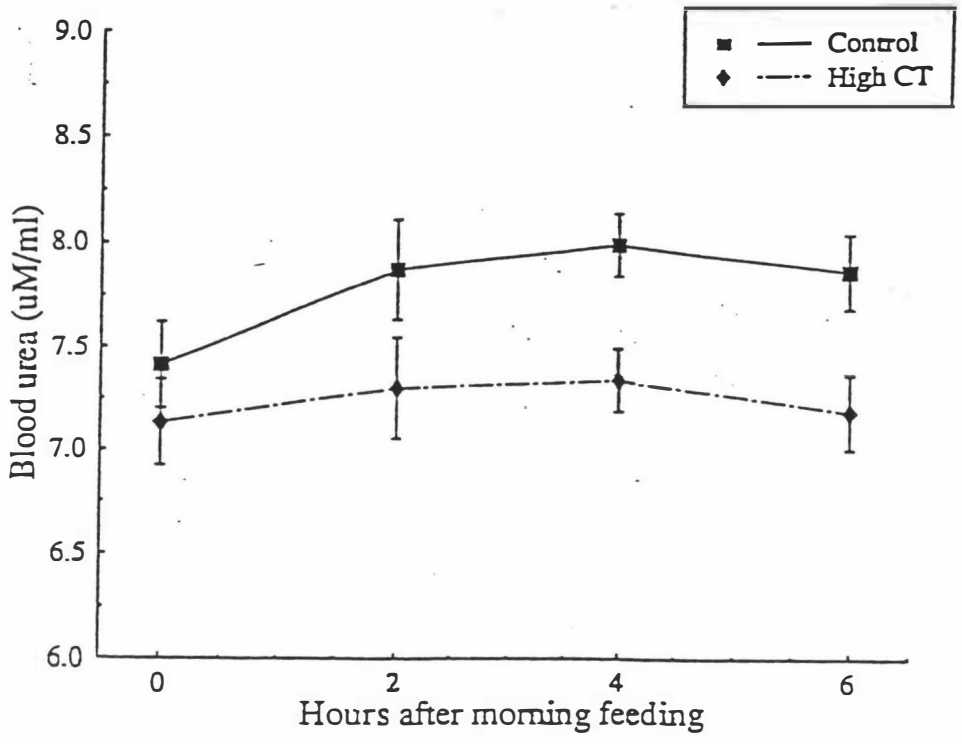


Figure 4.5. The variations in blood urea concentration between treatments at various times of measurement.

4.12. Apparent digestibility of dry matter and nitrogen

Mean values for apparent digestibility of DM and N by sheep are presented in Table 4.12. There were no significant differences between control and high CT sheep in apparent digestibility of DM either in each period or in the whole period. Sheep drenched with the high CT had significantly lower values for apparent digestibility of nitrogen in the period I, but not in period II. The interaction between treatment and period was not significant for apparent digestibility of DM but it was significant for the apparent digestibility of nitrogen.

Table 4.12. Mean values (± sem) for apparent digestibility of dry matter and nitrogen (%) for the two treatments; control (no CT) and the high level of Mimosa bark (high CT).

Apparent digestibiity	Control	High CT[1]	Level of significance for difference between treatment
Dry matter			
Period I	82.51 (±1.46)	78.54 (±1.35)	NS
Period II	81.45 (±1.37)	80.31 (±1.41)	NS
Whole period	81.98 (±0.96)	79.43 (±0.96)	NS
Nitrogen			
Period I	87.78 (±1.27)	83.16 (±1.17)	*
Period II	86.62 (±1.19)	88.16 (±1.22)	NS
Whole period	87.20 (±0.84)	85.66 (±0.84)	NS

* and NS refer to the foot of table 4.3
[1] refers to the foot of table 4.8

CHAPTER 5
DISCUSSION

5.1. Chemical analysis of the Mimosa bark extract

In the present study the Mimosa bark extract, as analysed by Wattle Bark Laboratory, contained 67.5 to 71.5% tannins which were characterised as condensed tannin (CT) (Annonymous, 1994). However the total CT concentration measured by the Massey University Nutrition laboratory was lower (50.69%) than the manufacturer's specification. The difference in concentrations between the manufacturer and Massey University nutrition laboratory analysis may be due to the different methods of determination. Another possible factor is that the University's sample of Mimosa bark extract was analysed about 10 months after the end of experiment and some of the tannin may have been oxidised through exposure to air (Feng yu *et al.*, 1996).

5.2. Concentration of condensed tannin administered to cows and sheep

Two dose rates were used in the present study with cows; 50 g and 100 g Mimosa bark extract powder per cow daily. Using the manufacturer's analysis of 70% CT, these were equivalent to 35 and 70 g CT (0.24 and 0.48% CT in DM eaten). These quantities were chosen because of the cost of the powder ($ 3 per kg), and the main interest of the sponsors was to test the ability of the powder to prevent bloat when given in commercially realistic dosages. In addition, when mixed with water, the powder formed a relatively thick viscous suspension. It was made up at 28 g powder per 100 ml of suspension, so that 100 g powder per day required 180 ml of drench to be given per cow twice daily.

Sheep were dosed at 6.66 g Mimosa bark extract powder per sheep daily. This level of dose corresponds to the highest level used in the cow's experiment (0.48% CT in DM eaten).

5.3. Dry matter intakes in cows and sheep

In the present study, all cows were grazed together so that there were no data for apparent dry matter intake for each treatment separately. The group average apparent daily dry matter intake of each cow was 17.0, 20.2, and 16.1 kgDM/cow for weeks I, II and III respectively (Table 4.2). The value for week II was higher than those for weeks I and III, perhaps due to the heavy rainfall in week II, that may have affected the measurement of pre-grazing and residual herbage mass by the Ellinbank plate meter.

Daily ME requirements for the cows in experiment 1 were estimated to be 173 MJ ME/cow (ARC,1980). Given the very high digestibility values for pasture (approximately 85% DOMD) this corresponds to a DM intake of 14 kg DM/day. The intake estimated using the Ellinbank Plate Meter were higher than this at 17.8 kg DM/day. This and the relatively high daily yield of milksolids (1.8 kg/day) implies that energy was not limiting for milk production.

The similar dry matter intakes for the control and high CT groups (Table 4.8) in the sheep experiment indicated that the level of CT drenched to the sheep did not affect intake. These observations agree with Waghorn and Shelton (1992) with *Lotus pedunculatus*, Waghorn *et al.* (1987) with *Lotus corniculatus* and McNabb *et al.* (1993) with *Lotus pedunculatus* with different CT concentration.

Waghorn and Shelton (1992) conducted an experiment on wethers aged about 15 months. One trial involved *Lotus pedunculatus* fed with pastures and the other involved *Lotus corniculatus* fed with pastures, and Polyethylene glycol (PEG) was infused to the wethers in order to inactivate CT by displacing protein from a pre-formed CT protein complex. There were no differences in dry matter intake (1.37 v 1.35 kg/d) between active CT (mixture of immature of *Lotus pedunculatus* and pastures, containing 2% CT in DM diet) and inactive CT sheep (mixture of immature of *Lotus pedunculatus* and pastures, administrated with PEG). There was also no difference between the active CT and inactive CT sheep in dry matter intake (1.25 vs 1.24 kg/d) on a mixture of *Lotus corniculatus* and pasture (containing 0.7 % CT in DM). Waghorn *et al.* (1987) reported

no differences between active CT sheep and inactive CT sheep in dry matter intake (1.40 v 1.46 kg/d) on a diet of *Lotus corniculatus* (containing 2.2% CT in DM).

However, for *Lotus pedunculatus* with 5% CT in DM, dry matter intake was reduced by 12% in sheep fed on the herbage with the active CT when compared to the inactive CT, due perhaps to a slower rate of microbial fermentation in the rumen, indicated by the larger rumen pool size, slower turn over and lower concentration of VFA (Waghorn *et al.*,1994).

5.4. Dry matter disappearance measured by the *in sacco* method in sheep

In the present study, CT of Mimosa bark extract drenched to the sheep did not decrease dry matter degradation in the rumen. This is indicated by the lack of significant differences in dry matter disappearance rates at all times and also the lack of significant differences in the instantly soluble fraction (A), proportional disappearance in time t (B), rate of disappearance (C) and potential dry matter disappearance (A+B) between control and high CT group. These results suggest that CT of Mimosa bark extract did not inhibit microbial enzyme activities in the rumen and therefore dry matter disappearance was not depressed .

In contrast, Chiquette *et al.* (1988) carried out an experiment on *Lotus corniculatus* containing 0.1-4% CT and 0.3-17.8% CT in DM using the *in sacco* method. They reported that average dry matter loss from four replicated nylon bags was lower for the high tannin than for the low tannin ration after 8, 12, and 24 hours of incubation in the rumen.

Unfortunately, protein disappearance was not directly determined in the present study. According to Voight and Piat Kowski (1987), cited by Rajcevix and Stekar (1993) a connection exists between degradability of crude protein and dry matter. Moreover, increasing the degradability of dry matter also leads to consequential increases in protein degradability. The present data suggest that the action of CT of Mimosa bark extract

was unlikely to reduce protein disappearance in the rumen although it did reduced rumen ammonia concentration.

Another factor that could influence the lack of effect of the CT of Mimosa bark extract upon dry matter disappearance in the present study may be that the CT of Mimosa bark extract was drenched via the mouth rather than contained in the forage. Therefore, the CT may not have mixed with the test sample of pasture in the nylon bag so effectively, leading to reduced effects of CT on the rate of DM disappearance in the nylon bag. However, adding Mimosa bark extract directly to the pasture material in the 3 polyester bags removed at 8 hours to produce a concentration of 17.5% CT in DM, showed no effect on DM disappearance (52% v 56% in the untreated bags).

5.5. Rumen ammonia concentrations in sheep

The rumen ammonia concentrations were significantly lower for the high CT sheep only at 2 hours after feeding. However, the significant decrease in rumen ammonia concentration of the high CT group compared with the control, as analysed for the whole period, provides evidence that the drenched CT did reduce protein breakdown in the rumen. These observations agree with that of Waghorn and Shelton (1992) with *Lotus pedunculatus* and *Lotus corniculatus*, Wang *et al.* (1995a) with *Lotus corniculatus*, Waghorn *et al.* (1994) with *Lotus pedunculatus*, Waghorn *et al.* (1994) on *Lotus pedunculatus* with different CT concentrations.

Waghorn *et al.* (1994) carried out an experiment on sheep given *Lotus pedunculatus* as sole diet (5.5% CT in DM). They reported that rumen ammonia concentrations in the active CT sheep were lower than in the inactive CT on day 21 (175 vs 458 μg NH_3N/ml) and at slaughter (283 vs 507 μg NH_3N/ml). They argued that the lower rumen ammonia concentration was associated with the reduced microbial growth and therefore reducing proteolysis of Lotus protein in the rumen caused by the CT.

In the present study, sheep drenched with the high CT consistently tended to have lower rumen ammonia concentrations at all times of measurements, but the values for rumen ammonia concentration remained above 50 mg/l which is the minimal concentration suggested for maximal microbial protein synthesis (Satter and Roffler, 1975).

5.6. Blood urea concentrations in cows and sheep

The concentration of urea in blood is closely related to the concentration of urea in the rumen, therefore the use of blood urea to predict rumen ammonia level has been suggested by Minson (1990). In the present study, cows in the high CT group (0.48% CT) had significantly lower blood urea concentrations than those in the control and low CT groups (Table 4.3). Sheep drenched with the CT of Mimosa bark at the high level (0.48% CT) also had significantly lower blood urea concentrations than the control group, analysed over the whole period. This indicated that at the high level of treatment, the establishment of CT-protein bonding in the rumen reduced the rate of protein degradation in the rumen, and therefore reduced the blood urea concentration. Similarly, West *et al.* (1993) reported that serum urea concentration declined linearly with increasing CT in the peanut skins. They suggested that the reduced serum urea indicated lower protein availability and reduced NH_3 transfer into the blood for conversion to urea and subsequent urinary excretion. These results also agree with Waghorn and Shelton (1995) and Wang *et al.* (1995b) on sheep. Waghorn and Shelton (1995) carried out an experiment on sheep given a mixture of grass and *Lotus pedunculatus* containing 1.8% CT in DM (active CT sheep) compared with those given inactive CT (by PEG infusion). They reported that in both immature and mature forages, the plasma urea concentration was significantly lower in the active CT sheep than in the inactive CT sheep (immature : 8.65 vs 9.86 mmol/lt; mature : 6.36 vs 8.32 mmol/lt). They suggested that the low plasma urea concentration in the active CT sheep indicated a lower absorption of rumen ammonia and lower absorption and or catabolism of absorbed amino acids.

5.7. Apparent digestibility of dry matter and nitrogen in sheep

In the present study there were no differences between the control and high CT group in apparent digestibility of dry matter and nitrogen (Table 4.12). This is consistent with the work of Waghorn *et al.* (1987) with *Lotus corniculatus* but not with Waghorn and Shelton (1995) with *Lotus pedunculatus* and Wang *et al.* (1995a) with *Lotus corniculatus* at various CT concentrations in apparent digestibility of dry matter. Apparent digestibility of nitrogen results are contrary to those Waghorn *et al.* (1987) and Wang *et al.* (1995a) with *Lotus corniculatus* at various CT concentrations (see Table 5.1).

Waghorn *et al.* (1987) reported that apparent digestibility of dry matter was similar for active CT sheep and inactive CT sheep (69% vs 71%) but the apparent digestibility of nitrogen was lower in the active CT sheep (70% and 78%) for *Lotus corniculatus* (2.2% CT in DM). In contrast, the apparent digestibility of dry matter and total N in *Lotus corniculatus* (2.7% CT) was significantly lower in active CT than in inactive CT sheep (Wang *et al.*, 1995a) (see Table 5.1).

Table 5.1. The effects of CT in pasture on apparent digestibility of dry matter and nitrogen.

References	Pasture	Level of CT in DM	Digestibility	
			Dry matter	Nitrogen
Waghorn *et al* (1987)	*Lotus corniculatus*	active CT (2.2%)	69%[a]	70%[a]
		inactive CT	71%[a]	78%[b]
Waghorn and Shelton (1995)	*Grass with Lotus p.*	active CT (5.2%)	72%[a]	65%[a]
		inactive CT	75%[b]	78%[b]
Wang *et al.* (1995a)	*Lotus corniculatus*	active CT (2.7%)	77%[a]	72%[a]
		inactive CT	79%[b]	80%[b]

a and b means within a coloumn with unlike superscripts differ ($P<0.05$)

Mean values for apparent digestibility of dry matter and nitrogen by the control group were slightly higher than for the high CT group in the present study. Reduction of apparent digestibility of nitrogen was found to be due to inhibition of enzyme activities (Wang *et al.*, 1995a). The rapidly increasing pH along the small intestine may enable CT-protein complexes to be reformed to protect proteins against hydrolysis by endogenous enzymes and the dissociated CT may bind to gut epithelium and affect amino acid absorption. This phenomenon could reduce the apparent nitrogen digestion in the postruminal digestive tract and therefore reduce apparent nitrogen digestibility.

5.8. Yield of milk, milk fat, milk protein and milk lactose in cows

In the present study, drenching with Mimosa bark extract to supply 0.24 and 0.48% CT in dry matter eaten had no significant effect on the yields of milk, milk fat, milk protein and milk lactose (Table 4.4).

In contrast, West *et al.*(1993) conducted an experiment on lactating Jersey cows offered a diet containing 8% and 24% peanut skins to determine the effect of the tannin component of peanut skins on cow performance. They reported that although crude protein intake was not different between treatments (3.50 and 3.67 kg/day) the yields of milk, milk fat and protein from cows fed the diet containing 6.2% tannin (24% peanut skins) were lower than for the diet containing 3.93% tannin (8% peanut skins). This was probably due to a higher protein availability in the small intestine resulted from increased crude protein digestibility in the lower tannin group (63 v 61%). Wang *et al.* (1994) conducted an experiment on the effect of CT in *Lotus corniculatus* (4% CT in DM) on lactation performance of ewes rearing twin lambs. They reported that although the organic matter intake for the active CT and the inactive CT groups were similar, the action of CT in *Lotus corniculatus* significantly increased the yields of milk, protein and lactose. They argued that the effect of CT in increasing milk volume and milk protein yield was probably due to its effect in increasing EAA (essential amino acids) absorption from small intestine.

Very little information is available about the effect of CT on milk production in grazing cows. However, several studies which investigated the effect of protein protection, using formaldehyde and fish meal with a variety of basal diets, have failed to detect significant improvements in dairy cow performance (Wachira et al., 1974; Broderick and Lane, 1978; Brookes, 1984; Penno and Carruthers, 1995).

In the present study, two factors may have contributed to the absence of any effects of the CT on milk yields. Firstly, the actual amounts of CT drenched daily were relatively low, equivalent to about 0.24% CT or 0.48% CT daily DM eaten. These values are much lower than the concentration in, for example *Lotus corniculatus* (4% in DM, Wang *et al.*1995b) or peanut skins (6% in DM, West, *et al.* 1993). Secondly, the CT were contained in Mimosa bark extract, which was drenched to the cows twice daily, which is different from the situation where the CT are contained in the forage itself. These factors may result in protein which was not protected so fully although in the high CT group blood urea concentration was reduced.

The absence of an increase in milk protein synthesis (Table 4.4 and 4.6) in the present investigation may also have been because sufficient amino acids were provided from microbial protein synthesis in the control cows, or because the pattern of extra amino acids produced may not have been appropriate for milk synthesis.

The beneficial effects of CT in reducing dietary protein degradation in the rumen, as indicated by decreasing blood urea concentration in the high CT group, however, were not accompanied by increased milk production. One possible reason is that the protein which was not degraded in the rumen by the CT group, did increase the quantity of protein that passed to the small intestine but may have decreased the quantity of microbial protein that was synthesised in the rumen. Clark *et al.* (1992) suggested this may result in only small differences in the total nitrogen that passes to the small intestine, when compared to use of unprotected proteins. Because microbial protein supplies a large proportion of the total amino acids passing to the small intestine, differences between treatments in the present study in the flow of individual amino acids into the small intestine were probably small,leading to no differences in milk production.

5.9. Milk composition in cows

In the present study, there were few significant differences between treatment groups in concentration of milk fat, milk protein and milk lactose. Among treatments, cows drenched with high CT had consistently lower lactose concentrations than the low CT group but similar to the control group (Table 4.5).

Wang *et al.* (1995b), who conducted an experiment on the effect of CT in *Lotus corniculatus* (4% CT in DM) on the performance of ewes rearing twin lambs, reported that lactose concentration increased in CT sheep although the fat concentration declined. They argued that the action of CT in *Lotus corniculatus* markedly increased lactose synthesis in the mammary gland. In addition increased amino acids availability from the action of CT increased secretion of lactose as well as protein.

5.10. Liveweight and body condition score in cows

In the present study, there were no significant differences between treatment groups in the initial, final and change in both liveweight and condition score. Similarly, Wang *et al.* (1994) who carried out an experiment on *Lotus corniculatus* (4% CT in DM) given to ewes reported that there was no significant differences in liveweight change of ewes between the active CT group and the inactive CT group.

It is well known that during early lactation dairy cows of high genetic merit lose weight, because they mobilize body reserves to produce milk (NRC, 1985). The results of the present study (3 - 7 week after lactation) were consistent with this pattern where losses in liveweight were experienced by all treatment groups during the experiment.

Orskov *et al.*(1977) postulated that a higher dietary protein concentration is required when animals are in negative energy balance and additional protein including by-pass protein appears to facilitate greater mobilization of body fat. In the present study, CT (low and high) treatment group tended (but not significantly) to lose more liveweight

than the control group suggesting that the action of CT may have facilitated the mobilization of body fat.

CHAPTER 6
CONCLUSION

The most significant findings of the experiments with cows and sheep in the present study were that the drenching of CT in Mimosa bark extract did cause reduced blood urea concentration in both studies (Table 4.3 and 4.11) and it reduced rumen ammonia concentration in sheep (Table 4.10). This indicates that the CT did have some biological effect in the rumen, namely a reduced protein degradation in the rumen. This result is similar to other studies with CT (Waghorn and Shelton, 1995; West et al.,1993; Wang et al., 1995a). However, assuming there is a high correlation between the degradability of protein and degradability of dry matter in the rumen (Rajcevix and Stekar, 1993), this effect was not consistent with the lack effect on the rate of disappearance of dry matter in the rumen (Table 4.12).

The ability of CT to improve nutritive value of pasture by protecting dietary protein from rumen degradation depends upon the types or sources of CT (Waghorn and Shelton, 1995). With Lotus CT, levels of 2 - 4% in DM have been recommended to provide beneficial effects (Barry, 1989), while a slightly higher level would be expected to be most effective for Sainfoin.

In the present study, the level of CT from Mimosa bark extract of 0.48% CT in DM has reduced rumen ammonia concentration and blood urea concentration in the sheep experiment. The ideal concentration of CT to reduce protein degradation in the rumen in sheep is expected to be different from the ideal concentration for dairy cows because there are differences between these species in the retention time of feed in the rumen, amount of feed eaten per unit body weight and the extent of chewing (Chalupa, 1988). In the present study with cows, the CT in Mimosa bark extract at the level of 0.48% CT did reduce blood urea concentration, but had no effect on milk production and milk composition. However, because this is the first report on the effect of CT in dairy cows drenched with Mimosa bark extract upon milk production and milk composition, and as very high level of CT (4% CT in DM) has been shown to increase milk production and milk composition in ewes fed *Lotus corniculatus* (Wang et al., 1995b), it seems that

further study is required in terms of the delivery of NAN (non ammonia nitrogen) to the abomasum and absorption of amino acids from the intestine as well as milk production using higher concentrations of dietary CT.

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APPENDIX 1

Table 1. Data for the nitrogen analysis of the faeces of the sheep (percentage of dry matter)

Sheep no	Treatment	Period	% Nitrogen
1	control	1	2.80
2	control	1	2.85
3	control	1	2.61
4	high CT	1	3.24
5	high CT	1	2.84
6	high CT	1	2.79
1	high CT	2	3.27
2	high CT	2	2.95
3	high CT	2	3.41
4	control	2	2.93
5	control	2	2.95
6	control	2	3.20